



Transplant International



Nurturing a Sustainable Transplantation Journey:
The Best of the ESOT Congress 2025



Transplant International



frontiers

| Publishing Partnerships

EDITOR-IN-CHIEF

Thierry Berney

DEPUTY EDITORS-IN-CHIEF

Oriol Bestard

Nina Pilat

Stefan Schneeberger

Maria Irene Bellini

(and Social Media Editor)

Núria Montserrat

(Honorary)

EXECUTIVE EDITORS

Frederike Ambagtsheer,
Rotterdam

Olivier Aubert,
Paris

Federica Casiraghi,
Bergamo

John Forsythe,
London

Nazia Selzner,
Toronto

Olivier Thaumat,
Lyon

ASSOCIATE EDITORS

Emin Baris Akin, Istanbul

Chiara Becchetti, Niguarda

Louise Benning, Heidelberg

Marina Berenguer, Valencia

Ekaterine Berishvili, Tbilisi

Marius Berman, Cambridge

Saskia Bos, Leuven

Ana Cristina Breithaupt-Faloppa,
São Paulo

Eline M. Bunnik, Rotterdam

Jadranka Buturovic-Ponikvar,
Ljubljana

Laurens J. Ceulemans, Leuven

Ligia Camera Pierrotti, São Paulo

Lionel Couzi, Bordeaux

Fabienne Dobbels, Leuven

Fabian Eibensteiner, Vienna

Laure Elkrief, Tours

Juliet Emamaullee, Rochester

Constança Figueiredo, Hanover

Maddalena Giannella, Bologna

Lorenzo Giovannico, Bari

Nicholas Gilbo, Liège

Hermien Hartog, Groningen

Sarah Hosgood, Cambridge

Nichon Jansen, Leiden

Marta Jimenez-Blanco, Madrid

Nassim Kamar, Toulouse

Nicos Kessar, London

Katja Kotsch, Berlin

L. Leonie van Leeuwen,

Groningen

Wai H. Lim, Perth

Mehdi Maanaoui, Lille

Olivier Manintveld, Rotterdam

Oriol Manuel, Lausanne

Beat Moeckli, Geneva

Letizia Morlacchi, Milan

Anna Mrzljak, Zagreb

Greg Nowak, New York City

Haizam Oubari, Somerville

Fernanda Ortiz, Helsinki

David Paredes-Zapata, Barcelona

Lorenzo Piemonti, Milan

Axel Rahmel, Frankfurt am Main

Juliano Riella, Atlanta

Elvana Rista, Tirana

Hanne Scholz, Oslo

Norihisa Shigemura, Pittsburgh

Piotr Socha, Warsaw

Donzilia Sousa Silva, Porto

Richard Taubert, Hanover

Rachel Thomas, Edinburgh

Christian Toso, Geneva

Pablo Daniel Uva, Buenos Aires

Pedro Ventura-Aguiar, Barcelona

Tiffany Wong, Hong Kong

Dafna Yahav, Ramat Gan

EDITOR-IN-CHIEF EMERITUS

Ferdinand Mühlbacher, Vienna

STATISTICAL EDITOR

Silvia Pineda, Madrid

Thomas Neyens, Leuven

EDITORIAL FELLOWS

Antonio Citro,
Milan

Delphine Kervella,
Barcelona

Emilien Seizilles De Mazancourt,
Paris

Arianna Trizzino,
Pisa

Andrea Zajacova,
Leuven

SOCIAL MEDIA EDITORS

Christophe Masset, Nantes

Marco Pascale, Rome

ESOT Project Manager

Ketevan Rukhadze

GRAPHIC DESIGNERS

Salome Tvildiani

Levan Kutateladze

Editorial Office

Nathan Masters

Richard Hales

ti@frontierspartnerships.org

Transplant International Book Copyright Statement

The copyright in the text of individual articles in this eBook is the property of their respective authors or their respective institutions or funders. The copyright in graphics and images within each article may be subject to copyright of other parties. In both cases this is subject to a license granted to Frontiers. The compilation of articles constituting this eBook is the property of Frontiers.

Each article within this eBook, and the eBook itself, are published under the most recent version of the Creative Commons CC-BY licence. The version current at the date of publication of this eBook is CC-BY 4.0. If the CC-BY licence is updated, the licence granted by Frontiers is automatically updated to the new version.

When exercising any right under the CC-BY licence, Frontiers must be attributed as the original publisher of the article or eBook, as applicable.

Authors have the responsibility of ensuring that any graphics or other materials which are the property of others may be included in the CC-BY licence, but this should be checked before relying on the CC-BY licence to reproduce those materials. Any copyright notices relating to those materials must be complied with.

Copyright and source acknowledgement notices may not be removed and must be displayed in any copy, derivative work or partial copy which includes the elements in question.

All copyright, and all rights therein, are protected by national and international copyright laws. The above represents a summary only. For further information please read Frontiers' Conditions for Website Use and Copyright Statement, and the applicable CC-BY licence.

ISSN 1432-2277

ISBN 978-2-8325-8715-7

DOI 10.3389/978-2-8325-8715-7

Generative AI statement

Any alternative text (Alt text) provided alongside figures in the articles in this eBook has been generated by Frontiers with the support of artificial intelligence and reasonable efforts have been made to ensure accuracy, including review by the authors wherever possible. If you identify any issues, please contact us.

Nurturing a Sustainable Transplantation Journey: The Best of the ESOT Congress 2025

Special Issue Editors

Olivier Thauinat — Université de Lyon; Université Claude Bernard Lyon 1, France

Colin Wilson — Newcastle University and Cambridge University, United Kingdom

Thierry Berney — Editor-in-Chief

Maria Irene Bellini — Deputy Editor-in-Chief and Social Media Editor

Nina Pilat — Deputy Editor-in-Chief

Oriol Bestard — Deputy Editor-in-Chief

Stefan Schneeberger — Deputy Editor-in-Chief



Table of contents

Editorial

- 10 **Nurturing a Sustainable Transplantation Journey: the best of ESOT Congress 2025**
DOI: 10.3389/ti.2026.17127
Olivier Thaumat and Colin Wilson on behalf of the Scientific Program Committee

Review

- 15 **Back in Circulation: A Review of the Implementation of Thoracoabdominal Normothermic Regional Perfusion in Donation After Circulatory Death in Lung Transplantation**
DOI: 10.3389/ti.2026.15272
Anna Niroomand, Stephanie Chang and Sandra Lindstedt
This review covers the critical foundations of the institution of thoracoabdominal normothermic regional perfusion. It covers the conceptualization of the technique, current practices, and reviews the literature on its use in lung transplantation.
- 21 **%dd-cfDNA: The New Frontier for Heart/Lung Transplant Surveillance?**
DOI: 10.3389/ti.2025.15555
Sean Agbor-Enoh, Ethan Fraser, Nitin Nadella, Temesgen E. Andargie and Muhtadi Alnababteh
Percent donor-derived cell-free DNA offers non-invasive rejection surveillance in heart and lung transplantation; our review synthesizes performance versus current standards, clarifies blind spots and confounders, and outlines future assays and pragmatic pathways for balanced clinical adoption.
- 32 **From Banff 1991 to Today: The Changing Landscape of the v-Lesion in Kidney Transplant Rejection**
DOI: 10.3389/ti.2025.14818
Karolien Wellekens, Priyanka Koshy, Candice Roufosse and Maarten Naesens
Informed by evolving clinical and molecular insights, this review calls for an update of the Banff classification to better define the isolated v-lesion phenotype.

- 45 **Interactions Between Immunosuppressive Regimens and Cytomegalovirus Infection After Solid-Organ Transplantation**
DOI: 10.3389/ti.2026.15987
Lucas Milo Bellier, Hannah Kaminski, Pierre Merville and Lionel Couzi
Immunosuppressive regimens shape CMV risk after solid-organ transplantation by weakening—or occasionally supporting—CMV-specific cellular immunity. ATG and belatacept most strongly predispose to clinically significant CMV, whereas mTOR inhibitors uniquely curb CMV through combined antiviral and immune-reinforcing effects.
- 57 **Immune Monitoring Goes Viral – Torque Teno Virus for Immunologic Risk Stratification After Kidney Transplantation**
DOI: 10.3389/ti.2025.15074
Konstantin Doberer, Sebastian Kapps, Frederik Haupenthal and Gregor Bond
TTV is a highly prevalent and non-pathogenic virus that reflects the host's immune status. It has been proposed for immunologic monitoring. This review emphasizes practical aspects of TTV-guided monitoring and highlights recent advancements in the field of kidney transplantation.

Original Research

- 67 **The UK National Appeals Panel Safely Extends Access to Liver Transplantation for Candidates Beyond Standard Listing Criteria**
DOI: 10.3389/ti.2026.15573
Abdul Rahman Hakeem, Sahil Gupta, Rhiannon Taylor, Tassos Grammatikopoulos, Steven Masson, Raj Prasad, Doug Thorburn, Krishna Menon, Derek Manas and Varuna Aluvihare
The UK National Appeals Panel ensures equitable and safe access to liver transplantation for patients beyond standard listing criteria, achieving excellent graft and patient outcomes across diverse and high-risk indications.
- 78 **Novel Allocation Strategies Can Boost Kidney Exchange Programs: A Monte Carlo Simulation**
DOI: 10.3389/ti.2026.15423
Mattheüs F. Klaassen, Marry de Klerk, Marije C. Baas, Hanneke Bouwsma, Laura B. Bungener, Maarten H. L. Christiaans, Twan Dollevoet, Kristiaan Glorie, Sebastiaan Heidt, Aline C. Hemke, Margriet F. C. de Jong, Judith A. Kal-van Gestel, Marcia M. L. Kho, Jeroen D. Langereis, Karlijn A. M. I. van der Pant, Claudia M. Ranzijn, Dave L. Roelen, Eric Spierings, Christina E. M. Voorter, Jacqueline van de Wetering, Arjan D. van Zuilen, Joke I. Roodnat and Annelies E. de Weerd
Kidney exchange programs can double transplant rates of difficult-to-match patients, by allowing incompatible matches for them. For compatible pairs, kidney exchange participation resulted in half of them being matched with fewer HLA-mismatches, while simultaneously enabling transplants for incompatible patients.

- 90 No Kidney Left Behind: Rescuing Unused Donor Kidneys for Transplant at the First Centralized Assessment and Repair Center**
DOI: 10.3389/ti.2025.15424
Christopher L. Jaynes, William C. Goggins, Matthew L. Holzner, Jacqueline Garonzik-Wang and Henri G. D. Leuvenink
In the United States, 30% of all kidneys recovered with the intent to transplant go unused annually. The first centralized assessment and repair center (ARC) offering sub-normothermic acellular perfusion (SNAP) services addresses this disparity with a 90% transplant rescue rate.
- 100 Nationwide Hypothermic Machine Perfusion for ECD and DCD Kidney Transplantation in Belgium: One-Year Outcomes and Impact on Transplant Rates and Budget Impact Analysis**
DOI: 10.3389/ti.2025.15282
Tom Darius, Ina Jochmans, Maxime Foguene, Eric Hoste, Caren Randon, Bart Bracke, Geert Roeyen, Nicholas Gilbo, Laurent Weekers, Daniel Jacobs-Tulleneers-Thevissen, Karl Martin Wissing, Tineke Bogaerts, Dimitri Mikhalski, Jean-Philippe De Wilde, Joël Daems and Jacques Pirenne
The national implementation of a centralized HMP service in Belgium led to excellent one-year transplant outcomes, increased ECD/DCD kidney utilization rate, and substantial healthcare cost savings. These findings support HMP(02) as the standard of care for kidney preservation in Belgium.
- 112 Duodenoduodenostomy as an Attractive Option for Exocrine Drainage in Pancreas Transplantation: Insights From a Single-Center Cohort**
DOI: 10.3389/ti.2025.15430
Alba Torroella, Rongrong Hu Zhu, Carlos Castillo-Delgado, Marco Pavesi, Ramón Rull, Emma Folch-Puy, Rocío García, Clara Bassaganyas, Carles Pérez-Serrano, Pedro Ventura-Aguiar, Enrique Montagud-Marrahi, Víctor Emilio Holguin, Antonio J. Amor, Fritz Diekmann, Ángeles García-Criado, Juan Carlos García-Valdecasas, Josep Fuster and Joana Ferrer-Fàbrega
Duodenoduodenostomy offers a technically favorable approach for exocrine drainage in pancreas transplantation, with the added advantage of enabling both percutaneous and endoscopic biopsy access, making it a safe, versatile, and contemporary option among current surgical techniques.
- 125 Five Years Follow-up of Imlifidase Desensitized Kidney Transplant Recipients**
DOI: 10.3389/ti.2025.15425
Tomas Lorant, Bonnie E. Lonze, Robert A. Montgomery, Niraj M. Desai, Christophe Legendre, Torbjörn Lundgren, Bengt von Zur-Mühlen, Ashley A. Vo, Kristoffer Sjöholm, Anna Runström, Jan Tollemar and Stanley C. Jordan
Five-years follow-up in HLA-incompatible kidney transplant recipients after desensitization with imlifidase reveals a patient survival of 90% and graft survival of 82%. Kidneys have good function with a mean eGFR of 50 mL/min/1.73 m² and decreasing DSA levels over time.

- 134 Blockade of CD155 and CD276 by Monoclonal Antibodies Fosters Immune Tolerance and Promotes Stable Engraftment of iPSC-Derived Islets in Allogeneic Humanized Mice**
DOI: 10.3389/ti.2025.15433
G. Siracusano, F. Deambrogio, V. Sordi, M. Malnati, L. Piemonti and R. Chimienti
Blocking CD276 and CD155 enhances survival of stem cell-derived pancreatic grafts by limiting NK and monocyte activation and promoting regulatory T cell expansion in humanized mice.
- 148 Efficacy of Intravenous Immunoglobulin in Eliminating De Novo Donor-Specific Antibodies After Lung Transplantation: Importance of Early Intervention**
DOI: 10.3389/ti.2025.15350
Maximilian Vorstandlechner, Philip Degenfelder, Gökce Yavuz, Olaf M. Glueck, Julia R. Kovács, Julia Walter, Andrea Dick, Sebastian Michel, Christian P. Schneider, Michael Zoller, Jürgen Barton and Teresa Kauke
Preemptive initiation of IVIG therapy before clinical onset of antibody-mediated rejection predicts donor-specific antibody clearance after lung transplantation, supporting early therapeutic intervention strategies.
- 157 Donor-Specific Blood Transfusion Induces a Transfusion-Related Early Protective Effect in Murine Lung Transplantation**
DOI: 10.3389/ti.2026.15409
Xin Jin, Charlotte Hooft, Balin Özsoy, Jan Van Slambrouck, Janne Kaes, Cedric Vanluyten, Annalisa Barbarossa, Marianne S. Carlon, Greetje Vande Velde, Mélanie Guyot, Karen Moermans, Steve Stegen, Robin Vos, Bart Vanaudenaerde, Jacques Pirenne and Laurens J. Ceulemans
Donor-specific blood transfusion (DSBT) induces systemic myeloid recalibration, expanding pro-reparative monocytes and cDC2s to provide a critical 7-day window of immunological protection against early lung transplant rejection.
- 169 Cellular Immunity in Chronic Kidney Disease and Changes After Kidney Transplantation**
DOI: 10.3389/ti.2026.15622
Georgios Lioulis, Eleni Moisidou, Michalis Christodoulou, Efstratios Kasimatis, Alikì Xochelli, Evaggelos Memmos, Stamatia Stai, Maria Stangou and Asimina Fylaktou
Kidney transplantation may restore total lymphocytes counts, already during the first year. However, long term graft survival comes at a cost of increased senescent CD28- T cells, independently of age.

182 Association Between Cytomegalovirus Viremia Clearance and Post-Solid Organ Transplant Mortality in Patients With Refractory Cytomegalovirus Infection: SOLSTICE Post Hoc Analysis

DOI: 10.3389/ti.2025.15331

Nassim Kamar, Robin K. Avery, Tien Bo, Joan Gu, Deepali Kumar and Oliver Witzke

All-cause mortality in solid organ transplant recipients with refractory cytomegalovirus infection was assessed at Week 20 based on cytomegalovirus clearance at Week 8. Mortality was higher in recipients who did not achieve clearance than those who did ($p = 0.0024$).

190 Evaluating Post-Transplant Outcomes in Elderly Liver Recipients Over 70: A Propensity-Score Matching Analysis

DOI: 10.3389/ti.2025.15429

Edoardo Prosperi, Enrico Prosperi, Matteo Serenari, Chiara Bonatti, Guido Fallani, Alberto Stocco, Giorgia Radi, Federica Mirici Cappa, Cristiana Laici, Antonio Siniscalchi, Maria Cristina Morelli, Matteo Ravaioli and Matteo Cescon

Age alone doesn't predict liver transplant success. Well-selected patients aged ≥ 70 can achieve survival similar to younger recipients when evaluated with a comprehensive, individualized selection process.



Are You a Member?

Join us to help shape and nurture the future of ESOT!

Learn more about membership benefits, access, discounts, including reduced publishing fees in Transplant International

ESOT Membership

TOP



NEVER STOP

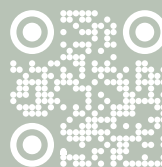
ESOTTRANSPLANTLIVE.ORG

ESOTTRANSPLANTLIVE.ORG

In transplantation, there is no time to waste. And yet your expertise has to stay current. The redesigned Transplant Live supports the way you work, with simplified login, clearer navigation, optimised search tools, open access to accredited courses, multidisciplinary case discussions and expert-led webinars.

Transplant Live

The online education platform of the European Society for Organ Transplantation



WHERE EXPERTISE EVOLVES



OPEN ACCESS

*CORRESPONDENCE

Olivier Thauvat,
✉ olivier.thauvat@inserm.fr

RECEIVED 12 June 2026
REVISED 12 June 2026
ACCEPTED 25 June 2026
PUBLISHED 14 July 2026
CORRECTED 28 August 2026

CITATION

Thauvat O and Wilson C (2026) Nurturing a Sustainable Transplantation Journey: the best of ESOT Congress 2025. *Transpl. Int.* 39:17127. doi: 10.3389/ti.2026.17127

COPYRIGHT

© 2026 Thauvat and Wilson. This is an open-access article distributed under the terms of the [Creative Commons Attribution License \(CC BY\)](https://creativecommons.org/licenses/by/4.0/). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

Nurturing a Sustainable Transplantation Journey: the best of ESOT Congress 2025

Olivier Thauvat^{1,2,3*} and Colin Wilson^{4,5,6} on behalf of the Scientific Program Committee

¹CIRI, Centre International de Recherche en Infectiologie, Université de Lyon, INSERM U1111, Université Claude Bernard Lyon 1, CNRS, UMR5308, ENS de Lyon, Lyon, France, ²Department of Nephrology, Transplantation and Clinical Immunology, Hospices Civils de Lyon, Groupement Hospitalier Centre, Lyon, France, ³Lyon-Est Medical Faculty, Claude Bernard University (Lyon 1), Lyon, France, ⁴National Institute of Health and Care Research Blood and Transplant Research Unit, Newcastle University and Cambridge University, Newcastle upon Tyne, United Kingdom, ⁵Translational and Clinical Research Institute, Newcastle University, Newcastle upon Tyne, United Kingdom, ⁶Institute of Transplantation, Freeman Hospital, Newcastle upon Tyne, United Kingdom

Introduction

This editorial introduces the special issue dedicated to the ESOT London Congress 2025, providing a conceptual and scientific synthesis framed around the “transplantation journey” and the emerging paradigm of sustainable and robust transplantation.

By curating 17 key contributions from the congress, it highlights how recent advances across the entire transplant pathway (from access to long-term graft stewardship) are collectively reshaping contemporary transplant practice.

A central contribution of this congress was the introduction of “robustness” as a solution to improve the sustainability of transplant systems that need not only to be efficient and innovative but also resilient to demographic shifts, biological complexity, and organizational constraints.

Overall, this special issue outlines a forward-looking roadmap for transplantation, bridging technological innovation with responsible implementation and offering a coherent vision for the next era of transplant research and patient care.

From innovation to responsibility: a new vision for transplantation

The ESOT Congress 2025, held in London from June 29 to July 2, brought together the global transplantation community around a shared vision for the future of the field. The remarkable success of the meeting reflected the enthusiasm of the international community for this ambition. The congress welcomed 3,192 delegates from 91 countries, received 1,655 abstract submissions, generated 989 social media posts using #ESOTCongress, and attracted coverage in more than 1,300 press articles. These figures testify not only to the vitality of transplantation research but also to the growing recognition that transplantation has an important role to play in addressing some of the broader challenges facing modern healthcare systems.

The London congress was organized around a theme that was both timely and transformative: “Nurturing the Sustainable Transplantation Journey.” More than a slogan, this theme embodied a collective commitment to reimagining transplantation through the lens of environmental responsibility, and long-term societal impact. The striking animated cover artwork of this special issue captures this vision with remarkable clarity, illustrating the congress philosophy that progress in transplantation should be measured not only by scientific advances but also by our ability to deliver those advances responsibly. However, as highlighted by the

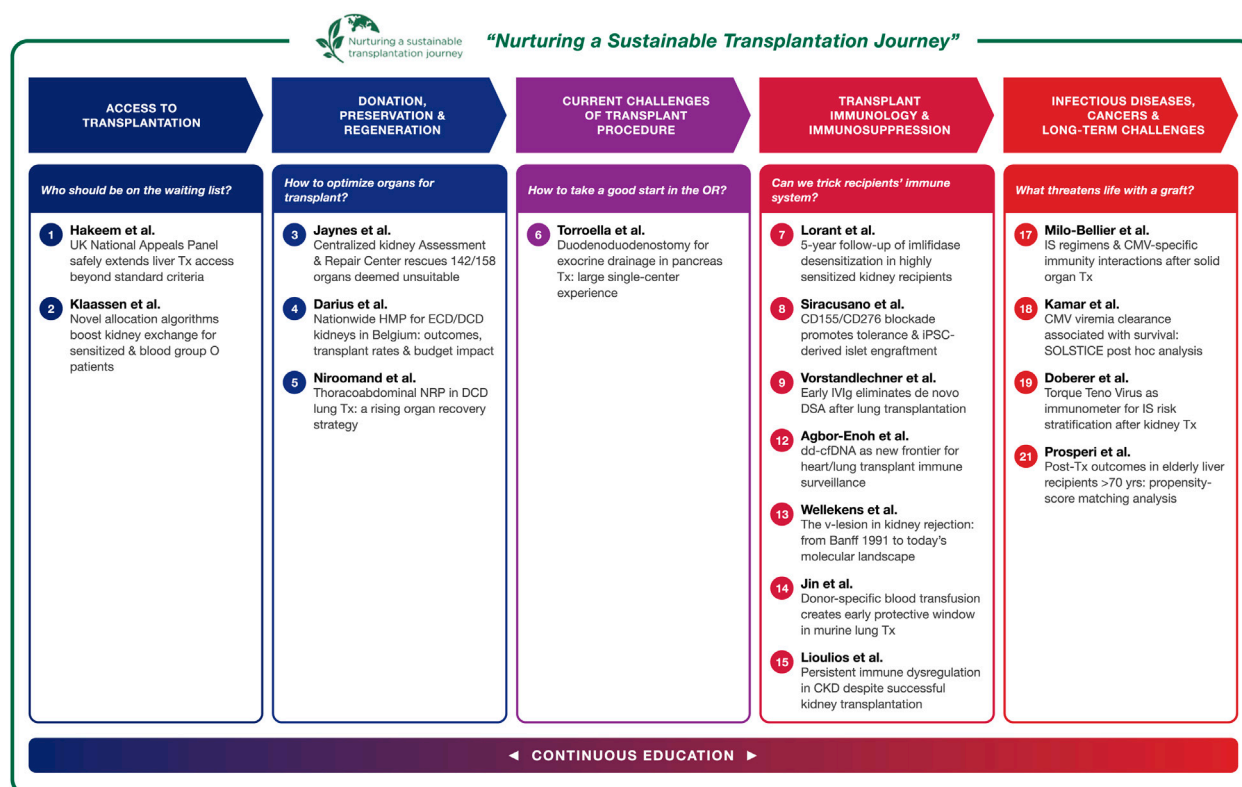


FIGURE 1

The 17 best abstracts selected from the ESOT Congress 2025 (London, 29 June – 2 July), distributed across the five scientific tracks of the Transplantation Journey. The overarching congress theme—Nurturing a Sustainable Transplantation Journey—Numbers correspond to references cited in the editorial text. References 10–11 (Charmetant et al.) are contextual citations, not congress abstracts. Abbreviations: HMP: hypothermic machine perfusion; NRP: normothermic regional perfusion; DCD: donation after circulatory death; IS: immunosuppression; CMV: cytomegalovirus; DSA: donor-specific antibodies; dd-cfDNA: donor-derived cell-free DNA; CKD: chronic kidney disease; Tx: transplantation.

Congress Presidents in their welcome address, sustainable transplantation extends far beyond reducing the ecological footprint of healthcare. It encompasses every stage of the transplant pathway—from donor identification and organ procurement to recipient care and lifelong graft stewardship. Central to this vision was the concept of *robustness*, introduced at the congress as the operational counterpart of sustainability. European healthcare systems now prioritize efficiency and productivity—directly eroding human robustness and resilience. A truly sustainable transplantation system must also be a robust one: capable of withstanding disruption, adapting to demographic and epidemiological changes, and delivering reliable outcomes across diverse clinical contexts and healthcare settings. In this perspective, robustness must be a systemic attribute embedded throughout the entire transplantation journey.

This special issue brings together seventeen outstanding contributions selected from the congress. Organized according to the “Transplantation Journey” framework that structured the scientific programme, these articles illustrate how the field continues to evolve—from expanding access to transplantation and optimizing organ preservation to advancing immunological monitoring and addressing the long-term challenges faced by transplant recipients (Figure 1). Together, they provide a compelling snapshot of a discipline committed not only to

innovation, but also to building a more sustainable and robust future for transplantation.

Expanding access to transplantation

The transplantation journey begins long before surgery. Ensuring equitable access to transplantation remains one of the most important challenges facing the field.

Hakeem et al [1] provide a compelling evaluation of the United Kingdom National Appeals Panel for liver transplantation. Their analysis demonstrates that carefully governed exception pathways can safely extend transplantation to patients who fall outside conventional listing criteria. Despite the complexity and severity of many approved cases, long-term outcomes remained excellent, illustrating how expert review mechanisms can reconcile equity with responsible stewardship of scarce organs.

Similarly, Klaassen et al [2] address another persistent barrier to access: the limited opportunities available to highly sensitized and blood group O patients within kidney exchange programs. Through sophisticated Monte Carlo simulations, they show how innovative allocation algorithms incorporating ABO-incompatible and low-level HLA-incompatible matching can substantially increase transplantation rates for difficult-to-match candidates. Their

findings illustrate how intelligent redesign of allocation systems can unlock opportunities without requiring additional donor organs.

Together, these studies remind us that sustainability begins with fairness. Maximizing the benefit derived from every donated organ requires allocation systems capable of adapting to increasingly complex patient populations while maintaining transparency and public trust.

Optimizing organs: preservation, assessment, and regeneration

The second stage of the transplantation journey focuses on ensuring that every potentially transplantable organ reaches a recipient in optimal condition.

Perhaps no article embodies this philosophy more directly than the report by Jaynes and colleagues [3] describing the first centralized kidney Assessment and Repair Center in the United States. By combining subnormothermic acellular perfusion with centralized viability assessment, the program successfully rescued 142 of 158 kidneys initially considered unsuitable for transplantation. This remarkable achievement demonstrates how novel organizational models may dramatically reduce organ discard while simultaneously improving logistics and organ sharing.

The nationwide Belgian experience reported by Darius and collaborators [4] further illustrates the transformative potential of machine perfusion technologies. Following implementation of a nationally reimbursed hypothermic machine perfusion service, Belgium observed excellent clinical outcomes, increased utilization of DCD kidneys, and substantial healthcare savings. Importantly, the study demonstrates how preservation technologies can generate benefits extending beyond graft outcomes to healthcare system sustainability.

Expanding the donor pool also requires innovation in organ procurement. In their comprehensive review, Niroomand et al [5] examine thoracoabdominal normothermic regional perfusion in donation after circulatory death. By restoring circulation after death determination, this approach mitigates ischemic injury while enabling functional organ assessment. The growing experience with this technique suggests it may become a key component of future strategies aimed at increasing organ availability without compromising quality.

These contributions collectively highlight a major trend emerging across transplantation: the transition from passive preservation toward active organ management. Organs are increasingly viewed not as static biological products but as dynamic systems that can be assessed, optimized, repaired, and even regenerated before transplantation.

Refining transplantation procedures

Technical excellence remains fundamental to successful transplantation, and advances in surgical practice continue to improve outcomes.

Torroella et al [6] revisit one of the enduring challenges in pancreas transplantation: the optimal method for exocrine drainage. Their large single-center experience with duodenoduodenostomy demonstrates excellent graft and patient survival with acceptable

complication rates. Beyond the technical details, this work illustrates how continuous refinement of surgical techniques can incrementally improve outcomes and better reproduce physiological conditions.

Such studies remind us that innovation in transplantation is not limited to breakthrough technologies. Careful reassessment of established practices remains an essential driver of progress.

Understanding and controlling alloimmunity

The largest group of contributions in this special issue focuses on transplant immunology, reflecting the central role of immune-mediated injury in determining long-term graft survival.

Lorant and colleagues [7] provide encouraging five-year follow-up data on imlifidase-enabled kidney transplantation in highly sensitized recipients. Patients traditionally considered among the most difficult to transplant achieved excellent patient and graft survival despite their extreme immunological risk. These results demonstrate how targeted desensitization strategies can expand access while maintaining favorable long-term outcomes.

At the other end of the spectrum, Siracusano et al [8] offer a glimpse into the future of regenerative transplantation. Using a humanized mouse model, they show that dual blockade of CD155 and CD276 promotes tolerance and facilitates engraftment of iPSC-derived pancreatic islets. As cell therapies move closer to clinical application, such targeted immunomodulatory approaches may become essential for achieving durable graft acceptance without broad immunosuppression.

The complexity of alloimmune responses is further explored through several complementary studies. Vorstandlechner and colleagues [9] demonstrate that early intravenous immunoglobulin therapy may facilitate clearance of donor-specific antibodies after lung transplantation. If these findings are confirmed and are not merely the consequence of the spontaneous disappearance of the dnDSA induced by the inverted direct pathway [10, 11], they would support a proactive intervention before clinical manifestations emerge. Agbor-Enoh and collaborators [12] review the growing role of donor-derived cell-free DNA as a non-invasive biomarker capable of transforming rejection surveillance in thoracic transplantation.

Meanwhile, Wellekens et al [13] revisit one of the most debated lesions in transplant pathology: intimal arteritis. Their historical and contemporary analysis challenges simplistic interpretations of the v-lesion and advocates a more nuanced understanding that incorporates evolving molecular and clinical insights.

Jin et al [14] provide intriguing experimental evidence that donor-specific blood transfusion may transiently reshape the early inflammatory environment after lung transplantation. Although insufficient alone to prevent chronic rejection, this strategy may create a therapeutic window for additional immunomodulatory interventions.

Finally, Lioulis et al [15] remind us that transplantation occurs against a backdrop of profound immune dysregulation associated with chronic kidney disease [16]. Their analysis reveals persistent alterations in regulatory and senescent immune populations even after successful transplantation, highlighting the complexity of immune recovery and adaptation.

Taken together, these studies demonstrate that modern transplant immunology is moving beyond the traditional dichotomy of rejection versus tolerance. Increasingly sophisticated tools are allowing clinicians to characterize, monitor, and manipulate immune responses with unprecedented precision.

Long-term challenges: infection, monitoring, and aging recipients

The transplantation journey does not end with graft implantation. Long-term success depends on maintaining a delicate balance between immune suppression and immune competence.

Several contributions address cytomegalovirus, one of the most important infectious complications in transplantation. Milo-Bellier et al [17] provide a timely review of the complex interactions between immunosuppressive agents and CMV-specific immunity. Their work emphasizes that infectious risk reflects the cumulative impact of immunosuppression rather than the effects of individual drugs.

Kamar et al [18] extend this discussion through a *post hoc* analysis of the SOLSTICE trial. Their findings demonstrate a striking association between CMV clearance and survival, with no deaths observed among patients achieving viral eradication. These results reinforce the importance of effective antiviral management as a determinant of long-term outcomes.

Doberer et al [19] explore another promising avenue for individualized care through the use of Torque Teno virus as a putative “immunometer” (i.e., biomarker of net immunosuppression [20]). Although challenges remain before widespread implementation, this approach exemplifies the broader movement toward precision immunosuppression guided by objective biological markers.

Finally, Prosperi et al [21] address an increasingly relevant demographic challenge: transplantation in older recipients. Their propensity-matched analysis demonstrates that carefully selected patients over 70 years of age can achieve outcomes comparable to younger recipients. As populations age worldwide, such findings will become increasingly important for ensuring equitable access while maximizing the societal benefits of transplantation.

Looking forward

The articles collected in this special issue collectively illustrate a field in transition. Across every stage of the transplantation journey, researchers and clinicians are seeking ways to deliver better outcomes while using resources more efficiently, reducing waste, expanding access, and personalizing care.

The concept of sustainable transplantation that defined ESOT 2025 extends far beyond environmental considerations. It encompasses sustainable allocation systems that improve equity, sustainable preservation strategies that reduce organ discard, sustainable immunological monitoring that minimizes unnecessary interventions, and sustainable long-term care that preserves both graft function and patient wellbeing.

The studies highlighted here demonstrate that these goals are no longer aspirational. They are already shaping clinical practice and research priorities across the transplantation community.

As transplantation enters a new era characterized by machine perfusion, regenerative medicine, precision immunology, and data-driven decision making, the challenge will be to ensure that innovation remains aligned with responsibility. The success of ESOT 2025 suggests that the community is ready to embrace this challenge.

The future of transplantation will not be defined solely by what we can achieve scientifically, but by how wisely, equitably, and sustainably we apply those achievements for the benefit of patients worldwide. This special issue offers a glimpse of that future—and it is an inspiring one.

Group members of scientific program committee

Anya Adair (United Kingdom), Varuna Aluvihare (United Kingdom), Oriol Bestard (Spain), Ekaterine Berishvili (Switzerland), Gregor Bond (Austria), Saskia Bos (United Kingdom), Philippe Compagnon (Switzerland), Lionel Couzi (France), Lucy Dames (United Kingdom), Caroline Dudreuilh (United Kingdom), Giuseppe Feltrin (Italy), Hermien Hartog (Netherlands), Speranta Iacob (Romania), Sophie Limou (France), Sandra Lindstedt (Sweden), Maarten Naesens (Belgium), Gianluca Rompianesi (Italy), Jelena Stojanovic (United Kingdom), Olivier Thaunat (ESOT President, Co-Chair, France), Fabio Vistoli (Italy), Robin Vos (Belgium), Colin Wilson (Co-Chair, United Kingdom).

Funding

The author(s) declared that financial support was not received for this work and/or its publication.

Acknowledgements

The authors are grateful to the ESOT Office for its instrumental role in the organization of the congress, as well as to their friends and colleagues from the Local Organizing Committee for their invaluable contribution and support: Menna Clatworthy (Cambridge), Andrew Fisher (Newcastle), Paul Johnson (Oxford), Maria Kaiser (Oxford), Krish Menon (London), Katie Morley (Cambridge), Gavin Pettigrew (Cambridge), Stephen Pettit (Cambridge), Rommel Ravanani (Bristol), Candice Roufosse (London), Niloufar Safinia (London), Karen Stevenson (Glasgow), Raj Thuraisingham (London), Steven White (Newcastle).

Correction note

A correction has been made to this article. Details can be found at: [10.3389/ti.2026.17532](https://doi.org/10.3389/ti.2026.17532).

Generative AI statement

The author(s) declared that generative AI was used in the creation of this manuscript. ChatGPT (GPT-5.3-mini) was used to support the revision of the manuscript by improving grammar, spelling, and

overall linguistic clarity, as well as to assist in the generation of the figure. The final version of the article was reviewed and approved by all authors.

Any alternative text (alt text) provided alongside figures in this article has been generated by Frontiers with the

support of artificial intelligence and reasonable efforts have been made to ensure accuracy, including review by the authors wherever possible. If you identify any issues, please contact us.

References

- Hakeem AR, Gupta S, Taylor R, Grammatikopoulos T, Masson S, Prasad R, et al. The UK national appeals panel safely extends access to liver transplantation for candidates beyond standard listing criteria. *Transpl Int* (2026) 39:15573. doi: 10.3389/ti.2026.15573
- Klaassen MF, De Klerk M, Baas MC, Bouwsma H, Bungener LB, Christiaans MHL, et al. Novel allocation strategies can boost kidney exchange programs: a monte carlo simulation. *Transpl Int* (2026) 39:15423. doi: 10.3389/ti.2026.15423
- Jaynes CL, Goggins WC, Holzner ML, Garonzik-Wang J, Leuvenink HGD. No kidney left behind: rescuing unused donor kidneys for transplant at the first centralized assessment and impact on transplant rates and budget impact analysis. *Transpl Int* (2025) 38:15424. doi: 10.3389/ti.2025.15424
- Darius T, Jochmans I, Foguene M, Hoste E, Randon C, Bracke B, et al. Nationwide hypothermic machine perfusion for ECD and DCD kidney transplantation in Belgium: one-year outcomes and impact on transplant rates and budget impact analysis. *Transpl Int* (2025) 38:15282. doi: 10.3389/ti.2025.15282
- Niroomand A, Chang S, Lindstedt S. Back in circulation: a review of the implementation of thoracoabdominal normothermic regional perfusion in donation after circulatory death in lung transplantation. *Transpl Int* (2026) 39:15272. doi: 10.3389/ti.2026.15272
- Torroella A, Zhu RH, Castillo-Delgado C, Pavesi M, Rull R, Folch-Puy E, et al. Duodenoduodenostomy as an attractive option for exocrine drainage in pancreas transplantation: insights from a single-center cohort. *Transpl Int* (2025) 38:15430. doi: 10.3389/ti.2025.15430
- Lorant T, Lonze BE, Montgomery RA, Desai NM, Legendre C, Lundgren T, et al. Five years Follow-up of imlifidase desensitized kidney transplant recipients. *Transpl Int* (2025) 38:15425. doi: 10.3389/ti.2025.15425
- Siracusano G, Deambrogio F, Sordi V, Malnati M, Piemonti L, Chimienti R. Blockade of CD155 and CD276 by monoclonal antibodies fosters immune tolerance and promotes stable engraftment of iPSC-Derived islets in allogeneic humanized mice. *Transpl Int* (2025) 38:15433. doi: 10.3389/ti.2025.15433
- Vorstandlechner M, Degenfelder P, Yavuz G, Glueck OM, Kovács JR, Walter J, et al. Efficacy of Intravenous Immunoglobulin in Eliminating *de novo* Donor-Specific Antibodies After Lung Transplantation: importance of Early Intervention. *Transpl Int* (2025) 38:15350. doi: 10.3389/ti.2025.15350
- Charmetant X, Pettigrew GJ, Thaunat O. Alloreognition unveiled: integrating recent breakthroughs into the current paradigm. *Transpl Int* (2024) 37:13523. doi: 10.3389/ti.2024.13523
- Charmetant X, Chen CC, Hamada S, Goncalves D, Saison C, Rabeyrin M, et al. Inverted direct alloreognition triggers early donor-specific antibody responses after transplantation. *Sci Transl Med* (2022) 14(663):eabg1046. doi: 10.1126/scitranslmed.abg1046
- Agbor-Enoh S, Fraser E, Nadella N, Andargie TE, Alnababteh M. dd-cfDNA: the new frontier for heart/lung transplant surveillance? *Transpl Int* (2025) 38:15555. doi: 10.3389/ti.2025.15555
- Wellekens K, Koshy P, Roufosse C, Naesens M. From banff 1991 to today: the changing landscape of the v-Lesion in kidney transplant rejection. *Transpl Int* (2025) 38:14818. doi: 10.3389/ti.2025.14818
- Jin X, Hooft C, Özsoy B, Van Slambrouck J, Kaes J, Vanluyten C, et al. Donor-specific blood transfusion induces a transfusion-related early protective effect in murine lung transplantation. *Transpl Int* (2026) 39:15409. doi: 10.3389/ti.2026.15409
- Lioulis G, Moissidou E, Christodoulou M, Kasimatis E, Xochelli A, Memmos E, et al. Cellular immunity in chronic kidney disease and changes after kidney transplantation. *Transpl Int* (2026) 39:15622. doi: 10.3389/ti.2026.15622
- Espi M, Charmetant x, Fusil F, Mathieu C, Legras M, Pelletier C, et al. Chronic Kidney Disease-Associated Defect in Humoral Immune Response Is Driven by Inflammation. *Toxins* (2026) 18 (2):104. doi: 10.3390/toxins18020104
- Bellier LM, Kaminski H, Merville P, Couzi L. Interactions between immunosuppressive regimens and cytomegalovirus infection after solid-organ transplantation. *Transpl Int* (2026) 39:15987. doi: 10.3389/ti.2026.15987
- Kamar N, Avery RK, Bo T, Gu J, Kumar D, Witzke O. Association between cytomegalovirus viremia clearance and post-solid organ transplant mortality in patients with refractory cytomegalovirus infection: SOLSTICE post hoc analysis. *Transpl Int* (2025) 38:15331. doi: 10.3389/ti.2025.15331
- Doberer K, Kapps S, Haupenthal F, Bond G. Immune monitoring goes viral – torque teno virus for immunologic risk stratification after kidney transplantation. *Transpl Int* (2025) 38:15074. doi: 10.3389/ti.2025.15074
- Chauvelot L, Barba T, Saison C, Siska E, Kulifaj D, Bakker S. J. L., et al. Longitudinal monitoring of torque teno virus DNAemia in kidney transplant recipients correlates with long-term complications of inadequate immunosuppression. *J. Med. Virol.* (2024) 96 (e29806). doi: 10.1002/jmv.29806
- Prosperi E, Prosperi E, Serenari M, Bonatti C, Fallani G, Stocco A, et al. Evaluating post-transplant outcomes in elderly liver recipients over 70: a propensity-score matching analysis. *Transpl Int* (2025) 38:15429. doi: 10.3389/ti.2025.15429



Back in Circulation: A Review of the Implementation of Thoracoabdominal Normothermic Regional Perfusion in Donation After Circulatory Death in Lung Transplantation

Anna Niroomand^{1,2}, Stephanie Chang¹ and Sandra Lindstedt^{2*}

¹Department of Cardiothoracic Surgery, New York University Langone Health, New York, NY, United States, ²Department of Cardiothoracic Surgery and Transplantation, Skåne University Hospital, Lund, Sweden

In the face of a growing mismatch between candidates awaiting transplantation and the supply of conventional donor organs, attention has shifted toward novel methods to increase the donor pool, including the use of donation after circulatory death (DCD) and the refinement of procurement techniques that safeguard graft quality. Thoracoabdominal normothermic regional perfusion (TA-NRP) has emerged as a new strategy, leveraging extracorporeal support to curtail warm-ischemic injury while permitting *in situ* functional assessment. This review covers the rationale behind the use of TA-NRP, while outlining its use during procurement and the current body of evidence gathered on its implementation in lung transplantation specifically.

Keywords: DCD, donation after circulatory death, lung transplantation, TA-NRP, thoracoabdominal normothermic regional perfusion

OPEN ACCESS

*Correspondence

Sandra Lindstedt,

✉ sandra.lindstedt_ingemansson@med.lu.se

Received: 14 July 2025

Revised: 01 December 2025

Accepted: 23 March 2026

Published: 28 April 2026

Citation:

Niroomand A, Chang S and Lindstedt S (2026) Back in Circulation: A Review of the Implementation of Thoracoabdominal Normothermic Regional Perfusion in Donation After Circulatory Death in Lung Transplantation. *Transpl. Int.* 39:15272. doi: 10.3389/ti.2026.15272

INTRODUCTION AND OVERVIEW OF TA-NRP METHODS AND IMPLEMENTATION

In an era in which the need for transplant heavily outweighs the availability of suitable donors, the field of transplantation has turned to novel methods to increase the donor pool. Tactics to do so focus on increasing the quantity of donors and broadening the range of organs considered suitable, such as the utilization of extended criteria lungs. Aside from improving donor management and liberalizing donor criteria such as age or smoking history, the inclusion of donation after circulatory death (DCD) has gained considerable traction in recent years. While historically cadaveric lungs have come from donation after brain death (DBD), the use of DCD lungs has started to rise. These lungs were previously excluded due to concern over quality, however numerous studies have shown no significant difference in survival with DCD lungs. For donors where direct implant of the DCD allograft may be concerning for the transplant team, methods like normothermic regional perfusion (NRP) and *ex vivo* lung perfusion (EVLP) have enabled the safe evaluation and effective use of DCD lungs. Also known as non-heart-beating donors or as donation after circulatory determination of death, DCD is classified by the Maastricht classification system which differentiates between “uncontrolled” donors for whom cardiac arrest was unexpected and in which resuscitation was unsuccessful and “controlled” donors where care is withdrawn resulting in cardiac arrest [1, 2]. There are differences in the geographic distribution of DCD vs. DBD cases globally, with a higher prevalence of DCD use in Australia and Europe compared to the United States, though rates of

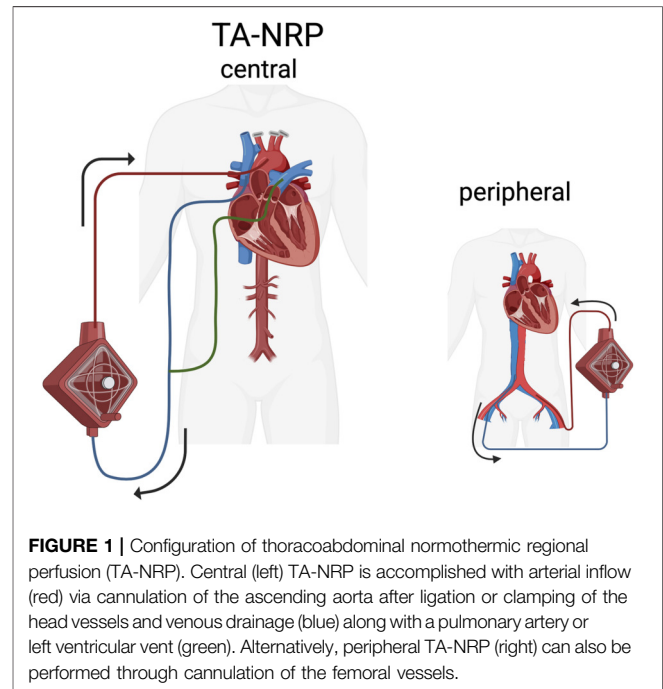
utilization in the US are steadily increasing [3]. In controlled DCD, from which most organs are procured, life-sustaining support is withdrawn leading to a period of warm ischemia during which organs are not perfused but not yet cooled. The implementation of supportive systems following procurement, i.e., NRP, may mitigate the effects of warm ischemia, which is thought to be particularly damaging to the organs. This review will focus on thoracoabdominal NRP (TA-NRP) and its use in donor organ procurement, with a highlight on how its implementation has impacted lung transplantation.

Procurement With TA-NRP

When procuring organs from the donor, organs can be recovered via the traditional direct procurement with cold flush and storage or alternatively by NRP whereby an extracorporeal membrane oxygenation (ECMO) circuit or another extracorporeal circuit like cardiopulmonary bypass (CPB) perfuses the organs. The use of a controlled reperfusion strategy both limits warm ischemic time and simultaneously allows for *in situ* evaluation of graft function [4–6]. The primary motivation behind the development of TA-NRP was the desire to expand heart transplantation to DCD donors, a population previously excluded due to irreversible myocardial warm ischemia. Conventional abdominal NRP maintained perfusion to abdominal organs but excluded the thoracic cavity, precluding safe cardiac recovery. By extending regional perfusion to include both thoracic and abdominal organs while isolating cerebral circulation, TA-NRP allowed for physiologic reperfusion, assessment, and recovery of the DCD heart.

Early use of NRP dates back to the 1980s and 1990s when Spanish groups performed high numbers of uncontrolled DCD procurement in liver transplantation. Unlike in other regions, the majority of DCD livers grew to be recovered with NRP and showed benefits in post-transplant outcomes such as biliary complications and graft loss compared to standard rapid recovery [7]. The use of abdominal NRP became routine in Spain and the UK, and early experiences at the Royal Papworth Hospital and within other UK centers pushed forward the development of TA-NRP [8, 9]. Adoption across the US has followed, with early experiences documented at Colorado, NYU and Vanderbilt accompanied by a growing popularity across centers in recent years [10].

From a technical standpoint, TA-NRP can be initiated via central or peripheral cannulation (**Figure 1**). The femoral artery and vein are most commonly accessed for peripheral cannulation. This approach has the primary advantage of being able to cannulate prior to the procurement sternotomy. Central cannulation is most commonly initiated via the ascending aorta and through the right atrium, which is considered the standard cannula placement [11]. While there is no one codified technical approach to TA-NRP, a consensus statement on technical standards was published in August of 2024 drafted by a working group made of members from several surgical societies [11]. Standard of care as prescribed by that group stipulates that cerebral blood flow should be prevented before systemic perfusion starts. Cerebral reperfusion is typically impeded by the placement of clamps or ligation of all brachiocephalic vessels. Perfusion can be established with either an ECMO or CPB circuit, with preference depending on experience and availability. At its core, any circuit that is used will need to have



a reservoir, oxygenator, pump, tubing and cannulas. It has however been noted that a CPB circuit does have some advantages including being able to unload the heart through venting, improving myocardial reperfusion, and the ability to correct derangements like hyperkalemia and metabolic acidosis which can occur with circulatory death [12]. Cardiac venting can be accomplished with a pulmonary artery vent which also facilitates lung procurement or with a left ventricular or a left atrial vent [11]. Many centers using ECMO for TA-NRP have modified the ECMO circuit to include a reservoir to allow for cardiac venting. Weaning off bypass/ECMO can be done with assessment of the graft's function under a more normal physiologic setting to come to a better idea of if the heart can be accepted for transplant [12]. Regardless of whether CPB or ECMO is used, there are several critical factors that should be considered for every TA-NRP case. Importantly for the lung, the ventilatory parameters should be protective and include recruitment maneuvers to prevent perioperative lung injury. The length of time on TA-NRP is not yet standardized and there is no established ideal duration. If only thoracic organs are being procured, the duration of NRP should be limited to less than 60 min [11]. Organs can and should be assessed while off TA-NRP, particularly to evaluate the native cardiac function and can include pulmonary vein gas samples for donor lung evaluation. TA-NRP concludes with placement of the aortic cross-clamp, and start of the cold preservation time.

The risks of initiating TA-NRP are tied to the speed with which the chest should be entered, which could increase the likelihood of injury to major vessels like the inferior vena cava. Injury to the aorta could lead to a hematoma or dissection during cannulation, and the speed at which TA-NRP is initiated could lead to laceration of the right ventricle or lungs during the sternotomy and pericardiotomy [13]. There is also a risk of coronary air embolism with rapid

cannulation which could lead to predonation arrhythmia [13]. These risks are mitigated by experience, which remains perhaps the biggest barrier to the use of TA-NRP along with the extent of resources it requires. As Moazami et al noted, the combined efforts of experienced cardiac anesthesiologists, transplant cardiologists, perfusionists, operating room personnel, and transplant surgeons are all needed to ensure the best chance at successful resuscitation of organs on bypass and for final acceptance [12]. As the recent consensus document notes, the surgeon performing TA-NRP should be experienced given the rapidity of cannulation and the need to initiate regional perfusion in a timely fashion. They suggest a credentialing process that includes an operative log of at least five cases for those with previous formal training or proctored recoveries for those without formal training. As the utilization of DCD organs becomes more commonplace, there are greater opportunities for providers and hospital systems to become familiar with the requirements and implementation of TA-NRP, which will eventually reduce impediments to its initiation.

USE OF TA-NRP IN LUNG TRANSPLANTATION

TA-NRP can facilitate the procurement of several organs, with a prominent focus on cardiac and pulmonary harvest. For the lungs, the benefit is to quickly and efficiently re-initiate perfusion, which should mitigate effects of warm ischemia [14]. In the Colorado experience, the right atrium and distal innominate artery are cannulated, with a pulmonary artery vent placed at the level of arterial transection to allow for heart and lung splitting [15]. Through their technique, they report in a retrospective review that the median time from asystole to reperfusion was 9.5 min (IQR 8.75–12.5 min) with a total perfusion time of 86.5 min (IQR 79.75–92.5 min) [13]. From this cohort, they experienced only one instance of primary graft dysfunction (PGD) grade 2 in a single transplant recipient compared to 7 of 8 patients who had PGD grade 1 or less. Additionally, the median post-operative $\text{PaO}_2/\text{FiO}_2$ (PF) ratio increased over the course of post-operative days 0–2 from a starting point of 280.5 (236–255.5) to a peak of 370 (316.0–436.0). In another case study series out of the Leuven group in Belgium, five TA-NRP and one abdominal-NRP (A-NRP) cases were performed over the course of a year [16]. From this, two resulted in double lung transplants, though in contrast five hearts were accepted and transplanted. Of these two, one developed PGD grade 3 on post-operative day 2 and the other PGD grade 2 on post-operative day 3. Lungs which were not accepted after TA-NRP were rejected due to medical reasons.

In the NYU protocol, following cannulation and reperfusion, the patient is reintubated, bronchoscopy is performed, a left atrial vent is placed and after 30 minutes, the donor is weaned off support with an arterial blood gas drawn. NRP is then resumed until the aortic cross-clamp is ready to be applied and was run using either CPB or an ECMO circuit with a reservoir. With this, after a series of 8 lung transplants and 3 heart-lung procurements on TA-NRP, 100% of organs were utilized and there were no instances of grade 3 PGD at 72 h [17]. Compared to DBD donors, there were no significant differences in the PF ratios or short or long-term outcomes. From

the Spanish experience which combined the results from several centers in the country, TA-NRP was performed in 28 lung cases and directly compared to 255 abdominal NRP (A-NRP) [18]. The TA-NRP group had no cases of PGD grade 3 at 72 h and had overall significantly lower rates of any grade of PGD compared to A-NRP. There were no differences in the rates or duration of mechanical ventilation, intensive care unit and hospital stays or in survival or readmission. In another retrospective analysis of transplanted lungs after TA-NRP from Vienna, there were no differences in rates of PGD or post-operative mortality when comparing concomitant heart-lung transplant to lung transplant alone [19]. Similarly, an analysis of the Scientific Registry of Transplant Recipients showed that among cardiac DCD donors, lung usage was similar between TA-NRP and direct procurement cases [20]. These case studies highlight the importance of monitoring post-operative PGD as well as organ utilization rates when trying to understand the impact of initiating TA-NRP as routine practice. The process of TA-NRP does not immediately appear to be correlated with high rates of severe PGD, however, the published papers are case series with limited numbers of implanted grafts. As experience with TA-NRP grows, any apparent relationship should become more evident. Experience will only grow if TA-NRP is deemed a useful tool in recovering organs, which appears to be the case given the utilization rates recorded across these case series. While utilization rate ranges from 64% in the 41 lungs transplanted from 70 lungs in the Spanish experience to the use of 100% in the NYU case series, TA-NRP appears to be a viable method of procurement to increase the donor pool.

Larger registry analyses have tried to find overarching relationships across comparison groups in multicenter data, finding suitable results within cases of TA-NRP pointing to no significant differences when compared to other forms of procurement. Across a query of the United Network for Organ Sharing (UNOS) database, of 627 DCD cases aimed at heart procurement, there were utilization rates of 14.9% of lungs in NRP cases compared to 13.8% in direct procurement [21]. Furthermore, there were no differences amongst in-hospital outcomes or short-term survival amongst the recipients in either group. Another UNOS registry data review from the same year looked at 434 DCD transplants, 17 of which were recovered by this classification with TA-NRP and demonstrated a lower likelihood of ventilation greater than 48 h and a non-statistically significant trend towards shorter hospital stays. Of 146 total TA-NRP donors in the pool the low number of 17 transplants equates to an 11.6% utilization rate, which is far lower than the 75.3% for cardiac transplant or 9.8% for kidney transplants [22]. In the STAR-OPTN dataset, 987 DCD lungs were analyzed, of which 92 underwent NRP without distinguishing between TA-NRP or A-NRP with no changes in short-term survival, PGD grade 3 or length of stay either. An important caveat to all three studies is that the TA-NRP groups consist of donors who are inferred to have gone through this type of procurement [23]. Each study relied on the time from asystole to cross clamp falling above a certain threshold of minutes to infer which were TA-NRP cases, which confers a degree of uncertainty as to the true status of each donor.

Use of TA-NRP has been criticized by some who cite concerns that its use can lead to pulmonary edema. Recommendations to

minimize this complication including both donor management and technique. The Colorado experience notes that suitable donors are those with adequate urine output or on diuresis (with a suggestion of furosemide 20–40 mg every 8 h) to combat resulting pulmonary edema from both TA-NRP and cold storage [13]. They also for this reason limit their TA-NRP runs to 45–60 min in length, while considering extensions to 90–120 min if abdominal procurement requires more time. Other considerations in surgical technique include the placement of early pulmonary arterial venting and as the consensus document notes, drainage of donor blood after placing the venous cannula to reduce intracardiac pressures and combat venous congestion before TA-NRP perfusion starts [11]. The placement of the vent, while considered optional in the circuit for some, is noted by others to play an important role in reducing hydrostatic pressure [13]. One preclinical study has so far attempted to investigate the effects of TA-NRP on procurement through a porcine model by comparing TA-NRP to direct procurement via a post-procurement run on EVLP [24]. While the mean PA pressure increased during TA-NRP, the edema evaluated during EVLP and the lung injury scoring of the histopathology were comparable between TA-NRP and direct procurement groups.

EVALUATION OF TA-NRP WITH EVLP

EVLP on its own has been discussed as a post-procurement method of evaluating and potentially rehabilitating donor organs both in DBD and DCD cases [25, 26]. Within the context of TA-NRP, EVLP has been proposed as an adjunctive method to further facilitate the evaluation of donor allografts and as a platform to deliver therapies [27]. In an abstract and subsequent article from Vanderbilt, a series of DCD lungs were put on EVLP at centralized centers and then categorized based on whether they were procured with TA-NRP, A-NRP or directly [28, 29]. The conversion rate following EVLP was found to be higher in TA-NRP compared to A-NRP but comparable to direct procurement. Across all of these groups, the need for post-operative ECMO was low at either 0 or 2 cases, however the length of hospital stay was significantly higher in both NRP groups. Other uses of EVLP with TA-NRP has been from case reports, including a study of a DCD recovery with TA-NRP run on EVLP for transport instead of cold storage. There was no finding of PGD following transplant and the authors concluded that the combination of TA-NRP with mobile EVLP served to reduce logistic challenges given the distance between the donor and the recipient [30]. These publications serve as limited proof that EVLP after TA-NRP can be both feasible and clinically useful, however, highlight a need for greater study of any synergistic effects.

CONCLUSION

The current literature regarding TA-NRP suggests that it is a useful tool before the procurement of DCD organs. TA-NRP allows for transplant groups to leverage an intraoperative opportunity to mitigate the effects of warm ischemia while

also evaluating organs *in situ*. Several case series from various groups internationally have both demonstrated the feasibility of TA-NRP as well as set the stage for standardization of the technique. Registry level analyses have also shown the use of TA-NRP leads to results equivalent to those of DBD donors in experienced centers. As Van Raemdonck et al have noted, however, a key tenet to maintaining good outcomes is good communication between teams involved in organ retrieval [31]. Additionally, presently, there is only a limited amount of data about TA-NRP and the controversial ethics surrounding TA-NRP demands the collection and analysis of more in-depth evidence to support its implementation. Registries should be modified to note when a donor case has proceeded with TA-NRP so that future studies do not infer the status from time intervals. As experience grows, single and multicenter retrospective analyses can create a clearer picture of clinical outcomes. These data sets will create the ability for centers to also further refine donor management, which has already demonstrated the need for certain practices like diuresis, venting during perfusion, and creating guidelines around the duration of TA-NRP. There may be opportunities to pair TA-NRP with other interventions such as EVLP to help facilitate logistic and clinical concerns. Prospective and adequately powered trials will always be necessary to validate long-term outcomes and understand the impact that these interventions have on rejection and survival metrics. Nevertheless, TA-NRP presents as a rapidly growing technique that certainly has its merits in organ procurement, with growing evidence demonstrating its utility in lung transplantation.

AUTHOR CONTRIBUTIONS

All authors contributed to the conceptualization, literature review, and critical analysis of the topic. AN drafted the initial manuscript. SC and SL provided substantial revisions and contributed key references and expertise. All authors contributed to the article and approved the submitted version.

FUNDING

The author(s) declared that financial support was not received for this work and/or its publication.

CONFLICT OF INTEREST

The authors(s) declared that this work was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

GENERATIVE AI STATEMENT

The author(s) declared that generative AI was not used in the creation of this manuscript.

Any alternative text (alt text) provided alongside figures in this article has been generated by Frontiers with the support of artificial intelligence and reasonable efforts have been

made to ensure accuracy, including review by the authors wherever possible. If you identify any issues, please contact us.

REFERENCES

1. Thuong M, Ruiz A, Evrard P, Kuiper M, Boffa C, Akhtar MZ, et al. New Classification of Donation After Circulatory Death Donors Definitions and Terminology. *Transpl Int* (2016) 29(7):749–59. doi:10.1111/tri.12776
2. Kootstra G, Daemen JH, Oomen AP. Categories of Non-Heart-Beating Donors. *Transpl Proc* (1995) 27(5):2893–4.
3. Holm AM, Courtwright A, Olland A, Zuckermann A, Van Raemdonck D. ISHLT Position Paper on Thoracic Organ Transplantation in Controlled Donation After Circulatory Determination of Death (Cdcd). *J Heart Lung Transpl* (2022) 41(6):671–7. doi:10.1016/j.healun.2022.03.005
4. Hessheimer AJ, Garcia-Valdecasas JC, Fondevila C. Abdominal Regional *in-situ* Perfusion in Donation After Circulatory Determination of Death Donors. *Curr Opin Organ Transpl* (2016) 21(3):322–8. doi:10.1097/MOT.0000000000000315
5. Jochmans I, Hessheimer AJ, Neyrinck AP, Paredes D, Bellini MI, Dark JH, et al. Consensus Statement on Normothermic Regional Perfusion in Donation After Circulatory Death: Report From the European Society for Organ Transplantation's Transplant Learning Journey. *Transpl Int* (2021) 34(11):2019–30. doi:10.1111/tri.13951
6. Minambres E, Royo-Villanova M, Dominguez-Gil B. Normothermic Regional Perfusion Provides a Great Opportunity to Maximize Organ Procurement in Donation After the Circulatory Determination of Death. *Crit Care Med* (2022) 50(11):1649–53. doi:10.1097/CCM.00000000000005645
7. Hessheimer AJ, Gastaca M, Minambres E, Colmenero J, Fondevila C, in representation of the SETH Working Group on DCD. Donation After Circulatory Death Liver Transplantation: Consensus Statements from the Spanish Liver Transplantation Society. *Transpl Int* (2020) 33(8):902–16. doi:10.1111/tri.13619
8. Messer S, Page A, Colah S, Axell R, Parizkova B, Tsui S, et al. Human Heart Transplantation From Donation After Circulatory-Determined Death Donors Using Normothermic Regional Perfusion and Cold Storage. *J Heart Lung Transpl* (2018) 37(7):865–9. doi:10.1016/j.healun.2018.03.017
9. Mehta V, Taylor M, Hasan J, Dimarakis I, Barnard J, Callan P, et al. Establishing a Heart Transplant Programme Using Donation After Circulatory-Determined Death Donors: A United Kingdom Based Single-Centre Experience. *Interact Cardiovasc Thorac Surg* (2019) 29(3):422–9. doi:10.1093/icvts/ivz121
10. Hoffman JRH, McMaster WG, Rali AS, Rahaman Z, Balsara K, Absi T, et al. Early US Experience with Cardiac Donation After Circulatory Death (DCD) Using Normothermic Regional Perfusion. *J Heart Lung Transpl* (2021) 40(11):1408–18. doi:10.1016/j.healun.2021.06.022
11. Hoffman JRH, Hartwig MG, Cain MT, Rove JY, Siddique A, Urban M, et al. Consensus Statement: Technical Standards for Thoracoabdominal Normothermic Regional Perfusion. *Transplantation* (2024) 108(8):1669–80. doi:10.1097/TP.00000000000005101
12. Moazami N, Smith D, Galloway A. Logistics for Expanding Heart Transplantation from Donation After Circulatory Death Using Normothermic Regional Perfusion. *JTCVS Tech* (2022) 12:110–2. doi:10.1016/j.xjtc.2022.01.014
13. Cain MT, Park SY, Schafer M, Hay-Arthur E, Justison GA, Zhan QP, et al. Lung Recovery Utilizing Thoracoabdominal Normothermic Regional Perfusion During Donation After Circulatory Death: The Colorado Experience. *JTCVS Tech* (2023) 22:350–8. doi:10.1016/j.xjtc.2023.09.027
14. Kukreja J, Campo-Canaveral de la Cruz JL, Van Raemdonck D, Cantu E, Date H, D'Ovidio F, et al. The 2024 American Association for Thoracic Surgery Expert Consensus Document: Current Standards in Donor Lung Procurement and Preservation. *J Thorac Cardiovasc Surg* (2025) 169(2):484–504. doi:10.1016/j.jtcvs.2024.08.052
15. Wang L, Cain MT, Minambres E, Hoffman JRH, Berman M. Thoracoabdominal Normothermic Regional Perfusion-Approaches to Arch Vessels and Options of Cannulation Allowing Donation After Circulatory Death multi-organ Perfusion and Procurement. *Ann Cardiothorac Surg* (2025) 14(1):70–2. doi:10.21037/acs-2025-dcd-28
16. Boelhouwer C, Vandendriessche K, Van Raemdonck D, Jochmans I, Monbaliu D, Degezelle K, et al. Lung Transplantation Following Donation After thoracoabdominal Normothermic Regional Perfusion (TA-NRP): A Feasibility Case Study. *J Heart Lung Transpl* (2022) 41(12):1864–7. doi:10.1016/j.healun.2022.09.011
17. Chang SH, Geraci TC, Piper GL, Chan J, James L, Paone D, et al. Outcomes of Lung and Heart-Lung Transplants Utilizing Donor After Circulatory Death With Thoracoabdominal Normothermic Regional Perfusion. *JHLT Open* (2024) 4:100058. doi:10.1016/j.jhlto.2024.100058
18. Minasyan A, de la Torre M, Rosado Rodriguez J, Jauregui Abularach A, Romero Roman A, Novoa Valentin N, et al. Outcomes of Controlled DCDD Lung Transplantation After Thoraco-Abdominal Vs Abdominal Normothermic Regional Perfusion: The Spanish Experience. *J Heart Lung Transpl* (2025) 44(5):697–705. doi:10.1016/j.healun.2024.09.018
19. Schwarz S, Gokler J, Moayedifar R, Atteneder C, Bocchialini G, Benazzo A, et al. Prioritizing Direct Heart Procurement in Organ Donors After Circulatory Death Does Not Jeopardize Lung Transplant Outcomes. *JTCVS Tech* (2022) 16:182–95. doi:10.1016/j.xjtc.2022.08.032
20. Alderete IS, Pontula A, Halpern SE, Patel KJ, Klapper JA, Hartwig MG. Thoracoabdominal Normothermic Regional Perfusion and Donation After Circulatory Death Lung Use. *JAMA Netw Open* (2025) 8(2):e2460033. doi:10.1001/jamanetworkopen.2024.60033
21. Malas J, Chen Q, Thomas J, Emerson D, Megna D, Esmailian F, et al. The Impact of Thoracoabdominal Normothermic Regional Perfusion on Early Outcomes in Donation After Circulatory Death Lung Transplantation. *J Heart Lung Transpl* (2023) 42(8):1040–4. doi:10.1016/j.healun.2023.04.009
22. Zhou AL, Ruck JM, Casillan AJ, Larson EL, Shou BL, Karius AK, et al. Early United States Experience with Lung Donation After Circulatory Death Using Thoracoabdominal Normothermic Regional Perfusion. *J Heart Lung Transpl* (2023) 42(6):693–6. doi:10.1016/j.healun.2023.03.001
23. Williams JE, Trahanas JM, Klapper JA, Demarest C, Lagisetty KH, Chang AC, et al. Use of Normothermic Regional Perfusion in Circulatory Death Donors for Lung Transplantation in the United States. *Clin Transpl* (2025) 39(3):e70135. doi:10.1111/ctr.70135
24. Ribeiro RVP, Reynolds FA, Sarrafian TL, Spadaccio C, Colby C, Richman A, et al. Impact of Normothermic Regional Perfusion During DCD Recovery on Lung Allograft Function: A Preclinical Study. *JHLT Open* (2023) 2:100009. doi:10.1016/j.jhlto.2023.100009
25. Amarelli C, Bello I, Aigner C, Berman M, Boffini M, Clark S, et al. European Society of Organ Transplantation (ESOT) Consensus Statement on Machine Perfusion in Cardiothoracic Transplant. *Transpl Int* (2024) 37:13112. doi:10.3389/ti.2024.13112
26. Niroomand A, Nita GE, Lindstedt S. Machine Perfusion and Bioengineering Strategies in Transplantation-Beyond the Emerging Concepts. *Transpl Int* (2024) 37:13215. doi:10.3389/ti.2024.13215
27. Gilbo N, Blondeel J, Pirenne J, Romagnoli R, Camussi G, Monbaliu D. Organ Repair and Regeneration During *Ex Situ* Dynamic Preservation: The Future Is Nano. *Transpl Int* (2023) 36:11947. doi:10.3389/ti.2023.11947
28. Hoetzenecker Kz B, Popa S, Messer R, von Kessler K, Stokes J, Lambright E, et al. Conversion Rates of EVLP Are Similar Between TA-NRP and Directly Procured DCD Lungs. In: *American Association for Thoracic Surgery 105th Annual Meeting* (2025).
29. Demarest C, Zofkie B, Popa S, Stokes J, Messer R, von Kessler K, et al. Impact of Normothermic Regional Perfusion on EVLP Outcomes in DCD Lung Donors.

- J Thorac Cardiovasc Surg* (2025) 171:792–800.e2. doi:10.1016/j.jtcvs.2025.10.014
30. Urban M, Bishawi M, Castleberry AW, Markin NW, Chacon MM, Um JY, et al. Novel Use of Mobile *Ex-Vivo* Lung Perfusion in Donation After Circulatory Death Lung Transplantation. *Prog Transpl* (2022) 32(2):190–1. doi:10.1177/15269248221087437
31. Van Raemdonck D, Brouckaert J, Van Slambrouck J, Vandendriessche K, Ceulemans LJ, Rega F. The Thoracic Surgeon perspective-lung Transplantation in Controlled Donation After Circulatory Determination of Death: Any

Conflict with the Heart? *Ann Cardiothorac Surg* (2024) 13(6):516–8. doi:10.21037/acs-2024-dcd-0032

Copyright © 2026 Niroomand, Chang and Lindstedt. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.



%dd-cfDNA: The New Frontier for Heart/Lung Transplant Surveillance?

Sean Agbor-Enoh^{1,2,3,4*}, Ethan Fraser^{2,4}, Nitin Nadella^{2,5}, Temesgen E. Andargie^{1,2} and Muhtadi Alnababteh^{1,2,4,6}

¹Genomic Research Alliance for Transplantation (GRAFT), Bethesda, MD, United States, ²Laboratory of Applied Precision Omics, National Heart, Lung, and Blood Institute (NHLBI), NIH, Bethesda, MD, United States, ³Department of Medicine, School of Medicine, Johns Hopkins University, Baltimore, MD, United States, ⁴Critical Care Medicine Department (CCMD), NIH, Bethesda, MD, United States, ⁵University of Missouri-Kansas City School of Medicine, University of Missouri, Kansas City, Kansas City, MO, United States, ⁶Department of Medicine, University of Maryland Medical Center, Baltimore, MD, United States

Transplantation improves survival and quality of life, but rejection remains a major threat to allograft longevity. Current surveillance relies heavily on protocols with clinically indicated biopsies, which are invasive, carry procedure-related risks, and have variable sensitivity due to sampling and interpretation limitations. Percent donor-derived cell-free DNA (%dd-cfDNA) has emerged as a noninvasive blood-based biomarker for allograft injury and a potential rule-out test for rejection. Centralized commercial assays are increasingly used in clinical practice; however, published studies report heterogeneous performance and reveal important blind spots and confounders. This review synthesizes the evidence for %dd-cfDNA in thoracic transplantation, delineates its limitations, and outlines emerging cfDNA methodologies that may reduce reliance on invasive biopsies and enable more individualized monitoring strategies.

Keywords: cfDNA, acute rejection, diagnosis, transplantation, allograft injury

INTRODUCTION

Acute rejection (AR) remains a critical vulnerability in thoracic transplantation. Clinicians rely on traditional biopsies of the allograft to detect AR and two classic phenotypes: acute cellular rejection (ACR) and antibody-mediated rejection (AMR). The traditional one-size-fits-all monitoring protocol performs repeated surveillance biopsies to detect and treat early forms of AR before irreversible allograft injury, chronic rejection, and allograft failure develop. Testing often necessitates intricate coordination among various specialties, procedural services, and advanced care planners [1, 2]. This complex model places a substantial burden on healthcare systems and patients alike, ultimately imposing significant socioeconomic across a wide spectrum of care [1, 3, 4]. Moreover, the low sensitivity and high inter-rater variability of biopsy further compromise transplant outcomes [5, 6].

In light of these challenges, donor-derived cell-free DNA (dd-cfDNA) has emerged as a highly sensitive and non-invasive alternative to biopsy. Cell-free nucleic acids are circulating DNA and RNA fragments (cfDNA and cfRNA, respectively) that are released from nuclear, mitochondrial, or microbial genomes into the peripheral bloodstream at the time of cell injury and/or death. In transplant patients, both the recipient and donor contribute to the circulating cell-free nucleic acid pool (rd-cfDNA and dd-cfDNA, respectively).

Cohort studies among transplant patients demonstrate excellent diagnostic performance of dd-cfDNA, with high negative predictive values when used to screen for acute rejection, primary graft dysfunction, and chronic rejection in clinically stable patients [7]. With the availability of

OPEN ACCESS

*Correspondence

Sean Agbor-Enoh,
✉ sean.agbor-enoh@nih.gov

Received: 08 September 2025

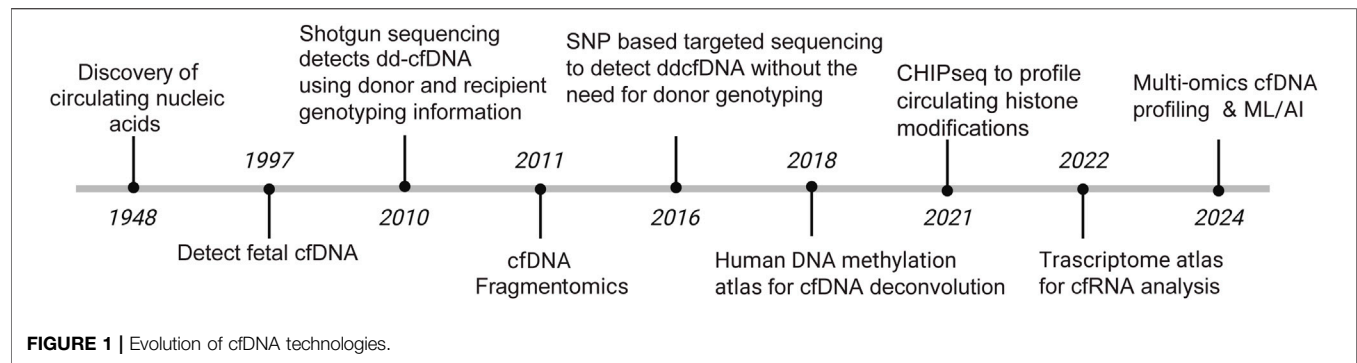
Revised: 14 November 2025

Accepted: 09 December 2025

Published: 29 December 2025

Citation:

Agbor-Enoh S, Fraser E, Nadella N, Andargie TE and Alnababteh M (2025) %dd-cfDNA: The New Frontier for Heart/Lung Transplant Surveillance? *Transpl. Int.* 38:15555. doi: 10.3389/ti.2025.15555



commercial testing, %dd-cfDNA has been increasingly adopted in routine clinical care at US and European Centers, particularly large academic institutions [8–10]. Clinical experiences, however, have been mixed: some centers report consistent performance aligning with early cohort findings, while others exhibit less favorable results alongside challenges with interpretation [7, 11, 12]. To optimize integration into routine clinical care, it is paramount to address the blind spots of %dd-cfDNA and move beyond the one-size-fits-all monitoring paradigm in thoracic transplant populations.

This review aims to highlight the strengths, blind spots, and novel approaches using cfDNA to address these dd-cfDNA gaps.

HISTORICAL BASIS: THE ADVENT OF CELL-FREE DNA TECHNOLOGY

Transplantation restores organ function and creates a donor–recipient genomic admixture wherein measurement of cell-free DNA (cfDNA) provides a noninvasive window into allograft health. Half a century after the discovery of cfDNA in human plasma, Denis Lo first reported on the presence of fetal DNA in maternal plasma in 1997 [13]. Despite these initial findings, cfDNA adoption in transplantation was initially slow. In 2010, Dr. Stephen Quake published a SNP-based approach that leveraged the unique transplant genomic admixture [14]. This 1st generation assay genotyped transplant donors and recipients to identify informative donor-recipient single nucleotide polymorphisms (SNPs). Post-transplant plasma was then subjected to cfDNA isolation and whole genome sequencing and reads were analyzed using SNPs to assign donor and recipient cfDNA fragments. Percent dd-cfDNA (%dd-cfDNA) was then computed as the donor-to-total (donor plus recipient) cfDNA percentage, which has become the standard reported value in transplant populations. Since then, commercial %dd-cfDNA tests are increasingly available for routine clinical care. These SNP-based assays used a targeted approach and imputed donor and recipient SNPs without the need for genotype data. **Figure 1** summarizes the modern evolution of cfDNA technologies.

Novel cfDNA approaches (3rd generation) have emerged in the last decade and while they do not fix the listed weaknesses,

they offer mechanisms that utilize epigenetic fingerprints on cfDNA to better characterize tissue-specific contributions and highlight molecular mechanisms of action [15]. In theory, these approaches could unveil known and unknown pathobiological information of AMR and ACR using a single vial of blood. We summarize these different epigenetic technologies at the end of this review.

CURRENT KNOWLEDGE

Cell-Free DNA to Detect Acute Rejection

Table 1 summarizes seminal studies on the use of cfDNA in thoracic transplant, highlighting the wide range of %dd-cfDNA cutoffs used in initial validation studies and the ongoing work that must be done before %dd-cfDNA is widely adopted. The Stanford Genome Transplant Dynamics (GTD) team launched the initial transformative studies in both heart and lung transplants. The NHLBI-funded Genomic Research Alliance for Transplantation (GRAfT) consortium has since built on these initial studies.

Lung Transplant

In 2015, using the 1st generation SNP-based assay to measure % dd-cfDNA, De Vlaminc et al. reported excellent diagnostic performance with a $\geq 1\%$ dd-cfDNA threshold used as indicator of AR when compared to traditional metrics of AR detection [22]. Lower grade ACR and AMR (A1 and A2) were not included.

Building on this initial data, GRAfT replicated and validated the cfDNA detection methods established by the Stanford GTD, enhancing the reliability and clinical applicability of %dd-cfDNA in assessing AR, which now included AMR and lower grades of ACR [27]. This early work resulted in a seminal publication by Jang et al. who proposed two %dd-cfDNA rejection detection thresholds of 1% and 0.5% dd-cfDNA indicative of a high and low risk patients, respectively [23]. This work set the stage for further clinical testing and validation studies, many of which have proposed different diagnostic thresholds to maximize the sensitivity and specificity of dd-cfDNA [25, 28–31].

In 2022, Keller et al. from the GRAfT consortium reported % dd-cfDNA performance as part of routine clinical care using a

TABLE 1 | Seminal Studies in the Validation of dd-cfDNA as a marker for thoracic organ rejection.

Transplant type	Author	Study design	Sample size (n)	Biomarker threshold	Data Collection methodology	AUROC	Specificity (%)	Sensitivity (%)	PPV (%)	NPV (%)
Heart transplantation	De Vlaminc et al. [16]	Single-center prospective cohort study	65	Dd-cfDNA $\geq 0.25\%$ to detect acute cellular rejection (ISHLT $\geq 2R/3A$) or AMR	SNP-based shotgun sequencing	0.83	58	93	-	-
	Khush et al. [17]	Multicenter prospective cohort study + single center cohort study	773	ddcfDNA $\geq 0.2\%$ to detect acute rejection	Allosure	0.64	80	44	8.9	97.1
	Agbor-Enoh et al. [18]	Multicenter prospective cohort study	171	Day 28 ddcfDNA $\geq 0.25\%$ to detect biopsy-positive acute rejection	SNP-based shotgun sequencing	0.92	81	85	19.6	99.2
	Knuttgen et al. [19]	Single-center prospective cohort study	87	ddcfDNA $\geq 0.35\%$ to detect severe rejection (ISHLT 1R/2R)	Therasure transplant monitor	0.81	83	76	31	97
	Kim et al. [20]	Observational single-center cohort study	223	ddcfDNA $\geq 0.15\%$ to detect acute rejection	Prospera	0.86	76.9	78.5	25.1	97.3
				Absolute quantity ddcfDNA ≥ 13 cp/mL to detect acute rejection		0.88	82.5	84	32.2	98.1
	Bohmer et al. [21]	Multicenter observational prospective cohort study	94 (24 children/70 adults)	Absolute quantity ddcfDNA ≥ 25 cp/mL to detect biopsy-confirmed rejection	SNP-based shotgun sequencing approach	0.87	80.7	94.1	8.6	99.9
				ddcfDNA $\geq 0.09\%$ to detect biopsy-confirmed rejection		0.75	49.3	88.2	3.1	99.6
	De Vlaminc et al. [22]	Single-center prospective cohort study	51	ddcfDNA $\geq 1.0\%$ to detect moderate-to-severe acute rejection	SNP-based shotgun sequencing	0.9	100	73	-	-
	Jang et al. [23]	Multicenter prospective cohort study	148	Day 45 ddcfDNA $\geq 0.5\%$ to detect acute rejection	SNP-based shotgun sequencing	0.89	65	95	51	96
Lung transplantation				Day 45 ddcfDNA $\geq 1.0\%$ to detect acute rejection		-	84	77	64	90
	Keller et al. [24]	Multicenter retrospective cohort study	175	ddcfDNA $\geq 1.0\%$ to detect acute lung allograft dysfunction	SNP-based shotgun sequencing	0.79	70	76.2	66.7	79.2
	Rosenheck et al. [25]	Single-center prospective cohort study	104	Day 45 ddcfDNA $\geq 1.0\%$ to detect acute rejection	Prospera	0.91	89.1	82.9	51.9	97.3
				Day 45 ddcfDNA $\geq 1.0\%$ to detect combined allograft injury (ACR + AMR + CLAD/ NRAD + INFxN)		0.76	59.9	83.9	-	-
	Ju et al. [26]	Single-center retrospective cohort study	188	Prediction score based upon ddcfDNA and mNGS ≥ 0.2781 to detect rejection	Allodx (NGS ddcfDNA system based upon analysis of 6200 SNPs)	0.986	94.7	98.2	88.7	99.2

home-based surveillance program and thresholds from Jang et al. %dd-cfDNA demonstrated excellent diagnostic performance for detection of acute lung allograft dysfunction (ALAD—defined in this study as a composite endpoint of either acute rejection or infection) and its use successfully avoided 80% of bronchoscopies, which aligned with the GRAFT and other cohort study experiences [24, 31].

However, in 2024, Sindu et al. used the same commercial testing platforms and %dd-cfDNA thresholds but observed unsatisfactory sensitivity for detecting ACR or respiratory infection [11]. These diverging experiences are potentially valid and highlight the need to better understand the

performance of the test in different patient populations. Future studies should also address multiple reported confounders that limit the assay performance [32–34].

Comparing the fidelity of %dd-cfDNA to more established rejection markers, %dd-cfDNA has a significantly higher sensitivity to detect rejection compared to FEV1 changes, 95% vs. 60%, while offering a more detailed injury map than traditional inflammatory markers such as ESR/CRP [23, 35]. Future studies would benefit from comparing the distinguishing performance of dd-cfDNA compared to ESR/CRP as studied within kidney transplantation [36].

Heart Transplant

The Stanford GTD published initial proof of concept for use of %dd-cfDNA in heart populations and the first seminal studies in their single center cohort [16]. In 2019, Khush et al. studied 740 heart transplant patients across 26 centers, pairing them with events of biopsy-proven rejection [17]. Using a 0.2% dd-cfDNA threshold, they reported a 97% NPV for detecting AR. Their findings indicated that %dd-cfDNA detected AR across a broad heart transplant population, not just in lung transplants.

Following those seminal studies, Agbor-Enoh et al. ran a prospective cohort study of 171 subjects through the GRAfT cohort [18]. Notably, AR showed higher %dd-cfDNA compared to controls, with elevations detectable 0.5–3.2 months before histopathologic diagnosis of both ACR and AMR via endomyocardial biopsy. A 0.25% threshold yielded a 99% NPV and could have avoided 81% of endomyocardial biopsies over the study period. Since these initial studies, multiple additional cohorts have emerged to validate diagnostic testing thresholds in AR [19, 20, 37]. We summarize seminal studies in **Table 1** and the differing diagnostic thresholds for detecting AR in these cohorts across both heart and lung transplantation.

While most heart transplantation societies do not recommend routine AR screening with troponin/BNP/ESR given their low sensitivity, further studies are needed to directly compare these easily available biomarkers with %dd-cfDNA [2]. When compared directly with endomyocardial biopsy and cardiac MRI, %dd-cfDNA shows sensitivity to detect AR as high as 88% which is higher than MRI alone (85% sensitivity) and EMBx (as low as 58% sensitivity depending on technique) [6, 12, 38]. In the GRAfT cohort, EMB was positive in less than 20% of instances with positive %dd-cfDNA. Of note, %dd-cfDNA was shown to notably not distinguish between patients with angiographic cardiac allograft vasculopathy (CAV) post-transplant and those without, highlighting a particular weakness given the high frequency of and mortality associated with CAV [39].

Like with lung transplantation, experiences have been mixed across centers with highlighting the challenges that still remain with using %dd-cfDNA routinely in heart transplantation. Institutions have reported inconsistent sensitivities for AR across a range of % cutoffs, high rates of non-rejection causes of elevated dd-cfDNA, and even that patients with elevated dd-cfDNA and negative biopsies had worse outcomes, highlighting areas for future studies [19, 40, 41].

Cell-Free DNA for Risk Stratification

Studies involving the long-term risk stratification ability of cfDNA have primarily focused on lung transplant populations within the GRAfT consortium, with cfDNA demonstrating consistent predictive performance throughout the transplant journey. In the pre-transplant period, Balasubramanian et al. evaluated 186 lung transplant candidates and reported variable n-cfDNA levels that were two-fold higher than those for healthy controls and were correlated with a patient's Lung Allocation Score as well as other markers of disease severity [42]. Patients with high levels pre-transplant

had higher risks of primary graft dysfunction and death post-transplant. The risk was highest in patients with elevated neutrophil-derived n-cfDNA, suggesting a role for pre-transplant n-cfDNA monitoring for risk evaluation and assessment.

High Early Injury after Transplantation (HEIT) also demonstrates predictive value, particularly injury in the early post-transplant period. In 2016, Agbor-Enoh et al. analyzed a cohort of 108 patients and reported variable %dd-cfDNA in the early post-transplant period. Patients with elevated %dd-cfDNA levels (upper tertile) showed higher rates of AMR, CLAD, and death when compared to those in the lower two tertiles [43]. Alnababteh et al. published a follow up study of rd-cfDNA in 215 patients and found that patients in the upper tertile had lower lung function post-transplant and an increased risk of death and AR when compared to the lower two tertiles [44]. Along a similar vein, Keller et al. evaluated the prognostic role of extreme molecular injury (EMI - measured as %dd-cfDNA above 5%) and found that all episodes of EMI were associated with an increased risk of severe CLAD or death [45]. Put together, there appears to be a close interplay between the allograft and the host which sets the stage for subsequent allograft function, rejection, and other poor outcomes.

Beyond the early post-transplant period, %dd-cfDNA drawn at the diagnosis of multiple acute post-transplant complications has predictive utility. In patients with respiratory pathogens, Bazemore K et al. showed that patients with %dd-cfDNA levels of 1% or higher showed increased risk of CLAD and death [32]. Keller et al. reported that patients with values above 1% at diagnosis of ACR demonstrated increased risk of CLAD and death [46, 47]. %dd-cfDNA levels at the diagnosis of organizing pneumonia and other acute complications are also predictive of CLAD and early death [48].

Cell-Free DNA to Monitor Infection

Microbial cfDNA (mcfDNA) is found alongside human cfDNA in peripheral blood at lower concentrations and metagenomic sequencing of mcfDNA is an emerging tool that enables unbiased pathogen detection. Currently, there is a commercial clinical-grade mcfDNA sequencing test called the Karius Test that identifies over 1,250 clinically relevant bacteria, DNA viruses, fungi, and parasites non-invasively [49]. Studies have leveraged microbial cfDNA to detect new pathogens in transplant populations and assess a patient's immunosuppression status [50, 51]. Although this approach has a limitation in identifying colonization versus active infection, it has the potential to detect unculturable and emerging microbes as well as help to distinguish AR from active infection—a known limitation of %dd-cfDNA.

Monitoring Immunosuppression

De Vlamincq et al. demonstrated a close association between plasma cfDNA and a patient's degree of immunosuppression post-transplant using plasma anellovirus abundance as a surrogate marker of immunosuppression [50]. Adequate immunosuppression is poised to reduce allograft injury and the risk of AR. Thus, %dd-cfDNA could theoretically assist

clinicians in understanding the relative degree of immunosuppression when interpreted alongside traditional laboratory markers. Charya et al. recently tested this hypothesis in the GRAfT cohort. They showed a significant inverse correlation of %dd-cfDNA with both tacrolimus trough concentrations and anellovirus abundance, a recognized surrogate marker of global immunosuppression over time [52]. Percent dd-cfDNA identified episodes of inadequate immunosuppression with higher performance compared to both tacrolimus troughs and anellovirus abundance.

ADOPTION OF %DD-CFDNA IN ROUTINE CLINICAL PRACTICE

Three CLIA-approved centralized commercial dd-cfDNA tests are available in the US and Europe: AlloSure (CareDx), Prospera (Natera), and TRAC (Eurofins Viracor) [20, 41]. These tests perform %dd-cfDNA testing without the need for prior genotyping. While these assays show considerable agreement in detecting rejection, the cutoff values are different [53]. CareDx also markets a more decentralized testing kit (CE-IVDD) that utilizes custom SNP panels and PCR. Direct comparison of CE-IVDD with a centralized assay demonstrated positive correlation and reproducibility [10]. However, CE-IVDD has a higher assay detection limit, which could reduce its sensitivity, particularly for heart transplants where lower threshold values are needed for diagnosis of AR.

Clinical adopters of %dd-cfDNA often follow variable monitoring protocols given the lack of consensus standards. A recent editorial summarizes common monitoring protocols used in recent years including the ALARM study, which used %dd-cfDNA thresholds of 0.5% and 1% [24, 54]. They found that %dd-cfDNA values above 1% were highly suggestive of AR and served as an “alarm signal”, or a trigger to biopsy and perform additional testing to identify a cause of the derangement. On the other hand, values below 0.5% provide reassurance as an “all clear” signal. Values between 0.5% and 1% represent a gray zone and could serve as an indication for careful monitoring to detect early or impending forms of complications. In light of these results, a recent meta-analysis showed consistency upon review, giving users more guidance on application of %dd-cfDNA although further studies to describe optimal testing windows are still needed [55].

GAPS IN KNOWLEDGE

Despite the robust performance of %dd-cfDNA in cohort studies, reports from routine clinical practice show conflicting %dd-cfDNA results, which suggest unaddressed gaps [20, 41, 56]. For example, while replicate analysis demonstrates reproducibility across laboratories, technicians, and platforms, %dd-cfDNA unfortunately presumes stable rd-cfDNA levels post-transplant, which is its first blind spot [57]. This blind spot is particularly problematic, as rd-cfDNA levels can surge and show variable levels after transplantation [21, 58–60]. Any

variability therefore results in false-negative or false-positive %dd-cfDNA values independent of the state of allograft injury. Some centers have included absolute dd-cfDNA levels in addition to % to minimize this concern. However, there are significant interindividual differences in dd-cfDNA levels, which can limit its utility [18, 29, 31, 61, 62].

Commercial assays use different %dd-cfDNA thresholds for AR detection, making it challenging to compare results across commercial assays - a 2nd blind spot [10, 63]. There are also no internal control standards to enable comparison between commercial tests. These limitations, plus the paucity of consensus clinical guidelines limit uniform %dd-cfDNA adoption across centers, a 3rd blind spot [20, 41]. Therefore, clinicians and scientists are left to determine their own significant %dd-cfDNA cutoffs for research and clinical purposes. Despite a growing body of evidence for use of %dd-cfDNA, there still remains no uniformly accepted decision-making process published to guide clinician use [2, 8, 64]. Clearly, a standardized approach to research methodology and data validation is required to implement dd-cfDNA beyond its current state, highlighting a key next step towards widespread adoption for transplant care.

%dd-cfDNA testing also lacks specificity for AMR and ACR or between AR and infection—a 4th blind spot. This is a critical shortcoming, as the therapeutic approach to manage ACR, AMR, and various infectious processes differs substantially, with delays or misclassification leading to irreversible allograft injury [23]. A future-ready cfDNA platform could overcome this limitation by coupling quantitative measures from multiple cfDNA compartments - including donor-derived, recipient-derived, and the novel cfDNA testing outlined below - with molecular fingerprints of etiology to produce separate probability scores for AMR and ACR compared to active infection.

Given the limitations outlined above, the emerging field of recipient-derived cfDNA offers a particularly promising avenue for a more holistic approach to post-transplant monitoring. This process may elucidate differences of %dd-cfDNA performance between cohorts and provide inferences to personalize test performance. Only a handful of studies have examined this dimension including our own recent work demonstrating that elevated recipient-derived cfDNA in the early post-transplant period is strongly associated with mortality, AR, and impaired lung function—likely reflecting a systemic injury phenotype that influences the host immune response [44, 65]. In the future, integrating donor and recipient-derived cfDNA into a unified graft–host injury map could quantify both local immune assault and broader physiologic stress, identifying patients at the highest risk for complications such as primary graft dysfunction, secondary infections, or chronic allograft dysfunction long before overt clinical decline.

Do we need randomized control trials (RCTs) in the cfDNA space? There is fear that RCTs, given their high cost, difficulty in achieving enrollment and study benchmarks, could divert resources away from other important discoveries. Well-designed cohort studies have often produced reliable clinical data, particularly in rare diseases as transplantation, without

the need for RCTs. However, in the case of %dd-cfDNA, mixed clinical experience compels the need for randomized trials to provide guidelines. A proposed study design for such a trial has been proposed but not yet clinically validated [66]. Any future RCT should ideally address the well-characterized blind spots of %dd-cfDNA to guide proper implementation and adoption into routine clinical practice.

NEXT GENERATION CFDNA APPROACHES COMING TO TRANSPLANT MEDICINE

Cell-free Nucleic Acids

The human body is complex and composed of various cells, tissues, and organ types, each with specialized functions. Single-cell genetic, transcriptomic, and epigenetic profiling have enabled the comprehensive characterization of cell populations in multiple tissue types—including rare cell types—during both physiologic and diseased states. Advances in next-generation sequencing technologies and computational tools have revolutionized the characterization of the genome, epigenome, and transcriptomic profiles of circulating nucleic acids. This allows researchers to better understand different diseases and pathways related to the disease, and aid in establishing diagnostic methods and therapeutic targets. Cell-free DNA carries genetic, epigenetic, and fragmentomic information related to tissues-of-origin and disease biology.

Cell-Free RNA

Plasma cfRNA opens a window to capture systemic response, systemic injury, and molecular mechanisms [67]. In addition to traditional RNAs, circulating cfRNA consists of a variety of cfRNA molecules such as microRNAs (miRNAs), short noncoding RNAs (sncRNAs), long non-coding RNAs (lncRNAs) and others that regulate gene expression. Recent studies used circulating messenger RNA to identify risk of preeclampsia in pregnant women and phenotype cancer subtypes [68–70]. There are limited studies on the use of cfRNA in transplantation. Previous studies focused on miRNAs and the results are conflicting [71, 72]. Nonetheless, we believe plasma cfRNA characterization may reveal pathological processes in previously inaccessible organs [73, 74].

DNA Methylation

Cells show unique epi-methylation markers that play vital roles in genetic regulation with patterns that are unique and stable to each cell type [75]. Recent studies have leveraged cell or tissue specific DNA methylomic markers to identify the tissue origin of cfDNA [76–78]. Microarray- or whole-genome bisulfite sequencing (WGBS) based methods have also successfully been used [79, 80]. While microarrays require high sample input and cover a small percentage of the total methylation site of interest, WGBS, after bisulfite conversion, surveys the methylation state of all cytosines residues and is considered the gold standard. Reference-based deconvolution libraries have continued to grow from an atlas of 25 human cells or tissues to more than 39 cell or tissue types in a recent

study [79, 80]. Despite these advances, studies exploring differential methylation regions from cfDNA in transplant settings are scarce. Furthermore, transplant patients are exposed to combinations of immunosuppressive drugs daily, which may cause epigenetic changes that may lead to undesirable outcomes. Therefore, cfDNA methylome analysis may be an additional tool to identify genes and pathways related to AR.

Cell-Free Histone Modifications

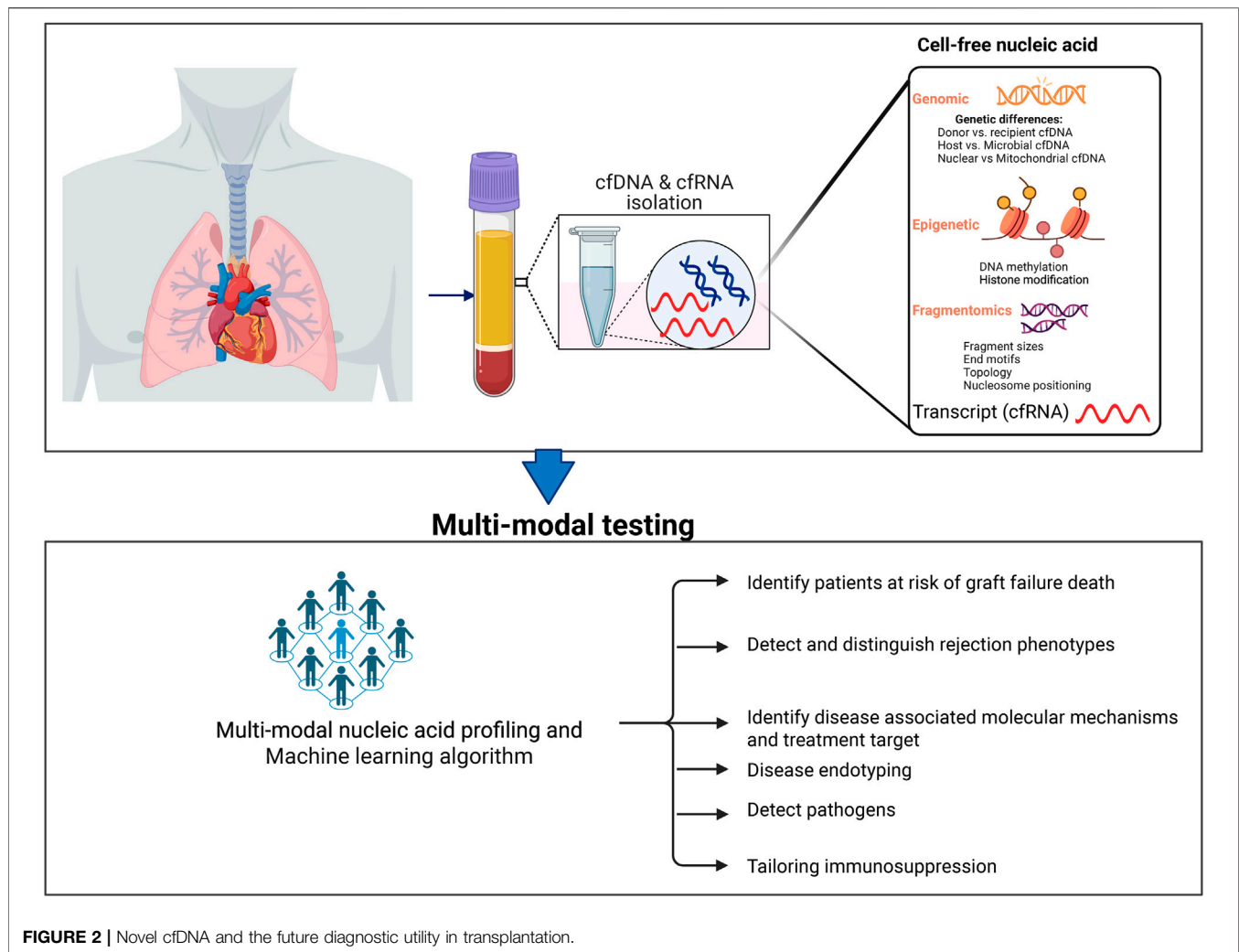
Plasma cfDNA from cell nuclei is wrapped around histone proteins H2A, H2B, H3, and H4 to form an octamer protein called nucleosome, a basic unit of chromatin. Epigenetic profiles of histones, such as mono-methylation of lysine 4 at histone H3 (H3K4me1; enhancer region), carry information related to tissues-of-origin and disease biology. Sadeh et al. recently performed cell-free chromatin immunoprecipitation sequencing (cfChIP-seq) targeting H3K4me3 to delineate gene expression patterns and subsequently enabled the identification of unique biological pathways linked to common disease conditions [81]. Similar studies in cancer biology report similar performance [82, 83]. In heart transplantation, cfChIP-seq demonstrated reliable gene expression signals for immunosuppression therapy including those in the calcineurin and mTOR signaling pathway [52]. This technique may hold significant potential to distinguish between ACR and AMR phenotypes and elucidate genes or molecular pathways associated with rejection for potential therapeutic targets.

Cell-Free DNA Fragmentomics

cfDNA fragmentation is non-random and regulated by chromatin structure and epigenetic modification. Fragmentation patterns also vary by the cfDNA tissue source and could infer disease biology [84]. Various cell death mechanisms—such as apoptosis, necrosis, autophagy, necroptosis, pyroptosis, ferroptosis, and NETosis—as well as active secretion, contribute distinct pools of cfDNA. Nucleosome positioning controls transcription by restricting access to the target DNA region [85]. Recent studies have leveraged nucleosome positioning to identify tissue origin of cfDNA and decipher gene expression profiles in cells contributing to cfDNA pools [86, 87]. Use of similar techniques in transplant patients could offer further insight into the unique transplant genetic environment and gene expression pathways.

Cell-Free Mitochondrial DNA

Mitochondria are found in varying numbers and shapes within human cells and differ among cell and tissue type, reflecting metabolic and bioenergetic demands. Mitochondrial DNA (mtDNA) is highly susceptible to oxidative damage due to its poor ability to repair its own DNA [88]. Therefore, during cellular injury, mtDNA is released into the extracellular environment as circulating mt-cfDNA and contribute to the circulating cfDNA pool [89]. A number of studies have reported that increased plasma levels of mtcfDNA in



transplant settings can serve as markers of mitochondrial or cell damage, as well as predict allograft dysfunction or episodes of rejection [90–93]. Additionally Ma et al.'s showed that both linear and circular mtDNA coexist in the plasma of liver transplant patients, and as such, both may provide different biological information [94]. Studies characterizing the mt-cfDNA fragment size distribution released from the allograft and recipient tissues may provide new disease-related information.

Future Directions

Figure 2 summarizes the diagnostic potential of %dd-cfDNA and novel technologies discussed. %dd-cfDNA has shown significant results in well-structured cohort studies [18, 43]. However, the mixed results from routine clinical experiences suggest the need for additional studies to address the blind spots and gaps in the dd-cfDNA test [15]. Notably, given the wide variability in host cfDNA levels between patients, it is essential to revisit the effectiveness of %dd-cfDNA across diverse populations, considering personal factors that may impact performance

[44]. There is also a need to establish decentralized testing with robust internal controls to enable reproducibility between labs.

Looking ahead, the promise of cfDNA lies in integrating different modalities. Embedding the readouts of these modalities into adaptive algorithms—especially when augmented by pharmacogenomics, immune monitoring, and AI-enabled prediction—could shift practice. For example, this approach could shift immunosuppression from empiric population-based regimens to an individualized, real-time management model. The ultimate vision is a precision-guided strategy where one test informs whether to intensify, taper, or redirect therapy, thereby reducing rejection, infection, and drug toxicity while improving long-term graft survival. While this promise may be a mere dream today, we have summarized novel cfDNA approaches that offer advantages to address these gaps. We anticipate that these new technologies could move transplant monitoring away from the one-size-fits-all paradigm towards a more individualized approach.

AUTHOR CONTRIBUTIONS

SA-E received invitation and defined content. S-AE, EF, NN, TA, and MA contributed to writing, and editing the manuscript. NN and TA produced Figures and Tables. All authors contributed to the article and approved the submitted version.

FUNDING

The author(s) declared that financial support was received for this work and/or its publication. SA-E Laboratory of Applied Precision Omics. This includes funding from the Cystic Fibrosis Foundation (AGBORE20QIO), the Anne Theodore Foundation, the National Institute of Health Distinguished Scholar Program and the National Heart, Lung, and Blood Institute Division of Intramural Research.

AUTHOR DISCLAIMER

This research was supported in part by the Intramural Research Program of the National Institutes of Health (NIH). The contributions of the NIH authors are considered Works of the

United States Government. The findings and conclusions presented in this paper are those of the authors and do not necessarily reflect the views of the NIH or the U.S. Department of Health and Human Services.

CONFLICT OF INTEREST

The author(s) declared that this work was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

GENERATIVE AI STATEMENT

The author(s) declared that generative AI was not used in the creation of this manuscript.

Any alternative text (alt text) provided alongside figures in this article has been generated by Frontiers with the support of artificial intelligence and reasonable efforts have been made to ensure accuracy, including review by the authors wherever possible. If you identify any issues, please contact us.

REFERENCES

- Christie JD, Van Raemdonck D, Fisher AJ. Lung Transplantation. *N Engl J Med* (2024) 391(19):1822–36. doi:10.1056/NEJMra2401039
- Velleca A, Shullo MA, Dhital K, Azeka E, Colvin M, DePasquale E, et al. The International Society for Heart and Lung Transplantation (ISHLT) Guidelines for the Care of Heart Transplant Recipients. *J Heart Lung Transpl* (2023) 42(5):e1–e141. doi:10.1016/j.healun.2022.10.015
- Lampert BC, Teuteberg JJ, Shullo MA, Holtz J, Smith KJ. Cost-Effectiveness of Routine Surveillance Endomyocardial Biopsy After 12 Months Post-Heart Transplantation. *Circ Heart Fail* (2014) 7(5):807–13. doi:10.1161/circheartfailure.114.001199
- Holzmann M, Nicko A, Kühl U, Noutsias M, Poller W, Hoffmann W, et al. Complication Rate of Right Ventricular Endomyocardial Biopsy via the Femoral Approach. *Circulation* (2008) 118(17):1722–8. doi:10.1161/CIRCULATIONAHA.107.743427
- Bhorade SM, Husain AN, Liao C, Li LC, Ahya VN, Baz MA, et al. Interobserver Variability in Grading Transbronchial Lung Biopsy Specimens After Lung Transplantation. *Chest* (2013) 143(6):1717–24. doi:10.1378/chest.12-2107
- Butler CR, Savu A, Bakal JA, Toma M, Thompson R, Chow K, et al. Correlation of Cardiovascular Magnetic Resonance Imaging Findings and Endomyocardial Biopsy Results in Patients Undergoing Screening for Heart Transplant Rejection. *J Heart Lung Transpl* (2015) 34(5):643–50. doi:10.1016/j.healun.2014.12.020
- Jiménez-Coll V, El Kaaoui El Band J, Llorente S, González-López R, Fernández-González M, Martínez-Banaclocha H, et al. All that Glitters in cfDNA Analysis Is Not Gold or Its Utility Is Completely Established Due to Graft Damage: A Critical Review in the Field of Transplantation. *Diagnostics (Basel)* (2023) 13(12):1982. doi:10.3390/diagnostics13121982
- Nikolova A, Agbor-Enoh S, Bos S, Crespo-Leiro M, Ensminger S, Jimenez-Blanco M, et al. European Society for Organ Transplantation (ESOT) Consensus Statement on the Use of Non-Invasive Biomarkers for Cardiothoracic Transplant Rejection Surveillance. *Transpl Int* (2024) 37:12445. doi:10.3389/ti.2024.12445
- Aubert O, Ursule-Dufait C, Brousse R, Gueguen J, Racapé M, Raynaud M, et al. Cell-Free DNA for the Detection of Kidney Allograft Rejection. *Nat Med* (2024) 30(8):2320–7. doi:10.1038/s41591-024-03087-3
- Loupy A, Certain A, Tangprasertchai NS, Racapé M, Ursule-Dufait C, Benbadi K, et al. Evaluation of a Decentralized Donor-Derived Cell-Free DNA Assay for Kidney Allograft Rejection Monitoring. *Transpl Int* (2024) 37:13919. doi:10.3389/ti.2024.13919
- Sindu D, Bay C, Grief K, Walia R, Tokman S. Clinical Utility of Plasma Percent Donor-Derived Cell-Free DNA for Lung Allograft Surveillance: A Real-World Single-Center Experience. *JHLT Open* (2024) 6:100141. doi:10.1016/j.jhlto.2024.100141
- Kim PJ, Olympios M, Sideris K, Tseliou E, Tran TY, Carter S, et al. A Two-Threshold Algorithm Using Donor-Derived Cell-Free DNA Fraction and Quantity to Detect Acute Rejection After Heart Transplantation. *Am J Transplant* (2025) 25(9):1895–905. doi:10.1016/j.ajt.2025.04.021
- Lo YM, Corbetta N, Chamberlain PF, Rai V, Sargent IL, Redman CW, et al. Presence of Fetal DNA in Maternal Plasma and Serum. *Lancet* (1997) 350(9076):485–7. doi:10.1016/s0140-6736(97)02174-0
- Snyder TM, Khush KK, Valentine HA, Quake SR. Universal Noninvasive Detection of Solid Organ Transplant Rejection. *Proc Natl Acad Sci U S A* (2011) 108(15):6229–34. doi:10.1073/pnas.1013924108
- Sebastian A, Silvy M, Coiffard B, Reynaud-Gaubert M, Magdinier F, Chiaroni J, et al. A Review of Cell-Free DNA and Epigenetics for Non-Invasive Diagnosis in Solid Organ Transplantation. *Front Transplant* (2024) 3:1474920. doi:10.3389/frtra.2024.1474920
- De Vlaminck I, Valentine HA, Snyder TM, Strehl C, Cohen G, Luikart H, et al. Circulating Cell-Free DNA Enables Noninvasive Diagnosis of Heart Transplant Rejection. *Sci Transl Med* (2014) 6(241):241ra77. doi:10.1126/scitranslmed.3007803
- Khush KK, Patel J, Pinney S, Kao A, Alharethi R, DePasquale E, et al. Noninvasive Detection of Graft Injury After Heart Transplant Using Donor-Derived Cell-Free DNA: A Prospective Multicenter Study. *Am J Transplant* (2019) 19(10):2889–99. doi:10.1111/ajt.15339
- Agbor-Enoh S, Shah P, Tunc I, Hsu S, Russell S, Feller E, et al. Cell-Free DNA to Detect Heart Allograft Acute Rejection. *Circulation* (2021) 143(12):1184–97. doi:10.1161/circulationaha.120.049098

19. Knüttgen F, Beck J, Dittrich M, Oellerich M, Zittermann A, Schulz U, et al. Graft-Derived Cell-Free DNA as a Noninvasive Biomarker of Cardiac Allograft Rejection: A Cohort Study on Clinical Validity and Confounding Factors. *Transplantation* (2022) 106(3):615–22. doi:10.1097/tp.0000000000003725
20. Kim PJ, Olymbios M, Siu A, Wever Pinzon O, Adler E, Liang N, et al. A Novel Donor-Derived Cell-Free DNA Assay for the Detection of Acute Rejection in Heart Transplantation. *J Heart Lung Transpl* (2022) 41(7):919–27. doi:10.1016/j.healun.2022.04.002
21. Böhmer J, Wählander H, Tran-Lundmark K, Odermarsky M, Alpman MS, Asp J, et al. Absolute Quantification of Donor-Derived Cell-Free DNA Following Pediatric and Adult Heart Transplantation. *J Heart Lung Transpl* (2025) 44:1638–47. doi:10.1016/j.healun.2025.04.024
22. De Vlaminck I, Martin L, Kertesz M, Patel K, Kowarsky M, Strehl C, et al. Noninvasive Monitoring of Infection and Rejection After Lung Transplantation. *Proc Natl Acad Sci U S A*. (2015) 112(43):13336–41. doi:10.1073/pnas.1517494112
23. Jang MK, Tunc I, Berry GJ, Marboe C, Kong H, Keller MB, et al. Donor-Derived Cell-Free DNA Accurately Detects Acute Rejection in Lung Transplant Patients, a Multicenter Cohort Study. *J Heart Lung Transpl* (2021) 40(8):822–30. doi:10.1016/j.healun.2021.04.009
24. Keller M, Sun J, Mutebi C, Shah P, Levine D, Aryal S, et al. Donor-Derived Cell-Free DNA as a Composite Marker of Acute Lung Allograft Dysfunction in Clinical Care. *J Heart Lung Transpl* (2022) 41(4):458–66. doi:10.1016/j.healun.2021.12.009
25. Rosenheck JP, Ross DJ, Botros M, Wong A, Sternberg J, Chen YA, et al. Clinical Validation of a Plasma Donor-Derived Cell-Free DNA Assay to Detect Allograft Rejection and Injury in Lung Transplant. *Transplant Direct* (2022) 8(4):e1317. doi:10.1097/txd.0000000000001317
26. Ju C, Wang L, Xu P, Wang X, Xiang D, Xu Y, et al. Differentiation Between Lung Allograft Rejection and Infection Using Donor-Derived Cell-Free DNA and Pathogen Detection by Metagenomic Next-Generation Sequencing. *Heliyon* (2023) 9(11):e22274. doi:10.1016/j.heliyon.2023.e22274
27. Agbor-Enoh S, Jackson AM, Tunc I, Berry GJ, Cochrane A, Grimm D, et al. Late Manifestation of Alloantibody-Associated Injury and Clinical Pulmonary Antibody-Mediated Rejection: Evidence from Cell-Free DNA Analysis. *J Heart Lung Transpl* (2018) 37(7):925–32. doi:10.1016/j.healun.2018.01.1305
28. Ju C, Xu X, Zhang J, Chen A, Lian Q, Liu F, et al. Application of Plasma Donor-Derived Cell Free DNA for Lung Allograft Rejection Diagnosis in Lung Transplant Recipients. *BMC Pulm Med* (2023) 23(1):37. doi:10.1186/s12890-022-02229-y
29. Khush KK, De Vlaminck I, Luikart H, Ross DJ, Nicolls MR. Donor-Derived, Cell-Free DNA Levels by Next-Generation Targeted Sequencing are Elevated in Allograft Rejection After Lung Transplantation. *ERJ Open Res* (2021) 7(1):00462–2020. doi:10.1183/23120541.00462-2020
30. Noda K, Snyder ME, Xu Q, Peters D, McDyer JF, Zeevi A, et al. Single Center Study Investigating the Clinical Association of Donor-Derived Cell-Free DNA with Acute Outcomes in Lung Transplantation. *Front Transplant* (2023) 2:1339814. doi:10.3389/frtra.2023.1339814
31. Trindade AJ, Chapin KC, Gray JN, Furuya Y, Mullican A, Hoy H, et al. Relative Change in Donor-Derived Cell-Free DNA Is Superior to Absolute Values for Diagnosis of Acute Lung Allograft Dysfunction. *Transplant Direct* (2023) 9(6):e1487. doi:10.1097/txd.0000000000001487
32. Bazemore K, Permpalung N, Mathew J, Lemma M, Haile B, Avery R, et al. Elevated Cell-Free DNA in Respiratory Viral Infection and Associated Lung Allograft Dysfunction. *Am J Transplant* (2022) 22(11):2560–70. doi:10.1111/ajt.17125
33. Smith JB, Peterson RA, Pomponio R, Steele M, Gray AL. Donor Derived Cell Free DNA in Lung Transplant Recipients Rises in Setting of Allograft Instability. *Front Transplant* (2024) 3:1497374. doi:10.3389/frtra.2024.1497374
34. Novo M, Nordén R, Westin J, Dellgren G, Böhmer J, Ricksten A, et al. Donor Fractions of Cell-Free DNA Are Elevated During CLAD But Not During Infectious Complications After Lung Transplantation. *Transpl Int* (2024) 37:12772. doi:10.3389/ti.2024.12772
35. Van Muylem A, Mélot C, Antoine M, Knoop C, Estenne M. Role of Pulmonary Function in the Detection of Allograft Dysfunction After Heart-Lung Transplantation. *Thorax* (1997) 52(7):643–7. doi:10.1136/thx.52.7.643
36. Wijnltiet V, Plaeke P, Abrams S, Hens N, Gielis EM, Hellemans R, et al. Donor-Derived Cell-Free DNA as a Biomarker for Rejection After Kidney Transplantation: A Systematic Review and Meta-Analysis. *Transpl Int* (2020) 33(12):1626–42. doi:10.1111/tri.13753
37. Richmond ME, Deshpande SR, Zangwill SD, Bichell DP, Kindel SJ, Mahle WT, et al. Validation of Donor Fraction Cell-Free DNA with Biopsy-Proven Cardiac Allograft Rejection in Children and Adults. *J Thorac Cardiovasc Surg* (2023) 165(2):460–8.e2. doi:10.1016/j.jtcvs.2022.04.027
38. Han D, Miller RJH, Otaki Y, Gransar H, Kransdorf E, Hamilton M, et al. Diagnostic Accuracy of Cardiovascular Magnetic Resonance for Cardiac Transplant Rejection. *JACC: Cardiovasc Imaging* (2021) 14(12):2337–49. doi:10.1016/j.jcmg.2021.05.008
39. Moeller CM, Oren D, Fernandez Valledor A, Rubinstein G, DeFilippis EM, Rahman S, et al. Elevated Donor-Derived Cell-Free DNA Levels Are Associated with Reduced Myocardial Blood Flow but Not Angiographic Cardiac Allograft Vasculopathy: The EVIDENT Study. *Circ Heart Fail* (2025) 18(1):e011756. doi:10.1161/circheartfailure.124.011756
40. Moeller CM, Fernandez Valledor A, Oren D, Rahman S, Baranowska J, Hertz A, et al. Significance of Elevated Donor-Derived Cell-Free DNA in Heart Transplant Recipients with Negative Endomyocardial Biopsies: A Dawn of a New Era. *Circ Heart Fail* (2025) 18:e012787. doi:10.1161/circheartfailure.125.012787
41. Jiménez-Blanco M, Crespo-Leiro MG, García-Cosío Carmena MD, Gómez Bueno M, López-Vilella R, Ortiz-Bautista C, et al. Donor-Derived Cell-Free DNA as a New Biomarker for Cardiac Allograft Rejection: A Prospective Study (FreeDNA-CAR). *J Heart Lung Transpl* (2025) 44(4):560–9. doi:10.1016/j.healun.2024.11.009
42. Balasubramanian S, Richert ME, Kong H, Fu S, Jang MK, Andargie TE, et al. Cell-Free DNA Maps Tissue Injury and Correlates with Disease Severity in Lung Transplant Candidates. *Am J Respir Crit Care Med* (2024) 209(6):727–37. doi:10.1164/rccm.202306-1064OC
43. Agbor-Enoh S, Wang Y, Tunc I, Jang MK, Davis A, De Vlaminck I, et al. Donor-Derived Cell-Free DNA Predicts Allograft Failure and Mortality After Lung Transplantation. *EBioMedicine* (2019) 40:541–53. doi:10.1016/j.ebiom.2018.12.029
44. Alnababteh M, Keller MB, Kong H, Phipps K, Namian J, Ponor L, et al. Early Post-Transplant Recipient Tissue Injury Predicts Allograft Function, Rejection, and Survival in Lung Transplant Recipients, Evidence from Cell-Free DNA. *Eur Respir J* (2025):2402537. doi:10.1183/13993003.02537-2024
45. Keller MB, Newman D, Alnababteh M, Ponor L, Shah P, Mathew J, et al. Extreme Elevations of Donor-Derived Cell-Free DNA Increases the Risk of Chronic Lung Allograft Dysfunction and Death, Even Without Clinical Manifestations of Disease. *J Heart Lung Transpl* (2024) 43(9):1374–82. doi:10.1016/j.healun.2024.04.064
46. Keller MB, Tian X, Jang MK, Meda R, Charya A, Berry GJ, et al. Higher Molecular Injury at Diagnosis of Acute Cellular Rejection Increases the Risk of Lung Allograft Failure: A Clinical Trial. *Am J Respir Crit Care Med* (2024) 209(10):1238–45. doi:10.1164/rccm.202305-0798OC
47. Keller MB, Sun J, Alnababteh M, Ponor L, P DS, Mathew J, et al. Baseline Lung Allograft Dysfunction After Bilateral Lung Transplantation Is Associated with an Increased Risk of Death: Results from a Multicenter Cohort Study. *Transplant Direct* (2024) 10(7):e1669. doi:10.1097/txd.0000000000001669
48. Keller MB, Tian X, Jang MK, Meda R, Charya A, Ozisik D, et al. Organizing Pneumonia Is Associated with Molecular Allograft Injury and the Development of Antibody-Mediated Rejection. *J Heart Lung Transpl* (2024) 43(4):563–70. doi:10.1016/j.healun.2023.11.008
49. Blauwkamp TA, Thair S, Rosen MJ, Blair L, Lindner MS, Vilfan ID, et al. Analytical and Clinical Validation of a Microbial Cell-Free DNA Sequencing Test for Infectious Disease. *Nat Microbiol* (2019) 4(4):663–74. doi:10.1038/s41564-018-0349-6
50. De Vlaminck I, Khush KK, Strehl C, Kohli B, Luikart H, Neff NF, et al. Temporal Response of the Human Virome to Immunosuppression and Antiviral Therapy. *Cell* (2013) 155(5):1178–87. doi:10.1016/j.cell.2013.10.034
51. Shah JR, Sohail MR, Lasco T, Goss JA, Mohajer MA, Khalil S. Clinical Utility of Plasma Microbial Cell-Free DNA Sequencing in Determining Microbiologic Etiology of Infectious Syndromes in Solid Organ Transplant

- Recipients. *Ther Adv Infect Dis* (2024) 11:20499361241308643. doi:10.1177/20499361241308643
52. Charya AV, Jang MK, Kong H, Park W, Tian X, Keller M, et al. Donor-Derived Cell-Free DNA Is Associated with the Degree of Immunosuppression in Lung Transplantation. *Am J Transplant* (2025) 25:1906–15. doi:10.1016/j.ajt.2025.04.011
 53. Hsi B, Van Zyl J, Alam K, Shakoor H, Farsakh D, Alam A, et al. Tale of Two Assays: Comparison of Modern Donor-Derived Cell-Free DNA Technologies. *JHLT Open* (2024) 4:100090. doi:10.1016/j.jhlto.2024.100090
 54. Mackintosh JA, Chambers DC. Genomic Lung Allograft Surveillance-Is It Primer Time? *J Heart Lung Transpl* (2022) 41(4):467–9. doi:10.1016/j.healun.2022.01.016
 55. Li Y, Liang B. Circulating Donor-Derived Cell-Free DNA as a Marker for Rejection After Lung Transplantation. *Front Immunol* (2023) 14:1263389. doi:10.3389/fimmu.2023.1263389
 56. Bloom RD, Bromberg JS, Poggio ED, Bunnapradist S, Langone AJ, Sood P, et al. Cell-Free DNA and Active Rejection in Kidney Allografts. *J Am Soc Nephrol* (2017) 28(7):2221–32. doi:10.1681/asn.2016091034
 57. Agbor-Enoh S, Tunc I, De Vlaminck I, Fideli U, Davis A, Cuttin K, et al. Applying Rigor and Reproducibility Standards to Assay Donor-Derived Cell-Free DNA as a Non-Invasive Method for Detection of Acute Rejection and Graft Injury After Heart Transplantation. *J Heart Lung Transpl* (2017) 36(9):1004–12. doi:10.1016/j.healun.2017.05.026
 58. Paunel-Görgülü A, Wacker M, El Aita M, Hassan S, Schlachtenberger G, Deppe A, et al. cfDNA Correlates with Endothelial Damage After Cardiac Surgery with Prolonged Cardiopulmonary Bypass and Amplifies NETosis in an Intracellular TLR9-Independent Manner. *Scientific Rep* (2017) 7(1):17421. doi:10.1038/s41598-017-17561-1
 59. Schütz E, Asendorf T, Beck J, Schauerte V, Mettenmeyer N, Shipkova M, et al. Time-Dependent Apparent Increase in dd-cfDNA Percentage in Clinically Stable Patients Between One and Five Years Following Kidney Transplantation. *Clin Chem* (2020) 66(10):1290–9. doi:10.1093/clinchem/hvaa175
 60. Filippone EJ, Farber JL. The Monitoring of Donor-Derived Cell-Free DNA in Kidney Transplantation. *Transplantation* (2021) 105(3):509–16. doi:10.1097/tp.0000000000003393
 61. Shah P, Agbor-Enoh S, Lee S, Andargie TE, Sinha SS, Kong H, et al. Racial Differences in Donor-Derived Cell-Free DNA and Mitochondrial DNA After Heart Transplantation, on Behalf of the GRAFT Investigators. *Circ Heart Fail* (2024) 17(4):e011160. doi:10.1161/circheartfailure.123.011160
 62. Keller MB, Meda R, Fu S, Yu K, Jang MK, Charya A, et al. Comparison of Donor-Derived Cell-Free DNA Between Single Versus Double Lung Transplant Recipients. *Am J Transplant* (2022) 22(10):2451–7. doi:10.1111/ajt.17039
 63. Oellerich M, Sherwood K, Keown P, Schütz E, Beck J, Stegbauer J, et al. Liquid Biopsies: Donor-Derived Cell-Free DNA for the Detection of Kidney Allograft Injury. *Nat Rev Nephrol* (2021) 17(9):591–603. doi:10.1038/s41581-021-00428-0
 64. Schultz J, Stehlik J. Emergence of Donor-Derived Cell-Free DNA Monitoring for Heart Transplant: Future of Rejection Surveillance. *Am Coll Cardiol* (2022).
 65. Zajacova A, Alkhourri M, Ferrao G, Guney M, Rezac D, Vyskocilova K, et al. Early Post-Lung Transplant Cell-Free DNA Levels are Associated with Baseline Lung Allograft Function. *Transpl Immunol* (2025) 92:102245. doi:10.1016/j.trim.2025.102245
 66. Keller M, Ross D, Bhorade S, Agbor-Enoh S. (1187) Study Design for a Randomized Control Trial of Lung Allograft Monitoring with Blood Donor-Derived Cell-Free DNA Assessments (LAMBDA 001). *The J Heart Lung Transplant* (2023) 42(4, Suppl. ment):S509. doi:10.1016/j.healun.2023.02.1398
 67. Vorperian SK, Moufarrej MN, Quake SR. Cell Types of Origin of the Cell-Free Transcriptome. *Nat Biotechnol* (2022) 40(6):855–61. doi:10.1038/s41587-021-01188-9
 68. Albrecht LJ, Höwner A, Griewank K, Lueong SS, von Neuhoff N, Horn PA, et al. Circulating Cell-Free Messenger RNA Enables Non-Invasive Pan-Tumour Monitoring of Melanoma Therapy Independent of the Mutational Genotype. *Clin Transl Med* (2022) 12(11):e1090. doi:10.1002/ctm2.1090
 69. Larson MH, Pan W, Kim HJ, Mauntz RE, Stuart SM, Pimentel M, et al. A Comprehensive Characterization of the Cell-Free Transcriptome Reveals Tissue- and Subtype-Specific Biomarkers for Cancer Detection. *Nat Commun* (2021) 12(1):2357. doi:10.1038/s41467-021-22444-1
 70. Moufarrej MN, Vorperian SK, Wong RJ, Campos AA, Quaintance CC, Sit RV, et al. Early Prediction of Preeclampsia in Pregnancy with Cell-Free RNA. *Nature* (2022) 602(7898):689–94. doi:10.1038/s41586-022-04410-z
 71. Shah P, Agbor-Enoh S, Bagchi P, deFilippi CR, Mercado A, Diao G, et al. Circulating microRNAs in Cellular and Antibody-Mediated Heart Transplant Rejection. *J Heart Lung Transpl* (2022) 41(10):1401–13. doi:10.1016/j.healun.2022.06.019
 72. Coutance G, Racapé M, Baudry G, Lécuyer L, Roubille F, Blanchart K, et al. Validation of the Clinical Utility of microRNA as Noninvasive Biomarkers of Cardiac Allograft Rejection: A Prospective Longitudinal Multicenter Study. *J Heart Lung Transpl* (2023) 42(11):1505–9. doi:10.1016/j.healun.2023.07.010
 73. Dominguez CC, Xu C, Jarvis LB, Rainbow DB, Wells SB, Gomes T, et al. Cross-Tissue Immune Cell Analysis Reveals Tissue-Specific Features in Humans. *Science* (2022) 376(6594):eabl5197. doi:10.1126/science.eabl5197
 74. Zhuang J, Ibarra A, Acosta A, Karns AP, Aballi J, Nerenberg M, et al. Survey of Extracellular Communication of Systemic and Organ-Specific Inflammatory Responses Through Cell Free Messenger RNA Profiling in Mice. *EBioMedicine* (2022) 83:104242. doi:10.1016/j.ebiom.2022.104242
 75. Portela A, Esteller M. Epigenetic Modifications and Human Disease. *Nat Biotechnology* (2010) 28(10):1057–68. doi:10.1038/nbt.1685
 76. Andargie TE, Roznik K, Redekar N, Hill T, Zhou W, Apalara Z, et al. Cell-Free DNA Reveals Distinct Pathology of Multisystem Inflammatory Syndrome in Children. *J Clin Invest* (2023) 133(21):e171729. doi:10.1172/jci171729
 77. Cheng AP, Cheng MP, Gu W, Sasing Lenz J, Hsu E, Schurr E, et al. Cell-Free DNA Tissues of Origin by Methylation Profiling Reveals Significant Cell, Tissue, and Organ-Specific Injury Related to COVID-19 Severity. *Med* (2021) 2(4):411–22.e5. doi:10.1016/j.medj.2021.01.001
 78. Andargie TE, Tsuiji N, Seifuddin F, Jang MK, Yuen PS, Kong H, et al. Cell-Free DNA Maps COVID-19 Tissue Injury and Risk of Death and Can Cause Tissue Injury. *JCI Insight* (2021) 6(7):e147610. doi:10.1172/jci.insight.147610
 79. Moss J, Magenheimer J, Neiman D, Zemmour H, Loyfer N, Korach A, et al. Comprehensive Human Cell-Type Methylation Atlas Reveals Origins of Circulating Cell-Free DNA in Health and Disease. *Nat Communications* (2018) 9(1):1–12. doi:10.1038/s41467-018-07466-6
 80. Loyfer N, Magenheimer J, Peretz A, Cann G, Bredno J, Klochendler A, et al. A DNA Methylation Atlas of Normal Human Cell Types. *Nature* (2023) 613(7943):355–64. doi:10.1038/s41586-022-05580-6
 81. Sadeh R, Sharkia I, Fialkoff G, Rahat A, Gutin J, Chappleboim A, et al. ChIP-seq of Plasma Cell-Free Nucleosomes Identifies Gene Expression Programs of the Cells of Origin. *Nat Biotechnology* (2021) 39:1–13. doi:10.1038/s41587-020-00775-6
 82. Vad-Nielsen J, Meldgaard P, Sorensen BS, Nielsen AL. Cell-Free Chromatin Immunoprecipitation (cfChIP) from Blood Plasma Can Determine Gene Expression in Tumors from Non-Small-Cell Lung Cancer Patients. *Lung Cancer* (2020) 147:244–51. doi:10.1016/j.lungcan.2020.07.023
 83. Trier MC, Meldgaard P, Stougaard M, Nielsen AL, Sorensen BS. Cell-Free Chromatin Immunoprecipitation Can Determine Tumor Gene Expression in Lung Cancer Patients. *Mol Oncol* (2023) 17(5):722–36. doi:10.1002/1878-0261.13394
 84. Liu Y. At the Dawn: Cell-Free DNA Fragmentomics and Gene Regulation. *Br J Cancer* (2022) 126(3):379–90. doi:10.1038/s41416-021-01635-z
 85. Suárez-Álvarez B, Baragaño Raneros A, Ortega F, López-Larrea C. Epigenetic Modulation of the Immune Function: A Potential Target for Tolerance. *Epigenetics* (2013) 8(7):694–702. doi:10.4161/epi.25201
 86. Ulz P, Thallinger GG, Auer M, Graf R, Kashofer K, Jahn SW, et al. Inferring Expressed Genes by Whole-Genome Sequencing of Plasma DNA. *Nat Genetics* (2016) 48(10):1273–8. doi:10.1038/ng.3648
 87. Schübeler D. Function and Information Content of DNA Methylation. *Nature* (2015) 517(7534):321–6. doi:10.1038/nature14192
 88. Larsen NB, Rasmussen M, Rasmussen LJ. Nuclear and Mitochondrial DNA Repair: Similar Pathways? *Mitochondrion* (2005) 5(2):89–108. doi:10.1016/j.mito.2005.02.002
 89. Montier LLC, Deng JJ, Bai Y. Number Matters: Control of Mammalian Mitochondrial DNA Copy Number. *J Genetics Genomics* (2009) 36(3):125–31. doi:10.1016/s1673-8527(08)60099-5

90. Yoshino O, Wong BKL, Cox DRA, Lee E, Hepworth G, Christophi C, et al. Elevated Levels of Circulating Mitochondrial DNA Predict Early Allograft Dysfunction in Patients Following Liver Transplantation. *J Gastroenterol Hepatol* (2021) 36(12):3500–7. doi:10.1111/jgh.15670
91. Han F, Sun Q, Huang Z, Li H, Ma M, Liao T, et al. Donor Plasma Mitochondrial DNA Is Associated with Antibody-Mediated Rejection in Renal Allograft Recipients. *Aging (Albany NY)* (2021) 13(6):8440–53. doi:10.18632/aging.202654
92. Mallavia B, Liu F, Lefrançois E, Cleary SJ, Kwaan N, Tian JJ, et al. Mitochondrial DNA Stimulates TLR9-Dependent Neutrophil Extracellular Trap Formation in Primary Graft Dysfunction. *Am J Respir Cell Mol Biol* (2020) 62(3):364–72. doi:10.1165/rcmb.2019-0140OC
93. Sayah DM, Mallavia B, Liu F, Ortiz-Muñoz G, Caudrillier A, DerHovanessian A, et al. Neutrophil Extracellular Traps are Pathogenic in Primary Graft Dysfunction After Lung Transplantation. *Am J Respir Crit Care Med* (2015) 191(4):455–63. doi:10.1164/rccm.201406-1086OC
94. Ma ML, Zhang H, Jiang P, Sin STK, Lam WKJ, Cheng SH, et al. Topologic Analysis of Plasma Mitochondrial DNA Reveals the Coexistence of Both Linear and Circular Molecules. *Clin Chem*. (2019) 65(9):1161–70. doi:10.1373/clinchem.2019.308122

Copyright © 2025 Agbor-Enoh, Fraser, Nadella, Andargie and Alnababteh. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.



From Banff 1991 to Today: The Changing Landscape of the v-Lesion in Kidney Transplant Rejection

Karolien Wellekens^{1,2}, Priyanka Koshy^{1,3}, Candice Roufosse⁴ and Maarten Naesens^{1,2*}

¹Nephrology and Renal Transplantation Research Group, Department of Microbiology, Immunology and Transplantation, KU Leuven, Leuven, Belgium, ²Department of Nephrology and Renal Transplantation, University Hospitals Leuven, Leuven, Belgium, ³Department of Pathology, University Hospitals Leuven, Leuven, Belgium, ⁴Department of Immunology and Inflammation, Imperial College, London, United Kingdom

Intimal arteritis (v-lesion) has long been considered a hallmark of higher-grade T cell-mediated rejection (TCMR) in kidney transplantation, historically associated with poor graft survival and resistance to therapy. These associations have informed treatment strategies, often prompting intensified immunosuppression, including anti-thymocyte globulins (ATG). However, emerging evidence challenges the assumption that all v-lesions signify TCMR—particularly when they occur in isolation, without significant tubulo-interstitial inflammation. Recent observational studies and molecular analyses suggest that isolated v-lesions may instead reflect non-immune injury mechanisms, such as ischemia-reperfusion injury, particularly in the early post-transplant period. In addition, the shared nature of the v-lesion between TCMR and antibody-mediated rejection (AMR) raises concerns about overdiagnosis and potential overtreatment of “mixed rejection” phenotypes. Following advances in modern immunosuppression and improved donor-recipient matching, the clinical course of v-lesions may have evolved, with severe v3 presentations now rare—rendering historical comparisons less applicable to current practice. These insights highlight the need to revisit traditional paradigms and adopt a more nuanced, context-aware interpretation of v-lesions. This review integrates historical and contemporary perspectives, advocating for a reappraisal of the role of the v-lesion in kidney transplant biopsy evaluation.

Keywords: rejection, kidney transplant, TCMR, Banff classification, AMR

OPEN ACCESS

*Correspondence

Maarten Naesens,
✉ maarten.naesens@kuleuven.be

Received: 24 April 2025

Accepted: 19 June 2025

Published: 30 June 2025

Citation:

Wellekens K, Koshy P, Roufosse C and Naesens M (2025) From Banff 1991 to Today: The Changing Landscape of the v-Lesion in Kidney Transplant Rejection. *Transpl. Int.* 38:14818. doi: 10.3389/ti.2025.14818

INTRODUCTION

Despite ongoing advances in transplant medicine, nearly 40% of kidney allografts fail within 10 years [1–3], with rejection remaining the primary cause [4, 5]. Rejection diagnosis relies on histopathological evaluation guided by the Banff classification [6], which primarily distinguishes T cell-mediated rejection (TCMR) from antibody-mediated rejection (AMR). The severity of TCMR is determined by histological findings, with moderate to severe tubular (t2-3) and interstitial inflammation (i2-3) defining grade I, while the presence of intimal arteritis upgrades the diagnosis to grade IIa (v1), IIb (v2), or III (v3), regardless of the extent of tubulo-interstitial inflammation. Intimal arteritis (“v”) is defined by the presence of inflammatory cells in the subendothelial space of one or more arteries [7]. Banff guidelines recommend evaluating at least two arteries for adequate v-lesion scoring. Treatment strategies for TCMR vary, with many U.S. centers favoring corticosteroids for TCMR grade I and a combination of steroids and anti-thymocyte globulin (ATG) for grade II or higher [8, 9], whereas ATG use is less common in Europe [10]. These

strategies are largely informed by historical studies associating v-lesions with poorer graft survival and reduced treatment response [11–15].

Recent observational cohort studies [16–19] and molecular biopsy-based analyses [20, 21] have challenged the clinical significance of v-lesions. Emerging evidence suggests that isolated v-lesions—v-lesions occurring without substantial tubulo-interstitial inflammation—may not reflect the severe rejection phenotype traditionally defined by the Banff classification. This review examines the evolving understanding of TCMR and discusses how recent insights could inform and improve future strategies for diagnosing and managing rejection.

REVIEW

Historical Assumptions on Prognostic Significance of the v-Lesion

In the first Banff classification (1991) [22], the v-lesion was introduced as a defining feature of acute rejection. Mild-to-moderate intimal arteritis in at least one artery (v1) was classified as acute rejection grade II, while moderate-to-severe arteritis affecting more than one artery (v2) or severe intimal arteritis involving multiple arterial cross-sections and/or presenting with transmural arteritis, fibrinoid change, and smooth muscle necrosis (v3) was designated as grade III acute rejection. In this 1991 classification, significant interstitial inflammation (t2-3) with moderate tubulitis (t2) was designated as grade I acute rejection, while severe tubulitis (t3) also counted for grade II acute rejection (**Table 1; Figure 1**).

At the Banff 1995 meeting [32], it was proposed to differentiate grade II acute rejection based on tubulitis versus arteritis, as these forms were suggested to have distinct pathogenesis and prognostic implications. It was demonstrated [12, 13] that intimal arteritis was associated with poorer graft survival and reduced response to therapy. This refinement was formally implemented in the 1997 classification [33], where grade II and higher were reserved for cases with intimal arteritis.

Since then, the v-lesion was considered a key marker of severe rejection, with studies from the 1990s and early 2000s consistently demonstrating its negative prognostic impact on kidney allograft survival. Higher v grades were associated with increased rejection severity and worse graft outcomes (**Table 2**). Koopmans et al. [11] compared 42 biopsies with acute tubulointerstitial rejection, 18 with acute vascular rejection, and 7 with diffuse thrombosis, reporting that vascular rejection was associated with more frequent rejection episodes and higher allograft loss rates. Colvin et al. [12] found that vascular rejection (N = 24) resulted in worse clinical outcomes than rejection characterized by tubulitis with interstitial infiltration (N = 94). Nickleleit et al. [13] observed that patients with vascular rejection (N = 35) had a significantly lower response to steroid treatment compared to those with tubulitis and interstitial inflammation (N = 20), though both groups responded similarly to ATG and had comparable one-year graft failure rates.

Further supporting these findings, Mueller et al. [14] analyzed 35 biopsies (7 borderline, 11 TCMR grade I,

10 TCMR grade II, 7 TCMR grade III), demonstrating that TCMR grade I-III correlated with treatment unresponsiveness and increased graft failure risk. Minervini et al. (2000) [15] compared 36 cases each of t1, t2, and t3 tubulitis, as well as 36 v1, 18 v2, and 11 v3 cases, irrespective of t scores. These findings confirmed that v2/v3 intimal arteritis had the poorest prognosis, surpassing v1 and all tubulitis grades without concurrent intimal arteritis, while severe tubulitis (t3) had outcomes comparable to v1.

Several limitations of earlier studies warrant consideration. Many did not account for concomitant tubulointerstitial or microvascular inflammation—both of which can independently affect outcomes—and often failed to assess donor-specific antibodies (DSA). Inclusion criteria for acute rejection subtypes were inconsistent, with some studies not adhering to Banff-defined thresholds. Most lacked multivariable analyses to adjust for confounding clinical factors, were limited by small sample sizes, and frequently included repeat biopsies, complicating survival analysis by counting some patients multiple times. Additionally, follow-up durations were often insufficient to capture the long-term impact of rejection phenotypes.

The Concept of “Isolated v” as a Distinct Phenotype

At the Banff 2009 conference [43], a working group on isolated v was established to assess its significance in renal allograft biopsies. This initiative stemmed from growing uncertainty regarding the clinical relevance of isolated arteritis in biopsies that lacked significant tubulointerstitial inflammation and, therefore did not meet the Banff criteria for grade I TCMR. A study by Sellarès et al. [26] found no correlation between v-lesions and graft failure risk, whether analyzed as v0 (N = 88) versus v > 0 (N = 19) or stratified by intimal arteritis severity (v1-2-3). Additionally, microarray analyses [27] revealed minimal inflammatory gene expression, including low levels of T cell activation, IFN- γ , and chemokines, in isolated v biopsies.

These findings led to the initiation of a multicenter retrospective case-control study to further investigate the clinical implications of isolated v. Presented at the 2013 Banff conference [44], the study defined isolated v as intimal arteritis with minimal interstitial inflammation ($i \leq 1$) and tubulitis ($t \leq 1$) [28]. The results demonstrated that isolated v (N = 103) had comparable treatment response rates and graft survival to v-lesions with significant tubulo-interstitial inflammation (N = 101, v1 with t2-3, i2-3). The Banff 2013 meeting report suggested that “*most isolated v-lesions should be reported as type 2 (or 3) acute TCMR.*” [44]

However, several limitations should be acknowledged. Some patients had concurrent AMR or microvascular inflammation (MVI), likely confounding the findings. As a case-control study, it was also inherently prone to selection bias. Importantly, the study did not address cases with v1 in combination with either t2-3/i0-1 or t0-1/i2-3, leaving uncertainty about how to classify and manage such presentations. Moreover, the conclusion that “most” isolated v-lesions represent severe TCMR remains

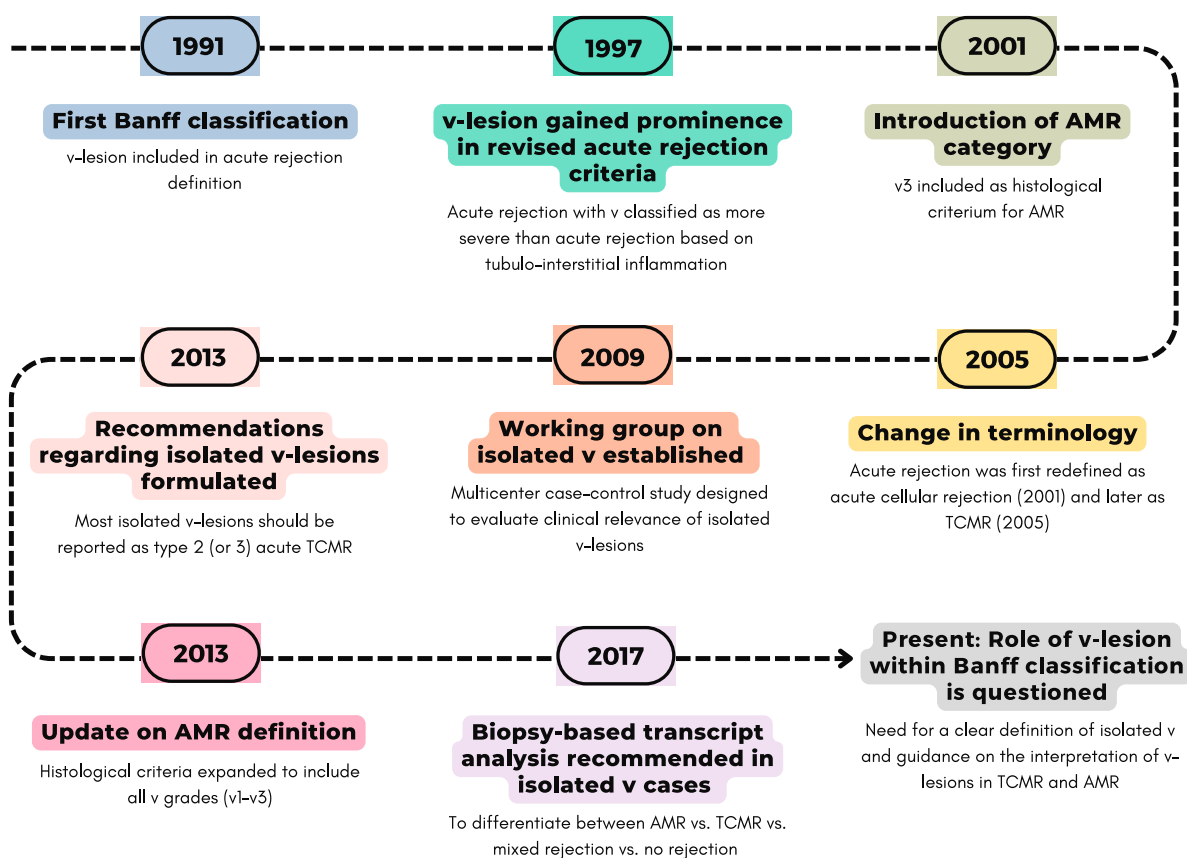
TABLE 1 | Detailed explanation of Banff updates on intimal arteritis (v-lesion).

TCMR			
Year	Change	Evidence supporting change	Update in definitions (changes are <u>underlined</u>)
1991	First Banff classification established	Meeting report by Solez et al. [22]	Definition of acute rejection: Grade I: significant interstitial inflammation (>25% of parenchyma, i2-3) with moderate tubulitis (>4 mononuclear cells/tubular cross section or group of 10 tubular cells, t2) Grade II: significant interstitial inflammation (>25% of parenchyma, i2-3) with severe tubulitis (>10 mononuclear cells/tubular cross section, t3) and/or mild-moderate intimal arteritis in at least one artery (v1) Grade III: moderate-severe (v2) or severe (v3) intimal arteritis in multiple arteries and/or with transmural arteritis, fibrinoid change and smooth muscle necrosis Refinement of acute rejection definition, emphasizing the inferior outcome associated with intimal arteritis: Grade IA: significant interstitial inflammation (>25% of parenchyma, i2-3) with moderate tubulitis (>4 mononuclear cells/tubular cross section or group of 10 tubular cells, t2) Grade IB: <u>significant interstitial inflammation (>25% of parenchyma, i2-3) with severe tubulitis (>10 mononuclear cells/tubular cross section, t3)</u> Grade IIA: <u>mild-moderate intimal arteritis (v1)</u> Grade IIB: severe intimal arteritis comprising >25% of the luminal area (v2) Grade III: transmural arteritis and/or arterial fibrinoid change and smooth muscle necrosis with accompanying lymphocytic inflammation (v3) <u>Additional change: v-grading was revised to be based on the most severely affected vessel due to the potential for sampling error in defining vasculitis</u> No changes to grading
1997	v-lesion gained more prominence in acute rejection criteria	Colvin et al. [12] (1997) and Nickleleit et al. [13] demonstrated that intimal arteritis was associated with worse therapy response and reduced graft survival	
2005	Acute (cellular) rejection redefined as TCMR	The introduction of AMR in 2001 distinguished acute cellular from antibody-mediated rejection. Studies presented at Banff 2003–2005 meetings identified tubulitis as the primary correlate of T-cell-mediated effects [23–25]	
2009	Working group on isolated v was established	Formed in response to increasing uncertainty about the clinical relevance of isolated arteritis [26, 27]	A multicenter retrospective case-control study was initiated
2013	Recommendations regarding isolated v-lesions were formulated	The study [28] demonstrated that isolated v-lesions had comparable treatment response and graft survival to cases with v-lesions and significant tubulo-interstitial inflammation	<u>"Most isolated v-lesions should be reported as type 2 (or 3) acute TCMR."</u>
Present	Role of v-lesion within Banff classification is challenged	Emerging evidence suggests isolated v may represent a non-rejection phenotype rather than TCMR Grade II–III [16–18, 20, 26, 29]	A potential change remains to be discussed
AMR 2001	Introduction of AMR category	Studies presented at the Banff 2001 congress highlighted the critical role of antibodies in rejection, showing that biopsies with C4d deposition had significantly lower graft survival [25]	Three criteria must be present for AMR diagnosis: 1. Histologic evidence of acute tissue injury - ATN-like minimal inflammation - Capillary and/or glomerular inflammation (ptc/g > 0) and/or thromboses - Arterial—v3 2. Evidence of antibody interaction with vascular endothelium 3. Serological evidence of donor-specific antibodies Three criteria must be present for AMR diagnosis: 1. Histologic evidence of acute tissue injury • g > 0 and/or ptc>0 • v>0 • Acute thrombotic microangiopathy • Acute tubular injury 2. Evidence of antibody interaction with vascular endothelium 3. Serologic evidence of donor-specific antibodies (Continued on following page)
2013	AMR definition expanded to include all v grades (v1–v3)	Lefaucheur et al. [30] demonstrated that AMR with v was associated with a worse prognosis than AMR without v.	

TABLE 1 | (Continued) Detailed explanation of Banff updates on intimal arteritis (v-lesion).

TCMR			
Year	Change	Evidence supporting change	Update in definitions (changes are <u>underlined</u>)
2017	Biopsy-based transcript analysis recommended in isolated v cases	Introduced in response to recurring diagnostic uncertainty in clinical practice [31]	Isolated v is a <u>recommended indication for the use of molecular diagnostics</u> in renal allograft biopsy interpretation to differentiate between AMR versus TCMR versus mixed rejection versus no rejection in cases of isolated v without MVI or TCMR, C4d-negative, with or without DSA.

Evolution of intimal arteritis (v-lesion) within the Banff classification

**FIGURE 1 |** Evolution of intimal arteritis (v-lesion) within Banff classification across different updates. A detailed explanation of each update is provided in **Table 1**.

vague, as no practical guidance was offered to support its implementation in clinical decision-making.

Recent Data on the Clinical Relevance of Isolated v-Lesions

Over the past decade, accumulating evidence suggests that isolated v may represent a benign phenotype, with multiple studies reporting favorable outcomes. Isolated v has been

associated with treatment responses and graft survival rates comparable to—or even better than—those observed in TCMR with significant tubulo-interstitial inflammation (**Table 2**). Salazar et al. [20] found that the presence of v-lesions (N = 49) did not increase the risk of graft failure compared to v-negative biopsies (N = 654). Novotny et al. [17] concluded that isolated v cases (N = 25) responded well to steroid treatment, showed no persistence of arteritis in follow-up biopsies, and had favorable graft outcomes. Similarly, Nankivell et al. [29]

TABLE 2 | Key studies on intimal arteritis (1996–2025).

Study	Phenotypic study definitions	Study type	Sample size	Main findings
Kooijmans et al. [11]	Acute tubulointerstitial rejection: tubulitis with focal or widespread mononuclear cell infiltrates in the interstitium Acute vascular rejection: endothelial proliferation/swelling with intimal edema and/or mononuclear cell infiltration and adherence of mononuclear cells to the endothelium Diffuse thrombosis: necrosis of small arteries/arterioles, thrombosis in hilar/arcuate arteries, and fibrin thrombi in glomerular/peritubular capillaries	Observational cohort	N = 42 acute tubulointerstitial rejection, N = 18 acute vascular rejection, N = 7 diffuse thrombosis	Acute vascular rejection and diffuse thrombosis were associated with more frequent rejection episodes and higher allograft loss rates than acute tubulointerstitial rejection
Colvin et al. [12]	Type I rejection: mononuclear infiltrate in $\geq 5\%$ of the cortex, tubulitis in ≥ 3 tubules and ≥ 2 of the following: edema, activated lymphocytes, or tubular injury Type II rejection: arterial, or arteriolar, endothelialitis, with or without type I features	Observational cohort	N = 94 type I, N = 24 type II	Type II rejection was clinically more severe than type I (peak serum creatinine $>120\%$ of baseline within 14 days after onset or incomplete response to antirejection therapy)
Nickeleit et al. [13]	Type I rejection: mononuclear infiltrate in $\geq 5\%$ of the cortex, tubulitis in ≥ 3 tubules and ≥ 2 of the following: edema, activated lymphocytes, or tubular injury Type II rejection: endarteritis (mononuclear cells in the subendothelial space), with or without type I features. Type III rejection: fibrinoid arterial necrosis or transmural inflammation	Observational cohort	N = 20 type I, N = 35 type II, N = 4 type III	Type II rejection had poorer steroid response compared to type I, but responded equally to ATG. Type II had similar one-year graft survival and renal function at 6 and 12 months post-biopsy as type I. Type III did not respond to therapy, with all cases leading to graft failure within 12 months
Mueller et al. [14]	Borderline TCMR: mild tubulitis (t1) with mild interstitial infiltrate (i1) TCMR grade I: interstitial infiltrate (i2–3) and tubulitis (t2–3) TCMR grade I: intimal arteritis (v1–2) TCMR grade I: severe intimal arteritis (v3)	Observational cohort	N = 7 borderline TCMR, N = 11 TCMR grade I, N = 10 TCMR grade II, N = 7 TCMR grade III	Acute rejection severity correlated with treatment unresponsiveness and increased graft failure risk within 18 months post-biopsy. Intimal arteritis was the only histomorphologic predictor of poor survival. TCMR grade I–III groups had significantly higher serum creatinine at 1 and 2 years post-transplantation than matched controls
Minervini et al. [15]	t1: maximum tubulitis score of t1 t2: maximum tubulitis score of t2 t3: t3 tubulitis, characterized by the presence of >10 mononuclear cells/tubular cross-section or ≥ 2 areas of basement membrane destruction with i2/i3 inflammation and t2 tubulitis elsewhere in the biopsy v1: v1 intimal arteritis, irrespective of tubulitis grade v2: v2 intimal arteritis, irrespective of tubulitis grade v3: v3 intimal arteritis, irrespective of tubulitis grade	Observational cohort	N = 36 t1, N = 36 t2, N = 36 t3, N = 36 v, N = 18 v2, N = 11 v3	Severe tubulitis (t3) was associated with worse graft outcomes than mild/moderate tubulitis (t1/t2), approaching the outcomes of v1 intimal arteritis. Intimal arteritis (v2/v3) had the poorest prognosis, exceeding that of v1 arteritis and all tubulitis grades without coexisting arteritis
Haas et al. [34]	Grade IIA: mild to moderate intimal arteritis (1) Grade IIB: severe intimal arteritis (v2)	Observational cohort	N = 102 grade IIA, N = 29 grade IIB	Grade IIB cases had worse responses to therapy and higher rates of graft failure compared to grade IIA. However, grade IIB cases had higher g + i + t score than grade IIA. Therapy response was associated with the extent of tubulitis and the composite g + i + t score (graft failure was not specifically analyzed in relation to g + i + t) (Continued on following page)

TABLE 2 | (Continued) Key studies on intimal arteritis (1996–2025).

Study	Phenotypic study definitions	Study type	Sample size	Main findings
Mueller et al. [27]	Intimal arteritis with minimal tubulointerstitial changes: $v > 0$, $t < 2$, $i < 2$	Transcriptomic profiling using microarray analysis	N = 143 biopsies +51 validation samples, <i>exact number of intimal arteritis with minimal tubulointerstitial changes not explicitly stated</i>	Biopsies with intimal arteritis and minimal tubulointerstitial changes exhibited low expression of pathogenesis-based transcript sets
Kozakowski et al. [35]	Intimal arteritis: $v > 0$	Immunohistochemical staining of immune cells	N = 34 biopsies with intimal arteritis	Biopsies of intimal arteritis consist of a mixed infiltrate of monocytes/macrophages and T-lymphocytes, with a median CD68/CD3 ratio of 1.03. There was no correlation between the cellular composition of arterial and interstitial infiltrates and the proportion of interstitial and arterial macrophages did not impact graft survival
Sellarés et al. [26]	v0: v-lesion negative $v \geq 1$: v-lesion positive (v1-v2-v3) Early: ≤ 1 year post-transplantation Late: > 1 year post-transplantation	Observational cohort	N = 88 v0, N = 13 v1, N = 5 v2, N = 1 v3. Number of early and late v-positive biopsies not reported	v-lesions were not associated with allograft loss in early or late biopsies
Sun et al. [36]	Intimal arteritis: $v > 0$	Immunohistochemical staining of immune cells	N = 5 allografts resected because of irreversible graft failure, all classified as AMR with intimal arteritis	Macrophages and T-lymphocytes were the predominant immune cells, with CD8 ⁺ cytotoxic T-cells comprising 45.4% of T-cells. Neutrophils and NK cells were also present, with a higher proportion of neutrophils in v3 vasculitis compared to v2 lesions
Reeve et al. [37]	Isolated v: $v > 0$ and ($i < 2$ and/or $t < 2$)	Transcriptomic profiling using microarray analysis	N = 403 biopsies, including N = 24 with isolated v	19 out of 24 biopsies (79%) with isolated v-lesions had low TCMR scores (≤ 0.1)
Halloran et al. [38]	Isolated v: $i < 2$ or $t < 2$ and $v > 0$	Transcriptomic profiling using microarray analysis	N = 300 biopsies, including N = 35 TCMR and N = 22 TCMR + AMR histological diagnoses	Among 35 TCMR cases, 11 were molecular score-negative/histology-positive, and among 22 TCMR + AMR cases, 5 were molecular score-negative/histology-positive. Of these, 2/11 TCMR and 1/5 TCMR + AMR discrepancies were attributed to TCMR diagnoses based on the isolated v-lesion criterion
Lefaucheur et al. [30]	TCMR/v-, TCMR/v+, AMR/v-, AMR/v+: classification based on hierarchical cluster and principal component analysis. Groups were defined using combined histological lesions, C4d and DSA status	Observational cohort	N = 139 TCMR/v-, N = 26 TCMR/v+, N = 73 AMR/v-, N = 64 AMR/v+	Risk of graft loss was similar between TCMR/v- and TCMR/v+ groups. AMR/v+ was associated with worse graft survival compared to AMR/v-
Sis et al. [28]	Isolated v: $v > 0$, $t \leq 1$, $i \leq 1$ TCMR grade I with v: $v > 0$, t2-3i2-3 Negative controls: v0, t0-1i0-1	Observational cohort	N = 103 isolated v, N = 101 TCMR grade I with v and N = 103 negative controls	Isolated v had similar treatment response rates and graft survival to TCMR grade I with v
Salazar et al. [20]	Isolated v: $v > 0$ and ($i < 2$ or $i < 2$), with/without AMR (as defined by Banff 2013 criteria) i-t-v lesions: $v > 0$ and ($i \geq 2$ and $t \geq 2$), with/without AMR Early: ≤ 1 year post-transplantation Late: ≤ 1 year post-transplantation Negative controls: v0	Observational cohort + transcriptomic profiling using microarray analysis	N = 28 isolated v, N = 21 i-t-v lesions, N = 654 v-negative. AMR in 17/49 v-positive biopsies. Early biopsy in 28/49, late in 21/49 v-positive biopsies	v-lesions did not increase the risk of graft failure compared to biopsies without v-lesions, regardless of the presence of concomitant AMR. Molecular TCMR scores were positive in 95% of i-t-v lesions and 21% of isolated v-lesions. Among 12 early isolated v-lesion biopsies, 9 showed no molecular rejection. Between 1 and 5 years, 4/5 had molecular rejection (1 TCMR, 1 AMR, 2 TCMR + AMR). Beyond 5 years, 9/11 had positive AMR scores, but only 1 had a positive TCMR score

(Continued on following page)

TABLE 2 | (Continued) Key studies on intimal arteritis (1996–2025).

Study	Phenotypic study definitions	Study type	Sample size	Main findings
Wu et al. [39]	sAMRV: suspicious AMR with v AMRV: AMR with v TMRV: TCMR with v All categories were defined according to the Banff 2013 criteria	Observational cohort	N = 37 sAMRV, N = 33 AMRV and N = 78 TCMRV. N = 80 v1, N = 51 v2 and N = 17 v3 cases (in total)	TCMRV, sAMRV, and AMRV showed similar responses to antirejection therapy, whereas v2 and v3 lesions were associated with significantly poorer outcomes than v1. Eight-year death-censored graft survival was higher in TCMRV than in AMRV, and significantly better in v1 compared to v2 or v3
Rabant et al. [16]	Isolated v: v1-3, $i \leq 1$ with any t-score, g + ptc ≤ 1 and C4d negative AMR with v: AMR criteria present with v-lesion	Observational cohort	N = 33 isolated v N = 54 AMR with v	Graft survival in AMR with v was significantly lower than in isolated v
Novotny et al. [17]	Isolated v: v1-3, $t < 2$, $i < 2$, g + ptc = 0, C4d0, DSA negative TCMR with v: v1-3, t0-3, i0-3, g + ptc = 0, C4d0, DSA negative Suspected AMR with v: v1-3, t0-3, i0-3, g + ptc 0-6, C4d 0-3, DSA negative/positive AMR with v: v1-3, t0-3, i0-3, g + ptc 0-6, C4d 0-3, DSA positive	Observational cohort	N = 25 isolated v, N = 18 TCMR with v, N = 36 suspected AMR with v and N = 19 AMR with v	Isolated v responded well to steroids, showed no persistence of arteritis in follow-up biopsies, and had favorable graft function and survival. AMR with v had significantly worse graft survival compared to isolated v
Mikhail et al. [18]	Isolated v1 i-t-v lesions <i>Criteria not explicitly stated</i>	Observational cohort	N = 50 v1, N = 28 i-t-v lesions	Isolated v1 had a better response to therapy and superior graft survival compared to i-t-v lesions
Novotny et al. [40]	AMR with v: v > 0 and presence of MVI $\pm$ C4d $\pm$ DSA AMR without v: presence of MVI $\pm$ C4d $\pm$ DSA	Observational cohort	N = 36 AMR with v, N = 102 AMR without v	AMR with v was a significant risk factor for the development of transplant glomerulopathy, regardless of DSA status, but was not associated with graft failure at 36 months
Nankivell et al. [29]	Isolated VR: v > 0, $i < 1$, $t < 1$ Inflamed VR: v > 0, $i \geq 1$, $t \geq 1$ irrespective of C4d and DSA Early: ≤ 1 month post-transplantation Late: >1 month post-transplantation	Observational cohort	N = 34 isolated VR, N = 66 inflamed VR; early VR in 51%, late VR in 49%	Isolated VR cases had better graft survival compared with inflamed VR. Early intimal arteritis was associated with delayed graft function and transient elevations in serum creatinine, but overall responded well to treatment. Late intimal arteritis was more frequently linked to chronic fibrosis and subsequent graft loss
Rosales et al. [41]	Intimal arteritis: v > 0	Transcriptomic profiling using NanoString B-HOT panel	N = 326 kidney transplant biopsies	Molecular TCMR scores correlated more strongly with t and i than with v
Sikosana et al. [42]	Intimal arteritis: v > 0	Transcriptomic profiling using microarray analysis	N = 1679 kidney transplant biopsies	Molecular TCMR scores were primarily associated with t and i, but not with v
Buxeda et al. [21]	Isolated endarteritis: v > 0, t0-1, i0-1, g + ptc 0-1, C4d negative Early: ≤ 1 month post-transplantation Late: >1 month post-transplantation AMR v+: v > 0 cases meeting 2019 Banff criteria for AMR, and those with isolated microvascular inflammation ≥ 2 C4d-negative TCMR v+ and mixed rejection v + <i>criteria not explicitly stated</i>	Observational cohort + transcriptomic profiling using NanoString B-HOT panel	N = 10 early isolated v, N = 13 late isolated v, N = 26 AMR v+, N = 10 TCMR v+, N = 23 mixed rejection v+	Early isolated v-lesions had higher delayed graft function rates and the worst 1-year death-censored graft survival compared to late isolated v-lesions and other rejection subtypes. Early isolated v showed increased early/acute injury gene expression but lower activation of rejection-related pathways, with reduced TCMR and AMR gene expression compared to TCMR, AMR, and mixed rejection
Wellekens et al. [19]	Isolated v: v > 0, t0-3i0 or t0i0-3, not meeting (p)AMR-MVI criteria) Borderline changes with v: v > 0, t1-3i1 or t1i1-3, not meeting (p)AMR-MVI criteria TCMR grade I with v: v > 0, t2-3i2-3, not	Observational cohort	N = 166 isolated v, N = 87 borderline changes with v, N = 66 TCMR grade I with v, N = 148 (p) AMR-MVI with v and N = 67 (12.5%) TCMR-I + (p)AMR-MVI with v	Although borderline changes with v showed slightly higher 10-year graft failure rates than isolated v-lesions, cases of TCMR-I, (p)AMR-MVI and TCMR-I + (p)AMR-MVI with v were associated with significantly worse

(Continued on following page)

TABLE 2 | (Continued) Key studies on intimal arteritis (1996–2025).

Study	Phenotypic study definitions	Study type	Sample size	Main findings
	meeting (p)AMR-MVI criteria (p)AMR-MVI with v: v > 0, t0-3i0-1 or t0-1i0-3, meeting Banff 2022 criteria ³ for (probable) AMR or DSA-negative C4d-negative MVI TCMR-I + (p)AMR-MVI with v: v > 0, t2-3i2-3, meeting (p)AMR-MVI criteria			outcomes. In a matched analysis of 534 v-positive cases and v-negative controls, v-lesions had no significant impact on graft outcomes

demonstrated that isolated v (N = 34) was associated with better graft survival than vascular rejection cases with concomitant tubulo-interstitial inflammation (N = 66). Also, Lefaucheur et al. [30] found no significant difference in outcomes up to 72 months post-transplant when comparing TCMR with v-lesions (N = 26) to TCMR without v-lesions (N = 139). In a multicenter observational cohort study [19] (707 v-lesion positive biopsies out of a total of 16,774 biopsies, corresponding to 534 transplants) we reported that isolated v-lesions were linked to superior 10-year graft survival compared to biopsies with t and i scores meeting or exceeding the borderline threshold, as well as biopsies fulfilling (probable) AMR/MVI criteria in the absence of significant tubulo-interstitial inflammation. Furthermore, when matching the 534 v-positive cases to v-negative controls, no significant impact of v-lesions on outcomes was observed.

A 2015 study [39] comparing 80 v1 cases to 51 v2 and 17 v3 cases concluded that higher-grade v-lesions (v2 and v3) were associated with significantly poorer response to rejection treatment and lower 8-year death-censored graft survival, with outcomes being more favorable in v1 cases. The study included patients who received kidney transplants between 1996 and 2012. It should be noted that the prevalence of v-lesions in recent observational cohort studies was relatively low, reported at, 2.7% [29], 4% [19, 30], 7% [20] and 9.7% [17], with the majority classified as mild (v1). The rarity of v3 lesions has generally limited the ability to perform meaningful analyses of this severe phenotype in recent studies.

Several factors likely contribute to the low detection rate of v-lesions in kidney allograft biopsies. Most biopsies contain only one to five arterial cross-sections, as arteries represent a small fraction of the total biopsy area compared to tubules and glomeruli. Moreover, rejection-related inflammation is often focal, and arteritis may affect only a segment of the arterial circumference, reflecting a patchy distribution. Together, these factors increase the risk of sampling bias. In addition, reproducibility may also be limited by interobserver variability, as some pathologists rely solely on morphology, while others incorporate immunohistochemical staining.

Nonetheless, the vascular rejection phenotype described in earlier studies may not reflect what is commonly observed today. Historically, vascular rejection was often hyperacute, characterized by severe arteritis, thrombosis, and frequent graft loss. Advances in immunosuppressive therapies [45, 46] and improved donor-recipient matching—particularly the avoidance of recipients with pre-transplant donor-specific HLA antibodies [47]—have significantly reduced the incidence of such severe presentations.

Moreover, the current Banff definition of intimal arteritis has a notably low diagnostic threshold, requiring only a single inflammatory cell in the subendothelial space to classify a biopsy as v1 (TCMR grade IIA) [7]. This raises the question of whether the v-lesions reported in older studies represent the same pathological process examined in more recent research.

Clinical Factors Related to Isolated v-Lesions

Timing post-transplantation is a key factor in the interpretation of isolated v-lesions. Rabant et al. [16] (N = 33) reported a median of 27 days post-transplantation for biopsies demonstrating isolated v-lesions. Nankivell et al. [29] found that early intimal arteritis (≤ 1 month, N = 51) was associated with delayed graft function (DGF) and transient elevations in serum creatinine, but generally responded well to therapy. In contrast, late-onset arteritis (> 1 month, N = 49) was more frequently associated with chronic fibrosis and graft loss. Buxeda et al. [21] reported that early (≤ 1 month) isolated v-lesions (N = 10) were associated with the poorest 1-year death-censored graft survival when compared to late isolated v-lesions (N = 13), or to TCMR with v (N = 10), AMR with v (N = 26), and mixed rejection with v (N = 23). In this cohort, 75% of early isolated v cases were associated with DGF, 60% progressed to primary non-function, and all graft losses in this group were due to this early event. However, the study lacked long-term follow-up, and the high proportion of expanded criteria donor kidneys likely increased vulnerability to delayed graft function [48–50]. In contrast, Sellarés et al. [26] (N = 19) compared the prognostic significance of intimal arteritis in biopsies performed within the first year (≤ 1 year) versus after 1 year (> 1 year). Unlike studies focused on the immediate post-transplant period (e.g., ≤ 1 month), this analysis did not find an association between v-lesions and allograft loss.

In addition, early isolated v-lesions are frequently identified in the absence of donor-specific anti-HLA antibodies (DSA). Several studies [16, 19–21] have reported low DSA positivity rates in early isolated v-lesions, in contrast to higher DSA prevalence in late-onset v-lesions or in cases associated with AMR or mixed rejection.

These findings have led to the hypothesis that early isolated v-lesions may reflect non-immune vascular injury—such as vascular trauma or ischemia-reperfusion injury related to the transplantation procedure—rather than alloimmune rejection [16, 17, 20].

Finally, arteritis is not pathognomonic for rejection. Arterial inflammation may also result from recurrent or *de novo* vasculitis

(e.g., ANCA-associated vasculitis, cryoglobulinemia, IgA vasculitis) or appear adjacent to vascular thrombosis, even in the absence of histological rejection.

v-Lesions in the Context of AMR

While the definitions by the Banff Working Group of “isolated v” were developed in relation to full TCMR [28], another important aspect of intimal arteritis in kidney transplant biopsies, is its non-specificity for TCMR. When AMR was first defined in 2001, v3 was already included among the histological criteria (**Table 1; Figure 1**). At the 2013 Banff meeting [44], it was further concluded that v1 and v2 should also be considered part of the AMR histologic criteria. This decision was based on findings by Lefaucheur et al. [30], who reported that AMR with v was associated with a worse prognosis than AMR without v. However, only eight out of 64 HLA-DSA-positive cases in their study had v-lesions as the only histologic feature of AMR, making it impossible to assess outcomes specifically in this subgroup. Additionally, AMR with v was associated with a higher incidence of t- and i-lesions, which correlated with graft loss. Since the primary analysis comparing AMR with and without v did not adjust for concomitant tubulointerstitial inflammation, it remains unclear whether the worse outcomes in AMR with v cases were driven by the v-lesion itself or by coexisting tubulointerstitial inflammation.

Since then, the relationship between AMR and intimal arteritis, in the absence of TCMR, has been studied only scarcely (**Table 2**). Rabant et al. [16] found that graft survival up to eight years after the index biopsy was significantly lower in AMR with v (N = 54) compared to isolated v cases (N = 33). Similarly, Novotny et al. [17] reported that AMR with v (N = 19) was associated with worse three-year graft survival compared to isolated v (N = 25). In addition, the presence of intimal arteritis in AMR was a significant risk factor for progression to transplant glomerulopathy, irrespective of DSA status, but was not associated with an increased risk of graft failure at 36 months [40]. Salazar et al. [20] also concluded that the presence of v-lesions did not increase the risk of graft failure when analyzed in biopsies diagnosed with AMR (N = 17). In a recent multicenter observational cohort study (707 v-lesion positive biopsies, derived from 534 transplants) [19], we observed no difference in 10-year graft survival between cases with a Banff category 2 diagnosis and a v-lesion and those with a category 2 diagnosis without a v-lesion. The study also highlighted the risk of overinterpreting the presence of a v-lesion in (probable)AMR/MVI cases as indicative of mixed rejection when significant tubulointerstitial inflammation is absent. Notably, cases diagnosed as “mixed rejection” solely due to the presence of a v-lesion had better clinical outcomes than those with both (probable)AMR/MVI and marked tubulo-interstitial inflammation—classified in the study as true “mixed rejection.”

Nevertheless, it is important to note that in studies using biopsies obtained before 2013, v1 and v2 lesions were not included in the diagnostic criteria for AMR. As a result, v-lesions in the context of AMR may have been interpreted and managed as TCMR, in accordance with the Banff classification at that time.

The Pathogenesis of Intimal Arteritis

Since 2017, the Banff classification has recommended biopsy-based transcript analysis to support the differential diagnosis of AMR, TCMR, mixed rejection in isolated v cases that lack MVI and TCMR features, are C4d-negative, and may or may not have DSA [31]. This recommendation underscores the added value of molecular tools to clarify ambiguous cases.

Reeve et al. [37] developed a TCMR score using microarray analysis of 403 kidney transplant biopsies and found that while the score correlated strongly with interstitial inflammation (i) and tubulitis (t), 79% of isolated v-lesions had low TCMR scores. A prospective validation study [38] (N = 300) confirmed that discordance between histology and molecular findings often stemmed from TCMR diagnoses based solely on isolated v-lesions. In a third study, the same team [20] compared 28 isolated v-lesions to 21 biopsies with i-t-v lesions and found that 95% of the latter had positive TCMR scores, whereas only 21% of isolated v-lesions did. Most early (≤ 1 year post-transplantation) isolated v-lesions lacked molecular rejection signals, whereas those occurring beyond 1 year more frequently exhibited molecular AMR signatures. Logistic regression models trained on molecular diagnoses confirmed that TCMR scores were primarily driven by i and t, but not v [42]. Similarly, studies using the NanoString Banff Human Organ Transplant (B-HOT) panel [51] showed that TCMR-associated gene sets correlated strongly with i and t [41], but not with v [21]. Moreover, early isolated v-lesions (≤ 1 month post-transplant) demonstrated increased expression of early injury-response genes compared to late-onset cases (> 1 month), further supporting a non-immune injury mechanism in the early post-transplant period. While advances in molecular diagnostics have enhanced our understanding of these biopsies, the focal nature of intimal arteritis may still lead to false-negative results due to sampling limitations [52].

Nevertheless, the underlying pathogenesis and cellular composition of intimal arteritis remain poorly characterized. While earlier studies primarily associated intimal arteritis with CD4⁺ and CD8⁺ T cell infiltration, more recent evidence suggests a broader immune response involving both T lymphocytes and macrophages [35, 36]. A recent study [53] using deconvolution of bulk transcriptomic data found that v-lesions were most strongly associated with innate immune cells—such as NK cells and monocytes/macrophages—similar to other vascular lesions like peritubular capillaritis and glomerulitis.

These findings suggest that innate immunity may contribute to the pathogenesis of intimal arteritis. In particular, early isolated v-lesions may reflect endothelial stress and innate immune activation following ischemia-reperfusion injury. This process induces endothelial and parenchymal damage, releasing damage-associated molecular patterns (DAMPs) [54–57] engage pattern recognition receptors (PRRs), such as Toll-like receptors (TLRs), on endothelial and immune cells. PRR signaling promotes cytokine release, interferon production, and regulated cell death, amplifying local inflammation and recruiting additional immune cells. Concomitantly, endothelial activation upregulates adhesion molecules (e.g., P-selectin, ICAM-1), promoting platelet adhesion and degranulation. Platelet-derived mediators amplify inflammation and recruit monocytes, neutrophils and T cells

[58]. In parallel, stress-induced changes in HLA expression—such as downregulation of classical HLA-I and upregulation of non-classical HLA-I and NKG2D ligands (e.g., MICA, MICB, ULBPs)—can trigger NK cell-mediated cytotoxicity [59, 60]. Persistent injury may also expose cryptic self-antigens (e.g., AT1R, perlecan, collagen V), triggering non-HLA autoantibody responses [61, 62]. These antibodies can form immune complexes that activate Fc receptors or complement pathways, amplifying cytokine release, endothelial damage, and vascular inflammation. However, whether these mechanisms directly contribute to the pathogenesis of intimal arteritis remains uncertain and warrants further investigation.

Given their association with favorable outcomes, early isolated v-lesions are presumed to potentially resolve spontaneously if the inciting insult wanes and immune activation diminishes. However, it remains unclear whether and how this resolution occurs, and whether there is a place for specific therapeutic interventions.

The mechanisms underlying intimal arteritis in TCMR also remain incompletely understood. Donor HLA antigens activate recipient T cells via direct or indirect allorecognition [63], promoting T cell-endothelium interactions through upregulated HLA expression. This may lead to cytokine release and direct cytotoxicity via perforin/granzyme or Fas/FasL pathways [64, 65]. Alternatively, v-lesions—like peritubular capillaritis—may result from increased leukocyte trafficking in areas of interstitial inflammation, without direct endothelial targeting. In contrast, the mechanisms driving arteritis in AMR are better defined: DSAs bind activated endothelium, triggering complement activation and antibody-dependent cellular cytotoxicity (ADCC), primarily mediated by NK cells or monocytes via Fc receptor engagement [66]. In both TCMR and AMR, immune infiltration into the subendothelial space gives rise to intimal arteritis, which can progress to transmural inflammation, smooth muscle necrosis, fibrin deposition, and vascular occlusion in severe cases.

While much remains unknown, v-lesions are clearly a heterogeneous finding whose clinical significance depends on timing post-transplant, immunologic risk (e.g., HLA-DSA status), and coexisting features suggestive of AMR, TCMR, or recurrent disease. Traditional histology offers limited specificity in isolated v-lesions, but emerging tools—such as (spatial) transcriptomics, integrated within multi-omics frameworks—may offer new biological insights. Refining the diagnostic role of v-lesions in the Banff classification will require a context-aware approach that accounts for both immune and non-immune causes of vascular injury.

DISCUSSION

The Banff classification has provided a crucial framework for diagnosing rejection in kidney transplantation, enabling consistency in histopathological assessment across centers worldwide. However, as transplant medicine evolves, so too must our understanding of the clinical significance of specific histological lesions.

Long regarded as a hallmark of moderate-to-severe T cell-mediated rejection (TCMR), the v-lesion is now recognized as a heterogeneous entity. Emerging evidence challenges the assumption that all v-lesions signify TCMR, particularly when they occur in isolation without substantial tubulo-interstitial inflammation. Furthermore, advances in immunosuppressive therapy and improved donor-recipient matching may have fundamentally altered the clinical course of intimal arteritis, raising doubts about whether historical interpretations remain applicable to contemporary transplant populations.

A clear and consistent definition of “isolated v” is essential before its role within the Banff classification can be meaningfully reconsidered. The 2009 Banff isolated v working group [28, 43] proposed defining “isolated v” as intimal arteritis with minimal interstitial inflammation ($i \leq 1$) and tubulitis ($t \leq 1$). However, cases fulfilling the Banff criteria for borderline TCMR ($t1i1$), and having v lesions, should likely not be called isolated v, as this would lead to the contradiction that “borderline TCMR” is often treated [9, 10], while the term “isolated v” designates a potentially more benign phenotype. In recent study [19] we proposed a definition more aligned with current Banff thresholds, distinguishing isolated v ($v > 0$, $t0-3i0$ or $t0i0-3$) from borderline TCMR ($v0$, $t1-3i1$ or $t1i1-3$), TCMR I ($v0$, $t2-3i2-3$), and TCMR II–III ($v > 0$, $t1-3i1-3$). While classifying $t2-3i0$ or $t0i2-3$ as isolated v may seem counterintuitive, such cases were rare (2.8% of v-lesion positive biopsies) in our study. In these instances, case-level pathology review is essential to determine whether tubulitis or interstitial inflammation is truly isolated. Tools such as the activity indices by Vaulet et al. [67] or molecular diagnostics such as the B-HOT panel [51] may help refine classification and inform treatment decisions in these exceptional cases.

Second, the prior definitions used by the Banff Working Group [28] did not account for the relationship between intimal arteritis and AMR, leaving it unclear whether v-lesions in AMR represent concurrent TCMR (“mixed rejection”). This is further complicated by the lack of a clear Banff definition for mixed rejection, making shared lesions like v difficult to interpret and potentially leading to overdiagnosis and overtreatment of mixed phenotypes [19]. In such cases, the v-lesion may be more appropriately interpreted as a manifestation of AMR, particularly when other histologic features of TCMR are absent. A more coherent approach would classify these as (probable) AMR/MVI with v, and reserve “isolated v” for biopsies lacking both AMR/MVI and tubulo-interstitial criteria for (borderline) TCMR. Refining the role of the v-lesion within the Banff classification does not mean abandoning its significance but rather integrating new insights to enhance diagnostic precision. Importantly, clinical practice is already evolving, as a recent survey indicated that many European transplant centers no longer treat isolated v-lesions with ATG [10].

The Banff process has always been one of evolution, shaped by emerging data and expert consensus. The challenge now is how best to incorporate these new insights while maintaining a classification system that remains clinically relevant and widely applicable. Over a decade ago, Salazar et al. [20] cautioned that “isolated v-lesions have been seriously misinterpreted for over 20 years, potentially leading to inappropriate treatment decisions.” With accumulating clinical and molecular evidence,

the Banff classification may need to adapt and reconsider the specific definition of an “isolated v” phenotype.

AUTHOR CONTRIBUTIONS

KW and MN drafted the manuscript. All authors contributed to the article and approved the submitted version.

FUNDING

The author(s) declare that financial support was received for the research and/or publication of this article. This work was supported by The Research Foundation Flanders (FWO); KW

holds a FWO fellowship grant (11P1524N) and MN is a senior clinical investigator of FWO (1842919N).

CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

GENERATIVE AI STATEMENT

The author(s) declare that no Generative AI was used in the creation of this manuscript.

REFERENCES

- Hariharan S, Johnson CP, Bresnahan BA, Taranto SE, McIntosh MJ, Stablein D. Improved Graft Survival after Renal Transplantation in the United States, 1988 to 1996. *N Engl J Med* (2000) 342(9):605–12. doi:10.1056/nejm200003023420901
- Hariharan S, Israni AK, Danovitch G. Long-Term Survival after Kidney Transplantation. *N Engl J Med* (2021) 385(8):729–43. doi:10.1056/NEJMra2014530
- Coemans M, Susal C, Dohler B, Anglicheau D, Giral M, Bestard O, et al. Analyses of the Short- and Long-Term Graft Survival after Kidney Transplantation in Europe between 1986 and 2015. *Kidney Int* (2018) 94(5):964–73. doi:10.1016/j.kint.2018.05.018
- Van Loon E, Senev A, Lerut E, Coemans M, Callemeyn J, Van Keer JM, et al. Assessing the Complex Causes of Kidney Allograft Loss. *Transplantation* (2020) 104(12):2557–66. doi:10.1097/tp.0000000000003192
- Mayrdorfer M, Liefeldt L, Wu K, Rudolph B, Zhang Q, Friedersdorff F, et al. Exploring the Complexity of Death-Censored Kidney Allograft Failure. *J Am Soc Nephrol* (2021) 32(6):1513–26. doi:10.1681/asn.2020081215
- Banfffoundation. Banff Classification for Renal Allograft Pathology (2022). Available online at: <https://banfffoundation.org/central-repository-for-banff-classification-resources-3> (Accessed 02, 2023).
- Roufosse C, Simmonds N, Clahsen-van Groningen M, Haas M, Henriksen KJ, Horsfield C, et al. A 2018 Reference Guide to the Banff Classification of Renal Allograft Pathology. *Transplantation* (2018) 102(11):1795–814. doi:10.1097/tp.0000000000002366
- Thiyagarajan UM, Ponnuswamy A, Bagul A. Thymoglobulin and its Use in Renal Transplantation: A Review. *Am J Nephrol* (2013) 37(6):586–601. doi:10.1159/000351643
- Sood P, Cherikh WS, Toll AE, Mehta RB, Hariharan S. Kidney Allograft Rejection: Diagnosis and Treatment Practices in USA- A UNOS Survey. *Clin Transpl* (2021) 35(4):e14225. doi:10.1111/ctr.14225
- Koshy P, Furian L, Nickerson P, Zaza G, Haller M, de Vries APJ, et al. European Survey on Clinical Practice of Detecting and Treating T-Cell Mediated Kidney Transplant Rejection. *Transpl Int* (2024) 37:12283. doi:10.3389/ti.2024.12283
- Kooijmans-Coutinho MF, Hermans J, Schrama E, Ringers J, Daha MR, Bruijn JA, et al. Interstitial Rejection, Vascular Rejection, and Diffuse Thrombosis of Renal Allografts. Predisposing Factors, Histology, Immunohistochemistry, and Relation to Outcome. *Transplantation* (1996) 61(9):1338–44. doi:10.1097/00007890-199605150-00009
- Colvin RB, Cohen AH, Saiontz C, Bonsib S, Buick M, Burke B, et al. Evaluation of Pathologic Criteria for Acute Renal Allograft Rejection: Reproducibility, Sensitivity, and Clinical Correlation. *J Am Soc Nephrol* (1997) 8(12):1930–41. doi:10.1681/asn.V8I121930
- Nickeleit V, Vamvakas EC, Pascual M, Poletti BJ, Colvin RB. The Prognostic Significance of Specific Arterial Lesions in Acute Renal Allograft Rejection. *J Am Soc Nephrol* (1998) 9(7):1301–8. doi:10.1681/asn.V9I71301
- Mueller A, Schnuelle P, Waldherr R, van der Woude FJ. Impact of the Banff '97 Classification for Histological Diagnosis of Rejection on Clinical Outcome and Renal Function Parameters after Kidney Transplantation. *Transplantation* (2000) 69(6):1123–7. doi:10.1097/00007890-200003270-00017
- Minervini MI, Torbenson M, Scantlebury V, Vivas C, Jordan M, Shapiro R, et al. Acute Renal Allograft Rejection with Severe Tubulitis (Banff 1997 Grade IB). *Am J Surg Pathol* (2000) 24(4):553–8. doi:10.1097/00000478-200004000-00009
- Rabant M, Boulenger F, Gnemmi V, Pellé G, Glowacki F, Hertig A, et al. Isolated V-Lesion in Kidney Transplant Recipients: Characteristics, Association with DSA, and Histological Follow-Up. *Am J Transpl* (2018) 18(4):972–81. doi:10.1111/ajt.14617
- Novotny M, Hrubá P, Vichová P, Malusková J, Honsova E, Viklický O, et al. Isolated V-Lesion Represents a Benign Phenotype of Vascular Rejection of the Kidney Allograft - a Retrospective Study. *Transpl Int* (2018) 31(10):1153–63. doi:10.1111/tri.13286
- Mikhail D, Chan E, Sharma H, Kleinsteinuber D, Wei J, Rim C, et al. Clinical Significance of Isolated V1 Arteritis in Renal Transplantation. *Transpl Proc* (2021) 53(5):1570–5. doi:10.1016/j.transproceed.2021.03.027
- Wellekens K, Coemans M, Koshy P, Valet T, Cleenders E, Debyser T, et al. The Impact of the Banff V-Lesion on Rejection Classification and Outcomes: Insights from a Multicenter Study. *Am J Transpl* (2025). doi:10.1016/j.ajt.2025.04.023
- Salazar ID, Merino López M, Chang J, Halloran PF. Reassessing the Significance of Intimal Arteritis in Kidney Transplant Biopsy Specimens. *J Am Soc Nephrol* (2015) 26(12):3190–8. doi:10.1681/asn.2014111064
- Buxeda A, Crespo M, Chamoun B, Gimeno J, Torres IB, Redondo-Pachón D, et al. Clinical and Molecular Spectrum of V-Lesion. *Am J Transpl* (2024) 24:2007–21. doi:10.1016/j.ajt.2024.07.025
- Solez K, Axelsen RA, Benediktsson H, Burdick JF, Cohen AH, Colvin RB, et al. International Standardization of Criteria for the Histologic Diagnosis of Renal Allograft Rejection: The Banff Working Classification of Kidney Transplant Pathology. *Kidney Int* (1993) 44(2):411–22. doi:10.1038/ki.1993.259
- Jabs WJ, Sedlmeyer A, Ramassar V, Hidalgo LG, Urmson J, Afrouzian M, et al. Heterogeneity in the Evolution and Mechanisms of the Lesions of Kidney Allograft Rejection in Mice. *Am J Transpl* (2003) 3(12):1501–9. doi:10.1046/j.1600-6135.2003.00269.x
- Pratt JR, Basheer SA, Sacks SH. Local Synthesis of Complement Component C3 Regulates Acute Renal Transplant Rejection. *Nat Med* (2002) 8(6):582–7. doi:10.1038/nm0602-582
- Racusen LC, Colvin RB, Solez K, Mihatsch MJ, Halloran PF, Campbell PM, et al. Antibody-Mediated Rejection Criteria - an Addition to the Banff

- 97 Classification of Renal Allograft Rejection. *Am J Transpl* (2003) 3(6): 708–14. doi:10.1034/j.1600-6143.2003.00072.x
26. Sellarés J, de Freitas DG, Mengel M, Sis B, Hidalgo LG, Matas AJ, et al. Inflammation Lesions in Kidney Transplant Biopsies: Association with Survival Is Due to the Underlying Diseases. *Am J Transpl* (2011) 11(3): 489–99. doi:10.1111/j.1600-6143.2010.03415.x
27. Mueller TF, Einecke G, Reeve J, Sis B, Mengel M, Jhangri GS, et al. Microarray Analysis of Rejection in Human Kidney Transplants Using Pathogenesis-Based Transcript Sets. *Am J Transpl* (2007) 7(12):2712–22. doi:10.1111/j.1600-6143.2007.02005.x
28. Sis B, Bagnasco SM, Cornell LD, Randhawa P, Haas M, Lategan B, et al. Isolated Endarteritis and Kidney Transplant Survival: A Multicenter Collaborative Study. *J Am Soc Nephrol* (2015) 26(5):1216–27. doi:10.1681/asn.2014020157
29. Nankivell BJ, Shingde M, P'Ng CH. The Pathological and Clinical Diversity of Acute Vascular Rejection in Kidney Transplantation. *Transplantation* (2022) 106(8):1666–76. doi:10.1097/tp.0000000000004071
30. Lefaucheur C, Loupy A, Vernerey D, Duong-Van-Huyen JP, Suberbielle C, Anglicheau D, et al. Antibody-Mediated Vascular Rejection of Kidney Allografts: A Population-Based Study. *Lancet* (2013) 381(9863):313–9. doi:10.1016/s0140-6736(12)61265-3
31. Haas M, Loupy A, Lefaucheur C, Roufosse C, Glotz D, Seron D, et al. The Banff 2017 Kidney Meeting Report: Revised Diagnostic Criteria for Chronic Active T Cell-Mediated Rejection, Antibody-Mediated Rejection, and Prospects for Integrative Endpoints for Next-Generation Clinical Trials. *Am J Transpl* (2018) 18(2):293–307. doi:10.1111/ajt.14625
32. Solez K, Benediktsson H, Cavallo T, Croker B, Demetris AJ, Drachenberg C, et al. Report of the Third Banff Conference on Allograft Pathology (July 20–24, 1995) on Classification and Lesion Scoring in Renal Allograft Pathology. *Transpl Proc* (1996) 28(1):441–4.
33. Racusen LC, Solez K, Colvin RB, Bonsib SM, Castro MC, Cavallo T, et al. The Banff 97 Working Classification of Renal Allograft Pathology. *Kidney Int* (1999) 55(2):713–23. doi:10.1046/j.1523-1755.1999.00299.x
34. Haas M, Kraus ES, Samaniego-Picota M, Racusen LC, Ni W, Eustace JA. Acute Renal Allograft Rejection with Intimal Arteritis: Histologic Predictors of Response to Therapy and Graft Survival. *Kidney Int* (2002) 61(4):1516–26. doi:10.1046/j.1523-1755.2002.00254.x
35. Kozakowski N, Böhmig GA, Exner M, Soleiman A, Huttary N, Nagy-Bojarszky K, et al. Monocytes/macrophages in Kidney Allograft Intimal Arteritis: No Association with Markers of Humoral Rejection or with Inferior Outcome. *Nephrol Dial Transpl* (2009) 24(6):1979–86. doi:10.1093/ndt/gfp045
36. Sun HJ, Zhou T, Wang Y, Fu Y, Jiang Y, Zhang L, et al. Macrophages and T Lymphocytes Are the Predominant Cells in Intimal Arteritis of Resected Renal Allografts Undergoing Acute Rejection. *Transpl Immunol* (2011) 25(1):42–8. doi:10.1016/j.trim.2011.04.002
37. Reeve J, Sellarés J, Mengel M, Sis B, Skene A, Hidalgo L, et al. Molecular Diagnosis of T Cell-Mediated Rejection in Human Kidney Transplant Biopsies. *Am J Transpl* (2013) 13(3):645–55. doi:10.1111/ajt.12079
38. Halloran PF, Pereira AB, Chang J, Matas A, Picton M, De Freitas D, et al. Potential Impact of Microarray Diagnosis of T Cell-Mediated Rejection in Kidney Transplants: The INTERCOM Study. *Am J Transpl* (2013) 13(9): 2352–63. doi:10.1111/ajt.12387
39. Wu K, Budde K, Schmidt D, Neumayer HH, Rudolph B. The Relationship of the Severity and Category of Acute Rejection with Intimal Arteritis Defined in Banff Classification to Clinical Outcomes. *Transplantation* (2015) 99(8): e105–14. doi:10.1097/tp.0000000000000640
40. Novotny M, Hrubá P, Kment M, Voska L, Kabrtová K, Slavcev A, et al. Intimal Arteritis and Microvascular Inflammation Are Associated with Inferior Kidney Graft Outcome, Regardless of Donor-Specific Antibodies. *Front Med (Lausanne)* (2021) 8:781206. doi:10.3389/fmed.2021.781206
41. Rosales IA, Mahowald GK, Tomaszewski K, Hotta K, Iwahara N, Otsuka T, et al. Banff Human Organ Transplant Transcripts Correlate with Renal Allograft Pathology and Outcome: Importance of Capillaritis and Subpathologic Rejection. *J Am Soc Nephrol* (2022) 33(12):2306–19. doi:10.1681/asn.2022040444
42. Sikosana MLN, Reeve J, Madill-Thomsen KS, Halloran PFINTERCOMEX Investigators. Using Regression Equations to Enhance Interpretation of Histology Lesions of Kidney Transplant Rejection. *Transplantation* (2023) 108:445–54. doi:10.1097/tp.0000000000004783
43. Sis B, Mengel M, Haas M, Colvin RB, Halloran PF, Racusen LC, et al. Banff '09 Meeting Report: Antibody Mediated Graft Deterioration and Implementation of Banff Working Groups. *Am J Transpl* (2010) 10(3): 464–71. doi:10.1111/j.1600-6143.2009.02987.x
44. Haas M, Sis B, Racusen LC, Solez K, Glotz D, Colvin RB, et al. Banff 2013 Meeting Report: Inclusion of C4d-Negative Antibody-Mediated Rejection and Antibody-Associated Arterial Lesions. *Am J Transpl* (2014) 14(2):272–83. doi:10.1111/ajt.12590
45. Halloran PF. Immunosuppressive Drugs for Kidney Transplantation. *N Engl J Med* (2004) 351(26):2715–29. doi:10.1056/NEJMra033540
46. Nankivell BJ, Alexander SI. Rejection of the Kidney Allograft. *N Engl J Med* (2010) 363(15):1451–62. doi:10.1056/NEJMra0902927
47. Tait BD, Süsal C, Gebel HM, Nickerson PW, Zachary AA, Claas FHH, et al. Consensus Guidelines on the Testing and Clinical Management Issues Associated with HLA and Non-HLA Antibodies in Transplantation. *Transplantation* (2013) 95(1):19–47. doi:10.1097/TP.0b013e31827a19cc
48. Filiopoulos V, Boletis JN. Renal Transplantation with Expanded Criteria Donors: Which Is the Optimal Immunosuppression? *World J Transpl* (2016) 6(1):103–14. doi:10.5500/wjt.v6.i1.103
49. Fijter JW, Mallat MJK, Doxiadis IIN, Ringers J, Rosendaal FR, Claas FHH, et al. Increased Immunogenicity and Cause of Graft Loss of Old Donor Kidneys. *J Am Soc Nephrol* (2001) 12(7):1538–46. doi:10.1681/asn.V1271538
50. Zhang R, Trotter PB, McCaffrey J, Fitzroy R, Trivioli G, Stewart BJ, et al. Assessment of Biological Organ Age Using Molecular Pathology in Pre-Transplant Kidney Biopsies. *Kidney Int* (2024) 106:302–16. doi:10.1016/j.kint.2024.03.028
51. Mengel M, Loupy A, Haas M, Roufosse C, Naesens M, Akalin E, et al. Banff 2019 Meeting Report: Molecular Diagnostics in Solid Organ Transplantation—Consensus for the Banff Human Organ Transplant (B-HOT) Gene Panel and Open Source Multicenter Validation. *Am J Transpl* (2020) 20(9):2305–17. doi:10.1111/ajt.16059
52. Randhawa PS. The Molecular Microscope Diagnostic System (MMDx) in Transplantation: A Pathologist's Perspective. *Am J Transpl* (2020) 20(8): 1965–6. doi:10.1111/ajt.15887
53. Vaulet T, Callemeyn J, Lamarthée B, Antoranz A, Debyser T, Koshy P, et al. The Clinical Relevance of the Infiltrating Immune Cell Composition in Kidney Transplant Rejection. *J Am Soc Nephrol* (2024) 35(7):886–900. doi:10.1681/asn.0000000000000350
54. DeWolf SE, Kasimsetty SG, Hawkes AA, Stocks LM, Kurian SM, McKay DB. DAMPs Released from Injured Renal Tubular Epithelial Cells Activate Innate Immune Signals in Healthy Renal Tubular Epithelial Cells. *Transplantation* (2022) 106(8):1589–99. doi:10.1097/tp.00000000000004038
55. Kasimsetty SG, McKay DB. Ischemia as a Factor Affecting Innate Immune Responses in Kidney Transplantation. *Curr Opin Nephrol Hypertens* (2016) 25(1):3–11. doi:10.1097/mnh.0000000000000190
56. Land WG, Agostinis P, Gasser S, Garg AD, Linkermann A. Transplantation and Damage-Associated Molecular Patterns (DAMPs). *Am J Transpl* (2016) 16(12):3338–61. doi:10.1111/ajt.13963
57. Li Q, Lan P. Activation of Immune Signals during Organ Transplantation. *Signal Transduct Target Ther* (2023) 8(1):110. doi:10.1038/s41392-023-01377-9
58. Kirk AD, Morrell CN, Baldwin WM, 3rd. Platelets Influence Vascularized Organ Transplants from Start to Finish. *Am J Transpl* (2009) 9(1):14–22. doi:10.1111/j.1600-6143.2008.02473.x
59. Koenig A, Chen CC, Marçais A, Barba T, Mathias V, Sicard A, et al. Missing Self Triggers NK Cell-Mediated Chronic Vascular Rejection of Solid Organ Transplants. *Nat Commun* (2019) 10(1):5350. doi:10.1038/s41467-019-13113-5
60. Duygu B, Olieslagers TI, Groeneweg M, Voort CEM, Wieten L. HLA Class I Molecules as Immune Checkpoints for NK Cell Alloreactivity and Anti-Viral Immunity in Kidney Transplantation. *Front Immunol* (2021) 12:680480. doi:10.3389/fimmu.2021.680480
61. Zhang Q, Reed EF. The Importance of Non-HLA Antibodies in Transplantation. *Nat Rev Nephrol* (2016) 12(8):484–95. doi:10.1038/nrneph.2016.88

62. Cardinal H, Dieudé M, Hébert MJ. The Emerging Importance of Non-HLA Autoantibodies in Kidney Transplant Complications. *J Am Soc Nephrol* (2017) 28(2):400–6. doi:10.1681/asn.2016070756
63. Duneton C, Winterberg PD, Ford ML. Activation and Regulation of Alloreactive T Cell Immunity in Solid Organ Transplantation. *Nat Rev Nephrol* (2022) 18(10):663–76. doi:10.1038/s41581-022-00600-0
64. Krupnick AS, Kreisel D, Popma SH, Balsara KR, Szeto WY, Krasinskas AM, et al. Mechanism of T Cell-Mediated Endothelial Apoptosis. *Transplantation* (2002) 74(6):871–6. doi:10.1097/00007890-200209270-00022
65. Halloran PF, Urmson J, Ramassar V, Melk A, Zhu LF, Halloran BP, et al. Lesions of T-Cell-Mediated Kidney Allograft Rejection in Mice Do Not Require Perforin or Granzymes A and B. *Am J Transpl* (2004) 4(5):705–12. doi:10.1111/j.1600-6143.2004.00421.x
66. Böhmig GA, Eskandary F, Doberer K, Halloran PF. The Therapeutic Challenge of Late Antibody-Mediated Kidney Allograft Rejection. *Transpl Int* (2019) 32(8):775–88. doi:10.1111/tri.13436
67. Vaulet T, Divard G, Thaumat O, Lerut E, Senev A, Aubert O, et al. Data-Driven Derivation and Validation of Novel Phenotypes for Acute Kidney Transplant Rejection Using Semi-Supervised Clustering. *J Am Soc Nephrol* (2021) 32(5):1084–96. doi:10.1681/asn.2020101418

Copyright © 2025 Wellekens, Koshy, Roufosse and Naesens. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.



Interactions Between Immunosuppressive Regimens and Cytomegalovirus Infection After Solid-Organ Transplantation

Lucas Milo Bellier^{1,2*}, Hannah Kaminski^{1,2,3}, Pierre Merville^{1,2,3} and Lionel Couzi^{1,2,3}

¹Centre Hospitalier Universitaire de Bordeaux, Bordeaux, France, ²UFR des Sciences Médicales, Université de Bordeaux, Talence, France, ³Immuno ConcEpT, CNRS-UMR 5164, Université de Bordeaux, Talence, France

Cytomegalovirus (CMV) remains a major infectious complication after solid-organ transplantation, driven by immunosuppressive therapies that alter CMV-specific cell-mediated immunity. Antithymocyte globulin induces profound and prolonged T-cell depletion, transiently impairing CMV-specific cell-mediated immunity and increasing CMV risk in seropositive recipients. Calcineurin inhibitors suppress cytokine production, notably IL-2 and IFN- γ , without significantly impairing cytotoxic function, while mycophenolate mofetil limits lymphocyte proliferation but preserves effector capacity. In contrast, mTOR inhibitors exert dual antiviral and immunomodulatory effects by directly inhibiting CMV replication and enhancing CMV-specific T-cell memory formation. Belatacept, through CD28–CD80/CD86 blockade, may predispose to late, severe, or relapsing CMV disease, particularly in elderly or D⁺/R[−] recipients. Corticosteroids broadly inhibit NK cell cytotoxicity and CMV-specific T-cell responses, but clinical data on steroid withdrawal remain inconsistent. Overall, CMV risk is determined less by a single drug than by the cumulative depth of immunosuppression. Integrating immune monitoring tools, such as CMV-specific T-cell assays, could enable tailored immunosuppressive regimens balancing antiviral protection with graft survival.

OPEN ACCESS

*Correspondence

Lucas Milo Bellier,
✉ lucas-milo.bellier@etu.u-bordeaux.fr

Received: 02 December 2025

Revised: 16 February 2026

Accepted: 23 March 2026

Published: 09 April 2026

Citation:

Bellier LM, Kaminski H, Merville P and Couzi L (2026) Interactions Between Immunosuppressive Regimens and Cytomegalovirus Infection After Solid-Organ Transplantation. *Transpl. Int.* 39:15987. doi: 10.3389/ti.2026.15987

Keywords: cytomegalovirus (CMV), immunosuppressant, infection, kidney transplant, review of literature

INTRODUCTION

Cytomegalovirus (CMV) is a major opportunistic pathogen in solid-organ transplantation (SOT), where immunosuppressive therapy promotes viral reactivation and disease.

In immunocompetent hosts, early recognition of CMV by dendritic cells and macrophages through Toll-like receptors triggers type I interferon and cytokine production, activating natural killer (NK) cells and limiting initial viral replication.

Long-term viral control, however, relies on adaptive immunity, particularly CMV-specific CD8⁺ T cells. Upon antigen presentation, these cells expand and differentiate into effector and memory subsets that clear infected cells via cytotoxic mechanisms (perforin, granzymes) and cytokine release (IFN- γ , TNF- α). Persistent infection drives “memory inflation,” characterized by the progressive accumulation of highly differentiated, functional CD8⁺ T cells targeting dominant antigens (IE1, pp65) [1]. These cells are crucial for maintaining viral latency, and their deficiency correlates with uncontrolled replication in immunocompromised patients. Functional monitoring of CMV-specific T-cell responses can be achieved using intracellular cytokine staining (ICS), CMV-ELISPOT, or

interferon-gamma release assays (e.g., QuantiFERON®-CMV), which differ in sensitivity and clinical applicability [2].

Although $\alpha\beta$ CD8⁺ and CD4⁺ T cells dominate adaptive control, $\gamma\delta$ T cells—especially nonV δ 2Vg9 subsets—also expand during infection and exhibit cytotoxic, IFN- γ -producing effector functions, compensating when conventional T-cell immunity is impaired [3], together with NK CD57⁺ NKG2C (high) NK cells [4].

Understanding immune dynamics is critical to assess how immunosuppressive therapies influence CMV susceptibility and clinical outcomes. This review examines the impact of each immunosuppressant class on CMV risk, adopting a structured approach according to risk level.

HIGH-RISK AGENTS FOR CMV INFECTION

This section identifies immunosuppressive agents that substantially increase CMV infection risk through profound immunosuppression, requiring heightened vigilance and tailored management strategies.

Antithymocyte Globulin: The Most Depleting Agent Mechanisms of Action

Antithymocyte globulins (ATG) cause profound T-cell depletion by targeting multiple surface antigens. Their main mechanisms include complement-dependent lysis, apoptosis, and opsonization with phagocytosis. Beyond depletion, ATG modulate immune responses by downregulating adhesion molecules and chemokine receptors, disrupting leukocyte trafficking [5]. ATG induces rapid and profound depletion of naive CD4⁺ T cells, more pronounced than that observed for CD8⁺ T cells. Notably, CD4⁺ lymphopenia often persists at 12 months post-transplantation [6].

ATG has been widely associated with profound immunosuppression and an increased incidence of cytomegalovirus (CMV) infection [7–11]. Since its initial use, it has been recognized that ATG may promote CMV infection by depleting CMV-specific cell-mediated immunity (CMI) [12].

Impact According to Serological Status

According to the prospective multicenter observational study by Kumar et al., very few D+R- SOTRs exhibit detectable CMV-specific CMI, as measured by ELISPOT assay, at the time of transplantation [13]. However, this finding has been challenged by a single-center Spanish cohort study, which reported that approximately 25% of D⁺/R⁻ patients had detectable CMV-specific CMI at transplantation [14]. These differences may, at least in part, be explained by differences in CMV prevalence worldwide. Furthermore, most CMV-seronegative recipients do not develop a detectable T-cell response during the prophylactic period [12]. Consequently, at the end of prophylaxis, only a small proportion of D⁺/R⁻ SOTRs exhibit CMV-specific T-cell CMI [13]. Specifically, only 12.1% of D+R- patients have a positive QuantiFERON-CMV assay at the end of prophylaxis [15], a

number that can rise to 20.9% when measured by ELISPOT assay [16]. Following these immunological observations, it has been demonstrated that ATG induction therapy, compared to anti-IL-2 receptor antagonists (IL-2RA), did not confer an additional risk of CMV DNAemia or disease in D⁺/R⁻ kidney transplant recipients [17]. As the increased risk of CMV associated with ATG is probably related to its depleting effect on CMV-specific T lymphocytes, this risk does not increase in patients who mostly lack CMV-specific CMI. Nevertheless, this treatment may exacerbate infection severity or recurrence rate, a possibility that requires further investigation.

In CMV-seropositive recipients (R+), most of the SOTRs have a CMV-specific CMI both at the time of transplantation and at the end of prophylaxis but this response is highly heterogeneous between the patients [13–18]. Consistent with these findings, it has been demonstrated that ATG induction therapy, compared with IL-2RA, confers an additional risk of CMV DNAemia and disease exclusively in R+ kidney transplant recipients [17]. Specifically, ATG increased the risk of CMV DNAemia in R+ recipients with detectable pretransplant CMI while no difference was observed among R+ recipients lacking detectable CMI, highlighting the pivotal role of CMV-CMI in the prevention of CMV infection (**Figure 1**). Furthermore, longitudinal analysis of CMV-specific CMI kinetics revealed that R+CMI+ recipients receiving rATG experienced a more pronounced suppression of their pre-existing CMV-specific immunity during the first two months post-transplantation compared to those treated with anti-IL-2RA, whereas no significant impact was observed in R+ CMI- recipients.

Recovery of CMV-Specific Immunity

The recovery of CMV-specific CMI after ATG has been studied in few studies. At one-month post-transplantation, approximately 50% of R+ SOTRs receiving ATG recover a detectable CMV-specific CMI, as measured by the QuantiFERON-CMV assay [19]. By 3 months post-transplant using ELISPOT, CMV-specific immune recovery is comparable between ATG-treated and non-ATG-treated patients [17], and among patients with detectable pretransplant CMV-specific CMI, immune responses recovered by 6 months post-transplant to levels similar to those observed prior to transplantation [18].

Taken together, these findings suggest that ATG transiently impairs CMV-specific T-cell responses in R+ SOTRs, while allowing for subsequent immune reconstitution over time [12]. The transient loss of CMV-specific CMI in these patients is highly correlated with the risk of CMV disease. Few data are available about ATG effect on $\gamma\delta$ T cells and NK cells.

Belatacept: The Special Case of Late and Atypical Infections Clinical Evidence

Belatacept, a costimulation blocker targeting the CD28–CD80/CD86 pathway, has demonstrated efficacy in preventing alloimmune responses while preserving renal function. Clinical trials showed that belatacept used from transplantation did not increase CMV risk compared to cyclosporine. In the BENEFIT

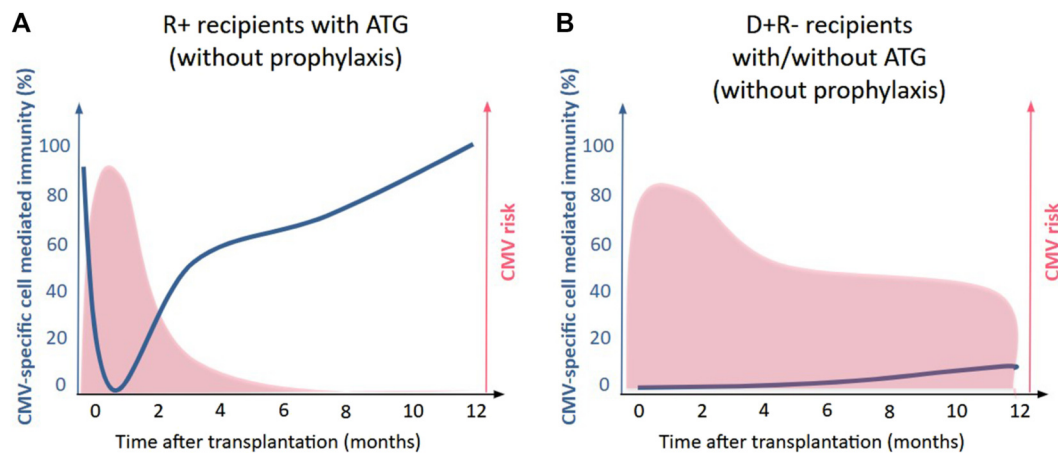


FIGURE 1 | Effect of rabbit anti-thymocyte globulin (rATG) on CMV-specific immunity according to recipient serostatus. This figure is a schematic representation of the restoration of CMV-specific immunity. **(A)** In CMV-seropositive (R^+) recipients, rATG transiently depletes CMV-specific T cells, increasing CMV disease risk until immune reconstitution occurs. **(B)** In CMV-seronegative (D^+/R^-) recipients, CMV risk is high with or without rATG, as CMV-specific cell mediated immunity is largely absent at baseline.

and BENEFIT-EXT trials, CMV infection rates were similar (6%–9% and 11%–14%, respectively) between belatacept and cyclosporine groups [20, 21]. In a smaller trial, Mannon et al. [22] observed more CMV viremia in the belatacept arm (20.7%), but differences were not statistically significant. Similarly, Woodle et al. [23] found comparable CMV rates (~11%) across tacrolimus and two belatacept regimens. Switching from Calcineurin inhibitors (CNIs) to belatacept did not also appear to increase CMV disease incidence. Budde et al. [24] and Divard et al. [25] reported no rise in CMV complications after conversion, even in high-risk patients.

On the opposite, several retrospective studies have highlighted an increased risk of CMV infection in patients receiving belatacept. Bertrand et al. reported a higher incidence of late-onset CMV disease, following conversion to belatacept [26]. A subsequent study by the same group confirmed that both CMV-seropositive (R^+) and high-risk CMV D^+/R^- recipients were affected [27]. Similarly, Chavarot et al. described an increased incidence of atypical, often severe CMV disease—sometimes with intestinal or ocular involvement—occurring several years after transplantation (median 43.6 months post-conversion) [28]. These infections were particularly common among elderly recipients (>70 years), patients with impaired graft function, or those who underwent late conversion to belatacept. Moreover, CMV infections in belatacept-treated recipients frequently relapse despite initial antiviral therapy, occasionally necessitating discontinuation of belatacept [29]. A U.S. cohort by Karadkhele and colleagues similarly found higher CMV disease rates in CMV high-risk patients on belatacept, reinforcing that this association is not restricted to specific geographic regions or clinical settings [30].

Pathophysiological Mechanisms

Xu et al. demonstrated that belatacept's immunosuppressive effects are more pronounced in naïve T cells but attenuate as

T cells mature [31]. Belatacept trough levels vary widely among kidney transplant recipients, ranging from 1.4 to 24.8 $\mu\text{g/L}$, with a mean of $8.4 \pm 3.9 \mu\text{g/L}$ [32]. *In vitro*, belatacept demonstrates strong immunosuppressive effects on alloreactive T cells, with an IC_{50} of 4 ng/mL for IL-2 production [33]. However, CMV-specific T cells are less susceptible to belatacept's antiproliferative effect compared to alloreactive T cells [31]. *In vivo*, CMV-specific CD4^+ and CD8^+ T-cell responses remain stable during the first year after switching to belatacept, with no significant decline in IFN- γ or TNF- α secretion [34] (Figure 2).

The immunopathogenesis of CMV reactivation under belatacept likely stems from its targeted blockade of the $\text{CD28}^-\text{CD80/CD86}$ costimulatory pathway, which is essential for the activation of naïve T cells. Regarding antiviral function, belatacept reduces IFN-gamma production by 50% in peripheral blood mononuclear cells (PBMCs) stimulated with a CMV peptide pool [33]. This inhibition dampens the primary CMV-specific T cell response, a particularly critical defect in seronegative recipients of CMV-positive organs (D^+/R^-). Consistent with this, Kleiboeker et al. reported a case of failed CMV-specific cellular immunity development in a high-risk D^+/R^- patient treated with belatacept in combination with low-dose tacrolimus, MPA, and steroids [35].

Belatacept does not impair the frequency of IL-2/TNF- α /IFN- γ triple-positive CD28^+ memory CMV-specific T cells, indicating that cytokine production by these memory cells remains largely preserved [31]. However, beyond its effects on naïve T cells, belatacept may indirectly impair CMV-specific memory T cells that are CD28-independent. Under physiological conditions, CD80 interacts in cis with PD-L1 on antigen-presenting cells, thereby limiting PD-1/PD-L1 engagement. Because belatacept has a high binding affinity for CD80, it can disrupt CD80/PD-L1 heterodimers [36]. This displacement could release free PD-L1, enhancing trans interactions between PD-1 and PD-L1, and thereby promoting functional exhaustion of PD1-expressing

Agent	Early activation (CD69↑)	IL-2 production	Proliferation	IFN- γ production	Cytotoxic potential
Calcineurin inhibitors	$\leftrightarrow$	$\downarrow\downarrow$	$\leftrightarrow$	$\downarrow\downarrow$ (CD4 ⁺ >CD8 ⁺)	$\leftrightarrow$
Belatacept	$\leftrightarrow$ / $\downarrow$ (memory/naïve)	$\leftrightarrow$ / $\downarrow$ (memory/naïve)	$\leftrightarrow$	$\leftrightarrow$ / $\downarrow$ (memory/naïve)	$\leftrightarrow$
Mycophenolic acid	$\leftrightarrow$	$\downarrow$	$\downarrow\downarrow\downarrow$	$\leftrightarrow$	$\leftrightarrow$
Everolimus / mTORi	$\leftrightarrow$	$\downarrow$	$\downarrow$	$\uparrow$	$\leftrightarrow$
Steroids	$\downarrow$	$\downarrow\downarrow$	$\downarrow$	$\downarrow\downarrow$	$\downarrow\downarrow$

FIGURE 2 | Simplified *in-vitro* effects of major immunosuppressive agents on CMV-specific T-cell functions. Assessed functions include early activation (CD69 upregulation), IL-2 production, proliferation, IFN- γ secretion, and cytotoxic potential (perforin/granulysin expression and target-cell killing). Data are derived from comparative *in vitro* studies using clinically relevant drug concentrations [31, 33, 51].

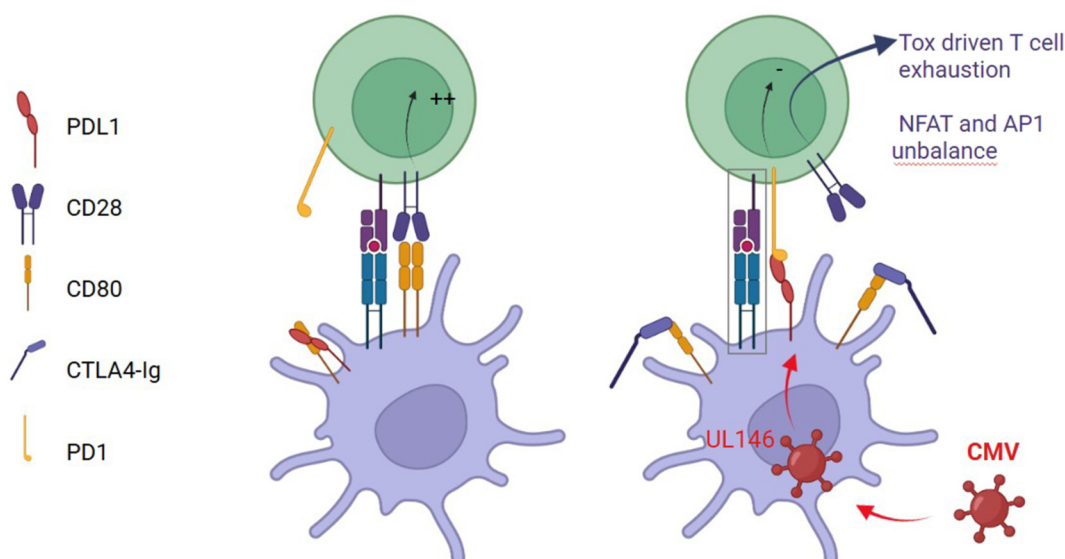


FIGURE 3 | Proposed mechanisms by which belatacept promotes CMV-specific T-cell exhaustion. Belatacept disrupts CD80–PD-L1 cis interactions on antigen-presenting cells, increasing free PD-L1 and enhancing PD-1/PD-L1 inhibitory signaling. CMV amplifies this pathway via UL146-mediated PD-L1 upregulation. Downstream, NFAT/AP-1 unbalance promotes TOX-driven exhaustion of CMV-specific CD8⁺ T cells (adapted from [37]).

CMV-specific CD8⁺ T cells [37]. CMV infection itself may further amplify this inhibitory axis. The viral UL146 gene product (vCXCL1) has been shown to upregulate PD-L1 expression on infected hepatic cells, thereby strengthening PD-1/PD-L1-mediated suppression [38]. Moreover, CD28 blockade alters downstream signaling balance between NFAT and AP-1, fostering TOX-driven transcriptional programs associated with T-cell exhaustion. Consistent with this mechanism, Long et al. demonstrated that abatacept—another CD28 pathway inhibitor—induced T-cell exhaustion, supporting a class effect of CD28 blockade [39]. Finally, rare human cases of inherited

CD28 deficiency are characterized by susceptibility to chronic viral infections, providing genetic evidence of the central role of this pathway in antiviral immunity [40] (**Figure 3**).

Management Strategies

Given the increased CMV risk observed under belatacept, several strategies have been proposed to optimize its use while minimizing infection. In a recent study by Del Bello et al., conversion from MPA to an mTOR inhibitor in belatacept-treated kidney transplant recipients during or after a first CMV episode, led to a marked reduction in CMV replication

or recurrence rates. In this study, the incidence of CMV DNAemia was 0.072 per month of exposure in patients treated with belatacept-MPA, compared with 0.035 in patients treated with belatacept-mTOR inhibitor ($p = 0.0002$) [41]. High-risk populations—particularly D^+/R^- recipients, elderly patients—could also benefit early initiation of antiviral prophylaxis or pre-emptive therapy for at least 6 months following the initiation of belatacept therapy [26]. In the future, combining belatacept with agents that preserve CMV-specific immunity may help maintain the delicate balance between graft protection and antiviral defense [42].

NEUTRAL OR MODERATE RISK AGENTS

This section examines immunosuppressive agents that do not independently increase CMV risk when used as monotherapy, but may contribute to CMV susceptibility through cumulative immunosuppression when combined with other agents.

Calcineurin Inhibitors (CNI): Tacrolimus and Cyclosporine

Mechanisms and Clinical Data

CNIs target specific cytoplasmic proteins—cyclophilins for cyclosporine and FK-binding proteins for tacrolimus—thereby inhibiting calcineurin, a key phosphatase involved in the activation of lymphocytes. This inhibition prevents the transcription of cytokines such as IL-2, TNF- α , and IFN- γ , which are critical for the immune response following alloantigen recognition.

CNIs remain the cornerstone of immunosuppressive therapy, and most CNI-sparing strategies have been compared to regimens based on mTOR inhibitors or belatacept. This makes it particularly challenging to isolate the specific effect of CNIs on the risk of CMV infection after transplantation. However, a few studies have attempted to assess the risk of CMV viremia and/or infection in KTRs undergoing CNI withdrawal compared to those maintained on CNI therapy. In the Cochrane meta-analysis by Karpe et al. [43], which reviewed 83 studies—including 30 that specifically reported on CMV-related disease incidence—a trend toward a protective effect of CNI withdrawal was observed. However, this trend did not reach statistical significance. Therefore, the clinical impact of CNI on CMV infection has not been definitively documented in clinical trials. Another important question is whether tacrolimus and cyclosporine have a similar impact on CMV infection. In the pivotal trials comparing the two CNIs, the incidence of CMV infection was found to be similar [44, 45]. In the SYMPHONY study, Ekberg et al. [46] reported CMV infection rates of 14.6% in the standard-dose cyclosporine group, 11% in the low-dose cyclosporine group, and 9.7% in the low-dose tacrolimus group. More recently, a retrospective comparison between the EVERCMV study and a *post hoc* analysis by Tedesco-Silva and colleagues showed comparable CMV DNAemia rates between patients treated with cyclosporine and those treated with tacrolimus, whether combined with everolimus (EVR) (39.2%

vs. 43.3%) or with mycophenolic acid (MPA) (77.8% vs. 77.9%) [47, 48]. Additionally, the type of CNI (tacrolimus versus cyclosporine) was not associated with viral eradication in the VICTOR study [49]. These findings support the hypothesis that both the incidence and severity of CMV infection are largely independent of the type of CNI used.

Impact on CMV-Specific Immunity

CNIs have been shown to exhibit direct antiviral properties, including the ability to impair viral replication. A recent study demonstrated that CNIs exert antiproliferative effects on CMV *in vitro*, with a sixfold increase in anti-CMV activity when combined with MPA. However, these effects were observed at drug concentrations exceeding those typically achieved *in vivo* based on pharmacokinetic data [50].

Additionally, several studies have investigated the impact of CNI on CMV-specific lymphocyte responses. In a recent study by Krueger et al., low-dose tacrolimus (5 ng/mL) did not significantly reduce early activation of $CD4^+$ and $CD8^+$ T cells upon stimulation with CMV pp65, as measured by CD69 expression [51]. However, tacrolimus alone significantly suppressed IL-2 production by both CMV-specific $CD4^+$ and $CD8^+$ T cells. Tacrolimus showed high potency in suppressing IL-2, with an IC_{50} of approximately 1 ng/mL [33]. However, this IL-2 inhibition had little impact on the proliferative capacity of CMV-specific T cells, suggesting that another cytokine such as IL-15 could act as a substitute [51]. Cyclosporin has been tested on the proliferation capacity of both conventional CMV-specific and $\gamma\delta$ T cells. A decreased proliferation capacity was observed at doses as low as 25 ng/mL [52].

Tacrolimus also reduces the production of IFN- γ , a key antiviral cytokine that limits CMV replication and dissemination. This effect is particularly pronounced in the $CD4^+$ CMV-specific T cell subset [51]. Additional studies have confirmed that CNIs also suppress IFN- γ production in virus-specific memory $CD8^+$ T cells [53]. Even at very low concentrations (1.5 ng/mL), tacrolimus was shown to reduce IFN- γ production by 80%–90% in CMV-specific T cells stimulated with CMV peptide pools for 72 h [33]. Furthermore, tacrolimus inhibits T cell responses in both CMV-naïve and CMV-immune individuals [31]. However, tacrolimus does not appear to significantly impair cytolytic function, as it does not reduce the expression of perforin or granzyme in CMV pp65-stimulated PBMC [51].

Collectively, these findings highlight the nuanced impact of tacrolimus on CMV-specific T cell responses—sparing early activation and cytolytic potential, while strongly suppressing key cytokines like IFN- γ , which is essential for antiviral control (Figure 2).

Mycophenolate Mofetil (MPA): Risk Related to Cumulative Effects

Mechanisms and Clinical Data

MPA acts as a noncompetitive, selective, and reversible inhibitor of inosine monophosphate dehydrogenase, the rate-limiting enzyme in *de novo* guanosine nucleotide synthesis [54].

Because lymphocytes depend exclusively on this pathway for purine synthesis, MPA profoundly inhibits T- and B-cell proliferation while sparing other cell types that can utilize salvage pathways.

In patients with lupus nephritis, MPA therapy showed no higher incidence of CMV infection compared to cyclophosphamide or azathioprine, both during induction and maintenance phases [55, 56]. The absence of CMV disease in these patients likely reflects the absence of allograft-derived viral reintroduction or post-transplant-specific “hits,” such as ischemia–reperfusion injury, which are known triggers of CMV infection [57]. In renal transplantation, early landmark trials found no significant increase in CMV infection with MPA/cyclosporine regimens compared with azathioprine or placebo [58–60]. Later, Miller et al. reported that MPA (1–2 g/day) in combination with tacrolimus did not result in higher CMV infection rates than azathioprine-based therapy [61]. In the VICTOR trial, the use of MPA had no significant influence on viral eradication [49]. More recent retrospective analyses confirm that MPA was not an independent risk factor for CMV recurrence or late CMV DNAemia [62, 63]. Together, these clinical data suggest that MPA use, *per se*, does not increase CMV replication risk.

Impact on CMV-Specific Immunity

Among immunosuppressive agents, MPA displays the most selective antiviral actions against CMV *in vitro*. In combination with tacrolimus, MPA demonstrated synergistic lethality against CMV in cell culture models, although these effects occurred at concentrations exceeding those achieved clinically [50].

MPA induces broad suppression of adaptive immunity by depleting CD4⁺, CD8⁺, and CD19⁺ lymphocytes, in part through apoptosis [54]. It downregulates CD25 expression, thereby impairing IL-2–driven T-cell activation and proliferation. *In vitro*, MPA decreases IFN- γ production, not by impairing cytokine synthesis per cell, but by reducing the total lymphocyte pool capable of activation. Consequently, the antiviral arm of the immune response may be dampened in proportion to overall lymphocyte depletion. Recent studies have examined the effects of MPA on CMV-specific T-cell responses. Krueger et al. reported that activation of CD4⁺ and CD8⁺ T cells by CMV pp65 antigen is not significantly affected by MPA alone [51]. MPA had high IC50 values at 3 ng/mL for IL-2. However, MPA strongly reduces the proliferation of purified CMV-specific T cells and $\gamma\delta$ T cells *in vitro* [52], indicating a selective antiproliferative effect without full functional suppression. Indeed, Egli et al. showed that MPA induced a moderate reduction in IFN- γ production by CMV peptide–stimulated T cells [33], and Krueger et al. found no significant reduction in IFN- γ –producing CMV-specific T cells upon MPA exposure [51]. Moreover, MPA does not impair cytotoxic T-cell function, since Krueger et al. observed preserved perforin and granzyme production and intact cytolytic activity of CMV-specific T cells exposed to MPA (Figure 2) [51].

In conclusion, MPA has not been shown to independently increase CMV replication or recurrence in SOT recipients.

However, it is frequently the first immunosuppressive agent to be reduced or discontinued during CMV infection, mainly because of its myelosuppressive effects, its strong inhibitory impact on T-cell proliferation critical for CMV control, and its contribution to the overall burden of immunosuppression. Reducing or withdrawing MPA may therefore help limit cytopenias and promote immune recovery during CMV infection.

Corticosteroids

Maintenance steroid dose has been shown to significantly associate with death with a functioning graft due to cardiovascular disease or infection during years 2–5 after kidney transplantation [64]. Glucocorticoids inhibit NK cell cytotoxicity and may impair antigen presentation by reducing the expression of major histocompatibility complex (MHC) class II molecules on circulating monocytes, thereby diminishing T-helper cell activation [65]. In addition, prednisolone (at a high dose of 0.5 μ g/mL) exerts broad inhibitory effects on CMV-specific T cells, including decreased activation, reduced IL-2 production, lower frequencies of CMV-specific IFN- γ –producing T cells, decreased production of perforin and granzyme, and inhibition of cytotoxic activity [51] (Figure 2). Consistent with these findings, the 2-year cumulative incidence of CMV infection in simultaneous pancreas–kidney transplant recipients was significantly lower in those managed with a prednisone-free protocol compared to standard steroid-based regimens, particularly among high-risk recipients (D⁺/R⁻ or D⁺/R⁺) (18% vs. 36%, $p < 0.05$) [66]. However, this clinical observation remains isolated in the literature, as subsequent randomized trials did not demonstrate a reduced incidence of CMV infection with rapid steroid withdrawal [67, 68], nor did the 2016 Cochrane meta-analysis confirm such an effect [69].

Although corticosteroids impair immune function, evidence that steroid withdrawal reduces CMV risk remains inconsistent. This discrepancy likely reflects the higher doses used *in vitro* compared with the much lower maintenance doses used clinically.

THE PROTECTIVE EXCEPTION: MTOR INHIBITORS

Multiple randomized trials and meta-analyses demonstrated a lower incidence of CMV infection and disease in patients receiving *de novo* mTOR inhibitors compared to CNI-based or MPA-containing regimens [70]. Trials comparing CNI + EVR to CNI + MPA consistently report fewer CMV events in the mTORi groups [71–79]. Prospective studies designed with CMV infection as a primary endpoint confirmed that CMV DNAemia and clinically significant infections were substantially reduced in EVR + low-dose CNI regimens, particularly among CMV-seropositive recipients [47, 77]. Head-to-head comparisons between EVR and sirolimus demonstrated comparable protection against CMV [78]. Even in high-risk patients receiving ATG, EVR was associated with fewer CMV events compared to MPA [71, 79]. Accordingly, the

Fourth International Consensus Guidelines for CMV in SOT recipients recommend preemptive or close clinical monitoring in CMV-seropositive kidney transplant patients receiving *de novo* mTOR-based immunosuppression [80].

Data in D⁺/R⁻ kidney transplant recipients remain limited but suggest reduced CMV disease with CNI-EVR compared with CNI-MPA regimens. In a monocentric study, basal and peak viral loads were significantly lower under CNI-EVR [81], consistent with the TRANSFORM study findings [82]. However, other studies found no significant difference in CMV DNAemia [83], warranting further investigation in this subgroup.

Similar reductions in CMV incidence under mTORi therapy have been observed in heart transplantation [84–88], pediatric renal transplantation [87], lung transplantation [88–90], and liver transplantation [91]. Meta-analyses and systematic reviews further confirm that mTOR inhibitors reduce CMV infection rates across various organ types [92, 93].

Conversion to mTOR inhibitors immediately after treatment of a first CMV episode significantly reduces the risk of recurrence in R⁺ kidney transplant recipients [94]. However, a retrospective analysis including heterogeneous cohorts did not show a significant difference in recurrence [95], highlighting the need for prospective data in D⁺/R⁻ high-risk population. Interestingly, in belatacept-treated patients, conversion from MPA to mTORi reduced CMV replication and recurrence [41], supporting the protective effect of mTOR inhibition even in settings of costimulation blockade.

CMV replication depends on both rapamycin-sensitive and rapamycin-resistant mTORC1 activity [96]. mTOR activation supports the translation of viral proteins and the maintenance of protein synthesis during infection [96, 97]. *In vitro*, inhibition of mTORC1 prevents the accumulation of immediate early, early, and late CMV proteins [98]. During late infection phases, sustained mTOR activation is required for synthesis of late viral proteins such as pUL44 and pp65. Rapamycin strongly suppressed CMV replication three to five days post-infection in macrophages, confirming that mTOR is essential for viral replication in myeloid cells [99]. Pre-infection treatment with everolimus suppresses viral spread and reduces the number of infected cells *in vitro* [100]. These findings collectively indicate that mTOR inhibition can directly impair CMV replication, particularly in the late stages of the viral cycle, by interfering with viral protein translation and host cell metabolism.

mTOR plays also a central role in T-cell differentiation and memory formation. mTOR inhibition has been shown to enhance both the quantity and functional quality of virus-specific CD8⁺ T cells, promoting the generation of long-lived memory populations [101, 102]. Under sirolimus monotherapy, antigen-specific CD8⁺ responses are suppressed toward allografts but augmented against viral or bacterial pathogens. *In vitro*, activation of CD4 and CD8⁺ T cells by CMV pp65 antigen is not significantly inhibited by EVR alone [51]. Sirolimus inhibits IL-2 production at low IC₅₀ concentrations (~2 ng/mL) [33], and EVR reduces CMV-specific T-cell proliferation [51]. Nevertheless, *in vivo*, switching from cyclosporine-MPA to EVR-based therapy increases circulating CMV-specific CD8⁺

T-cell counts [103]. These data indicate that mTOR inhibition transiently dampens proliferation *in vitro* but enhances functional antiviral T-cell responses *in vivo* (Figure 2).

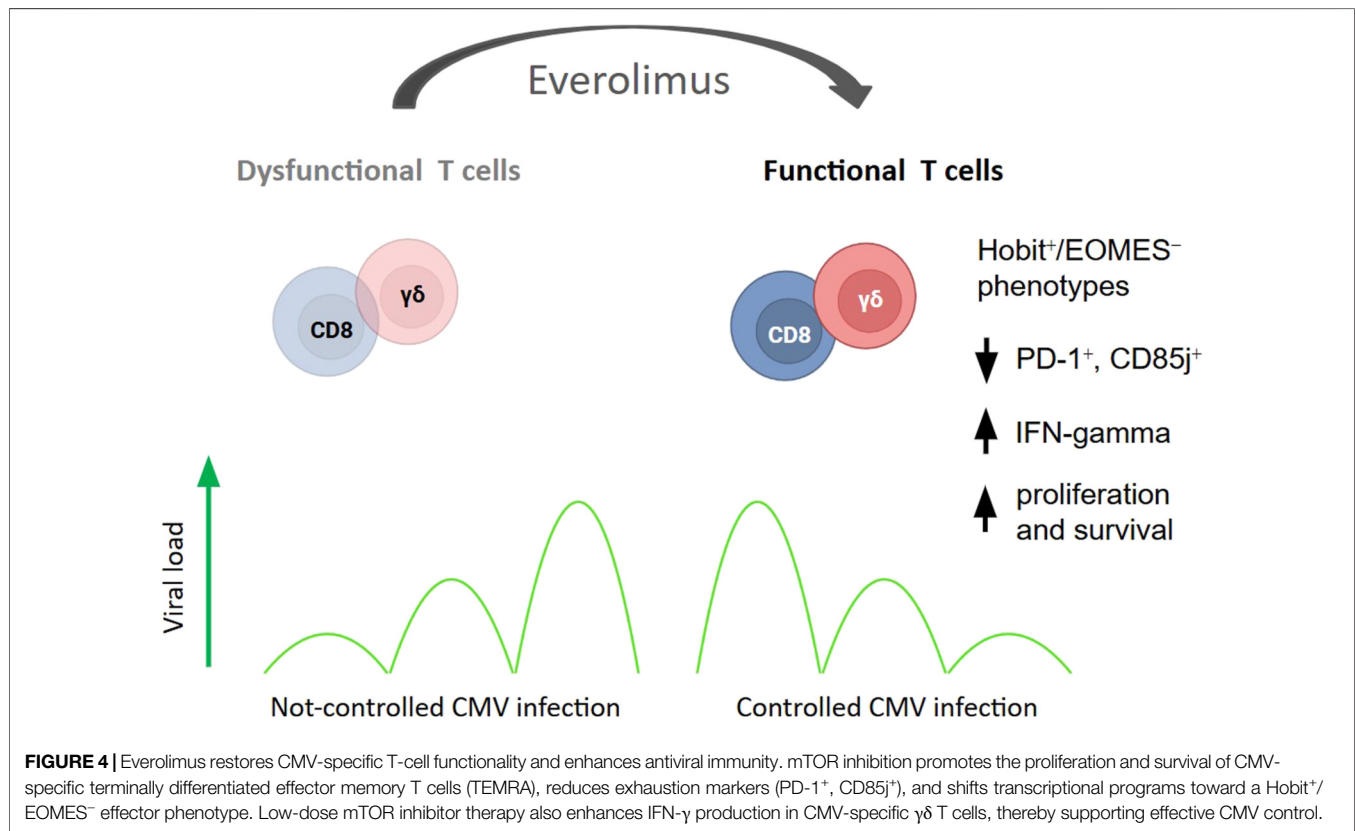
Sirolimus markedly reduces IFN- γ production *in vitro* [33]. However, EVR does not significantly impair IFN- γ production by CMV-specific T cells stimulated with pp65 antigen [51]. Such discrepancies may reflect differences in experimental conditions or drug concentrations. Indeed, in renal transplant recipients, mTORi therapy has been associated with higher frequencies of IFN- γ -producing CMV-specific cells compared with MPA [104]. Mechanistically, mTOR inhibition converts dysfunctional CMV-specific T cells into functional effector cells, enhancing $\gamma\delta$ and $\alpha\beta$ T-cell responses and correlating with lower CMV incidence [52]. mTORi therapy improves the proliferation and survival of terminally differentiated effector memory cells (TEMRA), reduces exhaustion markers (PD-1⁺, CD85j⁺), and shifts transcriptional profiles toward Hobit⁺/EOMES⁻ phenotypes. Low-dose mTOR inhibition enhances T-cell receptor signaling and IFN- γ production in $\gamma\delta$ T cells when compared to MPA [52]. In the EVER-CMV trial, continuous EVR therapy maintained improved CMV-specific T-cell functionality, while discontinuation led to loss of this benefit [47] (Figure 4).

Furthermore, EVR does not inhibit cytotoxic function: CMV pp65-stimulated PBMCs maintain perforin and granzyme production, and EVR-treated CMV-specific T cells preserve cytotoxicity against infected fibroblasts [51].

Overall, mTOR inhibition represents a dual-action strategy—providing effective immunosuppression while conferring viro-immunologic protection against CMV in R⁺ patients. Nevertheless, mTOR inhibitors are discontinued in approximately one third of patients because of intolerance, which may subsequently re-expose these patients to an increased risk of CMV infection.

THE CUMULATIVE RISK PARADIGM

CNI alone or MPA alone have not been shown to independently increase the risk of CMV infection in SOT recipients. Therefore, the increased risk observed is likely attributable to transplant-related factors such as the risk of primary infection in D⁺/R⁻ patients and the overall degree of immunosuppression. A prospective study by De Weerd et al. found no statistically significant differences in CMV replication between patients receiving tacrolimus monotherapy and those on a tacrolimus-MPA regimen in low-risk kidney transplant recipients without corticosteroids [105]. However, an earlier study by Shapiro et al.—one of the first to introduce the concept of triple combination regimens to prevent allograft rejection—reported a twofold higher incidence of CMV infection when MPA was added to a tacrolimus/prednisone regimen (8.5% vs. 16.7% at 15 months) [106]. In line with this finding, CNIs appear to affect viral eradication during antiviral therapy. In the VICTOR trial, patients on dual



immunosuppressive therapy (versus triple) and those with lower CNI concentrations (≤ 150 ng/mL for cyclosporine and ≤ 5 ng/mL for tacrolimus) demonstrated significantly higher rates of viral clearance, with odds ratios of 2.55 and 5.52, respectively [49]. These clinical observations are further supported by recent experimental data from Krueger et al., who showed that activation, IL-2 production, proliferation, IFN- γ secretion, and cytotoxicity of CMV pp65-specific CD4⁺ and CD8⁺ T cells were significantly reduced in the presence of Tacrolimus + EVR + Prednisolone or Tacrolimus + MPA + Prednisolone compared with Tacrolimus or MPA alone [51]. Taken together, the cumulative burden of immunosuppressive therapy, rather than any single agent, appears to drive CMV susceptibility and delayed viral clearance.

CONCLUSION

In summary, CMV infection results from the combined impact of immunosuppressive agents on both innate and adaptive immunity. While ATG exert the strongest inhibitory effects on CMV-specific T-cell function, CNIs and MPA contribute mainly through cumulative immunosuppression rather than direct viral promotion. In contrast, mTOR inhibitors show protective antiviral and immunomodulatory properties. Belatacept carries a specific risk of late and atypical CMV disease. Overall, optimizing immunosuppressive balance and incorporating

immune monitoring remain essential to prevent CMV complications.

AUTHOR CONTRIBUTIONS

LC, HK, and PM designed the figures. All authors contributed to the article and approved the submitted version.

FUNDING

The author(s) declared that financial support was not received for this work and/or its publication.

CONFLICT OF INTEREST

The author(s) declared that this work was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

GENERATIVE AI STATEMENT

The author(s) declared that generative AI was used in the creation of this manuscript. During the preparation of this work, the

author(s) used: - Mistral AI, Le Chat for: text translation, academic synthaxis - OpenEvidence, 2025: bibliographic research.

Any alternative text (alt text) provided alongside figures in this article has been generated by Frontiers with the support

of artificial intelligence and reasonable efforts have been made to ensure accuracy, including review by the authors wherever possible. If you identify any issues, please contact us.

REFERENCES

1. Khairallah C, Déchanet-Merville J, Capone M. $\gamma\delta$ T Cell-Mediated Immunity to Cytomegalovirus Infection. *Front Immunol* (2017) 8:105. doi:10.3389/fimmu.2017.00105
2. Bestard O, Kaminski H, Couzi L, Fernández-Ruiz M, Manuel O. Cytomegalovirus Cell-Mediated Immunity: Ready for Routine Use? *Transpl Int* (2023) 36:11963. doi:10.3389/ti.2023.11963
3. Kaminski H, Couzi L, Eberl M. Unconventional T Cells and Kidney Disease. *Nat Rev Nephrol* (2021) 17(12):795–813. doi:10.1038/s41581-021-00466-8
4. Lopez-Vergès S, Milush JM, Schwartz BS, Pando MJ, Jarjoura J, York VA, et al. Expansion of a Unique CD57+NKG2Chi Natural Killer Cell Subset During Acute Human Cytomegalovirus Infection. *PNAS* (2011) 108:14725–32. doi:10.1073/pnas.1110900108
5. Mohty M. Mechanisms of Action of Antithymocyte Globulin: T-Cell Depletion and Beyond. *Leukemia* (2007) 21:1387–94. doi:10.1038/sj.leu.2404683
6. Gurkan S, Luan Y, Dhillon N, Allam SR, Montague T, Bromberg JS, et al. Immune Reconstitution Following Rabbit Antithymocyte Globulin. *Am J Transpl* (2010) 10(9):2132–41. doi:10.1111/j.1600-6143.2010.03210.x
7. Brennan DC, Daller JA, Lake KD, Cibrik D, Castillo DD, Thymoglobulin Induction Study Group. Rabbit Antithymocyte Globulin Versus Basiliximab in Renal Transplantation. *N Engl J Med* (2006) 355(19):1967–77. doi:10.1056/NEJMoa060068
8. Hardinger KL, Brennan DC, Klein CL. Selection of Induction Therapy in Kidney Transplantation. *Transpl Int* (2013) 26:662–72. doi:10.1111/tri.12043
9. Luan FL, Samaniego M, Kommareddi M, Park Jm., Ojo Ao. Choice of Induction Regimens on the Risk of Cytomegalovirus Infection in Donor-Positive and Recipient-Negative Kidney Transplant Recipients. *Transpl Infect Dis* (2010) 12(6):473–9. doi:10.1111/j.1399-3062.2010.00532.x
10. Lebranchu Y, Bridoux F, Büchler M, Meur YL, Etienne I, Toupance O, et al. Immunoprophylaxis with Basiliximab Compared with Antithymocyte Globulin in Renal Transplant Patients Receiving MMF-Containing Triple Therapy. *Am J Transpl* (2002) 2(1):48–56. doi:10.1034/j.1600-6143.2002.020109.x
11. Webster AC, Ruster LP, McGee RG, Matheson SL, Higgins GY, Willis NS, et al. Interleukin 2 Receptor Antagonists for Kidney Transplant Recipients. *Cochrane Database Syst Rev* (2010) (1):CD003897. doi:10.1002/14651858.CD003897.pub3
12. Abate D, Saldan A, Fison M, Cofano S, Paciolla A, Furian L, et al. Evaluation of Cytomegalovirus (CMV)-Specific T Cell Immune Reconstitution Revealed that Baseline Antiviral Immunity, Prophylaxis, or Preemptive Therapy but Not Antithymocyte Globulin Treatment Contribute to CMV-Specific T Cell Reconstitution in Kidney Transplant Recipients. *J Infect Dis* (2010) 202(4):585–94. doi:10.1086/654931
13. Kumar D, Chin-Hong P, Kayler L, Wojciechowski D, Limaye AP, Osama Gaber A, et al. A Prospective Multicenter Observational Study of Cell-Mediated Immunity as a Predictor for Cytomegalovirus Infection in Kidney Transplant Recipients. *Am J Transpl* (2019) 19(9):2505–16. doi:10.1111/ajt.15315
14. Lúcia M, Crespo E, Melilli E, Cruzado JM, Luque S, Llaudó I, et al. Preformed Frequencies of Cytomegalovirus (CMV)-Specific Memory T and B Cells Identify Protected CMV-Sensitized Individuals Among Seronegative Kidney Transplant Recipients. *Clin Infect Dis* (2014) 59(11):1537–45. doi:10.1093/cid/ciu589
15. Manuel O, Husain S, Kumar D, Zayas C, Mawhorter S, Levi ME, et al. Assessment of Cytomegalovirus-Specific Cell-Mediated Immunity for the Prediction of Cytomegalovirus Disease in High-Risk Solid-Organ Transplant Recipients: A Multicenter Cohort Study. *Clin Infect Dis* (2013) 56(6):817–24. doi:10.1093/cid/cis993
16. Manuel O, Laager M, Hirzel C, Neofytos D, Walti LN, Hoenger G, et al. Immune Monitoring-Guided Versus Fixed Duration of Antiviral Prophylaxis Against Cytomegalovirus in Solid-Organ Transplant Recipients: A Multicenter, Randomized Clinical Trial. *Clin Infect Dis* (2024) 78(2):312–23. doi:10.1093/cid/ciad575
17. Kaminski H, Jarque M, Halfon M, Taton B, Di Ascia L, Pfirrmann P, et al. Different Impact of rATG Induction on CMV Infection Risk in D+R- and R+ KTRs. *J Infect Dis* (2019) 220(5):761–71. doi:10.1093/infdis/jiz194
18. Martín-Gandul C, Pérez-Romero P, Mena-Romo D, Molina-Ortega A, González-Roncero FM, Suárez M, et al. Kinetic of the CMV-Specific T-Cell Immune Response and CMV Infection in CMV-Seropositive Kidney Transplant Recipients Receiving Rabbit Anti-Thymocyte Globulin Induction Therapy: A Pilot Study. *Transpl Infect Dis* (2018) 20(3):e12883. doi:10.1111/tid.12883
19. Páez-Vega A, Gutiérrez-Gutiérrez B, Agüera ML, Facundo C, Redondo-Pachón D, Suárez M, et al. Immunoguided Discontinuation of Prophylaxis for Cytomegalovirus Disease in Kidney Transplant Recipients Treated with Antithymocyte Globulin: A Randomized Clinical Trial. *Clin Infect Dis* (2022) 74(5):757–65. doi:10.1093/cid/ciab574
20. Vincenti F, Charpentier B, Vanrenterghem Y, Rostaing L, Bresnahan B, Darji P, et al. A Phase III Study of Belatacept-Based Immunosuppression Regimens Versus Cyclosporine in Renal Transplant Recipients (BENEFIT Study). *Am J Transpl* (2010) 10(3):535–46. doi:10.1111/j.1600-6143.2009.03005.x
21. Durrbach A, Pestana JM, Pearson T, Vincenti F, Garcia VD, Campistol J, et al. Phase III Study of Belatacept Versus Cyclosporine in Kidney Transplants from Extended Criteria Donors (BENEFIT-EXT Study). *Am J Transpl* (2010) 10(3):547–57. doi:10.1111/j.1600-6143.2010.03016.x
22. Mannon RB, Armstrong B, Stock PG, Mehta AK, Farris AB, Watson N, et al. Avoidance of CNIs and Steroids Using Belatacept – Results of the Clinical Trials in Organ Transplantation 16 Trial. *Am J Transpl Off J Am Soc Transpl Am Soc Transpl Surg* (2020) 20(12):3599–608. doi:10.1111/ajt.16152
23. Woodle ES, Kaufman DB, Shields AR, Leone J, Matas A, Wiseman A, et al. Belatacept-Based Immunosuppression with Simultaneous Calcineurin Inhibitor Avoidance and Early Corticosteroid Withdrawal: A Prospective, Randomized Multicenter Trial. *Am J Transpl* (2020) 20(4):1039–55. doi:10.1111/ajt.15688
24. Budde K, Prashar R, Haller H, Rial MC, Kamar N, Agarwal A, et al. Conversion from Calcineurin Inhibitor- to Belatacept-Based Maintenance Immunosuppression in Renal Transplant Recipients: A Randomized Phase 3b Trial. *J Am Soc Nephrol* (2021) 32(12):3252–64. doi:10.1681/ASN.2021050628
25. Divard G, Aubert O, Debiais-Deschamps C, Raynaud M, Goutaudier V, Sablik M, et al. Long-Term Outcomes After Conversion to a Belatacept-Based Immunosuppression in Kidney Transplant Recipients. *Clin J Am Soc Nephrol* (2024) 19(5):628–37. doi:10.2215/CJN.0000000000000411
26. Bertrand D, Chavarot N, Gatault P, Garrouste C, Bouvier N, Grall-Jezequel A, et al. Opportunistic Infections After Conversion to Belatacept in Kidney Transplantation. *Nephrol Dial Transpl* (2020) 35:336–45. doi:10.1093/ndt/gfz255
27. Bertrand D, Terrec F, Etienne I, Chavarot N, Sberro R, Gatault P, et al. Opportunistic Infections and Efficacy Following Conversion to Belatacept-Based Therapy After Kidney Transplantation: A French Multicenter Cohort. *J Clin Med* (2020) 9(11):3479. doi:10.3390/jcm9113479
28. Chavarot N, Divard G, Scemla A, Amrouche L, Aubert O, Leruez-Ville M, et al. Increased Incidence and Unusual Presentations of CMV Disease in Kidney Transplant Recipients After Conversion to Belatacept. *Am J Transpl* (2021) 21(7):2448–58. doi:10.1111/ajt.16430
29. Magua W, Johnson AC, Karadkhele GM, Badell IR, Vasanth P, Mehta AK, et al. Impact of Belatacept and Tacrolimus on Cytomegalovirus Viral Load Control and Relapse in Moderate and High-Risk Cytomegalovirus Serostatus Kidney Transplant Recipients. *Transpl Infect Dis* (2022) 24:e13983. doi:10.1111/tid.13983

30. Karadkhele G, Hogan J, Magua W, Zhang W, Badell IR, Mehta A, et al. CMV High-Risk Status and Posttransplant Outcomes in Kidney Transplant Recipients Treated with Belatacept. *Am J Transpl* (2021) 21(1):208–21. doi:10.1111/ajt.16132
31. Xu H, Perez SD, Cheeseman J, Mehta AK, Kirk AD. The allo- and Viral-Specific Immunosuppressive Effect of Belatacept, but Not Tacrolimus, Attenuates with Progressive T Cell Maturation. *Am J Transpl Off J Am Soc Transpl Am Soc Transpl Surg* (2014) 14(2):319–32. doi:10.1111/ajt.12574
32. Chhun S, Trauchessec M, Melicine S, Nicolas F, Miele A, Lukic S, et al. A Validated LC-MS/MS Method for Performing Belatacept Drug Monitoring in Renal Transplantation. *Biomedicines* (2023) 11(11):2955. doi:10.3390/biomedicines11112955
33. Egli A, Kumar D, Broscheit C, O'Shea D, Humar A. Comparison of the Effect of Standard and Novel Immunosuppressive Drugs on CMV-Specific T-Cell Cytokine Profiling. *Transplantation* (2013) 95(3):448–55. doi:10.1097/TP.0b013e318276a19f
34. Schaenman J, Rossetti M, Pickering H, Sunga G, Wilhalme H, Elashoff D, et al. Preservation of Antiviral Immunologic Efficacy Without Alloimmunity After Switch to Belatacept in Calcineurin Inhibitor–Intolerant Patients. *Kidney Int Rep* (2023) 8(1):126–40. doi:10.1016/j.ekir.2022.10.015
35. Kleiboeker HL, Jorgenson MR, Smith JA. Belatacept May Result in Profound and Persistent Loss of Cytomegalovirus-Specific Cell-Mediated Immunity: A Case Report. *Transpl Infect Dis Off J Transpl Soc* (2022) 24(2):e13795. doi:10.1111/tid.13795
36. Tekguc M, Wing JB, Osaki M, Long J, Sakaguchi S. Treg-Expressed CTLA-4 Depletes CD80/CD86 by Troglucytosis, Releasing Free PD-L1 on Antigen-Presenting Cells. *Proc Natl Acad Sci* (2021) 118(30):e2023739118. doi:10.1073/pnas.2023739118
37. Zuber J, Leon J, Déchanet-Merville J, Kaminski H. Belatacept-Related Cytomegalovirus Infection: Advocacy for Tailored Immunosuppression Based on Individual Assessment of Immune Fitness. *Am J Transpl* (2025) 25(2):277–83. doi:10.1016/j.ajt.2024.09.035
38. Hu L, Wen Z, Chen J, Chen Y, Jin L, Shi H, et al. The Cytomegalovirus ULI46 Gene Product vCXCL1 Promotes the Resistance of Hepatic Cells to CD8+ T Cells Through Upregulation of PD-L1. *Biochem Biophys Res Commun* (2020) 532(3):393–9. doi:10.1016/j.bbrc.2020.08.060
39. Long SA, Muir VS, Jones BE, Wall VZ, Ylescupidez A, Hocking AM, et al. Abatacept Increases T Cell Exhaustion in Early RA Individuals Who Carry HLA Risk Alleles. *Front Immunol* (2024) 15:1383110. doi:10.3389/fimmu.2024.1383110
40. Béziat V, Rapaport F, Hu J, Titeux M, Claustres MBd, Bourgey M, et al. Humans with Inherited T Cell CD28 Deficiency Are Susceptible to Skin Papillomaviruses but Are Otherwise Healthy. *Cell* (2021) 184(14):3812–28.e30. doi:10.1016/j.cell.2021.06.004
41. Del Bello A, Cachoux J, Abravanel F, Prudhomme T, Kamar N. The Conversion from Mycophenolic Acid to Mammalian Target of Rapamycin Inhibitor Reduces the Incidence of Cytomegalovirus Replication in Belatacept-Treated Kidney-Transplant Recipients. *Kidney Int Rep* (2024) 9(6):1912–5. doi:10.1016/j.ekir.2024.02.1433
42. Kirk AD, Adams AB, Durrbach A, Ford ML, Hildeman DA, Larsen CP, et al. Optimization of De Novo Belatacept-Based Immunosuppression Administered to Renal Transplant Recipients. *Am J Transpl* (2021) 21(5):1691–8. doi:10.1111/ajt.16386
43. Karpe KM, Talaulikar GS, Walters GD. Calcineurin Inhibitor Withdrawal or Tapering for Kidney Transplant Recipients. *Cochrane Database Syst Rev* (2017) 2017(7):CD006750. doi:10.1002/14651858.CD006750.pub2
44. Pirsch JD, Miller J, Deierhoi MH, Vincenti F, Filo RS. A Comparison of Tacrolimus (FK506) and Cyclosporine for Immunosuppression After Cadaveric Renal Transplantation. FK506 Kidney Transplant Study Group. *Transplantation* (1997) 63(7):977–83. doi:10.1097/00007890-199704150-00013
45. Mayer AD, Dmitrevski J, Squifflet JP, Besse T, Grabensee B, Klein B, et al. Multicenter Randomized Trial Comparing Tacrolimus (FK506) and Cyclosporine in the Prevention of Renal Allograft Rejection: A Report of the European Tacrolimus Multicenter Renal Study Group. *Transplantation* (1997) 64(3):436–43. doi:10.1097/00007890-199708150-00012
46. Ekberg H, Tedesco-Silva H, Demirbas A, Vitko S, Nashan B, Gürkan A, et al. Reduced Exposure to Calcineurin Inhibitors in Renal Transplantation. *N Engl J Med* (2007) 357(25):2562–75. doi:10.1056/NEJMoa067411
47. Kaminski H, Kamar N, Thaunat O, Bouvier N, Caillard S, Garrigue I, et al. Incidence of Cytomegalovirus Infection in Seropositive Kidney Transplant Recipients Treated with Everolimus: A Randomized, Open-Label, Multicenter Phase 4 Trial. *Am J Transpl* (2022) 22(5):1430–41. doi:10.1111/ajt.16946
48. Basso G, Felipe CR, Cristelli MP, Mansur Siliano J, Viana L, Ferreira Brígido AN, et al. The Effect of Anti-Thymocyte Globulin and Everolimus on the Kinetics of Cytomegalovirus Viral Load in Seropositive Kidney Transplant Recipients Without Prophylaxis. *Transpl Infect Dis Off J Transpl Soc* (2018) 20(4):e12919. doi:10.1111/tid.12919
49. Asberg A, Jardine AG, Bignamini AA, Rollag H, Pescovitz MD, Gahlemann CC, et al. Effects of the Intensity of Immunosuppressive Therapy on Outcome of Treatment for CMV Disease in Organ Transplant Recipients. *Am J Transpl Off J Am Soc Transpl Am Soc Transpl Surg* (2010) 10(8):1881–8. doi:10.1111/j.1600-6143.2010.03114.x
50. Carretero-Ledesma M, Aguilar-Guisado M, Berastegui-Cabrera J, Balsera-Manzanero M, Pachón J, Cordero E, et al. Antiviral Activity of Immunosuppressors Alone and in Combination Against Human Adenovirus and Cytomegalovirus. *Int J Antimicrob Agents* (2024) 63(5):107116. doi:10.1016/j.ijantimicag.2024.107116
51. Krueger MB, Bonifacius A, Dragon AC, Santamorenna MM, Nashan B, Taubert R, et al. In Vitro Profiling of Commonly Used Post-Transplant Immunosuppressants Reveals Distinct Impact on Antiviral T-Cell Immunity Towards CMV. *Transpl Int* (2024) 37:12720. doi:10.3389/ti.2024.12720
52. Kaminski H, Marseres G, Yared N, Nokin MJ, Pitard V, Zouine A, et al. Mtor Inhibitors Prevent CMV Infection Through the Restoration of Functional $\alpha\beta$ and $\gamma\delta$ T Cells in Kidney Transplantation. *J Am Soc Nephrol JASN* (2022) 33(1):121–37. doi:10.1681/ASN.2020121753
53. Kühne JF, Neudörfl C, Beushausen K, Keil J, Malysheva S, Wandrer F, et al. Differential Effects of Belatacept on Virus-Specific Memory Versus De Novo Allo-Specific T Cell Responses of Kidney Transplant Recipients and Healthy Donors. *Transpl Immunol* (2020) 61:101291. doi:10.1016/j.trim.2020.101291
54. Krawczyk A, Kravčenia B, Maślanka T. Mycophenolate Mofetil: An Update on Its Mechanism of Action and Effect on Lymphoid Tissue. *Front Immunol* (2025) 15:1463429. doi:10.3389/fimmu.2024.1463429
55. Appel GB, Contreras G, Dooley MA, Ginzler EM, Isenberg D, Jayne D, et al. Mycophenolate Mofetil Versus Cyclophosphamide for Induction Treatment of Lupus Nephritis. *J Am Soc Nephrol JASN* (2009) 20(5):1103–12. doi:10.1681/ASN.2008101028
56. Houssiau FA, D'Cruz D, Sangle S, Remy P, Vasconcelos C, Petrovic R, et al. Azathioprine Versus Mycophenolate Mofetil for Long-Term Immunosuppression in Lupus Nephritis: Results from the MAINTAIN Nephritis Trial. *Ann Rheum Dis* (2010) 69(12):2083–9. doi:10.1136/ard.2010.131995
57. Zhang Z, Qiu L, Yan S, Wang JJ, Thomas PM, Kandpal M, et al. A Clinically Relevant Murine Model Unmasks a “Two-Hit” Mechanism for Reactivation and Dissemination of Cytomegalovirus After Kidney Transplant. *Am J Transpl* (2019) 19(9):2421–33. doi:10.1111/ajt.15376
58. European Mycophenolate Mofetil Cooperative Study Group. Placebo-Controlled Study of Mycophenolate Mofetil Combined with Cyclosporin and Corticosteroids for Prevention of Acute Rejection. *The Lancet* (1995) 345(8961):1321–5. doi:10.1016/S0140-6736(95)92534-1
59. Sollinger HW. Mycophenolate Mofetil for the Prevention of Acute Rejection in Primary Cadaveric Renal Allograft Recipients. *Transplantation* (1995) 60(3):225–32. doi:10.1097/00007890-199508000-00003
60. Browne BJ. The Tricontinental Mycophenolate Mofetil Trial. *Transplantation* (1996) 62(11):1697. doi:10.1097/00007890-199612150-00033
61. Miller J, Mendez R, Pirsch JD, Jensik SC, Group for the FDRKTS. Safety and Efficacy of Tacrolimus in Combination with Mycophenolate Mofetil (MMF) in Cadaveric Renal Transplant Recipients. *Transplantation* (2000) 69(5):875–80. doi:10.1097/00007890-200003150-00035
62. Natori Y, Humar A, Husain S, Rotstein C, Renner E, Singer L, et al. Recurrence of CMV Infection and the Effect of Prolonged Antivirals in Organ Transplant Recipients. *Transplantation* (2017) 101(6):1449–54. doi:10.1097/TP.0000000000001338
63. Viot B, Garrigue I, Taton B, Bachelet T, Moreau JF, Dechanet-Merville J, et al. Two-Year Post-Transplantation Cytomegalovirus DNAemia in Asymptomatic Kidney Transplant Recipients: Incidence, Risk Factors, and

- Outcome. *Transpl Infect Dis Off J Transpl Soc* (2015) 17(4):497–509. doi:10.1111/tid.12408
64. Opelz G, Döhler B. Association Between Steroid Dosage and Death with a Functioning Graft After Kidney Transplantation. *Am J Transpl* (2013) 13(8):2096–105. doi:10.1111/ajt.12313
 65. Chastain DB, Spradlin M, Ahmad H, Henao-Martínez AF. Unintended Consequences: Risk of Opportunistic Infections Associated with Long-Term Glucocorticoid Therapies in Adults. *Clin Infect Dis* (2024) 78(4):e37–56. doi:10.1093/cid/ciad474
 66. Axelrod D, Leventhal JR, Gallon LG, Parker MA, Kaufman DB. Reduction of CMV Disease with Steroid-Free Immunosuppression in Simultaneous Pancreas–Kidney Transplant Recipients. *Am J Transpl* (2005) 5(6):1423–9. doi:10.1111/j.1600-6143.2005.00855.x
 67. Vincenti F, Schena FP, Paraskvas S, Hauser IA, Walker RG, Grinyo J, et al. A Randomized, Multicenter Study of Steroid Avoidance, Early Steroid Withdrawal or Standard Steroid Therapy in Kidney Transplant Recipients. *Am J Transpl* (2008) 8(2):307–16. doi:10.1111/j.1600-6143.2007.02057.x
 68. Thomsch O, Wiesener M, Opgenoorth M, Pascher A, Woitas RP, Witzke O, et al. Rabbit-ATG or Basiliximab Induction for Rapid Steroid Withdrawal After Renal Transplantation (Harmony): An Open-Label, Multicentre, Randomised Controlled Trial. *Lancet Lond Engl* (2016) 388(10063):3006–16. doi:10.1016/S0140-6736(16)32187-0
 69. Haller MC, Royuela A, Nagler EV, Pascual J, Webster AC. Steroid Avoidance or Withdrawal for Kidney Transplant Recipients. *Cochrane Database Syst Rev* (2016) 2016(8):CD005632. doi:10.1002/14651858.CD005632.pub3
 70. Mallat SG, Tanios BY, Itani HS, Lotfi T, McMullan C, Gabardi S, et al. CMV and BKPyV Infections in Renal Transplant Recipients Receiving an mTOR Inhibitor-Based Regimen Versus a CNI-Based Regimen: A Systematic Review and Meta-Analysis of Randomized, Controlled Trials. *Clin J Am Soc Nephrol CJASN* (2017) 12(8):1321–36. doi:10.2215/CJN.13221216
 71. Silva JHT, Cibrik D, Johnston T, Lackova E, Mange K, Panis C, et al. Everolimus plus Reduced-Exposure CsA Versus Mycophenolic Acid plus Standard-Exposure CsA in Renal-Transplant Recipients. *Am J Transpl* (2010) 10(6):1401–13. doi:10.1111/j.1600-6143.2010.03129.x
 72. Cibrik D, Silva HTJ, Vathsala A, Lackova E, Cornu-Artis C, Walker RG, et al. Randomized Trial of Everolimus-Facilitated Calcineurin Inhibitor Minimization over 24 Months in Renal Transplantation. *Transplantation* (2013) 95(7):933–42. doi:10.1097/TP.0b013e3182848e03
 73. Brennan DC, Legendre C, Patel D, Mange K, Wiland A, McCague K, et al. Cytomegalovirus Incidence Between Everolimus Versus Mycophenolate in De Novo Renal Transplants: Pooled Analysis of Three Clinical Trials. *Am J Transpl* (2011) 11(11):2453–62. doi:10.1111/j.1600-6143.2011.03674.x
 74. Qazi Y, Shaffer D, Kaplan B, Kim DY, Luan FL, Peddi VR, et al. Efficacy and Safety of Everolimus Plus Low-Dose Tacrolimus Versus Mycophenolate Mofetil plus Standard-Dose Tacrolimus in De Novo Renal Transplant Recipients: 12-Month Data. *Am J Transpl* (2017) 17(5):1358–69. doi:10.1111/ajt.14090
 75. Pascual J, Berger SP, Witzke O, Tedesco H, Mulgaonkar S, Qazi Y, et al. Everolimus with Reduced Calcineurin Inhibitor Exposure in Renal Transplantation. *J Am Soc Nephrol* (2018) 29(7):1979–91. doi:10.1681/ASN.2018010009
 76. Sommerer C, Suwelack B, Dragun D, Schenker P, Hauser IA, Witzke O, et al. An Open-Label, Randomized Trial Indicates that Everolimus with Tacrolimus or Cyclosporine Is Comparable to Standard Immunosuppression in De Novo Kidney Transplant Patients. *Kidney Int* (2019) 96(1):231–44. doi:10.1016/j.kint.2019.01.041
 77. Tedesco-Silva H, Felipe C, Ferreira A, Cristelli M, Oliveira N, Sandes-Freitas T, et al. Reduced Incidence of Cytomegalovirus Infection in Kidney Transplant Recipients Receiving Everolimus and Reduced Tacrolimus Doses. *Am J Transpl* (2015) 15(10):2655–64. doi:10.1111/ajt.13327
 78. Toniato de Rezende Freschi J, Cristelli MP, Viana LA, Ficher KN, Nakamura MR, Proença H, et al. A Head-to-Head Comparison of De Novo Sirolimus or Everolimus plus Reduced-Dose Tacrolimus in Kidney Transplant Recipients: A Prospective and Randomized Trial. *Transplantation* (2024) 108(1):261–75. doi:10.1097/TP.0000000000004749
 79. Cervera C, Cofan F, Hernandez C, Soy D, Marcos MA, Sanclemente G, et al. Effect of Mammalian Target of Rapamycin Inhibitors on Cytomegalovirus Infection in Kidney Transplant Recipients Receiving Polyclonal Antilymphocyte Globulins: A Propensity Score-Matching Analysis. *Transpl Int* (2016) 29(11):1216–25. doi:10.1111/tri.12848
 80. Gardiner BJ, Westall GP, Sester M, Torre-Cisneros J, Kotton CN. Immune Monitoring Assays: Predicting Cytomegalovirus and Other Infections in Solid Organ Transplant Recipients. *Transplantation* (2025) 109(3):395–8. doi:10.1097/TP.0000000000005218
 81. Devresse A, Leruez-Ville M, Scemla A, Avettand-Fenoel V, Morin L, Lebreton X, et al. Reduction in Late Onset Cytomegalovirus Primary Disease After Discontinuation of Antiviral Prophylaxis in Kidney Transplant Recipients Treated with De Novo Everolimus. *Transpl Infect Dis* (2018) 20(2):e12846. doi:10.1111/tid.12846
 82. Tedesco-Silva H, Pascual J, Viklicky O, Basic-Jukic N, Cassuto E, Kim DY, et al. Safety of Everolimus with Reduced Calcineurin Inhibitor Exposure in De Novo Kidney Transplants: An Analysis from the Randomized TRANSFORM Study. *Transplantation* (2019) 103(9):1953–63. doi:10.1097/TP.0000000000002626
 83. Cristelli MP, Esmeraldo RM, Pinto CM, Sandes-Freitas TV, Felipe C, Lobo CF, et al. The Influence of mTOR Inhibitors on the Incidence of CMV Infection in High-Risk Donor Positive–Recipient Negative (D+/R–) Kidney Transplant Recipients. *Transpl Infect Dis* (2018) 20(4):e12907. doi:10.1111/tid.12907
 84. Kobashigawa J, Ross H, Bara C, Delgado JF, Dengler T, Lehmkuhl Hb., et al. Everolimus Is Associated with a Reduced Incidence of Cytomegalovirus Infection Following De Novo Cardiac Transplantation. *Transpl Infect Dis* (2013) 15(2):150–62. doi:10.1111/tid.12007
 85. Díaz Molina B, Velasco Alonso E, Lambert Rodríguez JL, Rodríguez Bernardo MJ, Rozado Castaño J, Padrón ER, et al. Effect of Early Conversion to Everolimus Together with Prophylaxis with Valganciclovir in the Prevention of Cytomegalovirus Infection in Heart Transplant Recipients. *Transpl Proc* (2015) 47(1):130–1. doi:10.1016/j.transproceed.2014.11.026
 86. Durante-Mangoni E, Andini R, Pinto D, Iossa D, Molaro R, Agrusta F, et al. Effect of the Immunosuppressive Regimen on the Incidence of Cytomegalovirus Infection in 378 Heart Transplant Recipients: A Single Centre, Prospective Cohort Study. *J Clin Virol* (2015) 68:37–42. doi:10.1016/j.jcv.2015.04.017
 87. Höcker B, Zencke S, Pape L, Krupka K, Köster L, Fichtner A, et al. Impact of Everolimus and Low-Dose Cyclosporin on Cytomegalovirus Replication and Disease in Pediatric Renal Transplantation. *Am J Transpl* (2016) 16(3):921–9. doi:10.1111/ajt.13649
 88. Rittà M, Costa C, Solidoro P, Sidoti F, Libertucci D, Boffini M, et al. Everolimus-Based Immunosuppressive Regimens in Lung Transplant Recipients: Impact on CMV Infection. *Antivir Res* (2015) 113:19–26. doi:10.1016/j.antiviral.2014.10.016
 89. Strueber M, Warnecke G, Fuge J, Simon AR, Zhang R, Welte T, et al. Everolimus Versus Mycophenolate Mofetil De Novo After Lung Transplantation: A Prospective, Randomized, Open-Label Trial. *Am J Transpl* (2016) 16(11):3171–80. doi:10.1111/ajt.13835
 90. Ghassemieh B, Ahya VN, Baz MA, Valentine VG, Arcasoy SM, Love RB, et al. Decreased Incidence of Cytomegalovirus Infection with Sirolimus in a Post Hoc Randomized, Multicenter Study in Lung Transplantation. *J Heart Lung Transpl* (2013) 32(7):701–6. doi:10.1016/j.healun.2013.04.010
 91. Sheng L, Jun S, Jianfeng L, Lianghui G. The Effect of Sirolimus-Based Immunosuppression Vs. Conventional Prophylaxis Therapy on Cytomegalovirus Infection After Liver Transplantation. *Clin Transpl* (2015) 29(6):555–9. doi:10.1111/ctr.12552
 92. Bowman LJ, Brueckner AJ, Doligalski CT. The Role of mTOR Inhibitors in the Management of Viral Infections: A Review of Current Literature. *Transplantation* (2018) 102(2S):S50. doi:10.1097/TP.0000000000001777
 93. Ueyama H, Kuno T, Takagi H, Alvarez P, Asleh R, Briasoulis A. Maintenance Immunosuppression in Heart Transplantation: Insights from Network Meta-Analysis of Various Immunosuppression Regimens. *Heart Fail Rev* (2022) 27(3):869–77. doi:10.1007/s10741-020-09967-3
 94. Viana LA, Cristelli MP, Basso G, Santos DW, Dantas MTC, Dreige YC, et al. Conversion to mTOR Inhibitor to Reduce the Incidence of Cytomegalovirus Recurrence in Kidney Transplant Recipients Receiving Preemptive Treatment: A Prospective, Randomized Trial. *Transplantation* (2023) 107(8):1835–45. doi:10.1097/TP.0000000000004559
 95. Kaminski H, Belanger J, Mary J, Garrigue I, Acquier M, Déchanet-Merville J, et al. Effect of mTOR Inhibitors During CMV Disease in Kidney Transplant

- Recipients: Results of a Pilot Retrospective Study. *Microbiol Immunol* (2020) 64(7):520–31. doi:10.1111/1348-0421.12794
96. Moorman NJ, Shenk T. Rapamycin-Resistant mTORC1 Kinase Activity Is Required for Herpesvirus Replication. *J Virol* (2010) 84(10):5260–9. doi:10.1128/JVI.02733-09
 97. Kudchodkar SB, Yu Y, Maguire TG, Alwine JC. Human Cytomegalovirus Infection Induces Rapamycin-Insensitive Phosphorylation of Downstream Effectors of mTOR Kinase. *J Virol* (2004) 78(20):11030–9. doi:10.1128/JVI.78.20.11030-11039.2004
 98. Clippinger AJ, Maguire TG, Alwine JC. The Changing Role of mTOR Kinase in the Maintenance of Protein Synthesis During Human Cytomegalovirus Infection. *J Virol* (2011) 85(8):3930–9. doi:10.1128/JVI.01913-10
 99. Poglitsch M, Weichhart T, Hecking M, Werzowa J, Katholnig K, Antlanger M, et al. CMV Late Phase-Induced mTOR Activation Is Essential for Efficient Virus Replication in Polarized Human Macrophages. *Am J Transpl* (2012) 12(6):1458–68. doi:10.1111/j.1600-6143.2012.04002.x
 100. Tan L, Sato N, Shiraki A, Yanagita M, Yoshida Y, Takemura Y, et al. Everolimus Delayed and Suppressed Cytomegalovirus DNA Synthesis, Spread of the Infection, and Alleviated Cytomegalovirus Infection. *Antivir Res* (2019) 162:30–8. doi:10.1016/j.antiviral.2018.12.004
 101. Araki K, Turner AP, Shaffer VO, Gangappa S, Keller SA, Bachmann MF, et al. mTOR Regulates Memory CD8⁺ T-Cell Differentiation. *Nature* (2009) 460(7251):108–12. doi:10.1038/nature08155
 102. Ferrer IR, Araki K, Ford ML. Paradoxical Aspects of Rapamycin Immunobiology in Transplantation. *Am J Transpl* (2011) 11(4):654–9. doi:10.1111/j.1600-6143.2011.03473.x
 103. Havenith SHC, Yong SL, van Donselaar-van der Pant KAMI, van Lier RAW, ten Berge IJM, Bemelman FJ. Everolimus-Treated Renal Transplant Recipients Have a More Robust CMV-Specific CD8⁺ T-Cell Response Compared with Cyclosporine- or Mycophenolate-Treated Patients. *Transplantation* (2013) 95(1):184–91. doi:10.1097/TP.0b013e318276a1ef
 104. Hauser IA, Marx S, Sommerer C, Suwelack B, Dragun D, Witzke O, et al. Effect of Everolimus-Based Drug Regimens on CMV-Specific T-Cell Functionality After Renal Transplantation: 12-Month ATHENA Subcohort-Study Results. *Eur J Immunol* (2021) 51(4):943–55. doi:10.1002/eji.202048855
 105. de Weerd AE, Fatly ZA, Boer-Verschagen M, Kal-van Gestel JA, Roelen DL, Dieterich M, et al. Tacrolimus Monotherapy Is Safe in Immunologically Low-Risk Kidney Transplant Recipients: A Randomized-Controlled Pilot Study. *Transpl Int* (2022) 35:10839. doi:10.3389/ti.2022.10839
 106. Shapiro R, Jordan ML, Scantlebury VP, Vivas C, Marsh JW, McCauley J, et al. A Prospective, Randomized Trial of Tacrolimus/Prednisone Versus Tacrolimus/Prednisone/Mycophenolate Mofetil in Renal Transplant Recipients*. *Transplantation* (1999) 67(3):411–5. doi:10.1016/S0022-5347(01)62219-5

Copyright © 2026 Bellier, Kaminski, Merville and Couzi. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.



Immune Monitoring Goes Viral – Torque Teno Virus for Immunologic Risk Stratification After Kidney Transplantation

Konstantin Doberer[†], Sebastian Kapps[†], Frederik Hauptenthal[†] and Gregor Bond^{*†}

Division of Nephrology and Dialysis, Department of Medicine III, Medical University of Vienna, Vienna, Austria

Using biomarkers to tailor immunosuppressive therapy after kidney transplantation was proposed to improve clinical care. Timely and individual adaptations of immunosuppression could reduce therapy-related side effects, such as infections, cardiovascular morbidity and malignancy, and further lower the risk of allograft rejection. Despite promising preliminary studies, evidence for implementing such a biomarker in clinical care is insufficient. Prominent candidates for immunologic monitoring after kidney transplantation include donor human leukocyte antigen-specific antibodies, donor-derived cell-free DNA, urinary chemokines and peripheral transcriptomics. In addition, the quantification of Torque Teno virus, a highly prevalent and non-pathogenic virus that was shown to associate with outcomes linked to immunocompetence, has been proposed for immunologic monitoring. This review summarises the prospects and limitations of Torque Teno virus for immunologic risk stratification after kidney transplantation in the context of current state-of-the-art. It will focus on cut-off values of plasma Torque Teno virus load that might be useful to guide immunosuppression in the clinical care of kidney transplant recipients, and highlights recently proposed indications of Torque Teno virus-guided immunosuppression.

Keywords: torque teno virus, immune monitoring, biomarker, kidney transplantation, risk stratification

OPEN ACCESS

*Correspondence

Gregor Bond,

✉ gregor.bond@meduniwien.ac.at

[†]ORCID:

Konstantin Doberer

orcid.org/0000-0002-8036-7042

Sebastian Kapps

orcid.org/0009-0002-4874-4757

Frederik Hauptenthal

orcid.org/0000-0002-7708-9045

Gregor Bond

orcid.org/0000-0003-0440-3053

Received: 10 June 2025

Revised: 19 August 2025

Accepted: 15 October 2025

Published: 14 November 2025

Citation:

Doberer K, Kapps S, Hauptenthal F and Bond G (2025) Immune Monitoring Goes Viral – Torque Teno Virus for Immunologic Risk Stratification After Kidney Transplantation. *Transpl. Int.* 38:15074. doi: 10.3389/ti.2025.15074

INTRODUCTION TO THE CLINICAL PROBLEM

Organ transplantation, which prolongs and improves the lives of patients with end-stage renal disease [1, 2], is complicated by the need to dampen the hosts' immune response. Patients on life-long immunosuppressive drug regimens are at increased risk of infections, cancer and cardiovascular morbidity [3, 4]. In addition, even modern immunosuppressive regimens cannot entirely abrogate alloimmune processes [5, 6]. Therefore, biomarker-guided immunosuppression was proposed to reduce complications caused by extensive or insufficient immunosuppression. Many candidate markers have been studied in recent decades with some very promising results [7, 8]. However, their transition into clinical use is faltering, and scientific evidence for integrating immunologic monitoring markers into routine care remains insufficient. While there are many reasons, they

Abbreviations: ABMR, Antibody-mediated rejection; BKV, BK polyomavirus; c/mL, copies/mL; CMV, Cytomegalovirus; CNI, Calcineurin inhibitor; dd-cfDNA, Donor-derived cell-free DNA; DSA, Donor-specific antibodies; IVDR, *In Vitro* Medical Devices Regulation; MPA, Mycophenolic acid; mTORi, Mammalian target of rapamycin inhibitor; TAC, Tacrolimus; TCMR, T cell-mediated rejection; TTV, Torque Teno virus; qPCR, Quantitative polymerase chain reaction; RCT, Randomised controlled trial.

nearly always come down to a lack of validation in interventional trials, potentially compounded by high costs and a lack of reimbursement. Currently, the most promising candidates for immunologic monitoring after kidney transplantation are donor-specific antibodies (DSAs), donor-derived cell-free DNA (dd-cfDNA), urinary chemokines and peripheral transcriptomics.

Unlike the abovementioned biomarkers, which mainly reflect organ damage or alloreactivity, the Torque Teno virus (TTV) – a highly prevalent and non-pathogenic virus of the family Anelloviridae – was shown to be associated with outcomes linked to both excessive and insufficient immunosuppression. Therefore, TTV may serve as a biomarker indicating the net state of the immune system and thus could complement existing injury and alloreactivity markers. A gradual deviation from an ‘optimal’ TTV load toward a high or low load was shown to correlate with an increased risk of infections and allograft rejection [9]. More recent data show associations with malignancy and the immune response after vaccination [10]. The introduction of a quantitative polymerase chain reaction (qPCR) assay has made TTV a readily measurable parameter. However, the framework for implementing it in routine clinical care requires further definition. One multicentre interventional trial assessing the clinical value of TTV-guided immunosuppression in kidney transplant recipients was successfully concluded in May 2025 (TTVguideIT, EUCT number: 2022-500024-30-00) [11], and another started recruiting in April 2025 (TAOIST, ClinicalTrials.gov ID: NCT06829719).

This article will reflect on the role of TTV as an immune marker in the clinical care of kidney transplant recipients, focusing on relevant data that have emerged over the last 3 years. Previous relevant studies will only be mentioned briefly, as they have been discussed in detail earlier [9]. The role of TTV from the clinical virologist’s perspective has been reviewed by others recently [12, 13]. Biomarkers other than TTV will only be discussed briefly, as current reviews cover peripheral blood gene expression, dd-cfDNA, urinary chemokines and other emerging candidates [8, 14, 15].

CURRENT STATUS OF IMMUNE MONITORING IN CLINICAL CARE OF KIDNEY TRANSPLANT RECIPIENTS

Despite a long history of immune monitoring research in kidney transplantation, no biomarker is supported by sufficient evidence to enter clinical practice. Standardised assays, rigorous validation and interventional trials demonstrating the clinical value of biomarkers when added to the standard of care are prerequisites for broad implementation. Lately, some studies yielded promising results and a recent consensus statement of the European Society of Organ Transplantation cautiously discussed recommendations in favour of biomarker monitoring in certain settings while urgently calling for interventional trials [16]. However, the study design in this context is complicated. Endpoints commonly used in interventional clinical trials, such as allograft rejection or loss, have low incidences, necessitating the inclusion of high-risk patients or large populations. Consequently,

a randomised controlled trial (RCT) assessing *de novo* DSA-triggered optimisation of immunosuppression suffered from lower-than-expected rejection and biopsy rates and failed to show improved allograft survival [17]. Similarly, a multicentre RCT evaluating urinary monitoring of C-X-C motif chemokine ligand 10 (CXCL10) in addition to the standard of care could not demonstrate a reduction in rejection rates during the first year post-transplantation, at least partly due to low biopsy and rejection rates [18].

Besides sufficient power, outcome selection is crucial in biomarker trial design. For example, while dd-cfDNA was developed as an injury marker, several studies focused on dd-cfDNA as a tool to rule out rejection and avoid invasive diagnostics. This demands close and longitudinal monitoring in large patient cohorts, which, even with a high negative predictive value, may easily become very resource-demanding. A more effective approach might be ruling in rejection in high-risk patients. Consistent with this approach, the first positive results came from a single-centre RCT demonstrating an accelerated diagnosis of late antibody-mediated rejection (ABMR) in patients with *de novo* DSA if dd-cfDNA was added to the standard of care [19]. Following these first promising results, validation in large and diverse multicentre cohorts is now eagerly awaited.

The nature of the biomarker should also be considered carefully in the study design and outcome selection phase. For example, injury markers might not be useful for predicting potential future adverse events [20]. Biomarkers reflecting the state of immunosuppression could overcome this problem. TTV is a promising candidate due to its link to both extensive and insufficient immunosuppressive burden before adverse events manifest. Quantifying immunocompetence using TTV may serve as a complement to markers of graft injury (e.g. dd-cfDNA) and alloreactivity (e.g. DSA).

Alternative approaches to quantify the net state of the immune system rely on cellular assays. One proposed assay was the ImmuKnow, which did not enter clinical practice despite promising results within a single centre RCT [21]. Recently, another RCT including paediatric patients showed promising data for Tvis [22], an assay that relies on quantifying virus-specific T cells. Because of the complex logistical procedures, most of the patients (86%) were randomised only at one study site. Multicentre validation will be crucial as the complexity of the assay might pose an obstacle to standardisation and implementation.

TORQUE TENO VIRUS – FROM DISCOVERY TO IMMUNE MONITORING

TTV is a small, circular, non-enveloped, single-stranded DNA virus that was first described in 1997. It is characterised by high genetic diversity, and 26 species are currently classified among the genus *Alphatorquevirus* within the family Anelloviridae. To date, no causal association with any disease has been demonstrated. Its high prevalence in the general population [23, 24], replication in almost all studied body tissues and liquids [25], and high positivity rates among infants [26] have given rise to the

hypothesis of TTV being a non-pathogenic, commensal virus. As such, it was attributed to the human virome [27].

Epidemiological analyses revealed higher TTV prevalences in patients with diseases causing reduced immunocompetence or chronic inflammation [10, 28]. As early as 2001, studies linked TTV prevalence to overall immunocompetence [29, 30]. In the same year, Maggi and Bendinelli speculated on the potential healthcare benefits of monitoring TTV in organ transplantation [31]. In 2003, Moen and colleagues observed steep increases in TTV load upon initiation of immunosuppression in kidney transplant recipients [32]. A decade later, associations between TTV and adverse outcomes in transplant patients became evident. In a systems biology approach, De Vlaminc and colleagues analysed the human plasma virome in patients after heart and lung transplantation, and observed not only a marked expansion of TTV after the initiation of immunosuppression but also an overall lower viral load in patients with allograft rejection [33]. Associations between TTV load and the occurrence of infections were demonstrated shortly after [34]. These findings were subsequently reproduced in a variety of different cohorts with broad consistency among studies and across different types of transplanted organs [9, 35].

Evidence for TTV as an immune marker has also emerged from studies involving non-transplant patients [36]. In patients with antineutrophil cytoplasmic antibody vasculitis, a retrospective analysis of the RAVE RCT [37] showed that those who experienced relapses had lower peripheral blood TTV loads at month 4 after therapy start, potentially reflecting insufficient immunosuppression (unpublished data from the Medical University of Innsbruck). In patients with rheumatoid arthritis, TTV loads were lower in those with persistent rheumatoid arthritis activity despite initiation of disease-modifying anti-rheumatic drug therapy, indicating an insufficient immunosuppressive effect [38].

TTV has also been proposed for triage in visits at emergency medicine outpatient clinics. Patients with a SARS-CoV-2-infection were shown to be at particularly increased risk of admission to the intensive care unit or death if TTV loads in nasopharyngeal swabs were high [39]. Promising results of TTV quantification in patients with oncologic disease were reported and recently summarised [40]. In women with ovarian cancer, those with unfavourable outcomes were found to have higher TTV loads [41]. In patients receiving chimeric antigen receptor T-cell therapy for lymphoproliferative disease, TTV dynamics were associated with therapy response and immune effector cell-associated neurotoxicity syndrome [42].

Altogether, a high TTV load has been associated with host factors and clinical conditions linked to compromised immunocompetence across heterogeneous study populations. In patients receiving immunosuppressive therapy, TTV load may be used to identify those with insufficient immune system control and increased risk of adverse outcomes. Therefore, TTV is a promising biomarker to not only quantify the net state of the immune system but also guide clinical decision-making. Ongoing and future interventional RCTs will help define the value of TTV in the clinical care of kidney transplant recipients.

NOVEL DATA ON TORQUE TENO VIRUS QUANTIFICATION TECHNIQUES

In clinical applications, TTV load is most commonly quantified in plasma; however, it can also be quantified in various body fluids, including serum, whole blood, urine, nasopharyngeal swabs and bronchoalveolar lavage. Quantifying TTV in whole blood and serum yields higher loads than in plasma. Recently, Truffot and colleagues systematically quantified this difference, analysing 216 consecutive paired samples from 68 kidney transplant recipients. They observed a mean TTV load difference of 0.4 log₁₀ copies/mL (c/mL) between whole blood and plasma, with a high correlation between paired samples [43]. Unpublished data from the University of Strasbourg shows a mean TTV load difference of 0.18 log₁₀ c/mL between serum and plasma among 40 solid organ transplant recipients with 169 paired samples. It is well established that viral PCRs for viruses such as cytomegalovirus (CMV) or BK polyomavirus (BKV) can show differences of up to one log between whole blood and plasma or serum, with plasma and serum providing comparable results. Future studies should investigate these relationships for TTV. Until then, we recommend quantifying TTV in plasma, as most proposed cut-offs are based on this matrix.

The most extensively reported systems for quantifying TTV load are qPCR-based and use either published primers and probes developed by Maggi and colleagues [44] or a commercially available *In Vitro* Medical Devices Regulation (IVDR)-labelled assay (TTV R-GENE®, bioMérieux, France) [45]. Notably, TTV loads can differ significantly between applied assays. A recent study compared the in-house PCR developed by Maggi and colleagues and the commercially available PCR in 342 samples from 314 patients, revealing a mean difference of 1.38 log₁₀ c/mL (95% confidence interval: 1.30–1.46) [46]. Notably, the assays showed a high and almost linear correlation, allowing one method to be extrapolated to the other. Besides primers and probes, differences in extractors, cyclers, consumables and local standards might also lead to significant differences in TTV load quantification. Indeed, a comparison of TTV loads quantified with the same PCR assay in Fabrizio Maggi's laboratory in Italy and at the Center for Virology at the Medical University of Vienna showed a difference of 1.0 log₁₀ c/mL (unpublished data). Given these findings, it is evident that locally obtained TTV PCR results need to be cross-validated and adapted to proposed TTV load cut-offs accordingly. Such a process can be facilitated by customised quality assessment programmes like those offered by Quality Control for Molecular Diagnostics (Glasgow, UK).

For the clinical implementation of any TTV qPCR assay, a low inter- and intra-centre variability is desirable. A recent analysis by the clinical virologists of the TTVguideTX consortium demonstrated that this can be achieved for the commercial PCR using standard testing platforms [47]. In preparation for the multicentre TTVguideIT trial, the PCR was set up locally in 13 recruiting centres across Europe. Applying an internal quality control demonstrated excellent accuracy, with an inter-laboratory standard deviation of 0.19 log₁₀ c/mL and an intra-laboratory standard deviation of 0.07–0.18 log₁₀ c/mL. External quality assessment and linearity panels similarly showed small variability. Implementation of qPCR assays might be further

TABLE 1 | TTV load cut-offs proposed based on an IVDR-labelled qPCR assay to guide immunosuppression after kidney transplantation.

Clinical setting	Time of TTV quantification	TTV threshold
Clinically overt graft rejection ^a (single centre, cohort) [49]	Month 4–12 post-transplant	<4.6 log ₁₀ c/mL ^{b,c}
Subclinical rejection ^a (single centre, cohort) [52]	Month 4–12 post-transplant	<4.6 log ₁₀ c/mL ^{b,c}
Underimmunosuppression ^d (single centre, cross-sectional) [53]	Month 12–36 post-transplant	<3.8 log ₁₀ c/mL ^e
Clinically overt graft rejection ^a (single centre, cross-sectional) [54]	Median of 6 years post-transplant	<3.6 log ₁₀ c/mL ^{b,c}
Vaccine response ^f (retrospective analysis of a multicentre RCT) [10]	Median of 7 years post-transplant	<4.6 log ₁₀ c/mL ^{b,c}
Infection ^g (single centre, cohort) [49]	Month 4–12 post-transplant	>6.6 log ₁₀ c/mL ^{b,c}
Malignant disease ^h (single centre cohort, unpublished)	Month 4–12 post-transplant	>6.6 log ₁₀ c/mL ^{b,c}
Overimmunosuppression ⁱ (single centre, cross-sectional) [53]	Month 12–36 post-transplant	>5.1 log ₁₀ c/mL ^e
CMV-DNAemia >3.0 log ₁₀ c/mL (single centre, paediatric cohort) [55]	Year 2 (median) to year 5 (median) post-transplant	>6.3 log ₁₀ c/mL ^{b,c}
BKV-DNAemia >3.0 log ₁₀ c/mL (single centre, paediatric cohort) [55]	Year 2 (median) to year 5 (median) post-transplant	>5.0 log ₁₀ c/mL ^{b,c}

Abbreviations: ABMR, antibody-mediated rejection; BKV, BK polyomavirus; CMV, cytomegalovirus; c/mL, copies/mL; RCT, randomised controlled trial; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

^abiopsy-proven borderline, cellular and ABMR, according to the respective Banff meeting report.

^bto facilitate the interpretation, TTV cut-off values obtained using an in-house PCR assay have been recalculated to match the results obtained with the TTV R-GENE[®] qPCR assay according to the comparative study by Görzer et al. who described a mean difference of 1.38 log₁₀ c/mL (95% confidence interval 1.30–1.46) between PCR methods [46].

^cplasma was used for TTV load quantification.

^dbased on comparison with a healthy collective and antibody response to vaccination.

^eserum was used for TTV load quantification.

^fhumoral and cellular.

^gdefined as need for hospitalisation, anti-microbial treatment or reduction of immunosuppression.

^hexcluding basaloma and carcinoma in situ.

ⁱdefined by the occurrence of serious infections or malignoma.

supported by using automated test systems. In this regard, a study by Spezia and colleagues might be of special interest. They compared the conventional in-house PCR with an automated approach in 112 samples and found a high concordance rate [48]. Automated qPCR systems could facilitate shorter turnaround times, reduced workload and higher throughput.

PROPOSED TORQUE TENO VIRUS CUT-OFF VALUES TO GUIDE IMMUNOSUPPRESSION

Most data on TTV in patients receiving immunosuppressive therapy was generated in kidney transplant recipients. Findings from initial retrospective studies have since been validated by non-interventional prospective studies [49, 50]. Today, independent and robust associations have been described between low TTV loads and all types of kidney graft rejection, including clinically overt T cell-mediated rejection (TCMR), ABMR, borderline TCMR and subclinical rejection. Higher TTV loads were found prior to a broad range of infectious events, including opportunistic infections by CMV and BKV, as well as bacterial urinary tract infections. Notably, linear associations showed step-wise increasing risk constellations for both infection and rejection with increasing or decreasing TTV load, respectively, as described in a 2022 review by our group [9] and a 2023 meta-analysis by van Rijn and colleagues [51].

Consequently, quantifying TTV loads was proposed for risk stratification of insufficient or excessive immunosuppression in kidney transplant recipients, and the research focus shifted towards defining clinically relevant TTV load cut-offs. TTV load cut-offs that have been proposed for the care of kidney transplant recipients are presented in **Table 1**; **Figure 1**. To facilitate the interpretation

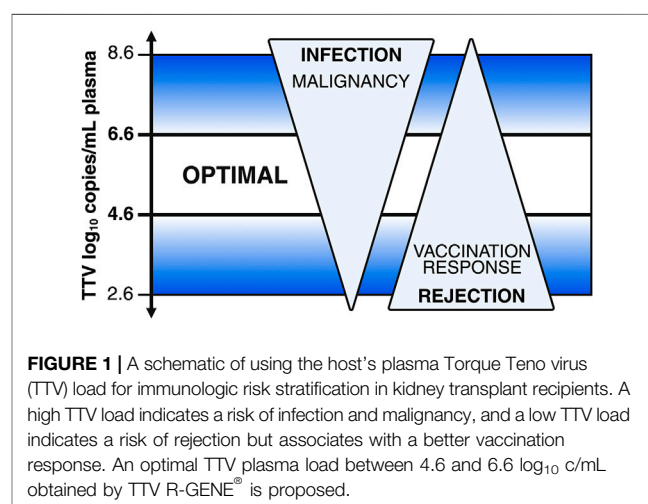


FIGURE 1 | A schematic of using the host's plasma Torque Teno virus (TTV) load for immunologic risk stratification in kidney transplant recipients. A high TTV load indicates a risk of infection and malignancy, and a low TTV load indicates a risk of rejection but associates with a better vaccination response. An optimal TTV plasma load between 4.6 and 6.6 log₁₀ c/mL obtained by TTV R-GENE[®] is proposed.

of these cut-offs, we converted TTV loads obtained by in-house qPCR assays to those of the commercially available qPCR assay. Using data from prospective cohort studies, a plasma TTV load between 4.6 and 6.6 log₁₀ c/mL was proposed as an optimal trade-off between the risks of rejection and infection 4–12 months after kidney transplantation [49]. Few studies have investigated TTV loads in patients beyond the first year post-transplantation. Schiemann et al. described an increased risk of rejection in patients with TTV loads <3.6 log₁₀ c/mL at a median of 6 years after transplantation in a cross-sectional study (**Table 2**) [54]. Chauvelot and colleagues proposed a range of 3.8–5.1 log₁₀ c/mL for patients between one and 4 years after transplantation and validated their findings in a prospective cohort (**Table 1**) [53].

It is important to note, that validation of the proposed cut-off values guiding immunosuppressive therapy in an interventional

TABLE 2 | A selection of studies that have recently expanded the indication of TTV-guided monitoring after kidney transplantation.

Study design	Cohort	TTV monitoring	Main outcome	Main finding
Paediatric cohort				
Retrospective analysis of single centre cohort [55]	71 KTX included 2y after TX; 3y FUP	every 4 to 8w for 3y	10 BKV / 7 CMV infections	High TTV load associates with CMV and BKV DNAemia
SARS-CoV-2 vaccination				
Retrospective analysis of multicentre RCT [10]	100 KTX with mRNA vaccination; 7y after TX	At first vaccination	31 seroconversions post 2 vaccinations	High TTV load associates with impaired vaccine response
Retrospective analysis of single centre cohort [56]	459 KTX with 2 or 3 mRNA vaccination; 6y after TX	At first vaccination	208 seroconversions post 2/ 130 post 3 vaccinations	High TTV load associates with impaired vaccine response
TTV load kinetic				
Retrospective analysis of single centre cohort [57]	48 KTX with isolated CNI dose adjustment; 1 year after TX	0, 30, and 60d after CNI dose adjustment	TTV load	TTV load changes detectable only 2m after CNI adaption
Retrospective analysis of single centre cohort [58]	43 KTX with 5w MPA withdrawal/ 33 continued MPA; 5y after TX	0, 1m after withdrawal, 2m post reintroduction	TTV load	TTV load decreases 1m after MPA withdrawal/ increases 2m after restart
Non-randomised, open label, controlled pilot [59]	18 KTX with 2w MPA withdrawal/ 22 continued MPA; 4y after TX	0, 2w after withdrawal, 1m post reintroduction	TTV load	TTV load decreases 2 w and 1 m after MPA withdrawal
Belatacept-based IS				
Retrospective analysis of single centre cohort [60]	68 KTX converted from TAC to belatacept; 4y after TX	0, 3, 6, and 12m after conversion	TTV load	No significant changes of TTV load post TAC-conversion
Retrospective analysis of 2 RCTs [61]	105 KTX converted from CNI to belatacept; 2y after TX	0, 6, and 12m after conversion	TTV load	No significant changes of TTV load post CNI-conversion
mTORi-based IS				
Cross-sectional, single centre [54]	715 KTX, 30 mTORi-based IS; 6y after TX	at screening	TTV load	Trend towards lower TTV load in mTORi-based IS
post-TX malignancy				
Retrospective analysis of a single centre cohort study [50]	221 KTX; 1 year FUP	0, 7d, 1, 3, 6, and 12m	54 opportunistic infections / 11 malignancies	High TTV load associates with opportunistic infection and malignancy
Retrospective analysis of single centre cohort ^a	428 KTX; 5y FUP	Every 3m for 5y	53 malignancies 2-5y after TX	High TTV load associates with malignancy
RCT				
1:1: TTV-guided TAC trough level vs. SOC [11]	13 EU centres; 260 low risk KTX randomised 4m after TX; 9m FUP	Every 6w for 9m	Death, graft loss, rejection, infection	TTVguideIT: last patient last visit May 2025; results expected 2026
1:1: TTV-guided IS vs. SOC ^b	4 French centres; 300 low risk KTX randomised 1-4y after TX; 3y FUP	Every 3m for 3y	Rejection, infection, cancer, graft loss, DSA	TAOIST: first patient first visit April 2025; results expected 2030

Abbreviations: BKV, BK polyomavirus; CMV, cytomegalovirus; CNI, calcineurin inhibitor; d, days; DSA, donor-specific antibodies; DSMB, data safety monitoring board; FUP, follow-up; IS, immunosuppression; KTX, kidney transplant recipients; MPA, mycophenolic acid; m, month(s); mRNA, messenger RNA; mTORi, mammalian target of rapamycin inhibitor; TX, transplantation; RCT, randomised controlled trial; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; TAC, tacrolimus; SOC, standard of care; w, weeks y, years.

^aunpublished data from a retrospective analysis of the prospective TTV-POET study (DRKS ID: DRKS00012335).

^bstudy protocol published on ClinicalTrials.gov (NCT06829719).

setting is necessary prior to clinical implementation. One completed (TTVguideIT, EUCT number: 2022-500024-30-00) and one ongoing (TAOIST, ClinicalTrials.gov ID: NCT06829719) RCT will help to clarify the clinical value of TTV-guided immunosuppression in post-transplant care. In addition, these multicentre RCTs will allow for the validation of TTV load cut-offs derived from single-centre studies. TTVguideIT was a multicentre, interventional, patient- and assessor-blinded RCT conducted in 13 European centres recruiting stable adult kidney transplant recipients with low immunological risk (Table 2). The corresponding study protocol and the statistical analysis plan have been previously published [11, 62]. Between 2022 and 2024,

260 patients were randomised at month four after transplantation to either standard of care or TTV-guided tacrolimus dosing at a 1:1 ratio. TTV load was quantified in both arms every 6 weeks for 9 months. In the interventional arm, the investigators followed a protocol to set tacrolimus target trough levels according to the actual TTV load. In the control arm, TTV load was concealed. The primary endpoint was a composite of the occurrence of infections, biopsy-proven allograft rejection, graft loss and death scored by central assessors blinded to the allocation sequence. The last patient concluded the trial in May 2025.

The TAOIST trial is an interventional, open-label, parallel-group RCT being conducted in four French

centres and recruiting adult kidney transplant recipients (**Table 2**). It aims to include 300 stable, low immunological-risk patients from months 12–48 after transplantation. The patients will be randomised to either standard of care or TTV-guided immunosuppression dosing for 36 months. TTV load will be quantified every 3 months. In the interventional arm, physicians will be free to change the dosing of immunosuppressive drugs to keep the TTV load within a predefined range. The primary outcome is a composite of *de novo* DSA, biopsy-proven rejection, infection, cancer or graft loss. The first patient was recruited in April 2025.

Notably, age, sex, and body mass index, which are associated with TTV load, do not confound or modify the association between TTV load and adverse effects related to over- and under-immunosuppression [23, 24, 63, 64]. Therefore, no adjustments to TTV cut-offs are necessary based on these characteristics. Conversely, it is plausible that patients at high risk for graft rejection - such as recipients with preformed DSA (and thus non-standard induction) or *de novo* DSA, re-transplantation, or a history of ABMR - as well as those at increased risk for infection - like older, frail recipients or individuals with comorbidities - may benefit from tailored immunosuppression. This could be reflected by higher or lower TTV cut-offs. Such a concept should be evaluated in phase three trials once the ongoing phase two studies, which recruit low-risk patients, demonstrate the safety of TTV-guided monitoring.

TIMING OF TORQUE TENO VIRUS-BASED IMMUNE MONITORING

TTV load cut-offs for the guidance of immunosuppression have been proposed to be useful from month 4 post-transplant. In the first 3–4 months after kidney transplantation, TTV load is not in a steady state, and thus the definition of clinically relevant cut-off values to guide immunosuppression is difficult [34, 49]. In contrast to the well-described dynamics of TTV load after initiation of immunosuppression, TTV kinetics following dose changes of immunosuppressive drugs were unknown until recently. Two cohort studies during the COVID-19 pandemic evaluating pausing antimetabolite treatment to enhance responses to SARS-CoV-2 mRNA vaccination produced the first insights (**Table 2**) [58, 59]. Both studies showed a reduction in TTV load between 4 and 6 weeks after pausing antimetabolite treatment, and TTV load reached baseline values within 2 months after reinitiation of antimetabolite therapy. This evidence was recently complemented by a single-centre study by Regele et al. [57], who examined 48 kidney allograft recipients with isolated calcineurin inhibitor (CNI) dosage changes from the TTV-POET study. No significant changes in TTV load were observed 1 month after the CNI dose changes. However, a median CNI dose reduction of 33% translated to a significant decrease in TTV load 2 months after the dosage change, and a 50% increase in CNI dosage caused a trend toward higher TTV

loads 2 months later (**Table 2**). Notably, no TTV load measurements were available after 2 months, making it impossible to analyse further changes beyond that time. Integrating the findings of these studies with the consistently described peak of TTV loads at around 3–4 months after initiating immunosuppression following transplantation, the optimal time frame for quantifying the TTV load may be assumed to be every 2–4 months. It is important to note, that assessing the effect of an immunosuppressive dose change on TTV load shortly afterward is not appropriate. Due to the time lag in TTV load changes following dose adjustments in immunosuppressive therapy, measuring TTV load should only be done approximately 2 months later.

TORQUE TENO VIRUS LOAD IN BELATACEPT- AND mTOR INHIBITOR-BASED IMMUNOSUPPRESSION

One question that has come up repeatedly in recent years is how TTV load cut-off values defined in patients treated with CNI-based immunosuppression can be translated to other immunosuppressive regimens. In a single-centre cross-sectional study with a limited number of patients, a higher TTV load was initially observed in those on belatacept-based ($n = 23$) compared to CNI-based immunosuppression [54]. Therefore, it was hypothesised that co-stimulation blockade might have led to more potent immunosuppression or insufficient formation of TTV-specific T-cells and thus directly influenced viral control. However, two recent studies challenged this earlier finding and did not show significant increases in TTV loads after conversion from CNIs to belatacept (**Table 2**) [60, 61, 65]. In a retrospective study in Grenoble that included 68 patients converted from CNI to belatacept at a median of 4 years after transplantation, TTV loads did not change significantly from baseline [60]. A retrospective analysis of two RCTs examined TTV loads in 105 patients randomised to either CNI continuation or conversion to belatacept at 6 and 12 months after conversion [61]. Those who switched to a belatacept-based regimen showed stable TTV loads and no significant differences in TTV dynamics compared to those who maintained CNI-based therapy at both time points. In both studies, while infection and rejection rates were low, precluding any meaningful analysis, TTV loads tended to be higher in patients with subsequent infections and lower before rejection episodes. These data suggest the potential application of TTV cut-off values defined using CNI-based regimens for risk stratification also in patients on belatacept-based immunosuppression.

Unlike belatacept-based immunosuppression, insufficient data is available for mammalian target of rapamycin inhibitor (mTORi)-based immunosuppression to make recommendations concerning the clinical value of TTV-guided immunosuppression. In only one retrospective cross-sectional single-centre study, the 30 patients receiving mTORi-based immunosuppression showed absolute but not statistically

significant lower TTV loads than those receiving CNI-based immunosuppression (Table 2) [54].

TORQUE TENO VIRUS MONITORING IN PAEDIATRIC KIDNEY TRANSPLANT RECIPIENTS

Until recently, cut-off values for TTV-guided immunosuppression were unavailable for paediatric kidney transplant recipients, and data were mainly derived from one cross-sectional study and one single-centre cohort study associating Anelloviridae trajectories with immunosuppression and graft rejection [66, 67]. Recently, a larger retrospective single-centre study by Eibensteiner and colleagues added to these data (Table 2). They retrospectively analysed all paediatric kidney transplant recipients followed between 2014 and 2020 at the Medical University of Vienna for TTV load at 4–8 weeks intervals in the context of CMV and BKV infections ($n = 71$) [55]. They described a higher TTV load in patients with subsequent CMV and BKV infections during a 3-year follow-up and defined cut-offs at 7.7 and 6.4 $\log_{10}$ c/mL, respectively, to predict subsequent clinically relevant viral DNAemia (Table 2). Recipients in the cohort showed a wide age range (IQR 3.5–13.2 years at transplantation), and it is well established that TTV load increases with age [24, 68]. Analyses from adult kidney transplant cohorts have demonstrated an association between TTV load and adverse effects related to both over- and under-immunosuppression across all age groups [49]. Future research should explore whether this relationship also applies to the full paediatric recipient population.

NOVEL ENDPOINTS FOR TORQUE TENO VIRUS-BASED MONITORING: MALIGNANCY AND VACCINATION RESPONSE

The consequences of intense immunosuppression go beyond an elevated incidence of infections, as such patients are also at an increased risk of oncologic disease. Until recently, only one single-centre cohort study that included 221 patients indicated higher TTV loads in kidney transplant recipients with subsequent oncologic disease. Notably, oncologic disease was analysed only within a combined endpoint that included mainly opportunistic infections (Table 2) [50]. An yet unpublished analysis of data from the prospective TTV-POET study (DRKS ID: DRKS00012335) showed that the cumulative TTV load in 428 patients from months 4–12 after transplantation was predictive for the development of malignoma (53 events) in the subsequent 4 years of follow-up. Using the Vienna in-house PCR, patients with a TTV load $>8 \log_{10}$ c/mL had a significantly higher risk of developing cancer than those with a TTV load $<8 \log_{10}$ c/mL. Notably, this TTV load cut-off is equivalent to the cut-off for defining patients at risk of

infection (Table 1). Therefore, it can be speculated that targeting the optimal TTV load range proposed to reduce infection might also reduce malignancy rates.

Another clinically relevant side effect of immunosuppression in kidney transplant recipients is a reduced response to vaccination, and recent studies have analysed TTV loads in this context. Graninger and colleagues retrospectively analysed 100 kidney transplant recipients from samples prospectively stored by an interventional German multicentre RCT, demonstrating that TTV loads were lower in serological responders than in non-responders after two doses of the SARS-CoV-2 mRNA vaccine. Those with TTV loads $>10^6$ c/mL showed no cellular immune response, and only 12% showed a serological response (Table 2) [10]. These findings were confirmed by a retrospective single-centre study involving 459 kidney transplant recipients receiving their second dose of the SARS-CoV-2 mRNA vaccine, of which half then received a third dose (Table 2) [56]. Notably, during the COVID-19 pandemic, temporal antimetabolite withdrawal was shown to enhance responses to SARS-CoV-2 vaccination in some [58, 69] but not all [59] RCTs. Therefore, a TTV-based approach to individualise both the amount and timing of reducing immunosuppression before planned vaccination could be an interesting design to evaluate in an interventional trial.

SCENARIOS WHERE TORQUE TENO VIRUS LOAD MIGHT NOT REFLECT IMMUNE FUNCTION ACCURATELY

Besides insufficient data on patients receiving mTORi-based immunosuppression, as mentioned above, there are other scenarios where TTV-guided immunosuppression might be challenging. In a few patients, the TTV load does not reach the detection limit of the commonly used qPCRs. In these cases, differentiating between non-infection and DNAemia below the detection limit is difficult, and the TTV load cannot be used for risk stratification of immunosuppression. Quantification of other genera of the Anelloviridae family might help in this situation. Recently, a study involving 168 solid organ transplant recipients showed that quantifying *Betatorquevirus* (formerly the Torque Teno Mini virus) and *Gammatorquevirus* (formerly the Torque Teno Midi virus) improved the prediction of the SARS-CoV-2 vaccination response [70].

TTV was shown to become almost undetectable following myeloablative conditioning regimens in hematopoietic stem cell transplantation patients [71, 72]. Similarly, TTV load significantly decreases during anti-thymocyte globulin treatment in solid organ transplant recipients [73]. In both settings, the TTV load cannot be used to quantify immunosuppression. Notably, TTV loads returned to baseline 1–2 weeks after anti-thymocyte globulin treatment and might reflect immunosuppression accurately again after that time point [73]. In concordance with the above mentioned findings leukocytes were proposed as a replication pool for TTV and it may be speculated upon whether the validity of TTV loads is also

reduced during episodes of significant leukopenia due to, for example, CMV infection or drug toxicity.

SUMMARY AND OUTLOOK

The association between TTV load and adverse outcomes linked to immune function in kidney transplant recipients is increasingly robust, and cut-offs for TTV-guided immunosuppression have been proposed. Recent studies involving paediatric cohorts, patients with belatacept-based immunosuppression and endpoints other than infection and rejection might broaden its potential clinical applications. Future studies must focus on knowledge gaps, including TTV loads in patients at later time points after transplantation and recipients on mTORi-based immunosuppression. Improved quantification methods of TTV load have been proposed to support implementation, which will depend on the results of ongoing interventional trials. Following results of ongoing interventional trials, TTV will have to position itself in the context of other emerging biomarkers for immunologic monitoring.

AUTHOR CONTRIBUTIONS

KD and GB wrote the manuscript, and SK and FH edited and critically revised the manuscript. All authors contributed to the article and approved the submitted version.

REFERENCES

- Wolfe RA, Ashby VB, Milford EL, Ojo AO, Ettenger RE, Agodoa LY, et al. Comparison of Mortality in All Patients on Dialysis, Patients on Dialysis Awaiting Transplantation, and Recipients of a First Cadaveric Transplant. *N Engl J Med* (1999) 341(23):1725–30. doi:10.1056/nejm199912023412303
- Yang J, Endo Y, Munir MM, Woldeesenbet S, Altaf A, Limkemann A, et al. Waitlist Time, Age, and Social Vulnerability: Impact on the Survival Benefit of Deceased Donor Kidney Transplantation Versus Long-Term Dialysis Among Patients With End-stage Renal Disease. *Transplantation* (2025) 109(1): e64–e74. doi:10.1097/tp.0000000000005125
- Hariharan S, Rogers N, Naesens M, Pestana JM, Ferreira GF, Requião-Moura LR, et al. Long-Term Kidney Transplant Survival Across the Globe. *Transplantation* (2024) 108(9):e254–e263. doi:10.1097/tp.0000000000004977
- Mayrdorfer M, Liefeldt L, Osmanodja B, Naik MG, Schmidt D, Duettmann W, et al. A Single Centre in-Depth Analysis of Death With a Functioning Kidney Graft and Reasons for Overall Graft Failure. *Nephrol Dial Transpl* (2023) 38(8): 1857–66. doi:10.1093/ndt/gfac327
- Coemans M, Süsal C, Döhler B, Anglicheau D, Giral M, Bestard O, et al. Analyses of the Short- and Long-Term Graft Survival After Kidney Transplantation in Europe Between 1986 and 2015. *Kidney Int* (2018) 94(5):964–73. doi:10.1016/j.kint.2018.05.018
- Budde K. How to Treat T Cell Mediated Rejection? -A Call for Action. *Transpl Int* (2024) 37:12621. doi:10.3389/ti.2024.12621
- Gupta G, Athreya A, Kataria A. Biomarkers in Kidney Transplantation: A Rapidly Evolving Landscape. *Transplantation* (2025) 109(3):418–27. doi:10.1097/tp.0000000000005122
- Westphal SG, Mannon RB. Biomarkers of Rejection in Kidney Transplantation. *Am J Kidney Dis* (2025) 85(3):364–74. doi:10.1053/j.ajkd.2024.07.018

FUNDING

The author(s) declare that financial support was received for the research and/or publication of this article. This work was funded by the Austrian Science Fund (grant number KLI1152; grant holder: GB) and the European Union Framework Programme for Research and Innovation Horizon 2020 (grant agreement ID: 896932; project coordinator: GB).

CONFLICT OF INTEREST

GB received fees from bioMérieux for preparing communication material for TTV-R-GENE®, an assay to quantify Torque Teno virus load sold by bioMérieux, and for a talk at a scientific conference. GB is coordinator of the European Commission-funded TTVguideTX project assessing the clinical value of Torque Teno virus-guided immunosuppression (grant agreement ID: 896932; consortium lead: Medical University of Vienna).

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

GENERATIVE AI STATEMENT

The author(s) declare that no Generative AI was used in the creation of this manuscript.

- Jaksch P, Görzer I, Puchhammer-Stöckl E, Bond G. Integrated Immunologic Monitoring in Solid Organ Transplantation: The Road Toward Torque Teno Virus-Guided Immunosuppression. *Transplantation* (2022) 106(10):1940–51. doi:10.1097/tp.0000000000004153
- Granger M, Stumpf J, Bond G, Görzer I, Springer DN, Kessel F, et al. Prediction of Humoral and Cellular Immune Response to COVID-19 mRNA Vaccination by TTV Load in Kidney Transplant Recipients and Hemodialysis Patients. *J Clin Virol* (2023) 162:105428. doi:10.1016/j.jcv.2023.105428
- Hauptenthal F, Rahn J, Maggi F, Gelas F, Bourgeois P, Hugo C, et al. A Multicentre, Patient- and Assessor-Blinded, Non-inferiority, Randomised and Controlled Phase II Trial to Compare Standard and Torque Teno virus-guided Immunosuppression in Kidney Transplant Recipients in the First Year After Transplantation: TTVguideIT. *Trials* (2023) 24(1):213. doi:10.1186/s13063-023-07216-0
- Brani P, Manzoer HZ, Spezia PG, Vigezzi A, Ietto G, Dalla Gasperina D, et al. Torque Teno Virus: Lights and Shades. *Viruses* (2025) 17(3):334. doi:10.3390/v17030334
- Spezia PG, Focosi D, Baj A, Novazzi F, Ferrante FD, Carletti F, et al. TTV and Other Anelloviruses: The Astonishingly Wide Spread of a Viral Infection. *Asp Mol Med* (2023) 1:100006. doi:10.1016/j.amolm.2023.100006
- Masset C, Danger R, Degauque N, Dantal J, Giral M, Brouard S. Blood Gene Signature as a Biomarker for Subclinical Kidney Allograft Rejection: Where Are We? *Transplantation* (2025) 109(2):249–58. doi:10.1097/tp.0000000000005105
- Budde K, Osmanodja B, Schrezenmeier E, Halleck F, Mayer KA, Böhmig GA, et al. Clinical Utility of Donor-Derived Cell-Free DNA Testing for Kidney Transplant Monitoring in Selected Patients. *Kidney Int* (2025) 107(5):792–6. doi:10.1016/j.kint.2024.09.023
- Park S, Sellares J, Tinel C, Anglicheau D, Bestard O, Friedewald JJ. European Society of Organ Transplantation Consensus Statement on Testing for Non-Invasive Diagnosis of Kidney Allograft Rejection. *Transpl Int* (2023) 36:12115. doi:10.3389/ti.2023.12115

17. Stringer D, Gardner L, Shaw O, Clarke B, Briggs D, Worthington J, et al. Optimized Immunosuppression to Prevent Graft Failure in Renal Transplant Recipients With HLA Antibodies (Outsmart): A Randomised Controlled Trial. *EClinicalMedicine* (2023) 56:101819. doi:10.1016/j.eclinm.2022.101819
18. Hirt-Minkowski P, Handschin J, Stampf S, Hopfer H, Menter T, Senn L, et al. Randomized Trial to Assess the Clinical Utility of Renal Allograft Monitoring by Urine CXCL10 Chemokine. *J Am Soc Nephrol* (2023) 34(8):1456–69. doi:10.1681/asn.0000000000000160
19. Akifova A, Budde K, Amann K, Buettner-Herold M, Choi M, Oellerich M, et al. Donor-Derived Cell-free DNA Monitoring for Early Diagnosis of Antibody-Mediated Rejection After Kidney Transplantation: A Randomized Trial. *Nephrol Dial Transpl* (2024) 40:1384–95. doi:10.1093/ndt/gfae282
20. Naesens M, Anglicheau D. Precision Transplant Medicine: Biomarkers to the Rescue. *J Am Soc Nephrol* (2018) 29(1):24–34. doi:10.1681/asn.2017010004
21. Ravaioli M, Neri F, Lazzarotto T, Bertuzzo VR, Di Gioia P, Stacchini G, et al. Immunosuppression Modifications Based on an Immune Response Assay: Results of a Randomized, Controlled Trial. *Transplantation* (2015) 99(8):1625–32. doi:10.1097/tp.0000000000000650
22. Ahlenstiel-Grunow T, Liu X, Schild R, Oh J, Taylan C, Weber LT, et al. Steering Transplant Immunosuppression by Measuring Virus-Specific T Cell Levels: The Randomized, Controlled IVIST Trial. *J Am Soc Nephrol* (2021) 32(2):502–16. doi:10.1681/asn.2020050645
23. Haloschan M, Bettesch R, Görzer I, Weseslindtner L, Kundi M, Puchhammer-Stöckl E. TTV DNA Plasma Load and Its Association with Age, Gender, and HCMV IgG Serostatus in Healthy Adults. *Age (Dordr)* (2014) 36(5):9716. doi:10.1007/s11357-014-9716-2
24. Focosi D, Spezia PG, Macera L, Salvadori S, Navarro D, Lanza M, et al. Assessment of Prevalence and Load of Torquetenovirus Viraemia in a Large Cohort of Healthy Blood Donors. *Clin Microbiol Infect* (2020) 26(10):1406–10. doi:10.1016/j.cmi.2020.01.011
25. Spandole S, Cimponeriu D, Berca LM, Mihăescu G. Human Anelloviruses: An Update of Molecular, Epidemiological and Clinical Aspects. *Arch Virol* (2015) 160(4):893–908. doi:10.1007/s00705-015-2363-9
26. Kaczorowska J, Cicilionytė A, Timmerman AL, Deijns M, Jebbink MF, van Goudoever JB, et al. Early-Life Colonization by Anelloviruses in Infants. *Viruses* (2022) 14(5):865. doi:10.3390/v14050865
27. Focosi D, Antonelli G, Pistello M, Maggi F. Torquetenovirus: The Human Virome From Bench to Bedside. *Clin Microbiol Infect* (2016) 22(7):589–93. doi:10.1016/j.cmi.2016.04.007
28. Davis JS, Chu G, Pathinayake P, Jones D, Giffard P, Macera L, et al. Seroprevalence of Torque Teno Virus in Hemodialysis and Renal Transplant Patients in Australia: A Cross-Sectional Study. *Transpl Infect Dis* (2020) 22(5):e13400. doi:10.1111/tid.13400
29. Touinssi M, Gallian P, Biagini P, Attoui H, Viallettes B, Berland Y, et al. TT Virus Infection: Prevalence of Elevated Viraemia and Arguments for the Immune Control of Viral Load. *J Clin Virol* (2001) 21(2):135–41. doi:10.1016/s1386-6532(01)00157-3
30. Shibayama T, Masuda G, Ajisawa A, Takahashi M, Nishizawa T, Tsuda F, et al. Inverse Relationship Between the Titre of TT Virus DNA and the CD4 Cell Count in Patients Infected with HIV. *Aids* (2001) 15(5):563–70. doi:10.1097/00002030-200103300-00004
31. Bendinelli M, Pistello M, Maggi F, Fornai C, Freer G, Vatteroni ML. Molecular Properties, Biology, and Clinical Implications of TT Virus, a Recently Identified Widespread Infectious Agent of Humans. *Clin Microbiol Rev* (2001) 14(1):98–113. doi:10.1128/cmr.14.1.98-113.2001
32. Moen EM, Sagedal S, Bjørø K, Degré M, Opstad PK, Grinde B. Effect of Immune Modulation on TT Virus (TTV) and TTV-Like-Mini-Virus (TLMV) Viremia. *J Med Virol* (2003) 70(1):177–82. doi:10.1002/jmv.10356
33. De VI, Khush KK, Strehl C, Kohli B, Luikart H, Neff NF, et al. Temporal Response of the Human Virome to Immunosuppression and Antiviral Therapy. *Cell* (2013) 155(5):1178–87. doi:10.1016/j.cell.2013.10.034
34. Görzer I, Haloschan M, Jaksch P, Klepetko W, Puchhammer-Stöckl E. Plasma DNA Levels of Torque Teno Virus and Immunosuppression After Lung Transplantation. *J Heart Lung Transpl* (2014) 33(3):320–3. doi:10.1016/j.healun.2013.12.007
35. Redondo N, Navarro D, Aguado JM, Fernández-Ruiz M. Viruses, Friends, and Foes: The Case of Torque Teno Virus and the Net State of Immunosuppression. *Transpl Infect Dis* (2022) 24(2):e13778. doi:10.1111/tid.13778
36. Kapps S, Haupenthal F, Bond G. Torque Teno Virus-guided Monitoring of Immunosuppressive Therapy. *Nephrol Dial Transpl* (2024) 39(12):1942–4. doi:10.1093/ndt/gfae149
37. Stone JH, Merkel PA, Spiera R, Seo P, Langford CA, Hoffman GS, et al. Rituximab Versus Cyclophosphamide for ANCA-Associated Vasculitis. *N Engl J Med* (2010) 363(3):221–32. doi:10.1056/NEJMoa0909905
38. Studenic P, Bond G, Kerschbaumer A, Bécède M, Pavelka K, Karateev D, et al. Torque Teno Virus Quantification for Monitoring of Immunomodulation with Biologic Compounds in the Treatment of Rheumatoid Arthritis. *Rheumatology (Oxford)* (2022) 61(7):2815–25. doi:10.1093/rheumatology/keab839
39. Feghoul L, Caillaud A, Peyrony O, Salmons M, Nere ML, Delaugerre C, et al. Respiratory Torque Teno Virus Load at Emergency Department Visit Predicts Intensive Care Unit Admission of SARS-CoV-2 Infected Patients. *J Med Virol* (2023) 95(12):e29319. doi:10.1002/jmv.29319
40. Tang JY, Chen TB, Kouznetsova VL, Tsigelny IF. Anelloviruses and Cancer. *J Infect Dis* (2025) 231(2):298–306. doi:10.1093/infdis/jiae626
41. Bartl T, Onoprienko A, Obermayr E, Zeilinger R, Concin N, Strassl R, et al. Pretherapeutic Torque Teno Virus (TTV) Counts Assessed by Liquid Biopsy as an Independent Prognostic Biomarker in Ovarian Cancer Patients. *Int J Gynecol Cancer* (2025) 35(2):101463. doi:10.1016/j.ijgc.2024.101463
42. Benzaquen A, Giménez E, Iacoboni G, Guerreiro M, Hernani R, Albert E, et al. Torque Teno Virus Plasma DNA Load: A Novel Prognostic Biomarker in CAR-T Therapy. *Bone Marrow Transpl* (2024) 59(1):93–100. doi:10.1038/s41409-023-02114-0
43. Truffot A, Cabezas L, Gnesotto M, Lupo J, Morand P, Jouve T, et al. Comparison of Torque Teno Virus DNA Load in Plasma and Whole Blood. *Kidney Int Rep* (2024) 10:2446–9. doi:10.1016/j.ekir.2025.04.033
44. Maggi F, Pifferi M, Fornai C, Andreoli E, Tempestini E, Vatteroni M, et al. TT Virus in the Nasal Secretions of Children with Acute Respiratory Diseases: Relations to Viremia and Disease Severity. *J Virol* (2003) 77(4):2418–25. doi:10.1128/jvi.77.4.2418-2425.2003
45. Macera L, Spezia PG, Medici C, Rofi E, Del Re M, Focosi D, et al. Comparative Evaluation of Molecular Methods for the Quantitative Measure of Torquetenovirus Viremia, the New Surrogate Marker of Immune Competence. *J Med Virol* (2022) 94(2):491–8. doi:10.1002/jmv.25488
46. Görzer I, Haupenthal F, Maggi F, Gelas F, Kulifaj D, Brossault L, et al. Validation of Plasma Torque Teno Viral Load Applying a CE-certified PCR for Risk Stratification of Rejection and Infection Post Kidney Transplantation. *J Clin Virol* (2023) 158:105348. doi:10.1016/j.jcv.2022.105348
47. Gore EJ, Gard L, Bourgeois P, Kulifaj D, McCulloch E, Spezia PG, et al. Validation, Implementation and Quality Control of a Torque Teno Virus qPCR in a Multinational Clinical Trial. *J Clin Virol* (2024) 175:105738. doi:10.1016/j.jcv.2024.105738
48. Spezia PG, Carletti F, Novazzi F, Specchiarello E, Genoni A, Drago Ferrante F, et al. Torquetenovirus Viremia Quantification Using Real-Time PCR Developed on a Fully Automated, Random-Access Platform. *Viruses* (2024) 16(6):963. doi:10.3390/v16060963
49. Doberer K, Schiemann M, Strassl R, Haupenthal F, Dermuth F, Görzer I, et al. Torque Teno Virus for Risk Stratification of Graft Rejection and Infection in Kidney Transplant recipients-A Prospective Observational Trial. *Am J Transpl* (2020) 20(8):2081–90. doi:10.1111/ajt.15810
50. Fernández-Ruiz M, Albert E, Giménez E, Ruiz-Merlò T, Parra P, López-Medrano F, et al. Monitoring of Alphatorquevirus DNA Levels for the Prediction of Immunosuppression-Related Complications After Kidney Transplantation. *Am J Transpl* (2019) 19(4):1139–49. doi:10.1111/ajt.15145
51. van Rijn AL, Roos R, Dekker FW, Rotmans JJ, Feltkamp M. Torque Teno Virus Load as Marker of Rejection and Infection in Solid Organ Transplantation - A Systematic Review and meta-analysis. *Rev Med Virol* (2023) 33(1):e2393. doi:10.1002/rmv.2393
52. Doberer K, Haupenthal F, Nackenhorst M, Bauernfeind F, Dermuth F, Eigenschink M, et al. Torque Teno Virus Load Is Associated With Subclinical Alloreactivity in Kidney Transplant Recipients: A Prospective Observational Trial. *Transplantation* (2021) 105(9):2112–8. doi:10.1097/tp.0000000000003619
53. Chauvelot L, Barba T, Saison C, Siska E, Kulifaj D, Bakker SJL, et al. Longitudinal Monitoring of Torque Teno Virus Dnaemia in Kidney Transplant Recipients Correlates With Long-Term Complications of Inadequate Immunosuppression. *J Med Virol* (2024) 96(7):e29806. doi:10.1002/jmv.29806

54. Schiemann M, Puchhammer-Stöckl E, Eskandary F, Kohlbeck P, Rasoul-Rockenschaub S, Heilos A, et al. Torque Teno Virus Load-Inverse Association with Antibody-Mediated Rejection After Kidney Transplantation. *Transplantation* (2017) 101(2):360–7. doi:10.1097/tp.0000000000001455
55. Eibensteiner F, Messner I, Uhl P, Bond G, Puchhammer-Stöckl E, Mueller-Sacherer T, et al. The Association of Torque Teno Viral Load with CMV and BKV Infection in Pediatric and Adolescent Kidney Transplant Patients. *J Clin Virol* (2024) 172:105673. doi:10.1016/j.jcv.2024.105673
56. Solis M, Benotmane I, Gallais F, Caillard S, Fafi-Kremer S. Torque Teno Virus Viral Load Predicts SARS-CoV-2 Vaccine Response in Kidney Transplant Recipients. *J Med Virol* (2023) 95(7):e28936. doi:10.1002/jmv.28936
57. Regele F, Hauptenthal F, Doberer K, Görzer I, Kapps S, Strassl R, et al. The Kinetics of Torque Teno Virus Plasma Load Following Calcineurin Inhibitor Dose Change in Kidney Transplant Recipients. *J Med Virol* (2024) 96(3):e29554. doi:10.1002/jmv.29554
58. Benning L, Reineke M, Bundschuh C, Klein JAF, Kühn T, Zeier M, et al. Quantification of Torque Teno Virus Load to Monitor Short-Term Changes in Immunosuppressive Therapy in Kidney Transplant Recipients. *Transplantation* (2023) 107(12):e363–e369. doi:10.1097/tp.0000000000004816
59. Regele F, Heinzel A, Hu K, Raab L, Eskandary F, Faé I, et al. Stopping of Mycophenolic Acid in Kidney Transplant Recipients for 2 Weeks Peri-Vaccination Does Not Increase Response to SARS-CoV-2 Vaccination-A Non-Randomized, Controlled Pilot Study. *Front Med (Lausanne)* (2022) 9:914424. doi:10.3389/fmed.2022.914424
60. Cabezas L, Truffot A, Germin R, Bugnazet M, Malvezzi P, Gnesotto M, et al. Evaluation of Torque Teno Virus DNA Load as a Predictive Biomarker in Kidney Transplant Recipients Converted from Calcineurin Inhibitors to Belatacept. *Kidney Int Rep* (2024) 9(6):1718–29. doi:10.1016/j.ekir.2024.03.006
61. Bredewold OW, Moest WT, de Fijter JW, Meijers E, Bruchfeld A, Skov K, et al. Attenuation of Torque Teno Viral Load Over Time in Kidney Transplantation Recipients Treated With Calcineurin Inhibitors Is Mitigated After Conversion to Belatacept. *J Med Virol* (2024) 96(9):e29905. doi:10.1002/jmv.29905
62. Herkner F, Hauptenthal F, Kapps S, Doberer K, Herkner H, König F, et al. Statistical analysis plan for TTVguideIT-a multicentre, patient- and assessor-blinded, non-inferiority, randomised and controlled phase II trial to compare standard and Torque Teno virus-guided immunosuppression in kidney transplant recipients in the first year after transplantation. *Trials*. (2025) 26: 432. doi:10.1186/s13063-025-09119-8
63. Herz CT, Kulterer OC, Kulifaj D, Gelas F, Franzke B, Hauptenthal F, et al. Obesity Is Associated with a Higher Torque Teno Viral Load Compared to Leanness. *Front Endocrinol (Lausanne)* (2022) 13:962090. doi:10.3389/fendo.2022.962090
64. Strassl R, Schiemann M, Doberer K, Görzer I, Puchhammer-Stöckl E, Eskandary F, et al. Quantification of Torque Teno Virus Viremia as a Prospective Biomarker for Infectious Disease in Kidney Allograft Recipients. *J Infect Dis* (2018) 218(8):1191–9. doi:10.1093/infdis/jiy306
65. Eskandary F, Bond G, Mohan K. Torque Teno Virus-Guided Immunosuppression in Kidney Transplantation: Expanding the Application. *Kidney Int Rep* (2024) 9(6):1568–70. doi:10.1016/j.ekir.2024.04.057
66. Eldar-Yedidia Y, Ben-Shalom E, Hillel M, Belostotsky R, Megged O, Freier-Dror Y, et al. Association of Post-Transplantation Anellovirus Viral Load With Kidney Transplant Rejection in Children. *Pediatr Nephrol* (2022) 37(8):1905–14. doi:10.1007/s00467-021-05336-w
67. Uhl P, Heilos A, Bond G, Meyer E, Böhm M, Puchhammer-Stöckl E, et al. Torque Teno Viral Load Reflects Immunosuppression in Paediatric Kidney-Transplanted Patients-a Pilot Study. *Pediatr Nephrol* (2021) 36(1):153–62. doi:10.1007/s00467-020-04606-3
68. Väisänen E, Kuisma I, Mäkinen M, Ilonen J, Veijola R, Toppari J, et al. Torque Teno Virus Primary Infection Kinetics in Early Childhood. *Viruses* (2022) 14(6):1277. doi:10.3390/v14061277
69. Schrezenmeier E, Rincon-Arevalo H, Jens A, Stefanski AL, Hammett C, Osmanodja B, et al. Temporary Antimetabolite Treatment Hold Boosts SARS-CoV-2 Vaccination-Specific Humoral and Cellular Immunity in Kidney Transplant Recipients. *JCI Insight* (2022) 7(9):e157836. doi:10.1172/jci.insight.157836
70. Cinti L, Spezia PG, Roberto P, Russo G, Lai Q, Carillo C, et al. Assessment of Torquetenominivirus (TTMV) and Torquetenomidivirus (TTMDV) as Complementary Biomarkers to Torquetenovirus (TTV). *Int J Mol Sci* (2025) 26(3):1022. doi:10.3390/ijms26031022
71. Maggi F, Focosi D, Albani M, Lanini L, Vatteroni ML, Petrini M, et al. Role of Hematopoietic Cells in the Maintenance of Chronic Human Torquetenovirus Plasma Viremia. *J Virol* (2010) 84(13):6891–3. doi:10.1128/jvi.00273-10
72. Albert E, Solano C, Pascual T, Torres I, Macera L, Focosi D, et al. Dynamics of Torque Teno Virus Plasma Dnaemia in Allogeneic Stem Cell Transplant Recipients. *J Clin Virol* (2017) 94:22–8. doi:10.1016/j.jcv.2017.07.001
73. Focosi D, Macera L, Boggi U, Nelli LC, Maggi F. Short-Term Kinetics of Torque Teno Virus Viraemia After Induction Immunosuppression Confirm T Lymphocytes as the Main Replication-Competent Cells. *J Gen Virol* (2015) 96(Pt 1):115–7. doi:10.1099/vir.0.070094-0

Copyright © 2025 Doberer, Kapps, Hauptenthal and Bond. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.



The UK National Appeals Panel Safely Extends Access to Liver Transplantation for Candidates Beyond Standard Listing Criteria

Abdul Rahman Hakeem^{1*}, Sahil Gupta¹, Rhiannon Taylor², Tassos Grammatikopoulos^{1,3}, Steven Masson⁴, Raj Prasad⁵, Doug Thorburn⁶, Krishna Menon¹, Derek Manas² and Varuna Aluvihare¹

¹Institute of Liver Studies, King's College Hospital NHS Foundation Trust, London, United Kingdom, ²NHS Blood and Transplant, Bristol, United Kingdom, ³Paediatric Liver, GI and Nutrition Centre, King's College Hospital NHS Foundation Trust, London, United Kingdom, ⁴Liver Unit, Newcastle NIHR Biomedical Research Centre, Newcastle Upon Tyne Hospitals NHS Foundation Trust and Translational and Clinical Research Institute, Faculty of Medical Sciences, Newcastle University, Newcastle upon Tyne, United Kingdom, ⁵Leeds Teaching Hospitals NHS Trust, Leeds, United Kingdom, ⁶Royal Free Hospital NHS Foundation Trust, London, United Kingdom

OPEN ACCESS

*Correspondence

Abdul Rahman Hakeem,
✉ abdul.hakeem1@nhs.net

Received: 11 September 2025

Revised: 11 December 2025

Accepted: 06 January 2026

Published: 27 January 2026

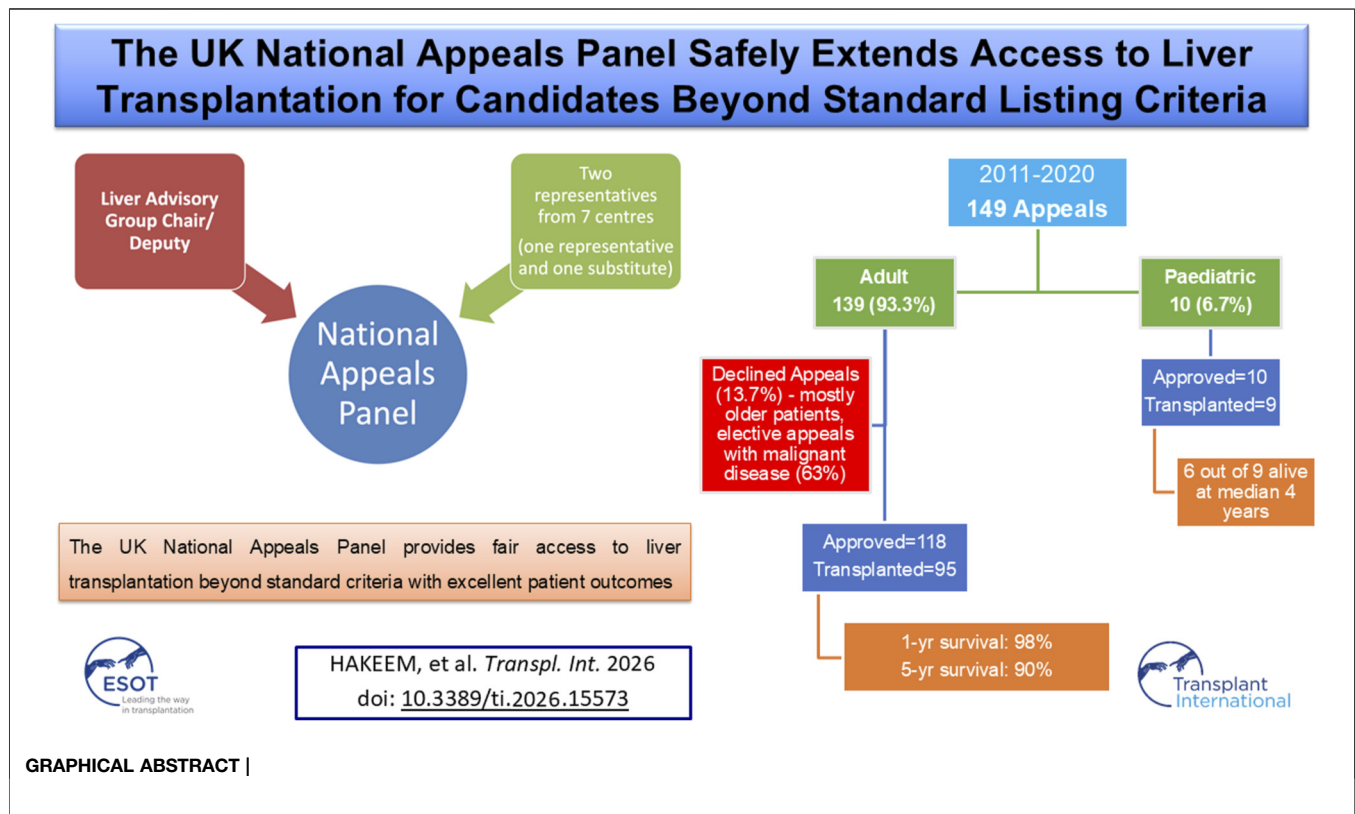
Citation:

Hakeem AR, Gupta S, Taylor R, Grammatikopoulos T, Masson S, Prasad R, Thorburn D, Menon K, Manas D and Aluvihare V (2026) The UK National Appeals Panel Safely Extends Access to Liver Transplantation for Candidates Beyond Standard Listing Criteria. *Transpl. Int.* 39:15573. doi: 10.3389/ti.2026.15573

Liver transplantation (LT) is the definitive treatment for selected acute and chronic liver diseases, yet standard national listing criteria do not encompass all clinical situations. To address this, the United Kingdom (UK) established the National Appeals Panel (NAP) in 2011 to review exceptional cases, aiming to ensure equitable access while safeguarding allocation of scarce donor organs. We conducted a retrospective analysis of all appeals submitted to the NAP between 2011 and 2020. 149 appeals were received: 139 (93.3%) adults and 10 (6.7%) paediatric patients. Overall, 128 (85.9%) appeals were approved, 19 (12.8%) declined, and 2 (1.3%) withdrawn. Approval was more frequent for adult super-urgent than elective requests (92.9% vs. 79.5%). Of 118 approved adults, 95 (80.5%) underwent LT, while 23 (19.5%) did not, most often due to deterioration on the waiting list. Transplanted adults included 46.3% super-urgent cases, with 20% ventilated and 25.3% on renal replacement therapy, yet achieved excellent outcomes with 98% one-year and 90% five-year survival. All 10 paediatric appeals were approved, with one child dying on the list and nine transplanted. Declined appeals mainly involved older patients with malignant indications. This review highlights the NAP's role in expanding LT access while ensuring equity and governance.

Keywords: declines, indications, liver transplant, national appeals panel, outcomes

Abbreviations: ACLF, Acute-on-chronic liver failure; ALF, Acute liver failure; AIH, Autoimmune hepatitis; ArLD, Alcohol-related liver disease; BMI, Body mass index; CCA, Cholangiocarcinoma; CLD, Chronic liver disease; DBD, Donation after brain death; DCD, Donation after circulatory death; GVHD, Graft-versus-host disease; HAT, Hepatic artery thrombosis; HCC, Hepatocellular carcinoma; IQR, Interquartile range; LAG, Liver Advisory Group; LDLT, Living donor liver transplantation; LT, Liver transplantation; MDT, Multidisciplinary team; NAP, National Appeals Panel; NET, Neuroendocrine tumour; NHSBT, NHS Blood and Transplant; NLRB, National Liver Review Board; NRP, Normothermic regional perfusion; QoL, Quality of life; RRT, Renal replacement therapy; SPSS, Statistical Package for the Social Sciences; SU, Super-urgent; UK, United Kingdom; UKELD, United Kingdom Model for End-stage Liver Disease.



INTRODUCTION

Liver transplantation (LT) remains the definitive treatment for selected acute and chronic liver diseases, offering improved survival and quality of life (QoL) [1]. Given the ongoing shortage of donor organs, patient selection must be based on transparent and equitable criteria to ensure fair allocation [2, 3]. In the United Kingdom (UK), the Liver Advisory Group (LAG), under NHS Blood and Transplant (NHSBT), defines standard listing criteria across four categories: acute liver failure (ALF), chronic liver disease (CLD), variant syndromes, and primary liver cancer. These aim to prioritise patients with the greatest need and likelihood of benefit [4].

However, some patients with urgent or complex presentations fall outside these criteria and are ineligible for routine listing [5, 6]. To address this, the UK established the National Appeals Panel (NAP) in January 2011, a multidisciplinary body that reviews appeals for such exceptional cases [7]. Through structured, case-by-case deliberation, the NAP ensures LT is not denied to individuals with rare but legitimate indications. This centralised process promotes consistency, accountability, and fairness in exception handling [8].

Despite the NAP's critical role in the UK transplant framework, limited evidence exists on its outcomes, particularly long-term graft and patient survival following successful appeals. Similarly, the characteristics and outcomes of declined cases or those not proceeding to transplant despite

approval are poorly described. Understanding these patterns is vital to evaluate the NAP's effectiveness and inform future revisions to national policy [8].

This retrospective study evaluates all appeals submitted to the NAP, analysing case characteristics, approval outcomes, transplant rates, and post-transplant patient and graft survival. We also explore reasons for declined appeals and recurring themes that may guide future policy development.

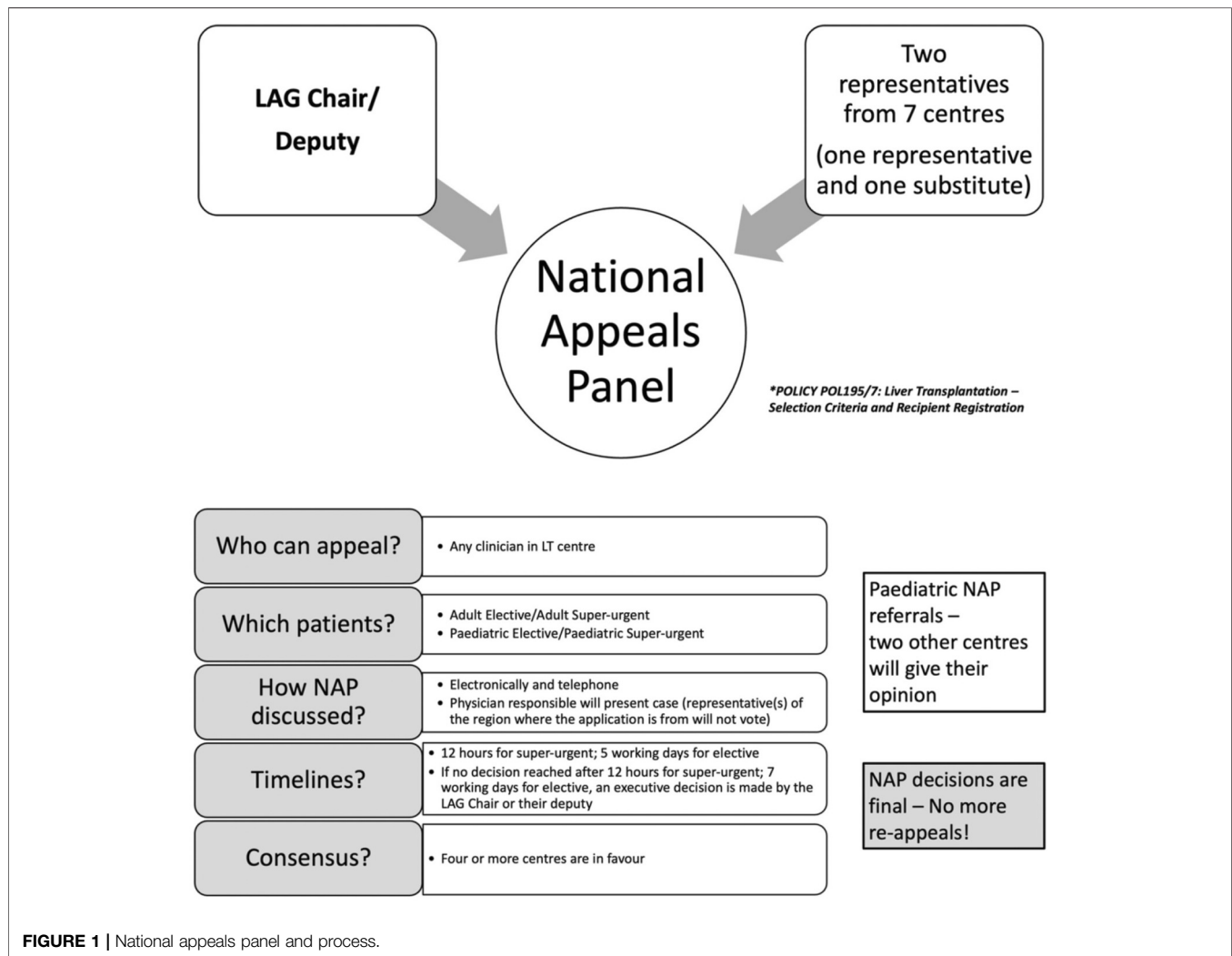
MATERIALS AND METHODS

Study Design and Data Source

This retrospective study included all appeals submitted to the UK NAP between January 2011 and December 2020 for adult and paediatric LTs. Clinicians from the seven UK LT centres submitted appeals using a standardised proforma (NHSBT's formal application form) for patients who did not meet the established national listing criteria, as outlined in Policy POL195/7 [6]. Data were extracted from the NHSBT database.

Structure and Function of the National Appeals Panel

The NAP, established by the LAG, provides a centralised and transparent mechanism for evaluating exceptional transplant candidates. The panel comprises an independent, non-voting



Chair (LAG Chair or their deputy) and two nominated representatives from each of the seven UK liver transplant centres, with one vote per centre (Figure 1). The submitting centre may not vote. Appeals are approved if four or more centres vote in favour.

The NAP may place a candidate on the transplant list if they do not meet any of the current criteria and, on the evidence provided to them, if one or more of the following conditions are met: (1) >50% probability of 5-year survival with acceptable QoL post-transplant, and (2) >9% 1-year mortality from liver disease without transplantation, or (3) poor QoL despite maximal therapy, expected to improve with LT. These thresholds follow UK national policy (Liver Advisory Group Policy POL195/7), which is based on long-term outcome modelling, published prognostic evidence, and expert consensus. Individual probabilities are estimated by each transplant centre using MDT assessment, disease-specific prognostic tools where available, and published survival data for complex indications [6]. Hepatocellular carcinoma (HCC) cases outside size/number limits may be considered if tumour biology supports favourable

outcomes. Paediatric cases require favourable responses from the two other paediatric transplant centres not involved in the patient's care.

Appeals Process

Appeals are submitted to the LAG Chair, who pre-screens for eligibility. Documentation is circulated electronically, and deliberations occur via email or telephone. For elective cases, decisions are issued within five working days. If no consensus is reached within 7 days, the Chair may make an executive decision. Outcomes include approved or declined or withdrawn, and are communicated to the referring centre. Re-submission is allowed only if new information emerges.

For super-urgent cases, centres submit to the Chair, who assesses validity. If approved, details are forwarded to the central NHSBT Hub Operations, who circulate the appeal to all centres. Responses are required within 12 h. Approval is granted with four affirmative votes or if no more than two centres object. Hub Operations then registers the patient as a super-urgent recipient [6] (Figure 1).

Patients approved under the NAP for high-risk or exceptional indications are listed within the UK national allocation system. Approval does not confer automatic prioritisation; patients remain subject to standard national allocation rules, including blood group and size matching. Centre-specific listing caps exist to prevent disproportionate allocation to any single centre, with oversight by national transplant authorities. Super-urgent cases are designated according to nationally agreed criteria and are offered organs on a priority basis via a national call system. This process ensures equitable organ allocation while allowing timely transplantation for patients at imminent risk.

Data Collection and Analysis

All consecutive NAP referrals were included. Extracted variables included patient demographics (age, gender, body mass index), clinical data (diagnosis, UK end-stage liver disease score, referring centre), and appeal-specific details (indication, outcome, rejection reason, transplant status). For patients who underwent transplantation, data on 90-day graft and patient survival, retransplantation, and complications (e.g., vascular and biliary complications, sepsis) were recorded. Narrative appeal texts were qualitatively reviewed to identify recurring themes.

Descriptive statistics were used to summarise patient demographics, clinical features, and outcomes. Continuous variables are reported as medians with interquartile ranges (IQRs) or means with standard deviation, depending on distribution. Categorical variables are presented as frequencies and percentages. Group comparisons were performed using chi-square or Fisher's exact test for categorical data, and t-test or Mann-Whitney U test for continuous data, as appropriate. Survival outcomes were analysed using the Kaplan-Meier methods. Survival time was calculated from the date of transplantation to either death or graft loss, with censoring at last clinic follow-up. The number of patients at risk at each time point is displayed below the Kaplan-Meier plot. All analyses were conducted using IBM SPSS Statistics for Windows, Version 27.0 (IBM Corp., Armonk, NY, USA). Narrative free-text appeal records were qualitatively reviewed to identify recurring clinical and ethical themes, particularly among declined or withdrawn appeals.

Ethical Considerations

The study was approved by NHSBT as part of its quality assurance programme. Data were extracted from the NHSBT registry in anonymised form, and no identifiable patient information was accessed. Formal research ethics committee approval was therefore not required, as per UK governance guidelines for registry-based service evaluations.

RESULTS

Appeals to the National Appeals Panel

Between January 2011 and December 2020, a total of 149 appeals were submitted to the NAP, of which 139 (93.3%) were for adult

TABLE 1 | Referrals to National Appeals Panel and their Outcomes.

Characteristic	N (%)
Total number of appeals from January 2011 to December 2020	149
Age group	
Adult patient appeals	139 (93.3%)
Paediatric patient appeals	10 (6.7%)
Type of appeals	
Adult super-urgent	56 out of 139 (40.3%)
Adult elective	83 out of 139 (59.7%)
Paediatric super-urgent	4 out of 10 (40.0%)
Paediatric elective	6 out of 10 (60.0%)
Outcomes of adult patient appeals	
Approved	118 (85.0%)
Declined	19 (13.7%)
Withdrawn	2 (1.4%)
Outcomes of paediatric patient appeals	
Approved	10 (100%)
Declined	0
Withdrawn	0
Success percentage based on urgency of appeals	
Adult super-urgent	92.9% (52 out of 56)
Adult elective	79.5% (66 out of 83)

patients and 10 (6.7%) for paediatric patients. Among adults, 56 appeals (40.3%) were super-urgent and 83 (59.7%) elective, while in paediatric patients 3 (30.0%) were super-urgent and 7 (70.0%) elective. Overall, 118 adult appeals (85.0%) were approved, 19 (13.7%) declined, and 2 (1.4%) withdrawn, whereas all 10 paediatric appeals (100%) were approved. Success rates were higher for super-urgent than elective appeals, with 92.9% (52/56) of adult super-urgent requests approved compared with 79.5% (66/83) of adult elective requests (Table 1).

Outcomes Following Approval From NAP for Adult Patients

Of 118 adult patients approved by the NAP, 95 (80.5%) underwent transplantation, while 23 (19.5%) did not proceed. The main reason was clinical deterioration after wait-listing (10 patients, 43.5%). Two patients (8.7%) died before an organ became available, and two (8.7%) were removed due to clinical improvement. In 115 approved adult cases, the decision was consistent with the urgency of the original request: super-urgent requests led to super-urgent listings, and elective requests to elective listings. Exceptions included one super-urgent request approved as elective, one variant syndrome listed through the CLD pathway, and one patient already eligible under standard criteria who was registered routinely. Another case involved a simultaneous liver-lung transplant for cystic fibrosis with liver disease. Although the UKELD score was <49, the presence of portal hypertension with varices supported the appeal, and the patient underwent elective transplantation (Table 1).

TABLE 2 | Demographics of adult patients transplanted after successful appeal.

Characteristic	N = 95
Recipient age (years) ^a	39 (27–53)
Recipient gender (female)	51 (53.7%)
Recipient BMI (kg/m ²) ^a	24 (21–28)
Recipient UK end-stage liver disease score (UKELD) ^a	50 (46–60)
Recipient blood group	
O group	46 (48.4%)
A group	35 (36.8%)
B group	12 (12.6%)
AB group	2 (2.1%)
Indications for transplant	
Post-liver transplant complications	20 (21.1%)
Cholestatic liver disease	20 (21.1%)
Acute liver failure	13 (13.7%)
Liver cancer	4 (4.2%)
Metabolic-dysfunction associated liver disease (MASLD)	5 (5.3%)
Metabolic diseases (Glycogen storage diseases, etc)	5 (5.3%)
Alcohol-related liver disease (ArLD)	1 (1.1%)
Chronic rejection	3 (3.1%)
Others	18 (18.8%)
Unknown	6 (6.3%)
Previous transplants	
1st transplant	75 (78.9%)
2nd transplant	18 (18.9%)
3rd transplant	1 (1.1%)
4th transplant	1 (1.1%)

^aMedian (inter-quartile range).

Demographics and Characteristics of Adult Patients Transplanted After Successful Appeal

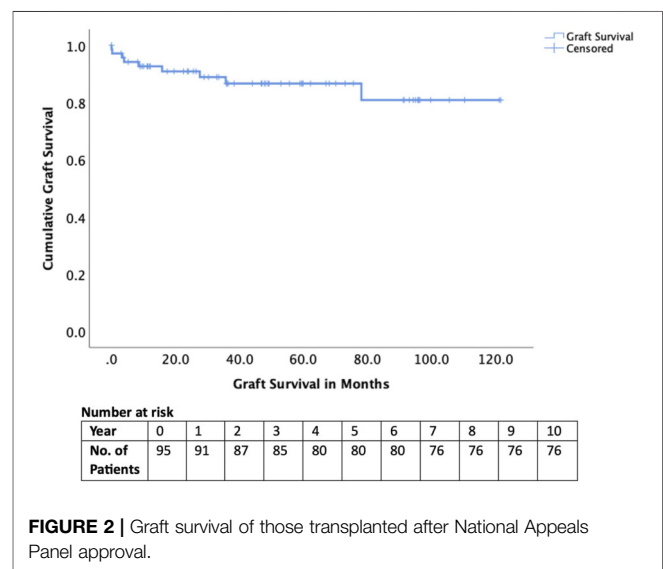
For those transplanted, the median age at listing was 39 years (IQR 27–53); 53.7% were female and 48.4% were blood group O. Median UKELD at referral was 50 (IQR 46–60). The most frequent indications were post-transplant complications (21.1%), cholestatic liver disease (21.1%), and ALF (13.7%). Of the 95 transplanted adults, 78.9% were first grafts and 21.1% regrafts, with 46.3% performed under super-urgent status. At the time of transplant, 20.0% were ventilated and 25.3% required renal replacement therapy (RRT). Most donor grafts were from donation after brain death (DBD; 93.7%), with 5.3% from donation after circulatory death (DCD) and one (1.1%) living donor transplant (Table 2).

Post-Transplant Complications and Outcomes for Adult Recipients

Early complications included hepatic artery thrombosis in four cases (4.2%), biliary complications in four cases (4.2%), and sepsis in 23 cases (24.1%). Two recipients (2.1%) experienced graft loss within 90 days, and one patient (1.1%) died within 90 days (Table 3). Long-term outcomes were excellent, with graft survival of 95% at 1 year and 85% at 5 years (Figure 2). Patient survival was 98% at 1 year and 90% at 5 years (Figure 3).

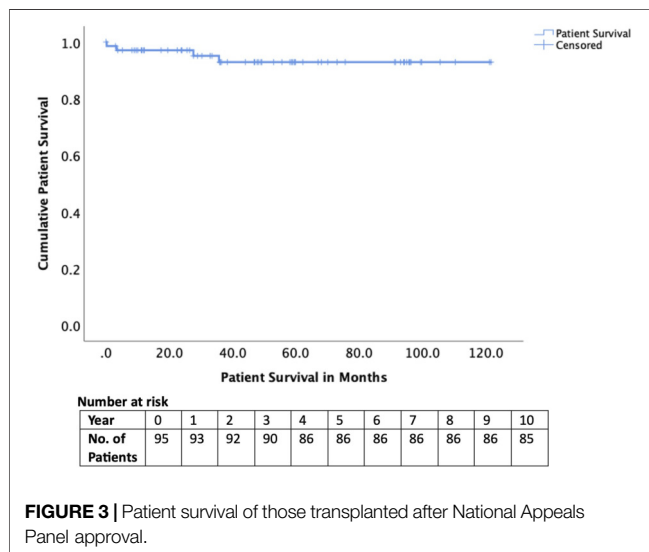
TABLE 3 | Graft and patient outcomes of adult patients transplanted after successful appeal.

Characteristic	N = 95
Urgency of transplant	
Super-urgent	44 (46.3%)
Ventilated at the time of transplant	19 (20.0%)
Renal support at the time of transplant	24 (25.3%)
Type of graft	
Donation after brain death (DBD) graft	89 (93.7%)
Donation after cardiac death (DCD) graft	5 (5.3%)
Living-donor liver transplant (LDLT) graft	1 (1.1%)
Complications post-transplant (90-day)	
Vascular complications	4 (4.2%) – all hepatic artery thrombosis
Biliary complications	4 (4.2%)
Sepsis	23 (24.2%)
Graft loss (90-day)	2 (2.1%)
Patient death (90-day)	1 (1.1%)
Graft survival	
1-year	95%
5-year	85%
Patient survival	
1-year	98%
5-year	90%

**FIGURE 2 |** Graft survival of those transplanted after National Appeals Panel approval.

Approved but Not Transplanted in the Adult Cohort

Among the 23 patients approved but not transplanted, the median age was slightly higher than the transplanted group (42 vs. 38 years; $p = 0.080$). A smaller proportion were female (43.5% vs. 53.7%; $p = 0.520$) and a greater proportion had blood group O (60.9% vs. 48.6%; $p = 0.400$). The urgency distribution was similar (super-urgent 47.8% vs. 46.3%; elective 52.2% vs. 53.7%; $p = 1.000$). Indication profiles were broadly comparable, though with some differences which were not statistically significant: post-transplant complications (30.4% vs. 21.1%; $p = 0.490$), cholestatic disease (8.7% vs. 21.1%; $p = 0.290$),



acute liver failure (8.7% vs. 13.7%; $p = 0.770$), metabolic/variant syndromes (34.8% vs. 33.7%; $p = 1.000$), and hepatocellular carcinoma/adenoma with favourable biology (17.4% vs. 10.5%; $p = 0.580$).

Outcomes in the non-transplanted group showed high attrition: 43.5% were removed for clinical deterioration, 8.7% removed for clinical improvement, 8.7% removed for unspecified reasons, and 8.7% died on the waiting list. At study closure, 30.4% had outcome not known (Table 4).

Declined Appeals in Adult Cohort

A total of 19 adult appeals (13.7%) were declined by the NAP. The majority of these involved unfavourable tumour biology, most commonly recurrent or advanced hepatocellular carcinoma, metastatic neuroendocrine tumours, or cholangiocarcinoma. Other reasons for decline included concerns about poor expected survival or situations where outcomes reported in the literature suggested futility (atypical ALF, graft-versus-host-disease). Compared with those approved, declined adult appeals represented an older population with a median age of 47 years ($p = 0.059$), were almost exclusively elective (89.5%; $p = 0.006$), and were dominated by malignant disease (63.0%; $p < 0.001$) (Table 5; Supplementary Table S1).

Withdrawn Appeals in Adult Cohort

Two appeals were withdrawn during the study period. One super-urgent appeal was withdrawn after donor pancreas histology, initially suspicious for malignancy, was confirmed benign on final pathology, negating the need for retransplantation. One was an elective referral for ArLD with decompensation, which was withdrawn by the referring centre prior to panel decision.

Paediatric Appeals

A total of 10 paediatric appeals were submitted to the NAP during the study period, and all were approved. Four were super-urgent appeals (40%), one elective with priority, and five were elective listings. The children ranged in age from

1 month to 17 years (median 11 years). Indications for transplantation included malignancy (3; hepatoblastoma, HCC, and undifferentiated sarcoma), acute liver failure (2; autoimmune and post-LT complications), metabolic liver disease (2; MSUD and OTC deficiency), sickle cell hepatopathy (2), and biliary atresia with decompensation (1). Of the 10 children, 9 underwent transplantation (all with DBD grafts), while one child with sickle cell hepatopathy died on the waiting list. Three deaths occurred following transplantation: two were due to disease relapse in the graft (sarcoma and autoimmune hepatitis with acute liver failure) and one with functioning graft (hepatoblastoma). At last follow-up, six children remain alive, with follow-up ranging from just under 2 years to more than 6 years post-transplant (Supplementary Table S2).

DISCUSSION

The present study provides the first comprehensive national analysis of the UK National Appeals Panel (NAP) for LT, covering a decade of experience since its inception in 2011. Our findings demonstrate that the NAP fulfils its intended purpose: extending access to transplantation for patients who fall outside conventional criteria while maintaining excellent graft and patient outcomes. The high approval rate, particularly for super-urgent appeals, alongside the outstanding post-transplant survival, highlights the effectiveness of this national mechanism in balancing equity, clinical benefit, and stewardship of scarce donor organs.

For context, national NHSBT outcome data from August 2025 report 1- and 5-year adult patient survival rates of approximately 95% and 83% for elective, first deceased-donor LTs performed for standard indications (noting that national figures include a small number of NAP cases) [9]. In comparison, NAP-approved patients in our cohort achieved excellent outcomes, with 1- and 5-year graft survival of 98% and 90%, respectively. These findings indicate that carefully selected exceptional-case candidates can achieve outcomes comparable to, and in some instances exceeding, national benchmarks despite falling outside conventional listing criteria.

Across the study period, nearly nine out of ten appeals were approved, reflecting effective triage at the time of submission and suggesting that centres use the NAP judiciously for well-selected patients. Approval was notably higher for super-urgent appeals (92.9%) compared with elective cases (79.5%). This difference aligns with the strict, nationally defined criteria governing super-urgent listing in the UK. According to NHSBT Policy POL195/7, super-urgent status is limited to patients with fulminant hepatic failure or rapidly progressive early graft failure, where death is expected within hours to days without transplantation [6]. Such cases undergo objective assessment against mandated biochemical and clinical thresholds and are used sparingly by centres for the most compelling, time-critical situations. The higher approval rate thus reflects the uniformly high mortality risk and stringent eligibility criteria, rather than any bias related to appeal status. NAP decisions for these cases remain based on established national criteria and evaluation of expected post-

TABLE 4 | Demographic and clinical characteristics of approved transplanted vs. approved not transplanted (died, removed from waiting list, suspended) adult patients.

Characteristic	Approved and transplanted (n = 95)	Approved but not transplanted (n = 23)	P value
Median age (years)	38 (IQR 26–52)	42 (IQR 29–55)	0.080
Gender (female)	51 (53.7%)	10 (43.5%)	0.520
Blood group O	46 (48.6%)	14 (60.9%)	0.400
Urgency at appeal	Super-urgent: 44 (46.3%) Elective: 51 (53.7%)	Super-urgent: 11 (47.8%) Elective: 12 (52.2%)	1.000
UKELD at referral (median, IQR)	51 (47–61)	48 (45–58)	0.699
Main indication categories	Post-LT complications: 20 (21.1%) Cholestatic: 20 (21.1%) ALF: 13 (13.7%) Other metabolic/variant: 32 (33.7%) HCC (favourable biology): 10 (10.5%) Transplanted: 95 (100%)	Post-LT complications: 7 (30.4%) Cholestatic: 2 (8.7%) ALF: 2 (8.7%) Other metabolic/variant: 8 (34.8%) HCC/adenoma (favourable biology): 4 (17.4%) Died on waiting list: 2 (8.7%) Removed (clinical deterioration): 10 (43.5%) Removed (unspecified): 2 (8.7%) Removed clinical (improvement): 2 (8.7%) Outcome not known: 7 (30.4%)	0.490 0.290 0.770 1.000 0.580 n/a

Abbreviations: ALF, acute liver failure; HCC, hepatocellular carcinoma; IQR, interquartile range; LT, liver transplantation; n, number; UKELD, UK end-stage liver disease score.

TABLE 5 | Demographic and clinical characteristics of approved vs. declined appeals in adult cohort.

Characteristic	Approved adult appeals (n = 118)	Declined adult appeals (n = 19)	P value
Median age (years)	39 (IQR 27–53)	47 (IQR 35–61)	0.059
Gender (female)	61 (51.4%)	8 (42.1%)	0.600
Blood group			n/a
O group	62 (53.4%)	Not available	
A group	35 (30.2%)	Not available	
B group	16 (13.8%)	Not available	
AB group	3 (2.6%)	Not available	
Urgency			0.006
Super-urgent	56 (47.5%)	2 (10.5%)	
Elective	62 (52.5%)	17 (89.5%)	
Indications – malignant	3 (2.5%) (HCC with favourable biology)	12 (63.0%) (HCC, recurrent HCC, CCA, NET)	<0.001
Indications – non-malignant	115 (97.5%) (ALF, cholestatic, metabolic, graft failure, etc.)	7 (37.0%) (ALF atypical, severe alcoholic hepatitis, AIH, GVHD, chronic rejection)	<0.001
Transplanted following appeal	95 (80.5%)	0	n/a
Waiting list outcome (non-Tx)	Died on list: 2 (8.7%) Removed: 14 (11.9%) Outcome not known: 7 (5.9%)	Not listed	n/a
1-year survival (if transplanted)	98%	n/a	n/a
5-year survival (if transplanted)	90%	n/a	n/a

Abbreviations: AIH, autoimmune hepatitis; ALF, acute liver failure; CCA, cholangiocarcinoma; GVHD, graft-versus-host disease; HCC, hepatocellular carcinoma; IQR, interquartile range; NET, neuroendocrine tumour; n/a, not applicable; n, number; Tx, transplantation.

The bold values demonstrate $P < 0.05$, which is statistically significant.

transplant benefit. In contrast, elective appeals involve more heterogeneous conditions with less predictable trajectories, prompting a more cautious appraisal by the panel [10, 11]. This distinction demonstrates a robust system in which both urgency and expected survival inform fair decision-making [12].

Patients approved and transplanted following appeal represented a broad spectrum of diagnoses, including post-transplant complications (21.1%), cholestatic liver disease (21.1%), acute liver failure (13.7%), and metabolic/variant conditions (33.7%). Notably, only a minority (10.5%) involved HCC with favourable biology [13, 14]. These patterns highlight the important role of the NAP in addressing rare, atypical, or post-transplant scenarios that are excluded from standard criteria

but nonetheless compatible with good outcomes. The inclusion of young patients with severe metabolic or vascular syndromes is particularly striking, as these groups often face devastating morbidity without transplantation but would not otherwise qualify under fixed listing criteria [15].

Despite the clinical severity of many cases, nearly half transplanted under super-urgent status, with 20.0% ventilated and 25.3% on renal support at the time of surgery, outcomes were excellent. Graft survival reached 95% at 1 year and 85% at 5 years, while patient survival was 98% and 90% respectively. These results are at least equivalent to, and in some instances superior to, national outcomes for standard indications, suggesting that NAP approval

successfully selects patients who are both high-need and high-benefit [16, 17]. The relatively low rates of vascular (4.2%) and biliary (4.2%) complications reinforce the appropriateness of these transplants, even under urgent or complex circumstances.

A substantial minority of approved patients (19.5%) did not undergo transplantation, most often because of clinical deterioration on the waiting list (43.5% of non-transplanted approvals). Others were removed for improvement, or unspecified reasons. This highlights the ongoing challenge of organ scarcity: even when approval is granted, timely transplantation is not guaranteed. Median UKELD was slightly lower among those not transplanted (48 vs. 51), but rates of malignancy indications were higher (17.4% vs. 10.5%). This pattern suggests that patients with cancer, despite favourable biology, may face greater difficulty securing an appropriate graft before disease progression [18, 19]. Strategies to optimise organ utilisation, including broader use of DCD with or without NRP, machine perfused marginal grafts, and LDLT, may help reduce waiting list mortality in this high-risk group [20–22].

The NAP declined 19 adult appeals (13.7%), the majority (63.0%) involving unfavourable oncological profiles such as recurrent or advanced HCC, cholangiocarcinoma (CCA), or metastatic neuroendocrine tumours (NET). These decisions align with international consensus that adverse tumour biology predicts poor post-transplant survival and unacceptable recurrence rates [23, 24]. A smaller number of non-malignant cases, such as severe alcoholic hepatitis, vanishing bile duct syndrome, and atypical ALF were declined due to uncertain benefit or lack of supporting precedent [25–27]. Although difficult, these rejections reflect the panel's essential gatekeeping role in protecting scarce organs from being used where futility or poor outcomes are likely. Importantly, the process provides transparency and consistency across the UK, avoiding *ad hoc* or centre-specific variation that could undermine equity [28, 29].

Transplant oncology is an evolving field, with expanding indications for LT in select high-risk malignancies, including unresectable colorectal liver metastases (CRLM), NETs and CCAs [30]. Recent studies have demonstrated that, in carefully selected patients, LT can offer survival benefits in these groups, leading to a cautious expansion of transplant criteria. These developments have raised complex ethical considerations regarding organ allocation, balancing potential survival benefits against scarcity of donor organs [31]. Within this context, the NAP has historically adopted a cautious approach to high-risk oncologic indications, reflecting the evolving evidence base and ethical deliberations. Our dataset captures this gradual adoption, highlighting how the appeals process accommodated these nuanced, high-risk cases during the study period, and providing insight into real-world clinical decision-making in transplant oncology.

The UK NAP shares similarities with the U.S. National Liver Review Board (NLRB) and comparable European mechanisms for exceptional case review [32, 33]. However, unlike regional or institutional committees operating in many European countries, the NAP represents the only nationally centralised, multidisciplinary structure with standardised governance and voting across all UK LT centres. Comparable frameworks

include the European Reference Network for Rare Liver Diseases (ERN-RARE-LIVER) and other regional appeal systems [34, 35].

Recent U.S. experience with the transition from regional review boards to a single NLRB has demonstrated reduced adjudication times, comparable waitlist outcomes between patients with and without exception scores, and greater process efficiency and equity, while maintaining rigorous scrutiny of exception requests [32]. Whereas many countries still rely on regional or centre-level discretion [34, 35], the UK ensures national consistency by requiring case triage by the Chair and then approval from at least four of seven transplant centres for each case. Our findings suggest this design achieves an appropriate balance: high approval rates for genuine exceptional need, but regular and principled rejections for cases with poor prognosis. This may serve as a model for other countries seeking to harmonise fairness with flexibility in organ allocation and to promote international standardisation of extended-criteria listing processes [36, 37].

Repeated successful appeals for certain conditions, such as acute intermittent porphyria, metabolic syndromes, and selected post-transplant complications, does raise the question of whether these should be incorporated into routine national listing criteria [38, 39]. Doing so would reduce the burden of appeals, expedite access for patients, and provide greater clarity for clinicians. Similarly, closer integration of tumour biology markers into listing criteria for HCC may refine the balance between excluding poor-risk cases and enabling access for those with favourable profiles beyond size/number thresholds [40].

The outcomes of paediatric appeals to the NAP demonstrate the effectiveness of the process, with all appeals approved and nine of ten children successfully proceeding to transplantation. This highlights that once approval was granted, access to transplantation was generally achieved, even in complex and high-risk cases. Importantly, six children remain alive with good medium- to long-term outcomes, including several beyond 5 years post-transplant, reflecting the durability of graft function in survivors. The spectrum of indications ranging from malignancy and acute liver failure to metabolic and haematological disorders, illustrates the capacity of the system to support the diversity in paediatric liver conditions, including urgent and super-urgent cases. These results underscore the value of a responsive appeals process and highlight the potential for excellent outcomes when timely listing is combined with close pre- and post-transplant monitoring [41, 42].

Acute-on-chronic liver failure (ACLF) represents a potential indication for NAP submission, particularly for patients at high risk of short-term mortality. Although the number of appeals explicitly recorded as ACLF in our dataset was small, several cases may have followed ACLF-like clinical trajectories, reflecting acute decompensation on chronic liver disease. During the study period, there was no national ACLF-specific listing policy; centres therefore occasionally utilised the NAP pathway to allow timely consideration of these high-risk patients. This highlights both the evolving spectrum of indications for

exceptional listing and the need for clear national guidance for ACLF in the context of transplant allocation.

This study has several strengths, including its national scope, comprehensive inclusion of all appeals, and robust survival analysis with long-term follow-up. By building on prior descriptive reports, it is the first to provide outcome data that clearly demonstrate the legitimacy and effectiveness of the NAP. Nonetheless, some limitations should be noted. Our analysis is descriptive and does not include a matched comparator cohort of patients listed through standard criteria. Therefore, while the NAP appears to facilitate access for well-selected patients, we cannot definitively conclude that it ensures equity. This limitation should be considered when interpreting our findings. Reliance on registry data restricted detail on QoL, functional outcomes, and precise reasons for non-transplantation, while incomplete or missing entries may have limited the depth of subgroup analyses. The relatively small number of declined cases reduced statistical power to explore this group, and the retrospective design precludes causal inference. Temporal bias may also have been introduced, as changes in listing policy, practice, panel, and organ availability over the 10-year study could have influenced case mix and outcomes. In addition, not all potential appeals may have reached the NAP stage, as some cases may have been resolved informally between centres or via the LAG. Future work should focus on prospective audit, capture patient-reported outcomes, and assess evolving trends to ensure equitable access and ongoing effectiveness of the appeals process.

This national review confirms the value of the NAP in ensuring fair and effective access to liver transplantation for exceptional cases. Despite high illness severity, transplanted patients achieved excellent long-term outcomes, validating the careful scrutiny applied during appeals. The panel's willingness to decline inappropriate cases further demonstrates its integrity as a gatekeeper of scarce donor organs. Refining national policy to incorporate conditions repeatedly approved at appeal, while continuing to monitor outcomes, will strengthen the system further. Ultimately, the NAP exemplifies how a centralised, multidisciplinary mechanism can reconcile compassion for individual patients with responsible stewardship of limited transplant resources.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving humans were approved by NHS Blood and Transplant, Bristol, UK. The studies were conducted in

accordance with the local legislation and institutional requirements. Written informed consent for participation was not required from the participants or the participants' legal guardians/next of kin in accordance with the national legislation and institutional requirements.

AUTHOR CONTRIBUTIONS

AH and VA conceptualised the study design. RT provided the NHSBT data. ARH performed statistical analysis and drafted the manuscript. SG prepared the tables. RT, TG, SM, RP, DT, KM, DM, and VA critically revised the manuscript and approved the final draft for submission. All authors contributed to the article and approved the submitted version.

FUNDING

The author(s) declared that financial support was not received for this work and/or its publication.

CONFLICT OF INTEREST

The author(s) declared that this work was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

GENERATIVE AI STATEMENT

The author(s) declared that generative AI was not used in the creation of this manuscript.

Any alternative text (alt text) provided alongside figures in this article has been generated by Frontiers with the support of artificial intelligence and reasonable efforts have been made to ensure accuracy, including review by the authors wherever possible. If you identify any issues, please contact us.

ACKNOWLEDGEMENTS

We thank NHS Blood and Transplant for access to registry data, the National Appeals Panel and Liver Advisory Group for their governance, and the clinicians and transplant teams across the seven UK centres for their contributions.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontierspartnerships.org/articles/10.3389/ti.2026.15573/full#supplementary-material>

REFERENCES

- Li ZX, Zeng JH, Zhong HL, Peng B. Liver Transplantation Improves Prognosis Across all Grades of Acute-On-Chronic Liver Failure Patients: A Systematic Review and Meta-Analysis. *World J Gastroenterol* (2025) 31(12):102007. doi:10.3748/wjg.v31.i12.102007
- Goldaracena N, Cullen JM, Kim DS, Eksler B, Halazun KJ. Expanding the Donor Pool for Liver Transplantation with Marginal Donors. *Int J Surg Lond Engl* (2020) 82S:30–5. doi:10.1016/j.ijsu.2020.05.024
- Lebret A. Allocating Organs Through Algorithms and Equitable Access to Transplantation—a European Human Rights Law Approach. *J L Biosci* (2023) 10(1):lsad004. doi:10.1093/jlb/lsad004
- Neuberger J. Organisational Structure of Liver Transplantation in the UK. *Langenbecks Arch Surg* (2015) 400(5):559–66. doi:10.1007/s00423-015-1296-9
- Puri P, Malik S. Liver Transplantation: Contraindication and Ineligibility. *J Clin Exp Hepatol* (2023) 13(6):1116–29. doi:10.1016/j.jceh.2023.04.005
- Liver Transplantation. Selection Criteria and Recipient Registration. POLICY POL195/7 (2018). Available online at: https://nhsbtdbe.blob.core.windows.net/umbraco-assets-corp/9440/pol195_7-liver-selection-policy.pdf (Accessed December 9, 2025).
- Neuberger J. An Update on Liver Transplantation: A Critical Review. *J Autoimmun* (2016) 66:51–9. doi:10.1016/j.jaut.2015.08.021
- Liver Advisory Group. Liver Advisory Group (ODT Clinical) (2025). Available online at: <https://www.odt.nhs.uk/transplantation/liver/liver-advisory-group/> (Accessed December 9, 2025).
- NHSBT Annual Report. NHSBT Annual Report on Liver Transplantation. Report for 2024/2025 (2025). Available online at: <https://nhsbtdbe.blob.core.windows.net/umbraco-assets-corp/37114/nhsbt-liver-transplantation-report-2425.pdf> (Accessed December 9, 2025).
- Latt NL, Niazi M, Pysopoulos NT. Liver Transplant Allocation Policies and Outcomes in United States: A Comprehensive Review. *World J Methodol* (2022) 12(1):32–42. doi:10.5662/wjm.v12.i1.32
- Gish RG, Wong RJ, Honerkamp-Smith G, Xu R, Osorio RW. United Network for Organ Sharing Regional Variations in Appeal Denial Rates with Non-Standard Model for End-Stage Liver Disease/Pediatric End-Stage Liver Disease Exceptions: Support for a National Review Board. *Clin Transpl* (2015) 29(6): 513–22. doi:10.1111/ctr.12544
- Gimson A. Development of a UK Liver Transplantation Selection and Allocation Scheme. *Curr Opin Organ Transpl* (2020) 25(2):126–31. doi:10.1097/MOT.0000000000000743
- BSG HCC. British Society of Gastroenterology Guidelines for the Management of Hepatocellular Carcinoma in Adults (2024). Available online at: <https://www.bsg.org.uk/getmedia/07619716-2735-45d5-804e-9f83edfa8691/HCC-gutjnl-2023-331695-full.pdf> (Accessed December 9, 2025).
- Halazun KJ, Sapisochin G, von Ahrens D, Agopian VG, Tabrizian P. Predictors of Outcome After Liver Transplantation for Hepatocellular Carcinoma (HCC) Beyond Milan Criteria. *Int J Surg Lond Engl* (2020) 82S:61–9. doi:10.1016/j.ijsu.2020.07.029
- Paklar N, Mijic M, Filipic-Kanizaj T. The Outcomes of Liver Transplantation in Severe Metabolic Dysfunction-Associated Steatotic Liver Disease Patients. *Biomedicines* (2023) 11(11):3096. doi:10.3390/biomedicines11113096
- Allen E, Taylor R, Gimson A, Thorburn D. Transplant Benefit-Based Offering of Deceased Donor Livers in the United Kingdom. *J Hepatol* (2024) 81(3): 471–8. doi:10.1016/j.jhep.2024.03.020
- Melehy A, Amara D, Gumate S, Ebaid S, Kaldas FM, Farmer DG, et al. Center-Level Variations are Associated with Disparate Waitlist Mortality and Probability of Liver Transplant in MELD 35 and Greater Patients. *Liver Transpl* (2025) 31(9):1100–10. doi:10.1097/LVT.0000000000000616
- Ishaque T, Massie AB, Bowring MG, Haugen CE, Ruck JM, Halpern SE, et al. Liver Transplantation and Waitlist Mortality for HCC and Non-HCC Candidates Following the 2015 HCC Exception Policy Change. *Am J Transpl Off J Am Soc Transpl Am Soc Transpl Surg* (2019) 19(2):564–72. doi:10.1111/ajt.15144
- Baker B, Aridi TG, Patel M, Carter A, Singleton C, Ross-Driscoll K, et al. Social Determinants Are Important Barriers to Completion of the Liver Transplant Pathway and Are Associated with Waitlisting and Mortality in Hepatocellular Carcinoma. *Dig Dis Sci* (2025). doi:10.1007/s10620-025-09278-3
- Meier RPH, King EA, Cameron AM, Malik S, Maluf DG, Bhati CS, et al. National Trends in Machine Perfusion and Living Donor Liver Transplantation: Complement or Competition? *Transplantation* (2025) 109: e722–e723. doi:10.1097/TP.0000000000005509
- Stahl CC, Aufhauser DD. Normothermic Regional Perfusion and Liver Transplant: Expanding the Donation After Circulatory Death Donor Pool. *Curr Opin Organ Transpl* (2025) 30(4):225–35. doi:10.1097/MOT.0000000000001230
- Li Z, Jones O, Magyar CTJ, Claassen MP, Ivanics T, Choi WJ, et al. Living Donor Availability Improves Patient Survival in a North American Center: An Intention-to-Treat Analysis. *Ann Surg* (2026) 283:326–34. doi:10.1097/SLA.0000000000006451
- Zhang X, Chen C, Wang Y, Xu J. Recurrence Risk Prediction Models for Hepatocellular Carcinoma After Liver Transplantation. *J Gastroenterol Hepatol* (2024) 39(11):2272–80. doi:10.1111/jgh.16693
- Ilmer M, Guba MO. Liver Transplant Oncology: Towards Dynamic Tumor-Biology-Oriented Patient Selection. *Cancers* (2022) 14(11):2662. doi:10.3390/cancers14112662
- Pérez González A, Martín López J, Rivera-Esteban J. Atypical and Fatal Presentation of Acute Liver Failure. *Eur J Intern Med* (2025) 140:106371. doi:10.1016/j.ejim.2025.05.036
- Rady ED, Anouti A, Thimphithaya C, Cotter TG. Liver Transplantation in Alcohol-Associated Liver Disease. *Clin Liver Dis* (2025) 29(2):165–84. doi:10.1016/j.cld.2024.12.001
- Fujiwara K, Yasui S, Yokosuka O. Autoimmune Acute Liver Failure: An Emerging Etiology for Intractable Acute Liver Failure. *Hepatol Int* (2013) 7(2):335–46. doi:10.1007/s12072-012-9402-3
- Ogundolie M, Chan N, McElroy LM. Equity in Liver Transplantation: Are we any Closer? *Curr Opin Organ Transpl* (2023) 28(4):259–64. doi:10.1097/MOT.0000000000001085
- Dale R, Cheng M, Pines KC, Currie ME. Inconsistent Values and Algorithmic Fairness: A Review of Organ Allocation Priority Systems in the United States. *BMC Med Ethics* (2024) 25(1):115. doi:10.1186/s12910-024-01116-x
- Gorji L, Brown ZJ, Limkemann A, Schenk AD, Pawlik TM. Liver Transplant as a Treatment of Primary and Secondary Liver Neoplasms. *JAMA Surg* (2024) 159(2):211–8. doi:10.1001/jamasurg.2023.6083
- Battistella S, Grasso M, Catanzaro E, D'Arcangelo F, Corrà G, Germani G, et al. Evolution of Liver Transplantation Indications: Expanding Horizons. *Medicina (Kaunas)* (2024) 60(3):412. doi:10.3390/medicina60030412
- Kwong AJ, Foutz J, Cafarella M, Biggins SW, Shah ND, Eason J, et al. Implementation of a National Liver Review Board for Exception Requests in the United States: A 2-Year Monitoring Report. *Liver Transpl* (2024) 30(4): 367–75. doi:10.1097/LVT.0000000000000248
- Kalra A, Biggins SW. New Paradigms for Organ Allocation and Distribution in Liver Transplantation. *Curr Opin Gastroenterol* (2018) 34(3):123–31. doi:10.1097/MOG.0000000000000434
- Bernts LHP, Jones DEJ, Kaatee MM, Lohse AW, Schramm C, Sturm E, et al. Position Statement on Access to Care in Rare Liver Diseases: Advancements of the European Reference Network (ERN) RARE-LIVER. *Orphanet J Rare Dis* (2019) 14(1):169. doi:10.1186/s13023-019-1152-z
- Di Maira T, Cailliez V, Domínguez-Gil B, Mahillo B, Álvarez M, Belli LS, et al. Regional Variations and Trends in Liver Transplantation Practices Across Europe. *JHEP Rep.* (2025) 7(8):101424. doi:10.1016/j.jhepr.2025.101424
- Jaure A, Jha V, McElroy LM, Muller E, Oniscu GC, Serper M, et al. Policy Innovations to Advance Equity in Solid Organ Transplantation. *Lancet Lond Engl* (2025) 406(10501):403–16. doi:10.1016/S0140-6736(25)00712-3
- Gómez-Orellana AM, Rodríguez-Perálvarez ML, Guijo-Rubio D, Gutiérrez PA, Majumdar A, McCaughan GW, et al. Gender-Equity Model for Liver Allocation Using Artificial Intelligence (GEMA-AI) for Waiting List Liver Transplant Prioritization. *Clin Gastroenterol Hepatol.* (2025). 23(12): 2187–2196. doi:10.1016/j.cgh.2024.12.010
- Younossi ZM, Stepanova M, Al Shabeeb R, Eberly KE, Shah D, Nguyen V, et al. The Changing Epidemiology of Adult Liver Transplantation in the United States in 2013–2022: The Dominance of Metabolic Dysfunction-Associated Steatotic Liver Disease and Alcohol-Associated Liver Disease. *Hepatol Commun* (2024) 8(1):e0352. doi:10.1097/HCP.0000000000000352
- Lissing M, Wang B, Wahlin S. Liver Transplantation and Primary Liver Cancer in Porphyria. *Liver Int Off J Int Assoc Study Liver* (2025) 45(3):e15894. doi:10.1111/liv.15894

40. Akabane M, Imaoka Y, Kawashima J, Pawlik TM. Advancing Precision Medicine in Hepatocellular Carcinoma: Current Challenges and Future Directions in Liquid Biopsy, Immune Microenvironment, Single Nucleotide Polymorphisms, and Conversion Therapy. *Hepatic Oncol* (2025) 12(1): 2493457. doi:10.1080/20450923.2025.2493457
41. Alnagar A, Daradka K, Kyrana E, Mtegha M, Palaniswamy K, Rajwal S, et al. Predictors of Patient and Graft Survival Following Pediatric Liver Transplantation: Long-Term Analysis of More than 300 Cases from Single Centre. *Pediatr Transpl* (2022) 26(1):e14139. doi:10.1111/petr.14139
42. Alnagar AM, Hakeem AR, Daradka K, Kyrana E, Methga M, Palaniswamy K, et al. Long-Term Outcomes of Pediatric Liver Transplantation in Acute Liver

Failure vs End-Stage Chronic Liver Disease: A Retrospective Observational Study. *World J Transpl* (2023) 13(3):96–106. doi:10.5500/wjt.v13.i3.96

Copyright © 2026 Hakeem, Gupta, Taylor, Grammatikopoulos, Masson, Prasad, Thorburn, Menon, Manas and Aluvihare. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.



Novel Allocation Strategies Can Boost Kidney Exchange Programs: A Monte Carlo Simulation

Mattheüs F. Klaassen^{1*}, Marry de Klerk¹, Marije C. Baas², Hanneke Bouwsma³, Laura B. Bungener⁴, Maarten H. L. Christiaans⁵, Twan Dollevoet⁶, Kristiaan Glorie⁷, Sebastiaan Heidt¹, Aline C. Hemke⁸, Margriet F. C. de Jong⁹, Judith A. Kal-van Gestel¹, Marcia M. L. Kho¹, Jeroen D. Langereis¹⁰, Karlijn A. M. I. van der Pant¹¹, Claudia M. Ranzijn¹², Dave L. Roelen¹³, Eric Spierings¹⁴, Christina E. M. Voorter¹⁵, Jacqueline van de Wetering¹, Arjan D. van Zuilen¹⁶, Joke I. Roodnat¹ and Annelies E. de Weerd¹

¹Department of Internal Medicine, Erasmus MC Transplant Institute, University Medical Center Rotterdam, Rotterdam, Netherlands, ²Department of Internal Medicine, Radboud University Medical Center, Nijmegen, Netherlands, ³Department of Internal Medicine, Leiden University Medical Center, Leiden, Netherlands, ⁴Department of Laboratory Medicine, University Medical Center Groningen, Groningen, Netherlands, ⁵Department of Internal Medicine, Maastricht University Medical Center, Maastricht, Netherlands, ⁶Erasmus School of Economics, Erasmus University Rotterdam, Rotterdam, Netherlands, ⁷Erasmus Q-Intelligence, Erasmus University Rotterdam, Rotterdam, Netherlands, ⁸Dutch Transplant Foundation, Leiden, Netherlands, ⁹Department of Internal Medicine, University Medical Center Groningen, Groningen, Netherlands, ¹⁰Department of Laboratory Medicine, Radboud University Medical Center, Nijmegen, Netherlands, ¹¹Department of Internal Medicine, Amsterdam University Medical Center, Amsterdam, Netherlands, ¹²Department of Immunogenetics, Sanquin Diagnostic Services, Amsterdam, Netherlands, ¹³Department of Immunology, Leiden University Medical Center, Leiden, Netherlands, ¹⁴Central Diagnostics Laboratory and Center of Translational Immunology, University Medical Center Utrecht, Utrecht, Netherlands, ¹⁵Department of Transplantation Immunology, Maastricht University Medical Center, Maastricht, Netherlands, ¹⁶Department of Internal Medicine, University Medical Center Utrecht, Utrecht, Netherlands

OPEN ACCESS

*Correspondence

Mattheüs F. Klaassen,

✉ m.f.klaassen@erasmusmc.nl

Received: 13 August 2025

Revised: 12 December 2025

Accepted: 22 January 2026

Published: 04 March 2026

Citation:

Klaassen MF, de Klerk M, Baas MC, Bouwsma H, Bungener LB, Christiaans MHL, Dollevoet T, Glorie K, Heidt S, Hemke AC, de Jong MFC, Kal-van Gestel JA, Kho MML, Langereis JD, van der Pant KAMI, Ranzijn CM, Roelen DL, Spierings E, Voorter CEM, van de Wetering J, van Zuilen AD, Roodnat JI and de Weerd AE (2026) Novel Allocation Strategies Can Boost Kidney Exchange Programs: A Monte Carlo Simulation. *Transpl. Int.* 39:15423. doi: 10.3389/ti.2026.15423

Kidney exchange programs (KEPs) enhance access to living donor kidney transplantation. Nonetheless, transplant rates in KEP remain low for highly immunized and blood type O patients. In the Netherlands, a novel allocation algorithm is being implemented, allowing ABO-incompatible matching for long waiting patients, next to prioritization and ‘low-level’ HLA-incompatible matching for selected highly immunized patients. We simulated this novel algorithm along with additional scenarios, by using a retrospective, 6-year cohort of Dutch KEP. For each scenario, 30 simulations were repeated with Monte Carlo technique. The novel algorithm increased median KEP transplant rate for incompatible pairs (53% versus 44%, $p < 0.001$) and for difficult-to-match subgroups. HLA-incompatible matching increased transplant rate for selected highly immunized patients significantly, while participation with multiple donors per recipient did not. In additional simulations, including all non-KEP unspecified donors ($n = 150$) for local KEP participation increased transplant rate for incompatible pairs up to 64% ($p < 0.001$). Simulating additional KEP participation by compatible pairs ($n = 149$), on the condition a KEP match should have fewer HLA mismatches, resulted in 58% being matched in KEP. In

Abbreviations: ABO, ABO blood type system; ABOi, ABO-incompatible; AM, Eurotransplant Acceptable Mismatch program; HI, Highly Immunized; HLA, Human Leukocyte Antigen; HLAi, HLA-incompatible; KEP, Kidney Exchange Program; LDKT, Living Donor Kidney Transplantation; LW, Long Waiting; MFI, Mean Fluorescence Intensity; sHI, selected Highly Immunized; UD, Unspecified Donor; vPRA, virtual Panel Reactive Antibodies.

conclusion, differential matching algorithms can boost KEP transplant rates, allowing incompatible matching for difficult-to-match subgroups, facilitating participation of unspecified donors, and optimizing the HLA matching of compatible pairs.

Keywords: allocation, kidney paired donation, kidney transplantation, living donor, simulation

INTRODUCTION

Living donor kidney transplantation (LDKT) is the preferred treatment for end-stage kidney disease, especially when performed pre-emptively [1–4]. For patients with a living donor, blood type (ABO) or Human Leukocyte Antigen (HLA) incompatibility poses barriers in roughly one-third of cases [5]. ABO-incompatible (ABOi) transplantation is common practice in many centers to overcome ABO incompatibility, but it is not feasible in case of high blood type antibody titers and it comes with additional treatment for the recipient [6]. While ABOi transplantation is favorable compared to waiting for a deceased donor transplantation, it is slightly inferior compared to compatible LDKT [7, 8]. HLA-incompatible (HLAi) transplantation is associated with higher graft failure rates despite desensitization protocols [9, 10]. Nonetheless, some degree of HLA incompatibility could be accepted to avoid long-term dialysis with associated morbidity and mortality [11].

Kidney exchange programs (KEPs) can facilitate compatible LDKT by making new donor exchange combinations between incompatible pairs [12, 13]. While single center KEPs can benefit from simplified logistics, multi-center KEPs have proven more effective due to the increased donor pool size [14–18]. The first national KEP was established in the Netherlands over

two decades ago [19]. During the years, KEP exchanges have evolved from two-way cycles to four-way cycles and domino chains [20–22]. Unspecified donors and compatible pairs nowadays participate in some KEPs [17, 23–26].

Despite these developments, transplant rates in KEP remain low for difficult-to-match subgroups. Worldwide, highly immunized and blood type O patients accumulate in KEP, leading to inequities in access to kidney transplantation [27–31]. A previous simulation study has shown benefit of allowing ABOi matching for highly immunized patients [24]. Studies from the United States have demonstrated the feasibility of prioritizing matches for difficult-to-match patients by assigning them priority points or weights [14, 32]. Other ways to enable more KEP transplants have been suggested, such as participating with multiple potential donors per recipient in KEP [33], or including compatible pairs who could improve their donor-recipient matching via KEP [25, 34–36].

ABOi matching is already allowed in some KEPs, for example in Spain, the United Kingdom, Scandiatransplant and Australia [20, 27, 37]. In the Netherlands, a new KEP algorithm is being implemented, allowing ABOi matching for long waiting candidates. In addition, selected highly immunized patients are prioritized, by maximizing the number of matches for them first, and are allowed for ABOi or ‘low-level’ HLAi matching [38]. In a

Novel allocation strategies can boost kidney exchange programs: a Monte Carlo simulation

Monte Carlo simulation



Dutch KEP 2018-2023

469 incompatible

23 unspecified

10,183 waitlist

Additional non-KEP

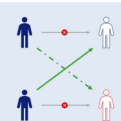
148 compatible

143 unspecified

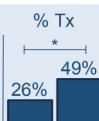
Scenario analyses

Difficult-to-match

Incompatible matching



selected Highly Immunized
Priority
HLAi ABOi



Long waiting
ABOi



One vs two donors

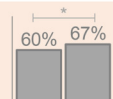
For difficult-to-match



selected Highly Immunized

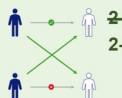


Long waiting



Compatible pairs

Better HLA-match



2-2-2
2-2-1
58%
Better match

+57 transplants for incompatible pairs
p<0.001

Unspecified donors

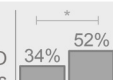
Domino chain



facilitate 1.3 domino transplants

+52 transplants for incompatible pairs
p<0.001

Blood type O paired patients



KLAASSEN, et al. *Transpl. Int.* 2026

doi: [10.3389/ti.2026.15423](https://doi.org/10.3389/ti.2026.15423)



GRAPHICAL ABSTRACT |

single center pilot of this novel algorithm, both the total number of transplants and the number of transplants for difficult-to-match candidates increased when compared to current KEP outcomes [39].

The incremental value of allowing ABOi and HLAi matching, priority allocation, and additional unspecified donor and compatible pair participation in a multi-center KEP has not yet been deciphered. We therefore conducted a simulation study using real-world patient data. The Dutch KEP served as a proxy to test several algorithms and varying numbers of KEP participants. By comparative analysis, we examined how different allocation strategies affect transplant rates across patient groups in KEP.

PATIENTS AND METHODS

The MONTEKEP study protocol was approved by the institutional review board of the Erasmus Medical Center (MEC-2023-0518). The need for informed consent was waived.

Retrospective Data Collection

All Dutch KEP participants from the seven different transplant centers, next to all Dutch patients on the Eurotransplant deceased donor waitlist, from January 2018 to December 2023, were included in the simulation database. We excluded patients with missing data or incomplete waitlist registration. Additionally, we included unspecified donors (UDs) that donated a kidney in their local transplant center outside the national KEP ('non-KEP UD'). To simulate additional KEP participation of compatible pairs, we added a sample of non-KEP compatible pairs from one center (Erasmus MC) who had been transplanted with their intended donor, with HLA A-B-DR mismatches ≥ 4 . This sample represents roughly one-fifth of the directed compatible transplants performed nationally with mismatches ≥ 4 .

Waitlist variables such as age, blood type, transplant center, dialysis vintage, HLA A-B-C-DR-DQ (and if available DP) phenotype, and unacceptable HLA antigens were collected. Virtual panel reactive antibodies (vPRA) were calculated from the unacceptable HLA antigens by using the Eurotransplant Reference Laboratory batch calculator¹. HLA mismatches were calculated on broad level for HLA-A/B and on split level for HLA-DR, in line with the Eurotransplant policy.

Definitions for Difficult-To-Match Patients

Selected Highly Immunized (sHI) was defined as vPRA $\geq 85\%$, dialysis vintage ≥ 2 years, and either ≥ 2 years participation in the Eurotransplant Acceptable Mismatch (AM) Program or being declined for the AM Program. Long Waiting (LW) was defined as dialysis vintage ≥ 2 years. Patients were retrospectively labeled as either LW, sHI, HI-other (vPRA $\geq 85\%$ without being LW or sHI), or regular.

Current and Novel Allocation Algorithms

For the current and novel algorithms, we refer to **Table 1**. In the novel algorithm, sHI patients are assigned priority and allowed for 'low-level MFI' HLAi matching, defined as Luminex SA Werfen Immucor® $< 8,000$ or Thermo Fischer One Lambda® $< 10,000$ and not being a repeated mismatch [40–42]. HLAi matching could be simulated for one center only, as MFI levels of HLA antibodies were only available for patients from one center. ABOi matching is allowed for LW and sHI patients with low IgG anti-A/B titers ($\leq 1:256$). We imputed the blood type antibody titers based on the cohort of difficult-to-match patients participating in the pilot KEP in Erasmus MC (**Supplementary Table S1**) [43]. Blood type A and B patients were assigned to have unacceptably high anti-A/B titers in 3% of cases, compared to 37% among blood type O patients.

In the novel algorithm, UD's can opt for local KEP participation if they are not willing to travel to another transplant center. Local participation of UD's is limited to matches with pairs from their local transplant center only, while these pairs can still trigger a national domino chain. The last donor kidney of UD-triggered domino chains (end-of-chain kidney) is allocated to the deceased donor waitlist. For this reason, waitlist patients are also assigned LW or sHI status if applicable.

We simulated the following scenarios:

1. Current KEP algorithm.
2. Novel algorithm (=ABOi matching for LW and sHI, and priority for sHI).
3. Novel algorithm + 'low-level MFI' HLAi matching for sHI (simulated for one center).
4. Novel algorithm + inclusion of two donors per LW and sHI KEP patient for simultaneous KEP participation.
5. Novel algorithm + inclusion of all UD's in KEP, with either:
 - a. Local participation (allowing UD's for matching within their local center only).
 - b. National participation (matching not limited to the local transplant center).
6. Novel algorithm + inclusion of extra compatible pairs with HLA A-B-DR mismatches ≥ 4 . At least one of the following criteria was obliged for matching:
 - i. Reduction in total number of HLA mismatches on A-B-DR, and ≤ 1 increase in HLA mismatches on DR locus.
 - ii. Reduction in HLA mismatches on DR locus with no increase in total number of HLA mismatches on A-B-DR.

Monte Carlo Simulation

Simulations were performed with the Monte Carlo method to predict the most likely effect of an intervention. For a detailed description we refer to the **Supplementary Material**. For each scenario, 30 simulations were performed. One KEP simulation consisted of 24 KEP match runs, similar to the 24 actual match runs (once every 3 months) in the Dutch KEP during the 6-year study period.

For each simulation, new pairs were created by randomly selecting a donor and patient from the database, after which

¹<https://www.etrl.org/FreqBatch.aspx>

TABLE 1 | Current and novel allocation algorithm of the Dutch kidney exchange program.

Current algorithm	Novel algorithm
1. Maximize the total number of transplants for pairs 2. Blood group identical over blood group compatible 3. Choose matches for patients with lowest match probability (% of HLA- and ABO-compatible donors in that run) 4. Minimize chain length 5. Maximize spread of matches over the centers (for logistical reasons) 6. Choose matches for patients with longest dialysis vintage	1. Maximize the total number of transplants for sHI (compatible → ABOi → HLAi → combined ABOi/HLAi) 2. Maximize the total number of transplants (compatible → ABOi for those allowed) 3. Choose matches for patients with higher vPRA (categorical: 85%-100% → 51%-84% → 6%-50% → 0%-5%) 4. Minimize the length of the longest chain/cycle 5. Choose matches for patients with longer dialysis vintage (categorical: >3 years → 1-3 years → <1 year)

ABOi = ABO-incompatible; HLA, human leukocyte antigen; HLAi = HLA-incompatible; sHI, selected Highly Immunized; vPRA, virtual Panel Reactive Antibodies.

TABLE 2 | Characteristics of patients in the retrospective database.

Characteristic	Paired KEP patients		Waitlist patients	Non-KEP compatible patients
	Incompatible	Compatible		
Number	469	18	10,183	148
Male (%)	232 (50)	10 (56)	6,278 (62)	88 (59%)
Median age (IQR) ^a	54 (45–63)	54 (36–63)	58 (46–66)	59 (49–65)
Blood type (%)				
A	118 (25)	8 (44)	3,963 (39)	65 (44)
B	54 (12)	4 (22)	1,334 (13)	15 (10)
O	290 (62)	5 (28)	4,469 (44)	65 (44)
AB	7 (1)	1 (6)	417 (4)	3 (2)
On dialysis (%)	275 (59)	13 (72)	5,503 (54)	51 (34)
Median days of patients on dialysis (IQR) ^b	752 (477–1,178)	308 (140–486)	784 (440–1,331)	464 (328–662)
Accepted for AM (%)	65 (14)	0 (0)	286 (3)	0 (0)
Immunized (%)	253 (54)	6 (35)	2,116 (21)	19 (13)
Median % vPRA of immunized (IQR) ^{b,c}	81 (56–99)	37 (23–61)	61 (30–89)	31 (9–56)
Status ^b (%)				
LW	92 (20)	NA	2,627 (26)	NA
sHI	54 (11)	NA	325 (3)	NA
HI-other	45 (10)	0 (0)	212 (2)	0 (0)
Regular	278 (59)	18 (100)	7,019 (69)	148 (100)

AM, eurotransplant acceptable mismatch program; HI, highly immunized; IQR, interquartile range; KEP, kidney exchange program; LW, long waiting; sHI, selected Highly Immunized; vPRA, virtual Panel Reactive Antibodies.

^aAt start of follow-up (1-1-2018) or at registration on the waitlist (if registered after 1-1-2018).

^bAt end of follow-up (20-12-2023) or at date of transplantation/waitlist removal.

^cCalculated with Eurotransplant Reference Laboratory calculator based on unacceptable antigens registered on the Eurotransplant deceased donor waitlist at the end of follow-up (20-12-2023) or at date of transplantation/waitlist removal.

they were stratified based on incompatibility (ABOi, HLAi, combined ABOi and HLAi) and patient's status (LW, sHI, HI-other or regular). Stratification percentages and numbers of KEP participants were based on the historical data provided by the Dutch Transplant Foundation (**Supplementary Table S1**). For the scenario of two donors per difficult-to-match patient (scenario 4), the HLA data of the non-KEP unspecified donors was used to simulate a broadening of the HLA donor pool.

Probabilities of renegeing (withdrawal from the program due to medical/non-medical reasons or transplantation outside KEP) and match failure (decline of KEP match due to positive cross-

match or medical/non-medical reasons) were taken into account (**Supplementary Table S1**), based on empirical findings in the Dutch KEP from 2003 to 2011 [24]. Compatible pairs and UD participants participated for a maximum of one KEP run in each scenario.

Outcome Measures

For each scenario, the median transplant rates of 30 simulations were calculated per subgroup, blood type and patient's status (LW or sHI). Quality of the matches was assessed by reporting the number of ABOi and/or HLAi matches. For the sake of comparison, we compared the outcome data of the Dutch KEP with a base-case simulation of the current Dutch KEP

TABLE 3 | Characteristics of donors in the retrospective database.

Characteristic	Paired KEP donors		KEP UD ^s	Non-KEP UD ^s	Non-KEP compatible donors
	Incompatible	Compatible			
Number	469	18	23	143	148
Median age (IQR) ^a	53 (44–61)	51 (40–54)	60 (46–69)	62 (50–68)	55 (48–63)
Blood type (%)					
A	270 (58)	6 (33)	13 (57)	62 (43)	36 (24)
B	76 (16)	1 (6)	2 (9)	14 (10)	2 (1)
O	114 (24)	11 (61)	8 (35)	62 (43)	110 (74)
AB	9 (2)	0 (0)	0 (0)	5 (3)	0 (0)
Ratio blood type O donors to O patients	0.4	2.2	NA	NA	1.7

IQR, interquartile range; KEP, kidney exchange program; UD, unspecified donor.

^aAt start of follow-up (1-1-2018) or at registration on the waitlist (if registered after 1-1-2018).

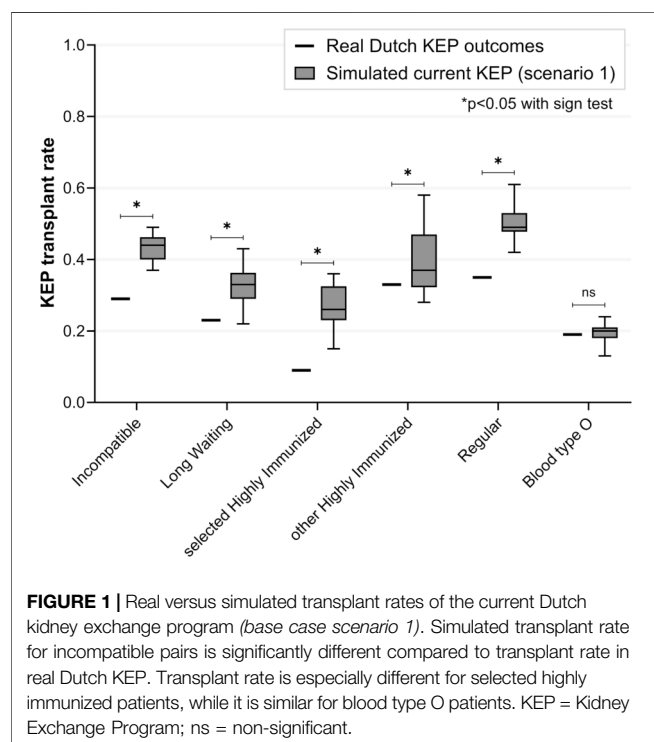


FIGURE 1 | Real versus simulated transplant rates of the current Dutch kidney exchange program (base case scenario 1). Simulated transplant rate for incompatible pairs is significantly different compared to transplant rate in real Dutch KEP. Transplant rate is especially different for selected highly immunized patients, while it is similar for blood type O patients. KEP = Kidney Exchange Program; ns = non-significant.

algorithm (scenario 1). The simulated current KEP was the comparator for the simulated novel algorithm (scenario 2). Variants in the novel algorithm and in the number of KEP participants (scenarios 3-6) were compared to the simulated novel algorithm (scenario 2).

Statistics

Donor and patient characteristics were reported with median and interquartile range (non-normally distributed data). Transplant rates in the real Dutch KEP were compared between the patient subgroups with the z-test. For simulated KEP scenarios, transplant rates were compared with the sign test. Simulated current KEP was compared to real Dutch KEP outcomes with the one sample sign test. P-values <0.05 were considered statistically

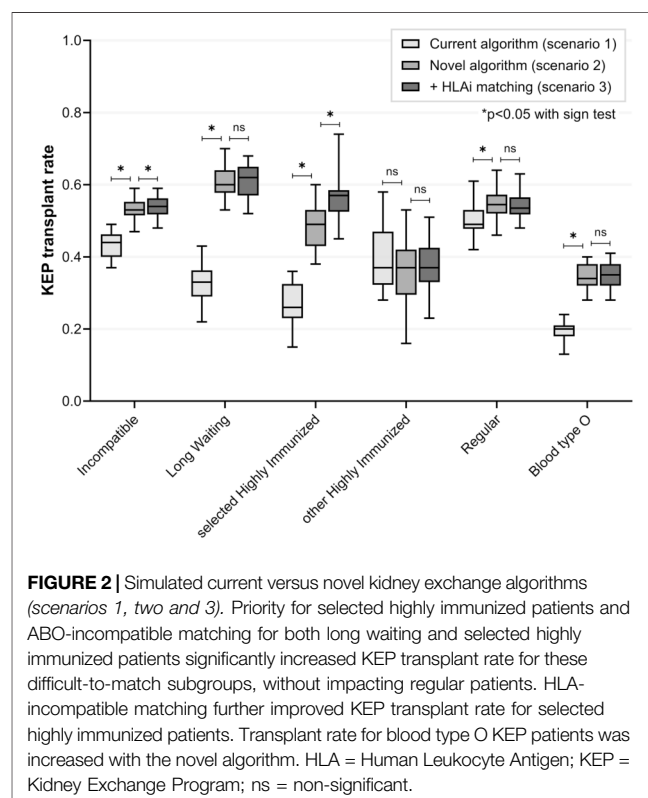


FIGURE 2 | Simulated current versus novel kidney exchange algorithms (scenarios 1, two and 3). Priority for selected highly immunized patients and ABO-incompatible matching for both long waiting and selected highly immunized patients significantly increased KEP transplant rate for these difficult-to-match subgroups, without impacting regular patients. HLA-incompatible matching further improved KEP transplant rate for selected highly immunized patients. Transplant rate for blood type O KEP patients was increased with the novel algorithm. HLA = Human Leukocyte Antigen; KEP = Kidney Exchange Program; ns = non-significant.

significant. Data was stored and processed in Microsoft Excel. Statistical calculations were performed with IBM SPSS version 28.0.1 software. Box-and-whisker plots were created with Graphpad Prism 10.1.2. Whiskers depict the minimum and maximum values.

RESULTS

Retrospective Cohort

Between 2018–2023, 500 pairs were registered in the national KEP registry, of which 487 were included in this study. Five

TABLE 4 | Number of simulated transplants for each scenario and patient subgroup.

Subgroup	Stratification settings (%)	Median number of transplants (median % of participants)					
		Current KEP	Novel KEP	+ HLAi matching	+ Two donors	+ UD _s local	+ UD _s national
		(1)	(2)	(3)	(4)	(5a)	(5b)
KEP pairs ^a	499 / 648	220 (44)	265 (53)	270 (54)	273 (55)	319 (64)	316 (63)
A patients	NA	109 (69)	118 (74)	121 (75)	121 (75)	122 (77)	126 (80)
B patients	NA	51 (82)	50 (80)	50 (81)	51 (84)	49 (81)	50 (83)
AB patients	NA	5 (83)	6 (88)	6 (91)	6 (88)	6 (96)	6 (90)
O patients	NA	54 (20)	92 (34)	93 (35)	95 (36)	142 (52)	136 (50)
A donors	NA	102 (36)	134 (47)	137 (48)	135 (49)	166 (61)	159 (58)
B donors	NA	67 (47)	83 (58)	86 (58)	85 (59)	94 (67)	95 (68)
AB donors	NA	5 (31)	6 (38)	6 (43)	8 (44)	11 (50)	12 (52)
O donors	NA	46 (78)	46 (82)	48 (83)	47 (79)	52 (81)	55 (83)
Incompatible ^a	480	210 (44)	256 (53)	259 (54)	263 (55)	308 (64)	307 (64)
LW	91 (19)	30 (33)	55 (60)	56 (62)	61 (67)	62 (68)	62 (68)
sHI	53 (11)	14 (26)	26 (49)	30 (57)	28 (52)	27 (50)	28 (53)
HI-other	43 (9)	16 (37)	16 (37)	16 (37)	17 (40)	17 (40)	18 (42)
Regular	293 (61)	145 (49)	159 (55)	157 (54)	159 (54)	206 (70)	197 (67)
Compatible	19 / 168	11 (58)	10 (53)	11 (58)	9 (47)	10 (53)	11 (58)
Unspecified donors	25 / 175	25 (100)	25 (100)	25 (100)	25 (100)	175 (100)	175 (100)
Waitlist ^a	1,440	25	25	25	25	175	175
LW	792 (55)	8	0	0	0	42	9
sHI	331 (23)	10	25	25	25	119	162
HI-other	216 (15)	5	0	0	0	9	4
Regular	101 (7)	1	0	0	0	6	1
A patients	NA	5	12	12	11	88	70
B patients	NA	17	10	10	10	37	34
AB patients	NA	3	2	2	2	10	10
O patients	NA	0	1	1	2	40	61

ABOi = ABO-incompatible; HI, highly immunized; HLAi = HLA-incompatible; KEP, kidney exchange program; LW, long waiting; NA, not applicable; sHI, selected Highly Immunized; UD, unspecified donor; vPRA, virtual Panel Reactive Antibodies.

^aMedian transplant rates of subgroups do not necessarily add up to total transplant rates, as the median of 30 simulations is reported for each subgroup.

pairs were excluded for missing data, and eight registrations were re-registrations of similar pairs. Of the 10,487 waitlist patients, 295 were excluded due to incomplete or pending registration. Of the sample of non-KEP compatible pairs, 148 (39%) had HLA mismatches ≥ 4 and were included in this simulation.

The majority of incompatible KEP patients had blood type O (Table 2). Ratio of blood type O donors versus O patients was 0.4 for incompatible KEP, 2.2 for compatible KEP, and 1.7 for the additional cohort of compatible pairs (Table 3). Of the 166 UD_s, the majority (n = 143; 86%) did not participate in KEP and donated in their local center.

When labelling patients as difficult-to-match, 20% of incompatible KEP patients and 26% of waitlist patients met the criteria for LW status, while this was 11% and 3% for sHI status, respectively (Table 2). sHI KEP pairs more often had blood type O donors than O patients (ratio 1.3), while LW KEP pairs and regular KEP pairs had less blood type O donors than O patients (ratio 0.4 and 0.2, respectively, Supplementary Table S2).

Reality Versus Simulated Current KEP

In reality, 500 pairs participated between 2018-2023, of which 152 (30%) were transplanted via KEP and 182 (36%) outside

national KEP (Supplementary Table S3). Transplant rates in KEP were significantly lower for difficult-to-match subgroups of LW (23%, $p = 0.029$) and sHI KEP patients (9%, $p < 0.001$) when compared to regular KEP patients (35%) (Supplementary Table S4). KEP transplant rate for blood type O patients was significantly lower when compared to other blood types (19% versus 47%, $p < 0.001$).

The simulated current KEP (scenario 1) yielded different transplant rates than real Dutch KEP (Figure 1). Total transplant rate for incompatible pairs was 44% in simulation versus 29% in real Dutch KEP ($p < 0.001$). Especially, transplant rate was higher in simulation for sHI KEP patients (26% versus 9%, $p < 0.001$) and blood type A and B patients (Supplementary Table S4). Transplant rate was similar for blood type O KEP patients (20% versus 19%, $p = 0.122$). All 25 participating UD_s were matched in simulation, compared to 11 of 23 in reality.

Scenario 2: Simulated Novel Algorithm

Compared to simulated current KEP (scenario 1), the simulated novel algorithm (with priority and ABOi matching) resulted in median 50 extra transplants for incompatible pairs (53% versus 44%, $p < 0.001$) (Figure 2; Table 4). For 98 (67%) of LW and sHI KEP patients, ABOi matching was allowed. ABOi matching and

TABLE 5 | Number of incompatible transplants resulting from each simulated scenario.

Subgroup	Median number of incompatible transplants (median % of all transplants) ^a					
	Novel KEP	+ HLAi matching	+ Two donors	+ UD _s local	+ UD _s national	+ Compatible pairs
	(2)	(3)	(4)	(5a)	(5b)	(6)
ABOi transplants ^b	50 (17)	49 (16)	52 (17)	82 (17)	92 (19)	50 (11)
LW KEP patients	38 (69)	38 (67)	40 (65)	36 (59)	37 (59)	39 (63)
sHI KEP patients	10 (38)	10 (31)	10 (34)	10 (37)	9 (32)	9 (34)
LW waitlist patients	0 (0)	0 (0)	0 (0)	3 (8)	0 (0)	0 (0)
sHI waitlist patients	2 (8)	2 (8)	2 (8)	33 (28)	47 (29)	2 (8)
A KEP patients	5 (4)	5 (4)	5 (4)	5 (4)	4 (3)	5 (3)
B KEP patients	3 (6)	3 (6)	3 (7)	5 (9)	5 (10)	3 (5)
AB KEP patients	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
O KEP patients	41 (42)	39 (42)	40 (42)	35 (25)	38 (27)	41 (23)
HLAi transplants ^b	NA	3 (1)	NA	NA	NA	NA
sHI KEP patients	NA	3 (9)	NA	NA	NA	NA
sHI waitlist patients	NA	0 (0)	NA	NA	NA	NA
Combined ABOi/HLAi ^b	NA	2 (1)	NA	NA	NA	NA
sHI KEP patients	NA	2 (7)	NA	NA	NA	NA
sHI waitlist patients	NA	0 (0)	NA	NA	NA	NA

ABOi = ABO-incompatible; DDWL, deceased donor wait list; HLAi = HLA-incompatible; KEP, kidney exchange program; LW, long waiting; NA, not applicable; sHI, selected Highly Immunized; UD, unspecified donor.

^aPercentages are calculated over the total number of transplants for all patients/for the subgroups of sHI/LW, and blood type.

^bMedian rates of subgroups do not necessarily add up to total rates, as the median of 30 simulations is reported for each subgroup.

priority resulted in increased transplant rate for difficult-to-match subgroups: 60% versus 33% ($p < 0.001$) for LW and 49% versus 26% ($p < 0.001$) for sHI KEP patients (Table 4). Median 50 (17% of total) transplants were ABOi (Table 5). Transplant rate remained similar for HI-other KEP patients ($p = 0.541$). More regular patients were transplanted with the novel algorithm (55% versus 49%, $p < 0.001$). Transplant rate for blood type O patients was increased in the novel compared to current algorithm (34% versus 20%, $p < 0.001$). More end-of-chain kidneys were allocated to sHI waitlist patients (25 versus 10, $p < 0.001$). Two sHI waitlist patients were matched ABOi.

Scenario 3: Additional HLAi Matching

For 14 (26%) of sHI KEP and 78 (24%) of sHI waitlist patients, MFI levels of HLA antibodies were available. Compared to scenario 2, allowing 'low-level MFI' HLAi matching for these patients increased total transplant rate for sHI KEP patients (57% versus 49%, $p < 0.001$). A median of 3 sHI patients were matched HLAi, and 2 patients both HLAi and ABOi (Table 5). Transplant rates for the other incompatible KEP patients did not change significantly (Figure 2; Table 4).

Scenario 4: Two Donors Per Difficult-To-Match Patient

Compared to scenario 2, participation of two potential donors per LW and sHI KEP patient increased transplant rate for incompatible KEP patients (55% versus 53%, $p = 0.031$) (Figure 3; Table 4). Transplant rate for LW KEP patients was slightly increased (67% versus 60%, $p < 0.001$), but transplant rate was not significantly different for sHI KEP patients (52% versus

49%, $p = 0.584$). The number of ABOi transplants remained similar ($p = 0.345$, Table 5).

Scenario 5: Participation by Extra Unspecified Donors

In scenario 5a, local KEP participation was simulated for 150 additional, non-KEP UD_s. All UD_s (median 100%) were matched after participating one KEP run (Figure 4; Table 4). Compared to scenario 2, local KEP participation by additional UD_s resulted in a median of 52 additional KEP transplants for incompatible pairs via domino chains (transplant rate 64% versus 53%, $p < 0.001$). Local UD participation facilitated more KEP transplants for blood type O KEP patients (52% versus 34%, $p < 0.001$). Transplant rate was increased for LW (68% versus 60%, $p < 0.001$) and regular KEP patients (70% versus 55%, $p < 0.001$), but remained similar for sHI KEP ($p = 1.000$) and HI-other KEP patients ($p = 0.345$). The number of ABOi transplants increased (82 versus 50, $p < 0.001$), but the proportion of total transplants was similar ($p = 0.678$). End-of-chain kidneys were mainly allocated to sHI waitlist patients (median 119, 68%) and to blood type A waitlist patients (median 88, 51%). A median of 33 sHI waitlist patients and 3 LW waitlist patients were matched ABOi.

National instead of local UD participation slightly increased transplant rate of highly immunized KEP patients (both sHI and HI-other KEP patients, Figure 4; Table 4). Transplant rate for blood type O KEP patients was reduced (136 versus 142, 50% versus 52%, $p < 0.001$). However, end-of-chain kidneys were more often allocated to blood type O waitlist patients (61 versus 40, $p < 0.001$) and sHI waitlist patients (162 versus 119, $p < 0.001$) with national UD participation. The proportion of ABOi transplants increased when compared to the local UD scenario (19% versus 17%, $p < 0.001$, Table 5).

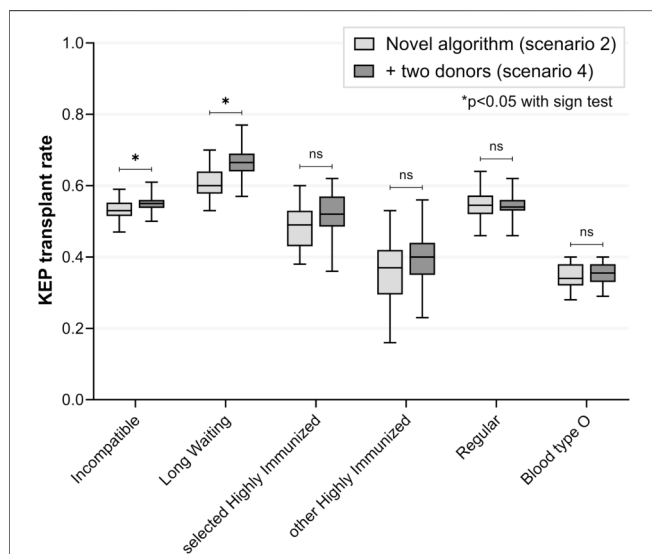


FIGURE 3 | Simultaneous participation by one versus two potential donors per difficult-to-match patient (*scenario 4 versus 2*). In both scenarios, the novel algorithm with priority and ABO-incompatible matching was simulated. In scenario 4, a second donor was added for each long waiting or selected highly immunized KEP recipient, to participate in KEP with two donors simultaneously. After one of two paired donors was matched, the remaining donor was excluded from the KEP pool. KEP transplant rate for long waiting patients was increased when including two donors versus one, while transplant rate for selected highly immunized patients was not significantly different between the scenarios. KEP = Kidney Exchange Program; ns = non-significant.

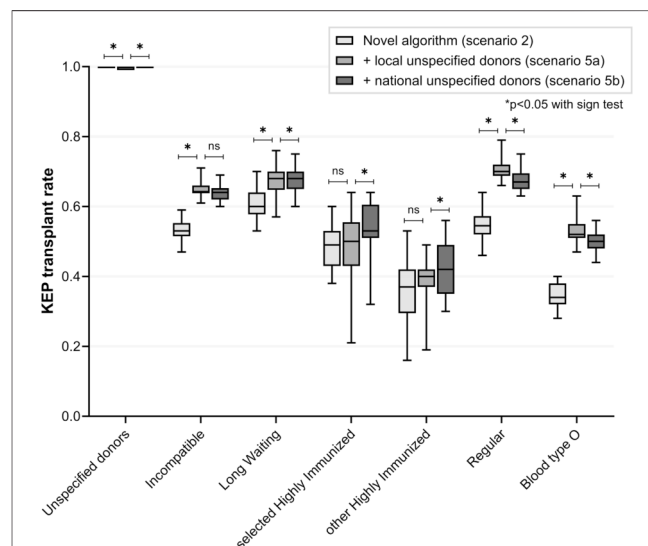


FIGURE 4 | Including all unspecified donors in the novel kidney exchange program (*scenario 5 versus 2*). In scenario 5a, matching of extra unspecified donors ($n = 150$) was restricted to a pair from the local center only, after which that pair could trigger a national chain. This local participation by unspecified donors allows them to remain at their evaluating transplant center, eliminating the need to travel to another center for donation (in Dutch KEP there is no organ shipment and the donor travels). In 5b, matching was allowed with pairs from all centers nationally. KEP transplant rate for incompatible pairs was significantly increased in both scenarios with extra unspecified donor participation, especially for blood type O patients and regular patients. National instead of local participation resulted in higher transplant rates for highly immunized subgroups. KEP = Kidney Exchange Program; ns = non-significant.

Scenario 6: Participation by Extra Compatible Pairs

Additional KEP participation was simulated for 149 compatible pairs, which represented roughly one-fifth of the national compatible directed transplants with HLA mismatches ≥ 4 . This resulted in 58% of compatible pairs being matched with a donor with fewer total HLA A-B-DR or fewer HLA DR mismatches, after participating one KEP run (**Figure 5; Table 4**). Compatible KEP participation increased transplant rate for incompatible pairs (median +57, 66% versus 53%, $p < 0.001$), and for the subgroups of LW KEP (68% versus 60%, $p < 0.001$), HI-other KEP (49% versus 37%, $p < 0.001$) and regular KEP patients (69% versus 55%, $p < 0.001$). Transplant rate for sHI KEP patients was similar (51% versus 49%, $p = 0.078$). Transplant rate for blood type O KEP patients increased (51% versus 34%, $p < 0.001$): explained by more transplants for both compatible and incompatible blood type O KEP patients (median 146 versus 91 for incompatible, $p < 0.001$). Number of ABOi transplants remained similar compared to scenario two ($p = 0.850$, **Table 5**).

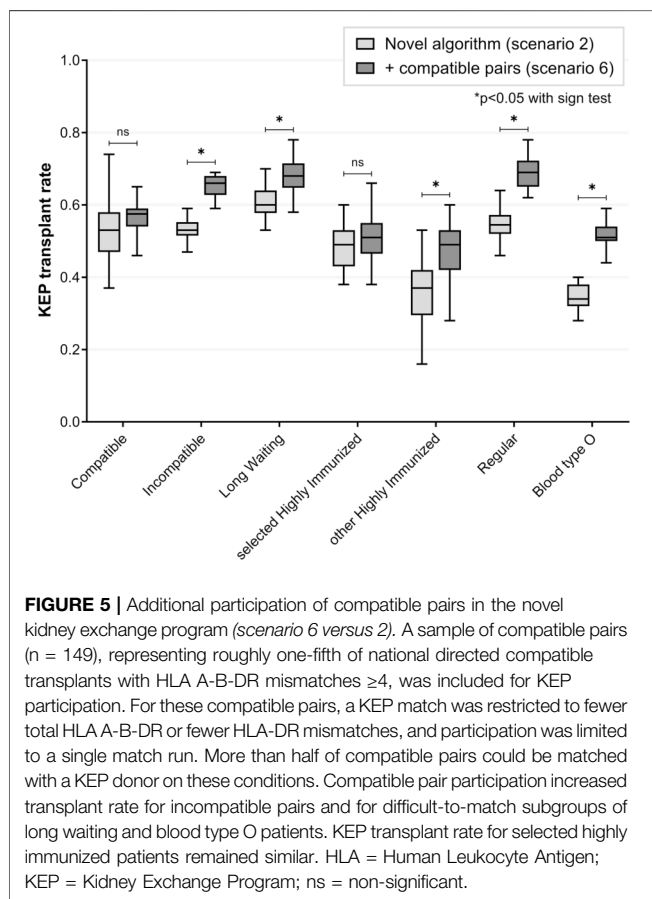
DISCUSSION

Kidney exchange programs facilitate living donor kidney transplantation, thereby reducing the waitlist and avoiding

long-term dialysis. Additionally, KEPs reduce incompatibilities between donor and recipient, mitigating the need for augmented immunosuppression and ultimately improving long-term graft survival [4, 13, 27–30, 39]. We simulated various KEP scenarios to optimize allocation strategies and solve common KEP problems, such as the congestion of highly immunized and blood type O patients.

In our study, ABOi matching for difficult-to-match patients resulted in a higher transplant rate for this group, while concomitantly increasing the transplant rate for regular patients. Scandiatransplant, which allows ABOi matching by default, has reported a transplant rate of 40% after 4 years [27]. This is in line with our simulation findings, as more HLAi pairs participate in Scandiatransplant and KEP volume is lower compared to the Netherlands. The United Kingdom Living Kidney Sharing Scheme also allows ABOi matching, but reports low rates of KEP ABOi transplants (seven over the past 4 years) [44]. Interestingly, Manook et al. described a trend in the United Kingdom to perform less direct, incompatible transplants, with a shift to more and longer KEP participation of incompatible pairs [45].

In line with previous reports, prioritizing highly immunized patients did not decrease the transplant rate for regular patients [32, 38]. Ashlagi et al. has simulated that, despite a priority point scale, the KEP transplant rate of patients with PRA $>98\%$ still lags behind



those of other immunized patients [14]. This is a well-known phenomenon in deceased donation programs as well, with a subgroup of very immunized patients not being transplanted despite priority [46, 47]. As priority alone would not suffice for this group, HLA-incompatible transplantation within KEP for lower-risk HLA antibodies might be the best alternative to dialysis or higher-risk incompatible transplantation outside KEP [47–49]. Our simulations demonstrate that HLA-incompatible matching, based on MFI cut-off values predicting a low risk of a positive crossmatch, would lead to more matching opportunities for this difficult-to-match group.

Compatible pairs can participate in KEP for altruistic reasons or to improve their immunological matching via KEP, by reducing the number of HLA mismatches or by avoiding repeated mismatches from previous transplants or pregnancies [50]. This can potentially improve long-term graft survival, reduce the risk of immunization, and prevent future re-transplantation [4]. In our simulations, an improved HLA match was found for more than half of compatible pairs, after participating one KEP run. In line with three other simulation studies [26, 34, 35], KEP participation by compatible pairs increased the transplant rate for incompatible pairs. Of note, Gentry et al. simulated two-way exchanges only [26], while the two other studies simulated a single KEP match run only. [34, 35]. In our simulation, participation of compatible pairs did not result in extra transplants for highly immunized patients, although the

immunized patients were prioritized. This unexpected finding, possibly related to changes in the KEP pool composition on the long term, demonstrates the importance of simulating multiple KEP runs over extended time periods. As we defined HLA matching on the antigen level, future studies on compatible KEP participation could test epitope matching for better immunological risk assessment [34, 51, 52].

In deceased donation, it has been demonstrated that internationally merged pools could enhance matching options for highly immunized patients [53]. In KEPs, it is known that a larger pool size allows for more and ‘better’ matches [18]. International cooperation between KEPs has been shown feasible and beneficial for KEP transplant rate [16, 27, 54], though logistical challenges must be overcome [15]. In this study, we tested enlarging the KEP donor pool via the inclusion of two potential donors per (difficult-to-match) recipient. We found no statistically significant difference for highly immunized patients, and only a modest benefit for long waiting patients. Although evaluation of multiple potential donors might increase costs and burden for donor candidates, the benefit may be more evident when including more additional donors or including donors with more diverse HLA genotypes.

Domino paired donation by unspecified donors has already been proven effective [23, 55–57]. Though logistically challenging, countries with low unspecified donation could consider deceased-donor initiated chains, that have a similar boosting effect [58–61]. For KEPs using donor travel as modality (such as in the Netherlands), local KEP participation of unspecified donors or compatible pairs might be a solution to increase KEP participation. Nonetheless, national instead of local participation will result in higher transplant rates for difficult-to-match subgroups. Clinicians should carefully counsel unspecified donors on local versus national KEP participation.

A persistent problem in KEPs is the shortage of blood type O donors [22, 31]. ABOi matching and KEP participation by unspecified donors or compatible pairs can reduce the blood type imbalance. Importantly, when unspecified donors with blood type O participate in KEP, the end-of-chain kidneys will mostly be of blood type A/B, resulting in less blood type O waitlist patients being matched via KEP chains. However, allowing ABOi matching for end-of-chain kidneys will reduce this disadvantage: in our simulations with ABOi matching and national unspecified donor participation, in which a median of 75 unspecified donors with blood type O were included, end-of-chain kidneys were matched (ABO-incompatible) to blood type O waitlist patients in median 61 cases. KEPs could consider prioritizing the matching of end-of-chain kidneys to blood type O waitlist patients, via an ABOi transplant, to further reduce blood type inequities on the waitlist. Of note, ABOi matching will not benefit all blood type O patients, as one-third had titers above threshold for ABOi transplantation in our pilot cohort [43].

We compared our base-case simulation of the current KEP to real Dutch KEP outcomes. There were significant differences: more patients, especially highly immunized patients and blood type A and B patients, were

transplanted in simulation. This might be caused by the different blood type distribution, as we did not stratify for blood types of simulated donor-recipient pairs; more blood type B donors were included compared to real Dutch KEP, and less blood type O donors. Additionally, renege or match failure rates might have been higher in reality: renege rate was set to 2% per month based on a historical estimate, while transplantation outside KEP with another living or deceased donor occurred in one-third of KEP pairs during the study period. Especially for unspecified donors, many were not transplanted in real Dutch KEP in this specific period, probably related to the COVID pandemic, while in simulation there was no renege of unspecified donors. Simulated transplant rates should therefore be interpreted with caution and relative to the base-case scenario.

In summary, we demonstrated the potential of adaptations in a national KEP and revealed the impact of these changes on different patient groups, by using a simulation model with real KEP data. This allows clinicians and policymakers to reflect on the challenges in their respective KEPs and to consider changes to the allocation strategy accordingly. Nevertheless, our study remains a simulation with practical simplifications and assumptions. Importantly, waiting time of ABOi pairs in KEP was not restricted in simulation, while in reality these pairs proceed with directed ABOi transplantation after participating unsuccessfully in one or two KEP runs. Next, our simulation output lacked parameters for comparing the HLA matching between the various scenarios. Additionally, our algorithm did not incorporate a center balance system, which ensures each center receives as many end-of-chain kidneys as the number of unspecified donors they contributed. Due to the retrospective nature of the data, our model did not account for waitlist dynamics, such as changes in dialysis vintage and vPRA over time. For HLAi matching, we simplified the delisting of antibodies by retrospectively applying a strict MFI cut-off, while a personalized approach is recommended [62].

In conclusion, a well-functioning KEP facilitates living donor kidney transplants, reduces incompatibilities and improves HLA matching. Differential matching constraints for difficult-to-match subgroups boost KEP transplant rate and mitigate current inequities. KEP participation by unspecified donors is pivotal and should be facilitated. Mismatched compatible pairs should be counseled to optimize their HLA matching via KEP. Novel allocation strategies should be tested in simulation to predict the effects and evaluate consequences for subgroups of participants.

DATA AVAILABILITY STATEMENT

Original datasets are available upon request to the corresponding authors.

ETHICS STATEMENT

The studies involving humans were approved by the institutional research review board of Erasmus MC,

Rotterdam, Netherlands. The studies were conducted in accordance with the local legislation and institutional requirements. The ethics committee/institutional review board waived the requirement of written informed consent for participation from the participants or the participants' legal guardians/next of kin because of the high number of participants, the risk of consent bias, and a part of the participants not being alive anymore.

AUTHOR CONTRIBUTIONS

MFK: Collected the data, performed the analysis, and wrote the manuscript; MdK and AH: Participated in the research design, collected the data, and reviewed the manuscript; MB, HB, LB, MC, SH, MJ, MMK, JL, KP, CR, DR, ES, CV, JW, AZ: Participated in the data collection and reviewed the manuscript; TD, KG: Performed the analysis and reviewed the manuscript; JG: Collected the data and reviewed the manuscript; JR: Drafted the idea, participated in the research design, and reviewed the manuscript; AW: Drafted the idea, participated in the research design and data collection, wrote and reviewed the manuscript. All authors contributed to the article and approved the submitted version.

FUNDING

The author(s) declared that financial support was received for this work and/or its publication. This study was funded by the foundation "Een nier voor Jeroen" and the Dutch Kidney Foundation (grant number 23OM+009).

CONFLICT OF INTEREST

The author(s) declared that this work was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

GENERATIVE AI STATEMENT

The author(s) declared that generative AI was not used in the creation of this manuscript.

Any alternative text (alt text) provided alongside figures in this article has been generated by Frontiers with the support of artificial intelligence and reasonable efforts have been made to ensure accuracy, including review by the authors wherever possible. If you identify any issues, please contact us.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontierspartnerships.org/articles/10.3389/ti.2026.15423/full#supplementary-material>

REFERENCES

- Yang J, Endo Y, Munir MM, Wollesenbet S, Altaf A, Limkemann A, et al. Waitlist Time, Age, and Social Vulnerability: Impact on the Survival Benefit of Deceased Donor Kidney Transplantation Versus Long-Term Dialysis Among Patients With End-Stage Renal Disease. *Transplantation* (2025) 109:e64–e74. doi:10.1097/TP.0000000000005125
- Wolfe RA, Ashby VB, Milford EL, Ojo AO, Ettenger RE, Agodoa LY, et al. Comparison of Mortality in all Patients on Dialysis, Patients on Dialysis Awaiting Transplantation, and Recipients of a First Cadaveric Transplant. *N Engl J Med* (1999) 341:1725–30. doi:10.1056/NEJM199912023412303
- Rana MR, Knight SR, Maggiore U, Lafranca JA, Dor F, Pengel LHM. What Are the Benefits of Preemptive Versus Non-preemptive Kidney Transplantation? A Systematic Review and Meta-Analysis. *Transpl Rev (Orlando)* (2023) 37:100798. doi:10.1016/j.trre.2023.100798
- Hariharan S, Israni AK, Danovitch G. Long-Term Survival After Kidney Transplantation. *N Engl J Med* (2021) 385:729–43. doi:10.1056/NEJMra2014530
- Roodnat JL, Kal-van Gestel JA, Zuidema W, van Noord MAA, van de Wetering J, Ijzermans JNM, et al. Successful Expansion of the Living Donor Pool by Alternative Living Donation Programs. *Am J Transpl* (2009) 9:2150–6. doi:10.1111/j.1600-6143.2009.02745.x
- Ma Y, Man J, Gui H, Niu J, Yang L. Advancement in Preoperative Desensitization Therapy for ABO Incompatible Kidney Transplantation Recipients. *Transpl Immunol* (2023) 80:101899. doi:10.1016/j.trim.2023.101899
- de Weerd AE, Betjes MGH. ABO-Incompatible Kidney Transplant Outcomes: A Meta-Analysis. *Clin J Am Soc Nephrol* (2018) 13:1234–43. doi:10.2215/CJN.00540118
- Scurt FG, Ewert L, Mertens PR, Haller H, Schmidt BMW, Chatzikyrkou C. Clinical Outcomes After ABO-Incompatible Renal Transplantation: A Systematic Review and meta-analysis. *Lancet* (2019) 393:2059–72. doi:10.1016/S0140-6736(18)32091-9
- Rennie TJW, Battle RK, Abel AA, McConnell S, McLaren R, Phelan PJ, et al. Comparison of Kidney Transplant Outcomes in HLA Compatible and Incompatible Transplantation: A National Cohort Study. *Nephrology (Carlton)* (2022) 27:962–72. doi:10.1111/nep.14102
- Tramper T, Roelen DL, Brand-Schaaf SH, Kal-van Gestel JA, Kho MML, Reinders MEJ, et al. The Detrimental Effect of Donor-Specific Antibodies Is Irrespective of Its Level in Highly-Immunized Living Donor Kidney Transplant Recipients: A Case-Control Series. *Front Immunol* (2022) 13:1093359. doi:10.3389/fimmu.2022.1093359
- Orandi BJ, Luo X, Massie AB, Garonzik-Wang JM, Lonze BE, Ahmed R, et al. Survival Benefit With Kidney Transplants From HLA-Incompatible Live Donors. *N Engl J Med* (2016) 374:940–50. doi:10.1056/NEJMoa1508380
- Park K, Moon JI, Kim SI, Kim YS. Exchange Donor Program in Kidney Transplantation. *Transplantation* (1999) 67:336–8. doi:10.1097/00007890-199901270-00027
- Ferrari P, de Klerk M. Paired Kidney Donations to Expand the Living Donor Pool. *J Nephrol* (2009) 22:699–707. Available online at: <https://www.ovid.com/journals/joneph/abstract/00052477-200911000-00003~paired-kidney-donations-to-expand-the-living-donor-pool?redirectionsource=fulltextview>
- Ashlagi I, Bingaman A, Burq M, Manshadi V, Gamarnik D, Murphey C, et al. Effect of Match-Run Frequencies on the Number of Transplants and Waiting Times in Kidney Exchange. *Am J Transpl* (2018) 18:1177–86. doi:10.1111/ajt.14566
- Matyasi L, Biró P. Testing re-optimisation Strategies in International Kidney Exchange Programmes by the ENCKEP Simulator. *Cent Eur J Operations Res* (2024) 32:989–1011. doi:10.1007/s10100-023-00880-2
- Druzsins K, Biró P, Klimentova X, Fleiner R. Performance Evaluation of National and International Kidney Exchange Programmes With the ENCKEP Simulator. *Cent Eur J Operations Res* (2024) 32:923–43. doi:10.1007/s10100-024-00914-3
- Cheng Y, Yang Z. Efficient Kidney Exchange With Dichotomous Preferences. *J Health Econ* (2021) 80:102536. doi:10.1016/j.jhealeco.2021.102536
- Ashlagi I, Cseh Á, Manlove D, Ockenfels A, Pettersson W. Designing a Kidney Exchange Program in Germany: Simulations and Recommendations. *Cent Eur J Operations Res* (2024) 33:1529–55. doi:10.1007/s10100-024-00933-0
- de Klerk M, Keizer KM, Claas FH, Witvliet M, Haase-Kromwijk BJ, Weimar W. The Dutch National Living Donor Kidney Exchange Program. *Am J Transpl* (2005) 5:2302–5. doi:10.1111/j.1600-6143.2005.01024.x
- Ferrari P, Weimar W, Johnson RJ, Lim WH, Tinkam KJ. Kidney Paired Donation: Principles, Protocols and Programs. *Nephrol Dial Transpl* (2015) 30:1276–85. doi:10.1093/ndt/gfu309
- Biró P, van de Klundert J, Manlove D, Pettersson W, Andersson T, Burnapp L, et al. Modelling and Optimisation in European Kidney Exchange Programmes. *Eur J Oper Res* (2021) 291:447–56. doi:10.1016/j.ejor.2019.09.006
- Glorie K, Haase-Kromwijk B, van de Klundert J, Wagelmans A, Weimar W. Allocation and Matching in Kidney Exchange Programs. *Transpl Int* (2014) 27:333–43. doi:10.1111/tri.12202
- Glorie K, Xiao G, van de Klundert J. The Health Value of Kidney Exchange and Altruistic Donation. *Value Health* (2022) 25:84–90. doi:10.1016/j.jval.2021.07.012
- Glorie KM, de Klerk M, Wagelmans AP, van de Klundert JJ, Zuidema WC, Claas FHJ, et al. Coordinating Unspecified Living Kidney Donation and Transplantation Across the Blood-Type Barrier in Kidney Exchange. *Transplantation* (2013) 96:814–20. doi:10.1097/tp.0b013e3182a132b7
- Chipman V, Cooper M, Thomas AG, Ronin M, Lee B, Flechner S, et al. Motivations and Outcomes of Compatible Living Donor-Recipient Pairs in Paired Exchange. *Am J Transpl* (2022) 22:266–73. doi:10.1111/ajt.16821
- Gentry SE, Segev DL, Simmerling M, Montgomery RA. Expanding Kidney Paired Donation Through Participation by Compatible Pairs. *Am J Transpl* (2007) 7:2361–70. doi:10.1111/j.1600-6143.2007.01935.x
- Duus WI, Andersson T, Birna Andrésdóttir M, Bengtsson M, Biglarnia A, Bistrup C, et al. ScandiTransplant Exchange Program (STEP): Development and Results From an International Kidney Exchange Program. *Transpl Direct* (2023) 9:e1549. doi:10.1097/TXD.0000000000001549
- Stepkowski SM, Mierzejewska B, Fumo D, Bekbolsynov D, Khuder S, Baum CE, et al. The 6-Year Clinical Outcomes for Patients Registered in a Multiregional United States Kidney Paired Donation Program - A Retrospective Study. *Transpl Int* (2019) 32:839–53. doi:10.1111/tri.13423
- Cantwell L, Woodroffe C, Holdsworth R, Ferrari P. Four Years of Experience With the Australian Kidney Paired Donation Programme. *Nephrology (Carlton)* (2015) 20:124–31. doi:10.1111/nep.12369
- Cole EH, Nickerson P, Campbell P, Yetzer K, Lahaie N, Zaltzman J, et al. The Canadian Kidney Paired Donation Program: A National Program to Increase Living Donor Transplantation. *Transplantation* (2015) 99:985–90. doi:10.1097/TP.0000000000000455
- Roodnat JI, van de Wetering J, Claas FH, Ijzermans J, Weimar W. Persistently Low Transplantation Rate of ABO Blood Type O and Highly Sensitized Patients Despite Alternative Transplantation Programs. *Transpl Int* (2012) 25:987–93. doi:10.1111/j.1432-2277.2012.01526.x
- Liu W, Melcher ML. Matching Kidneys With Priority in Kidney Exchange Programs. *Med Decis Making* (2019) 39:335–45. doi:10.1177/0272989X19849457
- Bray M, Wang W, Song PX, Kalbfleisch JD. Valuing Sets of Potential Transplants in a Kidney Paired Donation Network. *Stat Biosci* (2018) 10:255–79. doi:10.1007/s12561-018-9214-7
- Ferrari P, Cantwell L, Ta J, Woodroffe C, D'Orsogna L, Holdsworth R. Providing Better-Matched Donors for HLA Mismatched Compatible Pairs Through Kidney Paired Donation. *Transplantation* (2017) 101:642–8. doi:10.1097/TP.0000000000001196
- Tafulo S, Malheiro J, Dias L, Lobato L, Ramalheite L, Martinho A, et al. Improving HLA Matching in Living Donor Kidney Transplantation Using Kidney Paired Exchange Program. *Transpl Immunol* (2020) 62:101317. doi:10.1016/j.trim.2020.101317
- Basu A, Prieto M, Kosberg C, Mai ML, Khamash HA, Jadlowiec CC, et al. Ten Years of Kidney Paired Donation at Mayo Clinic: The Benefits of Incorporating ABO/HLA Compatible Pairs. *Transplantation* (2020) 104:1229–38. doi:10.1097/TP.0000000000002947
- Biró P, Haase-Kromwijk B, Andersson T, Ásgeirsson EI, Baltesová T, Boletis I, et al. Building Kidney Exchange Programmes in Europe-An Overview of Exchange Practice and Activities. *Transplantation* (2019) 103:1514–22. doi:10.1097/TP.0000000000002432
- de Klerk M, Kal-van Gestel JA, van de Wetering J, Kho ML, Middel-de Sterke S, Betjes MGH, et al. Creating Options for Difficult-To-Match Kidney Transplant Candidates. *Transplantation* (2021) 105:240–8. doi:10.1097/TP.0000000000003203

39. de Klerk M, Kal-van Gestel JA, Roelen D, Betjes MGH, de Weerd AE, Reinders MEJ, et al. Increasing Kidney-Exchange Options Within the Existing Living Donor Pool With CIAT: A Pilot Implementation Study. *Transpl Int* (2023) 36:11112. doi:10.3389/ti.2023.11112
40. Sullivan HC, Gebel HM, Bray RA. Understanding Solid-phase HLA Antibody Assays and the Value of MFI. *Hum Immunol* (2017) 78:471–80. doi:10.1016/j.humimm.2017.05.007
41. Oguz SR, Sinangil A, Barlas S, Ciftci HS, Ulusoy E, İzgi DK, et al. Correlation of Luminex-Based Single Antigen Based Results With Complement-Dependent Cytotoxicity Crossmatch and Flow Cytometry Crossmatch Results: A Single-Center Experience from Istanbul. *Transpl Proc* (2023) 55:303–8. doi:10.1016/j.transproceed.2023.02.005
42. Pandey P, Pande A, Mandal S, Marik A, Devra AK, Sinha VK, et al. Detection of Donor-Specific HLA Antibodies: A Retrospective Observation in 350 Renal Transplant Cases. *Transpl Immunol* (2023) 77:101783. doi:10.1016/j.trim.2022.101783
43. Klaassen MF, de Klerk M, Kal-van Gestel JA, Kho MML, van de Wetering J, Al Fatly Z, et al. Blood Group Antibody Titers in Long-Waiting Candidates for ABO-Incompatible Kidney Exchange Program Transplantation: A Retrospective Cohort Study. *Dutch Transpl Soc 'Bootcongres' 2024, 35th Annu Meet Dutch Transpl Found Utrecht, The Neth* (2024). Available online at: <https://www.transplantatievereniging.nl/wp-content/uploads/2024/03/Programma-Bootcongres-2024-def-2.pdf>.
44. NHS Blood and Transplant. Annual Report on Living Donor Kidney Transplantation: Report for 2023/2024 (2009).
45. Manook M, Johnson R, Robb M, Burnapp L, Fuggle SV, Mamode N. Changing Patterns of Clinical Decision Making: Are Falling Numbers of Antibody Incompatible Transplants Related to the Increasing Success of the UK Living Kidney Sharing Scheme? A National Cohort Study. *Transpl Int* (2021) 34:153–62. doi:10.1111/tri.13776
46. Heidt S, Haasnoot GW, van der Linden-van Oevelen MJH, Claas FHJ. Highly Sensitized Patients Are Well Served by Receiving a Compatible Organ Offer Based on Acceptable Mismatches. *Front Immunol* (2021) 12:687254. doi:10.3389/fimmu.2021.687254
47. Schinstock CA, Smith BH, Montgomery RA, Jordan SC, Bentall AJ, Mai M, et al. Managing Highly Sensitized Renal Transplant Candidates in the Era of Kidney Paired Donation and the New Kidney Allocation System: Is There Still a Role for Desensitization? *Clin Transpl* (2019) 33:e13751. doi:10.1111/ctr.13751
48. Mamode N, Bestard O, Claas F, Furian L, Griffin S, Legendre C, et al. European Guideline for the Management of Kidney Transplant Patients with HLA Antibodies: By the European Society for Organ Transplantation Working Group. *Transpl Int* (2022) 35:10511. doi:10.3389/ti.2022.10511
49. Pham TA, Lee JI, Melcher ML. Kidney Paired Exchange and Desensitization: Strategies to Transplant the Difficult to Match Kidney Patients with Living Donors. *Transpl Rev (Orlando)* (2017) 31:29–34. doi:10.1016/j.trre.2017.01.003
50. Jackson KR, Segev DL. Rethinking Incompatibility in Kidney Transplantation. *Am J Transpl* (2022) 22:1031–6. doi:10.1111/ajt.16826
51. Mattoo A, Jaffe IS, Keating B, Montgomery RA, Mangiola M. Improving Long-Term Kidney Allograft Survival by Rethinking HLA Compatibility: From Molecular Matching to Non-HLA Genes. *Front Genet* (2024) 15:1442018. doi:10.3389/fgene.2024.1442018
52. Niemann M, Lachmann N, Geneugelijk K, Spierings E. Computational Eurotransplant Kidney Allocation Simulations Demonstrate the Feasibility and Benefit of T-Cell Epitope Matching. *Plos Comput Biol* (2021) 17:e1009248. doi:10.1371/journal.pcbi.1009248
53. Mumford L, Fuggle SV, Martorell J, Slavcev A, Iniotaki A, Haasnoot GW, et al. A Europe Wide Acceptable Mismatch Program Will Enable Transplantation of Long Waiting Highly Sensitized Patients With a Compatible Donor. *Transpl Immunol* (2021) 64:101354. doi:10.1016/j.trim.2020.101354
54. Valentin MO, Garcia M, Costa AN, Bolotinha C, Guirado L, Vistoli F, et al. International Cooperation for Kidney Exchange Success. *Transplantation* (2019) 103:e180–e1. doi:10.1097/TP.0000000000002664
55. Roodnat JI, Zuidema W, Van De Wetering J, de Klerk M, Erdman RAM, Massey EK, et al. Altruistic Donor Triggered Domino-Paired Kidney Donation for Unsuccessful Couples from the Kidney-Exchange Program. *Am J Transpl* (2010) 10:821–7. doi:10.1111/j.1600-6143.2010.03034.x
56. Gentry SE, Montgomery RA, Swihart BJ, Segev DL. The Roles of Dominos and Nonsimultaneous Chains in Kidney Paired Donation. *Am J Transpl* (2009) 9:1330–6. doi:10.1111/j.1600-6143.2009.02622.x
57. Osburn N, Thomas AG, Ronin M, Cooper M, Flechner SM, Segev DL, et al. The Benefit to Waitlist Patients in a National Paired Kidney Exchange Program: Exploring Characteristics of Chain End Living Donor Transplants. *Am J Transpl* (2022) 22:113–21. doi:10.1111/ajt.16749
58. Verma U, Rangaraj N, Billa V, Usulumarty D. Long Term Simulation Analysis of Deceased Donor Initiated Chains in Kidney Exchange Programs. *Health Syst (Basingstoke)* (2024) 13:121–32. doi:10.1080/20476965.2023.2197023
59. Furian L, Nicolò A, Di Bella C, Cardillo M, Cozzi E, Rigotti P. Kidney Exchange Strategies: New Aspects and Applications With a Focus on Deceased donor-initiated Chains. *Transpl Int* (2020) 33:1177–84. doi:10.1111/tri.13712
60. Combe J, Hiller V, Tercieux O, Audry B, Baudet J, Malaquin G, et al. [Perspectives for Future Development of the Kidney Paired Donation Programme in France] Perspectives Pour Une Évolution Du Programme De Don Croisé De Reins En France. *Nephrol Ther* (2022) 18:270–7. doi:10.1016/j.nephro.2022.02.001
61. Wang W, Rees MA, Leichtman AB, Song PXX, Bray M, Ashby VB, et al. Deceased Donors as Nondirected Donors in Kidney Paired Donation. *Am J Transpl* (2021) 21:103–13. doi:10.1111/ajt.16268
62. Tambur AR, Campbell P, Claas FH, Feng S, Gebel HM, Jackson AM, et al. Sensitization in Transplantation: Assessment of Risk (STAR) 2017 Working Group Meeting Report. *Am J Transpl* (2018) 18:1604–14. doi:10.1111/ajt.14752

Copyright © 2026 Klaassen, de Klerk, Baas, Bouwsma, Bungener, Christiaans, Dollevoet, Glorie, Heidt, Hemke, de Jong, Kal-van Gestel, Kho, Langereis, van der Pant, Ranzijn, Roelen, Spierings, Voorter, van de Wetering, van Zuilen, Roodnat and de Weerd. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.



No Kidney Left Behind: Rescuing Unused Donor Kidneys for Transplant at the First Centralized Assessment and Repair Center

Christopher L. Jaynes^{1,2*†}, William C. Goggins^{3†}, Matthew L. Holzner^{4†},
Jacqueline Garonzik-Wang^{5†} and Henri G. D. Leuvenink^{1,2†}

¹Department of Surgery-Organ Donation and Transplantation, University Medical Center Groningen, Groningen, Netherlands, ²34 Lives, Public Benefit Company, West Lafayette, IN, United States, ³Division of Transplant Surgery, Department of Surgery, Indiana University School of Medicine, Indianapolis, IN, United States, ⁴Recanti/Miller Transplantation Institute, Icahn School of Medicine at Mount Sinai, New York, NY, United States, ⁵University of Wisconsin Department of Surgery, Madison, WI, United States

OPEN ACCESS

*Correspondence

Christopher L. Jaynes,
✉ c.jaynes@umcg.nl

†ORCID:

Christopher L. Jaynes
orcid.org/0000-0001-8214-2288
William C. Goggins
orcid.org/0000-0003-4296-9568
Matthew L. Holzner
orcid.org/0000-0002-4935-9968
Jacqueline Garonzik-Wang
orcid.org/0000-0002-2789-7503
Henri G. D. Leuvenink
orcid.org/0000-0001-5036-2999

Received: 13 August 2025

Revised: 05 October 2025

Accepted: 10 November 2025

Published: 28 November 2025

Citation:

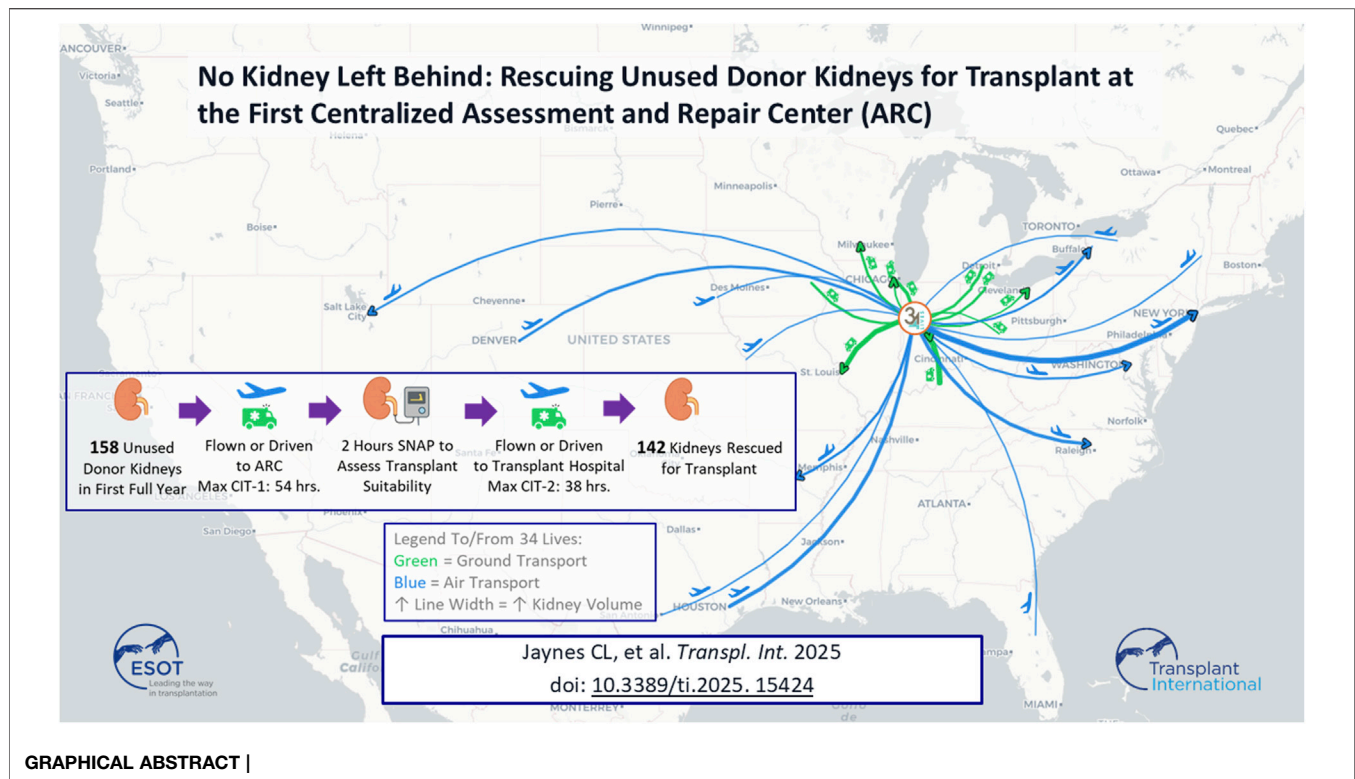
Jaynes CL, Goggins WC, Holzner ML, Garonzik-Wang J and Leuvenink HGD (2025) No Kidney Left Behind: Rescuing Unused Donor Kidneys for Transplant at the First Centralized Assessment and Repair Center. *Transpl. Int.* 38:15424. doi: 10.3389/ti.2025.15424

Rescue of non-used kidneys may be facilitated using sub-normothermic acellular machine perfusion (SNAP), which can prolong safe preservation times and provide additional viability assessment. While blood-based normothermic machine perfusion (NMP) has been utilized internationally, barriers to adoption of NMP in the US include limited availability, staffing and facility resource limitations, geographic distances between donor and recipient hospitals, blood shortages, and reliance on hypothermic machine perfusion (HMP). To overcome obstacles to adoption, the first centralized kidney Assessment and Repair Center (ARC) was established in West Lafayette, Indiana. Organized as an independent public benefit company, this center was designed to provide sub-normothermic acellular perfusion (SNAP) and assessment services to rescue unused/hard-to-place (HTP) kidneys. An acellular, human serum albumin-based perfusate was chosen, and a second cold ischemic time (CIT) was validated during pre-clinical testing. Between April 2024- May 2025, 158 unused deceased donor kidneys were transported to the ARC, resulting in 142 transplants (90%) after SNAP assessment. SNAP is feasible when performed by a centralized ARC and reduces kidney discard rates by providing objective viability assessment data. Moreover, SNAP allows for extended preservation times of nearly 70 h, enabling improved logistical planning and broader sharing of deceased donor kidneys.

Keywords: normothermic perfusion, NMP, ex vivo kidney, ARC, SNAP

INTRODUCTION

Kidney transplantation is a life-saving therapy for patients with end stage renal disease. As such, there are nearly 100,000 patients registered on the national kidney transplant waitlist in the US, with an average of 34 patients removed daily due to either dying or becoming too sick to receive a transplant. Due to the critical shortage of donor organs, kidneys are increasingly retrieved from non-ideal donors (older, more co-morbidities, etc.), leading to more uncertainty about organ quality, and resulting in escalating numbers of unused kidneys by the transplant centers (Figure 1) [1, 2].



GRAPHICAL ABSTRACT |

Hard-to-place kidneys (HTP) have been previously defined as those accepted in the United Network for Organ Sharing (UNOS) match run after being refused at least 165 times by transplant centers. These kidneys have statistically higher donor risk profiles including higher rates of the following: Donor Age >50, Death by cerebrovascular accident (CVA), diabetes, hypertension, Donation after Circulatory Death (DCD), Kidney Donor Profile Index (KDPI) >85%, cold ischemia time (CIT) >24 h, terminal serum creatinine ≥ 1.5 mg/dL, and static cold storage preservation [3]. While many of the factors common to HTP kidneys cannot be changed (e.g., donor age, KDPI, cause of death), factors such as preservation technique and transportation time, are modifiable and may be able to decrease HTP kidney non-utilization rates.

In addition to addressing the modifiable factors, post-procurement functional assessment would help to alleviate uncertainties about HTP kidneys. Unfortunately, the depressed metabolic rate at hypothermic temperatures using static ice or hypothermic machine perfusion (HMP) preservation does not permit comprehensive organ assessment. The few assessment parameters available during HMP include internal renal resistance (IRR), pressure, and flow rate, which have been shown to have varied associations with kidney allograft function [4–6].

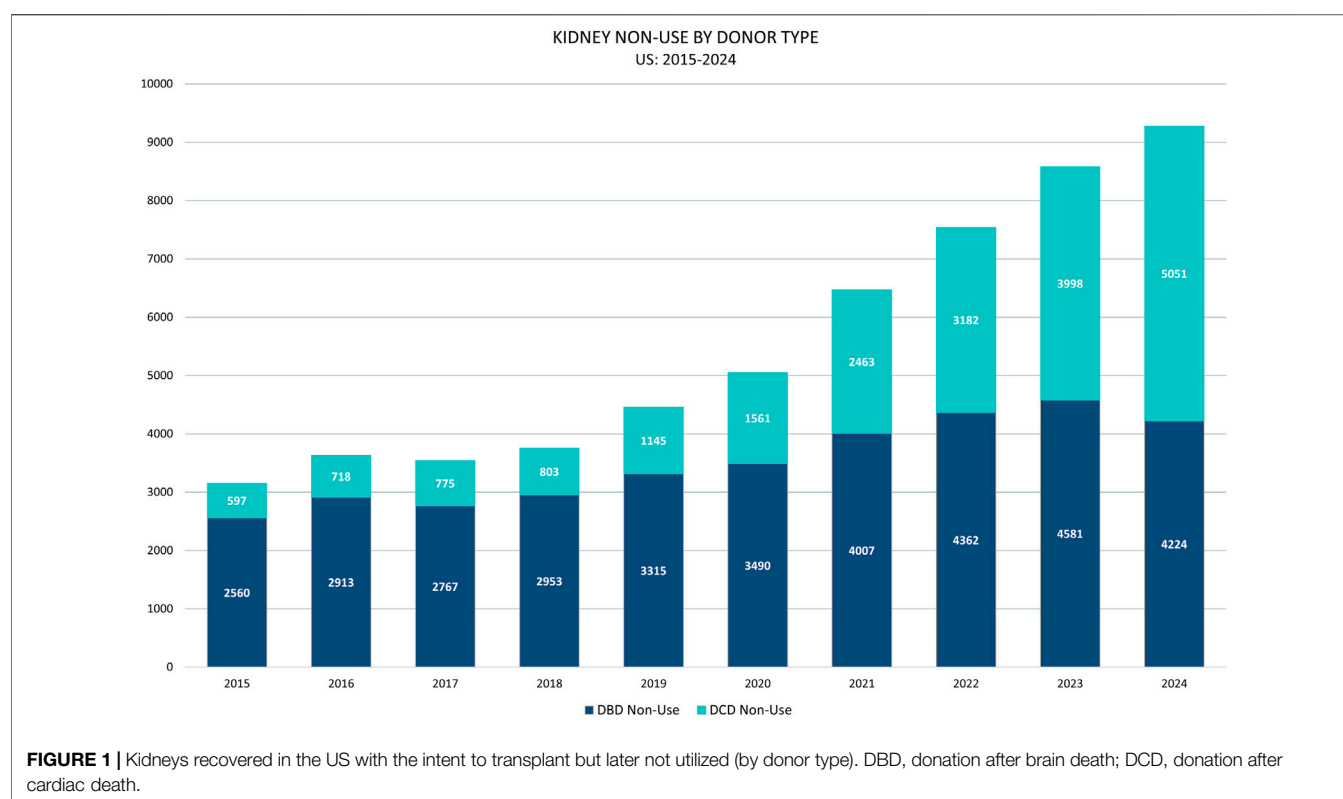
However, at temperatures approaching normothermia, kidney metabolism increases, and functional parameters can be assessed similarly as if the kidney was still *in vivo* in contrast to the other common preservation strategies (Table 1). The largest advancements in normothermic machine perfusion

(NMP) over the past decade have been made for lung, heart, and liver perfusion, contributing to an increased use of these organs for transplantation by 50% or more [7–9]. However, the use of kidney NMP has been limited, in part because of its higher tolerance to cold ischemia, logistical barriers, and widespread adoption of HMP [10]. Currently, the published clinical NMP experience comes from international transplant centers entirely outside of the US. Due to the increased complexities required to maintain organs on NMP, it has been suggested that regional organ assessment and repair centers (ARCs) may be the most effective and efficient in providing this specialized modality [11]. Here, we present our early experience with the initiation and implementation of the first US ARC.

MATERIALS AND METHODS

Facility

In 2022, a Public Benefit Company (“34 Lives, PBC”) was formed which built the first independent center in West Lafayette, Indiana, offering renal Sub-normothermic Acellular Perfusion (SNAP) assessment services to transplant hospitals and Organ Procurement Organizations (OPOs) to investigate the feasibility of an ARC in the US. This site was strategically chosen due to its proximity to Purdue University, a school with a strong reputation for engineering and an on-campus airport, but without a medical school or hospital, making it an ideal neutral location. In addition, the



geographic location makes it possible to reach 80% of the US population within a 12-h drive, or 2.5-h flight.

Perfusion Equipment

A two-room organ rescue laboratory (ORL) was constructed, in compliance with standard hospital operating room air exchanges and HEPA filtration, capable of providing SNAP to 4 kidneys simultaneously. The equipment platform has been described previously [12]. Briefly, a perfusion system was designed using a pediatric cardiopulmonary bypass

circuit, consisting of a centrifugal pump (Bio-pump, Medtronic) and a membrane oxygenator with integrated heat exchanger (Affinity Pixie, Medtronic). The hardware included a speed controller, flow transducer, temperature probe (Bio-Console 560, Medtronic), and a heater/cooler (HCU30, Jostra).

Sub-Normothermic Acellular Perfusion (SNAP) Protocol

After a thorough pre-clinical evaluation was carried out testing over 100 discarded human kidneys against several published NMP protocols [13–15], the SNAP protocol described by Minor *et al.* [16] was selected based on ease of use and repeatability of results. One of the major advantages of this protocol was the use of a non-blood-based, acellular perfusate, which is a safe alternative to erythrocyte-based perfusates at supraphysiological oxygen partial pressures [17]. Following the Minor protocol, the kidney is gently warmed from 8 °C to 35 °C using the oxygenated SNAP perfusate (composition, **Table 2**). At 90 min of SNAP, the kidney reaches its maximum temperature of 35 °C during which the first assessment occurs. A second assessment is completed after 120 min, allowing the observation of trends between timepoints. Kidneys were assessed for transplant suitability using a variety of validated and non-validated assessment parameters, including the Hosgood score [18], comprised of renal blood flow, urine output, and macroscopic appearance. Infrared thermal temperature measurements were taken to determine uniform

TABLE 1 | Functional assessment data provided by kidney preservation type.

Comparison between kidney preservation methods			
Renal Biomarker	SCS (4 °C)	HMP (4 °C)	SNAP (35 °C)
Renal flow rate		✓	✓
Renal pressure		✓	✓
Renal resistance		✓	✓
Perfusate pO ₂ /pCO ₂ /pH			✓
Urine pO ₂			✓
TCO ₂ /HCO ₃ ⁻ /Base excess			✓
Perfusate Na ⁺ /K ⁺ /Cl ⁻			✓
Urine Na ⁺ /K ⁺ /Cl ⁻			✓
Lactate/Glucose			✓
AST (marker of mitochondrial death)			✓
Oxygen consumption			✓
Hosgood transplant suitability score			✓

SCS, Static Cold Storage; HMP, Hypothermic Machine Perfusion; SNAP, Sub-normothermic Acellular Perfusion.

TABLE 2 | Sub-normothermic Acellular Perfusion (SNAP) perfusate composition.

Snap perfusate ingredient	Amount
Calcium chloride dihydrate	0.33 g
Calcium gluconate	0.74 g
Cefazolin	1 g
D (+) glucose monohydrate	1.09 g
Dextran 40	5 g
Human albumin	70 g
Magnesium dichloride hexahydrate	0.12 g
Potassium chloride	0.54 g
Sodium bicarbonate	1.89 g
Sodium chloride	9.7 g
Sodium dihydrogen phosphate dihydrate	9.4 mg
Sodium hydroxide (1M)	pH 7.4
Verapamil	5 mg
Water for injection (WFI)	Fill to 2 L

perfusion, instead of relying solely on macro appearance, as a surrogate due to the clear acellular perfusate.

Clinical Kidney Rescue

Clinical SNAP procedures began in April 2024, soon after the preclinical validation phase completed, and were allowed to proceed under the oversight of a Central Institutional Review Board (IRB). Three initial transplant hospitals (Indiana University Health, Mt. Sinai, and University of Wisconsin) piloted the centralized ARC service, ensuring both logistics and safety issues were appropriately considered. During this phase, HTP donor kidneys were offered to the ARC by OPOs after exhausting standard allocation efforts. Kidneys were subjected to pre-screening by the participating transplant program surgeons and nephrologists prior to accepting for SNAP assessment. Those with positive serologies (HIV, HCV, HBV), not on HMP, or with anatomical issues preventing SNAP were not evaluated. Beginning in October 2024, after the pilot phase, a total of 12 transplant hospitals, representing both large and small volume centers, participated and accepted SNAP-assessed kidneys through the remaining first year of ARC

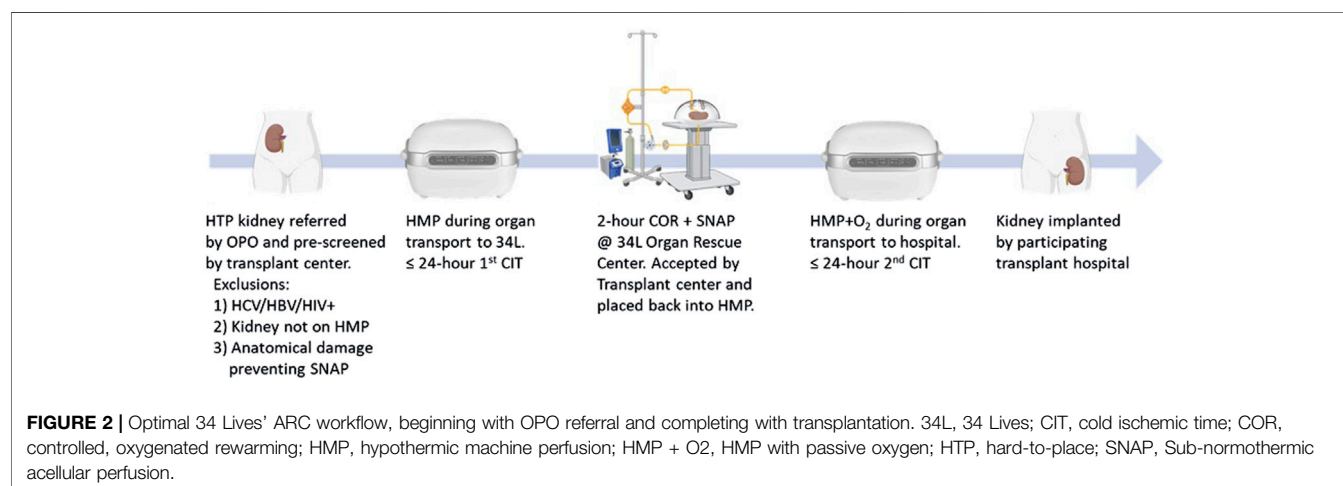
operation, ending in May 2025. After 2 hours of SNAP, kidneys with a Hosgood score of 1–3 (out of 5) were considered suitable for transplant, repackaged into a LifePort HMP device (ORS) using the passive bubble-oxygenated cassette pre-filled with Kidney Preservation Solution (KPS-1, ORS), and driven or flown by charter aircraft to the accepting transplant hospital (**Figure 2**). Each participating transplant hospital followed their own standard protocols for kidney implantation and immunosuppression. We hypothesized that sending HTP kidneys to a centralized ARC assessment facility utilizing peer-reviewed clinical SNAP protocols would result in a 50% or more success rate for allocation of these kidneys to a transplant center.

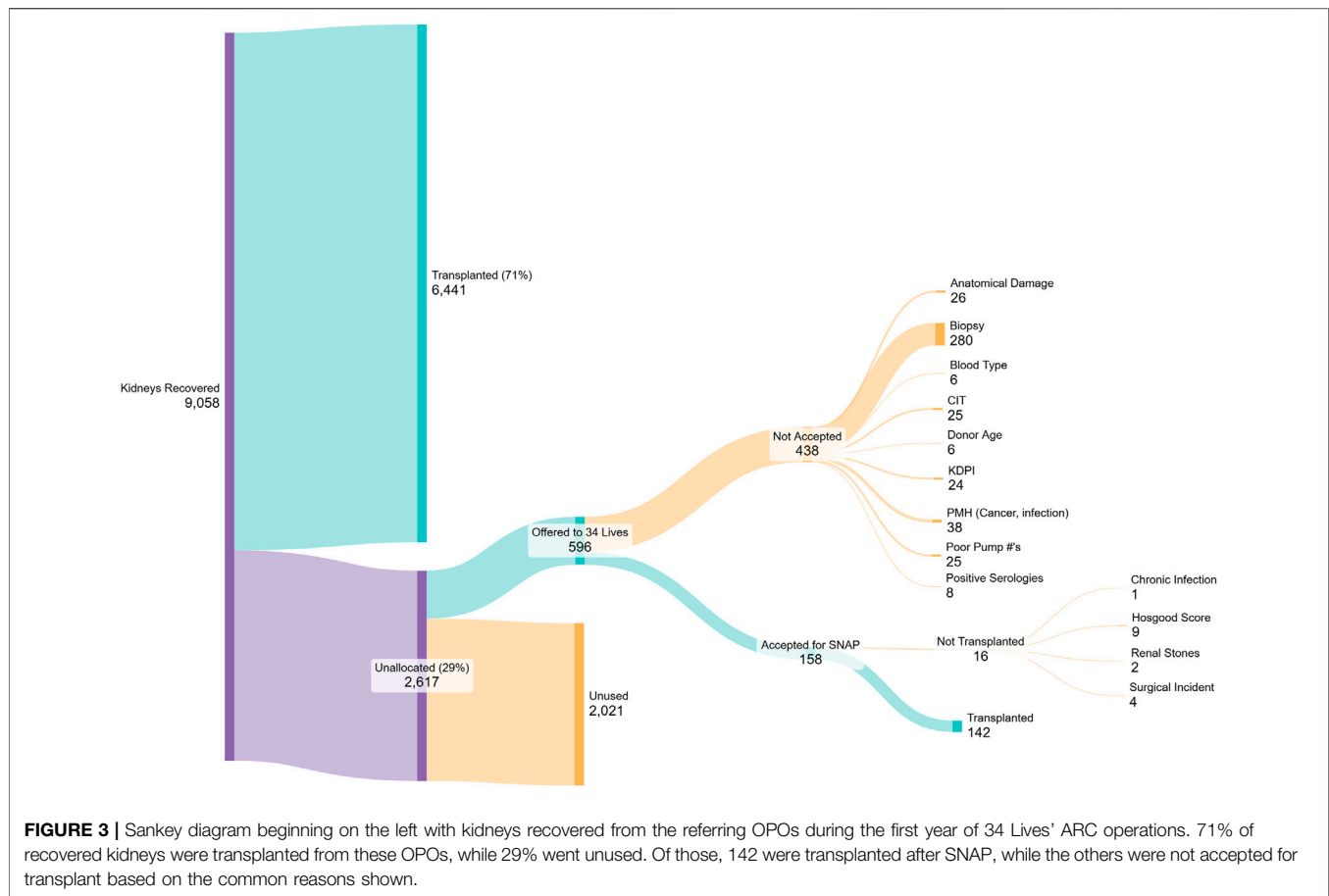
Statistical Analysis

Descriptive statistics are presented as means, standard deviations, medians and ranges for the continuous variables and as frequencies and percents for categorical variables. A two-sided alpha level of 5% and 95% confidence intervals were used for all subsequent descriptive comparisons and analyses, unless otherwise stated. Demographics and baseline characteristics are summarized for all donors in the study group and overall point estimates and corresponding 95% confidence intervals. The study hypothesis was tested using a one-sample proportion z test to determine if the rate of allocation is different from 50%, with a 95% confidence interval constructed around the observed allocation rate. A p-value <0.05 is considered significant. GraphPad Prism version 10.5.0 software (La Jolla, California, USA) was used for analysis.

Outcome Variables and Definitions

All outcome data was collected in a prospective manner. Donor variables were shared under a data use agreement by the participating OPOs via encrypted email and/or via DonorNet. CIT-1 is defined as the time from donor cross clamp to the beginning of SNAP. CIT-2 begins after SNAP stops until reperfusion in the recipient. Total out of body time starts at donor cross clamp and ends at recipient reperfusion. Additional variables collected at 90 (T90) and 120 (T120) minutes included arterial and venous blood gas samples, urine output (UO), renal





blood flow (RBF) intrarenal resistance (IRR), aspartate aminotransferase (AST) as a marker of mitochondrial dysfunction [19], and macroscopic kidney appearance (pink, patchy, mottled).

RESULTS

HTP Kidney Acceptance

Out of 158 HTP donor kidneys accepted and assessed with SNAP during the first full year, 142 (90%) were determined suitable by the accepting transplant physician and transplanted, surpassing the primary hypothesis of a 50% success rate. The median sequence number on the UNOS waiting list for patients receiving a SNAP rescued kidney was 3,302 (mean 5,164), further confirming that these kidneys were HTP. Reasons for non-acceptance and non-use can be found in **Figure 3**.

Donor Characteristics

Donor Characteristics are summarized in **Table 3**. Of note, more donors were females (56%), donating after circulatory death (DCD, 62%) due to anoxia (58%), with past medical histories including

hypertension (61%), smoking (74%), and a median KDPI of 71% (range 19%–98%).

HMP and SNAP Characteristics Total Preservation Time

In this protocol, there are two distinct periods of CIT which flank the SNAP assessment period. The first CIT (CIT-1) starts at donor cross clamp and ends when SNAP begins. Median CIT-1 was 21.15 h (range 10.45–54.37 h) prior to SNAP. CIT-2 starts when the kidney begins cool-down after SNAP for a second round of HMP and ends at kidney reperfusion in the recipient. Median CIT-2 was 14.5 h (range 2.87–37.75 h). The median time that HTP kidneys were preserved *ex vivo* using a combination of HMP and SNAP was 39.78 h (range 23–67.78 h). **Figure 4** depicts the distribution frequency of total out-of-body preservation times.

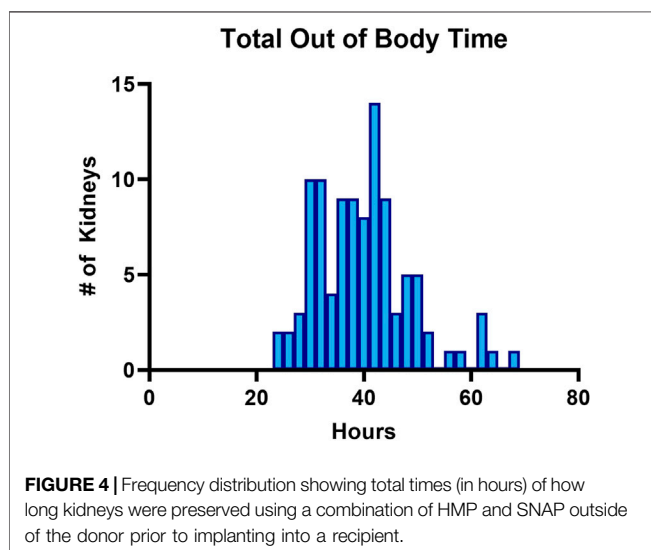
HMP Changes

The median IRR during HMP and prior to SNAP was 0.25 (range 0.07–1.03) with median flow rates of 106 mL/min (range 33–185). After SNAP, the compliance improved significantly (IRR 0.15, $p < 0.0001$).

TABLE 3 | Donor characteristics (n = 142).

Age (median years, range)	53 (15–73)
Sex (n, %)	
Males	62 (44%)
Females	80 (56%)
BMI (median kg/m ² , range)	30.63 (17.7–58.5)
Kidney laterality (n, %)	
Left	67 (47%)
Right	75 (53%)
Donor type (n, %)	
DBD	54 (38%)
DCD (no NRP)	57 (40%)
DCD + NRP	31 (22%)
Comorbidities (n, %)	
HTN	86 (61%)
Diabetes: A1C ≥6.5%	19 (13%)
Smoker	105 (74%)
Cause of death (n, %)	
Anoxia	82 (58%)
CVA/ICH	38 (27%)
Head trauma	19 (13%)
Other	3 (2%)
Inotropes (n, %)	
0 vasopressors	32 (22%)
1 vasopressor	110 (77%)
2 vasopressors	61 (43%)
3+ vasopressors	20 (14%)
Biopsies	
Glomerular sclerosis (median %, range)	7 (0–71)
Vascular changes (mild: 10%–24%)	39 (32.5%)
Vascular changes (moderate: 25%–50%)	2 (1.7%)
ATN (n, %)	13 (11%)
Creatinine (mg/dL; median, range)	
Peak	1.91 (0.5–11.09)
Terminal	1.24 (0.4–8.6)
KDPI (median %, range)	71 (19–98)

Unless otherwise specified, variables are expressed as actual counts. A1C, glycated hemoglobin; ATN, acute tubular necrosis; BMI, body mass index; DBD, donation after brain death; DCD, donation after cardiac death; CIT, cold ischemic time; CVA, cerebral vascular accident; ICH, intra-cerebral hemorrhage; HTN, hypertension; KDPI, kidney donor profile index; NRP, normothermic regional perfusion.



SNAP Changes

Once the temperature reached 30 °C, the kidney vasculature started to release a visible red effluent as shown in **Figure 5**. This phenomenon occurred in all HTP kidneys assessed during SNAP, despite a standard donor flush with UW or HTK solution and transport of up to 54 h on an HMP device. **Table 4** summarizes the assessment characteristics used to determine transplant suitability. Statistically significant ($p < 0.0001$) improvement in median assessment parameters at 90- and 120-min during SNAP includes flow rate (20% higher), IRR (15% lower), urine output (115% higher), oxygen consumption (16% higher), and mean Hosgood Score (14% lower). Statistically significant functional changes in median glucose (3% drop), lactate (120% increase), AST (19% increase) and weight gain (3% increase, $p = 0.0009$) were also observed, but did not thwart transplantation.

Centralized ARC Transportation Logistics

HTP kidneys accepted for SNAP with ≤6-h ground times to the 34 Lives' ARC were generally driven by medical couriers on LifePort HMP devices. If >6-h ground times were necessary, kidneys were typically flown by charter aircraft due to the commercial airline restrictions for liquids and lithium-ion batteries, common to HMP devices. During the first full year, 37 (26%) of the HTP kidneys were flown a total of 35,579 miles to the 34 Lives' ARC from the originating OPOs, while 105 (74%) were driven a combined 22,622 miles. Similarly, from the 34 Lives' ARC to the accepting transplant center, ground times ≤6-h were driven and >6-h were flown by charter aircraft to minimize CIT-2. During this time, 77 (54%) of the SNAP kidneys determined suitable for transplantation were flown from the 34 Lives' ARC to the accepting transplant centers for a total of 54,698 miles, while 65 (46%) were driven a combined 12,409 miles. One kidney in this study had a 6-h delay arriving at the 34 Lives' ARC due to the driver hitting a deer *en route*, but the kidney arrived safely and was successfully utilized after SNAP.

DISCUSSION

Novel solutions are needed to address the escalating US kidney discard rate to ensure that suitable kidneys are safely transplanted. While many unused kidneys cannot be rescued due to anatomical issues and donor histories (e.g., uncontrolled HTN, diabetes, cancers, infections), a meaningful number can be saved for transplantation following SNAP assessment. Our centralized ARC facilitated transplantation of 142/158 (90%) unused/HTP kidneys after SNAP during the first year of clinical operation, surpassing the initial 50% success rate hypothesis. This demonstrates feasibility and the promise for increased access to kidneys for thousands of patients awaiting transplantation.

In 2017, the National Kidney Foundation (NKF) convened a meeting of key opinion leaders with diverse backgrounds

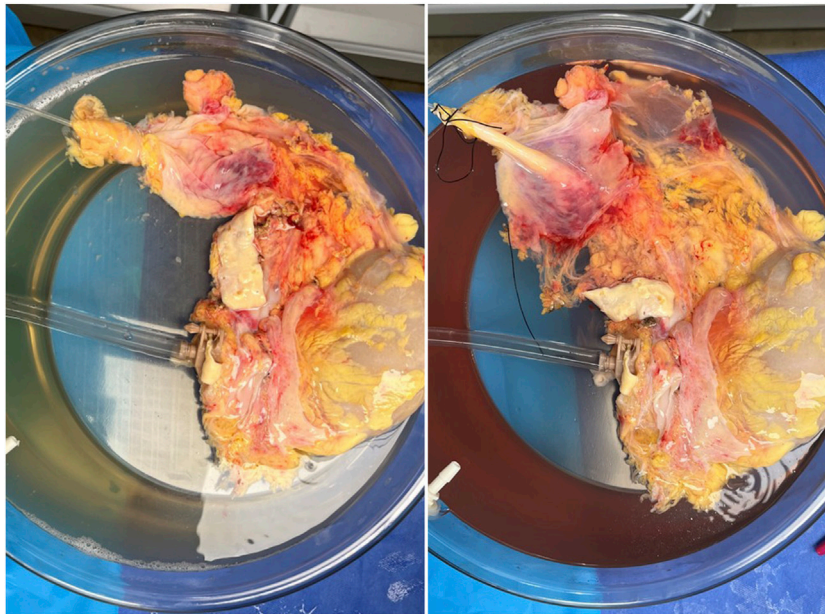


FIGURE 5 | Typical donor kidney at start (left) and end (right) of SNAP. As the kidney warms past 30 °C, the perfusate color turns from amber to red as the vasculature becomes more compliant.

TABLE 4 | HMP and SNAP characteristics (n = 142).

	Pre-SNAP	Post-SNAP	% Change	pValue	
HMP flow rate (mL/min)	106 (33–185)	103 (33–229)	–6%	0.42	ns
HMP pressure (mmHg)	32 (13–49)	20 (10–40)	–38%	<0.0001	****
HMP IRR (mmHg/mL/min)	0.25 (0.07–1.03)	0.15 (0.05–0.78)	–39%	<0.0001	****
Kidney weight (g)	478 (241–1,062)	493 (242–1,180)	3%	0.0009	***
	SNAP 90 min	SNAP 120 min	% Change	pValue	
SNAP flow rate: (mL/min)	560 (170–1,360)	670 (220–1,580)	20%	<0.0001	****
SNAP pressure (mmHg)	75 (75–75)	75 (75–75)	0%	1	ns
SNAP IRR (mmHg/mL/min)	0.13 (0.06–0.44)	0.11 (0.05–0.34)	–15%	<0.0001	****
Urine output (mL/hour)	13.5 (0–234)	29 (0–315)	115%	<0.0001	****
Arterial pO ₂ (mmHg)	570.2 (514–705)	571 (504.5–720)	–1%	0.0005	***
Venous pO ₂ (mmHg)	393 (120–594)	412.8 (210–587)	5%	0.0004	***
Venous pCO ₂ (mmHg)	43.5 (23–58.8)	42.2 (23–55.6)	–3%	<0.0001	****
Perfusate sodium (mEq/L)	142 (104–151)	144 (137–158)	1%	<0.0001	****
Perfusate potassium (mmol/L)	6.1 (4.3–12)	5.9 (4.1–12)	–3%	<0.3384	ns
Perfusate chloride (mEq/L)	116.5 (111–131)	117 (111–139)	0.4%	0.1508	ns
Perfusate glucose (mmol/L)	113 (74–164)	110 (73–153)	–3%	<0.0001	****
Perfusate lactate (mmol/L)	0.68 (0.3–4.1)	1.5 (0.3–4.24)	120%	<0.0001	****
Perfusate AST (U/L)	26 (9–278)	31 (10–340)	19%	<0.0001	****
Urine pO ₂ (mmHg)	183.1 (83–284)	190.4 (117–259)	4%	0.8246	ns
Urine sodium (mEq/L)	130 (85–153)	129 (83.5–147)	–0.8%	0.0252	*
Urine potassium (mmol/L)	10.64 (3.5–19.96)	11.3 (5.1–20)	6%	0.0961	ns
Urine chloride (mEq/L)	108.5 (65–123)	108.9 (60–128.6)	0.4%	0.1519	ns
Oxygen consumption (mLO ₂ /(min*125 cm [3]))	2.2 (0.45–6.25)	2.55 (0.75–6.55)	16%	<0.0001	****
Hosgood score (1–5)	2 (1–3)	2 (1–3)	0%	<0.0001	****
CIT-1 cross clamp to start of SNAP (hours, range)				21.15 (10.45–54.37)	
CIT-2 end of SNAP to recipient reperfusion (hours, range)				14.5 (2.87–37.75)	
Total out of body time (hours, range)				39.78 (23–67.78)	

Unless otherwise specified, variables are expressed as medians. AST, aspartate aminotransferase; CIT, cold ischemic time; IRR, intrarenal resistance; HMP, hypothermic machine perfusion; pO₂, partial pressure of oxygen; SNAP, sub-normothermic acellular perfusion.

TABLE 5 | NKF consensus group recommendations to decrease kidney discard rates.

Consensus recommendation	34 Lives' central ARC benefit
Create expedited placement pathways to directly offer organs at risk of discard to a small subset of centers that opt in to accept these organs	HTP kidneys offered by OPOs are assessed and accepted by participating transplant centers, increasing allocation success
Identify organs at risk of discard during standard allocation and shunt them to patients at transplant 'rescue' centers that utilize high-risk organs when standard placement is unsuccessful	HTP kidneys are offered sooner to the participating transplant 'rescue' centers when standard allocation efforts are unsuccessful, increasing odds of transplant
Standardize provision of gross photographs of procured kidneys to share on DonorNet	The 34 lives' central ARC provides photographs, videos, and digitized biopsy slides, highlighting kidney vasculature and including a ruler in the picture to help the surgeon determine scale
Develop decision and support tools to help surgeons evaluate the benefits and downstream risks of accepting or refusing an organ	The 34 lives' central ARC provides several additional assessment tools, enabling the transplant center to better evaluate kidney function data not currently available with static ice storage or HMP.
Optimize kidney recovery and management, including expanding research opportunities on normothermic <i>ex-vivo</i> perfusion to recondition organs from older donors or those with long cold ischemic times	The use of SNAP for HTP kidneys provides a standard platform for assessment of marginal organs due to extended CITs, poor initial biopsies, or from older donors and will provide new tools to assess advanced therapeutics and repair efforts in the future

including representatives from NKF, OPOs, transplant centers, the Centers for Medicare and Medicaid Services (CMS), the Health Resources and Services Administration (HRSA), the National Institutes of Health (NIH), the Scientific Registry of Transplant Recipients (SRTR), UNOS, and private health insurers. The goal of the conference was to develop actionable recommendations from key stakeholders to increase the use of more HTP kidneys and to decrease discard rates. The group made several recommendations [20], shown in **Table 5**, along with how each can be addressed by a centralized ARC.

SNAP provides more assessment data, including Hosgood Score, IRR, oxygen consumption and urine output, than is possible from static ice or HMP preservation, giving HTP kidneys extended time for a second look by transplant physicians. Using SNAP, we were able to preserve kidneys *ex vivo* for up to 68 h, which is significantly longer than the recommended standard of care of <24 h [21, 22]. This extended time allowed many transplant centers, including several that rejected the initial OPO offer for the same kidney, but later accepted after reviewing SNAP assessment data, to safely utilize these kidneys. While initial SNAP rescue rates seem promising, additional data will be necessary to verify the sustainability of SNAP provided by a centralized ARC and will be reported once long-term patient and graft survival data have been collected and analyzed.

While SNAP provides new tools to assess kidney function prior to transplantation, there still is much work to be done to decrease the rates of kidney non-use. From the Sankey diagram in **Figure 3**, the biggest potential improvement comes from encouraging OPOs to refer more HTP kidneys to the ARC (only 23% of the unallocated kidneys were referred to the ARC in the first full year from the participating OPOs). Additionally, decreasing the rate of kidneys not accepted for SNAP should include developing better assessment tools that discourage the current reliance on renal biopsy, KDPI and poor HMP pump numbers, and instead encourages trust in the functional assessment provided by SNAP.

SNAP is feasible when performed by an independent, centralized ARC and reduces kidney non-use rates by providing

objective viability assessment data, enabling up to a 90% success rate in transplant allocation. Transporting unused/HTP donor kidneys to a dedicated ARC provides transplant hospitals with additional time to locate and screen potential recipients and additional data to make better informed decisions not otherwise available with static ice or HMP preservation methods. Moreover, SNAP allows extended preservation times close to 70 h, enabling improved logistical planning and broader sharing of deceased donor kidneys. Increasing utilization of the current pool of available human donor kidneys is possible, offering an immediate solution to the current donor organ shortage, while providing significant and immediate benefits.

AUTHOR'S NOTE

These authors were responsible for the initial study conception, contributed to the article, served as the initial clinical investigators, provided data used in the submission, and approved the submitted version.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving humans were approved by WCG Clinical Services, Central IRB, as well as by the local IRBs from the participating transplant centers. The studies were conducted in accordance with the local legislation and institutional requirements. The ethics committee/institutional review board waived the requirement of written informed consent for participation from the participants or the participants' legal guardians/next of kin because Study was considered a non-significant risk to patients.

AUTHOR CONTRIBUTIONS

CJ, WG, MH, JG-W, and HL participated in the initial study conception, were the initial study clinical investigators, provided data used, and approved the submitted version. All authors contributed to the article and approved the submitted version.

FUNDING

The authors declare that no financial support was received for the research and/or publication of this article.

CONFLICT OF INTEREST

Author CJ was employed by and HL was a consultant to 34 Lives, Public Benefit Company.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

GENERATIVE AI STATEMENT

The authors declare that no Generative AI was used in the creation of this manuscript.

Any alternative text (alt text) provided alongside figures in this article has been generated by Frontiers with the support of

artificial intelligence and reasonable efforts have been made to ensure accuracy, including review by the authors wherever possible. If you identify any issues, please contact us.

ACKNOWLEDGEMENTS

This study wouldn't have been possible without the gracious gifts from the generous donor families and through collaborations with Kentucky Organ Donor Affiliates (a.k.a. Network for Hope), Life Connection of Ohio, Gift of Hope, Texas Organ Sharing Alliance, LifeGift, Indiana Organ Donor Network, OurLegacy, Arkansas Regional Organ Recovery, Gift of Life Michigan, DonorAlliance, Iowa Donor Network, Lifeline of Ohio, Mid-America Transplant, ConnectLife, Center for Donation & Transplant, Finger Lakes Donor Recovery Network, and LiveOn Nebraska. In addition to the authors, the following site investigators were critical to this project: Bonnie E. Lonze, MD, PhD; Andrew S. Barbas, MD; Liise K. Kayler, MD; Jason R. Wellen, MD; Reynold Lopez-Soler, MD, PhD; Dustin J. Carpenter, MD; Alvin C. Wee, MD; John R. Montgomery, MD; Jose M Figueiro, MD; Giselle Guerra, MD. Finally, the authors would like to thank Stephen Eilert, Nicholas Most, Haley Frost, and Paul Wiederhold for their efforts in validating the initial NMP protocols; Tim Hamelink, Veerle Lantinga, Leonie van Leeuwen, and Tom Taccini for their help designing and building the first ARC; and Kathleen St Jean and the entire 34 Lives' kidney preservation and coordination teams for extending these gifts of life.

REFERENCES

- Reese PP, Harhay MN, Abt PL, Levine MH, Halpern SD. New Solutions to Reduce Discard of Kidneys Donated for Transplantation. *J Am Soc Nephrol* (2016) 27(4):973–80. doi:10.1681/ASN.2015010023
- Heilman RL, Green EP, Reddy KS, Moss A, Kaplan B. Potential Impact of Risk and Loss Aversion on the Process of Accepting Kidneys for Transplantation. *Transplant* (2017) 101(7):1514–7. doi:10.1097/TP.0000000000001715
- Kayler LK, Nie J, Noyes K. Hardest to Place Kidney Transplant Outcomes in the United States. *AJT* (2021) 21:3663–72. doi:10.1111/ajt.16739
- Meister FA, Czigan Z, Reitzler K, Miller H, Reichelt S, Liu WJ, et al. Decrease of Renal Resistance During Hypothermic Oxygenated Machine Perfusion Is Associated with Early Allograft Function in Extended Criteria Donation Kidney Transplantation. *Nat Sci Rep* (2020) 10:17726. doi:10.1038/s41598-020-74839-7
- Tingle SJ, Figueiredo RS, Moir JA, Goodfellow M, Talbot D, Wilson CH. Machine Perfusion Preservation versus Static Cold Storage for Deceased Donor Kidney Transplantation. *Cochrane Database Syst Rev* (2019) 3(3):CD011671–78. doi:10.1002/14651858.CD011671.pub2
- Verstaeten L, Fieuws S, Hofker HS, Leuvenink HGD, Ploeg RJ, Pirenne J, et al. Consortium for Organ Preservation in Europe (COPE). Renal Resistance Trajectories During Hypothermic Machine Perfusion in Kidneys Donated After Circulatory Death: Associations with Donor Characteristics and Posttransplant outcomes- an Analysis of COMPARE Trial Data. *Am J Transpl* (2025). doi:10.1016/j.ajt.2025.06.014
- Tingle SJ, Dobbins JJ, Thompson ER, Figueiredo RS, Mahendran B, Pandanaboyana S, et al. Machine Perfusion in Liver Transplantation. *Cochrane Database Syst Rev* (2023) 12(19):CD014685. doi:10.1002/14651858.CD014685.pub2
- Schroder JN, Patel CB, DeVore AD, Casalinova S, Koomalsingh KJ, Shah AS, et al. Increasing Utilization of Extended Criteria Donor Hearts for Transplantation: The OCS Heart EXPAND Trial. *JACC Heart Fail* (2024) 12(3):438–47. doi:10.1016/j.jchf.2023.11.015
- Luo Q, Zhu L, Wang Y, Wang L, Wang LV, Hu J. The Conversional Efficacy of *Ex Vivo* Lung Perfusion and Clinical Outcomes in Patients Undergoing Transplantation of Donor Lungs by *Ex Vivo* Lung Perfusion: A Meta-Analysis. *Ann Transpl* (2019) 24:647–60. doi:10.12659/AOT.919242
- Moers C, Smits JM, Maathuis MHJ, Treckmann J, van Gelder F, Napieralski BP, et al. Machine Perfusion or Cold Storage in Deceased-Donor Kidney Transplantation. *NEJM* (2009) 360(1):7–19. doi:10.1056/NEJMoa0802289
- Whitson BA, Black SM. Organ Assessment and Repair Centers: The Future of Transplantation Is near. *World J Transpl* (2014) 4(2):40–2. doi:10.5500/wjt.v4.i2.40
- Hosgood SA, Nicholson ML. First-In-Man Renal Transplantation After *Ex-Vivo* Normothermic Perfusion. *Transplantation* (2011) 92(7):735–8. doi:10.1097/TP.0b013e31822d4e04
- Hosgood SA, Nicholson ML. The First Clinical Case of Intermediate *Ex Vivo* Normothermic Perfusion in Renal Transplantation. *AJT* (2014) 14:1690–2. doi:10.1111/ajt.12766
- Rijkse E, de Jonge J, Kimenai AN, Hoogduijn MJ, de Bruin RWF, van den Hoogen MWF, et al. Safety and Feasibility of 2 H of Normothermic Machine Perfusion of Donor Kidneys in the Eurotransplant Senior Program. *BJS Open* (2021) 5(1):zraa024. doi:10.1093/bjsopen/zraa024
- Mazilescu LI, Urbanellis P, Kim SJ, Goto T, Noguchi Y, Konvalinka A, et al. Normothermic *Ex Vivo* Kidney Perfusion for Human Kidney Transplantation: First North American Results. *Transplant* (2022) 106(9):1852–9. doi:10.1097/TP.0000000000004098

16. Minor T, von Horn C, Gallinat A, Kathis M, Kribben A, Treckmann J, et al. First-In-Man Controlled Rewarming and Normothermic Perfusion With Cell-Free Solution of a Kidney Prior to Transplantation. *Am J Transpl* (2020) 20: 1192–5. doi:10.1111/ajt.15647
17. von Horn C, Zlatev H, Lüer B, Malkus L, Ting S, Minor T. The Impact of Oxygen Supply and Erythrocytes During Normothermic Kidney Perfusion. *Sci Rep* (2023) 13(1):2021. doi:10.1038/s41598-023-29031-y
18. Hosgood SA, Barlow AD, Hunter JP, Nicholson ML. *Ex vivo* Normothermic Perfusion for Quality Assessment of Marginal Donor Kidney Transplants. *BJS* (2015) 102:1433–40. doi:10.1002/bjs.9894
19. Hunter JP, Faro LL, Rozenberg K, Dengu F, Ogbemudia A, Weissenbacher A, et al. Assessment of Mitochondrial Function and Oxygen Consumption Measured During *Ex Vivo* Normothermic Machine Perfusion of Injured Pig Kidneys Helps to Monitor Organ Viability. *Transpl Int* (2022) 35: 10420. doi:10.3389/ti.2022.10420
20. Cooper M, Formica R, Friedewald J, Hirose R, O'Connor K, Mohan S, et al. Report of National Kidney Foundation Consensus Conference to Decrease Kidney Discards. *Clin Transpl* (2019) 33(1):e13419. doi:10.1111/ctr.13419
21. Summers DM, Watson CJ, Pettigrew GJ, Johnson RJ, Neuberger JM, Bradley JA, et al. Kidney Donation After Circulatory Death (DCD): State of the Art. *Kid Intl* (2015) 88(2):241–9. doi:10.1038/ki.2015.88
22. Wood NL, VanDerwerken DN, Massie AB, Segev DL, Snyder JJ, Gentry SE. Diagnosing the Recent Decrease in Utilization of Deceased Donor Kidneys. *Transplantation* (2025) 109(3):496–503. doi:10.1097/TP.0000000000005178

Copyright © 2025 Jaynes, Goggins, Holzner, Garonzik-Wang and Leuvenink. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.



Nationwide Hypothermic Machine Perfusion for ECD and DCD Kidney Transplantation in Belgium: One-Year Outcomes and Impact on Transplant Rates and Budget Impact Analysis

Tom Darius^{1*}, Ina Jochmans², Maxime Foguene¹, Eric Hoste³, Caren Randon³, Bart Bracke⁴, Geert Roeyen⁴, Nicholas Gilbo⁵, Laurent Weekers⁵, Daniel Jacobs-Tulleneers-Thevissen⁶, Karl Martin Wissing⁶, Tineke Bogaerts⁶, Dimitri Mikhalski⁷, Jean-Philippe De Wilde⁷, Joël Daems⁸ and Jacques Pirenne²

¹Department of Surgery, Surgery and Abdominal Transplant Unit, Cliniques Universitaires Saint-Luc, Brussels, Belgium, ²Department of Surgery, Abdominal Transplant Surgery, UZ Leuven, Leuven, Belgium, ³Department of Intensive Care Medicine, Universitair Ziekenhuis Gent, Ghent, Belgium, ⁴Department of Hepatobiliary, Endocrine, and Transplantation Surgery, Universitair Ziekenhuis Antwerpen, Edegem, Belgium, ⁵Division of Abdominal Surgery and Transplantation, CHU de Liège, Liège, Belgium, ⁶Department of Surgical Oncology, Thoracic Surgery and Transplantation, Universitair Ziekenhuis Brussel, Brussels, Belgium, ⁷Department of Abdominal Surgery and Transplantation, Hopital Erasme, Brussels, Belgium, ⁸Rijksinstituut voor Ziekte- en Invaliditeitsverzekering, Brussels, Belgium

OPEN ACCESS

*Correspondence

Tom Darius,
✉ tom.darius@saintluc.uclouvain.be

Received: 17 July 2025

Revised: 26 September 2025

Accepted: 23 October 2025

Published: 13 November 2025

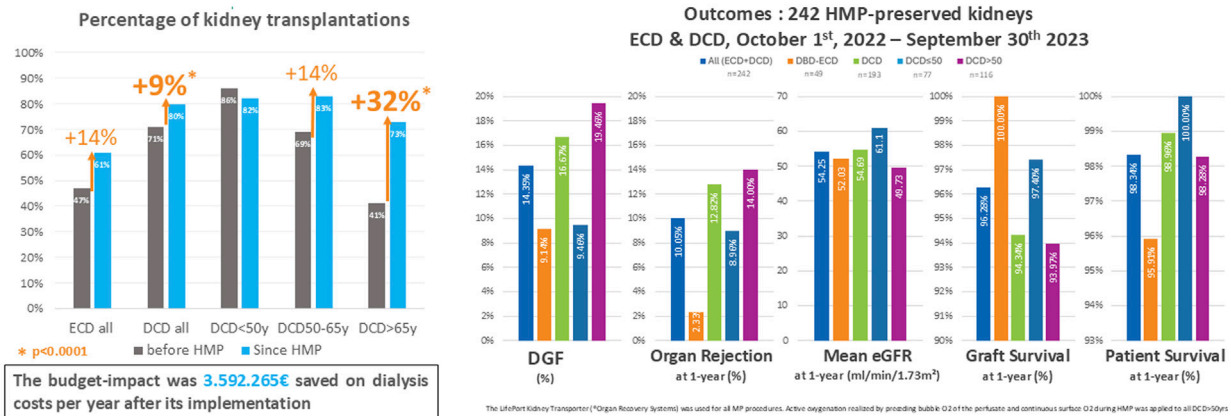
Citation:

Darius T, Jochmans I, Foguene M, Hoste E, Randon C, Bracke B, Roeyen G, Gilbo N, Weekers L, Jacobs-Tulleneers-Thevissen D, Wissing KM, Bogaerts T, Mikhalski D, De Wilde Jean-P, Daems J and Pirenne J (2025) Nationwide Hypothermic Machine Perfusion for ECD and DCD Kidney Transplantation in Belgium: One-Year Outcomes and Impact on Transplant Rates and Budget Impact Analysis. *Transpl. Int.* 38:15282. doi: 10.3389/ti.2025.15282

In September 2022, Belgium implemented a nationally reimbursed HMP service for all ECD and DCD kidneys procured and transplanted within the country. We retrospectively analyzed data from 242 kidney transplantations preserved with continuous HMP between October 2022 and September 2023. Active oxygenation (HMPO₂) was applied in DCD donors aged >50 years. One-year outcomes for all HMP kidneys included delayed graft function (DGF) in 14.4%, estimated glomerular filtration rate of 50 mL/min/1.73 m², 10.1% acute rejection, 96.3% death-censored graft survival, and 98.3% patient survival. DGF rates were lower in ECD kidneys (9.1%) and in DCD ≤50 years (9.5%), while higher in DCD >50 years (19.6%). National transplantation rates of DCD kidneys significantly increased from 90 to 175 per year (p < 0.0001), but not for ECD kidneys (from 45 to 54 per year (p = 0.2965) post-HMP implementation without affecting kidney export. The annual cost savings from reduced dialysis requirements were estimated at €3.59 million. The national implementation of a centralized HMP service in Belgium led to excellent one-year transplant outcomes, increased utilization of ECD and DCD kidneys, and substantial healthcare cost savings. These findings support HMP, and where appropriate HMPO₂, as the new standard of care for kidney preservation in Belgium, with potential implications for broader international collaboration.

Keywords: hypothermic machine perfusion, kidney transplantation (KT), hypothermic oxygenated machine perfusion, national implementation, budget impact analysis

National Implementation of HMP: Increases Local Kidney Utilisation Rate, Highly Cost-Effective, and Excellent Functional Outcome



Darius Tom, et al. *Transpl. Int.* 2025
doi: [10.3389/ti.2025.15282](https://doi.org/10.3389/ti.2025.15282)



GRAPHICAL ABSTRACT |

INTRODUCTION

Over the past 2 decades, hypothermic machine perfusion preservation (HMP) has gained traction as a method of donor kidney preservation. Continuous HMP, initiated immediately after procurement and maintained until implantation, has demonstrated superior transplant outcome compared with static cold storage (SCS) and endischemic application [1–6]. It reduces the risk of delayed graft function (DGF), irrespective of donor type and cold ischemia time [7–11]. Only a few publications reported an economic evaluation of the use of HMP as compared with SCS [12–14]. Two reports on the results of Euotransplant Machine Perfusion trial of Moers C et al confirmed cost savings in favor for HMP [12, 13]. One of the main reasons for the cost saving was the lower dialysis costs in the HMP group due to the decreased incidence of DGF.

Despite its proven benefits, only a few European countries (France, Netherlands and Switzerland), have implemented this technique at a national level through structured perfusion services. In Belgium, a major policy shift occurred in June 2021 when the National Institute for Health and Disability Insurance (NIHDI) approved reimbursement for HMP for all kidneys procured and transplanted in Belgium from donors after brain death (DBD) with expanded criteria donors (ECD) and donors after circulatory death (DCD), effective from September 2022. This initiative was initiated in particular to increase the national utilization of ECD and DCD kidneys. A dedicated national kidney HMP group successfully established a centralized national perfusion service (NPS) within a short timeframe.

This study has four primary objectives:

1. Discard Analysis and Feasibility: To assess overall kidney discard reasons and the feasibility of connecting transplantable kidneys to the HMP device.
2. Clinical Outcomes: To evaluate the one-year functional outcomes of kidneys preserved with continuous HMP during the first year of national implementation.
3. Economic Evaluation: To perform a budget impact analysis comparing dialysis costs with the costs associated with HMP.
4. Utilization Impact: To analyze the effect of HMP implementation on the national utilization rate of ECD and DCD kidneys for transplantation in Belgium, covering the period from October 2017 to March 2024.

Additionally, the study outlines the logistical and organizational challenges encountered during the implementation of the national HMP service and discusses future perspectives for its development.

MATERIALS AND METHODS

HMP Reimbursement Criteria, Lump-Sum and Kidney Allocation

The NIHDI approved reimbursement for HMP as preservation technique for all ECD and DCD kidneys that are procured and

transplanted in Belgium. ECDs are defined as brain-dead donors aged 50–60 with at least two of the following: arterial hypertension, serum creatinine above 1.5 mg/dL, or death due to a stroke, or any donor over 60 years old. All DCD donors are included regardless of age. The lump-sum of 3500€ per HMP-preserved kidney was calculated on the sum of the cost of the disposables of the HMP device (machine perfusion kit and accessories (connections tubes, canules, sterile drapes), a preservation specialist-fee and a transport from a central hub to the donor centrum and return transport. During the negotiation with the third party providing the HMP infrastructure, it was agreed that at the moment of initiation of this national HMP-program this lump-sum would be sufficient to cover most of the expenses for this project.

All seven Belgian kidney transplant centers agreed to a three-year pilot program (from September 2022 to August 2025) to use HMP as much as possible. HMP was not mandatory and preservation of these kidneys by SCS was allowed.

All kidneys were allocated according to the standard Eurotransplant allocation system with no exceptions.

Organization of the National Perfusion Service

The centralized NPS consists of a small team of 10 dedicated, well trained, preservation specialists (with a maximum capacity of 3 simultaneous multiorgan procurement procedures) supervised by experienced perfusionists, a central hub in Brussels where 6 machine perfusion devices are stored, and a 24/24h helpdesk. Activation of this service is done by the local donor transplant coordinator, preferably after the kidney is accepted by a Belgian transplantation center and full fitting the reimbursement criteria for HMP and before the start of the procurement procedure. This service consists of a priority transport by car of the preservation specialist and the machine perfusion (MP) device(s) to the donor hospital. The preservation specialist is responsible for the set-up of the MP device(s), support of the procurement surgeon connecting the kidney to the MP device and start of MP, and preparation of the HMP-kidney for non-accompanied transport to the recipient center. In case of device-related alarms during MP the 24/24h help desk is available to resolve them.

Based on the legally required European tender, Organ Recovery Systems (Diegem, Belgium) was selected as MP company together with the organization of the NPS. Therefore, the NPS is currently organized by Organ Recovery Systems (Diegem, Belgium) and the LifePort Kidney Transporter (®Organ Recovery Systems, Diegem, Belgium) was used for all MP procedures. The device pumps the perfusion fluid through the kidney vasculature in a pulsatile way (pressure-controlled) by arterial cannulation. The pressure was set at 30 mmHg, as most frequently used in clinical setting. Perfusate and kidney are cooled to 2 °C–8 °C using crushed ice outside the sterile container. Based on the results of the COMPARE trial, all kidneys from DCD donors above 50 years old were actively continuously oxygenated during HMP [15]. Oxygenation was realized by uploading the perfusate with oxygen via direct bubble oxygenation (at 0.5 L/min of O₂ for 15 min) followed by continuous surface oxygenation (at

0.2 L/min of O₂ throughout perfusion duration) during HMP [16, 17]. For this, a disposable oxygenation perfusion circuit (LKT-200X, Organ Recovery Systems, Diegem, Belgium) (CE marked since 2021) was used. Active oxygenation in younger DCD donors was possible in study context (ClinicalTrials.gov identifier: NCT05430620) and on surgeon's request of the recipient center.

Adverse events during total time of MP were real-time monitored by GPS connection and/or reported by the transplant coordinators/surgeons to the MP helpdesk service. In addition, to evaluate the safety and efficacy of the entire HMP service, a feedback evaluation sheet was introduced to report device- and logistic-related adverse events. The role of HMP preservation in the final timing of the transplant procedure (e.g., night to day surgery, emergency to semi-elective) was also polled on this evaluation sheet.

Study Period

A retrospective analysis was performed of all ECD and DCD donor procedures performed between October 1, 2022, and September 30, 2023, for which the NPS has been activated. Because HMP was considered by the health authorities as standard of care and with the obligation to provide a yearly evaluation by already obligatory outcome data collection on the Eurotransplant Kidney Registry, no ethical approval was necessary and written informed consent for participation was not required, in accordance with the national legislation and institutional requirements.

Donor and Recipient Demographics

The following donor demographics were collected: deceased donor type (DBD ECD versus DCD), age, body mass index (BMI), arterial hypertension, diabetes, cause of death, last serum creatinine, total donor warm ischemia time (defined in DCD as the time from withdrawal of mechanical ventilation until the start of *in-situ* cold perfusion). IGL-1 (Institut Georges Lopez, Lissieu, France) preservation solution was used in all donors. Standard procurement techniques were used in DBD and DCD donors. DCD donors were classified according to Belgian modified classification of Maastricht in which a category V is a DCD donor after euthanasia [18].

The following recipient demographics were retrospectively collected: age, BMI, dialysis status pretransplant, time on dialysis and number of kidney transplantation).

Perfusion Parameters During HMP

Renal flow (RF) and resistance (RR) were continuously registered by the MP device. The following device related parameters were collected: RF and RR at 2 min, 60 min and at the end of perfusion and total perfusion time.

Transplant Outcome

The following parameters were retrospectively collected to evaluate functional graft outcome: anastomosis time during graft implantation, total cold ischemia, primary nonfunction (PNF; defined as the permanent lack of graft function from time of transplantation when a kidney graft was well perfused

but never functioned, necessitating dialysis after kidney transplantation), DGF (defined as the need for dialysis during the first week after transplantation excluding dialysis for hyperpotassemia within the first 24 h after transplantation), acute rejection, estimated glomerular filtration rate (eGFR) (calculated by the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI creatinine equation) at 1 year, graft and death-censored graft- and patient survival at 1 year.

Impact on National Utilization Rate and Budget Impact Analysis

To evaluate the impact of the introduction of HMP on the national utilization rate of ECD and DCD kidneys procured and transplanted in Belgium, all isolated kidney and combined organ kidney transplantations originated from deceased donors in Belgium performed between October 2017 and September 2024 as registered in the Eurotransplant Database were retrospectively collected and analyzed as well as the impact on donor age and international exchange of kidneys.

In addition, a budget impact analysis for the NIDHI was performed comparing the expected cost of hemodialysis patients on the waiting list (44.932€/year/patient) versus the economy of reducing dialysis cost due to the net increase in the national use of ECD and DCD kidneys with HMP (3.500€/procedure) which would otherwise be rejected by using SCS preservation alone. As the transplantation costs (immunosuppressive drugs, staff time, ...) are identical for SCS and HMP (excluding the costs of the conservation method) this was not used for the budget analysis, nor was the relatively small saving in SCS-cost.

Statistical Analysis

Characteristics of the donor, the recipient and transplant outcome were described by classical descriptive statistics and comparison with historical data was performed by the chi-square test for differences in proportions. A p-value <0.05 was considered statistically significant. Continuous variables are provided as median and interquartile range. Statistical analysis and plots were accomplished with SAS (version 9.4M8) statistical software and Prism 10.5.0 (Graphpad Software, San Diego, CA, USA).

RESULTS

Activation of National Machine Perfusion Service and Donor Characteristics

Between 1 October 2022, and 30 September 2023, the national machine perfusion service was activated for 178 deceased donor procedures with a potential of 356 transplantable kidneys. Donor type, age, comorbidity and serum creatinine are shown in **Table 1**. In the DCD group 50 (36%), 31 (23%), 46 (33%) and 11 (8%) donors were in the age group of 0–50 years, 51–60 years, 61–70 years and above 71 years old, respectively. For DCD procedures, a rapid cannulation of the abdominal aorta and normothermic regional perfusion was performed before kidney procurement in 175 (98%) and 3 (2%), respectively.

Discarded Kidneys, Preservation Strategy and Transplantation Rate

A detailed overview of the kidney discard reasons, preservation strategy and transplantation rate is provided in **Figure 1**. In summary, forty-seven (13.2%) of the 356 potential donor kidneys were discarded before ($n = 4$), during ($n = 13$) and after the procurement ($n = 30$) because of medical contra-indications for transplantation which resulted in a total of 309 kidneys deemed transplantable. Of them, SCS was used instead of MP in 57 (18.5%) mainly for export outside Belgium since no reimbursement of HMP was foreseen in this setting ($n = 43$) and on request of the transplant surgeon in case of very short estimated cold ischemia time ($n = 9$). All these SCS-preserved kidneys were successfully transplanted.

In total, 252 kidneys were intended to be preserved by HMP of which 97% ($n = 245$) were successfully connected to the MP device. In three percent ($n = 7$), the procurement surgeon encountered a technical issue when connecting the kidney to the MP device or immediately after start of MP and organ preservation was immediately switched to SCS. Exact reasons are also detailed in **Figure 1**. Six of these 7 SCS-preserved kidneys were successfully transplanted.

Finally, 245 HMP-kidneys were transported from donor to the recipient center. In a minority of the cases the kidney procurement was in the same center as the transplant procedure. Two of them were reallocated afterwards (according to standard Eurotransplant kidney allocation rules) because the recipients were not transplantable due to unexpected medical reasons. Both kidneys were switched to SCS, awaiting acceptance of the reallocation procedure, and afterwards successfully transplanted, one in Belgium and one abroad. Three of the 245 HMP-kidneys were discarded in the recipient center by the transplant surgeon for the following reasons: anatomic reason ($n = 1$), postrenal artery stenosis ($n = 1$) and poor perfusion (by macroscopic evaluation) during HMP due to iatrogenic section of the renal artery at the hilus ($n = 1$). Of note, no kidney was discarded based on high renal resistance values.

The transplantation rate of ECD and DCD kidneys during this first year after the implementation of HMP was 86.23% ($n = 307$) of which 242, 63 and 2 were preserved by continuous HMP, SCS alone, and HMP switched to SCS preservation, respectively.

A retrospective analysis was performed on these 242 isolated HMP-perfused kidney transplantations of which 49 from ECD and 193 from DCD donors. Their donor characteristics are shown in **Table 2**.

Machine Perfusion Parameters and Adverse Events

Table 3 summarizes the renal resistance, renal flow, total perfusion time and adverse events during MP. The mean renal resistance of the 242 successfully transplanted kidneys was 0.42 (0.32–0.62) mmHg/ml/min, 0.25 (0.20–0.33) mmHg/ml/min and 0.19 (0.14–0.25) mmHg/ml/min, at 2 min, 30 min, and at the end of the perfusion period, respectively. The median total MP time was 5h15 (2h57 – 8h48).

TABLE 1 | Donor characteristics of the 178 deceased donor procedures for which the national machine perfusion service was activated in Belgium between 1 October 2022, and 30 September 2023.

Donor characteristics	178 deceased donor procedures
Type of donor, n (%)	
- DBD-ECD	36 (20.2%)
- DCD	
• DCDIII	139 (78.1%)
• DCDV	3 (1.7%)
Donor age, yrs	
- DBD-ECD	65 (61,25–68,75)
- DCD	
• DCDIII	57 (44–65)
• DCDV	54 (33–57)
Donor comorbidity, n (%)	
- Arterial hypertension	54 (30%)
- Diabetes mellitus	11 (6%)
Donor last serum creatinine before kidney procurement (mg/dL)	0.63 (0.5–0.76)
Total donor warm ischemia time for DCD donors (min)	12 (6–20)

Abbreviations: DBD, donation after brain death; DCD, donation after circulatory death; ECD, expanded criteria donors; HMP, hypothermic machine perfusion.

In total 10 adverse events during MP were recorded/reported. Details and action of handling to restart HMP are also illustrated in **Table 3**.

Recipient and Transplant Characteristics

The recipient and transplant characteristics are detailed in **Table 4**.

The anastomosis and total cold ischemia time were 30 (25–35) min and 8h33 (5h17–12h45), respectively.

1-Year Functional Outcome

The 1-year functional outcome, according to donor type, is shown in **Table 5**. Delayed graft function, median eGFR, organ rejection rate and overall graft-, death-censored graft- and patient survival at 1 year after transplantation for all the 242 ECD and DCD kidneys were 14.35%, 50 mL/min/1.73 m³, 10.05%, 96.28% and 98.24%, respectively. The reasons for graft loss (13/242) were primary nonfunction (n = 1), rejection (n = 1), donor-transmitted infection (n = 1), graft infection (n = 1), arterial thrombosis (n = 1), venous thrombosis (n = 2), unknown (n = 2) and patient death (n = 4).

Impact of HMP on the National Utilization Rate

Compared to historical data from October 2017 and March 2024, the national implementation of HMP resulted in a significant increase of the total number of kidney transplantations originating from Belgian DCD donors, regardless of donor age, from 90 before HMP implementation until 175 per year afterwards (p < 0.0001). ECD Kidney transplantation increased from 45 before, up to 54 per year after the implementation of HMP (p = 0.2965) (**Figure 2A**). The observed increase was more pronounced in the DCD donors above 50 years old and only significant for donor age above 65 years old, but not for DCD kidneys with donor age below the age of 50 (**Figure 2B**). The

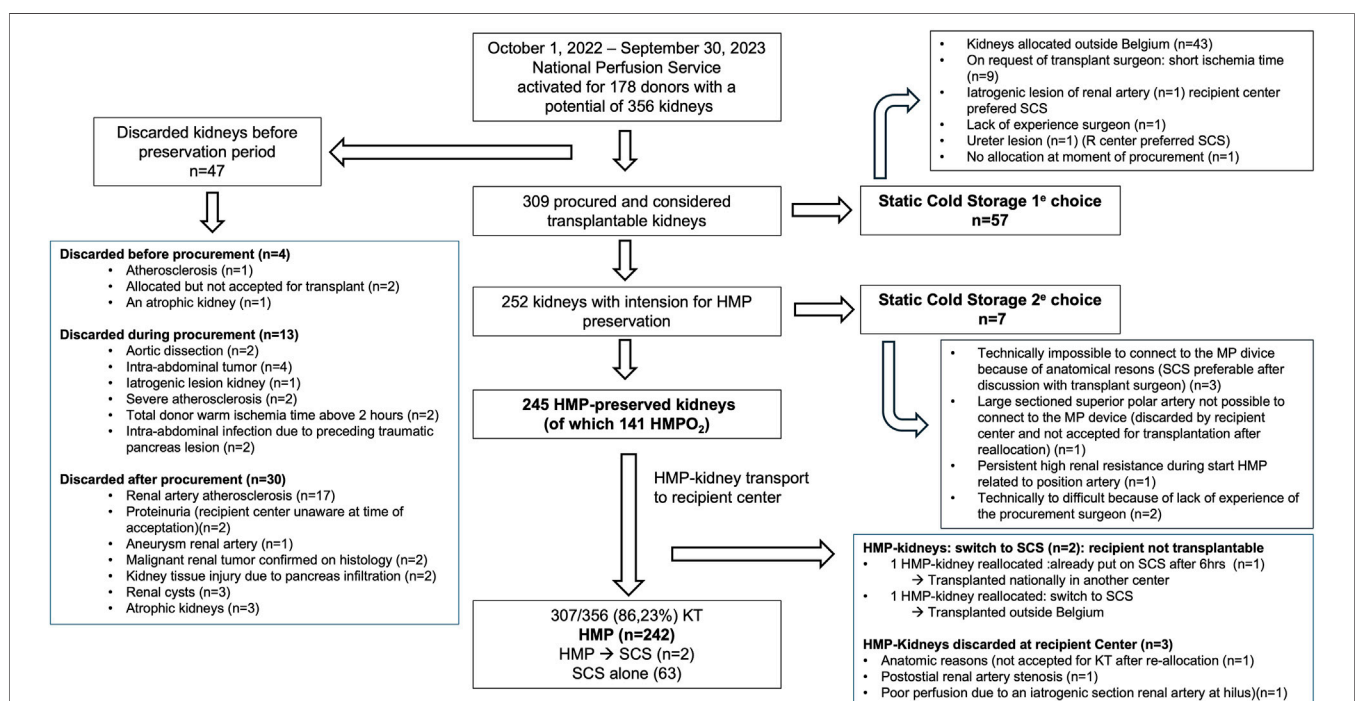


FIGURE 1 | Kidney discard reasons, preservation strategy and transplantation rate of the 356 potential kidneys of the 178 donors for which the national perfusion service was activated between 1 October 2022, and 30 September 2023.

TABLE 2 | Donor characteristics according to donor type of the 242 patients receiving an HMP-preserved kidney graft in Belgium between 1 October 2022, and 30 September 2023, procured from national ECD and DCD donors.

Donor type	All (ECD + DCD) (n = 242)	DBD ECD (n = 49)	DCD (n = 193)	DCD ≤ 50 years (n = 77)	DCD > 50 years (n = 116)
Donor age, yrs	58 (47–65)	64 (61–68)	55 (43–64)	42 (35–46)	61 (56–67)
Donor sex, male/female, n (%)	134 (55%)/108 (45%)	25 (51%)/ 24 (49%)	108 (56%)/ 85 (44%)	52 (68%)/25 (32%)	56 (48%)/60 (52%)
Donor body mass index	25 (23–28)	25 (24–28)	25 (22–29)	24 (21–28)	28 (23–29)
Donor comorbidity, n (%)					
- Arterial hypertension	68 (28%)	25 (51%)	43 (22%)	6 (8%)	37 (32%)
- Diabetes mellitus	15 (6%)	3 (6%)	12 (6%)	0 (0%)	12 (10%)
Donor last serum creatinine before kidney procurement (mg/dL)	0.6 (0.5–0.75)	0.72 (0.6–0.78)	0.57 (0.45–0.71)	0.57 (0.45–0.67)	0.54 (0.44–0.71)
Total donor warm ischemia time for DCD donors (min)	NA	NA	15 (11–21)	13 (11–23)	15 (12–21)

Abbreviations: DBD, donation after brain death; DCD, donation after circulatory death; ECD, expanded criteria donor; NA, not applicable; yrs, years.

TABLE 3 | Machine perfusion parameters and adverse events of the 242 HMP-preserved kidneys transplanted in Belgium between 1 October 2022, and 30 September 2023, procured from national ECD and DCD donors.

Machine perfusion parameters	242 HMP-preserved kidneys
Renal resistance, mmHg/mL/min	
- At 2 min	0.42 (0.32–0.62)
- At 30 min	0.25 (0.20–0.33)
- At end of perfusion	0.19 (0.14–0.25)
Renal flow, mL/min	
- At 2 min	60 (41–77)
- At 30 min	87 (72–102)
- At end of perfusion	105 (84–127)
Total perfusion time, h:m	5h15 (2h57 - 8h48)
Adverse events during MP	Details
- "Upstream bubble alarm" with stop MP (n = 4)	- Restart MP during transport (n = 2) or at arrival in recipient center (n = 2)
- "Leakage" at perfusion cannule with stop MP during organ transport (n = 1).	- Exploration in recipient center during bench preparation: due to a small aorta patch (pediatric donor) the cannule was not fixed tight enough
- "High resistance alarm" (n = 3)	- Movement during transport (n = 1) or during delivery of the kidney in the recipient center (n = 1): MP manually restarted
- "High pressure alarm" without interruption of MP (n = 1)	- Sudden peak (n = 1) of resistance spontaneously normalized without interruption of MP
- "High pressure alarm" with interruption of MP (n = 1)	- Unknown cause (probably transport-related) but alarm resolved by decreasing the pressure from 30 to 20 mmHg
	- Probably related to a kinking of the artery during transport: restart MP during transport

Abbreviations: DCD, donation after circulatory death; ECD, expanded criteria donors; HMP, hypothermic machine perfusion.

TABLE 4 | Recipient and transplant characteristics of the 242 patients receiving an HMP-preserved kidney graft and transplanted in Belgium between 1 October 2022, and 30 September 2023, procured from national ECD and DCD donors.

Recipient and transplant characteristics	HMP(O ₂) (n = 242)
Recipient characteristics	
Age, yrs	61 (53–68)
Body Mass index (BMI), n	26 (23–29)
Preemptive kidney transplant, n (%)	15 (6.20)
Time on dialysis, days	1,044 (617–1,497)
Number of kidney transplantation, n (%)	
- First kidney transplants	227 (93.8)
- Second kidney transplants	15 (6.2)
Transplant characteristics	
Anastomosis time, min	30 (25–35)
Total cold ischemia time	8h33 (5h17–12h45)

Abbreviations: DCD, donation after circulatory death; ECD, expanded criteria donors; HMP, hypothermic machine perfusion; HMPO₂, oxygenated HMP.

numbers of kidney transplantations from exported and imported kidneys in Belgium did not change after the introduction of HMP, **Figures 2C,D**, respectively. A mild increase of kidney combined organ-transplantation especially from ECD and DCD is illustrated in **Figure 2E**. As illustrated in **Figure 2F**, import in Belgium of a kidney for combined organ transplantation is rare.

Budget Impact Analysis of HMP Implementation

Based on the historical numbers of DCD and ECD kidney transplantations in Belgium as detailed in the previous paragraph, the routine use of HMP for DCD and ECD kidney resulted in a potential more or less 99 national extra DCD and ECD kidney per year as compared to the historical standard SCS preservation. Therefore, based on an estimated 240 procedures

TABLE 5 | One-year functional outcome of the 242 HMP-preserved kidneys successfully transplanted in Belgium between October 1, 2022, and September 30, 2023, procured from national ECD and DCD donors.

Donor type	All (ECD + DCD)	DBD ECD	DCD	DCD ≤ 50 years	DCD > 50 years
Type of kidney preservation (HMP, HMPO ₂)	HMP(O ₂) (n = 242)	HMP (n = 49)	HMP(O ₂) (n = 193)	HMP(O ₂) ^a (n = 77)	HMPO ₂ (n = 116)
Delayed graft function (%)	14.35	9.14	16.67	9.46	19.64
Primary nonfunction (%)	0.4	0	0	0	0.9
eGFR @1 year (ml/min/1.73 m ²)	50 (41–66)	49 (38–66)	50 (42–68)	61 (48–78)	48 (40–61)
Organ rejection @1 year (%)	10.05	2.33	12.82	8.96	14.00
Overall graft survival @1 year (%)	94.63	95.91	94.8	97.40	92.24
Death-censored graft survival @1 year (%)	96.28	100	94.34	97.40	93.97
Patient survival @1 year (%)	98.34	95.91	98.96	100	98.28

Abbreviations: DBD, donation after brain death; DCD, donation after circulatory death; ECD, expanded criteria donor; eGFR, estimated glomerular filtration rate; HMP, hypothermic machine perfusion; HMPO₂, oxygenated hypothermic machine perfusion; y, year; yrs, years.

^aHMP n = 63; HMPO₂ n = 14.

per year, the saving of dialysis costs for the health insurance was −3,592.265€ per year with a half-cycle correction (i.e., reducing the annual saving by 50% in order to correct for the timing effect of the transplant procedure during the year) of 1,376.132€ per year (based on costs of HMP (3,500€/procedure) and dialysis (44,932€/year/patient). The profit percentage accounts 163.8% versus the costs of machine perfusion.

DISCUSSION

This national cohort study demonstrates that the introduction of a centralized HMP service for all ECD and DCD donor in Belgium resulted in excellent one-year transplant outcomes. The implementation led to a substantial increase in the national utilization rate of ECD and DCD kidneys without impacting export rates. Furthermore, the approach proved to be highly cost-effective, with estimated annual savings of €3.59 million in dialysis-related costs.

Despite the well-documented advantages of HMP over SCS, including lower rates of DGF and improved graft survival, many countries have struggled to implement HMP at scale due to reimbursement and logistic barriers [1–11, 15]. The study of Brat A et al is the only study reporting that continuous nonoxygenated HMP preservation as a nationwide standard mode of preservation in the Netherlands of deceased donor kidneys is feasible and safe and resulted in an increase of DCD kidney transplantation, preemptive transplantation and retransplants as well as a significant reduction of DGF as compared with a historical cohort group [19]. The Belgian experience illustrates how national-level implementation is feasible through close collaboration between stakeholders, coordinated logistics, and proactive policy support. Key elements for success, such as stakeholder alignment, reimbursement mechanisms, and operational workflows, are summarized in **Figure 3**. The creation of a National Kidney HMP group was essential and pivotal for its implementation. Therefore, countries or regions who want to embark on a HMP program should take this as a key element for success. Another critical component of the implementation was the real-time communication and mutual trust between procurement and

transplant teams. In cases of anatomical anomalies or procurement complications, transplant centers were directly consulted to decide on preservation strategy, contributing to a low device failure or conversion rate (3%). This approach also limited unnecessary use of perfusion kits, optimizing budget.

The one-year functional outcomes in this cohort are in line with, and in some cases exceed, results reported in major randomized trials such as the COMPARE trial and the Eurotransplant MP trial [2, 15]. The overall DGF incidence in our cohort was 14.35%, and 19.64% among DCD donors older than 50 years—a notable improvement compared to 36% in the COMPARE trial's HMP groups. The use of continuous HMP from procurement to implantation, combined with short ischemia times, low associated donor comorbidity and uniform procedural standards across centers (e.g., standardisation of DCD procedures across all Belgian center (therapy withdrawal in OR, rapid procurement technique)), may explain this favourable outcome. These parameters reflecting the Belgian situation, and therefore, the expected results of a nationwide implementation of HMP in other countries will be influenced by national geography, local practice in kidney acceptance in case of high donor-associated comorbidity and local procurement strategies. However, our results are also in line with a *post hoc* analysis of the Eurotransplant MP trial demonstrating remarkably lower rates of DGF in kidneys transplanted after a very short cold ischemia time [11].

Since September 2022, oxygenated HMP (HMPO₂) has been applied to all DCD kidneys from donors aged >50 years, based on COMPARE trial findings. The rationale for HMPO₂ lies in maintaining aerobic metabolism and limiting ischemia-reperfusion injury by preventing accumulation of harmful metabolites [20–22]. While current evidence remains heterogeneous, recent studies and meta-analyses suggest that HMPO₂ may reduce severe adverse events and may be particularly beneficial in DCD kidneys and in continuous, rather than end-of-preservation, application (**Table 6**) [15, 23–28]. However, large-scale prospective data are still needed to conclusively define the added value of oxygenation.

Notably, in our cohort, no kidney was discarded solely due to high RR during HMP. While elevated RR is frequently cited as a reason for organ discard, especially in the US where 33% of

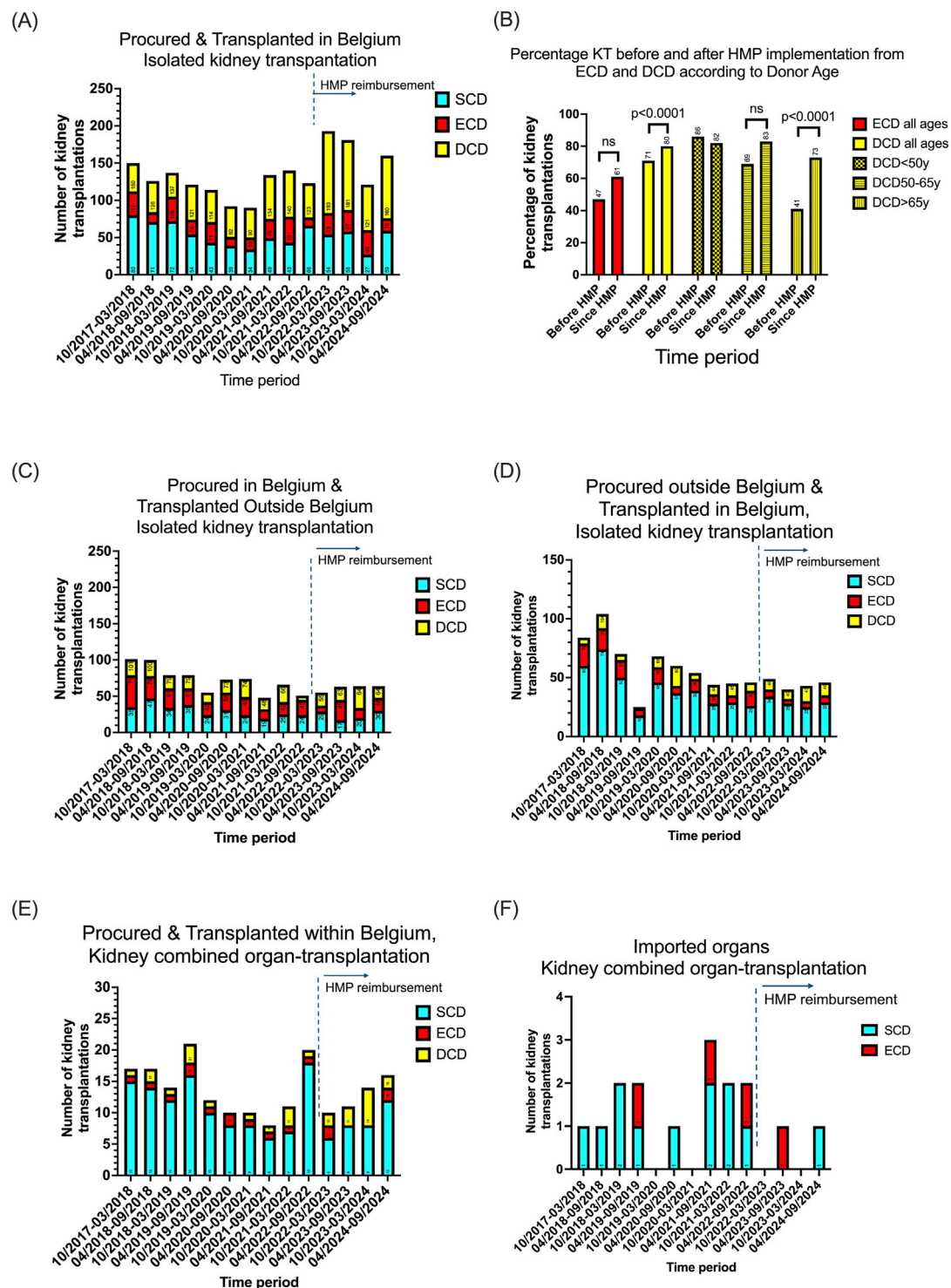
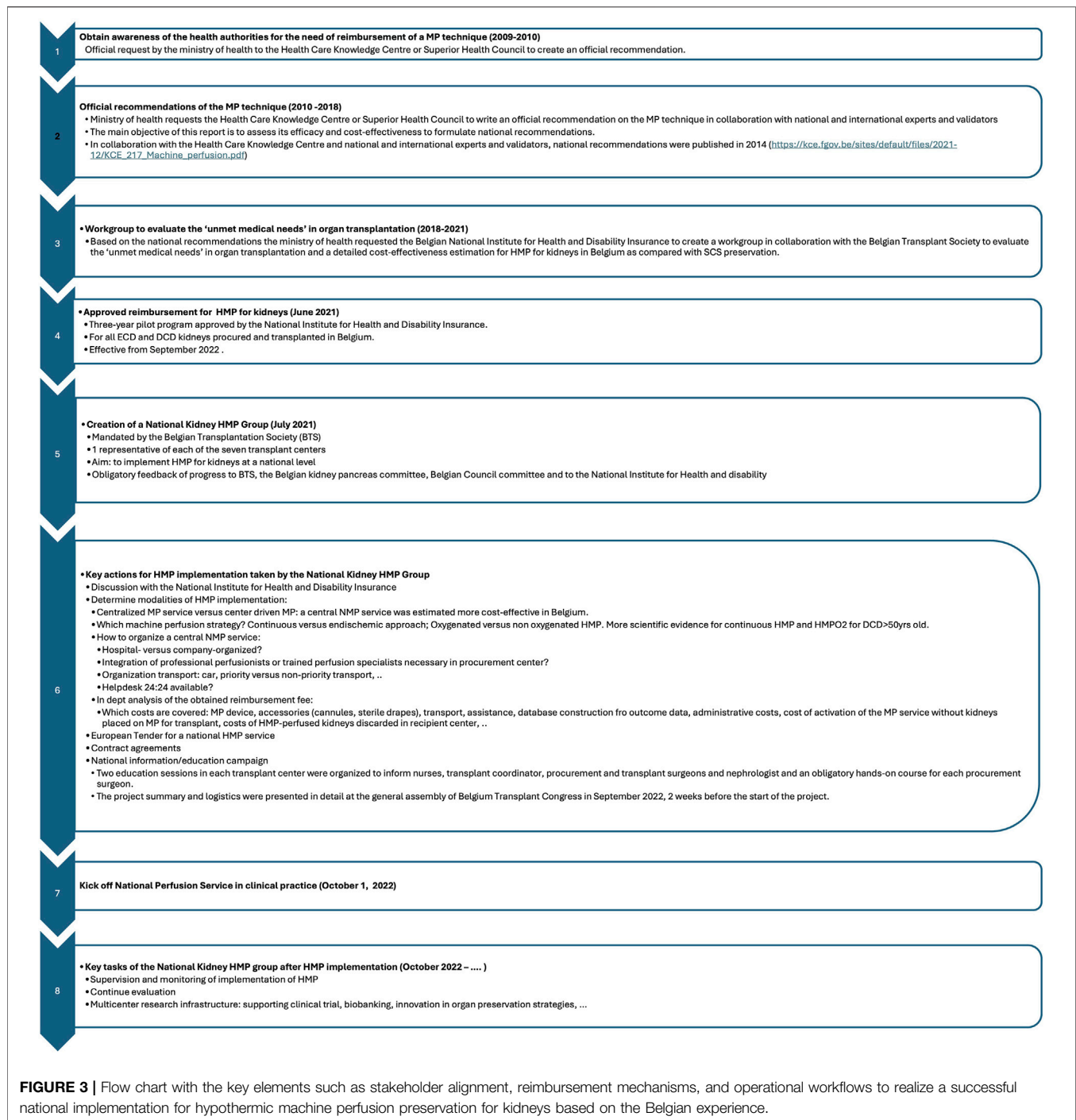


FIGURE 2 | The national implementation of HMP on kidney utilization rate in Belgium between October 2017 and March 2024 resulted in an increase of total number of kidney transplantations originated from Belgian DCD and ECD donors regardless of donor age (A). The observed increase was more pronounced in the donor age groups between 50 and 65 years old and above 65 years, but not for DCD kidneys with donor age below the age of 50 (B). The numbers of kidney transplantations from exported (C) and imported kidneys in Belgium (D) did not change after the introduction of HMP. A mild increase of kidney combined organ-transplantation from especially ECD and DCD was observed (E). Import in Belgium of a kidney for combined organ transplantation is rare (F).



pumped kidneys are not transplanted, our results support a more nuanced interpretation [29–33]. One kidney with persistently high RR due to the position of the renal artery was successfully transplanted after switching to SCS at the surgeon's discretion. This underscores that RR should not be used as a stand-alone viability criterion and must be interpreted within a clinical context. According to a recent meta-analysis, current evidence

does not support the use of isolated RR-thresholds for organ acceptance [29].

Although HMP implementation significantly increased the number of transplanted ECD/DCD kidneys, the total national kidney transplant volume did not rise proportionally. This reflects a broader trend of declining donor availability and increasing waiting lists and waiting list mortality. In addition,

TABLE 6 | Human clinical studies and series using oxygenated hypothermic machine perfusion preservation for kidneys between 2019 and June 2025.

Author, (year)	Study design	Population	DGF, n (%)	PFN, n (%)	eGFR (mL/min)	Graft survival (%)	Rejection, n (%)
Meister (2020)	RCT III Kidney Assist™	ECD:			@6 months	@6 months	
		HMPO ₂ n = 15	8 (53.0%)	1 (7.0%)	32 ± 14*	93.0%	NA
		SCS n = 30	10 (33.0%)	0 (0.0%)	38 ± 17*	100.0%	NA
Ravaioli (2020)	Non-RCT II	p-value	0.197	0.333	0.276	0.333	
		ECD:				@1y	@6 months
		cHMPO ₂ n = 10	2 (20.0%)	0 (0.0%)	NA	100.0%	1 (10.0%)
Jochmans (2020)	RCT III Kidney Assist™	SCS n = 30	12 (40.0%)	1 (3.3%)	NA	93.3%	2 (6.7%)
		p-value	0.607	0.948		0.894	
		DCD >50 years:			@1y	@1y	@1y
Husen (2021)	RCT III Kidney Assist™	cHMPO ₂ n = 83	38 (36.0%)	3 (3.0%)	50 ± 19	97.0%	15 (14.0%)
		cHMP n = 83	38 (36.0%)	5 (5.0%)	47 ± 17	90.0%	27 (26.0%)
		p-value	0.990	0.480	0.120	0.028	0.040
Pravisan (2022)	Retrospective cohort study Waves® LifePort® kidney transporter	ECD:			@1y	@1y	@1y
		SCS + eHMPO ₂ n = 127	30 (23.6%)	8 (6.3%)	40 ± 14	92.1%	23 (18.1%)
		SCS n = 135	38 (28.1%)	8 (5.9%)	41 ± 17	93.3%	18 (13.3%)
Dajti (2025)	RCT Vitasart®	p-value	0.400	0.900	0.630	0.630	0.290
		DBD:			sCreat (mg/dL)		
		SCS + eHMPO ₂ n = 51 (Waves®)	11 (21.5%)	0 (0.0%)	1.27 (1.09–1.67)	NA	6 (11.7%)
Darius (2025)	Retrospective belgian cohort LifePort® kidney transporter	SCS + eHMP n = 52 (LifePort®)	13 (25.0%)	0 (0.0%)	*	NA	4 (7.7%)
		p-value	0.580	1.000	1.40 (1.00–1.78)		0.520
					*		
Dajti (2025)	RCT Vitasart®	ECD:			0.319	@1y	@1y
		SCS + eHMPO ₂ (min 2h) n = 54	31 (57.4%)	3 (5.5%)	50 ± 10	83.6%	4 (7.3%)
		SCS n = 55	37 (67.3%)	4 (7.3%)	45 ± 14	88.9%	8 (14.8%)
Darius (2025)	Retrospective belgian cohort LifePort® kidney transporter	p-value	0.300	0.700	0.200	0.400	0.200
		DCD ≥50 years:			@1y	@1y	@1y
		cHMPO ₂ n = 116	22 (19.7%)	1 (0.9%)	50 (14.3–94.2)*	92.2%	14 (12.0%)

Abbreviations: cHMP, continuous hypothermic machine perfusion; cHMPO₂, continuous oxygenated hypothermic machine perfusion; DBD, donation after brain death; DCD, donation after circulatory death; DGF, delayed graft function; ECD, expanded criteria donors; eGFR, estimated glomerular filtration rate; eHMPO₂, endischemic oxygenated hypothermic machine perfusion; HMP, hypothermic machine perfusion; HMPO₂, oxygenated hypothermic machine perfusion; SCS, static cold storage.

Data are presented as mean ± range or mean (min–max) if indicated with*.

the overlapping COVID-19 period and the increased confidence in these kidneys because of the use of HMP during the study period are important parameters that might have influenced the utilization rate in addition to the HMP reimbursement itself. Nonetheless, the increased utilization of higher-risk organs maintained overall transplant activity, which may otherwise have declined. The excellent outcomes observed should help increase clinician confidence in accepting higher risk organs, potentially improving acceptance rates in the future.

The economic impact of HMP is supported by earlier evaluations of the Eurotransplant MP trial, which also showed cost savings through reduced DGF incidence [12, 13]. In our budget impact analysis—comparing the expected dialysis costs of patients on the waiting list (€44,932/year/patient) versus the economy of reducing dialysis cost due to the net increase in national use of ECD and DCD kidneys with HMP (€3,500/procedure) — the increase in transplant activity translated to an estimated 99 additional national kidney transplants per year, yielding substantial healthcare savings of €3.59 million per year.

We acknowledge the following limitations of this manuscript: 1) the lack of a historical control or propensity matched group, 2) the modified DGF definition that excludes one dialysis session

within the 24 h after transplantation could slightly underestimate the DGF rate as compared to studies including this in the DGF definition, 3) the retrospective design, 4) the short time follow-up, 5) the limited generalizability to other health systems because of the specific organizational structure in Belgium, and 6) the absence of a real cost-effectiveness analysis.

Based on these findings, the Belgian NIHD approved a five-year extension of the HMP reimbursement scheme (2025–2030), considering now HMP as standard of care for kidney preservation. The revised criteria now also include HMP for kidney combined-organ transplants, DBD donors <50 years with peak creatinine >1.5 mg/dL, and cross-border HMPO₂ import from the Netherlands. Implementation of cross-border HMP exchange will require harmonization of logistics, regulation, and reimbursement across Eurotransplant countries.

Finally, the national HMP platform offers a unique opportunity to evolve into a multicentre research infrastructure supporting clinical trials, biobanking, and innovation in organ preservation strategies. Future research will focus on refining viability assessment tools, comparing continuous versus end-HMPO₂, and long-term cost-effectiveness in real-world settings. Experience gained from this national effort will help to implement MP of other organs.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethics Committee of the University Clinics Saint-Luc, Brussels, Belgium. No ethical approval was necessary and written informed consent for participation was not required, in accordance with the national legislation and institutional requirements.

AUTHOR CONTRIBUTIONS

TD data collection and analysis/interpretation/concept/drafting article. IJ and JP: second drafting article. MF, EH, CR, BB, GR, NG, LW, DJ-T-T, KW, TB, DM, J-PD, and JD: critical revision of article. All authors contributed to the article and approved the submitted version.

FUNDING

The authors declare that no financial support was received for the research and/or publication of this article.

REFERENCES

1. Tingle SJ, Thompson ER, Figueiredo RS, Moir JA, Goodfellow M, Talbot D, et al. Normothermic and Hypothermic Machine Perfusion Preservation Versus Static Cold Storage for Deceased Donor Kidney Transplantation. *The Cochrane database Syst Rev* (2024) 7(7):Cd011671. doi:10.1002/14651858.CD011671.pub3
2. Moers C, Smits JM, Maathuis MH, Treckmann J, van Gelder F, Napieralski BP, et al. Machine Perfusion or Cold Storage in Deceased-Donor Kidney Transplantation. *The New Engl J Med* (2009) 360(1):7–19. doi:10.1056/NEJMoa0802289
3. O'Callaghan JM, Morgan RD, Knight SR, Morris PJ. Systematic Review and Meta-Analysis of Hypothermic Machine Perfusion Versus Static Cold Storage of Kidney Allografts on Transplant Outcomes. *The Br J Surg* (2013) 100(8):991–1001. doi:10.1002/bjs.9169
4. Deng R, Gu G, Wang D, Tai Q, Wu L, Ju W, et al. Machine Perfusion Versus Cold Storage of Kidneys Derived from Donation After Cardiac Death: A Meta-Analysis. *PloS one* (2013) 8(3):e56368. doi:10.1371/journal.pone.0056368
5. Martinez Arcos L, Fabuel Alcaniz JJ, Gomez Dos Santos V, Burgos Revilla FJ. Functional Results of Renal Preservation in Hypothermic Pulsatile Machine Perfusion Versus Cold Preservation: Systematic Review and Meta-Analysis of Clinical Trials. *Transpl Proc* (2018) 50(1):24–32. doi:10.1016/j.transproceed.2017.12.005
6. Peng P, Ding Z, He Y, Zhang J, Wang X, Yang Z. Hypothermic Machine Perfusion Versus Static Cold Storage in Deceased Donor Kidney Transplantation: A Systematic Review and Meta-Analysis of Randomized Controlled Trials. *Artif organs* (2019) 43(5):478–89. doi:10.1111/aor.13364
7. Gill J, Dong J, Eng M, Landsberg D, Gill JS. Pulsatile Perfusion Reduces the Risk of Delayed Graft Function in Deceased Donor Kidney Transplants, Irrespective of Donor Type and Cold Ischemic Time. *Transplantation* (2014) 97(6):668–74. doi:10.1097/01.TP.0000438637.29214.10

CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

GENERATIVE AI STATEMENT

The authors declare that no Generative AI was used in the creation of this manuscript.

Any alternative text (alt text) provided alongside figures in this article has been generated by Frontiers with the support of artificial intelligence and reasonable efforts have been made to ensure accuracy, including review by the authors wherever possible. If you identify any issues, please contact us.

ACKNOWLEDGEMENTS

We thank Ineke Tiekens, Remco Van Vliet and Andy van der Meij for their precious support of Eurotransplant data collection and recovery. We thank Gunther Vanwezer, Inès Van heesbeke, Peter De Muylder, Bert Theunis, and Bernard Cohen for their support of the machine perfusion data recovery and the organization of the national perfusion service. We thank JD for the help with the budget impact analysis. We thank Bastiaan Jansen for the statistical analysis.

8. Cannon RM, Brock GN, Garrison RN, Marvin MR, Franklin GA, Davis EG. Machine Perfusion: Not Just for Marginal Kidney Donors. *The Am surgeon* (2015) 81(6):550–6. doi:10.1177/000313481508100616
9. Jochmans I, O'Callaghan JM, Pirenne J, Ploeg RJ. Hypothermic Machine Perfusion of Kidneys Retrieved from Standard and High-Risk Donors. *Transpl Int* (2015) 28(6):665–76. doi:10.1111/tri.12530
10. Savoye E, Macher MA, Videcoq M, Gatault P, Hazzan M, Abboud I, et al. Evaluation of Outcomes in Renal Transplantation with Hypothermic Machine Perfusion for the Preservation of Kidneys from Expanded Criteria Donors. *Clin Transplant* (2019) 33(5):e13536. doi:10.1111/ctr.13536
11. Kox J, Moers C, Monbaliu D, Strelneice A, Treckmann J, Jochmans I, et al. The Benefits of Hypothermic Machine Preservation and Short Cold Ischemia Times in Deceased Donor Kidneys. *Transplantation* (2018) 102(8):1344–50. doi:10.1097/TP.0000000000002188
12. Garfield SS, Poret AW, Evans RW. The Cost-Effectiveness of Organ Preservation Methods in Renal Transplantation: US Projections Based on the Machine Preservation Trial. *Transpl Proc* (2009) 41(9):3531–6. doi:10.1016/j.transproceed.2009.10.004
13. Groen H, Moers C, Smits JM, Treckmann J, Monbaliu D, Rahmel A, et al. Cost-Effectiveness of Hypothermic Machine Preservation Versus Static Cold Storage in Renal Transplantation. *Am J Transplant* (2012) 12(7):1824–30. doi:10.1111/j.1600-6143.2012.04030.x
14. Tedesco-Silva HJ, Mello Offerni JC, Ayres Carneiro V, Ivani de Paula M, Neto ED, Brambate CLF, et al. Randomized Trial of Machine Perfusion Versus Cold Storage in Recipients of Deceased Donor Kidney Transplants with High Incidence of Delayed Graft Function. *Transplant Direct* (2017) 3(5):e155. doi:10.1097/TXD.0000000000000672
15. Jochmans I, Brat A, Davies L, Hofker HS, van de Leemkolk FEM, Leuvenink HGD, et al. Oxygenated Versus Standard Cold Perfusion Preservation in Kidney Transplantation (COMPARE): A Randomised, double-blind, Paired, Phase 3 Trial. *Lancet* (2020) 396(10263):1653–62. doi:10.1016/S0140-6736(20)32411-9

16. Darius T, Devresse A, Buemi A, Kanaan N, De Meyer M, Mourad M. First Clinical Kidney Transplantation Series Using Brief Bubble and Direct Surface Oxygenation as Alternative for Membrane Oxygenation During Hypothermic Machine Perfusion. *Artif organs* (2023) 47(4):777–85. doi:10.1111/aor.14475
17. Darius T, Nath J, Mourad M. Simply Adding Oxygen During Hypothermic Machine Perfusion to Combat the Negative Effects of Ischemia-Reperfusion Injury: Fundamentals and Current Evidence for Kidneys. *Biomedicine* (2021) 9(8):993. doi:10.3390/biomedicine9080993
18. Evrard P, Belgian Working Group on DCD National Protocol. Belgian Modified Classification of Maastricht for Donors After Circulatory Death. *Transpl Proc* (2014) 46(9):3138–42. doi:10.1016/j.transproceed.2014.09.169
19. Brat A, de Vries KM, van Heurn EWE, Huurman VAL, de Jongh W, Leuvenink HGD, et al. Hypothermic Machine Perfusion as a National Standard Preservation Method for Deceased Donor Kidneys. *Transplantation* (2022) 106(5):1043–50. doi:10.1097/TP.0000000000003845
20. Darius T, Vergauwen M, Smith TB, Gerin I, Joris V, Mueller M, et al. Brief O₂ Uploading During Continuous Hypothermic Machine Perfusion Is Simple yet Effective Oxygenation Method to Improve Initial Kidney Function in a Porcine Autotransplant Model. *Am J Transplant* (2020) 20:2030–43. doi:10.1111/ajt.15800
21. Jochmans I. Improving Organ Preservation: The Trick Is to Keep (Cells) Breathing. *Transplantation* (2022) 106(5):904–7. doi:10.1097/TP.0000000000003913
22. Calva Lopez A, Robles Garcia JE, Yanez Ruiz CA, Tapia Tapia MD, Talavera Cobo V, Muñoz BCA, et al. Does Oxygen Work? Evidence for Oxygenation During Kidney Graft Preservation: A Review. *J Clin Med* (2025) 14(6):1927. doi:10.3390/jcm14061927
23. Jia D, Guo S, Luo J, Zhang Y, Zhang H, Shengwei. Meta-Analysis and Clinical Guidance of Oxygenated Hypothermic Machine Perfusion for Kidney Transplantation. *Transpl Proc* (2025) 57(5):752–63. doi:10.1016/j.transproceed.2025.03.006
24. Ravaioli M, De Pace V, Angeletti A, Comai G, Vasuri F, Baldassarre M, et al. Hypothermic Oxygenated New Machine Perfusion System in Liver and Kidney Transplantation of Extended Criteria Donors: First Italian Clinical Trial. *Scientific Rep* (2020) 10(1):6063. doi:10.1038/s41598-020-62979-9
25. Husen P, Boffa C, Jochmans I, Krikke C, Davies L, Mazilescu L, et al. Oxygenated End-Hypothermic Machine Perfusion in Expanded Criteria Donor Kidney Transplant: A Randomized Clinical Trial. *JAMA Surg* (2021) 156(6):517–25. doi:10.1001/jamasurg.2021.0949
26. Pravisani R, Baccarani U, Molinari E, Cherchi V, Bacchetti S, Terrosu G, et al. PO(2) 21% Oxygenated Hypothermic Machine Perfusion in Kidney Transplantation: Any Clinical Benefit? *The Int J Artif organs* (2022) 45(8):666–71. doi:10.1177/03913988221107946
27. Dajti G, Vaccaro MC, Germinario G, Comai G, Caputo F, Odaldi F, et al. Hypothermic Oxygenated Perfusion in Extended Criteria Donor Kidney Transplantation-A Randomized Clinical Trial. *Clin Transplant* (2025) 39(5):e70166. doi:10.1111/ctr.70166
28. Meister FA, Czigany Z, Rietzler K, Miller H, Reichelt S, Liu WJ, et al. Decrease of Renal Resistance During Hypothermic Oxygenated Machine Perfusion Is Associated with Early Allograft Function in Extended Criteria Donation Kidney Transplantation. *Scientific Rep* (2020) 10(1):17726. doi:10.1038/s41598-020-74839-7
29. Buitrago LF, Verstraeten L, Fieuws S, Jochmans I. Renal Resistance During Hypothermic Machine Perfusion: A Scoping Review of Variability and Determinants, with a Meta-Analysis of Predictive Value for Transplant Outcomes. *Transpl Rev* (2025) 39:100956. doi:10.1016/j.trre.2025.100956
30. Jochmans I, Moers C, Smits JM, Leuvenink HG, Treckmann J, Paul A, et al. The Prognostic Value of Renal Resistance During Hypothermic Machine Perfusion of Deceased Donor Kidneys. *Am J Transplant* (2011) 11(10):2214–20. doi:10.1111/j.1600-6143.2011.03685.x
31. Sandal S, Paraskevas S, Cantarovich M, Baran D, Chaudhury P, Tchervenkov JJ, et al. Renal Resistance Thresholds During Hypothermic Machine Perfusion and Transplantation Outcomes - a Retrospective Cohort Study. *Transpl Int* (2018) 31(6):658–69. doi:10.1111/tri.13146
32. Verstraeten L, Jochmans I. Sense and Sensibilities of Organ Perfusion as a Kidney and Liver Viability Assessment Platform. *Transpl Int* (2022) 35:10312. doi:10.3389/ti.2022.10312
33. Werenski H, Stratta RJ, Sharda B, Garner M, Farney AC, Orlando G, et al. Knowing when to Ignore the Numbers: Single-Center Experience Transplanting Deceased Donor Kidneys with Poor Perfusion Parameters. *J Am Coll Surgeons* (2023) 236(4):848–57. doi:10.1097/XCS.0000000000000611

Copyright © 2025 Darius, Jochmans, Foguene, Hoste, Randon, Bracke, Roeyen, Gilbo, Weekers, Jacobs-Tulleneers-Thevisen, Wissing, Bogaerts, Mikhalski, De Wilde, Daems and Pirenne. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.



Duodenoduodenostomy as an Attractive Option for Exocrine Drainage in Pancreas Transplantation: Insights From a Single-Center Cohort

Alba Torroella^{1,2†}, Rongrong Hu Zhu^{1,2†}, Carlos Castillo-Delgado^{3†}, Marco Pavesi⁴, Ramón Rull^{1,2}, Emma Folch-Puy⁵, Rocío García^{1,2}, Clara Bassaganyas^{2,6}, Carles Pérez-Serrano^{2,6}, Pedro Ventura-Aguilar^{2,7,8}, Enrique Montagud-Marrahi^{2,7,8}, Víctor Emilio Holguin^{1,2,8}, Antonio J. Amor^{2,8,9}, Fritz Diekmann^{2,7,8}, Ángeles García-Criado^{2,6,8}, Juan Carlos García-Valdecasas^{1,2}, Josep Fuster^{1,2} and Joana Ferrer-Fàbrega^{1,2,8,10*}

¹Hepatobiliopancreatic Surgery and Liver and Pancreatic Transplantation Unit, Department of Surgery, Clinical Institute of Digestive and Metabolic Diseases (ICMDiM), Hospital Clínic Barcelona, Barcelona, Spain, ²University of Barcelona, Barcelona, Spain, ³Hepatobiliopancreatic Surgery and Abdominal Transplant Unit, Department of General Surgery, Hospital General Plaza de la Salud, Santo Domingo, Dominican Republic, ⁴Barcelona Clinical Coordinating Center (BCCC) - Fundació Mòn Clínic, Barcelona, Spain, ⁵Experimental Pathology Department, Institut d'Investigacions Biomèdiques de Barcelona-Consejo Superior de Investigaciones científicas (IIBB-CSIC), Barcelona, Spain, ⁶Radiology Department, Hospital Clínic Barcelona, Barcelona, Spain, ⁷Kidney Transplant Unit, Nephrology and Kidney Transplantation Department, Hospital Clínic Barcelona, Barcelona, Spain, ⁸August Pi i Sunyer Biomedical, Research Institute (IDIBAPS), Barcelona, Spain, ⁹Diabetes Unit, Endocrinology and Nutrition Department, Hospital Clínic Barcelona, Barcelona, Spain, ¹⁰Network for Biomedical Research in Hepatic and Digestive Diseases (CIBERehd), Barcelona, Spain

OPEN ACCESS

*Correspondence

Joana Ferrer-Fàbrega,
✉ joferrer@clinic.cat

[†]These authors have contributed
equally to this work

[‡]These authors share first authorship

Received: 13 August 2025

Accepted: 20 October 2025

Published: 03 November 2025

Citation:

Torroella A, Zhu RH, Castillo-Delgado C, Pavesi M, Rull R, Folch-Puy E, García R, Bassaganyas C, Pérez-Serrano C, Ventura-Aguilar P, Montagud-Marrahi E, Holguin VE, Amor AJ, Diekmann F, García-Criado Á, García-Valdecasas JC, Fuster J and Ferrer-Fàbrega J (2025) Duodenoduodenostomy as an Attractive Option for Exocrine Drainage in Pancreas Transplantation: Insights From a Single-Center Cohort. *Transpl. Int.* 38:15430. doi: 10.3389/ti.2025.15430

Techniques such as retroperitoneal graft placement have further enhanced the ability to replicate the physiology of the “native” pancreas. In our center, from January 2000, duodenojejunostomy (DJ) was the standard technique for exocrine drainage (n = 337). Herein, we report a series of 188 pancreas transplantations performed between May 2016 to July 2025, using a fully retrocolic graft position, systemic venous drainage and enteric drainage via duodenoduodenostomy. The primary endpoint was the assessment of intestinal events and their impact on graft and patient survival. A total of 14 patients (7.4%) experienced complications, including paralytic ileus (n = 2), intestinal obstruction (n = 4), duodenal dehiscence following pancreas transplantectomy (n = 1), anastomotic dehiscence (n = 5), and anastomotic bleeding (n = 2). Of these, 11 cases required relaparotomy for adhesiolysis (n = 2), internal hernia repair (n = 1), Hartmann's procedure (n = 1), transplantectomy (n = 2), primary leak closure (n = 3), and hemostasis with duodenal re-anastomosis (n = 2). After a median follow-up of 42.8 months [IQR 21.8–71.1], graft survival at 1 and 5 years was 87% and 83.4%, respectively

Abbreviations: BMI, Body mass index; CIT, Cold ischemia time; DBD, Donation after brain death; cDCD, Controlled donation after circulatory death; DD, Duodenoduodenostomy; DJ, Duodenojejunostomy; IGL-1, Institute Georges Lopez-1; PAK, Pancreas after kidney; PA, Pancreas alone; SPK, Simultaneous pancreas-kidney; US, Ultrasound; UW, University of Wisconsin.

($P = 0.688$ vs. DJ group), while patient survival was 100% and 98.2% ($P = 0.031$ vs. DJ group). Duodenoduodenostomy proved to be a feasible and effective technique, offering competitive outcomes in terms of graft and patient survival.

Keywords: pancreas transplantation, graft survival, exocrine drainage, duodenoduodenostomy, intestinal complications

INTRODUCTION

Pancreas transplantation has demonstrated its effectiveness in achieving normoglycemia and insulin independence, while also reducing metabolic instability and enhancing quality of life, with favorable long-term outcomes [1–3]. Since the first procedure in 1966, numerous refinements have been introduced, particularly concerning exocrine drainage, aiming to minimize morbidity and optimize results [4]. Initially, bladder drainage was the preferred method due to its utility in diagnosing rejection through urinary amylase monitoring [5]. However, its high complication rates and a substantial conversion rate to enteric drainage, reported to reach up to 40%, ultimately led to its decline [6, 7]. Today, enteric drainage is widely adopted, offering a more physiological approach and excellent clinical performance. Within this modality, several techniques have been developed, involving different segments of the small intestine. Two approaches have gained prominence: Roux-en-Y diversion and direct anastomosis to the jejunum or proximal ileal loop [8–10]. Additionally, alternative methods such as gastric anastomosis have also been described [11].

Although enteric diversion offers notable advantages, it is not without risks, presenting a considerable incidence of intra-abdominal adverse events and posing technical challenges in accessing the anastomosis and allograft for diagnostic and therapeutic purposes [1, 4].

Currently, various transplant centers have begun adopting direct duodenoduodenostomy (DD) as an alternative method for exocrine drainage, first introduced in 2007 [12]. In efforts to assess whether this approach offers improved graft survival and reduced postoperative morbidity, some case series have been published in recent years [13–23]. The benefits of endoscopic access for both diagnostic and therapeutic management have been highlighted, although interpreting duodenal biopsies remains challenging [24–27].

In line with the pursuit of safer and more reproducible surgical techniques that facilitate management of postoperative events, our team transitioned from duodenojejunostomy (DJ) to DD. This study aims to analyze the outcomes associated with DD, based on data collected from its implementation, focusing on two objectives. First, to assess intestinal events and their influence on graft and patient survival. Second, to compare these findings with

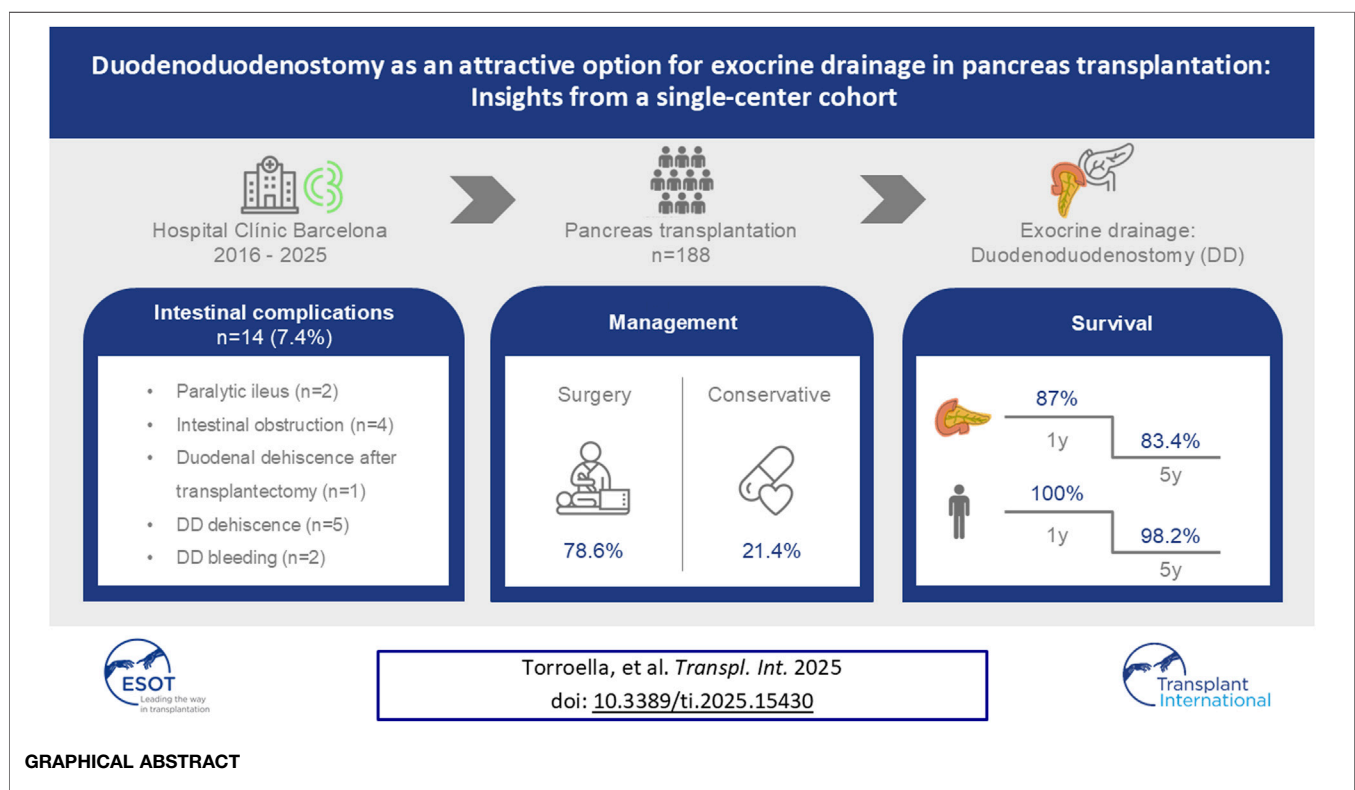




FIGURE 1 | Pancreas transplantation with retrocolic graft placement and duodenoduodenostomy for exocrine drainage. Esophagogastroduodenal transit study with Gastrografin (right) demonstrates proper contrast passage through the recipient's duodenum (arrowheads), the side-to-side duodenoduodenal anastomosis (white arrows), and the graft's duodenal segment (asterisks), with no evidence of leakage. Abdominal contrast-enhanced computed tomography of the same patient (left) shows a normoenhancing pancreatic graft (P), without collections or other complications.

the previously published historical series of DJ procedures performed at the same center, in order to evaluate the most appropriate surgical strategy for exocrine drainage moving forward.

MATERIALS AND METHODS

Study Design

Pancreas transplants performed at the Hospital Clínic of Barcelona since the inception of the program in 1989 have been recorded in a prospectively maintained database.

In May 2016, direct DD was adopted as the preferred method for managing the exocrine output of the graft. All procedures carried out from that date through July 2025 were subsequently analyzed. These findings were then compared with the outcomes of the previously published series using DJ for enteric diversion, performed between 2000 and April 2016 at the same institution [28].

All categories of pancreas transplantation were included in the analysis: simultaneous pancreas-kidney (SPK), pancreas after kidney (PAK), pancreas alone (PA), and retransplantation. Recipients underwent a standardized preoperative evaluation conducted by a multidisciplinary team, following institutional criteria for indications and contraindications previously published [29]. Donor selection adhered to the consensus guidelines for pancreas and islet transplantation established by the National Transplant Organization [30].

Prophylactic Therapy

Antibiotic prophylaxis consisted of ertapenem, vancomycin, and fluconazole, along with antiviral coverage for cytomegalovirus. Thromboprophylaxis was maintained using low molecular weight heparin and acetylsalicylic acid, following the protocol previously published elsewhere [28].

Immunosuppression Regimens

Routine immunosuppression followed institutional protocols, with regimens varying according to the date of transplantation [31]. During the SARS-CoV-2 pandemic, adjustments were made between April 2020 and May 2021 due to increased immunological risk. During this period, induction therapy with the anti-interleukin-2 monoclonal antibody (basiliximab) was preferred over rabbit anti-human polyclonal antibodies (thymoglobulin).

Surgical Technique

The pancreas is harvested *en bloc* along with the donor duodenum and spleen, and the graft is subsequently prepared on the back table. Arterial reconstruction is performed either by (a) end-to-end anastomosis between the splenic artery and the distal superior mesenteric artery, or (b) reconstruction with an iliac arterial "Y" graft. Additionally, the donor duodenum is transected with a mechanical stapler to a length of approximately 8–10 cm, and the edges are reinforced with either continuous or interrupted hand-sewn sutures using 3-0 non-absorbable material [32].

Recipient surgery was performed via midline laparotomy. Graft placement varied depending on the study period: (a) in the DD

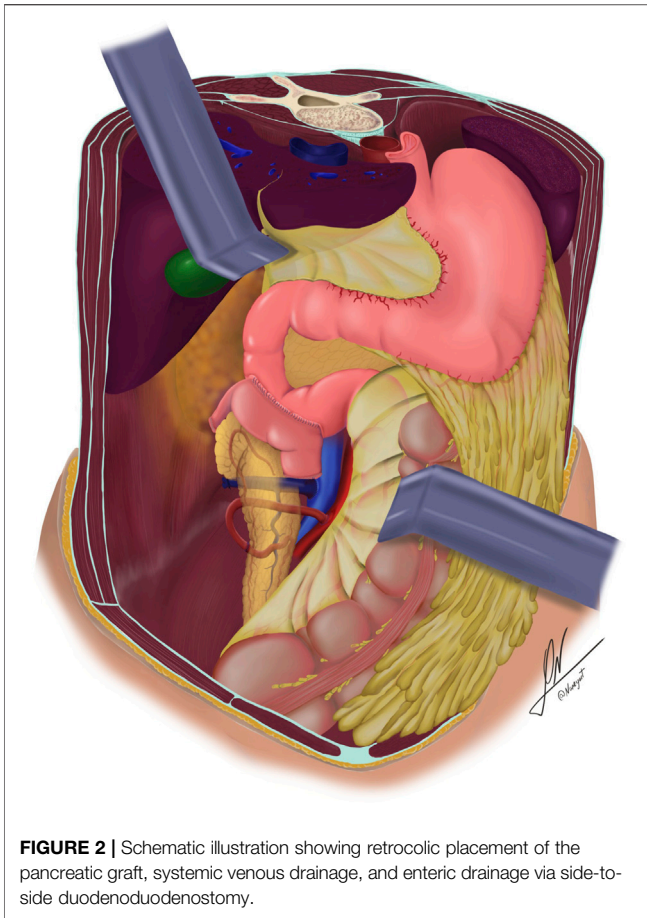


FIGURE 2 | Schematic illustration showing retrocolic placement of the pancreatic graft, systemic venous drainage, and enteric drainage via side-to-side duodenoduodenostomy.

group, the pancreas was positioned retrocolically on the right side of the pelvis following mobilization of the right colon using the Cattell-Braasch and Kocher maneuvers, with the duodenal segment oriented cephalad [21] (Figures 1, 2); or (b) in the DJ group, the graft was placed intraperitoneally, as previously described [28]. Venous drainage was directed into the systemic circulation through an anastomosis between the graft's portal vein and either the inferior vena cava or the recipient's right iliac vein. Arterial supply was established via an end-to-side anastomosis between the graft's superior mesenteric artery, or the Y-graft, depending on the reconstruction technique used during back-table preparation, and the recipient's common right iliac artery.

Following graft revascularization and achievement of haemostasis, the enteric anastomosis is performed. In the DD group, a side-to-side hand-sewn anastomosis is constructed using a double-layer technique: the outer layer with non-absorbable 3-0 sutures and the inner layer with absorbable 3-0 sutures, applied either continuously or with interrupted stitches (Figures 3, 4). In contrast, the DJ group underwent a side-to-side anastomosis without Roux-en-Y, positioned 60–80 cm distal to the ligament of Treitz. This was also performed using a double-layer hand-sewn technique, employing 3-0 non-absorbable sutures for the external layer and 3-0 absorbable sutures for the inner layer, as previously described [28].

Patient Progress and Follow-Up

Postoperative morbidity was defined as any complication occurring within 90 days following pancreas transplantation, and was categorized according to the Clavien-Dindo classification system [33]. Diagnostic approaches were also assessed. Doppler ultrasound (US) is the standard imaging technique used in the postoperative period. A mandatory US is performed within 24–48 h after transplantation to evaluate vascular patency, graft integrity, and potential indirect signs of intra-abdominal complications. In cases presenting with acute abdominal symptoms, graft dysfunction, or inconclusive ultrasound results, computed tomography or Angio-CT was employed. When acute rejection was suspected, based on biochemical markers, imaging findings, or poor glycemic control, graft biopsies were obtained. Rejection episodes were classified according to the Banff criteria [34].

Management of intestinal complications was tailored to the patient's clinical condition and the severity of the adverse event.

Statistical Analysis

Categorical variables are presented as absolute frequencies and percentages (%). Quantitative variables are expressed as median and interquartile range [IQR] (25th–75th percentiles). Categorical variables were analyzed by use of Fisher's exact or chi-square test, and continuous variables were analyzed by unpaired Student's t-test, Mann-Whitney U-test, or other nonparametric tests.

Pancreas graft survival was calculated from the time of transplant until the return to permanent insulin therapy dependency, or death/end of follow-up with a functioning graft. Patient survival was calculated from the time of transplant to death or the end of follow-up.

Patient and graft survival rates were compared between exocrine drainage technique groups by means of Kaplan-Meier curves and the Log-rank test. A Cox proportional hazards model was used to obtain a hazard ratio estimate for DD vs. DJ adjusting for the potential confounders of technique effects, i.e., those factors showing a clinically and statistically significant association with the technique group (Table 1) and with 10-year mortality (Supplementary Table S1) or 10-year graft survival (Supplementary Table S2).

A P value <0.05 was considered to indicate statistical significance. Data are collected and analyzed with SPSS statistical software (SPSS 20.0, 1989-1995; Chicago, IL, United States) and SAS v 9.4 software (SAS Institute, Cary, North Carolina, United States).

RESULTS

Donor and Recipient Demographic Data

Between January 2000 and July 2025, a total of 526 pancreas transplants were performed in our unit. From January 2000 to April 2016, DJ was the technique employed for exocrine drainage (n = 337). In May 2016, a transition to DD was implemented, with 188 transplants performed to date, which constitute the focus of this study. It is noteworthy that, in one case (excluded from the

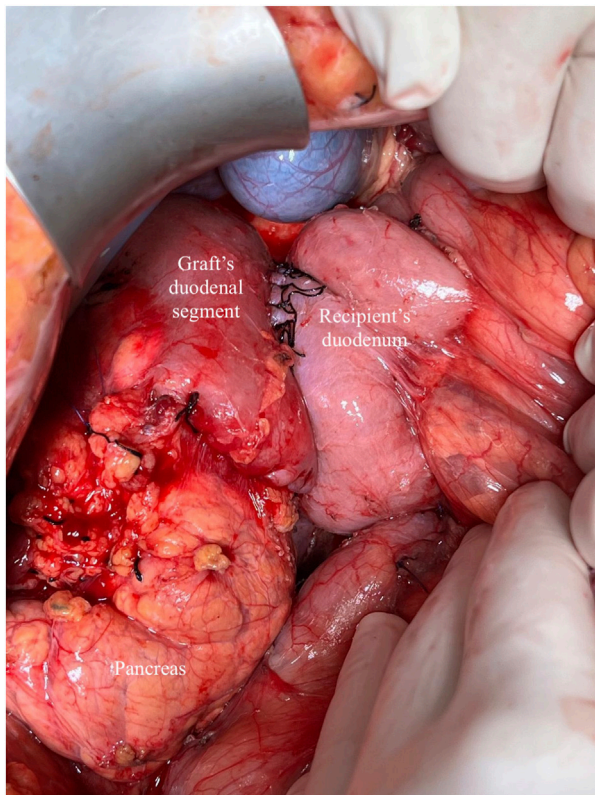


FIGURE 3 | Whole-Organ transplant with systemic venous and enteric exocrine drainage (cephalad position). Duodenoduodenostomy technique with side-to-side anastomosis between the duodenal segment and lower knee of the recipient's duodenum.

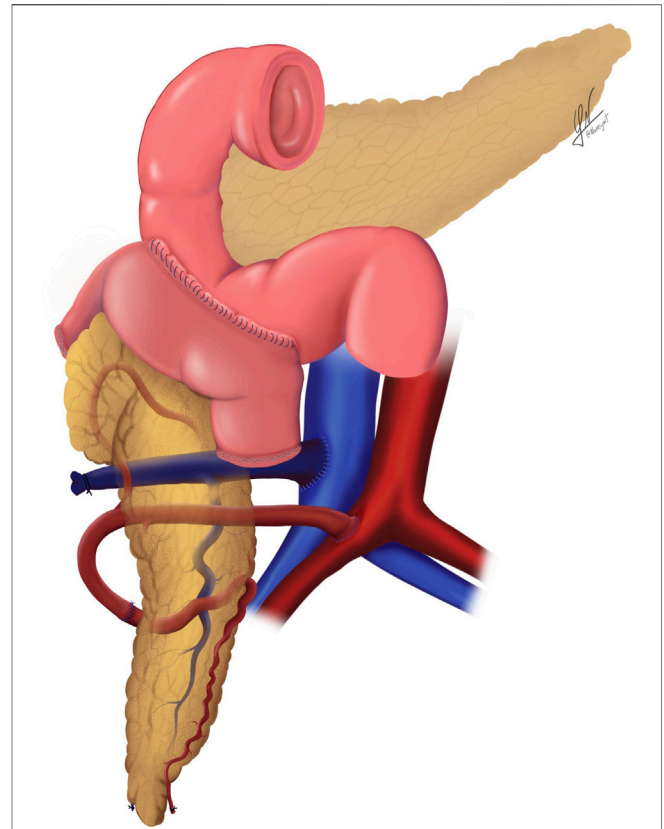


FIGURE 4 | Schematic illustration of the systemic venous drainage technique combined with duodenal enteric drainage.

analysis) during this period, a DJ was performed due to a technical challenge, as the inferior vena cava was positioned to the left of the aorta.

The characteristics of the donors and recipients are summarized in **Table 1**. Historically, all donors were donation after brain death (DBD). Controlled donation after circulatory death (cDCD) with normothermic regional perfusion was introduced at our center in 2019, with 32 cases performed to date, all within the DD group. The median donor age was significantly higher in the DD group ($P < 0.001$), with cerebrovascular disease being the leading cause of death (45.7%). In the DJ group, donor amylase and lipase levels were substantially elevated, with a trend toward statistical significance ($P = 0.059$ and $P = 0.079$, respectively). No significant differences were observed between groups regarding gender, body mass index (BMI), ICU stay duration, or Pancreas Donor Risk Index (P-PASS). Preservation solutions varied across groups and time periods. Institut Georges Lopez-1 (IGL-1) was predominantly used in the DD group (74.5%) following its introduction at our center in 2015 (31), whereas the University of Wisconsin (UW) solution was employed in 76% of DJ cases ($P < 0.001$). Cold ischemia time (CIT) for the pancreas was significantly shorter in the DD group [7.5 h (IQR 6–10)] compared to the DJ group [10.3 h (IQR 8–12)] ($P < 0.001$).

As for recipient demographics, age was slightly higher in the DD group ($P < 0.001$), with a predominance of male patients. Notably, the DD group exhibited a shorter duration of dialysis prior to transplantation ($P < 0.001$) and a longer history of diabetes mellitus ($P = 0.011$). Previous abdominal surgery was documented in nearly 40% of patients in the DD group. SPK transplantation was the most frequently performed procedure in both groups. For other transplant categories (PAK, PA, and retransplantation) the majority of cases belonged to the DJ group. Backtable vascular graft reconstruction using a splenic artery-superior mesenteric artery technique was performed in 85.6% of DD cases and 95.7% of DJ cases, with the remaining procedures utilizing a Y-graft configuration.

Post-reperfusion serum levels of amylase and lipase (measured at 24–48 h) were also analyzed. Amylase levels showed no significant difference between groups [DD: 168 (IQR 114.5–272.5) vs. DJ: 195 (IQR 111–349); $P = 0.176$], while lipase levels were significantly lower in the DD group [DD: 154.5 (IQR 84–268.5) vs. DJ: 183.5 (IQR 100–389.2); $P = 0.041$].

Postoperative Outcomes

Intestinal complications, a central focus of this study, were identified in 14 patients within the DD group (7.4%) (**Table 2**). Both mild and severe cases were included, encompassing patients managed conservatively as well as those

TABLE 1 | Donor and recipient characteristics.

	Total (n = 525)	DD group (n = 188)	DJ group (n = 337)	P Value
DONOR				
Type of donor	493 (93.9%)	156 (83%)	337 (100%)	<0.001 ^a
- DBD	32 (6.1%)	32 (17%)	-	
- cDCD III				
Cause of death				<0.001 ^b
- Trauma	251 (47.8%)	69 (36.7%)	182 (54%)	
- CVA	207 (39.4%)	86 (45.7%)	121 (35.9%)	
- Anoxic damage	41 (7.8%)	22 (11.7%)	19 (5.6%)	
- Euthanasia	4 (0.8%)	4 (2.1%)	-	
- Others	22 (4.2%)	7 (3.7%)	15 (4.5%)	
Age (years)	33 [21–42]	36 [23–46]	31 [21–40]	0.0004 ^c
Gender (M/F)	313 (59.6%)/212 (40.4%)	107 (56.9%)/81 (43.1%)	206 (61.1%)/131 (38.9%)	0.345 ^b
BMI (Kg/m ²)	23.4 [21.5–25.3]	23.5 [21.6–25.1]	23.4 [21.6–25.4]	0.583 ^c
ICU stay (days)	2 [1–5]	2 [1–5]	3 [1.7–7]	0.242 ^d
Amylase (IU/L)	80 [47–156.5]	75 [41–133]	84 [48.8–170]	0.059 ^d
Lipase (IU/L)	34.5 [17–95.2]	31 [16–64]	45 [17.5–115]	0.079 ^d
P-PASS total	16 [14–18]	17 [15.2–18]	16 [14–18]	0.208 ^c
Preservation solution	271 (51.6%)	15 (8%)	256 (76%)	<0.001 ^b
- UW	99 (18.9%)	32 (17%)	67 (19.9%)	
- CS	8 (1.5%)	1 (0.5%)	7 (2.1%)	
- HTK	147 (28%)	140 (74.5%)	7 (2.1%)	
- IGL-1				
Pancreas CIT ^e (hours)	10 [7–11.5]	7.5 [6–10]	10.3 [8–12]	<0.001 ^c
RECIPIENT				
Age (years)	41 [35–47]	43 [36–49]	40 [35–45]	<0.001 ^a
Gender (M/F)	330 (62.9%)/195 (37.1%)	110 (58.5%)/78 (41.5%)	220 (63.3%)/117 (34.7%)	0.123 ^b
BMI (Kg/m ²)	23 [21–25.6]	23 [21.1–25.3]	23 [20.8–25.9]	0.799 ^a
Type of DM				0.067 ^b
- DM 1	516 (98.3%)	183 (97.3%)	333 (98.8%)	
- DM 2	3 (0.6%)	3 (1.6%)	-	
- Others	6 (1.1%)	2 (1.1%)	4 (1.2%)	
DM vintage (years)	26 [21–32]	28 [22–34]	26 [21–31]	0.011 ^c
Dialysis vintage (months)	24 [13.9–35.7]	18 [11–27]	27.0 [19.4–37.7]	<0.001 ^c
Type of dialysis				<0.001 ^b
- Predialysis	63 (12%)	36 (19.1%)	27 (8%)	
- Peritoneal	108 (20.6%)	31 (16.5%)	77 (22.8%)	
- Hemodialysis	291 (55.4)	103 (54.8%)	188 (55.8%)	
- None	63 (12%)	18 (9.6%)	45 (13.4%)	
Transplant type				0.107 ^b
- SPK	444 (84.6%)	168 (89.4%)	276 (81.9%)	
- PAK	30 (5.7%)	7 (3.7%)	23 (6.8%)	
- PA	3 (0.6%)	-	3 (0.9%)	
- Retransplant	48 (9.1%)	13 (6.9%)	35 (10.4%)	

^aFisher Exact p-value.^bChi-Square p-value.^cEqual variance two sample t-test.^dKruskal-Wallis p-value.

Continuous variables are expressed as median [interquartile ranges (IQR)] and categorical variables as frequencies (percentages).

BMI, body mass index; cDCD, controlled donation after circulatory death; CS, celsior; CIT, cold ischemia time; CVA, cerebrovascular accident; DBD, donation after brain death; DD, duodenoduodenostomy; DJ, duodenojejunostomy; DM, diabetes mellitus; F, female; HTK, Histidine-Tryptophan-Ketoglutarate; IGL-1, Institut Georges Lopez-1; ICU, intensive care unit; M, male; PPASS, preprocurement pancreas suitability score; PAK, pancreas after kidney; PA, pancreas transplant alone; SPK, Simultaneous Pancreas-Kidney; UW, university of wisconsin.

^eCIT, is the interval between the initiation of organ perfusion with cold preservation solution in the donor and the onset of reperfusion in the recipient.

requiring more intensive interventions. All diagnoses were confirmed through radiological imaging, specifically computed tomography scan.

Two patients developed *postoperative paralytic ileus*, both successfully managed with a conservative approach involving nasogastric decompression and total parenteral nutrition. In both cases, peripheral venous thrombosis was also identified during routine US follow-up. This finding did not affect

pancreatic graft function and did not necessitate any modification to the standard anticoagulation protocol, which consists of low molecular weight heparin at a dose of 20 mg every 12 h, combined with acetylsalicylic acid at 50 mg once daily.

Four patients experienced *intestinal obstruction*. In two cases, the cause was adhesions involving the colon and small intestine secondary to graft pancreatitis, requiring reoperation for adhesiolysis. Another patient presented with an internal

TABLE 2 | Summary of intestinal complications.

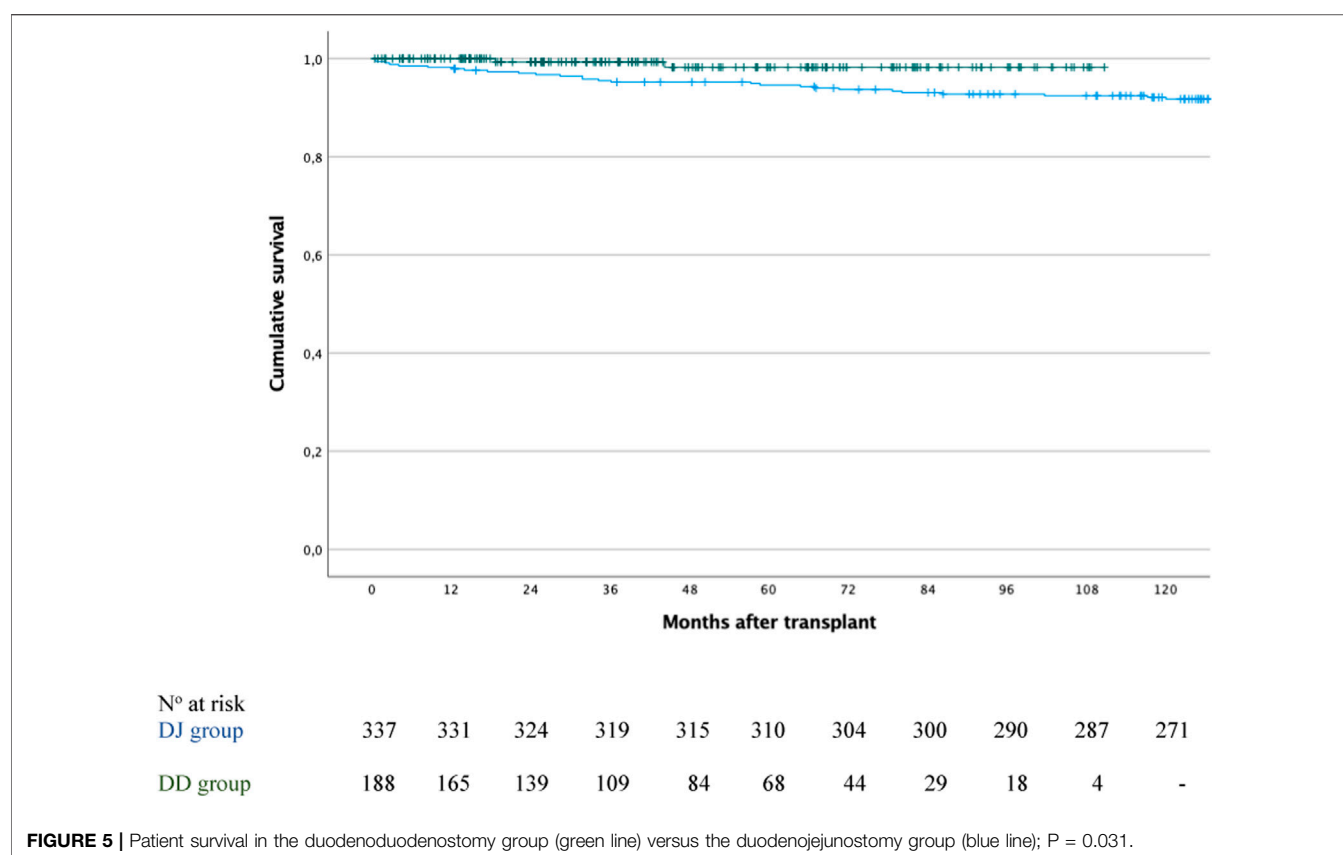
Transplant type (year)	Donor type/cause of death Gender/age (years)	CIT (hours)	Intestinal complication	Recipient gender/age (years)/BMI (Kg/m ²)	Treatment Post-transplant day of treatment	Graft/patient survival (months)
SPK (2018)	DBD/CVA Male/24	11.10	Intestinal obstruction <i>No vascular event</i> Clavien-Dindo: IIIb	Male/36/18.7	Surgery: Internal hernia repair (15° day)	85.8/85.8 Alive
Pancreas Retx (2018)	DBD/CVA Female/38	11.30	Recipient duodenum dehiscence after transplantectomy (<i>vascular thrombosis</i>) Clavien-Dindo: IIIa	Female/57/21.3	Endoscopic clip (72° day)	1.18/79.8 Alive
SPK (2019)	DBD/CVA Female/41	12	DD dehiscence <i>No vascular event</i> Clavien-Dindo: IIIb	Male/53/33.1	Transplantectomy (8° day)	0.85/18.6 Death (sepsis)
SPK (2019)	DBD/Trauma Male/36	9	Paralytic ileus <i>Venous (SMV) peripheral thrombosis^a</i> Clavien-Dindo: II	Female/43/24.9	Nasogastric tube TPN (8° day)	44.2/44.2 Death (Brest cancer)
SPK (2019)	DBD/CVA Female/56	6	DD dehiscence <i>Distal arterial thrombosis head of pancreas^a</i> Clavien-Dindo: IIIb	Male/42/22	Primary leak closure (11° day)	68.6/68.6 Alive
SPK (2020)	DBD/CVA Male/20	7.40	Intestinal obstruction <i>No vascular event</i> Clavien-Dindo: IIIb	Male/46/21.2	Adhesiolysis between the colon and pancreatic tail (12° day)	62.9/62.9 Alive
SPK (2020)	DBD/Trauma Male/24	12	Paralytic ileus <i>Venous (SV) peripheral thrombosis^a</i> Clavien-Dindo: II	Male/56/31.8	Nasogastric tube TPN (7° day)	56.2/56.2 Alive
SPK (2021)	DBD/Trauma Male/18	10.6	Intraluminal DD bleeding <i>No vascular event</i> Clavien-Dindo: IIIb	Female/45/23.9	Hemostasis and DD re-anastomosis (11° day)	16.6/43.9 Chronic rejection Alive
SPK (2022)	cDCD/ Neurodegenerative disorder Male/46	4.2	DD dehiscence Hemoperitoneum (1° day: reoperation) <i>No vascular event</i> Clavien-Dindo: IIIb	Male/43/27	Primary leak closure (7° day)	41.2/41.2 Alive
SPK (2022)	DBD/CVA Male/48	5.6	Intestinal obstruction 2° to pancreatitis <i>No vascular event</i> Clavien-Dindo: IIIb	Male/46/25.9	Adhesiolysis (11° day)	0.36/40.2 Primary Graft Dysfunction Alive
PAK (2022)	cDCD/CVA Male/46	4	Intestinal obstruction 2° to stercoral colitis Clavien-Dindo: IIIb	Male/45/25.2	Hartmann's procedure (37° day)	34.5/34.5 Alive
SPK (2022)	DBD/Trauma Male/40	11	Anastomotic bleeding <i>Venous (SV and SM) occlusive thrombosis</i> Clavien-Dindo: IIIb	Female/30/31.2	Thrombectomy and hemostasis and DD re-anastomosis (6° day)	33.7/33.7 Alive
SPK (2022)	DBD/CVA Female/50	5.2	DD dehiscence <i>No vascular event</i> Clavien-Dindo: IIIb	Male/46/21.6	Primary leak closure (15° day)	32.4/32.4 Alive
SPK (2024)	cDCD/Anoxia Male/36	5.2	DD dehiscence <i>Venous (SV and SM) occlusive thrombosis</i> Clavien-Dindo: IIIb	Male/42/29.7	Transplantectomy (7° day)	0.3/17.2 Alive

BMI, body mass index; cDCD, controlled donation after circulatory death; CIT, cold ischemia time; CVA, cerebrovascular accident; DBD, donation after brain death; DD, duodenoduodenostomy; PAK, pancreas after kidney; Retx, pancreas retransplantation; SPK, Simultaneous Pancreas-Kidney; SMV, superior mesenteric vein; SV, splenic vein; TPN, total parenteral nutrition.

^aAnticoagulation protocol [Low molecular weight heparin (20 mg/12 h), and acetylsalicylic acid (50 mg/24 h)].

hernia, which also necessitated surgical intervention. Lastly, a Hartmann's procedure was performed in a patient diagnosed with stercoral colitis on postoperative day 37. None of these cases were associated with vascular events.

A recipient duodenal leak following graft transplantectomy due to venous thrombosis was also observed. The diagnosis was made based on the bilious appearance of the abdominal drainage. The complication was managed endoscopically and successfully



sealed using the OVESCO system (OTSC®, Ovesco Endoscopy GmbH, Tübingen, Germany), with satisfactory clinical outcomes.

Anastomotic duodeno-duodenostomy dehiscence was diagnosed in five patients. One presented with abdominal pain, while the remaining cases were identified due to bilious output from abdominal drainage. Three patients underwent reoperation, with successful primary closure of the infracentimetric leak and reinforcement of the anastomosis using separate stitches, resulting in good postoperative recovery and preserved pancreatic graft function. The remaining two cases had previously undergone unsuccessful surgical attempts to repair the leak, ultimately requiring graft removal. One recipient had a BMI of 33 and a CIT of 12 h, with evidence of duodenal hypoperfusion observed at the time of reperfusion. The second case involved a marginal cDCD donor who had been supported with ECMO for 12 days and also presented with associated occlusive vascular thrombosis.

Bleeding from the duodeno-duodenostomy anastomosis was identified in two patients, prompting surgical intervention to revise the intestinal anastomosis and achieve duodenal hemostasis. Notably, one of these cases also presented with venous occlusive thrombosis, for which a thrombectomy was performed. The graft was successfully salvaged and remains functional at 33.7 months post-transplantation.

Overall, in the DD group, the median length of hospital stay was 12 days [IQR 10–20]. Patients who

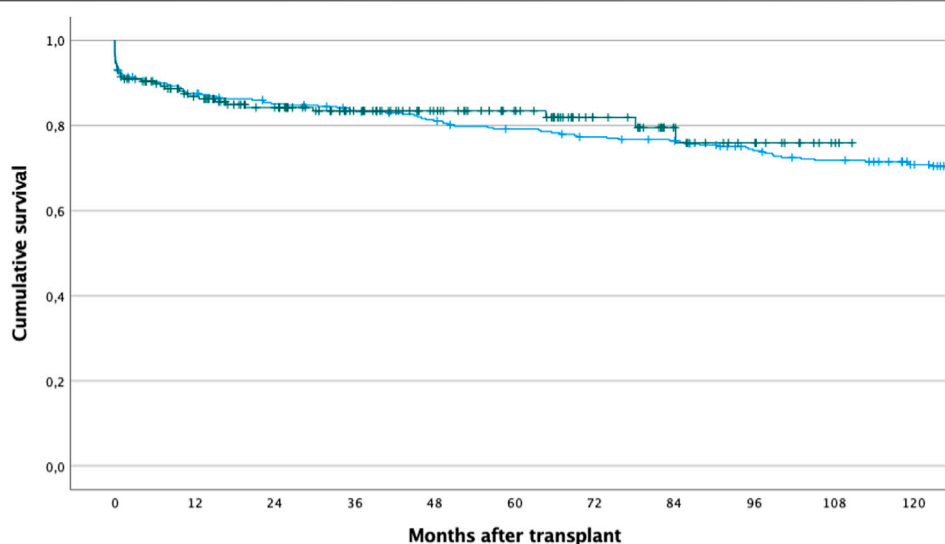
experienced intestinal complications had significantly longer hospitalizations, with a median of 27.5 days [IQR 16–38.5] ($P < 0.001$).

Patient and Graft Survival

After a median follow-up of 124.9 months [IQR: 49.6–205.1] for the entire cohort ($n = 525$), overall patient survival at 1, 3, 5, and 10 years was 98.8%, 96.7%, 95.8%, and 93.3%, respectively. When stratified by group, survival rates were 100%, 99.3%, 98.2% and 97.6% for the DD group, and 98.2%, 95.5%, 94.6% and 92.1% for the DJ group ($P = 0.031$; **Figure 5**).

Focusing on the DD group, after a median follow-up of 42.8 months [IQR 21.8–71.1], patient survival was 100% in both the intestinal complication and non-complication groups at 1 year. At 3 years, survival was 92.3% in the complication group versus 100% in the non-complication group, and at 5 and 10 years, 76.9% versus 100%, respectively ($P < 0.001$). Two patients died: one due to abdominal sepsis at 18.6 months post-transplant, and another from metastatic breast cancer at 44.2 months post-transplant.

Furthermore, among the 525 patients, death-censored pancreas graft survival at 1, 3, 5, and 10 years was 87.3%, 83.4%, 80% and 71.3%, respectively. When stratified by group, survival rates were 87%, 83.4%, 83.4% and 75.9% for the DD group, and 87.5%, 83.5%, 79.1% and 70.8% for the DJ group ($P = 0.688$; **Figure 6**).



N° at risk											
DJ group	337	292	280	272	262	253	245	240	226	217	205
DD group	188	141	118	92	71	58	36	23	12	3	-

FIGURE 6 | Pancreas graft survival in the duodenoduodenostomy group (green line) versus the duodenojejunostomy group (blue line); $P = 0.688$.

In the DD group, graft survival was significantly lower in patients with intestinal complications compared to those without: 71.4% versus 88.1% at 1 year, 64.3% versus 85% at 3 years and 5 years, and 64.3% versus 77.1% at 10 years ($P = 0.049$).

After adjusting the effect of surgical technique on patient survival for the potential confounders observed in **Table 1** and in **Supplementary Table S1** (donor age, amylase levels and pancreas CIT), the estimated risk for DD vs. DJ was a hazard ratio (HR) of 0.47 (95% CI 0.10–2.14; $P = 0.3264$).

The Cox proportional hazards model fitted for 10-year graft survival adjusted group effects for recipient age, type of dialysis, and for donor pancreas CIT. The estimated HR for DD vs. DJ was 1.05 (95% CI 0.67–1.64; $P = 0.8378$).

DISCUSSION

Exocrine drainage has undergone several variations throughout history. Bladder drainage, which was predominant until the mid 90s, allows for immunological monitoring of the graft through the determination of pancreatic enzymes in urine or by cystoscopic biopsy [5, 35]. However, reflux pancreatitis and urinary bicarbonate losses causing metabolic acidosis and dehydration led to its replacement by enteric drainage. Despite technical advances, no universally accepted surgical approach has been established to date. The choice of technique varies significantly across transplant centers, influenced by team experience, recipient characteristics, and institutional preferences. This heterogeneity reflects both the complexity of the procedure and the need to tailor

treatment to the clinical context. Traditionally, enteric anastomosis can be performed at the level of the proximal jejunum or distal ileum, either end-to-end, end-to-side, or side-to-side. Although Boggi et al [8] described excellent results using the Roux-en-Y side-to-side DJ, the direct side-to-side duodenojejunostomy is more prevalent [10, 28].

An attractive option arises when the pancreas is placed in the right retrocolic position, the direct side-to-side DD, which allows for a more physiological graft placement. The main advantage of DD is its endoscopic accessibility [24–27], as it facilitates pancreatic and duodenal biopsies for the diagnosis of rejection and allows for stenting of the pancreatic duct in cases of pancreatic fistulas.

A key element promoting the use of DD was its role in pancreas alone transplantation, which faces a higher risk of immunological graft loss compared to SPK transplantations due to the absence of monitoring tools for rejection without a sentinel kidney graft. Consequently, the endoscopic accessibility to the graft duodenum was a significant factor that set off the expansion of DD worldwide.

Beyond this potential advantage, parallel considerations must be taken into account by the fact that retrocolic placement of the graft raised concerns regarding restricted access for percutaneous biopsy, potentially complicating postoperative monitoring. However, recently, Bassaganyas et al. [36] demonstrated that percutaneous biopsy accessibility in retrocolic positioned grafts implanted via duodenoduodenal anastomosis is not only feasible but also associated with a high success rate in terms of histological diagnosis. Moreover, the accessibility rate was found to be even

higher than that observed in intraperitoneally positioned grafts (87.9% in DD group compared to 68.4% in DJ group). These findings challenge the previously held perception that retrocolic positioning might hinder access and instead support the use of this technique as a safe and effective tool for the post-transplant follow-up of pancreatic grafts. Due to the favorable outcomes achieved with percutaneous biopsy at our center, transduodenal biopsies have not been required thus far. Nonetheless, this does not exclude the possibility that such procedures may be necessary in future cases.

The first case reports performing DD were published in 2007 [13] and since then, many centers have adopted this technique [37]. In 2012, Gunasekaran et al [17] presented the first series of 21 cases of DD and compared its results with DJ, not finding significant differences between groups in terms of graft survival. In 2014, the German group from Bochum [18] published one of the largest series to date with 125 cases of DD. Survival rates were slightly better in the DD group than DJ group, although this difference was not statistically significant ($p = 0.202$). Graft thrombosis, anastomotic insufficiency, and the need for relaparotomy occurred less frequently in the DD group. These findings may be attributed to the relatively fixed nature of the anastomosis to the duodenum, which likely reduces the risk of graft vessel torsion or twisting. In the same year, Perosa et al. [20] described their first experience with 53 cases of DD. To date, the Brazilian group represents the largest reported Latin American series involving the duodenal drainage technique, comprising a total of 248 cases [38]. With over 25 years of experience and more than 1,000 transplants performed, the authors consider that combining DD anastomosis with venous drainage to the inferior vena cava, particularly when using a very short graft vein, constitutes an optimal surgical strategy. In fact, this is the technique currently employed at our center [21]. This configuration has been associated with low rates of thrombosis and anastomotic leakage, while preserving the advantages of easy access to the pancreatic graft [39].

Nevertheless, some transplant centers have discontinued the routine use of DD due to its limitations in clinical practice. The Norwegian group [40] reported low sensibility of duodenal graft biopsies for detecting pancreas graft rejection, while Ryu et al. [41], representing the Korean group, highlighted the greater technical complexity of DD compared to DJ. Notably, Ryu's team performed the first DD in South Korea and recently published their experience with 100 consecutive pancreas transplants, of which 86 were performed using DD and 14 using DJ. Their analysis reported a remarkably low incidence of surgical complications, including adhesions, technical failure, duodenal graft leakage, and vascular thrombosis, along with pancreatic graft survival rates of 91% at 1 year and 78.5% at 5 years. Despite these favorable outcomes, the group transitioned back to DJ, discontinuing routine use of DD in mid-2022 due to its greater technical complexity.

Our group reported the initial series of 10 cases employing duodenal drainage in 2017 [21], with no complications linked to the innovative surgical approach. These promising results laid the groundwork for its consolidation. Since then, DD has become the preferred method for enteric drainage.

In the current cohort of 188 procedures, an intestinal complication rate of 7.4% ($n = 14$) was observed, which is comparable to the rate reported in our historical series using DJ (6.8%; $p = 0.789$) [28].

Five cases of anastomotic dehiscence were recorded, with two requiring transplantectomy, likely due to impaired duodenal vascularization. Vascular events were identified in two of the three cases, including distal arterial thrombosis and venous occlusion, the latter ultimately leading to graft removal.

Potential disadvantages of DD include the difficulty of duodenal repair in the event of anastomotic dehiscence. Nevertheless, the most experienced groups in this type of anastomosis agree that DD is a safe procedure, with no significant increase in the incidence of intestinal complications [37]. In fact, it has been suggested that the retrocolic positioning of the graft may reduce the risk of peritonitis in the event of an intestinal leak, by limiting the intraperitoneal spread of duodenal contents [17, 23]. This observation aligns with the fact that, in three cases, DD dehiscence was successfully managed by primary closure while preserving graft function, a maneuver that, in the presence of peritonitis, would have posed greater technical challenges and likely resulted in less favorable outcomes. In the DJ cohort [28], 2 out of 4 cases of duodenum-jejunum anastomotic dehiscence required pancreas removal. Additional events compromising grafted duodenum integrity were also documented. Among these, three patients developed intestinal fistulas originating from the duodenal edge, without anastomotic failure; two underwent transplantectomy within 15 days post-surgery. Duodenal ischemia proved devastating in two cases, both necessitating graft excision. Furthermore, one individual required reoperation due to duodenal graft torsion, which was corrected by repositioning the graft medially. It is worth noting that intraperitoneal placement of the pancreas may facilitate twisting, thereby increasing the likelihood of venous thrombosis. Duodenal dehiscence following transplantectomy was also reported in one patient, which was successfully treated with endoscopic clip placement. This case highlights one of the advantages of DD, as it allowed for a more conservative approach to the management of complications, thereby avoiding the need for a surgical intervention. In contrast, a patient with prior DJ who presented with anastomotic dehiscence in the recipient jejunum after graft transplantectomy, required a repeated laparotomy for resection of the affected intestinal segment [28]. An additional benefit observed with DD is the low incidence of paralytic ileus, which occurred in only two patients in our series and was successfully managed conservatively. As previously noted, the retrocolic positioning of the graft contributes to this reduced rate, as ischemia-reperfusion-induced pancreatitis remains confined to the retroperitoneal space [17, 23]. This observation is further supported by comparison with the 21.7% incidence reported in our series of patients who received DJ [28], a figure that may in fact be underestimated due to the inclusion of individuals transplanted more than two decades ago. Four cases of intestinal obstruction were identified, although only two were attributed to postoperative adhesions. Additionally, one patient

developed stercoral colitis, which necessitated a Hartmann's procedure. Notably, only one case of internal hernia was documented. This low incidence may be attributed to the limited mobility of the graft in its retrocolic position, as previously described [18]. Interestingly, 7 out of 23 patients in the DJ group required surgery for adhesiolysis secondary to graft pancreatitis [28], a significantly higher proportion. A manual suture was employed in all DD cases and anastomotic bleeding was observed in two cases, both requiring reconnection of the duodenoduodenal anastomosis. In one case, the bleeding was concomitant with venous occlusive thrombosis requiring an additional thrombectomy procedure. In contrast, Gunasekaran et al [17] initially hypothesized a higher incidence of anastomotic site bleeding in the DD group but subsequent analyses attributed this complication primarily to the use of surgical staplers rather than the anastomotic technique itself. Although they perform the intestinal suturing manually, the German group [18] reported that gastrointestinal bleeding complications occurred more frequently in the DD group (11%) compared to DJ group (3%). Half of these cases were successfully treated with endoscopic haemostasis, although 28% required surgical intervention.

Despite the theoretical advantages of DD over DJ, findings from our cohort suggest that both techniques yield comparable outcomes, with similar rates of intestinal complications and competitive graft and patient survival. Notably, overall patient survival in the DD group was significantly higher compared to the historical DJ cohort ($P = 0.031$). It is also important to acknowledge that data collection for the DD group was considerably more comprehensive, whereas the DJ series corresponds to procedures performed prior to 2016. This discrepancy may introduce a degree of bias in the comparative analysis between both cohorts.

It should be emphasized that, beyond the surgical technique itself, multiple factors may predispose to early postoperative intestinal complications. These include ischemia of the duodenal cuff, impaired tissue repair due to immunosuppressive therapy, trauma to the donor duodenum during procurement or back-table preparation, and intra-abdominal infections, among others. Additionally, donor-related variables and triggers of post-reperfusion pancreatitis must not be overlooked. In this regard, the DD group included donors considered marginal on paper, such as cDCD, which may partly explain the slightly lower graft survival observed during the first year compared to the DJ group ($P = 0.688$).

To improve outcomes, logistical optimizations have been implemented at our center. Specifically, for recipients with a panel-reactive antibody (PRA) of 0% (at least 2 determinations in the past 6 months), a virtual crossmatch is performed. Hence the surgical procedure commences without awaiting the Complement-Dependent Cytotoxicity (CDC) or flow cytometry crossmatch results, leading to a reduction in the CIT. Furthermore, the protocol for lymph node transport from external donor hospitals has been refined. Lymph nodes are now harvested at the beginning of the donor procedure and

immediately transferred to our center, enabling crossmatch testing to be completed prior to organ arrival.

To our knowledge, this constitutes one of the largest published series involving retrocolic graft positioning combined with DD for exocrine drainage. We deliberately focused on complications directly related to the DD itself, aiming to provide a targeted analysis within this specific area of study. Although the study is retrospective, single-center, and non-randomized, it benefits from the consistent application of clinical management protocols and a standardized surgical technique, which contribute to the homogeneity of the cohort.

Ultimately, DD represents a technically viable alternative in pancreas transplantation, with clinical outcomes comparable to other enteric drainage modalities. Its adoption broadens the spectrum of surgical options, particularly in retransplantation scenarios. Owing to its favorable technical profile and the added benefit of facilitating graft access for biopsy when needed, both endoscopic and percutaneous, duodenoduodenostomy stands out as a contemporary and relevant strategy within the array of available surgical approaches.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

The study protocol received approval from the institutional review board (HCB/2020/0498) and was conducted in accordance with the ethical principles outlined in the Declaration of Helsinki (1975). The manuscript contains only non-identifiable images, as no names or other personal data are included that could lead to the identification of any patient.

AUTHOR CONTRIBUTIONS

JF-F, JF and JCG-V contributed to conception and design of the study. JF-F, AT, EF-P and RHZ organized the database. JF-F, AT, RHZ, CC-D, MP, RR, EF-P, RG, CB, CP, PV-A, EM, VH, AA, FD, AG-C, JCG-V, and JF and contributed to the acquisition of the data or analysis and interpretation of the data. JF-F, AT, RHZ, CC-D, JF and JCG-V drafted the manuscript and wrote sections of the manuscript. All authors revised the manuscript critically for essential intellectual content. All authors read and approved the final version to be published.

FUNDING

The author(s) declare that no financial support was received for the research and/or publication of this article.

CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

GENERATIVE AI STATEMENT

The author(s) declare that no Generative AI was used in the creation of this manuscript.

Any alternative text (alt text) provided alongside figures in this article has been generated by Frontiers with the support of artificial intelligence and reasonable efforts have been made to ensure accuracy, including review by the

authors wherever possible. If you identify any issues, please contact us.

ACKNOWLEDGMENTS

We thank Steve Illing, Cambridge RSA CELTA, for English language assistance.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontierspartnerships.org/articles/10.3389/ti.2025.15430/full#supplementary-material>

REFERENCES

- Boggi U, Vistoli F, Andres A, Arbogast HP, Badet L, Baronti W, et al. First World Consensus Conference on Pancreas Transplantation: Part II - Recommendations. *Am J Transpl* (2021) 21(Suppl. 3):17–59. doi:10.1111/ajt.16750
- Bellotti R, Brugger M, Oberhuber R, Cardini B, Resch T, Maglione M, et al. The Impact of Delayed Graft Function After Pancreas Transplantation on Clinical Outcomes and Survival. *Pancreatol* (2025) 25(4):534–9. doi:10.1016/j.pan.2025.04.019
- Folch-Puy E, Rull R, Ferrer-Fàbrega J. Recipient Risk Factors Impacting Outcomes After Pancreas Transplantation: Strategies for Optimization. *Curr Opin Organ Transpl* (2025) 30(4):289–303. doi:10.1097/MOT.0000000000001233
- Laftavi MR, Gruessner A, Gruessner R. Surgery of Pancreas Transplantation. *Curr Opin Organ Transpl* (2017) 22(4):389–97. doi:10.1097/MOT.0000000000000434
- Prieto M, Sutherland DER, Fernandez-Cruz L, Heil J, Najarian JS. Experimental and Clinical Experience with Urine Amylase Monitoring for Early Diagnosis of Rejection in Pancreas Transplantation. *Transplantation* (1987) 43(1):73–9. doi:10.1097/00007890-198701000-00017
- Siskind EJ, Amodu LI, Pinto S, Akerman M, Jonsson J, Molmenti EP, et al. Bladder Versus Enteric Drainage of Exocrine Secretions in Pancreas Transplantation: A Retrospective Analysis of the United Network for Organ Sharing Database. *Pancreas* (2018) 47(5):625–30. doi:10.1097/MPA.0000000000001043
- Jiménez-Romero C, Manrique A, Meneu JC, Cambra F, Andrés A, Morales JM, et al. Comparative Study of Bladder Versus Enteric Drainage in Pancreas Transplantation. *Transpl Proc* (2009) 41(6):2466–8. doi:10.1016/j.transproceed.2009.06.164
- Boggi U, Vistoli F, Signori S, Del Chiaro M, Amorese G, Vanadia Bartolo T, et al. Outcome of 118 Pancreas Transplants With Retroperitoneal Portal-Enteric Drainage. *Transpl Proc* (2005) 37(6):2648–50. doi:10.1016/j.transproceed.2005.06.081
- Ziaja J, Wullstein C, Woeste G, Bechstein WO. High Duodeno-Jejunal Anastomosis as a Safe Method of Enteric Drainage in Simultaneous Pancreas and Kidney Transplantation. *Ann Transpl* (2002) 7(3):46–9.
- Ferrer-Fàbrega J, Fernández-Cruz L. Exocrine Drainage in Pancreas Transplantation: Complications and Management. *World J Transpl* (2020) 10(12):392–403. doi:10.5500/wjt.v10.i12.392
- El-Hennawy H, Stratta RJ, Smith F. Exocrine Drainage in Vascularized Pancreas Transplantation in the New Millennium. *World J Transplant* (2016) 6(2):255–71. doi:10.5500/wjt.v6.i2.255
- De Roover A, Coimbra C, Detry O, Van Kemseke C, Squifflet JP, Honoré P, et al. Pancreas Graft Drainage in Recipient Duodenum: Preliminary Experience. *Transplantation* (2007) 84(6):795–7. doi:10.1097/01.tp.0000281401.88231.da
- Hummel R, Langer M, Wolters HH, Senninger N, Brockmann JG. Exocrine Drainage into the Duodenum: A Novel Technique for Pancreas Transplantation. *Transpl Int* (2008) 21(2):178–81. doi:10.1111/j.1432-2277.2007.00591.x
- De Roover A, Detry O, Coimbra C, Squifflet JP, Honoré P, Meurisse M. Exocrine Pancreas Graft Drainage in Recipient Duodenum Through Side-To-Side Duodeno-Duodenostomy. *Transpl Int* (2008) 21(7):707. doi:10.1111/j.1432-2277.2008.00684.x
- Schenker P, Flecken M, Vonend O, Wunsch A, Traska T, Viebahn R. *En bloc* Retroperitoneal Pancreas-Kidney Transplantation With Duodenoduodenostomy Using Pediatric Organs. *Transpl Proc* (2009) 41(6):2643–5. doi:10.1016/j.transproceed.2009.06.113
- Kornberg A, Küpper B, Bärthel E, Tannapfel A, Müller UA, Thrum K, et al. Combined *en-bloc* liver-pancreas Transplantation in Patients With Liver Cirrhosis and Insulin-Dependent Type 2 Diabetes Mellitus. *Transplantation* (2009) 87(4):542–5. doi:10.1097/TP.0b013e3181949cce
- Gunasekaran G, Wee A, Rabets J, Winans C, Krishnamurthi V. Duodenoduodenostomy in Pancreas Transplantation. *Clin Transpl* (2012) 26(4):550–7. doi:10.1111/j.1399-0012.2011.01563.x
- Walter M, Jazra M, Kykalos S, Kuehn P, Michalski S, Klein T, et al. 125 Cases of Duodenoduodenostomy in Pancreas Transplantation: A Single-Centre Experience of an Alternative Enteric Drainage. *Transpl Int* (2014) 27(8):805–15. doi:10.1111/tri.12337
- Khubutia M, Pinchuk A, Dmitriev I, Storozhev R. Simultaneous Pancreas-Kidney Transplantation With Duodeno-Duodenal Anastomosis. *Transpl Proc* (2014) 46(6):1905–9. doi:10.1016/j.transproceed.2014.05.070
- Perosa M, Noujaim H, Ianhez LE, Oliveira RA, Mota LT, Branez JR, et al. Experience With 53 Portal-Duodenal Drained Solitary Pancreas Transplants. *Clin Transpl* (2014) 28(2):198–204. doi:10.1111/ctr.12297
- Ferrer J, Molina V, Rull R, López-Boado MÁ, Sánchez S, García R, et al. Pancreas Transplantation: Advantages of a Retroperitoneal Graft Position. *Cir Esp* (2017) 95(9):513–20. doi:10.1016/j.ciresp.2017.05.004
- Ryu JH, Lee TB, Park YM, Yang KH, Chu CW, Lee JH, et al. Pancreas Transplant With Duodeno-Duodenostomy and Caval Drainage Using a Diamond Patch Graft: A Single-Center Experience. *Ann Transpl* (2017) 22:24–34. doi:10.12659/aot.901469
- Lindahl JP, Horneland R, Nordheim E, Hartmann A, Aandahl EM, Grzyb K, et al. Outcomes in Pancreas Transplantation with Exocrine Drainage Through a Duodenoduodenostomy Versus Duodenojejunosomy. *Am J Transpl* (2018) 18(1):154–62. doi:10.1111/ajt.14420
- Pinchuk AV, Dmitriev IV, Shmarina NV, Teterin YS, Balkarov AG, Storozhev RV, et al. Endoscopic Findings Following Retroperitoneal Pancreas Transplantation. *Clin Transpl* (2017) 31(7):e12989. doi:10.1111/ctr.12989
- Ryu DG, Kang DH, Choi CW, Choi BH, Kim HW, Park SB, et al. Endoscopic Visualization of Graft Status in Patients with Pancreas Transplantation. *Surg Endosc* (2022) 36(6):4057–66. doi:10.1007/s00464-021-08727-0
- Gunther Brockmann J, Butt A, AlHussaini HF, AlMana H, AlSaad K, Al-Awwami M, et al. Protocol Duodenal Graft Biopsies Aid Pancreas Graft

- Surveillance. *Transplantation* (2019) 103(3):622–9. doi:10.1097/TP.0000000000002412
27. Horneland R, Paulsen V, Lindahl JP, Grzyb K, Eide TJ, Lundin K, et al. Pancreas Transplantation with Enteroanastomosis to Native Duodenum Poses Technical Challenges-But Offers Improved Endoscopic Access for Scheduled Biopsies and Therapeutic Interventions. *Am J Transpl* (2015) 15(1):242–50. doi:10.1111/ajt.12953
 28. Ferrer-Fàbrega J, Cano-Vargas B, Ventura-Aguar P, Cárdenas G, García-Criado Á, López-Boado MA, et al. Early Intestinal Complications Following Pancreas Transplantation: Lessons Learned From Over 300 Cases - A Retrospective Single-Center Study. *Transpl Int* (2021) 34(1):139–52. doi:10.1111/tri.13775
 29. Ferrer Fàbrega J, Ricart MJ. Indicaciones Y Contraindicaciones Del Trasplante De Páncreas-Riñón Y Del Trasplante De Páncreas Aislado. Selección Del Candidato a Trasplante. In: *Guía De Trasplantes De Órganos Abdominales* (2017). p. 333.
 30. Organización nacional de trasplantes. Documento De Consenso Sobre Trasplante De Páncreas E Islotes (2005). Available online at: <https://www.ont.es/wp-content/uploads/2023/06/Documento-de-Consenso-sobre-Trasplante-de-Pancreas-e-Islotes.-Ano-2005-1.pdf> (Accessed August 12, 2025).
 31. Ferrer-Fàbrega J, Folch-Puy E, Lozano JJ, Ventura-Aguar P, Cárdenas G, Paredes D, et al. Current Trends in Organ Preservation Solutions for Pancreas Transplantation: A Single-Center Retrospective Study. *Transpl Int* (2022) 35:10419. doi:10.3389/ti.2022.10419
 32. Ferrer-Fàbrega J, Cárdenas G, Sapena V, García-Criado Á, Barrufet M, Pérez C, et al. Validation of the Back-Table Graft Arterial Anastomosis Between the Splenic Artery and Superior Mesenteric Artery: Arterial Complications After a 21-Year Single-Center Experience of Pancreas Transplantation. *Ann Surg* (2025) 281(5):843–51. doi:10.1097/SLA.00000000000006188
 33. Clavien PA, Barkun J, de Oliveira ML, Vauthey JN, Dindo D, Schulick RD, et al. The Clavien-Dindo Classification of Surgical Complications: Five-Year Experience. *Ann Surg* (2009) 250(2):187–96. doi:10.1097/SLA.0b013e3181b13ca2
 34. Loupy A, Haas M, Roufosse C, Naesens M, Adam B, Afrouzian M, et al. The Banff 2019 Kidney Meeting Report (I): Updates on and Clarification of Criteria for T Cell- and Antibody-Mediated Rejection. *Am J Transpl* (2020) 20(9):2318–31. doi:10.1111/ajt.15898
 35. Benedetti E, Najarian JS, Gruessner AC, Nakhleh RE, Troppmann C, Hakim NS, et al. Correlation Between Cystoscopic Biopsy Results and Hypoamylasuria in Bladder-Drained Pancreas Transplants. *Surgery* (1995) 118(5):864–72. doi:10.1016/s0039-6060(05)80277-6
 36. Bassaganyas C, Darnell A, Soler-Perromat A, Rafart G, Ventura-Aguar P, Cuatrecasas M, et al. Accessibility of Percutaneous Biopsy in Retrocolic-Placed Pancreatic Grafts with a Duodeno-Duodenostomy. *Transpl Int* (2024) 37:12682. doi:10.3389/ti.2024.12682
 37. Schenker P, Viebahn R. Duodenal Drainage: The European Experience (Germany). In: Gruessner RWG, Gruessner AC, editors. *Transplantation of the Pancreas*. Cham: Springer (2023). p. 429–32.
 38. Perosa M, Branez JR, Danziere FR, Zeballos B, Mota LT, Vidigal AC, et al. Over 1000 Pancreas Transplants in a Latin American Program. *Transplantation* (2025). doi:10.1097/TP.0000000000005421
 39. Perosa M, Danziere F, Branez J, Genzini T. Duodenal Drainage: The South American Experience (Brazil). In: Gruessner RWG, Gruessner AC, editors. *Transplantation of the Pancreas*. Cham: Springer (2023). p. 419–27.
 40. Nordheim E, Horneland R, Aandahl EM, Grzyb K, Aabakken L, Paulsen V, et al. Pancreas Transplant Rejection Episodes Are Not Revealed by Biopsies of the Donor Duodenum in a Prospective Study with Paired Biopsies. *Am J Transpl* (2018) 18(5):1256–61. doi:10.1111/ajt.14658
 41. Ryu JH, Yang KH, Horneland R, Ju SH, Choi BH. Experience from 100 Consecutive Pancreas Transplants Amidst Low National Transplant Activity: A Retrospective Study at a Single Center in Korea. *Clin Transpl Res* (2024) 38(3):203–11. doi:10.4285/ctr.24.0033

Copyright © 2025 Torroella, Zhu, Castillo-Delgado, Pavesi, Rull, Folch-Puy, García, Bassaganyas, Pérez-Serrano, Ventura-Aguar, Montagud-Marrahi, Holguin, Amor, Diekmann, García-Criado, García-Valdecasas, Fuster, Ferrer-Fàbrega. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.



Five Years Follow-up of Imlifidase Desensitized Kidney Transplant Recipients

Tomas Lorant^{1*†}, Bonnie E. Lonze^{2†}, Robert A. Montgomery^{2†}, Niraj M. Desai^{3†}, Christophe Legendre^{4†}, Torbjörn Lundgren⁵, Bengt von Zur-Mühlen¹, Ashley A. Vo^{6†}, Kristoffer Sjöholm^{7†}, Anna Runström^{7†}, Jan Tollemar⁷ and Stanley C. Jordan^{6†}

OPEN ACCESS

*Correspondence

Tomas Lorant,
✉ tomas.lorant@uu.se

†ORCID:

Tomas Lorant
orcid.org/0000-0002-2466-6259
Bonnie E. Lonze
orcid.org/0000-0002-0973-1657
Robert A. Montgomery
orcid.org/0000-0003-2728-4552
Niraj M. Desai
orcid.org/0000-0002-0457-3506
Christophe Legendre
orcid.org/0000-0002-1743-2948
Ashley A. Vo
orcid.org/0000-0003-4492-5331
Kristoffer Sjöholm
orcid.org/0000-0002-3763-9284
Anna Runström
orcid.org/0000-0001-9139-0283
Stanley C. Jordan
orcid.org/0000-0002-0456-8635

Received: 13 August 2025

Revised: 13 October 2025

Accepted: 28 October 2025

Published: 27 November 2025

Citation:

Lorant T, Lonze BE, Montgomery RA, Desai NM, Legendre C, Lundgren T, von Zur-Mühlen B, Vo AA, Sjöholm K, Runström A, Tollemar J and Jordan SC (2025) Five Years Follow-up of Imlifidase Desensitized Kidney Transplant Recipients. *Transpl. Int.* 38:15425. doi: 10.3389/ti.2025.15425

¹Department of Surgical Sciences, Section of Transplant Surgery, Uppsala University, Uppsala, Sweden, ²Department of Surgery, NYU Langone Transplant Institute, New York, NY, United States, ³Northwell Transplant Institute, Manhasset, NY, United States, ⁴Nephrology and Transplantation Unit, Hôpital Necker and Université Paris Cité, Paris, France, ⁵Department of Transplantation Surgery, Karolinska Institutet, Stockholm, Sweden, ⁶Cedars-Sinai Medical Center, Comprehensive Transplant Center, Los Angeles, CA, United States, ⁷Hansa Biopharma AB, Lund, Sweden

In four phase 2 clinical trials, patients underwent imlifidase-enabled kidney transplantation, converting a positive crossmatch (+XM) to negative. Here, we present data from 39 patients enrolled in the 5-year follow-up extension trial. Patient survival, graft survival, renal function, delayed graft function (DGF) antibody mediated rejection (AMR) frequency, safety and presence of donor specific antibodies (DSAs) are presented. Data from the long-term follow-up trial (17-HMedIdeS-14); NCT 03611621) with planned visits at 1, 2, 3, and 5 years after imlifidase were combined with up to 6-month phase 2 data from 4 pivotal trials (13-HMedIdeS-02, 13-HMedIdeS-03, 14-HMedIdeS-04, 15-HMedIdeS-06). Five-year patient survival was 90% with 3 deaths occurring between 6 months and 1 year, with no deaths occurring subsequently. Death-censored graft survival was 82% (with 3 early graft losses and 3 graft losses between 2 and 5 years). Mean eGFR at 5 years was 50.1 (MDRD), 55.8 (CKD-EPI, 2021) and 52.5 (KRS, 2023) mL/min/1.73 m² (N = 24 patients) among functioning grafts. DSA rebounded with levels progressively decreasing over time and no increase in DSA seen between 3 and 5 years. No AMR occurred between 3 and 5 years. Furthermore, no additional safety signals assessed as related to imlifidase were reported in this cohort. At 5 years, highly-HLA sensitized patients who underwent imlifidase desensitization prior to transplantation demonstrated excellent patient and graft survival, despite their high-risk immunological profile. Imlifidase offers a viable and effective treatment option for highly sensitized patients to achieve life-saving transplantation and continued optimism is warranted.

Clinical Trial: ClinicalTrials.gov (NCT02790437), EudraCT Number: 2016-002064-13.

Keywords: desensitization, highly sensitized patients, imlifidase, kidney transplantation, long-term follow-up

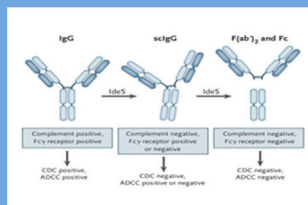
Five years follow-up of imlifidase desensitized kidney transplant recipients



Multiple Centers

- 39 XM+ Kidney transplants from pivotal trials
- 34 Patients entered the long-term follow up trial
- 24 Patients were followed for 5 years

Desensitization with imlifidase



Outcome at 5 years

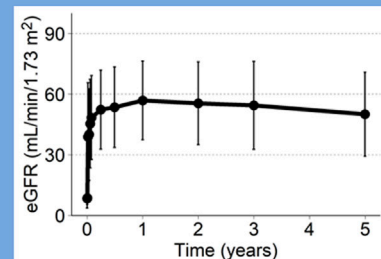
90% Patient Survival

82% Death-censored graft survival

No late AMRs occurred and no grafts were lost in patients adherent to a triple immunosuppressive protocol.

eGFR (MDRD) at 5 years

50.1 mL/min/1.73 m²



Kidneys have good function with a mean eGFR of 50.1 mL/min/1.73 m².



Tomas Lorant, et al. *Transpl. Int.* 2025

In press/ti.2025. 15425



GRAPHICAL ABSTRACT |

INTRODUCTION

Kidney transplantation is the preferred treatment option for patients with end-stage renal disease, however, it is not readily accessible to the highly sensitized patient population [1–3]. Prioritization within kidney allocation systems, transplantation through acceptable antigen mismatch programs, and living donor paired exchange programs have led to substantial increases in compatible transplants for all but the most highly sensitized [3]. Desensitization has been utilized as a strategy to increase the opportunity for transplantation in sensitized candidates who are biologically incompatible with a high percentage of available donors and therefore remain difficult to transplant, despite the different strategies. However, transplantation rates for patients with cPRA $\geq 99.9\%$ are so low that these patients are more likely to die or be removed from the transplant list than ever receiving a life-saving kidney transplant [1–3]. Since there is currently no standard of care for desensitization, patients endure extensive wait times, which increase morbidity and mortality. There is a clear and persistent need for therapies that can reduce or eliminate the immunologic barrier to transplantation posed by HLA sensitization. Current desensitization therapies often take months to years to even show minimal effects on HLA antibodies in the most sensitized patients with patients often needing multiple desensitization approaches [1–7]. In addition, the paucity of literature describing long-term outcomes of these patients, introduces uncertainty when evaluating the benefits of

desensitization transplantation versus the potential risks of a higher immunologic risk transplants [6, 7].

Imlifidase is a cysteine proteinase derived from immunoglobulin G (IgG)-degrading enzyme of *Streptococcus pyogenes* (IdeS), which effectively cleaves all preformed IgG, including anti-HLA antibodies, intravascular and extravascular, inhibiting complement-dependent cytotoxicity (CDC) and antibody-dependent cellular cytotoxicity (ADCC) within hours of administration. Thus, imlifidase opens up an antibody-free window to enable safe HLA-incompatible transplantation from both living and deceased donors [8–13]. An analysis at 3 years of the crossmatch positive population has been published previously, reporting on 39 patients receiving living- or deceased-donor kidney transplants across four pivotal phase 2 studies conducted in the United States and Europe [13]. Three years after imlifidase-enabled desensitization and transplantation, death-censored allograft survival was 84%, patient survival was 90% and mean eGFR (MDRD) was 50.1 mL/min/1.73 m². The results demonstrated a consistent safety profile with the highest risk of antibody mediated rejection (AMR) seen during the first weeks following imlifidase enabled transplantation. To date, imlifidase (Idefix®; Hansa Biopharma AB, Lund, Sweden), remains the only conditionally regulatory approved agent in EU/EEA, Australia, Israel, Switzerland and the United Kingdom for desensitization in adult kidney transplantation.

Here we report results of a 5-year long-term follow-up study in which these imlifidase-desensitized kidney transplant patients enrolled (17-HMedIdeS-14; NCT 03611621).

MATERIALS AND METHODS

A 5-year analysis of patient survival, graft survival, renal function, AMR frequency, safety and presence of donor specific antibodies (DSAs) is presented. From 2014–2017, 46 adult (18–70 years) patients at 6 transplant centers (Sweden 2, France 1 and US 3) received an imlifidase-enabled transplantation across four phase 2 clinical trials, with data collected up to 6-month post-transplant. Upon completion of the initial studies, patients were asked to participate in this non-interventional long-term follow up (LTF) study, in which collected standard of care follow-up data were collected annually. The LTF study (17-HMedIdeS-14; NCT 03611621) had planned data collection at 1, 2, 3, and 5 years after imlifidase and was combined with the phase 2, 6-month data. Only patients with a positive crossmatch (+XM) to their donor kidney prior to imlifidase treatment were included in the final analysis (N = 39 out of 46). All studies were conducted in accordance with the ethical principles that have their origins in the Declaration of Helsinki; all ethical and regulatory approvals were available before any patient was exposed to any study-related procedure. Each study was reviewed and approved by authorities and each center's institutional review board before study initiation with all patients providing written informed consent.

Outcomes

Details of the methodology for clinical outcomes of patient survival, graft survival, graft function, DSA levels, anti-drug antibodies (ADA), HLA characteristics of the pooled analysis have been previously published [13]. Biopsy proven AMR was diagnosed according to Banff 2017 criteria. Estimated glomerular filtration rate (eGFR) was calculated from local serum creatinine measurements using three different methodologies: the four-variable modification of diet in renal disease, MDRD¹, and the equations removing race as a variable, CKD-EPI (2021)², as well as the KRS (2023)³.

Statistical Methods

Survival analysis was performed using Kaplan-Meier methodology. Patients were censored at the last known visit or death. In addition, censoring was performed for loss to follow-up and death with a functioning graft with death only depicted in the graft-loss figures. DSA and eGFR were calculated according to linear model from 3 months up to 5 years. All statistical analyses were performed using R version 4.2.1 (R Core Team (2018), R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

Demographic and Baseline Characteristics

The four pivotal trials included 46 patients, among which 39 were XM+ and included in the analysis. Subsequent or prior to

completion of the parent trial, 12 patients did not actively enroll in the follow-up trial; 3 patients had died, 3 patients suffered graft loss in parent trial, 2 declined to participate in the LTF study and 4 could not be contacted. During years 2–5, 3 patients lost their grafts thus 24 patients could be followed up to 5 years.

The study population was predominantly Caucasian (77%), from the US (72%), EU (28%) who received a deceased or living donor kidney (DD, 82% and LD, 18%) (**Supplementary Table S1**). Mean age at transplant was 43.2 years (SD 13), with 69% of patients having had at least one transplant prior to imlifidase desensitization. Mean time on dialysis prior to the current transplant was 6.4 years (SD 5.6). All patients were highly sensitized, with a median cPRA of 99.62% (range 41.67%–100%).

Clinical Assessments

Three deaths occurred between 6 months and 1 year (one death was attributed to *pseudomonas* bacteremia/influenza, one due to cardiac arrest; while the cause of the third death was unknown), with no deaths occurring between 1 and 5 years. The 5-year patient survival was 90% (CI: 80%–100%, **Figure 1A**). No deaths were attributed to imlifidase or to graft failure.

Death censored graft survival at 5-year was 82% (CI: 70%–96%, **Figure 1B**) with 3 graft losses early in the studies attributed to one IgM mediated hyperacute rejection and two primary non-functioning grafts. No grafts were lost due to AMR throughout the trial period and DGF in patients with AMR was 47% compared to 42% in XM+ patients without AMR (**Supplementary Table S1**).

There was no difference in graft survival (death censored) between the patients experiencing AMR and the patient without (**Figure 2**). Two allograft losses occurred between 2 and 3 years, attributed to reduction of immunosuppression secondary to an infection in one patient and immunosuppression medication non-adherence in another. One graft loss occurred between 3 and 5 years due to a continuous decline in graft function over time from a transplant which initially struggled with acute tubular necrosis and delayed graft function. Data on 24 patients, was available who were followed for 5 years.

eGFR for the patients is presented in **Table 1**; **Figure 3**, using the MDRD, the CKD-EPI (2021) and the KRS equations. For patients with a functioning graft and with available data, mean eGFR estimation at 5 years was 50.1 (MDRD), and 55.8 (CKD-EPI, 2021), and 52.5 (KRS) mL/min/1.73 m² for the three equations respectively. At 5 years 79% (MDRD) of patients had an eGFR ≥30 mL/min/1.73 m². The slope of the eGFR remained stable over time with an initial increase seen earlier in the transplantation course (**Figure 3**). The eGFR did not differ significantly at the 5-year visit between patients who experienced AMR compared to patients without AMR (**Figure 4**; **Table 2**).

Occurrence of DSA at 5 years was 27% and was defined as any DSA with an MFI ≥2,000 (**Table 1**). As previously described, the MFI remained low for approximately 1 week after imlifidase treatment, then rebounded to approximately 80% of pre-treatment levels, with the peak occurring 14 days post-treatment (**Figure 5**). Immunodominant Class II DSA

¹https://www.acpjournals.org/doi/10.7326/0003-4819-145-4-200608150-00004?url_ver=Z39.88-2003&rft_id=ori:rid:crossref.org&rft_dat=cr_pub%20%200pubmed

²<https://www.nejm.org/doi/full/10.1056/NEJMoa2102953>

³<https://doi.org/10.1136/bmj-2022-073654>

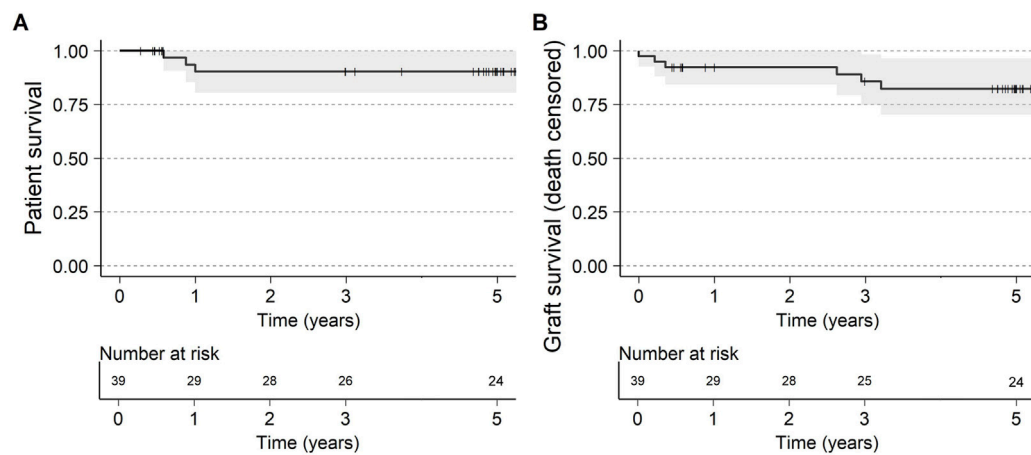


FIGURE 1 | (A) Patient survival and (B) Graft survival (death censored) up to 5 years after transplantation. Visit window of the 5-year visit was ± 6 months.

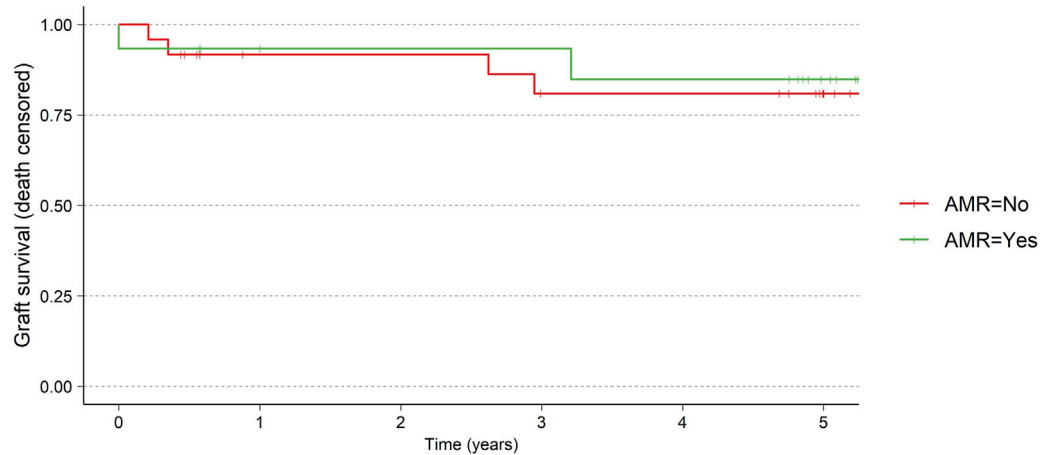


FIGURE 2 | Graft survival for AMR vs. no-AMR.

TABLE 1 | eGFR up to 5 years using MDRD, CKD-EPI (2021) and KRS (2023).

Timepoint	eGFR (mL/min/1.73 m ²)			iDSA (immunodominant DSA, MFI)		
	MDRD Mean (SD)	CKD-EPI Mean (SD)	KRS Mean (SD)	Immunodominant DSA Mean (SD)	Class I Mean (SD)	Class II Mean (SD)
Predose and Tx	8.4 (4.8), n = 22	8.8 (5.1), n = 22	8.5 (7.3), n = 22	9,772.7 (7,173.9), n = 22	8,884.7 (6,806.1), n = 10	10,512.7 (7,683.5), n = 12
Month 6	53.5 (19.9), n = 23	58.4 (20.6), n = 23	56.3 (17.7), n = 23	5,420.8 (6,247.3), n = 20	2,499.6 (2,164.1), n = 9	7,810.9 (7,513), n = 11
Year 1	56.9 (19.4), n = 24	63.1 (21.5), n = 24	58.7 (16.6), n = 24	1,427.3 (1,026.7), n = 6	1,886 (267.3), n = 2	1,198 (1,234), n = 4
Year 2	55.5 (20.5), n = 24	60.5 (21.5), n = 24	57.7 (18.0), n = 24	4,209.2 (4,285.3), n = 12	731 (661.1), n = 3	5,368.7 (4,369.4), n = 9
Year 3	54.5 (21.7), n = 23	59.5 (23.2), n = 23	56.5 (18.7), n = 23	1,783.2 (2,254.5), n = 17	907.2 (532.8), n = 8	2,561.9 (2,910), n = 9
Year 5	50.1 (20.8), n = 24	55.8 (24.5), n = 24	52.5 (18.7), n = 24	2,522.6 (4,379.3), n = 15	543.3 (289), n = 6	3,842.1 (5,349), n = 9

DSA is evaluated as the Immunodominant DSA and separated by the class of the immunodominant DSA. Presented in the table are mean, standard deviation and the number of subjects evaluated at each timepoint and analysis.

was more prevalent than Class I. In general, the Class II DSA strength was higher pre-dose and with rebound after imlifidase dosing (Figure 5). After initial peak at 14 days post-treatment,

the levels of DSA progressively decreased over time, which was sustained at 5 years with an MFI for Class I DSA below 1,000 and below 4,000 for Class II DSA. Patients who

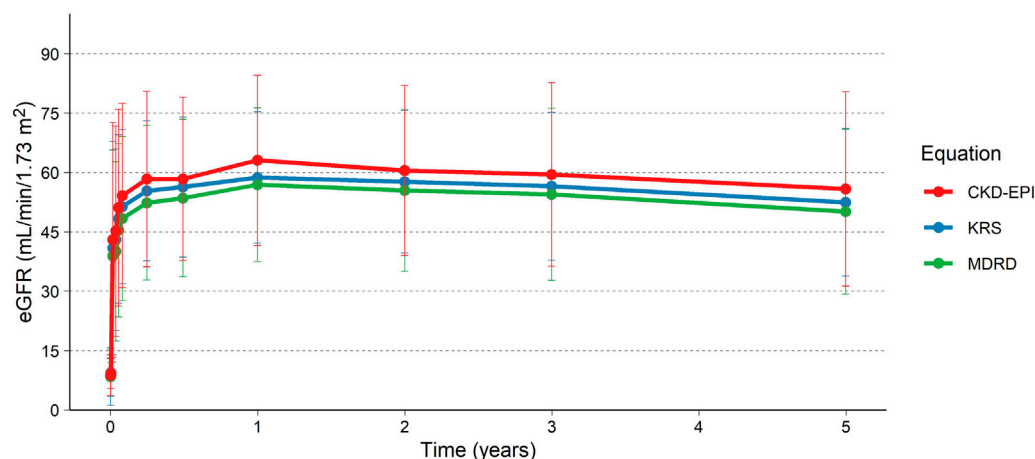


FIGURE 3 | eGFR up to 5 years using the MDRD, CKD-EPI and KRS (2023). Colored lines and points are the mean, and the error bars are the standard deviation. Only subjects with measurements after the initial studies, i.e., at 1 year or later, included in the analysis (N = 25).

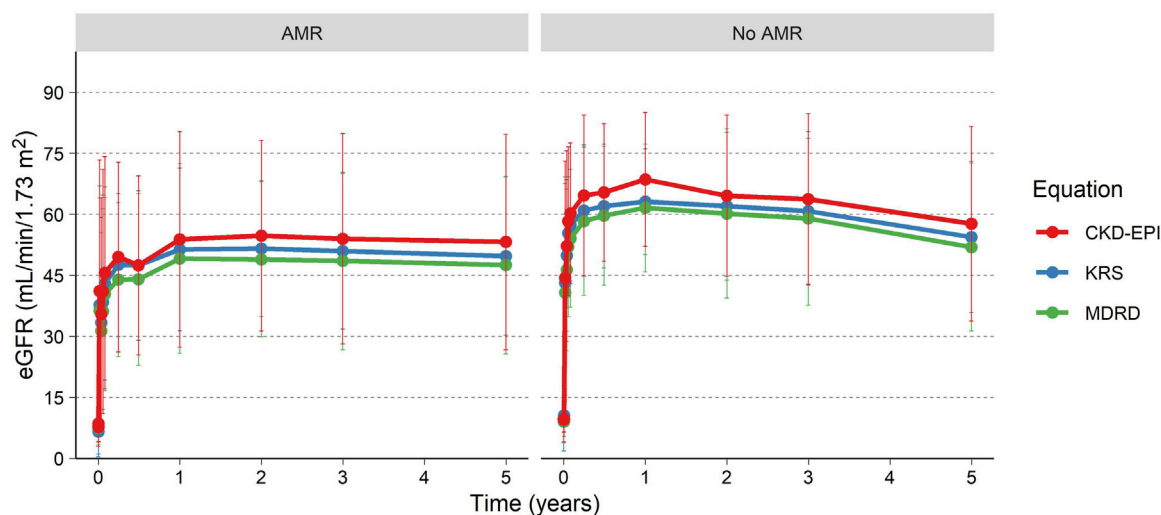


FIGURE 4 | eGFR over time for patients experiencing an AMR and patients without AMR.

demonstrated no DSA at 6 months remained DSA rebound-free during the 5-year follow up. Only one patient developed *de novo* DSA during the study. DSA levels did not differ significantly at the 5-year visit between patients that experienced AMR compared to patients without AMR, with a median (range) of 1,838 (348; 17,630) MFI and 948 (258; 4,130) MFI, respectively. This patient with *de novo* DSA developed these antibodies after an infection 3-year post-transplant which required reduction in immunosuppression, and unfortunately the graft was subsequently lost.

Antibody Mediated Rejection (AMR)

Allograft rejection was required to fulfill the criteria outlined in the Banff 2017 criteria throughout the assessed period (biopsy

TABLE 2 | eGFR at 5 years after transplantation for patients experiencing AMR vs. no-AMR.

eGFR equation (mean [SD])	AMR (N = 10)	No AMR (N = 14)	P-value
MDRD	47.5 (21.8)	52 (20.7)	0.546
CKD-EPI	53.2 (26.5)	57.7 (23.9)	0.703
KRS	49.8 (19.4)	54.4 (18.6)	0.667

P-value calculated using Mann-Whitney U test.

taken at time of graft rejection episode, histological evidence of AMR on pathology and presence of detectable DSA). The majority of AMR occurred within the first month after transplantation (N = 11), with 4 additional AMRs recorded

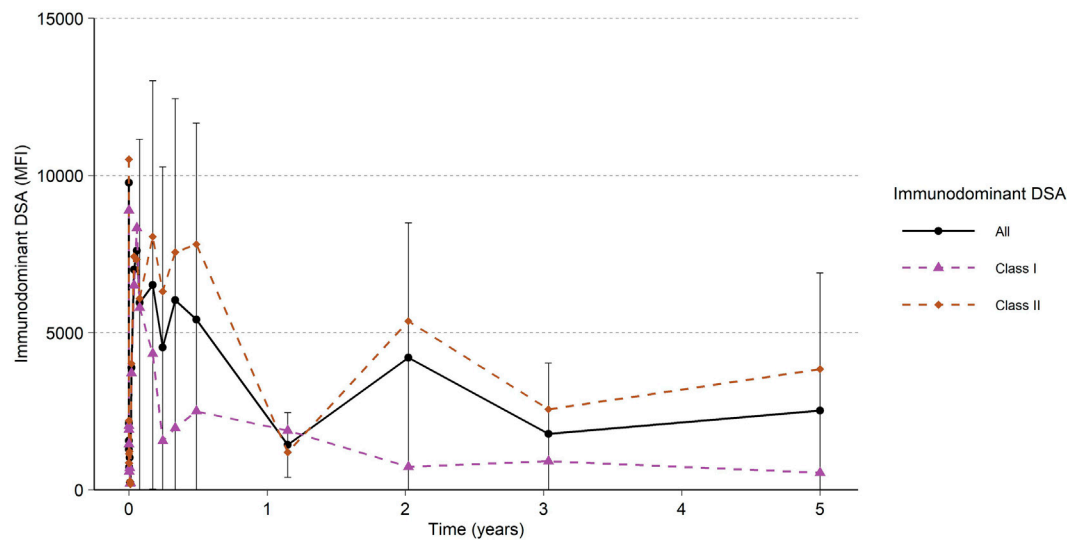


FIGURE 5 | All Immunodominant DSA and Immunodominant DSA per HLA class I and HLA class II. Black and colored lines and points are the mean, and the error bars are the standard deviation. Only subjects with centrally analyzed samples after the initial studies, i.e., at 1 year or later, included in the analysis (N = 22).

between 2 and 6 months from transplantation totaling an AMR rate in the first 6 months post-transplantation of 38%. In the extended follow up, 7 rejection episodes were reported, with 5 clinically suspected allograft rejections that did not meet study criteria (Banff 2017); one at 18 months post-transplant that was C4d negative/DSA negative, one at 24 months post-transplant that was C4d negative/DSA negative with pathology that did not meet criteria, one at 45 months post-transplant that was DSA negative and with inadequate biopsy for diagnosis, one at 57 months post-transplant diagnosed with no biopsy proven acute rejection (BPAR) with no additional data provided, and one at 60 months that was C4d negative/DSA negative which was not treated. Therefore, all histologically confirmed AMRs remained from the timeframe of the first few months following transplantation and were diagnosed and treated with standard available therapies as described previously [13]. The one biopsy confirmed acute rejection was recorded 9 months after transplantation and was a previous AMR recorded in the phase 2 trial. To date, no allograft losses attributable to AMR have been reported.

Safety

Adverse events assessed as related to imlifidase have been previously described and remain unchanged [10–13]. At 5 years, no additional safety signals assessed as related to imlifidase have been reported.

Comorbidities in the form of infections were reported in 18 of the 37 patients enrolled. The most common followed for 5 years, were urinary tract infection (37 occasions reported in 9 patients), nasopharyngitis (10 occasions reported in 3 patients), pyelonephritis (6 occasions reported in 3 patients), upper respiratory infection (5 occasions reported in 4 patients), and

COVID-19 infection (4 occasions reported in 4 patients). In addition, 2 patients reported 6 events of urosepsis, 2 patients reported 2 events of sepsis, and 2 patients reported 2 events of pneumonia. No death or graft loss occurred due to infection beyond 6 months after transplantation. No BK virus infections were reported up to 5 years follow-up and 23/24 tested negative in PCR evaluation (<500 copies) at 5 years. There have been no reported malignancies in the patients during the long-term follow-up (data on file).

DISCUSSION

Kidney allocation systems are continually being reviewed and refined to balance both equity and utility for the scarce resource of deceased donor kidneys, with efforts to prioritize sensitized candidates when a compatible donor organ becomes available [14–19]. What remains consistent is a cohort of the most highly sensitized patients whose statistical probability of finding a compatible offer is so low, that desensitization to facilitate an HLA-incompatible transplant is the only path to the survival benefit afforded by transplantation instead of dialysis.

The ability of imlifidase to effectively cleave and reduce the entire spectrum of preformed IgG anti-HLA antibodies, regardless of intensity or subclass, might open up a new era in desensitization in transplantation [20, 21]. Compared to other desensitization methods, imlifidase provides within hours a near complete IgG antibody-free window, even in patients with positive cytotoxic crossmatches, and thus enables transplantation in both the living and deceased donor settings [22, 23]. Initially some expressed reservation regarding the broader use of imlifidase and incompatible transplantation due

to speculation of early immunologically driven graft losses. Furthermore, the risk of increased cold ischemia time and thereby risk of higher DGF due to crossmatch evaluation before and after treatment, has been mentioned as a potential risk. DGF impact on long-term function remains unclear. However, in the present 5 years pooled data analysis this seems unlikely, as imlifidase-enabled transplant recipients continue to demonstrate sustained patient and graft survival with relative stability of allograft function despite the high-risk immunological profile, and in whom transplant may have not been possible otherwise.

Of the patients with a functioning graft and available data at 5 years, 79% had an eGFR above 30 mL/min/m² with a mean eGFR of 50.1 mL/min/1.73 m² (calculated by MDRD). Guidelines supporting the use of the most recent race-free transplant specific eGFR equation, eGFR calculations were also provided using the CKD-EPI (2021) and KRS equations for comparison [24, 25]. We believe this finding is important as many of the advantages of transplantation over dialysis are linked to adequacy of kidney allograft function and are lost with compromised eGFR [26–29]. In the current data, eGFR continues to be sustained, indicating low likelihood of chronic kidney disease progression or impending graft failure. The incidence of AMR was similar to those reported at 3 years and may be related to the overall pattern of declining DSA over time across both class I and class II antibodies. This is most probably due to continuous good immunosuppressive therapy, not triggering immunologic response and DSA increase which could be seen in a patient where immunosuppression was reduced, due to severe infection. Data up to 21 days post-transplantation confirms that when a rejection occurs, DSA rises during the early period of DSA rebound thus indicating AMR in a noninvasive test. The frequency or severity of early AMR was not substantially different from what is expected and reported in highly sensitized candidates receiving incompatible kidneys and few late AMRs were reported. Patients' AMR were treated with available standard available therapies, and no grafts were lost due to AMR. The current data indicate that the challenge of AMR in these patients is manageable, and no greater with imlifidase desensitization than with other approaches to transplantation in this at-risk group of highly sensitized patients. The long-term safety profile of imlifidase was consistent with that described previously with no increase in safety signals as it relates to infection or malignancy over time (data on file).

Limitations of this 5-year analysis of four 6-month pivotal trials are the lack of following relevant kidney function tests such as proteinuria and lack of long-term protocol biopsies. Although early AMRs were diagnosed and treated early and successfully, this does not preclude the presence of chronic active AMR (caAMR) and the incidence of this remains largely unknown over the time studied. Clinical parameters of interest to understand granular details of renal function over time including presence of proteinuria, and levels and type of immunosuppression were not part of the original trial design as long-term management of allografts will vary and are subject to much heterogeneity in real-world settings. An additional

limitation is the loss of data over time. As common with long-term follow up studies, attrition will inevitably occur and unfortunately, data for some outcomes of interest were unavailable for all patients.

In conclusion, efficacy and safety outcomes were assessed in imlifidase treated kidney-transplant recipients at 5 years after treatment. Data remains the best available long-term data in a cohort of patients who received a crossmatch positive, incompatible kidney at the time of transplantation. Imlifidase demonstrated outcomes at 5 years that are comparable to those seen with compatible transplantation and indicates imlifidase represents an important new therapy to create a temporary “antibody-free window” enabling HLA incompatible transplantation. Some challenges remain associated with this desensitization methodology, in particular the risk of early antibody rebound, but this does not invariably occur in all patients, and with proactive management, it can be managed successfully. This 5-year LTF study suggests good long-term outcomes are achievable and are certainly superior to those for HLA sensitized patients who remained on dialysis [28].

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Regional Ethical Review boards in Uppsala (Sweden), Necker Hospital Paris (France), Cedars Sinai Los Angeles, NYU New York and Johns Hopkins Baltimore (US). The patients/participants provided written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

TLo, BL, RM, ND, TLu, BZ, AV, and SJ participated as investigators in the trials, interpretation of the trial and creation, writing, reviewing and final approval of the manuscript. KS, AR, and JT Trial design and implementation and analysis and interpretation of data. Part of writing the manuscript and reviewing the manuscript.

FUNDING

The author(s) declare that financial support was received for the research and/or publication of this article. The authors declare that this study received funding from Hansa Biopharma. The funder had the following involvement with the study: Sponsor of the original trials.

CONFLICT OF INTEREST

Author TLo was employed by Hansa Biopharma 2019 to 2020. Author AR is employed by Hansa Biopharma since November 2012. Author JT is employed by Hansa Biopharma since August 2021. Author KS is employed by Hansa Biopharma since May 2018.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

GENERATIVE AI STATEMENT

The author(s) declare that no Generative AI was used in the creation of this manuscript.

Any alternative text (alt text) provided alongside figures in this article has been generated by Frontiers with the

support of artificial intelligence and reasonable efforts have been made to ensure accuracy, including review by the authors wherever possible. If you identify any issues, please contact us.

ACKNOWLEDGEMENTS

The authors would like to acknowledge the contributions of Hansa Biopharma researchers.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontierspartnerships.org/articles/10.3389/ti.2025.15425/full#supplementary-material>

REFERENCES

- Habal MV, Farr M, Restaino S, Chong A. Desensitization in the Era of Precision Medicine: Moving from the Bench to Bedside. *Transplantation* (2019) 103(8):1574–81. doi:10.1097/TP.0000000000002737
- Jackson KR, Holscher C, Motter JD, Desai N, Massie AB, Garonzik-Wang J, et al. Posttransplant Outcomes for CPRA-100% Recipients Under the New Kidney Allocation System. *Transplantation* (2020) 104(7):1456–61. doi:10.1097/TP.0000000000002989
- Schinstock CA, Smith BH, Montgomery RA, Jordan SC, Bentall AJ, Mai M, et al. Managing Highly Sensitized Renal Transplant Candidates in the Era of Kidney Paired Donation and the New Kidney Allocation System: Is There Still a Role for Desensitization? *Clin Transpl* (2019) 33(12):e13751. doi:10.1111/ctr.13751
- Vo AA, Petrozzino J, Yeung K, Sinha A, Kahwaji J, Peng A, et al. Efficacy, Outcomes, and Cost-Effectiveness of Desensitization Using IVIG and Rituximab. *Transplantation* (2013) 95(6):852–8. doi:10.1097/TP.0b013e3182802f88
- Jackson AM, Kraus ES, Orandi BJ, Segev DL, Montgomery RA, Zachary AA. A Closer Look at Rituximab Induction on HLA Antibody Rebound Following HLA-Incompatible Kidney Transplantation. *Kidney Int* (2015) 87(2):409–16. doi:10.1038/ki.2014.261
- Jackson KR, Covarrubias K, Holscher CM, Luo X, Chen J, Massie AB, et al. The National Landscape of Deceased Donor Kidney Transplantation for the Highly Sensitized: Transplant Rates, Waitlist Mortality, and Posttransplant Survival Under KAS. *Am J Transpl* (2018) 4:1129–38. doi:10.1111/ajt.15149
- Jackson KR, Motter JD, Kernodle A, Desai N, Thomas AG, Massie AB, et al. How Do Highly Sensitized Patients Get Kidney Transplants in the United States? Trends over the Last Decade. *Am J Transpl* (2020) 20:2101–12. doi:10.1111/ajt.15825
- Ge S, Chu M, Choi J, Louie S, Vo A, Jordan SC, et al. Imlifidase Inhibits HLA Antibody-Mediated NK Cell Activation and Antibody-Dependent Cell-Mediated Cytotoxicity (ADCC) in Vitro. *Transplantation* (2019) 104:1574–9. doi:10.1097/TP.0000000000003023
- Lorant T, Bengtsson M, Eich T, Eriksson BM, Winstedt L, Järnum S, et al. Safety, Immunogenicity, Pharmacokinetics, and Efficacy of Degradation of anti-HLA Antibodies by Ides (Imlifidase) in Chronic Kidney Disease Patients. *Am J Transpl* (2018) 18(11):2752–62. doi:10.1111/ajt.14733
- Jordan SC, Legendre C, Desai N, Lorant T, Bengtsson M, Lonze BE, et al. Imlifidase desensitization in crossmatch-positive, highly-sensitized kidney transplant recipients: results of an international phase 2 trial (Highdes). *Transplantation* (2021) 105:1808–1817. doi:10.1097/TP.0000000000003496
- Lonze BE, Tatapudi VS, Weldon EP, Min ES, Ali NM, Deterville CL, et al. Ides (Imlifidase): A Novel Agent that Cleaves Human IgG and Permits Successful Kidney Transplantation Across High-Strength Donor-Specific Antibody. *Ann Surg* (2018) 268:488–96. doi:10.1097/SLA.0000000000002924
- Jordan SC, Legendre C, Desai N, Lorant T, Bengtsson M, Lonze BE, et al. Imlifidase Desensitization in Crossmatch-Positive, Highly-Sensitized Kidney Transplant Recipients: Results of an International Phase 2 Trial (Highdes). *Transplantation* (2021) 105:1808–17. doi:10.1097/TP.0000000000003496
- Kjellman C, Maldonado AQ, Sjöholm K, Lonze BE, Montgomery RA, Runström A, et al. Outcomes at 3 Years Posttransplant in Imlifidase-Desensitized Kidney Transplant Patients. *Am J Transpl* (2021) 21:3907–18. doi:10.1111/ajt.16754
- Stewart DE, Kucheryavaya AY, Klassen DR, Turgeon NA, Formica RN, Aeder MI. Changes in Deceased Donor Kidney Transplantation One Year After KAS Implementation. *Am J Transpl* (2016) 16:1834–47. doi:10.1111/ajt.13770
- Hahn AB, Mackey M, Constantino D, Ata A, Chandolia N, Lopez-Soler R, et al. The New Kidney Allocation System Does Not Equally Advantage all Very High CPRA Candidates – A Single Center Analysis. *Hum Immunol* (2017) 78:37–40. doi:10.1016/j.humimm.2016.10.010
- Stewart DE, Wilk AR, Toll AE, Harper AM, Lehman RR, Robinson AM, et al. Measuring and Monitoring Equity in Access to Deceased Donor Kidney Transplantation. *Am J Transpl* (2018) 18:1924–35. doi:10.1111/ajt.14922
- Stewart DE, Wilk AR, Klassen DK. KAS Turns Four: The State of Deceased Donor Kidney Allocation in the U.S. *OBM Transplant* (2019) 3:1. doi:10.21926/obm.transplant.1901041
- Maldonado AQ, Bradbrook K, Sjöholm K, Kjellman C, Lee J, Stewart D. The Real Unmet Need: A Multifactorial Approach for Identifying Sensitized Kidney Candidates With Low Access to Transplant. *Clin Transplant* (2023) 37:e14946. doi:10.1111/ctr.14946
- Maldonado AQ, Sjöholm K, Lee J, et al. Beyond CPRA: Identifying Sensitized Kidney Candidates with Markedly Low Access to Deceased Donor Transplantation by Granular CPRA and Blood Type. *OBM Transplant* (2021) 5(2):143. doi:10.21926/obm.transplant.2102143
- Uhlin F, Szpirt W, Kronbichler A, Bruchfeld A, Soveri I, Rostaing L, et al. Endopeptidase Cleavage of Anti-Glomerular Basement Membrane Antibodies *In Vivo* in Severe Kidney Disease: An Open-Label Phase 2a Study. *J Am Soc Nephrol* (2022) 33:829–38. doi:10.1681/ASN.2021111460
- Rostaing L, Noble J, Malvezzi P, Jouve T. Imlifidase Therapy: Exploring Its Clinical Uses. *Expert Opin Pharmacother* (2023) 24(2):259–65. doi:10.1080/14656566.2022.2150965
- Schinstock CA, Tambur AR. Apples, Oranges, and Anything in Between: In Search of the Best Desensitization Therapy. *Am J Transpl* (2021) 21(12):3825–6. doi:10.1111/ajt.16808
- Schinstock C, Tambur A, Stegall M. Current Approaches to Desensitization in Solid Organ Transplantation. *Front Immunol* (2021) 12:686271. doi:10.3389/fimmu.2021.686271
- Inker LA, Eneanya ND, Coresh J, Tighiouart H, Wang D, Sang Y, et al. New Creatinine- and Cystatin C-Based Equations to Estimate GFR Without Race. *N Engl J Med* (2021) 385:1737–49. doi:10.1056/NEJMoa2102953

25. Kidney Disease: Improving Global Outcomes (KDIGO) CKD Work Group. KDIGO 2024 Clinical Practice Guideline for the Evaluation and Management of Chronic Kidney Disease. *Kidney Int* (2024) 105:S117–S314. doi:10.1016/j.kint.2023.10.018
26. Meier-Kriesche HU, Schold JD, Srinivas TR, Reed A, Kaplan B. Kidney Transplantation Halts Cardiovascular Disease Progression in Patients With End-Stage Renal Disease. *Am J Transpl* (2004) 4(10):1662–8. doi:10.1111/j.1600-6143.2004.00573.x
27. Meier-Kriesche HU, Baliga R, Kaplan B. Decreased Renal Function Is a Strong Risk Factor for Cardiovascular Death After Renal Transplantation. *Transplantation* (2003) 75(8):1291–5. doi:10.1097/01.TP.0000061602.03327.E2
28. Orandi BJ, Luo X, Massie AB, Garonzik-Wang JM, Lonze BE, Ahmed R, et al. Survival benefit of kidney transplants from HLA-incompatible live donors. *N Engl J Med* (2016) 374:940–50. doi:10.1056/NEJMoa1508380
29. Kasiske BL, Israni AK, Snyder JJ, Skeans MA, Patient Outcomes in Renal Transplantation PORT Investigators. The Relationship Between Kidney Function and Long-Term Graft Survival After Kidney Transplant. *Am J Kidney Dis* (2011) 57(3):466–75. doi:10.1053/j.ajkd.2010.10.054

Copyright © 2025 Lorant, Lonze, Montgomery, Desai, Legendre, Lundgren, von Zur-Mühlen, Vo, Sjöholm, Runström, Tollemar and Jordan. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.



Blockade of CD155 and CD276 by Monoclonal Antibodies Fosters Immune Tolerance and Promotes Stable Engraftment of iPSC-Derived Islets in Allogeneic Humanized Mice

G. Siracusano^{1,2*}, F. Deambrogio¹, V. Sordi¹, M. Malnati³, L. Piemonti^{1,2*†} and R. Chimienti^{1,2*†}

¹Diabetes Research Institute (DRI), IRCCS San Raffaele Scientific Institute, Milan, Italy, ²University Vita-Salute San Raffaele, Milan, Italy, ³Division of Immunology, Transplantation and Infectious Disease (DITID), IRCCS San Raffaele Scientific Institute, Milan, Italy

OPEN ACCESS

*Correspondence

G. Siracusano,
✉ siracusano.gabriel@hsr.it
L. Piemonti,
✉ piemonti.lorenzo@hsr.it
R. Chimienti,
✉ chimienti.raniero@hsr.it

[†]These authors have contributed
equally to this work

Received: 14 August 2025

Revised: 07 November 2025

Accepted: 14 November 2025

Published: 01 December 2025

Citation:

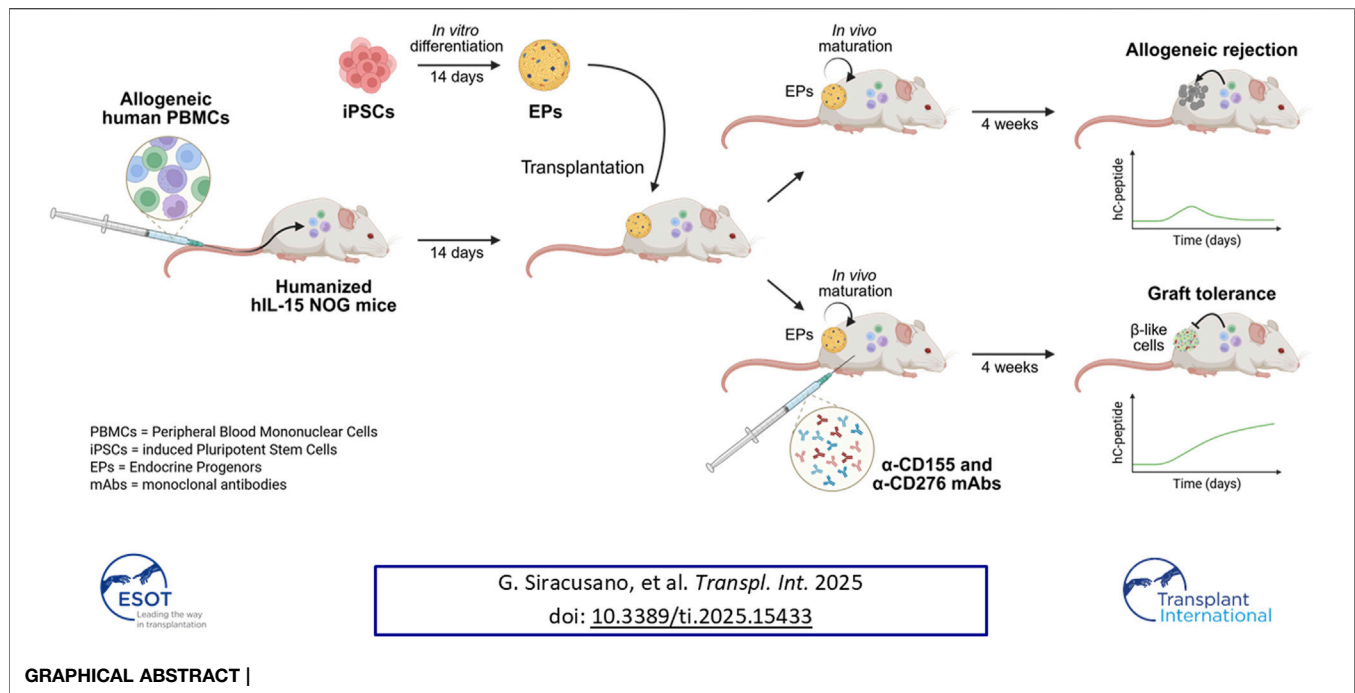
Siracusano G, Deambrogio F, Sordi V, Malnati M, Piemonti L and Chimienti R (2025) Blockade of CD155 and CD276 by Monoclonal Antibodies Fosters Immune Tolerance and Promotes Stable Engraftment of iPSC-Derived Islets in Allogeneic Humanized Mice. *Transpl. Int.* 38:15433. doi: 10.3389/ti.2025.15433

Induced pluripotent stem cell (iPSC)-derived pancreatic islets represent a promising therapeutic approach for restoring insulin independence in type 1 diabetes (T1D). However, their clinical success remains critically dependent on overcoming rejection mediated by innate and adaptive immune responses. Current immunosuppressive therapies pose significant long-term risks and only partially control alloimmune and autoimmune reactions. Targeted immunomodulation using monoclonal antibodies is a safer, more precise alternative. Here, we explored the impacts of blocking CD276 (B7-H3) and CD155 (PVR), activating ligands involved in immune recognition and regulation, on the survival and *in vivo* maturation of iPSC-derived endocrine progenitors (EPs) into functional pancreatic islets. Using a humanized mouse model, we demonstrated that dual blockade of CD276 and CD155 markedly reduced NK cell-mediated graft rejection, prevented CD14⁺ monocyte activation, and limited overall immune infiltration. In addition, CD155 blockade increased PD-1 levels on activated CD8⁺ T cells and significantly enhanced regulatory T cell (Treg) expansion and function, thereby promoting graft tolerance. Combined treatment prolonged engraftment and facilitated the maturation of EPs into functional, insulin-secreting cells, as indicated by increased human C-peptide levels and glucose responsiveness 4 weeks post-transplantation. Our findings highlight CD276/CD155 blockade as a novel immunomodulatory strategy to support tolerance and the functional maturation of iPSC-derived pancreatic grafts in T1D.

Keywords: monoclonal antibodies, immune tolerance induction, type 1 diabetes, allograft rejection, iPSC pancreatic derivatives

INTRODUCTION

Induced pluripotent stem cell (iPSC) technology has ushered in a new era of regenerative medicine for treating type 1 diabetes (T1D), providing a potentially unlimited source of insulin-producing cells for transplantation and aiming to restore insulin independence [1–4]. Despite recent progress, the success of iPSC-derived β -cell transplantation still hinges on controlling complex immune responses [5, 6], requiring chronic immunosuppression to prevent rejection [7, 8]. However, standard regimens



carry substantial long-term risks, including infections, malignancies, and metabolic complications [9], and they do not fully address the combined alloimmune and recurrent autoimmune responses typical of T1D recipients [10].

Innovative strategies have thus emerged to minimize or avoid chronic immunosuppression [11, 12]. These include genetically engineering stealthy, hypoimmunogenic stem cell-derived islets - which hold significant promise despite regulatory and safety challenges - and complementary approaches, such as the adoptive transfer of immunomodulatory cells or targeted induction therapies. Together, these methods aim to promote immune homeostasis and durable transplant tolerance by shifting from broad immunosuppression toward more precise immunomodulation.

Within this evolving landscape, monoclonal antibody (mAb) therapies that target specific immune pathways have long been considered an attractive strategy for preventing allograft rejection. For example, antibodies antagonizing the CD40/CD154 axis have been proposed to modulate alloimmune responses in preclinical animal transplant models [13, 14]. Although the early clinical development of anti-CD154 mAbs was halted after thromboembolic complications were reported in human studies [15], preclinical testing of anti-CD40 mAbs in rhesus macaques significantly reduced donor-specific antibody formation and prolonged islet survival [16]. Furthermore, anti-CD25 (basiliximab) is already used for induction therapy in islet transplantation for T1D patients [17, 18]. In renal allotransplantation, additional mAbs, such as anti-CD3 (OKT3) and anti-CD52 (alemtuzumab), have also reached clinical use, although with limited success [19, 20].

We previously demonstrated that genetically targeting the activating ligands CD276 (B7-H3) and CD155 (PVR) effectively

prevents the recognition of MHC class I-deficient pancreatic cells by Natural Killer (NK) cells [21]. However, NK cells also contribute to allograft rejection through missing-self recognition in the context of Killer-cell Immunoglobulin-like Receptor (KIR)-HLA mismatches, where insufficient inhibitory signals due to mismatches promote NK-mediated graft loss [22, 23]. Activating ligands, such as CD276 and CD155, are crucial not only in direct NK cell recognition [21, 24, 25] but also in broader immune regulation [26–28]. These ligands play essential roles in modulating dendritic cell maturation [29, 30], macrophage-mediated recruitment and engulfment [31, 32], and lymphocyte T-cell activity [26, 33, 34], and thereby potentially impacting the entire immune response network [35]. In this study, we used monoclonal antibodies against CD276 and CD155, taking advantage of their prior preclinical and clinical development in oncology [36–40]. We assessed whether short-term peri-transplant administration of these targeted antibodies could foster immune tolerance by modulating key activating pathways, thereby improving engraftment and supporting the maturation of iPSC-derived pancreatic progenitors into functional islets.

MATERIALS AND METHODS

Peripheral Blood Mononuclear Cell Isolation, iPSC Line Generation, and Differentiation

Peripheral blood mononuclear cells (PBMCs) for mouse humanization and iPSC generation were isolated from healthy donors after informed consent using Ficoll-Paque separation. Four iPSC lines (AMF70.10, NL83.01, RP84.03, and AG89.04)

were generated by reprogramming the donor-derived PBMCs with the CytoTune-iPS 2.0 Sendai Reprogramming Kit (Thermo Fisher Scientific). All lines were routinely screened for *Mycoplasma* using the MycoAlert Detection Kit (Lonza). Luciferase gene transduction was performed as previously described [21], and pancreatic differentiation followed established protocols [41].

NK Cell Isolation and Expansion

NK cells were isolated from freshly obtained donor PBMCs using the CD56⁺CD16⁺ NK Cell Isolation Kit (Miltenyi Biotec) and expanded for 12 days in NK MACS Medium (Miltenyi Biotec) supplemented with 5% human AB serum (Corning), 70 ng/mL IL-15, and 500 U/mL IL-2 (Peprotech).

KIR-HLA Interaction Scoring

We quantified donor NK cell-recipient iPSC compatibility using an additive score that integrates the inhibitory (I) and activating (S) KIR copy numbers with the recipient HLA-C group and Bw4 epitopes. Copy numbers were obtained from KIR genotyping (per gene), while the HLA-C group and Bw4-I80/T80 were derived from iPSC HLA typing. A positive total score ($S > 0.0$) indicates a net inhibitory match, a negative score ($S < 0.0$) indicates a net activating/missing-self-like mismatch, and a score of zero ($S = 0.0$) is neutral. The Bw4 weight was defined as:

$$W_{Bw4} = 1.5n_{I80} + 1.0n_{T80}$$

For C1/C1 recipients:

$$\begin{aligned} S_{C1/C1} = & +1K_{2DL2}n_{C1} + 0.5_{2DL3}n_{C1} - 1K_{2DL1}n_{C1} - 1K_{2DS2}n_{C1} \\ & + 1K_{2DS1}n_{C1} + 0.5K_{2DS5}n_{C1} + 1K_{3DL1}w_{Bw4} \\ & - 1K_{3DS1}w_{Bw4} - 0.1K_{2DS4f}n_{C*04:01} - 0.05K_{2DS4f}n_{A11} \\ & + 0.1K_{3DL2}n_{A11} \end{aligned}$$

For C2/C2 recipients:

$$\begin{aligned} S_{C2/C2} = & +1K_{2DL1}n_{C2} - 0.6K_{2DL2}n_{C2} - 0.1K_{2DL3}n_{C2} - 1K_{2DS1}n_{C2} \\ & + 1K_{2DS2}n_{C2} - 0.5K_{2DS5}n_{C2} + 1K_{3DL1}w_{Bw4} - 1K_{3DS1}w_{Bw4} \\ & - 0.1K_{2DS4f}n_{C*04:01} - 0.05K_{2DS4f}n_{A11} + 0.1K_{3DL2}n_{A11} \end{aligned}$$

Where “K” represents the copy number of the given KIR gene, and “n” represents the iPSC allele counts for the corresponding HLA determinant. Interaction weights were based on published KIR-HLA interactions and included a dedicated term for KIR2DS4-HLA-C*04:01 binding, along with additional terms for KIR2DS4 and KIR3DL2 in the presence of HLA-A*11:01.

In Vitro Cytotoxicity Assays

Endocrine progenitor (EP) clusters were cultured in suspension at 95 rpm in their specific medium, which was supplemented with 10 ng/mL IFN- γ and 50 ng/mL TNF- α (Peprotech). NK cells were pre-activated with 1,000 U/mL IL-2 and 20 ng/mL IL-12 (Peprotech). After overnight incubation, the iPSCs or the EP clusters were stained with 250 nM Incucyte Cytotox Green Dye (Sartorius) in complete NK MACS medium. The NK cell effectors were labeled with 5 μ M Cell Proliferation Dye eFluor670 (Thermo Fisher Scientific). The effector and target cells were

co-cultured at a 1:1 ratio in 96-well plates, with ≥ 3 target-only wells included to quantify basal cell death. The plates were then placed in the IncuCyte S3 Live-Cell Analysis System and analyzed using the associated software (Sartorius).

In Vivo Experiments

Female NOD-scid IL2R γ manull (NSG) mice (age: 6–8 weeks old; weight: 20–24 g) were obtained from Charles River Laboratories, Italy. Female hIL-15 NOG mice (NOD.Cg-Prkdc^{scid} Il2rg^{tm1Sug} Tg (CMV-IL2/IL15)1-1Jic/JicTac) (age: 6–8 weeks old; weight: 20–24 g) were purchased from Taconic Biosciences. Female mice were chosen because males more often develop spontaneous dermatitis, which could confound symptoms of graft-versus-host disease (GvHD). Additionally, female mice exhibit more consistent human immune cell engraftment. The mice were humanized via an intravenous injection of 2.5×10^6 human PBMCs, with PBMC labeling using the IVISense DiR 750 Fluorescent Cell Labeling Dye (XenoLight, Revvity, Inc.) performed as needed, following the manufacturer's instructions. After 14 days, the mice were infused with ~ 800 clusters (100–120 μ m in diameter) into the intermuscular space of their lower hindlimbs and were monitored for up to 4 weeks post-transplantation. All procedures were conducted under protocols approved and overseen by the Animal Care and Use Committee of the San Raffaele Scientific Institute.

In Vivo Imaging

D-luciferin potassium salt (PerkinElmer) was administered intraperitoneally to anesthetized mice at 150 mg/kg. The animals were imaged using the Lumina II IVIS system (PerkinElmer), acquiring both bioluminescence and fluorescence signals. Bioluminescence was quantified as maximum radiance expressed in photons/s/cm²/sr, whereas fluorescence was measured as average radiant efficiency ([photons/s/cm²/sr]/[μ W/cm²]). Signal intensities within the defined Regions of Interest (ROIs) were quantified using Aura software (Spectral Instruments Imaging).

Flow Cytometry

Clusters were dissociated with trypsin (Lonza), and live cells were identified using the LIVE/DEAD Fixable Violet kit (Thermo Fisher Scientific). Surface staining was performed by incubating cells with antibodies for 30 min at 4 °C in FACS buffer (DPBS with 2% FBS and 2 mM EDTA). For intracellular staining, cells were fixed with Cytofix/Cytoperm (BD Biosciences) and permeabilized with Phosflow Perm Buffer III (BD Biosciences), then incubated for 45 min at 4 °C with intracellular antibodies. For the phenotyping of humanized mice, 50 μ L of blood collected from the retro-orbital plexus was stained for 30 min at 4 °C, followed by red blood cell lysis with BD FACS™ Lysing Solution prior to analysis. The following conjugated antibodies were used: anti-B2M-APC (clone 2M2); anti-HLA-A, B, C-PE (clone W6/32), anti-CD45-PE/Dazzle™594 (clone HI30); anti-CD8-BV605 (clone SK1); anti-CD154-APC (clone 24–31) (all were obtained from Biolegend); anti-OCT3/4-AF647 (clone 40/Oct3); anti-CD184-PE (clone 12G5); anti-PDX-1-AF488 (clone 658A5); anti-NKX6.1-PE (clone R11-560); anti-insulin-AF647 (clone T56-706); anti-glucagon-BV421; anti-CD3-FITC/

BUV395 (clone SK7); anti-CD4-PB (clone RP-T4); anti-CD56-PE (clone NCAM1); anti-HLA-DR-BV480 (clone G46-6); α -PD-1-R718 (clone EH12.1); anti-CD38-APC (clone HIT2); anti-CD223-BUV395 (clone T47-530), anti-FoxP3-R718 (clone 259D/C7); anti-Helios-PE (clone 22F6); anti-CD107a-APC-H7 (clone H4A3) (all were obtained from BD Biosciences); anti-CD4-PE-Vio770 (clone REA623); anti-CD14-VioGreen (clone TÜK4) (both were obtained from Miltenyi Biotec); anti-CD16-SuperBright436 (clone 3G8) (obtained from Invitrogen); and anti-CD69-StarBright UltraViolet 510 (clone FN50) (obtained from BioRad). The cells were acquired on the CytoFLEX LX flow cytometer (Beckman Coulter) using CytExpert, and the data were analyzed using FlowJo v10.

Glucose Tolerance Test and C-Peptide Measurement

An intraperitoneal glucose tolerance test (ipGTT) was performed on day 28. After a 4-h fast, the mice received a 2 g/kg intraperitoneal glucose bolus, and blood glucose was monitored at 0, 30, 60, 90, and 120 min. At the 90-min time point, blood was collected and plasma was isolated by centrifugation and subsequently analyzed for human C-peptide using a Mercodia ELISA. Absorbance was measured on a BioRad microplate reader.

Immunohistochemistry

Explanted grafts were fixed, processed, and paraffin-embedded. Sections were cut at 5 μ m for histological analysis. Hematoxylin and eosin (H&E) staining was performed to visualize the grafts and assess their morphology. BOND™ Ready-To-Use Primary Antibody Insulin (Leica, clone 2D11-H5) was used to detect mature iPSC-derived islets. The slides were scanned using a Leica Aperio 200.

Statistical Analysis

All data are presented as the mean $\pm$ SEM unless otherwise specified. Comparisons between more than two independent groups were performed using one-way ANOVA with a Tukey's *post hoc* test or a Kruskal-Wallis with Dunn's test for non-parametric data. Longitudinal datasets were analyzed using two-way ANOVA followed by Holm-Šidák's correction. Survival analysis was conducted using the Kaplan-Meier method with the log-rank (Mantel-Cox) test. Pairwise comparisons were made using a two-tailed unpaired or paired Student's *t*-test, as appropriate. All analyses were performed using GraphPad Prism v10.

RESULTS

Blockade of CD276 and CD155 Dampens Missing-Self-Recognition and Killing of SC-Derived Pancreatic Endocrine Cells by KIR-HLA Mismatched NK Cells

First, we examined the contribution of the NKp30-CD276 and CD226-CD155 axes to missing-self recognition under KIR-HLA

class I mismatch conditions using *in vitro* NK cytotoxicity assays. The NK cells derived from eight KIR-genotyped donors were matched or mismatched with four HLA-typed iPSC lines. Undifferentiated iPSC lines were then co-cultured with the NK cells, and NK-mediated killing events were recorded via live cell microscopy in the presence or absence of blocking mAbs (**Figure 1A**). The list of characteristics of both donor NK cells and iPSC lines used in these experiments, along with the matched/mismatched pairs based on KIR gene copy number and HLA haplotype, is reported in **Supplementary Tables S1–S3**.

As expected, KIR-HLA mismatching resulted in a significant three-to-fourfold increase in NK-mediated killing compared to matched pairs ($11.1\% \pm 2.7\%$ vs. $36.9\% \pm 6.4\%$; $p < 0.0001$). Blocking either NKp30 or CD226 with specific mAbs reduced the killing of mismatched cells ($24.2\% \pm 4.2\%$ with α -NKp and $18.1\% \pm 2.6\%$ with α -CD226, respectively) (**Figure 1B**). Accordingly, blocking the activating ligands CD276 or CD155 on target cells reduced NK cell cytotoxicity by a similar amount as blocking their counterreceptors ($26.7\% \pm 8.9\%$ with α -CD276 and $16.5\% \pm 7.0\%$ with α -CD155, respectively). Remarkably, the combination of CD276 and CD155 blockade had a synergistic effect, reducing NK-mediated killing of the mismatched iPSCs to levels comparable to those of KIR-HLA-matched pairs ($11.1\% \pm 2.7\%$ for matched vs. $40.9\% \pm 8.9\%$ for mismatched vs. $9.6\% \pm 3.0\%$ with α -CD276+ α -CD155, respectively) (**Figure 1B**).

Previously, we demonstrated that both β 2-microglobulin (B2M) and HLA class I molecules are dynamically regulated during pancreatic differentiation [21]. Consistent with prior findings, iPSC lines exhibited the highest B2M and HLA-A/B/C expression levels, while a marked downregulation was observed at the posterior foregut (PF) and EP stages. SC-islets displayed an intermediate and more heterogeneous expression profile, with a broader range of HLA class I surface levels among cells (**Supplementary Figures S1A,B**).

Since EP clusters show low HLA expression, potentially limiting T cell immunogenicity, we hypothesized that they might be susceptible to missing-self-recognition by NK cells. **Supplementary Figures S1C–E** illustrate changes in key differentiation markers at each stage. OCT4 was highly expressed in iPSCs and was rapidly lost after definitive endoderm (DE) induction, while CXCR4 and FOXA2 increased. Progression to the EP stage was associated with increased expression of the pancreatic lineage markers PDX1 and NKX6.1, along with the early endocrine markers INS and GCG (**Supplementary Figure S1C**). Coexpression of PDX1 and NKX6.1 in EP cells was confirmed by flow cytometry (**Supplementary Figure S1D**), and EP cluster morphology was documented by bright-field imaging (**Supplementary Figure S1E**). To evaluate the susceptibility of EP clusters to NK cell infiltration and killing *in vitro*, we performed live-cell imaging cytotoxicity assays under both KIR-HLA matching and mismatching conditions. In mismatched pairs, missing-self-recognition occurred, as evidenced by increased NK cell infiltration and EP cell killing. This was measured by a progressive rise in green fluorescence over time (**Figures 1C,D**) and increased co-localization of red-labeled NK cells

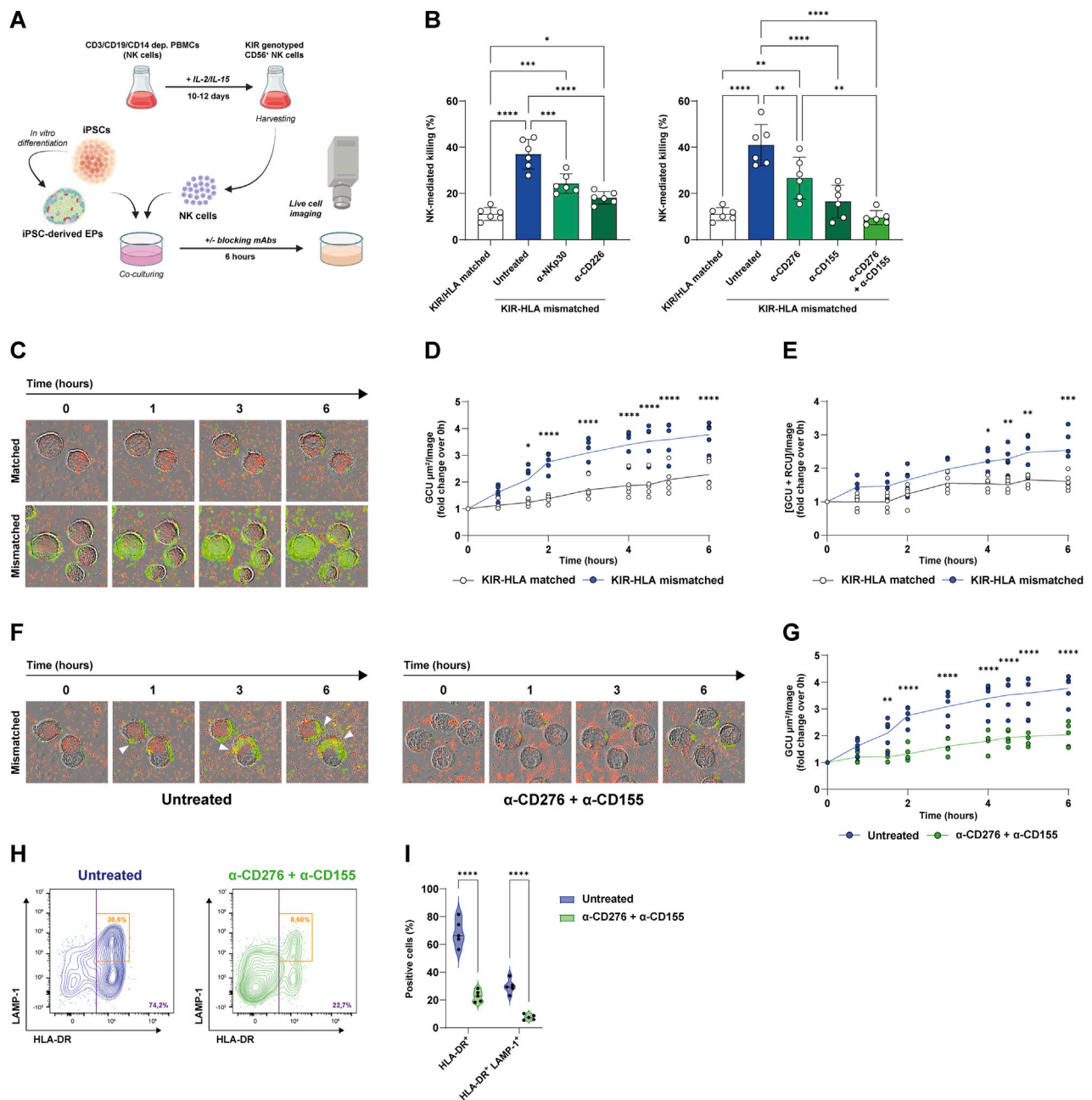


FIGURE 1 | Blockade of CD276 and CD155 reduces missing-self recognition and NK-mediated killing of iPSC-derived EPs. **(A)** Experimental workflow. Both iPSCs and iPSC-derived EP clusters were co-cultured with KIR-genotyped NK cells that were isolated and expanded from healthy donor PBMCs in the presence of IL-2 and IL-15. NK-mediated cytotoxicity was evaluated by live-cell imaging over 6 h, in the presence or absence of blocking mAbs targeting the NKp30/CD276 and CD226/CD155 axes. **(B)** Quantification of NK-mediated killing (% of dead target cells) under KIR-HLA-matched (white bars) or mismatched (colored bars) conditions, with the latter in the presence of α -NKp30, α -CD226, α -CD276, α -CD155, or both α -CD276 and α -CD155 mAbs. N = 6. * p < 0.05; ** p < 0.01; *** p < 0.001; **** p < 0.0001. **(C)** Representative time-lapse images of NK cell infiltration and EP cluster lysis under matched and mismatched conditions (green = dead cells, red = eFluor670-labeled human NK cells). **(D)** Kinetic analysis of dead cell area expressed as Green Calibrated Unit (GCU) per square micrometer per field of view, under matched versus mismatched conditions. The line represents the mean. N = 5. * p < 0.05; **** p < 0.0001 by two-way ANOVA followed by Šidák's *post hoc* multiple comparison test. **(E)** Kinetic analysis of NK cell-mediated infiltration and killing measured as the co-localization of GCU and Red Calibrated Unit (RCU) per field of view, under matched versus mismatched conditions. The line represents the mean. N = 5. * p < 0.05; ** p < 0.01; *** p < 0.001 by two-way ANOVA followed by Šidák's *post hoc* multiple comparison test. **(F)** Representative time-lapse images of NK cell infiltration and killing in untreated versus α -CD276 + α -CD155-treated EPs under mismatched conditions. **(G)** Kinetic analysis of dead cell area under untreated versus α -CD276 + α -CD155-treated EPs. The line represents the mean. N = 5. * p < 0.01; **** p < 0.0001 by two-way ANOVA followed by Šidák's *post hoc* multiple comparison test. **(H)** Flow cytometry plots showing NK cell activation (HLA-DR⁺) and degranulation (LAMP-1⁺) under untreated versus α -CD276 + α -CD155 treated conditions. **(I)** Violin plots representing the quantification of HLA-DR⁺ and HLA-DR⁺/LAMP-1⁺ NK cells. N = 5. **** p < 0.0001 by two-tailed unpaired Student's *t*-test.

with green fluorescence signals (**Figure 1E**). Importantly, treatment of HLA-mismatched EPs with α -CD276 and α -CD155 monoclonal antibodies significantly reduced cell death (**Figures 1F,G**), in addition to NK cell activation markers, including HLA-DR ($68.4\% \pm 9.7\%$ vs. $22.7\% \pm 4.2\%$; $p < 0.0001$) and the degranulation marker CD107a (LAMP-1) on activated HLA-DR⁺ cells ($29.7\% \pm 5.1\%$ vs. $7.5\% \pm 1.9\%$; $p < 0.0001$) (**Figures 1H,I**).

Dual Blockade of CD276 and CD155 Prevents Immune Rejection of EP Grafts in a Fully Humanized Mouse Model

Since CD276 and CD155 are expressed on both graft cells and immune cells, we next evaluated whether antagonizing them may provide graft tolerance by modulating immune cell activity other than direct graft targeting. NSG mice demonstrated sustained engraftment of T lymphocytes but limited NK cell and monocyte reconstitution (**Supplementary Figure S2**); therefore, we used hIL-15 NOG immunodeficient mice, which achieve superior humanization efficiency and improved engraftment of innate immune cells (**Supplementary Figure S3**). To evaluate the efficacy of blocking mAbs, mice were first injected intravenously with 2.5×10^6 human PBMCs and, 14 days later, transplanted with ~ 800 clusters of luciferase-expressing iPSC-derived EPs into the intermuscular space of the lower hindlimbs. The mAb treatment regimen consisted of 5 intraperitoneal injections every 3 days, starting 2 days before transplantation and continuing up to 10 days post-transplantation, as outlined in **Figure 2A**. We tested two doses of α -CD276 (1.25 and 15 mg/kg), one dose of α -CD155 (2.5 mg/kg), and a combined treatment consisting of the highest dose of α -CD276 (15 mg/kg) plus α -CD155 (2.5 mg/kg). Humanized untreated mice rejected EP grafts within 7–10 days (**Figures 2B,C**). Notably, increasing doses of α -CD276 prolonged graft survival for 2 weeks (**Figure 2C**), but were associated with increased mortality due to an earlier onset of GvHD, as confirmed by log-rank trend analysis ($p = 0.025$) (**Figure 2D**). In contrast, treatment with α -CD155, either alone or in combination with α -CD276, ensured graft survival for up to 4 weeks at levels comparable to non-humanized mice (**Figure 2C**). Moreover, co-administration of α -CD155 mitigated the severe GvHD effects observed with α -CD276 alone, as mortality in the α -CD155-treated groups was comparable to that of untreated humanized mice (**Figure 2D**).

In Vivo Imaging Reveals Reduced PBMC Accumulation at the Implant Site Under CD276/CD155 Blockade

The kinetics of immune cell infiltration and the impact of checkpoint blockade were longitudinally investigated by *in vivo* imaging combining bioluminescence (to track luciferase-expressing EP grafts) and fluorescence detection of DiR-labeled human PBMCs (**Figure 3A**). Humanized mice were treated intraperitoneally with α -CD276 (15 mg/kg), α -CD155 (2.5 mg/kg), or both antibodies in combination at the same doses.

As early as day 4, untreated humanized mice showed progressive PBMC accumulation at the graft site, with the fluorescence signal increasing from 13.2 ± 5.0 - to $66,537 \pm 59,820$ -fold over background by day 14. Mice treated with α -CD276 alone showed delayed yet substantial infiltration ($5,727 \pm 3,284$ fold), while α -CD155 alone more effectively reduced immune cell recruitment (208 ± 158 fold). Strikingly, the combined blockade of CD276 and CD155 limited the increase in PBMC-associated fluorescence on day 14 to ~ 69 -fold over the day 0 baseline. Although this signal remains higher than that observed in untransplanted (~ 19 -fold) and non-humanized (~ 1.8 -fold) mice at the same time point, the absence of further fluorescence amplification indicates that only a few immune cells reach the grafts, without evidence of sustained recruitment (**Figure 3B**).

No significant differences were observed between groups at early time points (day 4–10). On day 14, however, Kruskal-Wallis analysis revealed significant divergence in PBMC accumulation across experimental groups ($H = 11.48$, $p = 0.043$). Dunn's *post hoc* comparisons revealed that PBMC infiltration in untreated mice ($66,537 \pm 59,820$ -fold) was significantly higher than in non-humanized (1.81 ± 0.54 -fold; $p < 0.001$), untransplanted (19.2 ± 14.0 -fold; $p < 0.01$), and dual mAb-treated mice (69.6 ± 49.1 -fold; $p < 0.05$). Differences with α -CD276 ($5,727 \pm 3,284$ -fold) and α -CD155 (208 ± 158 -fold) were not statistically significant after correction, although a downward trend was noted in the α -CD155 group (**Figure 3B**).

Anti-CD276 Prevents Innate Immune Cell Activation and Migration, While the CD155 Blockade Enhances T cell Exhaustion and Promotes CD4⁺ Treg Expansion

Next, we sought to investigate the mechanisms underlying the protective effects of α -CD276 and α -CD155 mAb treatments and their impact on immune cell activation and migration. According to *in vitro* experiments, we found that both α -CD276 and α -CD155 prevented the overexpression of the tissue migration marker CD69 on circulating CD56^{dim}CD16⁺ NK cells (**Figure 4A**). Moreover, α -CD276, either alone or in combination with α -CD155, dampened CD14⁺ monocyte activation and migration, as confirmed by lower levels of HLA-DR⁺ and CD40L⁺ cells compared to the untreated group (**Figure 4B**), supporting the hypothesis that CD276 signaling contributes to monocyte infiltration into the grafts.

However, high-dose α -CD276 alone did not prevent CD8⁺ T cell activation (**Figure 3C**). In contrast, α -CD155 treatment alone significantly increased the frequency of exhausted PD-1⁺CD8⁺ T cells (**Figure 4C**) and LAG-3⁺CD4⁺ T cells (**Figure 4D**) 12 days post-transplantation. The combination of α -CD276 and α -CD155 led to a slight but significant reduction in CD38⁺HLA-DR⁺CD8⁺ T cells at 12 days post-transplantation (**Figure 4C**) and an early increase in LAG-3 expression on total CD4⁺ T cells compared to the untreated group (**Figure 4D**). In line with recent studies showing that CD226 signaling negatively affects Treg stability [42, 43], blockade of its ligand CD155 resulted in a twofold increase in the percentage of total

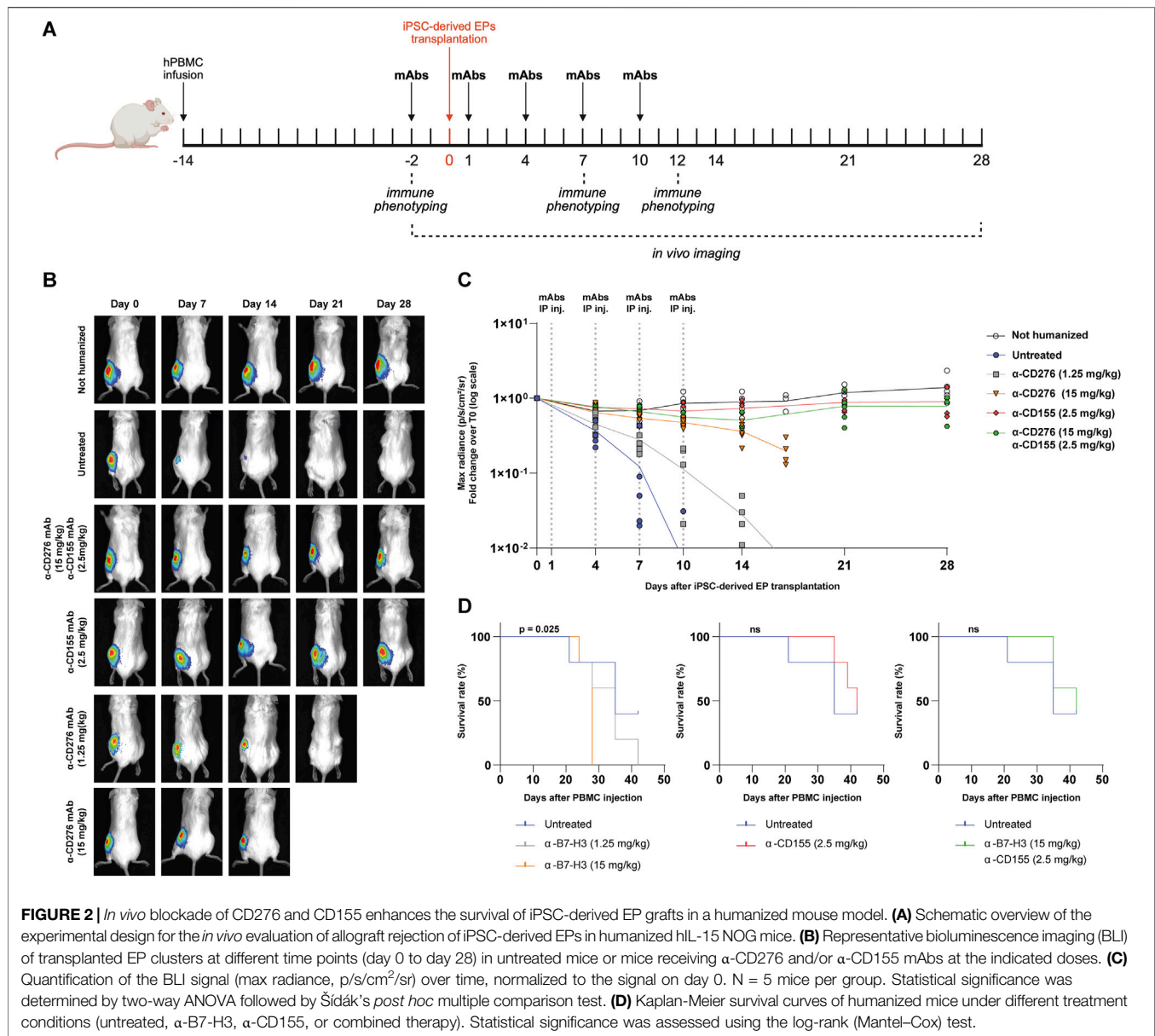


FIGURE 2 | *In vivo* blockade of CD276 and CD155 enhances the survival of iPSC-derived EP grafts in a humanized mouse model. **(A)** Schematic overview of the experimental design for the *in vivo* evaluation of allograft rejection of iPSC-derived EPs in humanized hIL-15 NOG mice. **(B)** Representative bioluminescence imaging (BLI) of transplanted EP clusters at different time points (day 0 to day 28) in untreated mice or mice receiving α -CD276 and/or α -CD155 mAbs at the indicated doses. **(C)** Quantification of the BLI signal (max radiance, p/s/cm²/sr) over time, normalized to the signal on day 0. N = 5 mice per group. Statistical significance was determined by two-way ANOVA followed by Šidák's *post hoc* multiple comparison test. **(D)** Kaplan-Meier survival curves of humanized mice under different treatment conditions (untreated, α -B7-H3, α -CD155, or combined therapy). Statistical significance was assessed using the log-rank (Mantel-Cox) test.

Tregs compared to the untreated mice (Figure 4E). Furthermore, evaluation of IL-2 Receptor α (IL-2R α /CD25) on FoxP3⁺Helios⁺CD4⁺ Tregs revealed a significant upregulation of CD25 surface expression in the α -CD155-treated group compared to mock controls (735 ± 66.4 vs. 488 ± 53.6 ; $p < 0.0001$) (Figures 4F,G), supporting the hypothesis of an enhanced Treg suppressive ability in response to CD155 blockade, consistent with previous reports [44, 45].

EP Grafts Properly Mature Into Glucose-Responsive, Insulin-Secreting β Cells in mAb-Treated Humanized Mice

To assess the maturation and function of the iPSC-derived EP grafts, we monitored plasma human c-peptide levels for 4 weeks

post-transplantation. A progressive increase in basal c-peptide levels was observed in both non-humanized and mAb-treated mice, indicating functional maturation. In stark contrast, humanized mice that did not receive CD276/CD155 blockade showed only a modest increase in c-peptide levels at 2 weeks (7.27 ± 8.11 pmol), followed by a complete loss at 4 weeks, consistent with the early rejection of endocrine cells and the failure of the grafts to mature (Figure 5A).

On day 28, an ipGTT was performed to evaluate glucose responsiveness. It should be noted that the glycemic curves shown are not intended to reflect graft functionality, as glycemia is predominantly controlled by endogenous murine islets in this setting. Both non-humanized and mAb-treated mice exhibited a significant rise in human c-peptide 90 min after glucose administration (196.5 ± 18.6 vs. 40.0 ± 5.88 pmol and $191.0 \pm$

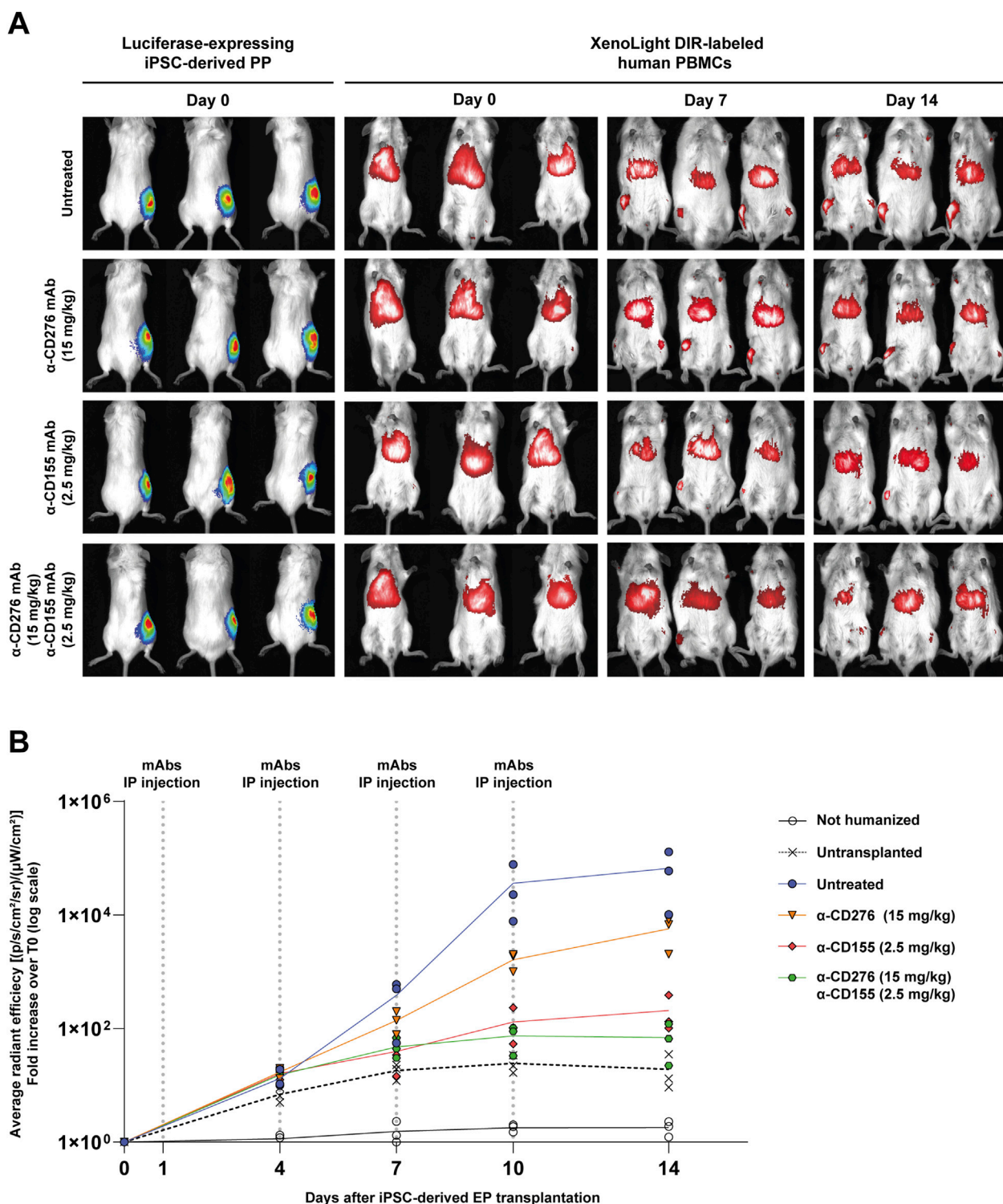


FIGURE 3 | *In vivo* tracking of human PBMCs after iPSC-derived EP transplantation under CD276/CD155 blockade. **(A)** Representative bioluminescence imaging (BLI) of luciferase-expressing iPSC-derived EPs and XenoLight DIR-labeled human PBMCs in hIL-15 NOG mice. Images were acquired before transplantation (TX) and 7 and 14 days after under untreated or α -CD276, α -CD155, and combined mAb treatment conditions. **(B)** The average radiant efficiency [(p/s/cm²/sr)/(μW/cm²)] of the graft area is expressed as a fold change compared to the signal recorded on day 0 to measure PBMC infiltration. Untransplanted humanized mice and non-humanized mice were used as controls. N = 3. Statistical significance between groups at each time point was assessed using a Kruskal-Wallis test followed by Dunn's *post hoc* multiple comparison test.

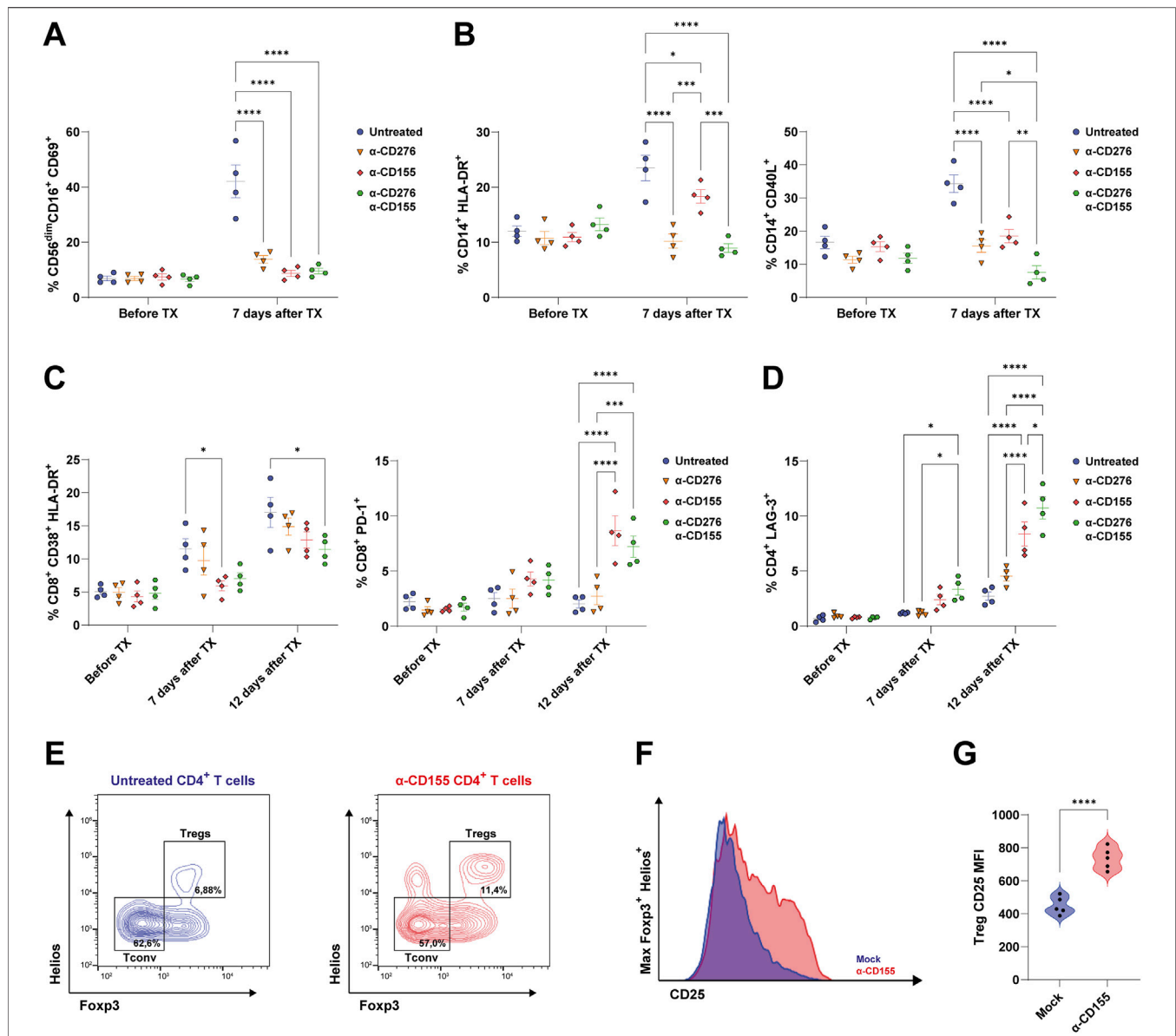


FIGURE 4 | Blockade of CD276 and CD155 modulates the activation of human immune cells. **(A)** Frequency of activated NK cells ($CD56^{dim}CD16^{+}CD69^{+}$) in the peripheral blood before and 7 days after EP TX in untreated or antibody-treated mice (α -CD276, α -CD155, or combination therapy). Data represent mean $\pm$ SEM. $N = 4$. **** $p < 0.0001$ by one-way ANOVA followed by Tukey's *post hoc* multiple comparison test. **(B)** Frequencies of activated monocytes, evaluated by expression of HLA-DR and CD40L, before and 7 days after TX. Data represent mean $\pm$ SEM. $N = 4$. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$ by one-way ANOVA followed by Tukey's *post hoc* multiple comparison test. **(C)** Frequencies of activated $CD8^{+}$ T cells ($CD8^{+}CD38^{+}HLA-DR^{+}$) and $PD-1^{+}CD8^{+}$ T cells before, 7 days, and 12 days after TX. Data represent mean $\pm$ SEM. $N = 4$. * $p < 0.05$, *** $p < 0.001$, **** $p < 0.0001$ by one-way ANOVA followed by Tukey's *post hoc* multiple comparison test. **(D)** Frequency of $CD4^{+}$ T cells expressing LAG-3 before and 7 and 12 days after TX. Data represent mean $\pm$ SEM. $N = 4$. * $p < 0.05$, *** $p < 0.001$, **** $p < 0.0001$ by one-way ANOVA followed by Tukey's *post hoc* multiple comparison test. **(E)** Representative flow cytometry plots of $CD4^{+}$ T cells showing Tconv (Foxp3 $^{-}$ Helios $^{+}$) and Treg (Foxp3 $^{+}$ Helios $^{-}$) subsets under untreated and α -CD155-treated conditions. **(F)** CD25 expression on Foxp3 $^{+}$ Helios $^{+}$ Tregs under untreated and α -CD155-treated groups. **(G)** Violin plots showing the quantification of the CD25 Mean Fluorescent Intensity (MFI) on Foxp3 $^{+}$ Helios $^{+}$ Tregs. $N = 5$. **** $p < 0.0001$ by two-tailed unpaired Student's *t*-test.

35.2 vs. 30.2 ± 13.4 pmol, respectively; $p < 0.0001$), while the untreated humanized group showed a negligible response at 90 min post-ipGTT (1.02 ± 0.79 pmol), indicating an absence of functional endocrine cells (Figure 5B).

Histological analysis showed preserved graft architecture in non-humanized and mAb-treated mice, whereas untreated mice

displayed tissue disorganization. Insulin-producing cells were found in both non-humanized and mAb-treated groups, but not in the untreated group, consistent with the c-peptide data. Moreover, human CD45 staining showed marked leukocyte infiltration in the grafts of the untreated mice, but not in the mAb-treated groups (Figure 5C). These findings indicate that

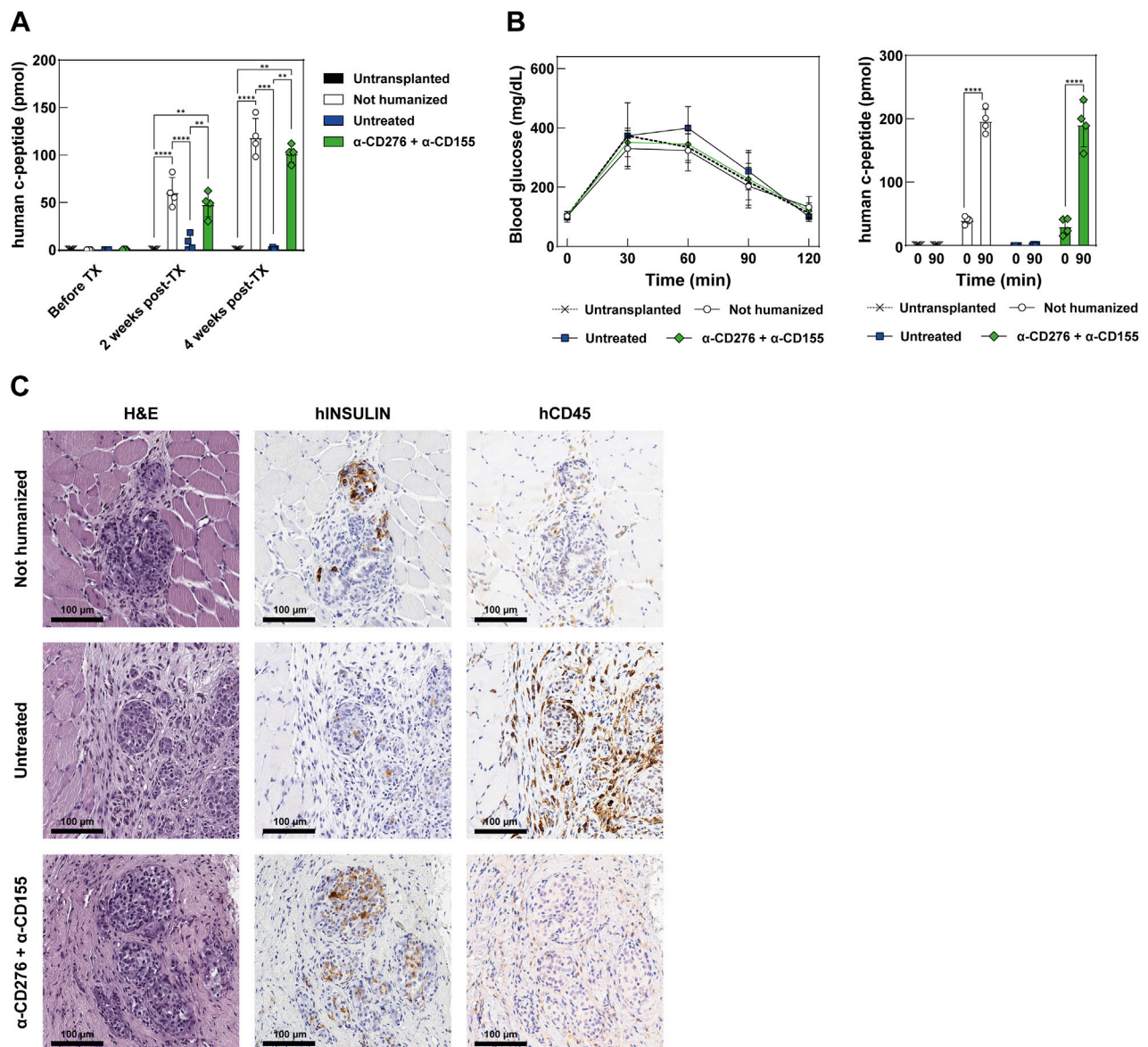


FIGURE 5 | Functional assessment and maturation of EP grafts after transplantation **(A)** Quantification of circulating human C-peptide in the plasma of recipient mice at baseline (before TX), 2 weeks, and 4 weeks post-TX. Data represent mean $\pm$ SD. $N = 4$. $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$ by one-way ANOVA followed by Tukey's *post hoc* multiple comparison test. **(B)** Intraperitoneal glucose tolerance test (ipGTT) at 28 days post-TX. The left panel shows blood glucose concentrations in mg/dL over time; the right panel shows human C-peptide secretion at baseline and 90 min post-glucose challenge. Data represent mean $\pm$ SD. $N = 4$. $^{****}p < 0.0001$ by two-tailed paired Student's *t*-test. **(C)** Representative histology of the graft site at 28 days post-TX in non-humanized, untreated, or treated mice receiving the combined α -CD276 and α -CD155 treatment. The H&E-stained section shows a pancreatic graft overlying host muscle fibers. Human insulin-positive endocrine cells and infiltrating CD45-positive immune cells are also visible. Scale bar: 100 μ m.

dual blockade of CD276 and CD155 protects the grafts from early immune rejection, enabling *in vivo* maturation of EP cells into functional, glucose-responsive β -like cells.

DISCUSSION

Treatment with α -CD276 and α -CD155 blocking monoclonal antibodies in humanized hIL-15 NOG mice transplanted with

iPSC-derived EPs significantly prevented immune activation and allojection. Our study provides the first preclinical proof-of-concept for targeting stress-inducible ligands such as CD276 (B7-H3) and CD155 (PVR), as a strategy to promote graft tolerance through selective immunomodulation. These findings expand on previous evidence showing that both ligands mediate NK cell-driven missing-self recognition [21, 46] and broadly shape immune crosstalk during inflammatory responses [47, 48].

CD276, a B7-family immune checkpoint, has been extensively investigated in oncology; however, its function remains debated due to its dual activating/inhibiting effects [49]. Originally identified as a co-stimulatory molecule that interacts with NKP30, CD276 can deliver both activating and inhibitory signals through the TLT-2 (TREM2) receptor [50, 51]. While the function of TLT-2 in NK cells remains uncertain [52], its expression on monocytes, macrophages, and granulocytes has been linked to enhanced phagocytic activity and IL-6 production [32, 53]. In transplantation, CD276 has been implicated in both acute and chronic islet allograft rejection [54], but in fully MHC-mismatched models, it has also been associated with a Th2 shift and prolonged graft survival [55].

CD155 (PVR), a nectin-like co-stimulatory molecule, balances immune activation through interaction with both activating (CD226/DNAM-1) and inhibitory (TIGIT, CD96) receptors on NK and T cells [56]. Naturally expressed at low levels on epithelial, endothelial, and antigen-presenting cells, CD155 is rapidly upregulated in response to stress and inflammation [57, 58], and localizes to interendothelial junctions, where it regulates the diapedesis of CD226⁺ leukocytes; blocking either CD155 or CD226 arrests monocytes and prevents their trans-endothelial migration [59]. In renal transplantation models, both CD155 and CD112 are constitutive, but their role in acute rejection has not been demonstrated [60].

Dual blockade of CD276 and CD155 almost completely prevented the increase in NK cytotoxicity observed under KIR–HLA mismatch, confirming these ligands as dominant checkpoints for missing-self recognition. In particular, CD155 inhibition curtailed early alloreactivity by dampening CD226-driven cytotoxicity and expanding FOXP3⁺Helios⁺ Tregs. It increased Treg frequency and CD25 expression, enhancing their IL-2-dependent suppressive ability, while also inducing PD-1/LAG-3 on effector T cells. These effects align with evidence that blocking CD226 signaling promotes Treg differentiation [42, 43, 45] which in the transplant setting is essential for both immune tolerance and tissue repair/engraftment [61].

These actions generate a more balanced immune milieu in which innate and effector T cell responses are attenuated, while Treg-mediated regulation could be reinforced. This creates a permissive niche that supports the engraftment and *in situ* maturation of iPSC-derived pancreatic tissues. Notably, these benefits are achieved without broad immunosuppression, thus preserving systemic immune competence and avoiding the detrimental effects that conventional drugs exert on lymphocyte and tolerogenic functions [62, 63].

In vivo, treatment with these targeted mAbs prolonged EP graft survival for up to 4 weeks, reduced innate immune infiltration, and enabled differentiation into functional insulin-producing β -like cells. The translational relevance of this approach is strengthened by the availability of fully human or humanized antibodies that have already been evaluated in oncology trials [36, 38], providing a feasible path toward

clinical applications. Importantly, by modulating immunity during the period when iPSC-derived pancreatic tissues temporarily downregulate HLA class I [21, 64], our approach safeguards the grafts without requiring lifelong immunosuppression. Since transient HLA class I downregulation also occurs in cardiac [65], hepatic [66], and neural derivatives [67], this strategy may have broad applicability across stem cell-based transplantation.

Nonetheless, some limitations must be acknowledged. First, the observation window was limited to 28 days due to the onset of GVHD in PBMC-humanized mice, which prevented long-term follow-up. As a result, we cannot assess long-term graft survival, chronic rejection, allo-sensitization, or the development of donor-specific antibodies. Alternative humanized models lacking murine MHC class I and II, such as NSG MHC I/II double-knockout mice [68], could help address these limitations by lowering GVHD incidence and enabling longer-term evaluation of graft outcomes.

Second, while our data demonstrate that CD276/CD155 blockade promotes the sustained engraftment and *in vivo* maturation of iPSC-derived EPs over 4 weeks, this early “tolerance-like” phenotype should not be interpreted as definitive evidence of long-term immune tolerance. Secondary challenges, such as donor-matched skin grafting or long-term re-exposure to antigen, will be needed to assess if durable, antigen-specific tolerance has been achieved.

Additionally, we observed that a high dose of α -CD276 mAb accelerated GvHD onset, highlighting a narrow therapeutic window requiring optimization in future dose-ranging studies. Given that the immunomodulatory effects of CD276/CD155 blockade are transient, the primary risk may lie in increased susceptibility to infection during the peri-transplantation period. However, the duration and extent of immune modulation after treatment withdrawal, especially regarding antiviral and anti-tumor surveillance, remain unclear, and pathogen-challenge or tumorigenicity assays will be essential to understanding the long-term effects of the immunomodulation.

In conclusion, dual blockade of CD276 and CD155 emerges as a rational, early tolerance-promoting strategy that tempers innate immune checkpoints while enhancing regulatory T cell function. This immunomodulatory platform supports the engraftment and functional maturation of stem cell-derived pancreatic grafts and may serve as a foundational component of next-generation cell therapies. Future studies should assess whether combining this approach with adoptive Treg therapy or gene-edited hypoinmunogenic iPSC derivatives can achieve durable, drug-free tolerance in large animal models and, ultimately, in clinical settings.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding authors.

ETHICS STATEMENT

The studies involving humans were approved by Institutional Ethics Committee. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study. The animal study was approved by Institutional Animal Care and Use Committee (IACUC) and authorized by the Italian Ministry of Health. The study was conducted in accordance with the local legislation and institutional requirements.

AUTHOR CONTRIBUTIONS

GS and RC performed immune cell isolation, *in vitro* cytotoxicity assays, animal experiments, data generation, and statistical analyses. They also contributed to the study's conceptualization and the manuscript preparation. FD and VS carried out stem cell pancreatic differentiation. MM contributed to the pairing of stem cell lines and donor-derived NK cells based on KIR genotype interpretation. LP and RC supervised the project, critically revised, and edited the manuscript. All authors contributed to the article and approved the submitted version.

FUNDING

The authors declare that financial support was received for the research and/or publication of this article. This study was supported by the European Association for the Study of Diabetes (EASD) through the EFSD/JDRF/Lilly European Program in Type 1 Diabetes Research (2021), awarded to LP and RC, and by Breakthrough T1D (formerly JDRF) under the Strategic Research Agreement no. 3-SRA-2022-1254-S-B awarded to LP, RC, and MM.

CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

REFERENCES

- Hogrebe NJ, Ishahak M, Millman JR. Developments in Stem Cell-Derived Islet Replacement Therapy for Treating Type 1 Diabetes. *Cell Stem Cell* (2023) 30: 530–48. doi:10.1016/j.stem.2023.04.002
- Fujikura J, Anazawa T, Toyoda T, Ito R, Kimura Y, Yabe D. Toward a Cure for Diabetes: Ipsc and ESC-Derived Islet Cell Transplantation Trials. *J Diabetes Invest* (2025) 16:384–8. doi:10.1111/jdi.14366
- Piemonti L. The Last Mile in Beta-Cell Replacement Therapy for Type 1 Diabetes: Time to Grow up. *Transpl Int Front Publishing Partnerships* (2025) 38:14565. doi:10.3389/ti.2025.14565
- Reichman TW, Markmann JF, Odorico J, Witkowski P, Fung JJ, Wijkstrom M, et al. Stem Cell-Derived, Fully Differentiated Islets for

GENERATIVE AI STATEMENT

The authors declare that no Generative AI was used in the creation of this manuscript.

Any alternative text (alt text) provided alongside figures in this article has been generated by Frontiers with the support of artificial intelligence and reasonable efforts have been made to ensure accuracy, including review by the authors wherever possible. If you identify any issues, please contact us.

ACKNOWLEDGEMENTS

We would like to acknowledge the Preclinical Imaging Facility at the San Raffaele Experimental Imaging Center for their essential support with optical imaging procedures, and the Animal Histopathology Facility at the San Raffaele Scientific Institute for their help with processing histological samples. We also thank the Flow Cytometry Resource and the Advanced Cytometry Technical Applications Laboratory (FRACTAL), along with the Advanced Light and Electron Microscopy BioImaging Center (ALEMBIC) at the San Raffaele Scientific Institute, for their technical support with FACS and imaging analysis, respectively.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontierspartnerships.org/articles/10.3389/ti.2025.15433/full#supplementary-material>

SUPPLEMENTARY TABLE S1 | Immunogenetic features of the iPSC lines used in the study.

SUPPLEMENTARY TABLE S2 | KIR genotyping of the NK cell donors.

SUPPLEMENTARY TABLE S3 | KIR-HLA compatibility matrix between donor-derived NK cells and iPSC lines.

SUPPLEMENTARY FIGURE S1 | HLA class I and differentiation marker expression dynamics during pancreatic commitment.

SUPPLEMENTARY FIGURE S2 | Human immune cell engraftment in hIL-15 NOG mice infused with total PBMCs.

SUPPLEMENTARY FIGURE S3 | Immune subset characterization in humanized hIL-15 NOG mice.

Type 1 Diabetes. *N Engl J Med* (2025) 393:858–68. doi:10.1056/NEJMoa2506549

- Kabakchieva P, Assyov Y, Gerasoudis S, Vasilev G, Peshevska-Sekulovska M, Sekulovski M, et al. Islet Transplantation-Immunological Challenges and Current Perspectives. *World J Transpl* (2023) 13:107–21. doi:10.5500/wjt.v13.i4.107
- Lemos JRN, Poggioli R, Ambut J, Bozkurt NC, Alvarez AM, Padilla N, et al. Impact of GAD65 and IA2 Autoantibodies on Islet Allograft Survival. *Front Clin Diabetes Healthc* (2023) 4:1269758. doi:10.3389/fcdhc.2023.1269758
- Shapiro AMJ, Ricordi C, Hering BJ, Auchincloss H, Lindblad R, Robertson RP, et al. International Trial of the Edmonton Protocol for Islet Transplantation. *N Engl J Med* (2006) 355:1318–30. doi:10.1056/NEJMoa061267

8. Hering BJ, Ballou CM, Bellin MD, Payne EH, Kandeel F, Witkowski P, et al. Factors Associated With Favourable 5 Year Outcomes in Islet Transplant Alone Recipients with Type 1 Diabetes Complicated by Severe Hypoglycaemia in the Collaborative Islet Transplant Registry. *Diabetologia* (2023) 66:163–73. doi:10.1007/s00125-022-05804-4
9. Lee AY, Jeong J, Heo K-N, Park S, Ah Y-M, Han JM, et al. Complications Associated With Immunosuppressive Agents in Solid Organ Transplant Recipients: A Nationwide Analysis. *J Clin Med* (2025) 14:3602. doi:10.3390/jcm14103602
10. Piemonti L, Everly MJ, Maffi P, Scavini M, Poli F, Nano R, et al. Alloantibody and Autoantibody Monitoring Predicts Islet Transplantation Outcome in Human Type 1 Diabetes. *Diabetes* (2013) 62:1656–64. doi:10.2337/db12-1258
11. Mu-u-min RBA, Diane A, Allouch A, Al-Siddiqi HH. Immune Evasion in Stem Cell-Based Diabetes Therapy—Current Strategies and Their Application in Clinical Trials. *Biomedicine* (2025) 13:383. doi:10.3390/biomedicine13020383
12. Deuse T, Schrepfer S. Progress and Challenges in Developing Allogeneic Cell Therapies. *Cell Stem Cell* (2025) 32:513–28. doi:10.1016/j.stem.2025.03.004
13. Gilson CR, Milas Z, Gangappa S, Hollenbaugh D, Pearson TC, Ford ML, et al. Anti-CD40 Monoclonal Antibody Synergizes with CTLA4-Ig in Promoting Long-Term Graft Survival in Murine Models of Transplantation. *J Immunol* (2009) 183:1625–35. doi:10.4049/jimmunol.0900339
14. Oura T, Yamashita K, Suzuki T, Fukumori D, Watanabe M, Hirokata G, et al. Long-Term Hepatic Allograft Acceptance Based on CD40 Blockade by ASKP1240 in Nonhuman Primates. *Am J Transpl* (2012) 12:1740–54. doi:10.1111/j.1600-6143.2012.04014.x
15. Kwun J, Kirk AD, Knechtle SJ. The Emerging Era of Organ Transplantation and anti-CD154mAb. *Am J Transpl* (2024) 24:1339–41. doi:10.1016/j.ajt.2024.04.008
16. Lowe M, Badell I, Thompson P, Martin B, Leopardi F, Strobert E, et al. A Novel Monoclonal Antibody to CD40 Prolongs Islet Allograft Survival. *Am J Transpl* (2012) 12:2079–87. doi:10.1111/j.1600-6143.2012.04054.x
17. Kenmochi T, Maruyama M, Saigo K, Akutsu N, Iwashita C, Otsuki K, et al. Successful Islet Transplantation From the Pancreata of Non-Heart-Beating Donors. *Transplant Proc* (2008) 40:2568–70. doi:10.1016/j.transproceed.2008.08.068
18. Caldara R, Tomajer V, Monti P, Sordi V, Citro A, Chimienti R, et al. Allo Beta Cell Transplantation: Specific Features, Unanswered Questions, and Immunological Challenge. *Front Immunol* (2023) 14:1323439. doi:10.3389/fimmu.2023.1323439
19. Sevnis S, Emiroglu R, Karakayali F, Yagmurdu MC, Dalgic A, Moray G, et al. OKT3 Treatment for Steroid-Resistant Acute Rejection in Kidney Transplantation. *Transplant Proc* (2005) 37:3016–8. doi:10.1016/j.transproceed.2005.07.052
20. van Vugt LK, van der Zwan M, Clahsen-van Groningen MC, van Agteren M, Hulleig-Peelen DM, De Winter BCM, et al. A Decade of Experience With Alemtuzumab Therapy for Severe or Glucocorticoid-Resistant Kidney Transplant Rejection. *Transpl Int* (2023) 36:11834. doi:10.3389/ti.2023.11834
21. Chimienti R, Baccega T, Torchio S, Manenti F, Pellegrini S, Cospito A, et al. Engineering of Immune Checkpoints B7-H3 and CD155 Enhances Immune Compatibility of MHC-I/- Ipscs for β Cell Replacement. *Cell Rep* (2022) 40:111423. doi:10.1016/j.celrep.2022.111423
22. Koenig A, Chen C-C, Marçais A, Barba T, Mathias V, Sicard A, et al. Missing Self Triggers NK Cell-Mediated Chronic Vascular Rejection of Solid Organ Transplants. *Nat Commun* (2019) 10:5350. doi:10.1038/s41467-019-13113-5
23. Callemeyn J, Senev A, Coemans M, Lerut E, Sprangers B, Kuypers D, et al. Missing Self-Induced Microvascular Rejection of Kidney Allografts: A Population-Based Study. *J Am Soc Nephrol* (2021) 32:2070–82. doi:10.1681/ASN.2020111558
24. Zhang Z, Wu N, Lu Y, Davidson D, Colonna M, Veillette A. DNAM-1 Controls NK Cell Activation via an ITT-like Motif. *J Exp Med* (2015) 212:2165–82. doi:10.1084/jem.20150792
25. Ziegler AE, Fittje P, Müller LM, Ahrenstorf AE, Hagemann K, Hagen SH, et al. The co-inhibitory Receptor TIGIT Regulates NK Cell Function and Is Upregulated in Human Intrahepatic CD56bright NK Cells. *Front Immunol* (2023) 14:1117320. doi:10.3389/fimmu.2023.1117320
26. Chapoval AI, Ni J, Lau JS, Wilcox RA, Flies DB, Liu D, et al. B7-H3: A Costimulatory Molecule for T Cell Activation and IFN-Gamma Production. *Nat Immunol* (2001) 2:269–74. doi:10.1038/85339
27. Zhao B, Li H, Xia Y, Wang Y, Wang Y, Shi Y, et al. Immune Checkpoint of B7-H3 in Cancer: From Immunology to Clinical Immunotherapy. *J Hematol Oncol* (2022) 15:153. doi:10.1186/s13045-022-01364-7
28. Paolini R, Molfetta R. CD155 and its Receptors as Targets for Cancer Therapy. *Int J Mol Sci* (2023) 24:12958. doi:10.3390/ijms241612958
29. Calmeiro J, Carrascal M, Gomes C, Falcão A, Cruz MT, Neves BM. Highlighting the Role of DC-NK Cell Interplay in Immunobiology and Immunotherapy. *Dendritic Cells* (2018). doi:10.5772/intechopen.78804
30. Liu L, You X, Han S, Sun Y, Zhang J, Zhang Y. CD155/TIGIT, a Novel Immune Checkpoint in Human Cancers (Review). *Oncol Rep* (2021) 45:835–45. doi:10.3892/or.2021.7943
31. Durlanik S, Fundel-Clemens K, Viollet C, Huber HJ, Lenter M, Kitt K, et al. CD276 Is an Important Player in Macrophage Recruitment into the Tumor and an Upstream Regulator for PAI-1. *Sci Rep Nat Publishing Group* (2021) 11:14849. doi:10.1038/s41598-021-94360-9
32. Cheng M, Chen S, Li K, Wang G, Xiong G, Ling R, et al. CD276-dependent Efferocytosis by Tumor-Associated Macrophages Promotes Immune Evasion in Bladder Cancer. *Nat Commun* (2024) 15:2818. doi:10.1038/s41467-024-46735-5
33. Sato K, Yamashita-Kanemaru Y, Abe F, Murata R, Nakamura-Shinya Y, Kanemaru K, et al. DNAM-1 Regulates Foxp3 Expression in Regulatory T Cells by Interfering With TIGIT Under Inflammatory Conditions. *Proc Natl Acad Sci* (2021) 118:e2021309118. doi:10.1073/pnas.2021309118
34. Huang M, Yu X, Wang Q, Jiang Z, Li X, Chen W, et al. The Immune Checkpoint TIGIT/CD155 Promotes the Exhaustion of CD8 + T Cells in TNBC Through Glucose Metabolic Reprogramming Mediated by PI3K/AKT/mTOR Signaling. *Cell Commun Signal* (2024) 22:35. doi:10.1186/s12964-023-01455-z
35. Zhang G, Hou J, Shi J, Yu G, Lu B, Zhang X. Soluble CD276 (B7-H3) Is Released From Monocytes, Dendritic Cells, and Activated T Cells and Is Detectable in Normal Human Serum. *Immunology* (2008) 123:538–46. doi:10.1111/j.1365-2567.2007.02723.x
36. Obeidat A, Atieh A, Vitenshtein A, Cinamon G, Paz K, Roviš T, et al. 474 First-in-Class Anti-PVR Mab NTX1088 Restores Expression of DNAM1 and Augments Antitumor Immunity. *J Immunother Cancer* (2022) A494. doi:10.1136/jitc-2022-SITC2022.0474
37. Zhang J, Zhou Z, Chen K, Kim S, Cho IS, Varadkar T, et al. A CD276-Targeted Antibody-Drug Conjugate to Treat Non-small Lung Cancer (NSCLC). *Cells* (2023) 12:2393. doi:10.3390/cells12192393
38. Shenderov E, De Marzo AM, Lotan TL, Wang H, Chan S, Lim SJ, et al. Neoadjuvant Enoblituzumab in Localized Prostate Cancer: A Single-Arm, Phase 2 Trial. *Nat Med Nat Publishing Group* (2023) 29:888–97. doi:10.1038/s41591-023-02284-w
39. Jung S, Schlenk RF, Hackenbruch C, Roldan Pinzon SSL, Bitzer M, Pflügler M, et al. Protocol of a First-In-Human Clinical Trial to Evaluate the Safety, Tolerability, and Preliminary Efficacy of the Bispecific CD276xCD3 Antibody CC-3 in Patients with Colorectal Cancer (CoRe_CC-3). *Front Oncol* (2024) 14:1351901. doi:10.3389/fonc.2024.1351901
40. Fu S, Wang H, Chen X, Wang J, Ding M, Guo C, et al. Preclinical Evaluation of the Efficacy of Anti-PVR Antibody-Drug Conjugates Combined With Anti-PD-1 Therapy for Bladder Cancer. *JCO* (2025) 43:e16547. doi:10.1200/JCO.2025.43.16_suppl.e16547
41. Barsby T, Ibrahim H, Lithovius V, Montaser H, Balboa D, Vähäkangas E, et al. Differentiating Functional Human Islet-Like Aggregates From Pluripotent Stem Cells. *STAR Protoc* (2022) 3:101711. doi:10.1016/j.xpro.2022.101711
42. Brown ME, Peters LD, Hanbali SR, Arnoletti JM, Sachs LK, Nguyen KQ, et al. Human CD4+CD25+CD226- Tregs Demonstrate Increased Purity, Lineage Stability, and Suppressive Capacity Versus CD4+CD25+CD127lo/- Tregs for Adoptive Cell Therapy. *Front Immunol* (2022) 13:873560. doi:10.3389/fimmu.2022.873560
43. Thirawatananond P, Brown ME, Sachs LK, Arnoletti JM, Yeh W-I, Posgai AL, et al. Treg-Specific CD226 Deletion Reduces Diabetes Incidence in NOD Mice by Improving Regulatory T-Cell Stability. *Diabetes* (2023) 72:1629–40. doi:10.2337/db23-0307
44. Permanyer M, Bošnjak B, Glage S, Friedrichsen M, Floess S, Huehn J, et al. Efficient IL-2R Signaling Differentially Affects the Stability, Function, and Composition of the Regulatory T-cell Pool. *Cell Mol Immunol* (2021) 18:398–414. doi:10.1038/s41423-020-00599-z

45. Brown ME, Thirawatananond P, Peters LD, Kern EJ, Vijay S, Sachs LK, et al. Inhibition of CD226 Co-Stimulation Suppresses Diabetes Development in the NOD Mouse by Augmenting Regulatory T Cells and Diminishing Effector T Cell Function. *Diabetologia* (2025) 68:397–418. doi:10.1007/s00125-024-06329-8
46. Bogomiakova ME, Sekretova EK, Anufrieva KS, Khabarova PO, Kazakova AN, Bobrovsky PA, et al. iPSC-Derived Cells Lack Immune Tolerance to Autologous NK-cells due to Imbalance in Ligands for Activating and Inhibitory NK-cell Receptors. *Stem Cell Res and Ther* (2023) 14:77. doi:10.1186/s13287-023-03308-5
47. Molfetta R, Zitti B, Lecce M, Milito ND, Stabile H, Fionda C, et al. CD155: A Multi-Functional Molecule in Tumor Progression. *Int J Mol Sci* (2020) 21:922. doi:10.3390/ijms21030922
48. Getu AA, Tigabu A, Zhou M, Lu J, Fodstad Ø, Tan M. New Frontiers in Immune Checkpoint B7-H3 (CD276) Research and Drug Development. *Mol Cancer* (2023) 22:43. doi:10.1186/s12943-023-01751-9
49. Li G, Quan Y, Che F, Wang L. B7-H3 in Tumors: Friend or Foe for Tumor Immunity? *Cancer Chemother Pharmacol* (2018) 81:245–53. doi:10.1007/s00280-017-3508-1
50. Hashiguchi M, Kobori H, Ritprajak P, Kamimura Y, Kozono H, Azuma M. Triggering Receptor Expressed on Myeloid Cell-like Transcript 2 (TLT-2) Is a Counter-Receptor for B7-H3 and Enhances T Cell Responses. *Proc Natl Acad Sci U S A* (2008) 105:10495–500. doi:10.1073/pnas.0802423105
51. Lee Y, Martin-Orozco N, Zheng P, Li J, Zhang P, Tan H, et al. Inhibition of the B7-H3 Immune Checkpoint Limits Tumor Growth by Enhancing Cytotoxic Lymphocyte Function. *Cell Res. Nat Publishing Group* (2017) 27:1034–45. doi:10.1038/cr.2017.90
52. Schwane V, Huynh-Tran VH, Vollmers S, Yakup VM, Sauter J, Schmidt AH, et al. Distinct Signatures in the Receptor Repertoire Discriminate CD56bright and CD56dim Natural Killer Cells. *Front Immunol* (2020) 11:568927. doi:10.3389/fimmu.2020.568927
53. Li J, Cao C, Xiang Y, Hong Z, He D, Zhong H, et al. TLT2 Suppresses Th1 Response by Promoting IL-6 Production in Monocyte Through JAK/STAT3 Signal Pathway in Tuberculosis. *Front Immunol* (2020) 11:2031. doi:10.3389/fimmu.2020.02031
54. Wang L, Fraser CC, Kikly K, Wells AD, Han R, Coyle AJ, et al. B7-H3 Promotes Acute and Chronic Allograft Rejection. *Eur J Immunol* (2005) 35:428–38. doi:10.1002/eji.200425518
55. Ueno T, Yeung MY, McGrath M, Yang S, Zaman N, Snawder B, et al. Intact B7-H3 Signaling Promotes Allograft Prolongation Through Preferential Suppression of Th1 Effector Responses. *Eur J Immunol* (2012) 42:2343–53. doi:10.1002/eji.201242501
56. Stanietzky N, Mandelboim O. Paired NK Cell Receptors Controlling NK Cytotoxicity. *FEBS Lett* (2010) 584:4895–900. doi:10.1016/j.febslet.2010.08.047
57. Escalante NK, von Rossum A, Lee M, Choy JC. CD155 on Human Vascular Endothelial Cells Attenuates the Acquisition of Effector Functions in CD8 T Cells. *Arterioscler Thromb Vasc Biol* (2011) 31:1177–84. doi:10.1161/ATVBAHA.111.224162
58. Li W, Deng C, Yang H, Tian X, Chen L, Liu Q, et al. Upregulation of the CD155-CD226 Axis Is Associated With Muscle Inflammation and Disease Severity in Idiopathic Inflammatory Myopathies. *Neurol Neuroimmunol Neuroinflamm* (2023) 10:e200143. doi:10.1212/NXI.0000000000200143
59. Reymond N, Imbert A-M, Devillard E, Fabre S, Chabannon C, Xerri L, et al. DNAM-1 and PVR Regulate Monocyte Migration Through Endothelial Junctions. *J Exp Med* (2004) 199:1331–41. doi:10.1084/jem.20032206
60. Kraus AK, Chen J, Edenhofer I, Ravens I, Gaspert A, Cippà PE, et al. The Role of T Cell Costimulation via DNAM-1 in Kidney Transplantation. *PLOS ONE Public Libr Sci* (2016) 11:e0147951. doi:10.1371/journal.pone.0147951
61. Arpaia N, Green JA, Moltedo B, Arvey A, Hemmers S, Yuan S, et al. A Distinct Function of Regulatory T Cells in Tissue Protection. *Cell* (2015) 162:1078–89. doi:10.1016/j.cell.2015.08.021
62. Wood KJ, Bushell A, Hester J. Regulatory Immune Cells in Transplantation. *Nat Rev Immunol* (2012) 12:417–30. doi:10.1038/nri3227
63. McCall M, Shapiro AMJ. Update on Islet Transplantation. *Cold Spring Harb Perspect Med* (2012) 2:a007823. doi:10.1101/cshperspect.a007823
64. Ramzy A, Thompson DM, Ward-Hartstonge KA, Ivison S, Cook L, Garcia RV, et al. Implanted Pluripotent Stem-Cell-Derived Pancreatic Endoderm Cells Secrete Glucose-Responsive C-peptide in Patients With Type 1 Diabetes. *Cell Stem Cell* (2021) 28:2047–61.e5. doi:10.1016/j.stem.2021.10.003
65. Nakamura Y, Miyagawa S, Yoshida S, Sasawatari S, Toyofuku T, Toda K, et al. Natural Killer Cells Impede the Engraftment of Cardiomyocytes Derived from Induced Pluripotent Stem Cells in Syngeneic Mouse Model. *Sci Rep Nat Publishing Group* (2019) 9:10840. doi:10.1038/s41598-019-47134-3
66. Gantier M, Ménoret S, Fourrier A, Delbos F, Nguyen TH, Anegón I. Human Pluripotent Stem Cell-Derived Hepatic Progenitors Exhibit a Partially Hypoimmunogenic Phenotype and Actively Inhibit Immune Responses. *Front Immunol* (2025) 16:1507317. doi:10.3389/fimmu.2025.1507317
67. Mehler VJ, Burns CJ, Stauss H, Francis RJ, Moore ML. Human iPSC-Derived Neural Crest Stem Cells Exhibit Low Immunogenicity. *Mol Ther - Methods and Clin Development* (2020) 16:161–71. doi:10.1016/j.omtm.2019.12.015
68. Brehm MA, Kenney LL, Wiles MV, Low BE, Tisch RM, Burzenski L, et al. Lack of Acute Xenogeneic Graft-Versus-Host Disease, but Retention of T-cell Function Following Engraftment of Human Peripheral Blood Mononuclear Cells in NSG Mice Deficient in MHC Class I and II Expression. *FASEB J* (2019) 33:3137–51. doi:10.1096/fj.201800636R

Copyright © 2025 Siracusano, Deambrogio, Sordi, Malnati, Piemonti and Chimienti. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.



Efficacy of Intravenous Immunoglobulin in Eliminating De Novo Donor-Specific Antibodies After Lung Transplantation: Importance of Early Intervention

Maximilian Vorstandlechner^{1*†}, Philip Degenfelder^{1†}, Gökce Yavuz¹, Olaf M. Glueck¹, Julia R. Kovács¹, Julia Walter^{1,2}, Andrea Dick³, Sebastian Michel^{4,5,6}, Christian P. Schneider^{1,5,6}, Michael Zoller⁷, Jürgen Barton^{2,6} and Teresa Kauke^{1,5,6}

¹Division of Thoracic Surgery, LMU University Hospital, Ludwig-Maximilians Universität (LMU) München, Munich, Germany, ²Department of Medicine V, LMU University Hospital, Ludwig-Maximilians Universität (LMU) München, Munich, Germany, ³Laboratory for Immunogenetics, Division of Transfusion Medicine, Cell Therapeutics and Haemostaseology, LMU University Hospital, Ludwig-Maximilians Universität (LMU) München, Munich, Germany, ⁴Department of Cardiac Surgery, LMU University Hospital, Ludwig-Maximilians Universität (LMU) München, Munich, Germany, ⁵Comprehensive Pneumology Center Munich (CPC-M), German Center for Lung Research (DZL), Munich, Germany, ⁶Transplantation Center, LMU University Hospital, Ludwig-Maximilians Universität (LMU) München, Munich, Germany, ⁷Department of Anesthesiology, LMU University Hospital, Ludwig-Maximilians Universität (LMU) München, Munich, Germany

OPEN ACCESS

*Correspondence

Maximilian Vorstandlechner,
✉ max.vorstandlechner@med.uni-muenchen.de

[†]These authors have contributed equally to this work and share first authorship

Received: 30 July 2025

Accepted: 20 October 2025

Published: 03 November 2025

Citation:

Vorstandlechner M, Degenfelder P, Yavuz G, Glueck OM, Kovács JR, Walter J, Dick A, Michel S, Schneider CP, Zoller M, Barton J and Kauke T (2025) Efficacy of Intravenous Immunoglobulin in Eliminating De Novo Donor-Specific Antibodies After Lung Transplantation: Importance of Early Intervention. *Transpl. Int.* 38:15350. doi: 10.3389/ti.2025.15350

The development of de novo donor-specific anti-HLA antibodies (dnDSA) after lung transplantation (LuTX) has been increasingly linked to the onset of antibody-mediated rejection (AMR), chronic lung allograft dysfunction (CLAD), and impaired long-term outcomes. However, the therapeutic impact of intravenous immunoglobulin (IVIg) therapy in patients with dnDSA remains unclear. We conducted a retrospective single-center study of LuTX recipients (2015–2019) who developed dnDSA post-transplantation and received IVIg-based therapy. Patients were classified as responders or non-responders based on post-treatment antibody clearance. Clinical, immunological and functional outcomes were compared. Among 47 patients with dnDSA and IVIg-based therapy, 23 (48.9%) achieved complete antibody elimination. Preemptive treatment, defined as initiation of IVIg therapy before onset of clinical symptoms, was found to be an independent predictor of antibody clearance (odds ratio 29.5; $p = 0.013$). Responders showed significantly lower baseline MFI. While differences in CLAD-free survival favored responders, they did not reach statistical significance. Preemptive IVIg therapy in

Abbreviations: ACR, Acute Cellular Rejection; AMR, Antibody-mediated Rejection; BOS, Bronchiolitis Obliterans Syndrome; CLAD, Chronic Lung Allograft Dysfunction; COPD, Chronic Obstructive Pulmonary Disease; dnDSA, De Novo Donor-Specific Antibody; DSA, Donor-Specific Antibody; ELD, End-Stage Lung Disease; FEV1, Forced Expiratory Volume in One Second; HLA, Human Leukocyte Antigen; ILD, Interstitial Lung Disease; IQR, Interquartile Range; ISHLT, International Society for Heart and Lung Transplantation; IVIg, Intravenous Immunoglobulin; LuTX, Lung Transplantation; MFI, Mean Fluorescence Intensity; MHC, Major Histocompatibility Complex; OR, Odds Ratio; SD, Standard Deviation; SSO, Sequence-Specific Oligonucleotide; TBB, Transbronchial Biopsy; tPE, Therapeutic Plasma Exchange.

asymptomatic dnDSA-positive LuTX recipients may enhance antibody clearance and reduce CLAD risk. These findings support early intervention strategies and underscore the need for prospective trials to define optimal therapeutic thresholds and timing.

Keywords: lung transplantation, dnDSA, AMR, ACR, CLAD

INTRODUCTION

Lung transplantation (LuTX) remains the only definitive therapeutic option for patients with end-stage lung diseases (ELD) who have exhausted all other treatment modalities [1–3]. While short-term outcomes have improved markedly over recent decades, largely due to advances and standardization in immunosuppressive regimens and perioperative care, long-term survival remains critically constrained [4–6]. One of the principal challenges in this field is the complex immunological interplay between donor and recipient, which manifests in various forms of allograft rejection and contributes significantly to graft failure and patient mortality [3, 7, 8]. Acute cellular rejection (ACR) is a well-recognized complication during the first year following transplantation. Although typically responsive to corticosteroid therapy and not often fatal in the acute phase, ACR has been linked to increased long-term risk of developing chronic lung allograft dysfunction (CLAD), the predominant cause of late graft failure [9–12].

Beyond cellular rejection, humoral responses have gained recognition as major contributors to graft injury. The development of donor-specific anti-HLA antibodies (DSA) post-transplant (*de novo* DSA) is increasingly implicated in the pathogenesis of CLAD and reduced allograft survival [13–15]. Notably, the presence of dnDSAs has been associated with increased risk for both acute

and chronic rejection, including the emergence of antibody-mediated rejection (AMR), a distinct and often insidious form of immune-mediated injury [16, 17]. The immunological basis for these processes lies in the human leukocyte antigen (HLA) system, a highly polymorphic set of genes that encodes the major histocompatibility complex (MHC) proteins. These molecules play an essential role in presenting antigens to T and B cells. Mismatches between donor and recipient HLA profiles are a potent trigger of alloimmune responses [18]. Formation of anti-HLA antibodies, whether preformed or *de novo*, can initiate a cascade of immune events involving complement activation, endothelial injury and eventual tissue destruction [19].

The diagnosis of pulmonary AMR remains difficult, in part due to its heterogeneous clinical presentation and the lack of definitive biomarkers. The consensus guidelines propose a multifactorial diagnostic framework involving the presence of DSAs, histological evidence of capillaritis, complement deposition (specifically C4d), evidence of graft dysfunction, and the exclusion of other etiologies [20]. Notably, subclinical AMR, defined by immunologic and histologic findings in the absence of functional impairment, is increasingly recognized as an early phase in the spectrum of humoral rejection [21]. Current therapeutic approaches for AMR are largely derived from treatment protocols in kidney and heart transplantation. These include intravenous immunoglobulin (IVIg), therapeutic plasma

Efficacy of Intravenous Immunoglobulin in Eliminating De Novo Donor-Specific Antibodies After

Lung Transplantation:

Importance of Early Intervention

- 2014–2019
- 47 patients with dnDSA
 - 23 Responders
 - 24 Non-responders

- IVIg-Treatment
 - 1g/kg initially, followed by 0.5 g/kg at four week intervals, three doses max.



- Antibody elimination was achieved in 23 patients (48.9%)
- IVIg-Therapy before onset of clinical antibody-mediated rejection is associated with successful dnDSA-clearance (OR:29.5; p=0.013)



Maximilian Vorstandlechner, Philip Degenfelder et al. *Transpl. Int.* 2025

doi: 10.3389/ti.2025.15350



GRAPHICAL ABSTRACT

exchange (tPE), B-cell depletion with rituximab, and more recently, proteasome inhibitors (carfilzomib) and complement-blocking agents (eculizumab) [22–24]. Despite these efforts, treatment outcomes remain inconsistent. Studies have demonstrated variable antibody clearance rates and a persistently high risk of CLAD and death in patients with clinically manifest AMR [25]. Emerging evidence suggests that preemptive intervention in patients with newly detected dnDSAs, before the onset of overt graft dysfunction, may hold promise [21, 26, 27]. These findings suggest that early immunologic intervention could interrupt the pathogenic cascade that leads to chronic dysfunction, marking a potential shift in clinical strategy from reactive to preventive care.

Despite this progress, many critical questions remain unanswered. There is no consensus on the optimal treatment regimen, and randomized controlled trials are lacking. Furthermore, predictive markers that could guide patient selection and treatment decisions remain elusive. The significant heterogeneity in clinical response underscores the need for further mechanistic studies and controlled trials to identify which patients are most likely to benefit from specific therapeutic interventions. Ultimately, improving long-term outcomes in lung transplantation will depend not only on controlling cellular rejection, but on understanding and modulating the humoral immune response with greater precision.

MATERIALS AND METHODS

This retrospective single-center study was conducted at the Division of Thoracic surgery, LMU University Hospital, Munich. It was approved by the institutional review board of the faculty of medicine, Ludwig-Maximilians University Munich (UE No. 22-0123) and conducted in accordance with the ethical principles of the declaration of Helsinki. Only adult patients who underwent LuTX between 2015 and 2019 were eligible for inclusion. For the purpose of this study, only individuals who developed dnDSA postoperatively were included in further analyses. Follow-up included routine clinical controls and HLA antibody screening at standardized intervals: 1, 3, 6, 12, 18, 24, 30, 36, and 48 months postoperatively. Immunosuppression followed a standard triple-drug regimen consisting of corticosteroids, tacrolimus and mycophenolate mofetil.

All included patients had undergone routine pre- and post-transplant immunological monitoring. Genotyping was performed using sequence-specific oligonucleotide probes (SSO; LabType, One Lambda, Canoga Park, CA, United States), targeting HLA-A, -B, -C, DRB1, -DRB3/4/5, and DQB1 loci. Antibody screening and identification were performed using Luminex-based bead assays (LABScreen™, One Lambda), enabling high-sensitivity detection of HLA class I and II IgG antibodies. Mean fluorescence intensity (MFI) values greater than 1,000 were considered positive.

Within the dnDSA-positive cohort, patients were retrospectively categorized based on their immunological response to IVIG therapy. The standard therapeutic approach

at our center for newly detected dnDSA is to initiate IVIG at 1 g/kg body weight, followed by three subsequent doses of 0.5 g/kg at 4-week intervals. The subset of patients who received IVIG-therapy, was divided into two groups: (1) those who achieved complete elimination of dnDSA following treatment (responders), and (2) those with persistent antibodies after therapy (non-responders). Complete antibody elimination was defined as the absence of previously detected donor-specific HLA antibodies in follow-up screenings, without subsequent recurrence.

Bronchoscopic surveillance was performed at regular intervals during the first 2 years post-transplant and subsequently based on clinical indication. Transbronchial biopsies (TBB) and histological diagnosis of ACR followed the International Society for Heart and Lung Transplantation (ISHLT) criteria, classifying rejection grades A0–A4 and lymphocytic bronchiolitis grades B0–B2R [28]. The 2016 ISHLT consensus report provided the basis for defining antibody-mediated rejection (AMR), distinguishing between clinical AMR, marked by detectable declines in lung function, which may occur without symptoms, and subclinical AMR, in which lung function remains preserved despite immunologic evidence of rejection [19, 20]. Lung function testing (forced expiratory volume in the first second, FEV1) was conducted in parallel with antibody assessments. To evaluate treatment efficacy, FEV1 measurements taken before therapy (baseline) were compared to post-treatment averages within 1 year after treatment initiation. CLAD was defined according to international consensus guidelines [29]. In this study, only bronchiolitis obliterans syndrome (BOS) was used as a clinical endpoint and diagnosed based on sustained FEV1 decline in the absence of alternative explanations [30]. Clinical, immunological, and procedural data were extracted from institutional records and the ET-database, including demographic information, transplant type, donor characteristics and intraoperative parameters (e.g., allograft ischemia time).

Statistical Analysis

Statistical analyses were conducted using R (version 4.0.5) and RStudio (version 1.4.1106). Comparisons between groups were performed using the chi-square test or Fisher's exact test for categorical variables and the Student's t-test or Wilcoxon–Mann–Whitney test for continuous variables, as appropriate. Time-dependent outcomes were analyzed using Kaplan–Meier curves with log-rank tests. Logistic regression models were applied to identify factors independently associated with antibody elimination. Statistical significance was defined as a two-tailed p-value ≤ 0.05 .

RESULTS

A total of 47 LuTX-recipients met the inclusion criteria and were enrolled in the analysis, among which 22 were female (46.8%) and 25 male (53.2%). The most common underlying disease was interstitial lung disease (ILD) (n = 21; 44.7%), followed by cystic

TABLE 1 | Non-Responders vs. Responders.

Non-responders and responders	Non-responders (n = 24)		Responders (n = 23)		(p)
	(mean)	(sd)	(mean)	(sd)	
Age (years)					
Recipient	51.2	12.1	46.8	15.2	0.28
Donor	44.7	16.9	48.8	15.6	0.4
Ischemia time (minutes)	522	125	510	128	0.75
	(n)	(%)	(n)	(%)	(p)
Sex					
Female	11	45.8%	11	47.8%	1.00
Male	13	44.2%	12	52.2%	
Underlying Diagnosis for LuTX					
ILD	11	45.8%	10	43.5%	1.00
COPD	4	16.7%	5	21.7%	
CF	6	25.0%	6	26.1%	
other	3	12.5%	2	8.7%	
One-year Survival	20	83.3%	21	91.3%	0.66
Lung Allograft Rejection					
ACR	8	33.3%	6	26.1%	0.75
Clinical AMR	16	66.7%	1	4.3%	<0.001
CLAD	7	29.2%	0	0.0%	0.009

ACR, acute cellular rejection; AMR, antibody-mediated rejection; CF, cystic fibrosis; CLAD, chronic lung allograft dysfunction; COPD, chronic obstructive pulmonary disease; LuTX, lung transplantation.

fibrosis (CF) (n = 12; 25.5%) and chronic obstructive pulmonary disease (COPD) (n = 9; 19.1%). The median follow-up time post-transplantation was 18 months (range: 3–48 months). Antibody elimination was achieved in 48.9% of all patients (n = 23; responder), while 51% (n = 24; non-responder) exhibited persistent dnDSA throughout follow-up.

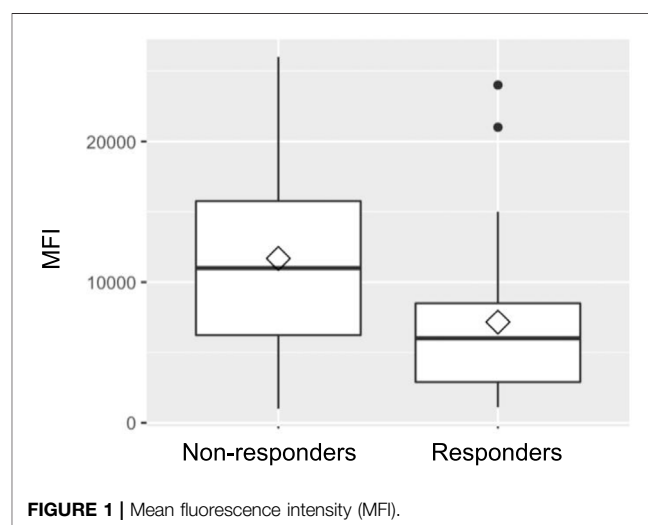
Responders vs. Non-Responders

As depicted in **Table 1**, comparison of demographic characteristics between responders and non-responders revealed no statistically significant differences in recipient or donor age, sex distribution, transplant indication or surgical approach. Both groups had similar proportions of bilateral versus single lung transplants and comparable ischemia times.

When analyzing HLA class distribution of dnDSA, it became apparent that non-responders were more likely to develop HLA class II antibodies (95.8%; n = 23 vs. 78.3%; n = 18 in the responder group); however, this difference did not reach statistical significance (p = 0.097). As shown in **Figure 1**, MFI prior to therapy was significantly higher in the non-responder group compared to responders (mean 11,683 ± 7,055 vs. 7,152 ± 5,912; p = 0.019). Notably, the time to development of dnDSA was also significantly longer in non-responders than in responders (median: 94 days (IQR 35–343) vs. 39 days (IQR 22–65); p = 0.0025).

Lung Function

Baseline lung function, assessed by FEV₁ prior to treatment, was retrospectively found to be significantly better in future responders (75.0% of predicted) than in non-responders (54.8%; p = 0.019). Lung function testing at 1 year following IVIG-therapy initiation, showed consistently higher FEV₁ values (78.8% vs. 63.3%) in responders, although the difference between the groups was no

**FIGURE 1 |** Mean fluorescence intensity (MFI).

longer statistically significant (p = 0.07). **Figures 2, 3** illustrate FEV₁ prior to and 1 year after IVIG-therapy.

Acute Cellular Rejection (ACR)

ACR was identified in a total of 14 patients, corresponding to 29.8% of the entire study cohort. Among these cases, the majority were categorized as grade A1 rejection, which is characterized histologically by minimal perivascular mononuclear infiltrates without evidence of tissue injury. These milder rejection episodes were evenly distributed across both study groups (6/23 in the responder group vs. 6/24 in the non-responder group). Notably, a single episode of grade A2 rejection and one episode of grade A3 rejection were each observed and both occurred within the non-responder group. When comparing the incidence of ACR between treatment groups, no statistically significant difference

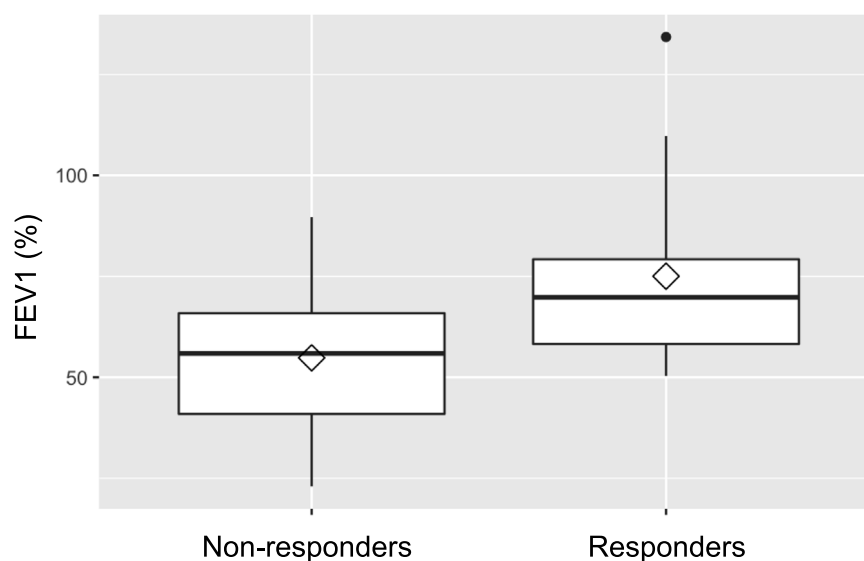


FIGURE 2 | FEV1 pre IVIG-therapy.

was observed: 6 out of 23 responders (26.1%) experienced ACR, compared to 8 out of 24 non-responders (33.3%) ($p = 0.75$). These findings suggest that the rate of ACR was comparable between groups, regardless of treatment response.

Antibody-Mediated Rejection (AMR)

The prevalence of AMR at the time of IVIG initiation differed between responders and non-responders (**Figure 4**). For clinical AMR, only 1 of 23 responders (4.3%) met the criteria for possible clinical AMR, compared to 16 of 24 non-responders (66.7%). In contrast, subclinical AMR was more frequent among responders: 22 responders were classified with subclinical AMR at baseline, whereas only 8 non-responders fell into this category. Clinical AMR was therefore predominantly observed in non-responders, while subclinical AMR was more commonly seen in responders. Multivariable logistic regression analysis identified preemptive treatment, defined as initiation of therapy during subclinical AMR prior to the onset of clinical symptoms, as a significant independent predictor of dnDSA clearance (odds ratio 29.5 (OR); $p = 0.013$). Other variables assessed, including age, sex, MFI levels, antibody class, and timing of dnDSA detection were not significantly associated with antibody elimination.

Chronic Lung Allograft Dysfunction (CLAD)

CLAD, defined according to standard criteria for BOS, was observed exclusively among patients in the non-responder group. A total of 7 non-responders (29.2%) were diagnosed with BOS during the observation period, whereas no responder developed this form of chronic allograft dysfunction. This difference was statistically significant ($p = 0.009$). Importantly, all cases of BOS were diagnosed prior to the initiation of IVIG therapy.

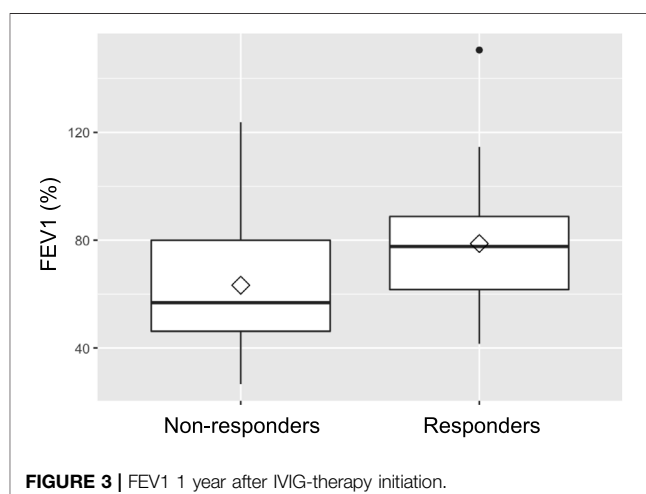


FIGURE 3 | FEV1 1 year after IVIG-therapy initiation.

Survival

One-year post-transplant survival in the overall cohort was 87.2%. Of the ten patients who died, eight were from the non-responder group and two from the responder group. Both deaths among responders were attributed to causes not directly related to the transplant (intracranial hemorrhage and cardiac failure). Although 1-year survival was numerically higher in responders compared to non-responders (91.3% vs. 83.3%), the difference was not statistically significant ($p = 0.66$). A similar trend was observed in 1-year survival following dnDSA detection: 91.3% in responders versus 71.4% in non-responders ($p = 0.13$). Survival 1 year after initiation of IVIG therapy was also higher in responders (90.9%) compared to non-responders (66.7%), although this difference did not reach statistical significance ($p = 0.11$). Kaplan–Meier analysis demonstrated non-

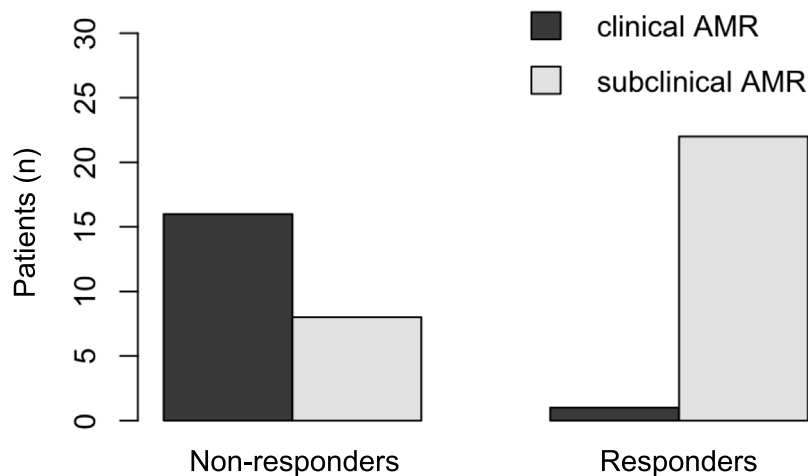


FIGURE 4 | Antibody-mediated rejection (AMR) pre IVIG-therapy.

significant differences in survival between groups following dnDSA detection (log-rank $p = 0.11$) and IVIG initiation (log-rank $p = 0.06$). Overall survival probability showed no differences between the two groups (log-rank $p = 0.2$).

DISCUSSION

The development of dnDSA following LuTX and their association with the onset of CLAD, impaired graft function and reduced survival have been well documented in numerous studies over recent years [13–16, 19, 31]. To date, the management of AMR remains heterogeneous, with no standardized protocols or universally accepted treatment strategies in place [21, 26, 32].

Our findings are consistent with growing evidence in literature that early immunological intervention can mitigate the development of chronic allograft dysfunction and extend graft survival [20, 33, 34]. While IVIG is well established in the treatment of AMR, its role in asymptomatic dnDSA or subclinical AMR is less clearly defined. We demonstrated that early intervention, particularly in asymptomatic patients with subclinical AMR, may significantly improve dnDSA clearance rates and associated clinical outcomes. Preemptive therapy, defined as the initiation of treatment before the onset of clinical symptoms, proved to be an independent predictor of successful dnDSA elimination in multivariable analysis (OR 29.5; $p = 0.013$). This observation aligns with data from Ius et al. (2018), who reported a 92% clearance rate following preemptive IVIG therapy in asymptomatic recipients, with graft survival comparable to DSA-negative controls [21]. Hachem et al. (2010) similarly found that preemptive therapy with IVIG solely or IVIG in combination with Rituximab resulted in DSA clearance in 65% of patients. Furthermore, patients witnessing successful antibody-depletion were less likely to develop BOS throughout the follow-up period [32]. Recent evidence presented by McDermott et al. provides important

insight [27]. In a cohort of asymptomatic LuTX-recipients with dnDSA, preemptive IVIG monotherapy resulted in substantial reduction in antibody strength, with complete clearance in more than half of the patients. Moreover, they observe a trend toward lower rates of subsequent ACR among patients achieving DSA clearance.

The timing of dnDSA detection also appeared to influence treatment success. In our cohort, responders exhibited significantly earlier detection and received treatment sooner (median 15.0 vs. 36.5 days), suggesting that patients with early-onset dnDSA may benefit more from antibody-directed intervention. These observations are supported by Ensor et al. (2017) and Vacha et al. (2017), who reported that earlier therapy initiation was associated with improved antibody clearance and better clinical outcomes. Delayed treatment may permit maturation of antibodies and acquisition of complement-binding properties, which are known to reduce responsiveness to desensitization [24, 25].

While MFI values were not independently predictive of treatment success, we observed significantly higher pre-treatment MFI levels in non-responders, with the median nearly double that of responders. This observation is consistent with findings by [21, 24], who reported that lower baseline MFI values were associated with a higher likelihood of antibody clearance [21, 24]. In contrast, Timofeeva et al. (2021) did not find a significant correlation between pre-treatment MFI levels and overall survival; however, they observed that lower post-treatment MFI values following therapeutic plasma exchange (tPE) were significantly associated with improved survival [35].

Clinically, lung function and survival outcomes tended to be more favorable in the responder group. However, these differences were not statistically significant, which may be attributable to small sample size and ceiling effects, as many responders already had preserved baseline FEV₁ values. Both groups showed only modest relative improvements in lung

function and therapy in responders was more likely aimed at preventing decline rather than reversing damage. Similar associations between DSA elimination and improved BOS-free survival have been reported in other studies [24, 32, 35].

This study has several limitations that should be considered when interpreting the findings. First, the retrospective and single-center design inherently limits the generalizability of the results and may introduce selection or information bias. Although the study was conducted at a high-volume transplant center over a 5-year period, the overall sample size remained relatively small, thereby limiting statistical power and increasing the risk of type II error, particularly in subgroup analyses and survival comparisons. Furthermore, the classification of treatment response was based solely on dnDSA elimination, without histopathologic correlates such as C1q-binding capacity or tissue deposition. Due to institutional standards, uniform C4d staining was not performed, which may limit the ability to fully characterize the histopathologic phenotype of AMR and subclinical graft injury. However, given the limited sensitivity and specificity of C4d staining in lung allografts and the variability in its expression across AMR phenotypes, as highlighted in the ISHLT consensus report and subsequent studies, we consider its absence to have limited clinical impact in the context of this study [19]. While our study focused on IVIg monotherapy, the heterogeneity of dnDSA profiles and immune status across patients suggests that individualized or combination therapies may be required, an area not addressed in this analysis.

All cases of BOS in our cohort occurred prior to IVIg initiation and exclusively among non-responders, which may reflect inherent biological or immunologic differences between patients who did and did not achieve dnDSA clearance. The question of whether this observation strengthens or weakens the association between IVIg therapy and DSA clearance cannot be answered in the retrospective analysis and requires prospective data. This temporal pattern limits the ability to directly attribute the absence of BOS in responders to the effect of IVIg therapy.

Given the retrospective nature of the study, it did not include a control group of dnDSA-positive patients who did not receive IVIg therapy. This design inherently limits the ability to fully distinguish treatment effects from the natural course of dnDSA evolution. Future prospective studies with appropriate control cohorts will be important to confirm the therapeutic impact of IVIg. Lastly, the observational nature of the study precludes causal inference, and although multivariable analysis was performed, unmeasured confounders may still influence the associations observed.

Taken together, our findings highlight the importance of early, preemptive intervention in lung transplant recipients with dnDSA, particularly those who are asymptomatic and have moderate antibody burden. While high MFI and class II specificity may suggest reduced clearance probability, they should not preclude therapeutic attempts when clinical stability allows. DSA elimination remains a critical therapeutic goal, not only to limit immunologic injury but also to preserve long-term graft function and survival. Our findings underscore the need for early detection and individualized intervention in dnDSA-positive patients. Prospective multicenter trials are

essential to validate risk-adapted treatment algorithms and define standardized thresholds for therapeutic initiation.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving humans were approved by the Institutional Review Board of the Faculty of Medicine, Ludwig-Maximilians University Munich. The studies were conducted in accordance with the local legislation and institutional requirements. The ethics committee/institutional review board waived the requirement of written informed consent for participation from the participants or the participants' legal guardians/next of kin because observational and non-interventional retrospective study.

AUTHOR CONTRIBUTIONS

Concept and design: MV, PD, TK, AD, and JW; Data collection: PD and TK; Data interpretation: MV, PD, GY, OG, JK, JW, AD, SM, CS, MZ, JB, and TK (all authors); Statistics: PD, JW; Writing and editing: MV, PC, GY, OG, JK, JW, AD, SM, CS, MZ, JB, and TK (all authors); Critical revision: MV, PC, GY, OG, JK, JW, AD, SM, CS, MZ, JB, and TK (all authors). All authors contributed to the article and approved the submitted version.

FUNDING

The author(s) declare that no financial support was received for the research and/or publication of this article.

CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

GENERATIVE AI STATEMENT

The author(s) declare that no Generative AI was used in the creation of this manuscript.

Any alternative text (alt text) provided alongside figures in this article has been generated by Frontiers with the support of artificial intelligence and reasonable efforts have been made to ensure accuracy, including review by the authors wherever possible. If you identify any issues, please contact us.

ACKNOWLEDGMENTS

The content of this manuscript is based on research data that was previously presented in the context of a doctoral thesis submitted to the Ludwig-Maximilians-Universität München. The thesis, entitled “Intravenöse Immunglobulin-Therapie bei Antikörper-vermittelter Abstoßungsreaktion nach Lungentransplantation Analyse prädiktiver Faktoren für die Elimination *de novo* donorspezifischer Antikörper” [36], includes the full dataset analyzed in this study. The thesis is publicly accessible through the university’s library repository and has not been published in any peer-reviewed journal. A full reference to the

thesis is included in the reference list of this manuscript. The use of generative AI in this manuscript was limited to language refinement and editing. No AI tools were used for data analysis, interpretation, or content generation. All use adheres to applicable ethical guidelines.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontierspartnerships.org/articles/10.3389/ti.2025.15350/full#supplementary-material>

REFERENCES

- Chambers DC, Cherikh WS, Goldfarb SB, Hayes D, Jr, Kucheryavaya AY, Toll AE, et al. The International Thoracic Organ Transplant Registry of the International Society for Heart and Lung Transplantation: Thirty-Fifth Adult Lung and Heart-Lung Transplant Report-2018; Focus Theme: Multiorgan Transplantation. *J Heart Lung Transpl* (2018) 37(10):1169–83. doi:10.1016/j.healun.2018.07.020
- Weill D, Benden C, Corris PA, Dark JH, Davis RD, Keshavjee S, et al. A Consensus Document for the Selection of Lung Transplant Candidates: 2014--An Update From the Pulmonary Transplantation Council of the International Society for Heart and Lung Transplantation. *J Heart Lung Transpl* (2015) 34(1):1–15. doi:10.1016/j.healun.2014.06.014
- Leard LE, Holm AM, Valapour M, Glanville AR, Attawar S, Aversa M, et al. Consensus Document for the Selection of Lung Transplant Candidates: An Update From the International Society for Heart and Lung Transplantation. *J Heart Lung Transpl* (2021) 40(11):1349–79. doi:10.1016/j.healun.2021.07.005
- Valapour M, Lehr CJ, Skeans MA, Smith JM, Miller E, Goff R, et al. OPTN/SRTR 2019 Annual Data Report: Lung. *Am J Transpl* (2021) 21(Suppl. 2):441–520. doi:10.1111/ajt.16495
- Dipchand AI, Kirk R, Edwards LB, Kucheryavaya AY, Benden C, Christie JD, et al. The Registry of the International Society for Heart and Lung Transplantation: Sixteenth Official Pediatric Heart Transplantation Report--2013; Focus Theme: Age. *J Heart Lung Transpl* (2013) 32(10):979–88. doi:10.1016/j.healun.2013.08.005
- Nelson J, Kincaide E, Schulte J, Hall R, Levine DJ. Immunosuppression in Lung Transplantation. *Handb Exp Pharmacol* (2022) 272:139–64. doi:10.1007/164_2021_548
- Niehaus H, Haverich A, Ius F. Current Status of Transplantation Medicine in the Field of Heart and Lung Transplantation: Legal Regulations and Clinical Implementation. *Anaesthesiologie* (2022) 71(9):727–38. doi:10.1007/s00101-022-01179-8
- Sato M. Chronic Lung Allograft Dysfunction After Lung Transplantation: The Moving Target. *Gen Thorac Cardiovasc Surg* (2013) 61(2):67–78. doi:10.1007/s11748-012-0167-3
- Royer PJ, Olivera-Botello G, Koutsokera A, Aubert JD, Bernasconi E, Tissot A, et al. Chronic Lung Allograft Dysfunction: A Systematic Review of Mechanisms. *Transplantation* (2016) 100(9):1803–14. doi:10.1097/TP.0000000000001215
- Bos S, Vos R, Van Raemdonck DE, Verleden GM. Survival in Adult Lung Transplantation: Where Are We in 2020? *Curr Opin Organ Transpl* (2020) 25(3):268–73. doi:10.1097/MOT.0000000000000753
- Burton CM, Iversen M, Carlsen J, Mortensen J, Andersen CB, Steinbrüchel D, et al. Acute Cellular Rejection Is a Risk Factor for Bronchiolitis Obliterans Syndrome Independent of Post-Transplant Baseline FEV1. *J Heart Lung Transpl* (2009) 28(9):888–93. doi:10.1016/j.healun.2009.04.022
- Khalifah AP, Hachem RR, Chakinala MM, Yusen RD, Aloush A, Patterson GA, et al. Minimal Acute Rejection After Lung Transplantation: A Risk for Bronchiolitis Obliterans Syndrome. *Am J Transpl* (2005) 5(8):2022–30. doi:10.1111/j.1600-6143.2005.00953.x
- Kauke T, Kneidinger N, Martin B, Dick A, Schneider C, Schramm R, et al. Bronchiolitis Obliterans Syndrome Due to Donor-Specific HLA-Antibodies. *Tissue Antigens* (2015) 86(3):178–85. doi:10.1111/tan.12626
- Safavi S, Robinson DR, Soresi S, Carby M, Smith JD. De Novo Donor HLA-Specific Antibodies Predict Development of Bronchiolitis Obliterans Syndrome After Lung Transplantation. *J Heart Lung Transpl* (2014) 33(12):1273–81. doi:10.1016/j.healun.2014.07.012
- Girnit AL, Duquesnoy R, Yousem SA, Iacono AT, Corcoran TE, Buzoianu M, et al. HLA-Specific Antibodies Are Risk Factors for Lymphocytic Bronchiolitis and Chronic Lung Allograft Dysfunction. *Am J Transpl* (2005) 5(1):131–8. doi:10.1111/j.1600-6143.2004.00650.x
- Lobo LJ, Aris RM, Schmitz J, Neuringer IP. Donor-Specific Antibodies Are Associated With Antibody-Mediated Rejection, Acute Cellular Rejection, Bronchiolitis Obliterans Syndrome, and Cystic Fibrosis After Lung Transplantation. *J Heart Lung Transpl* (2013) 32(1):70–7. doi:10.1016/j.healun.2012.10.007
- Snyder LD, Wang Z, Chen DF, Reinsmoen NL, Finlen-Copeland CA, Davis WA, et al. Implications for Human Leukocyte Antigen Antibodies After Lung Transplantation: A 10-Year Experience in 441 Patients. *Chest* (2013) 144(1):226–33. doi:10.1378/chest.12-0587
- Choo SY. The HLA System: Genetics, Immunology, Clinical Testing, and Clinical Implications. *Yonsei Med J* (2007) 48(1):11–23. doi:10.3349/yymj.2007.48.1.11
- Levine DJ, Glanville AR, Aboyou N, Belperio J, Benden C, Berry GJ, et al. Antibody-Mediated Rejection of the Lung: A Consensus Report of the International Society for Heart and Lung Transplantation. *J Heart Lung Transpl* (2016) 35(4):397–406. doi:10.1016/j.healun.2016.01.1223
- Roux A, Levine DJ, Zeevi A, Hachem R, Halloran K, Halloran PF, et al. Banff Lung Report: Current Knowledge and Future Research Perspectives for Diagnosis and Treatment of Pulmonary Antibody-Mediated Rejection (AMR). *Am J Transpl* (2019) 19(1):21–31. doi:10.1111/ajt.14990
- Ius F, Verboom M, Sommer W, Poyanmehr R, Knoefel AK, Salman J, et al. Preemptive Treatment of Early Donor-Specific Antibodies With IgA- and IgM-Enriched Intravenous Human Immunoglobulins in Lung Transplantation. *Am J Transpl* (2018) 18(9):2295–304. doi:10.1111/ajt.14912
- Bery AI, Hachem RR. Antibody-Mediated Rejection After Lung Transplantation. *Ann Transl Med* (2020) 8(6):411. doi:10.21037/atm.2019.11.86
- Messika J, Belousova N, Parquin F, Roux A. Antibody-Mediated Rejection in Lung Transplantation: Diagnosis and Therapeutic Armamentarium in a 21st Century Perspective. *Transpl Int* (2024) 37:12973. doi:10.3389/ti.2024.12973
- Ensor CR, Yousem SA, Marrari M, Morrell MR, Mangiola M, Pilewski JM, et al. Proteasome Inhibitor Carfilzomib-Based Therapy for Antibody-Mediated Rejection of the Pulmonary Allograft: Use and Short-Term Findings. *Am J Transpl* (2017) 17(5):1380–8. doi:10.1111/ajt.14222
- Vacha M, Chery G, Hulbert A, Byrns J, Benedetti C, Finlen Copeland CA, et al. Antibody Depletion Strategy for the Treatment of Suspected Antibody-Mediated Rejection in Lung Transplant Recipients: Does It Work? *Clin Transpl* (2017) 31(3):e12886. doi:10.1111/ctr.12886
- Halverson LP, Hachem RR. Antibody-Mediated Rejection: Diagnosis and Treatment. *Clin Chest Med* (2023) 44(1):95–103. doi:10.1016/j.ccm.2022.10.008

27. McDermott JK, Castaneda SJ, Mietz SM, Lawson CK, Gerlach JA, Hadley RJ, et al. Preemptive Treatment of De Novo Donor Specific Anti-hla Antibodies with IVIG Monotherapy After Lung Transplantation. *Transpl Int* (2024) 37. doi:10.3389/ti.2024.13431
28. Stewart S, Fishbein MC, Snell GI, Berry GJ, Boehler A, Burke MM, et al. Revision of the 1996 Working Formulation for the Standardization of Nomenclature in the Diagnosis of Lung Rejection. *J Heart Lung Transpl* (2007) 26(12):1229–42. doi:10.1016/j.healun.2007.10.017
29. Verleden GM, Vos R, Vanaudenaerde B, Dupont L, Yserbyt J, Van Raemdonck D, et al. Current Views on Chronic Rejection After Lung Transplantation. *Transpl Int* (2015) 28(10):1131–9. doi:10.1111/tri.12579
30. Meyer KC, Raghu G, Verleden GM, Corris PA, Aurora P, Wilson KC, et al. An International ISHLT/ATS/ERS Clinical Practice Guideline: Diagnosis and Management of Bronchiolitis Obliterans Syndrome. *Eur Respir J* (2014) 44(6):1479–503. doi:10.1183/09031936.00107514
31. Schmitzer M, Winter H, Kneidinger N, Meimarakis G, Dick A, Schramm R, et al. Persistence of De Novo Donor Specific HLA-Antibodies After Lung Transplantation: A Potential Marker of Decreased Patient Survival. *Hla* (2018) 92:24–32. doi:10.1111/tan.13306
32. Hachem RR, Yusen RD, Meyers BF, Aloush AA, Mohanakumar T, Patterson GA, et al. Anti-Human Leukocyte Antigen Antibodies and Preemptive Antibody-Directed Therapy After Lung Transplantation. *J Heart Lung Transpl* (2010) 29(9):973–80. doi:10.1016/j.healun.2010.05.006
33. Lefaucheur C, Nochy D, Andrade J, Verine J, Gautreau C, Charron D, et al. Comparison of Combination Plasmapheresis/IVIg/anti-CD20 Versus High-Dose Ivig in the Treatment of Antibody-Mediated Rejection. *Am J Transpl* (2009) 9(5):1099–107. doi:10.1111/j.1600-6143.2009.02591.x
34. Roux A, Bendib Le Lan I, Holifanjaniaina S, Thomas KA, Hamid AM, Picard C, et al. Antibody-Mediated Rejection in Lung Transplantation: Clinical Outcomes and Donor-Specific Antibody Characteristics. *Am J Transpl* (2016) 16(4):1216–28. doi:10.1111/ajt.13589
35. Timofeeva OA, Choe J, Alsammak M, Yoon EJ, Geier SS, Mathew L, et al. Guiding Therapeutic Plasma Exchange for Antibody-Mediated Rejection Treatment in Lung Transplant Recipients - A Retrospective Study. *Transpl Int* (2021) 34(4):700–8. doi:10.1111/tri.13825
36. Degenfelder P, Intravenöse Immunglobulin-Therapie Bei Antikörper-vermittelter Abstoßungsreaktion Nach Lungentransplantation Analyse Prädiktiver Faktoren Für Die Elimination De Novo Donorspezifischer Antikörper

Copyright © 2025 Vorstandlechner, Degenfelder, Yavuz, Glueck, Kovács, Walter, Dick, Michel, Schneider, Zoller, Barton and Kauke. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.



Donor-Specific Blood Transfusion Induces a Transfusion-Related Early Protective Effect in Murine Lung Transplantation

Xin Jin^{1,2}, Charlotte Hooft¹, Balin Özsoy^{1,2}, Jan Van Slambrouck^{1,2}, Janne Kaes¹, Cedric Vanluyten^{1,2}, Annalisa Barbarossa^{1,2}, Marianne S. Carlon¹, Greetje Vande Velde³, Mélanie Guyot⁴, Karen Moermans⁵, Steve Stegen⁵, Robin Vos^{1,6}, Bart Vanaudenaerde¹, Jacques Pirenne^{7,8} and Laurens J. Ceulemans^{1,2*}

¹Lab of Respiratory Diseases and Thoracic Surgery, Department of Chronic Diseases, Metabolism (CHROMETA), KU Leuven, Leuven, Belgium, ²Department of Thoracic Surgery, UZ Leuven, Leuven, Belgium, ³Department of Imaging and Pathology, KU Leuven, Leuven, Belgium, ⁴Laboratory of Angiogenesis and Vascular Metabolism, Department of Oncology, VIB-KU Leuven, Leuven, Belgium, ⁵Clinical and Experimental Endocrinology, Department of Chronic Diseases, Metabolism (CHROMETA), KU Leuven, Leuven, Belgium, ⁶Department of Respiratory Diseases, UZ Leuven, Leuven, Belgium, ⁷Department of Abdominal Transplantation, UZ Leuven, Leuven, Belgium, ⁸Lab of Abdominal Transplantation, Department of Microbiology, Immunology and Transplantation, KU Leuven, Leuven, Belgium

OPEN ACCESS

*Correspondence

Laurens J. Ceulemans,

✉ laurens.ceulemans@uzleuven.be

Received: 09 August 2025

Revised: 28 February 2026

Accepted: 06 April 2026

Published: 30 April 2026

Citation:

Jin X, Hooft C, Özsoy B, Van Slambrouck J, Kaes J, Vanluyten C, Barbarossa A, Carlon MS, Vande Velde G, Guyot M, Moermans K, Stegen S, Vos R, Vanaudenaerde B, Pirenne J and Ceulemans LJ (2026) Donor-Specific Blood Transfusion Induces a Transfusion-Related Early Protective Effect in Murine Lung Transplantation. *Transpl. Int.* 39:15409. doi: 10.3389/ti.2026.15409

Lung transplantation (LTx) remains limited by high immunogenicity and chronic rejection, yet the application of donor-specific blood transfusion (DSBT) in LTx has not been fully investigated. We established a murine orthotopic LTx model (BALB/c to C57BL/6N) to evaluate the safety and efficacy of DSBT administered 24 h prior to transplantation. Pre-transplant transfusion was well-tolerated showing no evidence of Transfusion-Related Acute Lung Injury (TRALI) or volume-overload injury. Radiographic analysis at POD 7 demonstrated that DSBT-treated grafts maintained significantly higher aerated lung volume compared to non-transfused controls. Flow cytometric analysis at the same time point revealed that these grafts were preferentially infiltrated by recipient-derived monocytes and type 2 conventional dendritic cells (cDC2s), while lymphoid cell counts remained comparable across groups in both the lung and spleen, indicating no systemic immune depletion. By POD 35, however, histological analysis revealed that the lung grafts were extensively destroyed by severe rejection. These findings demonstrate that a 24-h pre-transplant DSBT window improves early graft patency and modulating the localized myeloid landscape in a murine model. We conclude that DSBT serves as a safe and

Abbreviations: ALV, aerated lung volume; APC, antigen-presenting cell; CCR7, C-C chemokine receptor type 7; CT, computed tomography scan; DSBT, donor-specific blood transfusion; GMP, granulocyte-monocyte progenitor; H&E, hematoxylin and eosin staining; HSPC, hematopoietic stem and progenitor cell; IL-10, interleukin-10; ILC, innate lymphoid cell; INF- β , interferon beta; ISHLT, International Society for Heart and Lung Transplantation; LTx, lung transplantation; MDP, monocyte-dendritic cell progenitor; MFI, mean fluorescent intensity; MHC, major histocompatibility complex; NALV, non-aerated lung volume; NMR, neutrophil-to-monocyte ratio; PFA, paraformaldehyde; POD, post-operative day; TGF- β , transforming growth factor beta; TRALI, transfusion-related acute lung injury; Treg, regulatory T cell; TLV, total lung volume; WOFIE, window of opportunity for immunologic engagement; cDC, conventional dendritic cell; dLN, mediastinal draining lymph node; pDC, plasmacytoid dendritic cell.

effective induction strategy to mitigate early inflammatory consolidation, providing a predictable temporal window for secondary immunomodulatory interventions in lung transplantation.

Keywords: allograft rejection, donor-specific blood transfusion, immune regulation, immunosuppression, lung transplantation

INTRODUCTION

According to the latest report of the International Society for Heart and Lung Transplantation (ISHLT), the 5-year survival rate for lung transplantation is approximately 59%, which is the lowest among all solid organ transplants [1, 2]. Lung transplant patients require lifelong immunosuppression to suppress the intense immune response elicited by major histocompatibility complex (MHC) incompatibility, which is significantly more intense in the lung than in the other solid organs. Despite being the ultimate treatment for end-stage pulmonary disease, its long-term success is hindered by the fragile balance between the necessity for profound immunosuppression carrying risks of nephrotoxicity, malignancy, and opportunistic infection versus the persistent threat of chronic allograft rejection [3, 4]. The future of lung transplantation depends on strategies that expand the therapeutic window by promoting graft acceptance and simultaneously limiting the need for profound immunosuppression and its associated complications.

Donor-specific blood transfusion (DSBT), the infusion of fresh donor whole blood containing all blood cell types and plasma proteins into the recipient prior to transplantation, has been reported to improve graft acceptance and potentially induce a tolerogenic state [5]. Since its first application in canine allogeneic kidney transplantation in 1964 and in human

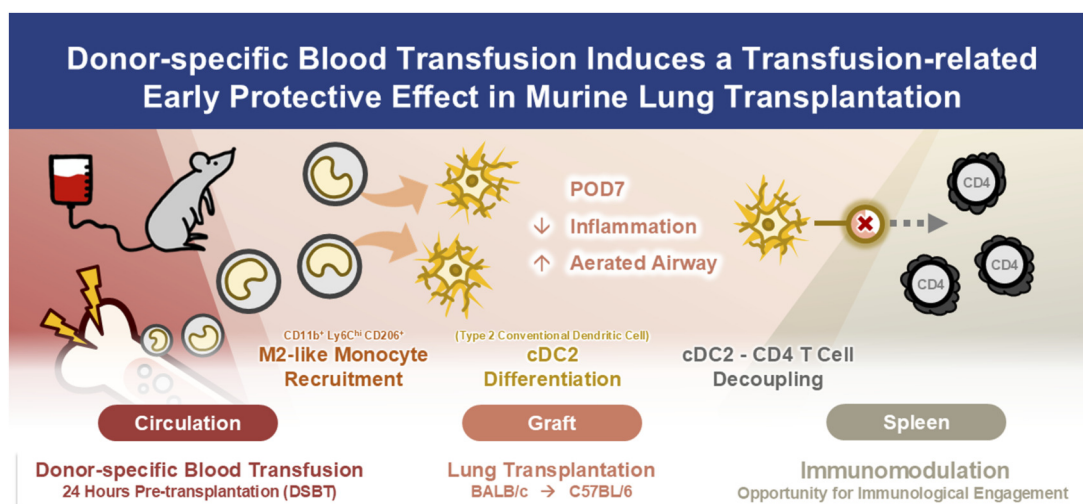
kidney transplantation in 1980, multiple studies including our Leuven Immunomodulatory Protocol in intestinal transplantation have demonstrated that DSBT can prolong graft survival and reduce the required dosage of postoperative immunosuppressants [6–9]. However, the application of DSBT remains unexplored in the context of LTx, and its underlying immunomodulatory mechanisms in the pulmonary environment remain largely elusive.

In this proof-of concept study, we first evaluated the safety of DSBT by investigating its impact on pulmonary integrity and systemic hematological profiles in a murine model. Furthermore, we established the first murine orthotopic LTx model of DSBT preconditioning to analyze its effects on graft and immune cell dynamics, providing a novel framework for understanding the regulation of lung allograft rejection.

MATERIALS AND METHODS

Blood Transfusion and Orthotopic Left LTx Mouse Model (Figure 1)

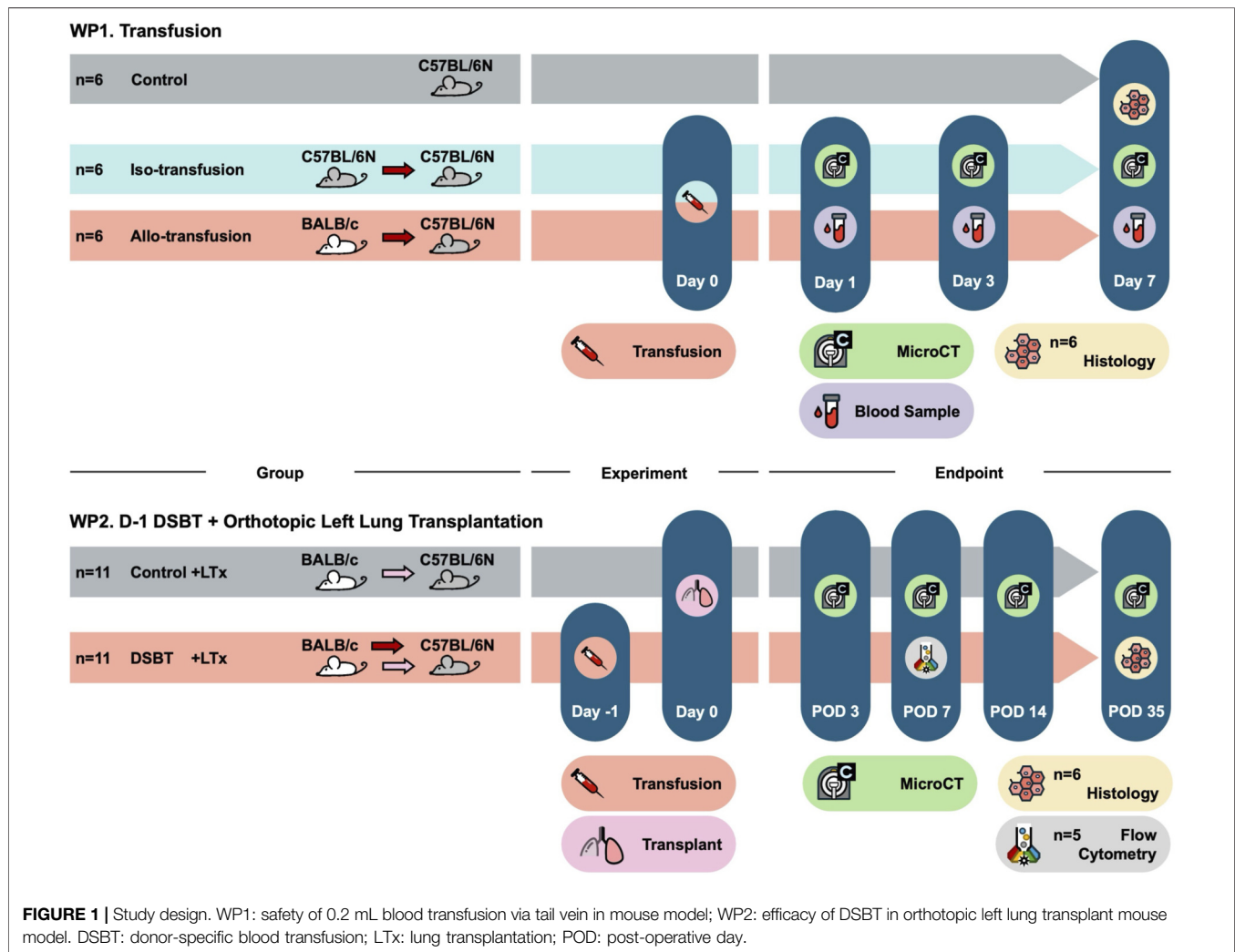
The project was approved by the Ethical Committee for Animal Research at KU Leuven (P015/2024). Eight-to 12-week-old BALB/c (H2K^d) and C57BL/6N (H2K^b) mice were purchased from Janvier Labs (Le Genest-Saint-Isle, France).



XIN JIN, et al. *Transpl. Int.* 2026
doi: [10.3389/ti.2026.15409](https://doi.org/10.3389/ti.2026.15409)



GRAPHICAL ABSTRACT |



All animals were housed in a conventional facility with individually ventilated cages and had access to standard chow and water *ad libitum*.

WP1. Transfusion:

Donor: C57BL/6N (iso-transfusion, n = 6)

or BALB/c (allo-transfusion, n = 6)

Recipient: C57BL/6N

Control: C57BL/6N non-transfused mice (n = 6)

Donor mice were anesthetized using 5.0% isoflurane (Iso-Vet, Piramal Critical Care, the Netherlands) in room air, intubated, and maintained under anesthesia with 2.0% isoflurane. Ventilation was provided using a microventilator (UNO microventilator, the Netherlands) at a respiratory rate of 120 breaths per minute with a tidal volume of 275–300 μ L. Inferior vena cava was exposed by laparotomy, and 0.8–1.0 mL of whole blood (for three recipients) was collected into a heparinized centrifuge tube which was stored at 4 °C fridge until transfusion.

Recipient transfusion mice were anesthetized with 3.0% isoflurane in room air. A total of 0.20 mL of whole blood was withdrawn into an insulin syringe (0.3 mL U-100 30 G, BD, USA) and administered via the lateral tail vein. The interval between blood collection and transfusion did not exceed 30 min.

All mice that received transfusion only were euthanized on day 7, and the left lung was procured for histological analysis.

WP2. D-1 DSBT + Orthotopic Left LTx:

Experiments were randomized.

Donor: BALB/c (blood and lung)

Recipient: C57BL/6N with DSBT 24 h prior to LTx (D-1 DSBT + LTx, n = 11)

Control: C57BL/6N non-transfused mice (Control + LTx, n = 11)

The surgical procedure for murine orthotopic left LTx was performed as previously described in our review on murine LTx [10]. Briefly, both donor and recipient mice were anesthetized with isoflurane and connected to a ventilator.

Procurement: A total of 100 μ L of heparin (LEO Pharma, Denmark) was injected into the inferior vena cava, followed by perfusion of the heart–lung bloc with 2–3 mL of sterile 4 °C saline via the pulmonary artery. The left pulmonary artery, bronchus, and pulmonary vein were carefully dissected and cuffed using 24-, 20-, and 22-gauge cuffs, respectively. The donor lung was stored at 4 °C fridge until implantation.

Transplantation: In the recipient, the left hilum was exposed, and the vessels were occluded using 9-0 slip knot sutures. The anastomoses were performed in the sequence of bronchus–vein–artery, deviating from the previously reported artery–bronchus–vein sequence, to optimize surgical outcomes. The second warm ischemic time (removal of the graft from cold storage to reperfusion) should be less than 20 min to maintain a low-inflammatory baseline and minimize confounding effects from ischemia-reperfusion injury. The native left lung was removed, and the thoracic incision was closed using 6-0 or 7-0 running sutures.

Postoperative care: For analgesia, buprenorphine (0.1 mg/kg Vetergesic, Ecuphar, Netherlands) was administered subcutaneously twice daily until postoperative day (POD) 3. To prevent graft rejection, recipients received subcutaneous injections of methylprednisolone (1.6 mg/kg; SoluMedrol, Pfizer, Belgium) and cyclosporine (10 mg/kg; Sandimmun, Novartis, Belgium) once daily starting from POD 1. All recipients were euthanized on POD 35, and the left lung grafts were procured for histological analysis.

Endpoints

Clinical Endpoints

During the follow-up period, all animals were monitored and weighed daily. Euthanasia was performed if any of the following endpoints were met: 1) irreversible body weight loss exceeding 20%; 2) complete loss of appetite or inability to drink; 3) severe respiratory or circulatory distress, including gasping, edema, or cyanosis of the extremities; or 4) abnormal behavior or movement, such as hyperactivity, lethargy, or abnormal muscle tone.

Blood Sample Analysis (WP1)

The complete blood count by facial vein phlebotomy collection was conducted using hematology analyzer (Element HT5, Heska, US) according to the machine manual on Day 1, 3 and 7 after transfusion.

In Vivo microCT Imaging (WP1&2)

For transfusion-only recipients, *in vivo* microCT scans were performed on Day 1, 3, and 7.

For transplant recipients, scans were conducted on POD 3, 7, 14, and 35. Recipients presenting with complete consolidation of the left lung graft within the first 3 days, suggestive for technical failure [primary graft dysfunction (PGD) or severe inflammation] were excluded and replaced by new animals.

During scanning, animals were anesthetized with 2.0% isoflurane in pure oxygen and positioned supine. Imaging was performed with SkyScan 1278 *in vivo* microCT scanner (Bruker,

Belgium). Scanning and reconstruction (NRecon, v1.7.0.4; CTan software, v1.16.8.0, Bruker, Belgium) parameters were set according to our protocol [11]. Pixel with a gray value of 0–80 is defined as aerated lung tissue. The number of voxels and mean gray value were further calibrated to volume in mL and density in Hounsfield Units (HU).

Histological Analysis (WP1&2, n = 6)

Histological analysis was performed on POD 35 according to our established protocol [11]. At the time of sacrifice, lungs were ventilated *in vivo* and perfused with saline via the pulmonary artery, followed by perfusion with 4% paraformaldehyde (PFA). The trachea was ligated under full inflation, and the lungs were fixed in 4% PFA at 4 °C for 12–24 h.

Lung grafts were processed into 4 μ m formalin-fixed paraffin-embedded sections and stained with hematoxylin and eosin [WP1, n = 6; WP2, n = 6 (Leica, Germany), H&E] and Masson's trichrome kit [WP2, n = 6 (Sigma-Aldrich, US)] following the manufacturer's instructions. All sections were blindly evaluated by experienced pulmonary pathologists according to ISHLT grading system for cellular rejection [12].

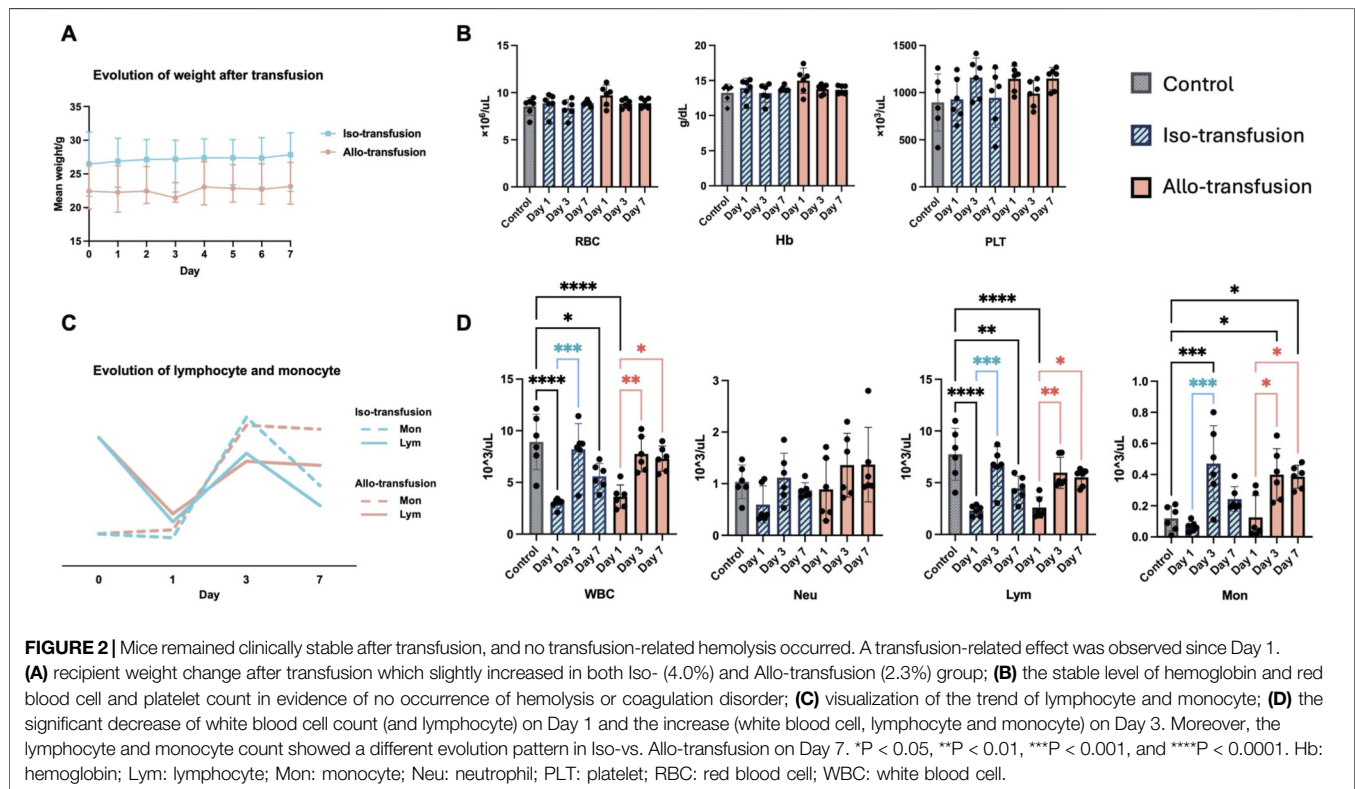
The percentage of collagen deposition in lung sections stained with Masson's trichrome was quantified using QuPath software (v0.6.0) with the Pixel Classifier Tool.

Flow Cytometry Analysis (WP2, n = 5)

Flow cytometry analysis was performed on POD 7 according to our established protocol [13]. At the time of euthanasia, the recipients were anesthetized via an intraperitoneal injection of xylazine (100 mg/kg; Xyl-M, VMD, Belgium) and ketamine (10 mg/kg; Nimatek, Dechra, Belgium). To distinguish marginated circulatory cells from tissue-resident cells, 50 μ L of CD45.2 antibody solution (0.2 mg/mL in PBS) was injected into the inferior vena cava. Five minutes later, the lung graft and spleen were harvested and processed into single-cell suspension. Absolute cell numbers in the lung and spleen were quantified using a flow cytometer (BD LSRFortessa™; Water Biosciences, US). The gating strategy (**Supplementary Material 1**) included total viable cells (Live/Dead negative), myeloid cells, lymphoid cells, and cell chimerism (H2K^{b/d}). Data were analyzed using FlowJo software (v10.6.0, Waters Biosciences, US).

Statistical Analysis

Data were analyzed using GraphPad statistical software (v10.2.1, Prism, US) and SPSS (v20.0.0, IBM, US). Mixed-effects model with multiple comparisons (mean $\pm$ SD, for longitudinal data), Mann-Whitney U tests [Median (Min-Max), for non-normal distribution data], Chi-square test (for categorical variables) and Kaplan-Meier analysis (for graft survival) were used to compare intergroup differences. A P-value <0.05 was considered significant. In cases where non-parametric analysis failed to reach significance but suggested a clear distributional trend, we applied bootstrapping techniques (10,000 iterations) to re-evaluate the comparative differences with increased statistical power.



RESULTS

Transfusion

Mice Remained Clinically Stable After Transfusion (Figure 2A)

Before transfusion, the weight of iso-transfusion mice was 26.58 ± 4.59 g, and that of allo-mice was 23.02 ± 3.03 g ($P = 0.180$). On Day 7, the weight of iso-mice increased by $4.0\% - 27.43 \pm 3.55$ g, and the weight of the allo-mice increased by $2.3\% - 23.50 \pm 2.82$ g ($P = 0.093$). All mice remained clinically stable during observation with no development of organ dysfunction or reaching humane endpoints.

No Transfusion-Related Hemolysis Occurred and a Transfusion Effect on White Blood Cells was Observed (Figures 2B–D)

Compared with the control group, there were no significant changes in red blood cell, platelet counts or hemoglobin level during the 7-day follow-up in either group.

White blood cells count decreased significantly on Day 1 from $8.922 \times 10^3/\mu\text{L}$ to $2.990 \times 10^3/\mu\text{L}$ in the iso-transfusion group ($P < 0.001$) and to $3.625 \times 10^3/\mu\text{L}$ in the allo-group ($P < 0.001$). By Day 3, white blood cells count recovered to $8.200 \times 10^3/\mu\text{L}$ in the iso-group ($P > 0.999$) and to $7.752 \times 10^3/\mu\text{L}$ in the allo-group ($P = 0.986$). On Day 7, white blood cells count in the allo-group remained comparable to baseline ($7.298 \times 10^3/\mu\text{L}$, $P = 0.834$), whereas a significant decline was observed in the iso-group ($5.595 \times 10^3/\mu\text{L}$, $P = 0.030$).

Lymphocytes count exhibited a similar trend, with a significant decrease on Day 1 (control: $7.738 \times 10^3/\mu\text{L}$; iso-:

$2.317 \times 10^3/\mu\text{L}$, $P < 0.001$; allo-: $2.602 \times 10^3/\mu\text{L}$, $P < 0.001$), a recovery on Day 3 (iso-: $6.573 \times 10^3/\mu\text{L}$, $P = 0.953$; allo-: $5.963 \times 10^3/\mu\text{L}$, $P = 0.512$), and a divergent trend on Day 7 (iso-: $4.458 \times 10^3/\mu\text{L}$, $P = 0.008$; allo-: $5.522 \times 10^3/\mu\text{L}$, $P = 0.196$).

Monocytes count did not change significantly on Day 1 (control: $0.118 \times 10^3/\mu\text{L}$; iso-: $0.067 \times 10^3/\mu\text{L}$, $P > 0.999$; allo-: $0.125 \times 10^3/\mu\text{L}$, $P > 0.999$) but increased on Day 3 (iso-: $0.470 \times 10^3/\mu\text{L}$, $P = 0.001$; allo-: $0.400 \times 10^3/\mu\text{L}$, $P = 0.013$). This increase persisted until Day 7 in the allo-group ($0.387 \times 10^3/\mu\text{L}$, $P = 0.021$) but returned to baseline in the iso-group ($0.243 \times 10^3/\mu\text{L}$, $P = 0.843$).

Neutrophil counts showed a pattern similar to monocyte, but this did not reach statistical significance at any time point.

No Transfusion-Related Acute Lung Injury Occurred (Figure 3)

MicroCT imaging revealed normal lung morphology at all time points in both the iso-transfusion and allo-transfusion groups (Figure 3A). No evidence of transfusion-related acute lung injury (TRALI) was observed in either group as lung grafts cellular rejection all graded as A0 (Figure 3B). There were no signs of consolidation indicative of edema, pneumonia, or pleural effusion, as reflected by stable left lung volumes (iso-vs. allo-: Day 1: 0.162 ± 0.012 mL vs. 0.149 ± 0.024 mL; Day 3: 0.160 ± 0.013 mL vs. 0.156 ± 0.022 mL; Day 7: 0.141 ± 0.016 mL vs. 0.152 ± 0.019 mL; all $P > 0.05$) and density (iso-vs. allo-: Day 1: -393.9 ± 28.6 HU vs. -423.4 ± 26.0 HU; Day 3: -421.9 ± 37.9 HU vs. -429.1 ± 28.4 HU; Day 7: -401.3 ± 12.2 HU vs. -397.7 ± 17.2 HU; all $P > 0.05$) throughout the follow-up period (Figure 3C).

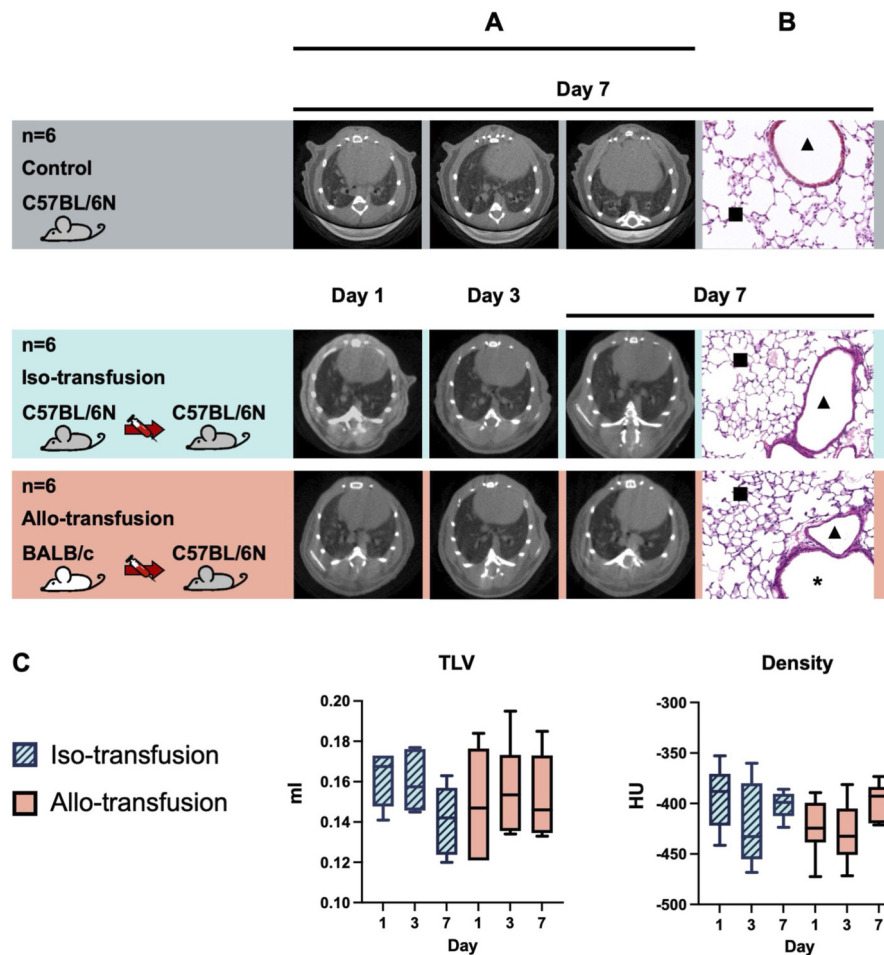


FIGURE 3 | No transfusion-related acute lung injury occurred after DSBT. Representative pictures of (A) *in vivo* microCT and (B) hematoxylin and eosin (H&E) stain, magnification $\times 20$, [▲] indicates vessels, [*] indicates airways, [■] indicates parenchyma; (C) evolution of total lung volume (TLV) and mean density of left lung.

D-1 DSBT + Orthotopic Left LTx The Protective Effect of DSBT Was Biphasic as the Most Obvious on POD 7 but not Observed by POD35 (Figures 4, 5)

The median interval between DSBT and LTx was 26.1 (24.2–27.9) hours. The cold ischemia time, defined as the duration from pulmonary artery flush to retrieval from refrigeration, was 69 (48–100) minutes in the control group and 55 (51–61) minutes in the DSBT group. Warm ischemia time, was similar between groups: 16.5 (10–20) minutes in the control group and 16.5 (14–20) minutes in the DSBT group ($P = 0.842$). The total surgical duration was comparable in both group [control: 65 (51–74) vs. DSBT: 64 (55–70) minutes ($P = 0.732$)].

Three surviving recipients in each group were excluded due to complete consolidation of the left lung graft observed by microCT on POD 3 and were replaced by no-consolidated mice. No animals reached criteria for euthanasia during the 35-day follow-up period.

On POD 7, microCT scans ($n = 6$, **Figure 4A**) revealed complete graft consolidation in 33.3% of mice in the control

group, whereas 100% of mice in the DSBT group showed well lung aeration ($P = 0.455$). By POD 14, the proportion of functional grafts remained higher in the DSBT group compared with the control group (83.3% vs. 50%, $P = 0.546$). On POD 35, 50% of the left lung grafts remained functional in both groups (**Figure 4B**, Kaplan-Meier curve $P = 0.489$).

Total left lung volume (TLV) is presented as side-by-side stacked bars, partitioned into aerated (ALV) and non-aerated (NALV) fractions in longitudinal assessment of microCT on POD 3, 7, 14, and 35 (**Figures 4C,D**). The DSBT group exhibited a significantly more stable TLV trajectory compared to the decline observed in the control group during the early post-operative phase (Interaction $P = 0.024$). Notably, at POD 7 and POD 14, DSBT-treated lungs maintained a higher proportion of functional ALV while effectively limiting the expansion of pathological NALV (Interaction $P = 0.034$).

Histological assessment ($n = 6$) on POD 35 showed comparable cellular rejection grades [control vs. DSBT: (A3 $n = 1$, A4 $n = 5$) vs. (A3 $n = 1$, A4 $n = 5$), $P = 1.000$;

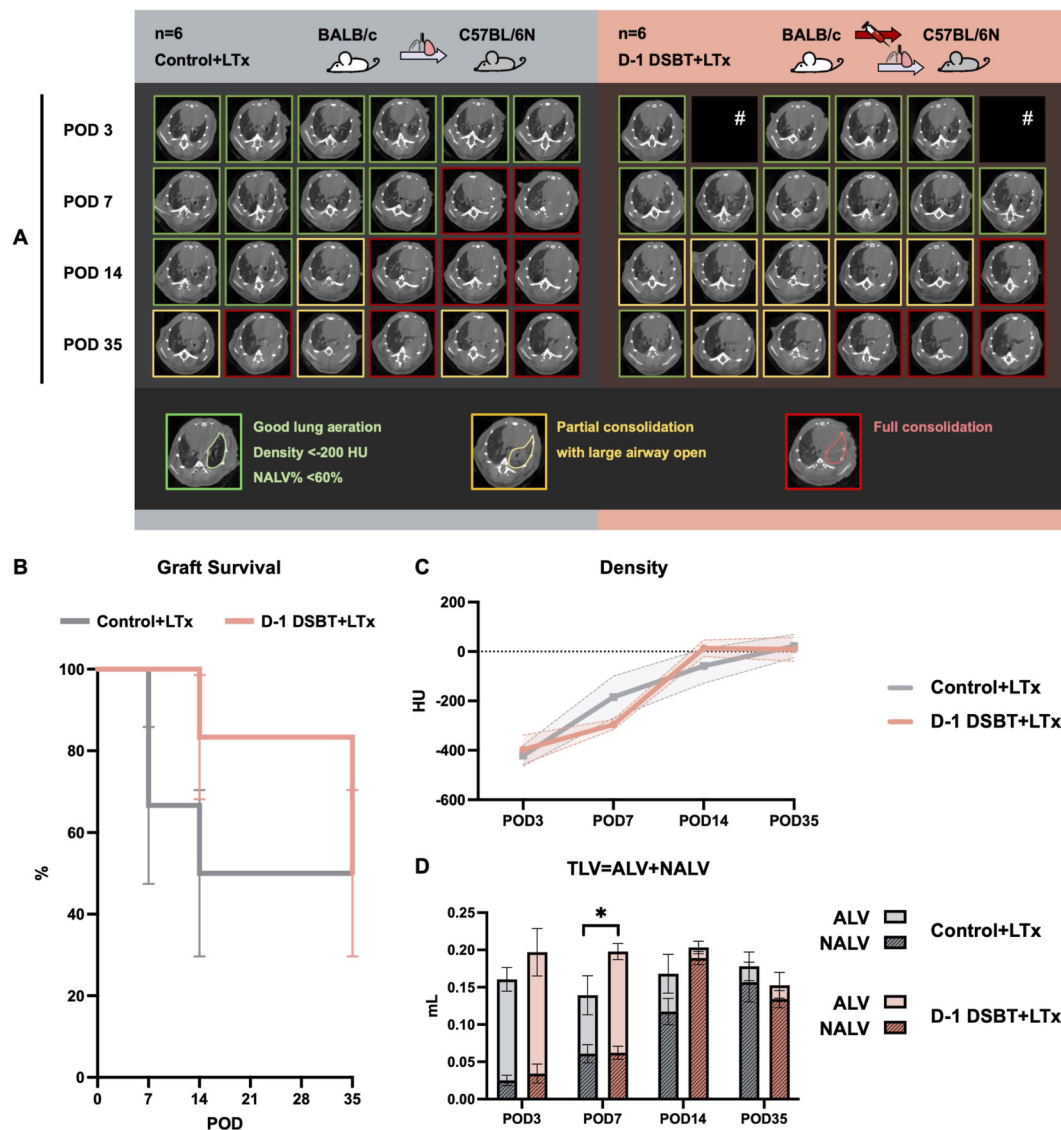


FIGURE 4 | The evolution of the density and volume of left lung graft on condition of no acute complication on POD 3[#]. The protective effect of DSBT was the most obvious on POD 7 but not observed by POD35 in mouse lung transplantation. **(A)** representative pictures of *in vivo* microCT image of each recipient on POD 7, 14 and 35, image with clear lung markings on the left side was labelled with green, partial consolidation with yellow and full consolidation with red. **(B)** (functional) graft survival if no full consolidation image on CT; evolution of left lung graft's **(C)** mean density and **(D)** volume. * $P < 0.05$. ALV: aerated lung volume; DSBT: donor-specific blood transfusion; NALV: non-aerated lung volume, TLV: total lung volume; POD: post-operative day. [#]POD3 images of two mice of DSBT group was missing due to a mechanical issue. They were still included in the study because of their stable status, no abnormal activity and good lung aeration images on POD7 (they would not recover if full consolidation happened on POD3).

Figure 5C] and similar fibrosis scores (collagen deposition, control vs. DSBT: 10.01% vs. 11.91%, $P = 0.699$; Figure 5D).

Increased Infiltration of Pro-Reparative Monocyte-cDC2 Axis With Concomitant Suppression of Systemic Immune Activation on POD 7

Given that the most pronounced protective effect of DSBT was observed on POD 7, we performed a detailed flow cytometric analysis of lung grafts and recipient spleens ($n = 5$ per group) to explore the underlying mechanisms. In the DSBT group, the total

count of intra-graft recipient-derived CD45⁺ immune cells was significantly higher than in control group (8.08×10^6 vs. 5.82×10^6 , $P = 0.032$, Figure 6A). This increase was primarily driven by a robust expansion of CD11b⁺ Ly6C^{hi} Ly6G[−] monocytes (2.53×10^5 vs. 1.05×10^5 , $P = 0.008$). Notably, these infiltrating monocytes were predominantly of the pro-reparative CD206⁺ M2-like subset (13.58×10^4 vs. 3.80×10^4 , $P = 0.008$). Beyond monocyte expansion, we observed a distinct lineage bias in the DSBT group: infiltrating myeloid cells preferentially differentiated into potentially immunoregulatory cDCs,

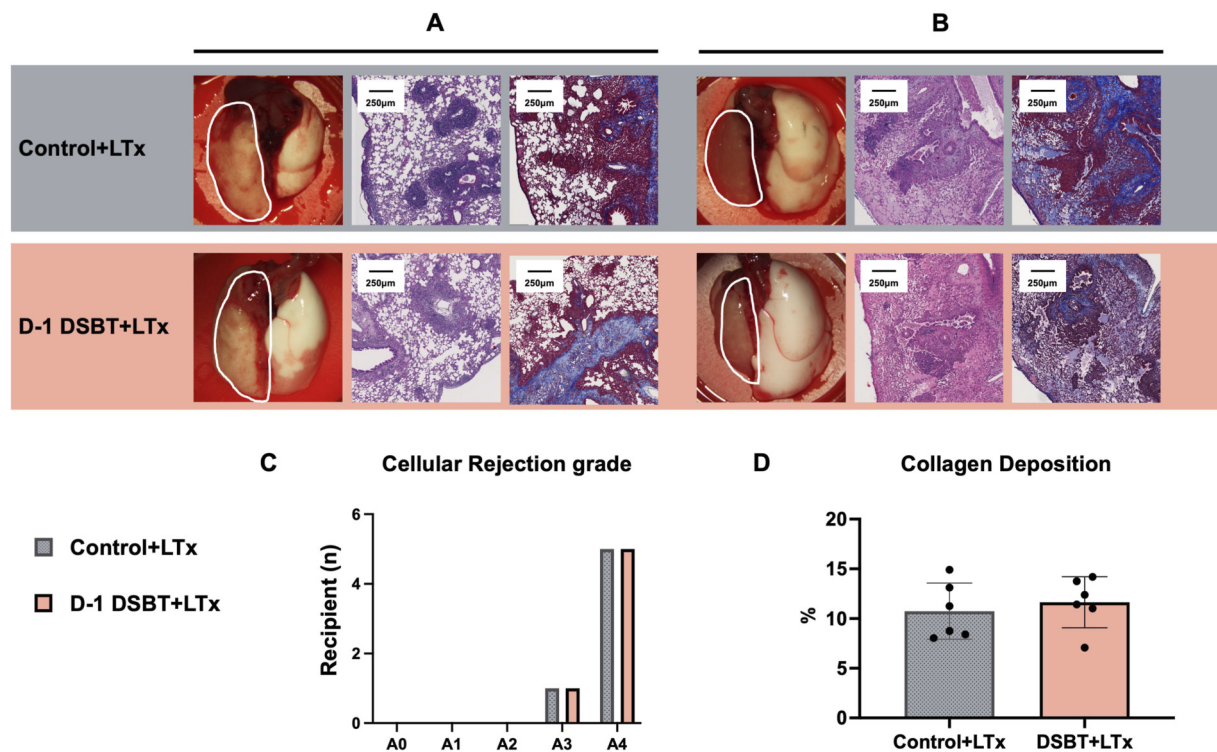


FIGURE 5 | Histological analysis of the left lung graft on POD 35 after lung transplantation. The protective effect of DSBT was not observed by POD 35. Representative pictures of macroscopic view (left, labelled with white frame), hematoxylin and eosin staining (middle) and Masson's trichrome (right, collagen dyed in blue) of (A) moderate rejection (cellular rejection grade A3) and (B) severe rejection in left lung graft (cellular rejection grade A4); (C) cellular rejection grade of left lung graft; (D) collagen deposition in left lung graft measured by QuPath.

specifically CD11b+ XCR1-cDC2s (3.58×10^5 vs. 1.15×10^5 , $P = 0.048$), rather than pro-inflammatory plasmacytoid dendritic cells (pDCs. Intra-graft pDC/cDC ratio: 0.008 vs. 0.020, $P = 0.008$, **Figure 6B**) [14]. Furthermore, the DSBT group exhibited a markedly lower neutrophil-to-monocyte ratio (NMR, 0.618 vs. 2.354, $P = 0.008$), suggesting a shifted inflammatory milieu that favors monocyte-mediated regulation over neutrophil-driven injury. Crucially, despite the increased graft cDC2 infiltration, the ratio of splenic CD4⁺ T cells-to-graft cDC2s was significantly reduced in the DSBT group (2.198 vs. 5.819, $P = 0.032$), indicating a functional cDC2-CD4⁺ T cell decoupling. No significant differences were observed in the absolute counts of other donor- or recipient-derived myeloid or lymphoid cell subsets (**Figure 6C, Supplementary Material 2**), underscoring the specificity of the DSBT-induced myeloid recalibration.

DISCUSSION

In this study, we established a novel preconditioning platform for murine LTx and identified a distinct biphasic immune response induced by DSBT, integrating the foundational DSBT principles of Fabre *et al.* with the surgical precision of Okazaki's cuff-

technique [15, 16]. Our data demonstrate that DSBT provides a transient period of graft protection characterized by preserved parenchymal aeration and a recalibrated myeloid landscape on POD 7. However, this effect eventually faded out by a rejection flare-up by POD 35. Rather than viewing the late failure merely as a therapeutic limitation, we propose that DSBT acts as a powerful spatiotemporal modulator that converts chaotic acute injury into a predictable "Window of Opportunity for Immunologic Engagement (WOFIE)," a conceptual framework to describe the essential engagement between the host immune system and the allograft required for tolerance induction [17].

From a translational perspective, the primary concern regarding DSBT is the potential induction of Transfusion-Related Acute Lung Injury (TRALI). According to the "two-hit" hypothesis, TRALI requires an initial priming of the host environment (first hit) followed by the activation of neutrophils by transfused components (second hit) [18]. In our model, the absence of evidence of diffuse alveolar damage suggests that DSBT, when administered 24 h prior to surgery, does not incite TRALI-like acute injury. This safety profile is likely attributed to the low-inflammatory threshold at the time of transfusion as prioritized in the Leuven Immunomodulatory Protocol [19, 20]. By bypassing systemic inflammatory priming during the transfusion window, DSBT appears to

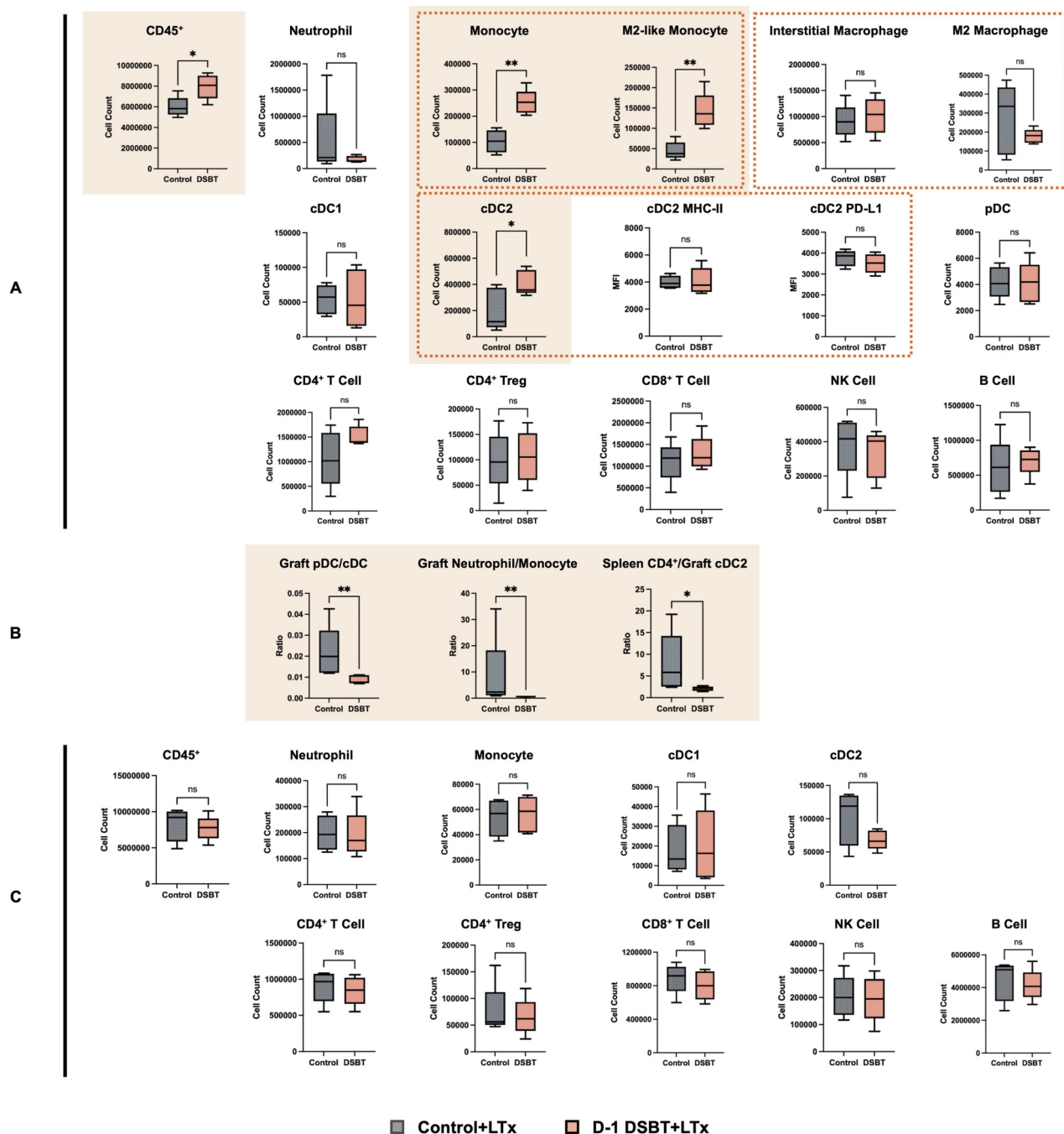


FIGURE 6 | DSBT treatment recalibrated the intra-graft myeloid landscape and induces functional immune decoupling by POD 7. Flow cytometric analysis was performed on left lung graft and recipient spleen on POD 7 ($n = 5$). **(A)** recalibration of the myeloid pool in lung grafts. DSBT treatment significantly expanded the recipient-derived $CD11b^{+} Ly6G^{+} Ly6G^{-}$ monocyte population, particularly the pro-reparative $CD206^{+}$ M2-like subset. Despite this influx, the downstream interstitial macrophage pool remained stable, and increased infiltrating $CD11b^{+} XCR1^{-}$ cDC2s remain functionally controlled without further upregulation of MHC-II or PD-L1 expression. **(B)** lineage bias in monocyte differentiation. Within the graft, infiltrating monocytes preferentially shifted toward a potentially immunoregulatory cDC2 fate rather than a pro-inflammatory pDC lineage (decreased pDC/cDC and NMR). **(C)** systemic immune quiescence in the spleen. The localized accumulation of cDC2s and M2-like monocytes in the graft did not translate into systemic $CD4^{+}$ or $CD8^{+}$ T cell activation * $P < 0.05$, ** $P < 0.01$, ns: no significance. cDC: conventional dendritic cell; pDC: plasmacytoid dendritic cell; LTx: lung transplantation; MFI: mean fluorescent intensity; NK cell: natural killer cell; Treg: regulatory T cell; NMR: neutrophil-to-monocyte ratio.

serve as a safe pre-conditioning intervention that favors homeostatic recalibration over hyperacute sensitization.

The 24-h interval between DSBT and LTx likely orchestrates the homing of DSBT donor cells to secondary lymphoid organs and a systemic recipient myeloid recalibration rooted in the concept of “trained immunity” [21]. As the primary biological filter for intravenous cargo, the spleen likely serves as the sensory hub for this process. While we observed quantitative stability in the splenic compartment (**Figure 6C**), its specialized microenvironment is evolutionarily optimized to process blood-borne antigens and initiate systemic tolerogenic signals. Recent evidence has identified a robust spleen-bone marrow axis. The splenic sensing of peripheral immunomodulation which could be the immunogenic components in DSBT in this study triggers the release of systemic mediators that migrate to the bone marrow to recalibrate hematopoietic stem and progenitor cells (HSPCs) [5, 22, 23]. This process likely biases the bone marrow output toward monocyte-dendritic cell progenitors (MDPs) over the pro-inflammatory granulocyte-monocyte progenitor (GMP) lineage [24, 25].

In contrast to the pro-inflammatory role of GMP-derived neutrophils inducing acute lung injury, MDP-derived cells appear predisposed toward a reparative and homeostatic phenotype following DSBT. This systemic recalibration manifests at POD 7 as a robust expansion of recipient-derived CD11b⁺ Ly6C^{hi} CD206⁺ M2-like monocytes ($P = 0.008$) and CD11b⁺ XCR1⁻ cDC2s ($P = 0.048$), coupled with a significant reduction in the intra-graft NMR ($P = 0.008$). Consistent with the findings of Menezes *et al.*, who demonstrated that the intrinsic heterogeneity of Ly6C^{hi} monocytes dictates their developmental fate toward either dendritic cells or macrophages [26], our data suggest that DSBT effectively “primes” the monocyte pool toward a cDC2-lineage bias. This deliberate expansion of the pro-reparative monocyte pool, coupled with the numerical stability of mature macrophage populations ($P = 0.548$), suggests that DSBT does not merely suppress inflammation, but rather re-engineers the graft’s cellular architecture. Building on the “niche occupancy” theory, we propose that these primed monocytes, rapidly recruited via the instantaneously re-established vascular inflow, likely compete with neutrophils for limited interstitial niches [27]. By effectively “out-crowding” pro-inflammatory effectors, this expanded monocyte pool fosters a transiently quiescent, tissue-reparative microenvironment.

We hypothesize that these cells may exist in a functionally quiescent or immature state. This is underscored by the striking numerical homeostasis of the intra-graft mature M2 macrophage population ($P = 0.691$), the significant expansion or stochastic surges of which is typically associated with clinical rejection [28, 29]. While the stable level of MHC-II and PD-L1 on surface of cDC2s suggests that cDC2 could maintain tissue stability through a “low-signal” phenotype [30], literature further indicates that downregulated costimulatory molecules (e.g., CD40, CD80, and CD86) or reduced CCR7 expression typically restricts cDC2 migration and limits “Signal 2” to T cells [31, 32]. Furthermore, specific subpopulation (such as IFNAR1^{hi} TNFR2⁺ cDC2s, iR2D2) are known to be potent sources of IL-10 and IFN- β , which can foster pulmonary homeostasis by

inducing regulatory T cells (Tregs), modulating T-helper cell polarization, or mediating antigen-specific T-cell hyporesponsiveness [8, 33]. Within our WOFIE framework, the expansion of this phenotypically stable high-volume cDC2 pool and the arrested differentiation of M2-like monocytes into mature macrophages likely functions as a “local buffer” that prevents early inflammatory signals from escalating into adaptive rejection on POD 7, effectively buying time before the subsequent lymphatic reconnection.

While pulmonary vasculature is restored immediately during surgery, the transection of lymphatic vessels creates a temporary state of “spatial immune decoupling” by precluding the migration of activated APCs to mediastinal draining lymph nodes (dLNs). This anatomical barrier initially favors the DSBT effect because donor antigens in DSBT circulate hematogenously prior to surgery, they preferentially home to the spleen and dLNs [34]. It establishes a regulatory baseline through sub-clinical desensitization. Following LTx, the paralysis of lymphatic efflux reinforces this protection by physically trapping mature APCs within the graft and preventing them from cascade amplifying immune response in dLNs. However, as lymphangiogenesis typically restores connections by POD 14, a “dual anatomical-molecular floodgate” is opened [35, 36]. The synergy of physical reconnection and the molecular upregulation of CCR7 on the backlog of intra-graft APCs triggers a synchronized surge into the dLNs. This concentrated influx eventually overwhelms the DSBT-mediated desensitization, leading to the fulminant adaptive response observed on POD 35.

The uniqueness of the lung’s immunological trajectory becomes particularly evident when evaluated against the homeostatic frameworks of other solid organs. Unlike the liver, which possesses an intrinsic filtration and tolerogenic system via Kupffer cells and LSECs rich in immunoregulatory cytokines like IL-10 and TGF- β [37, 38], or the intestine, which utilizes localized gut-associated lymphoid tissue (GALT) networks for *in situ* regulation [39], the lung relies heavily on extrinsic regulation via dLNs [40, 41]. This underscores why the reconnection of lymphatic vessels abruptly shut WOFIE in the lung unlike successful experience in the liver or intestine transplant. Furthermore, the decay of protection on POD 35 may reflect the rapid turnover kinetics of the systemic myeloid reservoir, where the initial DSBT-conditioned regulatory niche is eventually replaced by a secondary wave of recipient monocytes derived from *de novo* hematopoiesis, primed by persistent graft inflammatory signals.

Several limitations warrant consideration. First, the small sample size ($n = 5-6$) reflects the inherent technical complexity of the murine orthotopic LTx model. Second, while the 24-h preconditioning window presents logistical challenges, it remains manageable via hypothermic preservation strategy [42]. Third, future lineage-tracing and intravital microscopy are needed to definitively confirm the MDP origin and migration kinetics of the observed cDC2s. Furthermore, experiments involving a third-party (non-donor) mouse strain are necessary to confirm whether the observed protective effect is strictly donor-specific. Finally, the failure to induce permanent tolerance—as achieved in other organ

models—highlights the need for further optimization of DSBT parameters (e.g., timing, adjunct immunosuppression, and duration) specifically tailored to the lung's unique immunological environment.

In summary, our findings represent the first successful establishment of a DSBT preconditioning protocol in a murine orthotopic LTx model. We demonstrate that this strategy safely and effectively induces a transfusion-related early protective effect, characterized by preserved graft function and a recalibrated myeloid landscape during the critical first postoperative week. Our hypothesis-generating work establishes a critical baseline for the refinement of DSBT protocols, aiming to achieve sustained graft protection and minimize the requirement for long-term immunosuppression. The predictable nature of this 7-day temporal window provides a foundational platform and a novel translational framework for managing the high immunogenic hurdles inherent to clinical LTx.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

The animal study was approved by Ethical Committee for Animal Research at KU Leuven (P015/2024). The study was conducted in accordance with the local legislation and institutional requirements.

AUTHOR CONTRIBUTIONS

XJ, JV, RV, BV, JP, and LC designed the experiment; XJ, CH, BÖ, JK, MG and KM conducted the experiments and collected the data; XJ wrote the manuscript. All authors contributed to the article and approved the submitted version.

FUNDING

The author(s) declared that financial support was received for this work and/or its publication. XJ holds a predoctoral mandate for fundamental research from the Research Foundation - Flanders (FWO 11PGT24N), Belgium. CH holds a predoctoral mandate for fundamental research from the Research Foundation - Flanders (FWO 1152225N), Belgium. RV is supported as a

senior clinical research fellow by the Research Foundation - Flanders (FWO 1803521N), Belgium. LC is supported as a senior clinical research fellow by the Research Foundation - Flanders (FWO 18E2B24N), Belgium.

CONFLICT OF INTEREST

The author(s) declared that this work was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

GENERATIVE AI STATEMENT

The author(s) declared that generative AI was used in the creation of this manuscript. Declaration of Generative AI and AI-assisted technologies in the writing process: During the preparation of this work the authors used Gemini 3 Pro (Google) in order to improve readability and language. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

Any alternative text (alt text) provided alongside figures in this article has been generated by Frontiers with the support of artificial intelligence and reasonable efforts have been made to ensure accuracy, including review by the authors wherever possible. If you identify any issues, please contact us.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontierspartnerships.org/articles/10.3389/ti.2026.15409/full#supplementary-material>

SUPPLEMENTARY FIGURE S1 | Gating strategy for flow cytometric analysis of immune cells in the left lung graft and recipient spleen on POD7 after lung transplantation (n=5). Donor- or recipient-derived cells were separated via MHC-I (H2K^d/H2K^b) on cell surface. Because the alveolar macrophage is only in the lung graft, the gate of lung interstitial macrophage is the same for all macrophages in the spleen. cDC: conventional dendritic cell; pDC: plasmacytoid dendritic cell; ILC: innate lymphoid cell; LTx: lung transplantation; MHC: major histocompatibility complex; NK: natural killer cell; Treg: regulatory T cell.

SUPPLEMENTARY FIGURE S2 | Flow cytometric analysis of donor-derived cells in left lung graft and recipient spleen on POD7 after lung transplantation (n=5). No significant deviation between the control and DSBT group was observed by POD 7. Donor-derived immune cells in the **(A)** left lung graft and **(B)** spleen. *Donor- or recipient-derived alveolar macrophages are excluded from the study because these cells are autofluorescent, which were gated before separating circulating/tissue cells and BALB/c/C57BL/6N); **Cell population for median absolute count <50 is excluded from the study due to interference from the background noise. cDC: conventional dendritic cell; pDC: plasmacytoid dendritic cell; LTx: lung transplantation; Treg: regulatory T cell.

REFERENCES

- Bos S, Vos R, Van Raemdonck DE, Verleden GM. Survival in Adult Lung Transplantation: Where Are We in 2020? *Curr Opin Organ Transpl* (2020) 25: 268–73. doi:10.1097/MOT.0000000000000753
- Chambers DC, Cherikh WS, Harhay MO, Hayes D, Jr, Hsich E, Khush KK, et al. The International Thoracic Organ Transplant Registry of the International Society for Heart and Lung Transplantation: Thirty-Sixth Adult Lung and Heart-Lung Transplantation Report-2019; Focus Theme: Donor and Recipient Size Match. *J Heart Lung Transpl* (2019) 38:1042–55. doi:10.1016/j.healun.2019.08.001

3. Bharat A. A Need for Targeted Immunosuppression After Lung Transplantation. *Am J Respir Cell Mol Biol* (2019) 61:279–80. doi:10.1165/rcmb.2019-0100ED
4. Thabut G, Mal H. Outcomes After Lung Transplantation. *J Thorac Dis* (2017) 9:2684–91. doi:10.21037/jtd.2017.07.85
5. Jin X, Pirenne J, Vos R, Hooft C, Kaes J, Van Slambrouck J, et al. Donor-Specific Blood Transfusion in Lung Transplantation. *Transpl Int* (2024) 37:12822. doi:10.3389/ti.2024.12822
6. Halasz NA, Orloff MJ, Hirose F. Increased Survival of Renal Homografts in Dogs After Injection of Graft Donor Blood. *Transplantation* (1964) 2:453–8. doi:10.1097/00007890-196407000-00001
7. Salvatierra O, Jr., Vincenti F, Amend W, Potter D, Iwaki Y, Opelz G, et al. Deliberate Donor-specific Blood Transfusions Prior to Living Related Renal Transplantation. A New Approach. *Ann Surg* (1980) 192:543–52. doi:10.1097/0000658-198010000-00012
8. Ceulemans LJ, Braza F, Monbaliu D, Jochmans I, De Hertogh G, Du Plessis J, et al. The Leuven Immunomodulatory Protocol Promotes T-Regulatory Cells and Substantially Prolongs Survival After First Intestinal Transplantation. *Am J Transpl* (2016) 16:2973–85. doi:10.1111/ajt.13815
9. Kitade H, Kawai M, Rutgeerts O, Landuyt W, Waer M, Mathieu C, et al. Early Presence of Regulatory Cells in Transplanted Rats Rendered Tolerant by Donor-Specific Blood Transfusion. *J Immunol* (2005) 175:4963–70. doi:10.4049/jimmunol.175.8.4963
10. Jin X, Kaes J, Van Slambrouck J, Inci I, Arni S, Geudens V, et al. A Comprehensive Review on the Surgical Aspect of Lung Transplant Models in Mice and Rats. *Cells* (2022) 11:11. doi:10.3390/cells11030480
11. Kaes J, Pollenus E, Hooft C, Liu H, Aelbrecht C, Cambier S, et al. The Immunopathology of Pulmonary Rejection After Murine Lung Transplantation. *Cells* (2024) 13:13. doi:10.3390/cells13030241
12. Stewart S, Fishbein MC, Snell GI, Berry GJ, Boehler A, Burke MM, et al. Revision of the 1996 Working Formulation for the Standardization of Nomenclature in the Diagnosis of Lung Rejection. *J Heart Lung Transpl* (2007) 26:1229–42. doi:10.1016/j.healun.2007.10.017
13. Hooft C, Prenen F, Jin X, Kaes J, Pollenus E, Kerckhof P, et al. Investigating Lung Chimerism by Flow Cytometry in Mouse Allografts After Lung Transplantation. *The J Heart Lung Transpl* (2025) 44:S771–S2. doi:10.1016/j.healun.2025.02.1661
14. Saadeh D, Kurban M, Abbas O. Update on the Role of Plasmacytoid Dendritic Cells in Inflammatory/Autoimmune Skin Diseases. *Exp Dermatol* (2016) 25:415–21. doi:10.1111/exd.12957
15. Fabre JW, Morris PJ. The Effect of Donor Strain Blood Pretreatment on Renal Allograft Rejection in Rats. *Transplantation* (1972) 14:608–17. doi:10.1097/00007890-197211000-00013
16. Okazaki M, Krupnick AS, Kornfeld CG, Lai JM, Ritter JH, Richardson SB, et al. A Mouse Model of Orthotopic Vascularized Aerated Lung Transplantation. *Am J Transpl* (2007) 7:1672–9. doi:10.1111/j.1600-6143.2007.01819.x
17. Calne R. WOFIE Hypothesis: Some Thoughts on an Approach Toward Allograft Tolerance. *Transplant Proc* (1996) 28:1152.
18. Gilliss BM, Looney MR. Experimental Models of Transfusion-Related Acute Lung Injury. *Transfus Med Rev* (2011) 25:1–11. doi:10.1016/j.tmr.2010.08.002
19. Toy P, Lowell C. TRALI--Definition, Mechanisms, Incidence and Clinical Relevance. *Best Pract Res Clin Anaesthesiol* (2007) 21:183–93. doi:10.1016/j.bpa.2007.01.003
20. Mannem HC, Donahoe MP. Transfusion and Acute Respiratory Distress Syndrome: Clinical Epidemiology, Diagnosis, Management, and Outcomes. *Hematologic Abnormalities and Acute Lung Syndromes* (2017) 213–28. doi:10.1007/978-3-319-41912-1_11
21. Ochando J, Mulder WJM, Madsen JC, Netea MG, Duivenvoorden R. Trained Immunity - Basic Concepts and Contributions to Immunopathology. *Nat Rev Nephrol* (2023) 19:23–37. doi:10.1038/s41581-022-00633-5
22. Schluter T, van Elsas Y, Priem B, Ziogas A, Netea MG. Trained Immunity: Induction of an Inflammatory Memory in Disease. *Cell Res* (2025) 35:792–802. doi:10.1038/s41422-025-01171-y
23. Alvarez R, Oliver L, Valdes A, Mesa C. Cancer-Induced Systemic Myeloid Dysfunction: Implications for Treatment and a Novel Nanoparticle Approach for Its Correction. *Semin Oncol* (2018) 45:84–94. doi:10.1053/j.seminoncol.2018.05.001
24. Guak H, Al HS, Ma EH, Aldossary H, Al-Masri M, Won SY, et al. Glycolytic Metabolism Is Essential for CCR7 Oligomerization and Dendritic Cell Migration. *Nat Commun* (2018) 9:2463. doi:10.1038/s41467-018-04804-6
25. Steinman RM, Hawiger D, Nussenzweig MC. Tolerogenic Dendritic Cells. *Annu Rev Immunol* (2003) 21:685–711. doi:10.1146/annurev.immunol.21.120601.141040
26. Menezes S, Melandri D, Anselmi G, Perchet T, Loschko J, Dubrot J, et al. The Heterogeneity of Ly6C(hi) Monocytes Controls Their Differentiation Into Inos(+) Macrophages or Monocyte-Derived Dendritic Cells. *Immunity* (2016) 45:1205–18. doi:10.1016/j.immuni.2016.12.001
27. Williams M, Scott CL. Does Niche Competition Determine the Origin of Tissue-Resident Macrophages? *Nat Rev Immunol* (2017) 17:451–60. doi:10.1038/nri.2017.42
28. Schreurs I, Meek B, van Moorsel CHM, van Kessel DA, Luijk HD, Grutters JC. Monocyte Derived Macrophages From Lung Transplantation Patients Have an Increased M2 Profile. *Transplant Rep* (2020) 5:5. doi:10.1016/j.tpr.2019.100038
29. van den Bosch TP, Caliskan K, Kraaij MD, Constantinescu AA, Manintveld OC, Leenen PJM, et al. CD16+ Monocytes and Skewed Macrophage Polarization Toward M2 Type Hallmark Heart Transplant Acute Cellular Rejection. *Front Immunol* (2017) 8:346. doi:10.3389/fimmu.2017.00346
30. Santambrogio L, Strominger JL. The Ins and Outs of MHC Class II Proteins in Dendritic Cells. *Immunity* (2006) 25:857–9. doi:10.1016/j.immuni.2006.11.007
31. Koshiba T, Kitade H, Waer M, Mathieu C, Van Damme B, Pirenne J. Break of Tolerance Via Donor-specific Blood Transfusion by High Doses of Steroids: A Differential Effect After Intestinal Transplantation and Heart Transplantation. *Transpl Proc* (2003) 35:3153–5. doi:10.1016/j.transproceed.2003.10.042
32. Kitade H, Kawai M, Koshiba T, Giulietti A, Overbergh L, Rutgeerts O, et al. Early Accumulation of Interferon-Gamma in Grafts Tolerized by Donor-Specific Blood Transfusion: Friend or Enemy? *Transplantation* (2004) 78:1747–55. doi:10.1097/01.tp.0000147788.23922.5b
33. Mansouri S, Katikaneni DS, Gogoi H, Pipkin M, Machuca TN, Emtiazjoo AM, et al. Lung IFNAR1(hi) TNFR2(+) cDC2 Promotes Lung Regulatory T Cells Induction and Maintains Lung Mucosal Tolerance at Steady State. *Mucosal Immunol* (2020) 13:595–608. doi:10.1038/s41385-020-0254-1
34. Creusot RJ, Yaghoubi SS, Chang P, Chia J, Contag CH, Gambhir SS, et al. Lymphoid-Tissue-Specific Homing of Bone-Marrow-Derived Dendritic Cells. *Blood* (2009) 113:6638–47. doi:10.1182/blood-2009-02-204321
35. Outtz Reed H, Wang L, Kahn ML, Hancock WW. Donor-Host Lymphatic Anastomosis After Murine Lung Transplantation. *Transplantation* (2020) 104:511–5. doi:10.1097/TP.00000000000003041
36. Wong BW. Lymphatic Vessels in Solid Organ Transplantation and Immunobiology. *Am J Transpl* (2020) 20:1992–2000. doi:10.1111/ajt.15806
37. Patseas D, El-Masry A, Liu Z, Ramachandran P, Triantafyllou E. Myeloid Cells in Chronic Liver Inflammation. *Cell Mol Immunol* (2025) 22:1237–61. doi:10.1038/s41423-025-01324-4
38. Puri M, Sonawane S. Liver Sinusoidal Endothelial Cells in the Regulation of Immune Responses and Fibrosis in Metabolic Dysfunction-Associated Fatty Liver Disease. *Int J Mol Sci* (2025) 26:3988. doi:10.3390/ijms26093988
39. Morbe UM, Jorgensen PB, Fenton TM, von Burg N, Riis LB, Spencer J, et al. Human Gut-Associated Lymphoid Tissues (GALT): Diversity, Structure, and Function. *Mucosal Immunol* (2021) 14:793–802. doi:10.1038/s41385-021-00389-4
40. Trivedi A, Reed HO. The Lymphatic Vasculature in Lung Function and Respiratory Disease. *Front Med (Lausanne)* (2023) 10:1118583. doi:10.3389/fmed.2023.1118583
41. Zhang E, Zhao B, Liu Y, Liu G, Lu S, Xiong L, et al. Protocol for *In Situ* and *In Vivo* Dissection of Mouse Lung-Draining Lymph Nodes. *STAR Protoc* (2024) 5:103487. doi:10.1016/j.xpro.2024.103487
42. Cenik I, Van Slambrouck J, Provost AL, Barbarossa A, Vanluyten C, Boelhouwer C, et al. Controlled Hypothermic Storage for Lung Preservation: Leaving the Ice Age Behind. *Transpl Int* (2024) 37:12601. doi:10.3389/ti.2024.12601

Copyright © 2026 Jin, Hooft, Özsoy, Van Slambrouck, Kaes, Vanluyten, Barbarossa, Carlon, Vande Velde, Guyot, Moermans, Stegen, Vos, Vanaudenaerde, Pirenne and Ceulemans. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.



Cellular Immunity in Chronic Kidney Disease and Changes After Kidney Transplantation

Georgios Lioulios^{1*}, Eleni Moisidou², Michalis Christodoulou², Efstratios Kasimatis², Aliko Xochelli³, Evangelos Memmos⁴, Stamatia Stai², Maria Stangou² and Asimina Fylaktou³

¹Department of Nephrology, 424 General Military Hospital, Thessaloniki, Greece, ²First Department of Nephrology, Aristotle University of Thessaloniki, General Hospital of Thessaloniki "Hippokratio", Thessaloniki, Greece, ³National Peripheral Histocompatibility Center, Department of Immunology, General Hospital of Thessaloniki "Hippokratio", Thessaloniki, Greece, ⁴Department of Nephrology, "Papageorgiou" General Hospital, Thessaloniki, Greece

This study evaluates changes in cellular immunity components, from chronic kidney disease stage V (CKD-V) to long-term transplantation (IKTx). We applied flow cytometry to determine total, CD4⁺, CD8⁺, CD28⁺ T-lymphocytes, Natural killer cells (NK) and regulatory T-lymphocytes (Tregs), in peripheral blood of 56 patients with CKD-V, 207 patients on hemodialysis (HD), 149 recently transplanted (rKTx), 26 IKTx patients and 49 healthy volunteers as a control group (CG). Lymphocyte proportion decreased in CKD vs. CG [20.2 (14.4–25.8) vs. 29.7 (24.4–38.1)%, $p < 0.001$] without further deterioration in HD [19.9 (15.3–23.7)%, $p = 0.83$ vs. CKD-V] and increased in rKTx [24.7 (20–32.6)%, $p < 0.001$ vs. HD], and IKTx [25.5 (21.9–35)%, $p = 0.16$ vs. CG]. Similar kinetics were observed in CD4⁺ subpopulations, however CD8⁺ T-cells gradually increased from CG to IKTx. NK remained stable in CKD-V and HD, reduced in rKTx, and marginally increased in IKTx. Tregs gradually declined until HD and suboptimally improved with transplantation. CD28⁺ subpopulations largely increased in IKTx, compared with HD [CD4⁺CD28⁺: 12.6 (4.7–27.6) vs. 6 (2.1–13.2)%, $p = 0.006$, CD8⁺CD28⁺: 68.4 (54.4–90.3) vs. 45.5 (28.4–58.9)%, $p < 0.001$], independently of age for CD8⁺CD28⁺ ($p = 0.33$). Cellular immunity subpopulations show significant changes in the spectrum of CKD, with transplantation restoring total lymphocytes and CD4⁺ T-cells, but not Tregs and NK. LKTx was associated with a large increase in CD28⁺ subpopulations.

OPEN ACCESS

*Correspondence

Georgios Lioulios,
✉ pter43@yahoo.gr

Received: 21 September 2025

Revised: 01 December 2025

Accepted: 06 January 2026

Published: 03 February 2026

Citation:

Lioulios G, Moisidou E, Christodoulou M, Kasimatis E, Xochelli A, Memmos E, Stai S, Stangou M and Fylaktou A (2026) Cellular Immunity in Chronic Kidney Disease and Changes After Kidney Transplantation. *Transpl. Int.* 39:15622. doi: 10.3389/ti.2026.15622

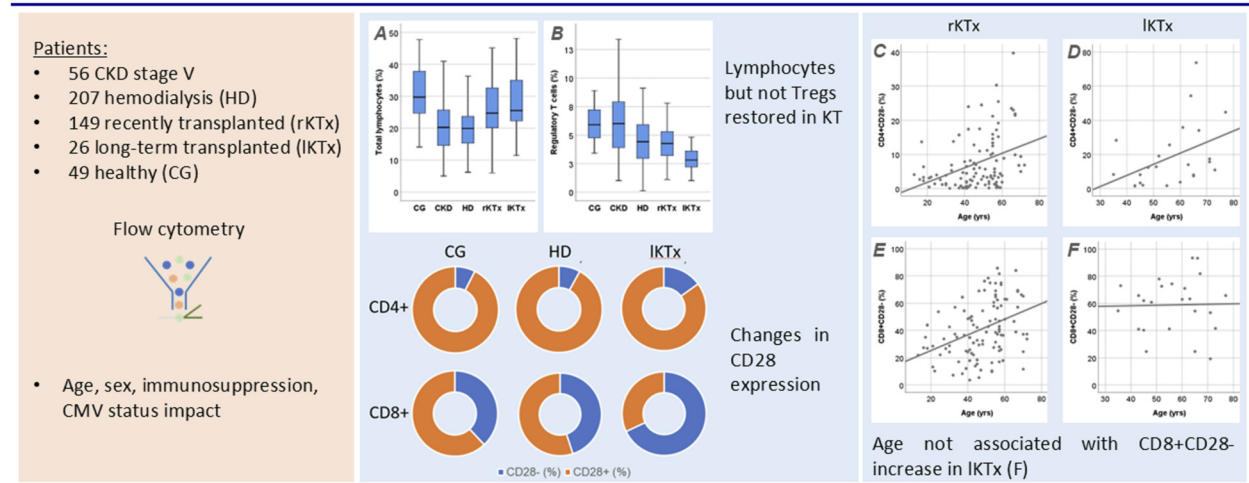
Keywords: CD28⁺ T cells, chronic kidney disease, dialysis, senescence, T cells, transplantation, regulatory T cells, natural killers

INTRODUCTION

Chronic kidney disease (CKD) is characterized by phenotypic and functional alterations of cellular elements of immune system, leading to relative immunodeficiency in these patients. This is clinically expressed as increased susceptibility in infections, decreased response to vaccination, as well as increased risk for neoplasia, potentially due to altered immune surveillance [1–3]. As a result, patients in hemodialysis (HD) have a greater mortality rate, with life expectancy being as low as 25%–30% compared to healthy individuals of the same age [1].

CKD has an impact on all cellular populations of acquired immunity, with T cells being the most well studied. One main alteration is the loss of CD28 expression on their surface [4]. CD28 is a

Cellular immunity in chronic kidney disease and changes after kidney transplantation



LIOLIOS, et al. *Transpl. Int.* 2026

doi: [10.3389/ti.2026.15622](https://doi.org/10.3389/ti.2026.15622)



GRAPHICAL ABSTRACT |

transmembrane protein that provides the second signal in the immune synapse between T cell and antigen-presenting cell and thus is indispensable for T cell activation [5]. Failure of CD28 to bind to its ligands CD80 and CD86, during T cell receptor recognition of antigen presented on HLA molecule, leads to impaired of T cell activation and anergy [6]. Moreover, other lymphocytic populations such as regulatory T cells (Tregs) and natural killers (NK) have been described to be altered in CKD patients [7].

The substitution of kidney function with kidney transplantation improves immune function, potentially partially restoring the above-described cellular alterations. However, these alterations have not been adequately described in the whole cohort of CKD (pre-HD, HD, short- and long-term kidney transplantation). The purpose of the present study was to evaluate changes in cellular immunity elements from non-dialysis stage V CKD to long lasting kidney transplantation.

PATIENTS AND METHODS

Study Design

This is an observational study conducted in the First Department of Nephrology of Aristotle University of Thessaloniki, in collaboration with the Department of Immunology, National Histocompatibility Center, of General Hospital of Thessaloniki “Hippokraton”, from June 2021 to June 2024. Four groups of patients were included in the study; patients with CKD stage V not yet in dialysis (estimated glomerular filtration rate - eGFR: <15 mL/min/1.73 m²) – CKD-V group; patients in

chronic maintenance hemodialysis for at least 1 year – HD group; recently transplanted patients (1 year since kidney transplantation) – rKTx group; long transplanted patients (>17 years since kidney transplantation) – IKTx group. The cut-off of 17 years was the average predicted 3rd quantile of living and deceased donors graft survival in years, as reported in a large study published in 2021 [8]. All transplanted patients had received an ABO and HLA compatible graft, with no detectable anti-HLA antibodies against donor's HLA molecules.

Demographic, clinical and laboratory characteristics, medication, and also, hemodialysis prescription data (for those on HD), were recorded on the day of enrollment. For all patients IgG antibodies levels against cytomegalovirus were measured. Patients eligible for the study were Caucasians, adults, with a minimum of 12-month follow-up in our department. All patients should be in stable treatment for the last 3 months, with well controlled hyperparathyroidism and anemia. Patients undergoing hemodialysis were on a chronic maintenance thrice a week program with Kt/V >1.2, with satisfactory regulation of biochemical parameters, according to international guidelines. For rKTx and IKTx patients, eGFR should be >30 mL/min/1.73 m² calculated using the equation CKD-EPI 2021 for creatinine. Patients were excluded in case of a recent infection or vaccination (<3 months), active malignancy, autoimmune, or hematologic disorder (<5 years), or prior immunosuppressive therapy within the past year (excluding steroids, MMF, and cyclosporine or tacrolimus for transplant patients). Moreover, we excluded transplanted patients receiving agents other than those mentioned in the following section, such as everolimus or belatacept, or patients on a corticosteroid sparing scheme. Forty-

nine age- and sex-matched healthy volunteers were used as a control group (CG). In all patient groups and the CG, we assessed immune phenotype with flow cytometry in peripheral blood samples, as described in the relevant section.

Treatment Protocols

All transplanted patients had received standardized immunosuppressive treatment according to the Transplant Clinic protocol. Induction therapy consisted of basiliximab or anti-thymocyte globulin (ATG), according to the following. The standard induction regimen consisted of two doses of basiliximab 20 mg (administered 60 min before and on day 4 post-transplant) and two doses of methylprednisolone 500 mg, 12 and 1 h before transplantation. Patients in medium immunological risk, for example patients with history of donor specific antibodies (DSA) positivity, high expected cold ischemia time or marginal donor, received induction therapy with 1.5 mg/kg ATG for 4 days to a total dose of 6 mg/kg. Maintenance therapy included methylprednisolone (125 mg intravenously on day one and then 16 mg orally until day 14, with a gradual tapering until day 42), tacrolimus (plasma levels 6–8 ng/mL for the first year), and mycophenolate mofetil (2 g per day until day 14 and then 1 g daily). Patients with T-cell mediated rejection episodes received ATG, whereas patients with antibody mediated rejection episodes received corticosteroids and/or plasma exchange, depending on the indication.

All study participants signed an informed consent prior to enrollment. The study was conducted in accordance with the Declaration of Helsinki and was approved by the Bioethics Committee of the Aristotle University of Thessaloniki (no. 2348/24-11-2019).

Flow Cytometry

Immune phenotype examination was performed with flow cytometry on whole blood samples collected in K2EDTA tubes, within a short period after collection and in any case in no more than 12 h. Analysis was performed on a Navios EX Flow Cytometer (Beckman Coulter), according to the manufacturer's instructions, using the following conjugated antibodies: CD45 PC7 (Clone J33), CD3 FITC (Clone UCHT1), CD16-CD56 PE [CD16: Clone 3G8, CD56: Clone N901(NKH-1)], CD4 APC (Clone 13B8.2), CD8 PC5.5 (Clone B9.11), CD4 FITC (Clone SF112T4D11), CD25 PC5 (Clone B1.49.9), and FOXP3 PE (Clone 259D, intracellular staining). Percentages of CD4⁺ (CD3⁺CD4⁺), CD8⁺ (CD3⁺CD8⁺), regulatory (CD4⁺CD25⁺FOXP3⁺) T lymphocytes as well as NK cells (CD3⁺CD16⁺CD56⁺) and CD28⁺ (CD4⁺CD28⁺ and CD8⁺CD28⁺) T cells were determined. The total lymphocyte count was also determined from a blood count in the same sample. Gating strategy is shown in **Figure 1** and was manually adjusted in each case.

Singlets discrimination was performed with plotting forward scatter integral (FS-INT) versus forward scatter Time of Flight (FS-TOF). Events deviating from the singlet line were considered cell aggregates and discarded from analysis. Compensation settings were determined before experimental analysis, by using single stained control samples. One sample for each conjugated antibody was analyzed separately to measure the spectral overlap of each fluorochrome's emission into other

detectors and mathematical compensation was applied and adjusted manually to correct the spillover. No viability check was needed, as the analysis was performed in fresh blood samples.

Statistical Analysis

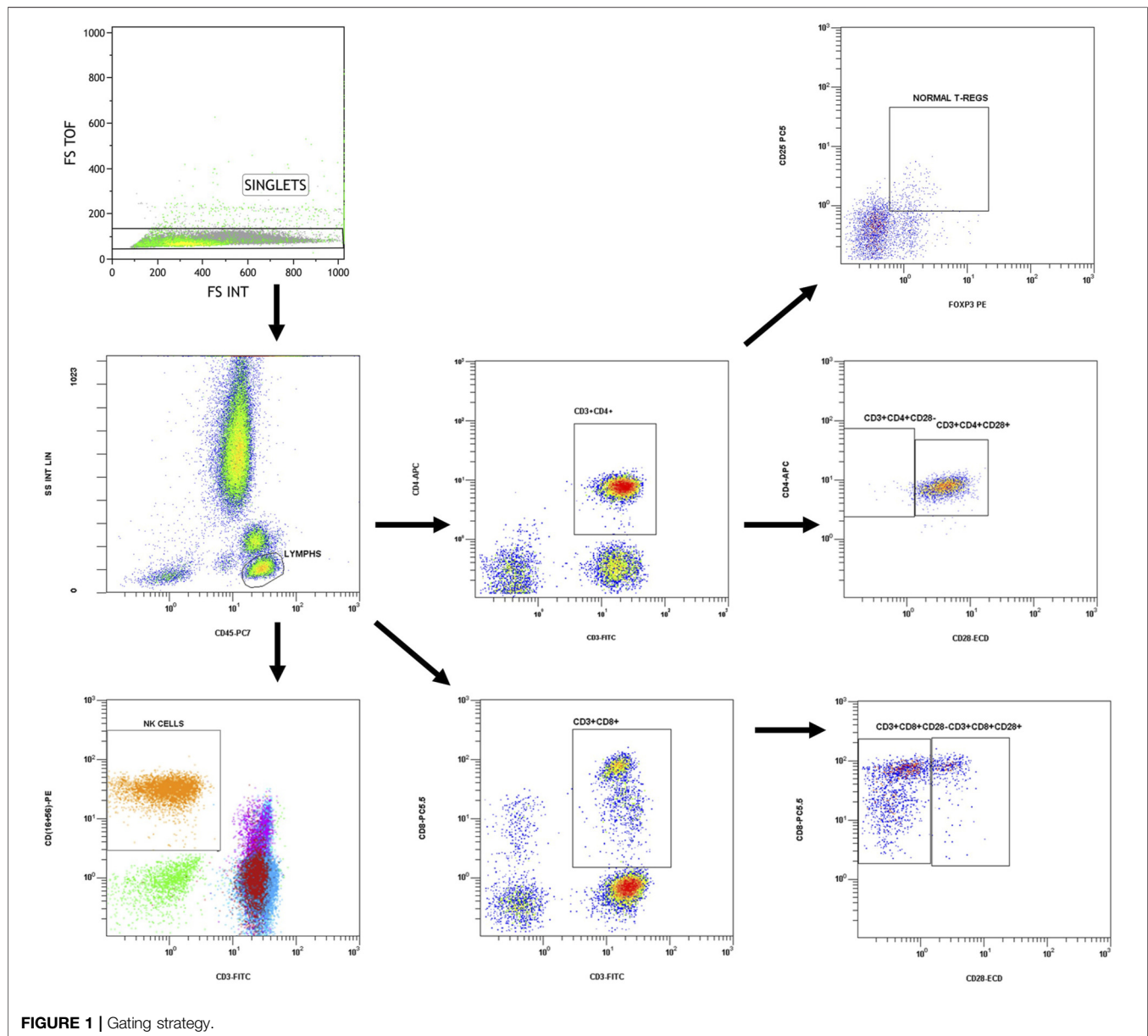
Statistical analysis was performed with Statistical Package for the Social Sciences (SPSS) for Windows, version 25 (IBM, NY, United States). Continuous variables are presented as median (25th – 75th percentile). Quantitative variables were compared with Mann-Whitney U-test between two groups, or with Kruskal-Wallis H-test among multiple groups. The χ^2 test was used to compare differences in non-quantitative variables between two groups. Correlation between two continuous variables was assessed using the Spearman test. The result was considered significant when R was greater than the critical value for the degrees of freedom. Linear univariate regression was used to predict simple models after logarithmic transformation of the data, and linear multivariate analysis was applied to find independent factors associated with a variable. Statistical significance was considered at a p value of <0.05. Finally, in cases of comparison of more than two different populations, the Bonferroni correction was applied.

Given the use of non-parametric tests between CG, HD and LKTx patients and for a significance level of 0.005 (after Bonferroni correction) with 80% power, the expected effect size for the variable “CD8⁺CD28⁺ T cells percentage” was estimated based on a previous similar study [9], corresponding to a moderate effect (Cohen's $d \approx 0.48$, corresponding to $r \approx 0.23$). Based on this effect size, the adequate sample size for the LKTx group was calculated to $N = 121$ patients. Due to practical limitations, the sample size for LKTx patients ($N = 26$) in this study is smaller than the calculated one. Accordingly, the findings of the present study referring to the LKTx group should be interpreted with caution and considered exploratory.

RESULTS

Patients' Demographics

In total, 438 patients were included in the study, aged 54 (42–62) years. Out of them 56 were patients with CKD-V, 207 patients on HD for at least 1 year, 149 rKTx patients (1 year post-transplantation), and 26 LKTx patients (>17 years post-transplantation). The sex and median age of the patients did not differ among groups [58 (39–69) years for CKD-V, 55 (43–62) years for HD, 51 (41–57) years for rKTx and 58 (45–66) years for LKTx patients, $p = 0.788$]. The control group included 49 healthy individuals aged 53 (39–64) years. Patients' demographics are demonstrated in **Table 1**. In the rKTx group, 22 patients had received ATG as an induction treatment, as described above. Moreover, seven patients had a rejection episode during the first year, 5 out of which had biopsy-proven T cell mediated rejection and received ATG for treatment. All these patients had received ATG for induction. The rest two patients had antibody mediated rejection and were treated with plasma exchange, corticosteroids and intravenous immunoglobulin. In the LKTx group, one patient had a T cell mediated rejection episode and he was treated with



ATG both for induction and rejection. Another one patient had an antibody mediated rejection and was also treated with corticosteroids and plasma exchange. Moreover, in the same group, two patients were not on calcineurin inhibitors at the time of flow cytometry analysis, for three and 5 years, because of chronic toxicity. Moreover, three of the patients in the rKTx cohort had a PCR proven CMV reactivation. All of these patients were already CMV seropositive at transplantation.

Differences in Lymphocytic Subpopulations Among Patients

Table 2 shows the differences of lymphocytic cell subsets in patients' groups, compared to the CG. In the following

comparisons of lymphocyte subpopulations, the level of statistical significance was set at $p < 0.005$, after Bonferroni correction. Total lymphocyte percentage was reduced in patients with CKD-V compared to CG, 20.2 (14.4–25.8) vs. 29.7 (24.4–38.1)%, respectively, $p < 0.001$, without further substantial deterioration after dialysis initiation [19.9 (15.3–23.7)% in HD, $p = 0.83$ compared to CKD-V]. Kidney transplantation resulted in an early increase in total lymphocyte percentage during the first year, 24.7 (20–32.6) vs. 19.9 (15.3–23.7)% in rKTx and HD respectively, $p < 0.001$, with no increase iKTx 25.5 (21.9–35)%, $p = 0.27$ compared to rKTx and $p = 0.16$ compared to CG (Table 2). Similar kinetics were observed in $CD4^+$ subpopulation proportion (Figure 2A), however, with $CD8^+$ T lymphocytes percentage presenting a

TABLE 1 | Patients' demographics.

Patients' characteristics		CKD-V	HD	rKTx	IKTx
Patients	N	56	207	149	26
Median age, years		58 (39–69)	55 (43–62)	51 (41–57)	58 (45–66)
Sex, male/female		32/24	123/84	99/50	12/14
<i>Cause of CKD</i>					
Primary glomerulonephritis		11	49	42	11
Hypertension		16	33	26	5
Diabetes mellitus		16	28	11	3
Polycystic kidney disease		5	21	27	4
Obstructive nephropathy		0	14	18	1
Other		0	12	11	0
Unknown		8	50	14	2
<i>Treatment modality</i>					
Online hemodiafiltration			54		
Median dialysis vintage			84 (19–126)	84 (24–126)	30 (16–65)
<i>Comorbidities</i>					
Hypertension		50	143	115	24
Cardiovascular disease		11	54	45	18
Diabetes mellitus		16	30	27	7
Dyslipidemia		21	77	64	20
History of kidney transplantation (%)		-	35	-	-
ATG treatment, N (%)				22 (14.7)	5 (19.2)
Preemptive Ktx				12	3
Live donor				44	16
Delayed graft function				22	2
Rejection episode				7	2
Retransplantation (%)				13 (8.7)	4 (15.3)
Pre-transplantation anti-HLA sensitization (PRA >5%)				56	11

Continuous variables are reported as median (25th – 75th percentile), CKD-V: chronic kidney disease stage V, HD: hemodialysis, rKTx: recent kidney transplantation, IKTx: long-term kidney transplantation.

gradual upward tendency which was significant in the rKTx group compared to CG, CKD-V and HD [29.6 (24.3–38.3) in rKTx vs. 21.9 (17.1–31.1)% in CG, $p < 0.001$, vs. 23.3 (20.1–31.9)% in CKD-V $p < 0.001$, vs. 24.8 (19.1–30.6)% in HD, $p < 0.001$, (**Figure 2B**). Similar kinetics were observed in the total counts of total lymphocytes and $CD4^+$ T cells, whereas the total count of $CD8^+$ T cells was reduced in HD in comparison to HC and peaked in the IKTx group (**Supplementary Table S1; Supplementary Figure S1**).

HD patients presented a significant increase of NK proportion compared to CG [17.6 (11.8–24.3) vs. 10.9 (9.4–15.8)% respectively, $p = 0.009$]. In the rKTx group, NK were found to have significantly decreased in comparison to HD [8.9 (5.6–14.9) vs. 17.6 (11.8–24.3)%, $p < 0.001$], without difference from CG ($p = 0.07$) and IKTx patients ($p = 0.70$) (**Figure 3A**). The count of NK cells was found to reach a nadir in the group of rKTx, but the fluctuations were not significant after Bonferroni correction (**Supplementary Table S1; Supplementary Figure S2**).

Treg percentage was found to be within normal range in CKD-V patients [6 (3.7–7.9) vs. 5.9 (4.4–7.4)%, for CKD-V and CG, respectively, $p = 0.76$] but showed a decline after HD initiation, 4.4 (2.9–5.9)%, $p < 0.001$ compared to CKD-V. One year after kidney transplantation, Treg percentage remained stable [4.2 (3.2–5.3)%, $p = 0.30$, in comparison to HD, but a further decline was observed in long-term transplantation 2.8

(1.7–3.7)%, $p < 0.001$ in comparison to rKTx (**Figure 3B**). The Treg count decreased gradually from CG to HD, following the total lymphocyte decrease. However, a slight but significant increase was observed in Treg count after transplantation, despite the drop in their proportion (**Supplementary Table S1; Supplementary Figure S2**).

The percentage of $CD28^-$ T cell subpopulations increased in HD patients compared to CG, with $CD8^+CD28^-$ increasing already before dialysis initiation, in the latest stages of CKD-V, 38 (24.4–49.8), 49.3 (24.2–63.7) and 45.5 (28.4–58.9)% for CG, CKD-V and HD respectively, $p = 0.049$ for CKD-V vs. CG and $p = 0.026$, for HD vs. CG. In the $CD4^+CD28^-$ subpopulation, the increase was observed only after HD initiation, 3.5 (1.4–6.8), 3.8 (1–11.9) and 6 (2.1–13.2)%, for CG, CKD-V and HD respectively, $p = 0.64$ for CKD-V vs. CG and $p = 0.02$ for CKD-V vs. HD. However, the above changes were not significant after Bonferroni correction. Kidney transplantation resulted in restoration of $CD4^+CD28^-$ and $CD8^+CD28^-$ to normal levels already from the first year after transplantation. However, in the group of long-term transplanted patients, an increase in both $CD28^-$ subpopulations was observed to levels considerably higher even than from those of HD patients, $CD4^+CD28^-$: 12.6 (4.7–27.6) vs. 6 (2.1–13.2)%, for IKTx and HD respectively, $p = 0.006$, $CD8^+CD28^-$: 68.4 (54.4–90.3) vs. 45.5 (28.4–58.9)% for IKTx and HD respectively, $p < 0.001$ (**Figure 4**). The steep increase of $CD28^-$ T cell subsets in IKTx patients was also

TABLE 2 | Differences in proportions of lymphocytic subpopulations among healthy controls and patient groups of different stage of chronic kidney disease.

Cell population, %	CG	CKD-V	KD	rKTx	IKTx	p
	N = 49	N = 56	N = 207	N = 149	N = 26	
Total lymphocytes	29.7 (24.4–38.1)	20.2 (14.4–25.8) ^a	19.9 (15.3–23.7) ^a	24.7 (20–32.6) ^{a,b,c}	25.5 (21.9–35) ^{b,c}	<0.001
CD4 ⁺	50.6 (45.1–55.1)	48.1 (38.7–52.8)	43.2 (37.4–52.1) ^a	45.3 (38.1–53.8)	53.1 (43.1–59) ^c	<0.001
CD8 ⁺	21.9 (17.1–31.1)	23.3 (20.1–31.9)	24.8 (19.1–30.6)	29.6 (24.3–38.3) ^{a,b,c}	27.3 (24.7–34.4)	<0.001
NK	10.9 (9.4–15.8)	12.2 (9.9–19.8)	17.6 (11.8–24.3)	8.9 (5.6–14.7) ^c	9.5 (5.1–14.3) ^c	<0.001
Tregs	5.9 (4.4–7.4)	6 (3.7–7.9)	4.4 (2.9–5.9) ^{a,b}	4.2 (3.2–5.3) ^{a,b}	2.8 (1.7–3.7) ^{a,b,c,d}	<0.001
CD4 ⁺ CD28 [−]	3.5 (1.4–6.8)	3.8 (1–11.9)	6 (2.1–13.2) ^a	4.6 (1.5–11.1)	12.6 (4.7–27.6) ^{a,b,c,d}	<0.001
CD8 ⁺ CD28 [−]	38 (24.4–49.8)	49.3 (24.2–63.7) ^a	45.5 (28.4–58.9) ^a	39.1 (26.7–59.9)	68.4 (54.4–90.3) ^{a,b,c,d}	<0.001

CG: control group, CKD-V: chronic kidney disease stage V, HD: hemodialysis, rKTx: recent kidney transplantation, IKTx: long-term kidney transplantation.

^aStatistical significance from CG.

^bStatistical significance from CKD-V.

^cStatistical significance from HD.

^dStatistical significance from rKTx. For the above comparisons statistical significance level has been set to <0.005 after Bonferroni correction.

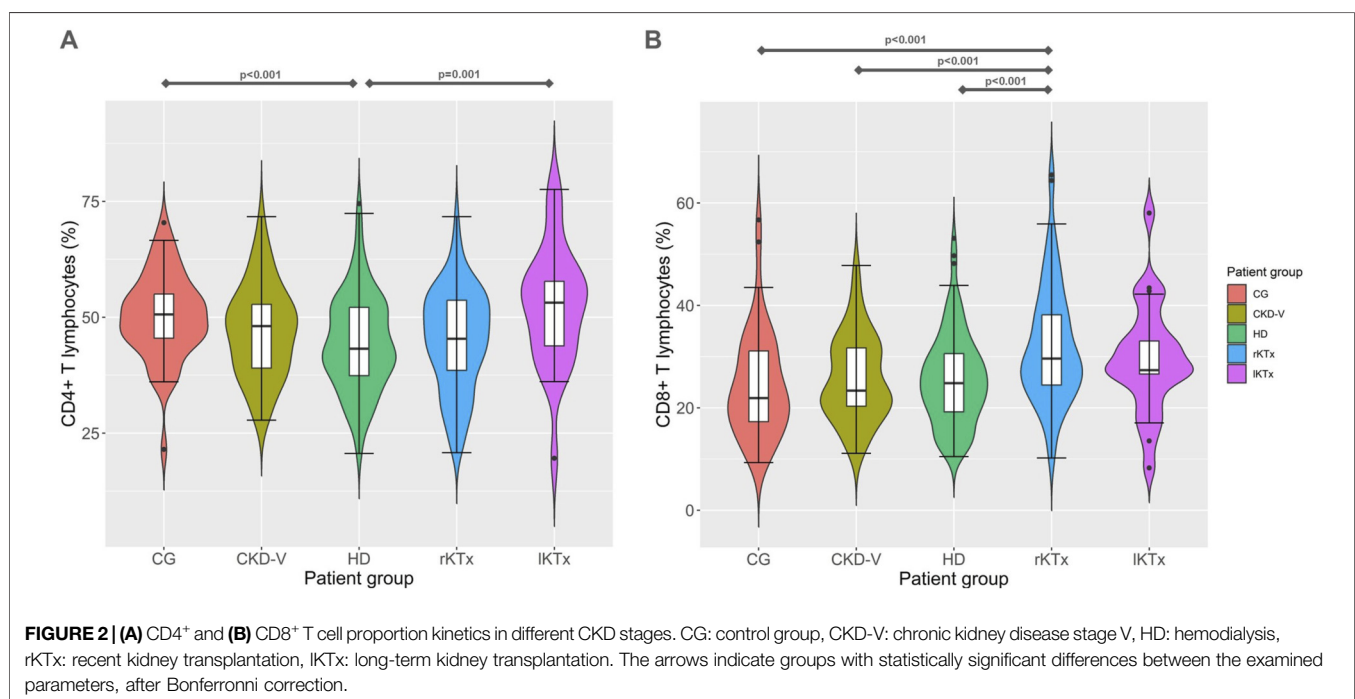


FIGURE 2 | (A) CD4⁺ and **(B)** CD8⁺ T cell proportion kinetics in different CKD stages. CG: control group, CKD-V: chronic kidney disease stage V, HD: hemodialysis, rKTx: recent kidney transplantation, IKTx: long-term kidney transplantation. The arrows indicate groups with statistically significant differences between the examined parameters, after Bonferroni correction.

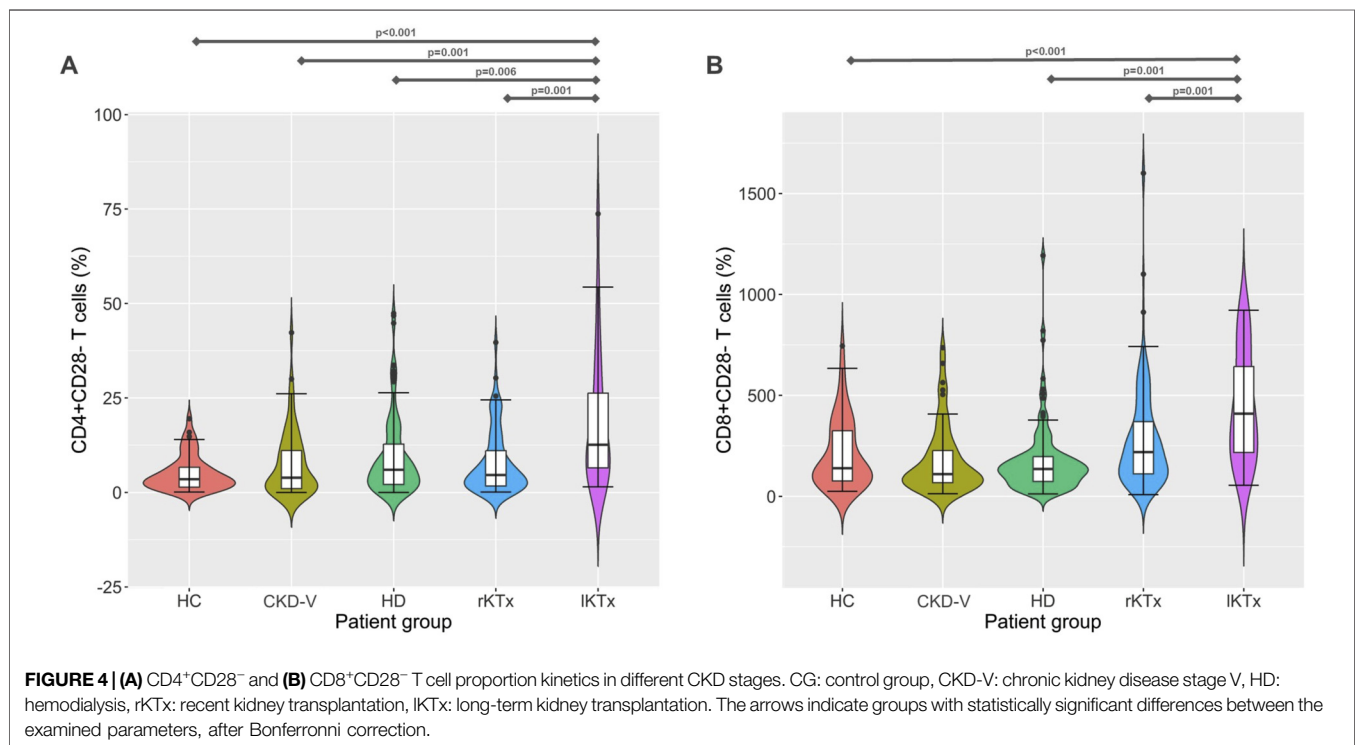
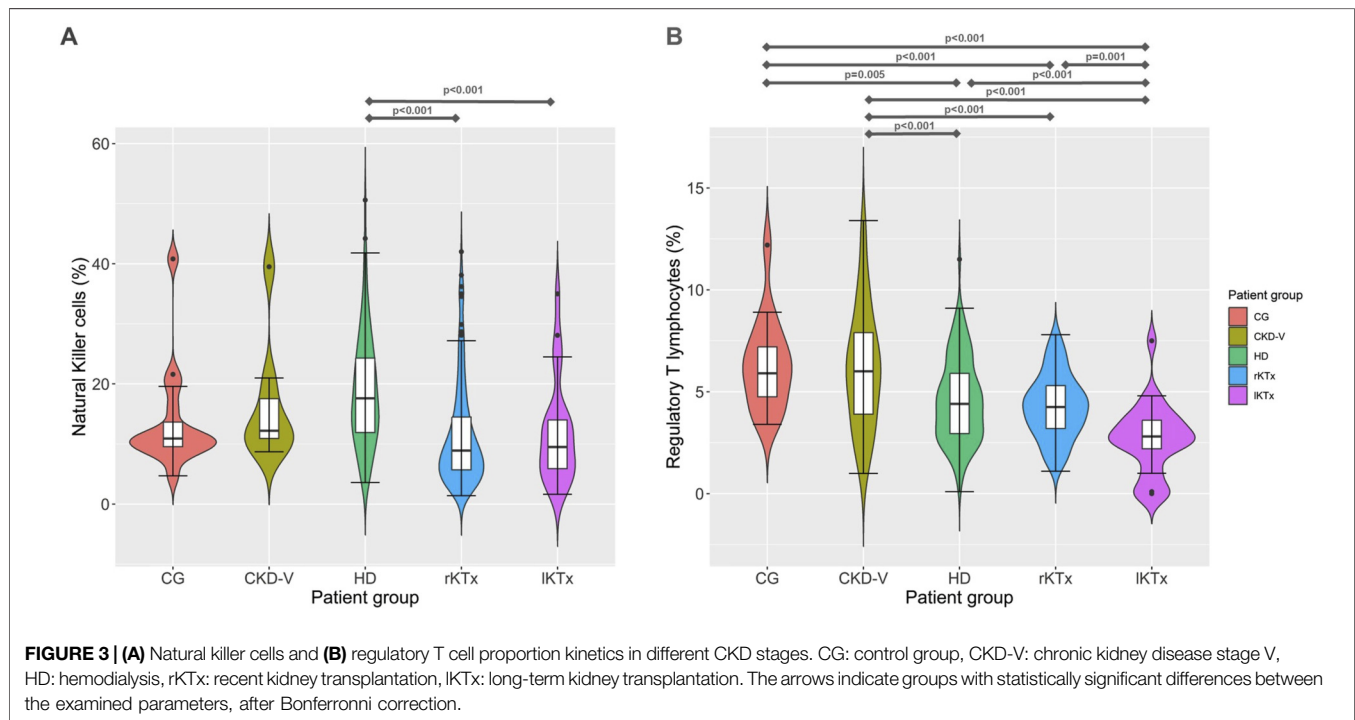
significant when total cell counts were examined (Supplementary Table S1).

Patients Sex, Age, Previous CMV Infection and Renal Function Were the Main Parameters Associated With the Immune Phenotype

Immune phenotype differed significantly between male and female patients in the HD group. Women had an increased total lymphocyte proportion in comparison to men [21.4 (17.5–25.9) vs. 18.1 (14.6–22.4)%, $p < 0.001$] which was due to increased CD4⁺ T cell proportion [46.5 (38.1–53.8) vs. 42.3 (36–49)%, $p = 0.038$], but not CD8⁺ T cells [24.3 (20.3–29.2) vs. 25.3 (18.1–31.6)%, $p = 0.99$]. Moreover, NK were decreased

in women on HD [14.5 (10.3–20.7) vs. 19.3 (14.4–26.6)%, $p < 0.001$], with no differences in the rest of analyzed cell subsets. Sex remained significant after inclusion of age and CMV status in a multivariate model only for total lymphocytes percentage (95% CI: 0.025–0.321, $p = 0.023$) and NK (95% CI: -0.587 – -0.071, $p = 0.013$) but not for CD4⁺ T cells percentage (95% CI: -0.034 – 0.182, $p = 0.178$). In comparison, in the CG, only CD4⁺ T cell proportion differed between male and female patients [46.2 (39.2–53.6) vs. 43.9 (37.5–47.9)% for females and males respectively, $p = 0.028$].

Patients' age was a significant predictor of the percentage of CD8⁺CD28[−] T cells in all patient groups studied, as well as in healthy volunteers, except for the IKTx group (Figure 5). The linear regression data are shown in Table 3. In kidney transplant patients, both rKTx and IKTx, age was an independent predictor



of the percentage of CD4⁺CD28⁻ T cells (Table 3). In the rKTx group, age was an independent predictor for several T cell populations, such as total lymphocytes percentage ($r = -0.31$, $R^2 = 0.097$, 95% CI: $-0.275 - -0.088$, $p < 0.001$), NK percentage ($r = 0.26$, $R^2 = 0.071$, 95% CI: $0.066 - 0.276$, $p = 0.002$) CD4⁺ T

lymphocytes count ($r = -0.4$, $R^2 = 0.16$, 95% CI: $-15.34 - -6.92$, $p < 0.001$) and Tregs count ($r = -0.31$, $R^2 = 0.09$, 95% CI: $-0.685 - -0.216$, $p < 0.001$). In the IKTx group, NK proportion depended also on the age ($r = 0.49$, $R^2 = 0.21$, 95% CI: $0.073 - 0.643$, $p = 0.016$).

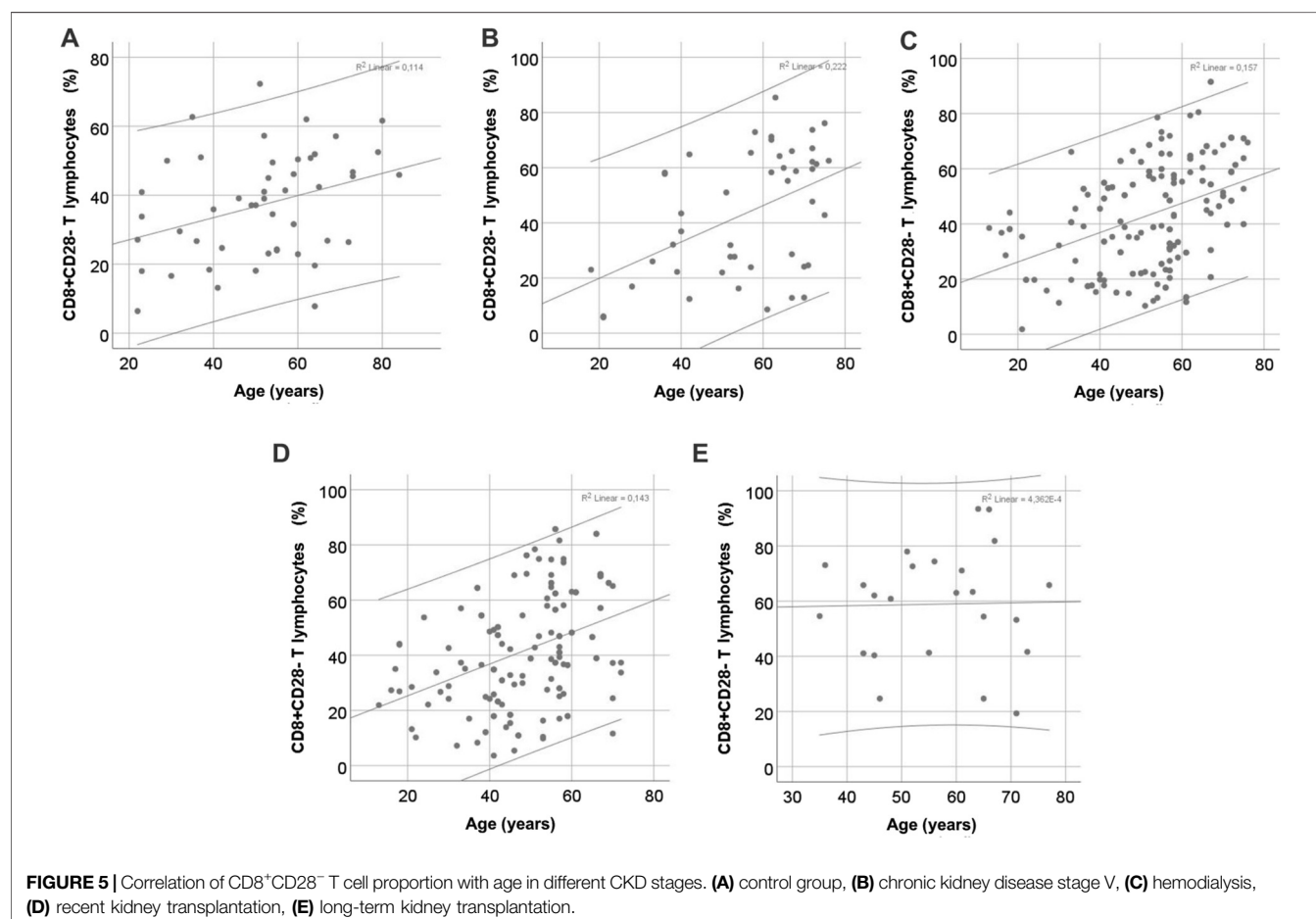


TABLE 3 | Uni- and multivariate linear regression for predictions of CD4⁺CD28⁻ and CD8⁺CD28⁻ T cell percentage, based on age, in patient groups of different stages of chronic kidney disease.

Patient group	Univariate linear regression				Multivariate linear regression		
	B	R ²	95% CI	p	B	95% CI	p
CD4 ⁺ CD28 ⁻							
CG	0.04	0.002	-0.018 – 0.024	0.791	–	–	–
CKD-V	0.31	0.1	0.002–0.048	0.036	0.56	-0.003 – 0.091	0.058
HD	0.09	0.008	-0.006 – 0.024	0.226	–	–	–
rKTx	0.30	0.09	0.015–0.046	<0.001	0.25	0.005–0.046	0.014
lKTx	0.47	0.22	0.009–0.083	0.018	0.48	0.007–0.085	0.024
CD8 ⁺ CD28 ⁻							
CG	0.33	0.11	0.002–0.02	0.02	–	–	–
CKD-V	0.48	0.23	0.009–0.029	0.001	0.68	-0.029 – 0.083	0.22
HD	0.37	0.14	0.01–0.02	<0.001	0.32	0.004–0.022	0.005
rKTx	0.33	0.11	0.008–0.022	<0.001	0.30	0.005–0.023	0.004
lKTx	0.04	0.002	-0.017 – 0.14	0.839	–	–	–

CG: control group, CKD-V: chronic kidney disease stage V, HD: hemodialysis, rKTx: recent kidney transplantation, lKTx: long-term kidney transplantation. In the multivariate linear regression models anti-CMV antibodies titres and eGFR were included.

Anti-CMV IgG antibodies levels could predict the percentage of CD4⁺CD28⁻ and CD8⁺CD28⁻ T lymphocytes in HD patients, ($r = 0.29$, $R^2 = 0.08$, 95% CI: 0.001–0.007, $p = 0.011$ and $r = 0.28$, $R^2 = 0.08$, 95% CI: 0.000–0.03, $p = 0.015$ respectively). However,

in multivariate regression including age, the antibody levels did not remain statistically significant for the prediction of CD8⁺CD28⁻ T lymphocytes and age remained the only independent factor. Correlation of anti-CMV IgG antibody

levels with the percentage of $CD4^+CD28^-$ T lymphocytes, ($r = 0.31$, $R^2 = 0.1$, 95% CI: 0.002–0.007, $p = 0.002$) was also observed in rKTx patients but not in the lKTx group ($r = 0.071$, $R^2 = 0.1$, 95% CI: -0.002 – 0.029, $p = 0.072$). In multivariate analysis, both antibody levels and age remained independent factors for the percentage of $CD4^+CD28^-$ T lymphocytes. Finally, eGFR was not a predictor of the percentage of $CD28^-$ T lymphocytes in either of the groups of transplanted patients.

Dialysis vintage (DV) was associated negatively to the total lymphocytes proportion in HD patients ($r = -0.18$, $R^2 = 0.035$, 95% CI: -0.002 – 0.000, $p = 0.016$), affecting mainly $CD4^+$ T cells ($r = -0.22$, $R^2 = 0.052$, 95% CI: -0.002 – 0.000, $p = 0.003$), but not $CD8^+$ T cells ($r = 0.06$, $R^2 = 0.004$, 95% CI: -0.001 – 0.001, $p = 0.468$). NK were positively associated to DV ($r = 0.19$, $R^2 = 0.038$, 95% CI: 0.000–0.004, $p = 0.017$), while Tregs decreased as DV increased ($r = -0.17$, $R^2 = 0.028$, 95% CI: -0.003 – 0.000, $p = 0.031$). $CD4^+CD28^-$ T cells proportion was also affected ($r = 0.17$, $R^2 = 0.03$, 95% CI: 0.000–0.009, $p = 0.035$), but not $CD8^+CD28^-$ T cells proportion ($r = -0.08$, $R^2 = 0.007$, 95% CI: -0.001 – 0.003, $p = 0.297$). However, in the multivariate models including sex, age and CMV status, DV was significant for none of the analyzed cell subsets. This effect of DV in total lymphocytes and $CD4^+$ T cells was also retained during the first year of transplantation, but was not significant in multivariate analysis or lKTx patients.

Estimated GFR was a predictor of $CD4^+$ and $CD8^+$ T cell proportion ($r = 0.21$, $R^2 = 0.045$, 95% CI: 0.001–0.005, $p = 0.012$ and $r = 0.17$, $R^2 = 0.029$, 95% CI: -0.006 – 0.000, $p = 0.043$, respectively) and Tregs count ($r = 0.21$, $R^2 = 0.047$, 95% CI: 0.002–0.012, $p = 0.01$) in rKTx patients, but this correlation did not remain significant in multivariate analysis including age and anti-CMV antibodies levels.

ATG Treatment Affects Immune Phenotype

History of ATG treatment in the transplant patient groups had impact on phenotype of T cells. In the rKTx groups $N = 22$ patients had received ATG for induction therapy. These patients had a lower $CD4^+$ T cell proportion in comparison to patients who had not received ATG [36.9 (29.6–45.9) vs. 47.7 (40.2–53.9)% respectively, $p = 0.003$], as well as more $CD8^+$ T cells [38 (25.9–46.3) vs. 28.9 (24.1–36.8)% respectively, $p = 0.037$], and more $CD8^+CD28^-$ T cells [49 (37–69) vs. 37 (24–57)% respectively, $p = 0.013$] (**Supplementary Table S2**). However, in the multivariate linear model with age, sex and CMV status as confounding factors, ATG treatment significantly affected only the $CD4^+$ T cell proportion (95% CI: -0.367 – -0.09, $p = 0.002$), along with age, but neither of $CD8^+$ or $CD8^+CD28^-$ T cells, and age was a significant factor for $CD8^+CD28^-$ T cells percentage. In the lKTx group no difference was found in the immune phenotype according to ATG treatment ($N = 5$ patients had received ATG, data on **Supplementary Table S2**). In order to increase the generalizability of the study and eliminate the impact of ATG treatment on lymphocytic subsets, we performed the comparisons of the examined subsets among the patients groups, after excluding the ATG treated patients. The kinetics of subpopulations were not substantially altered, as shown in the **Supplementary Table S3**.

DISCUSSION

In this study, changes in specific lymphocyte subpopulations were examined across the spectrum of CKD, from the pre-end stage to long-term transplantation. One of the main conclusions was that CKD, especially in the end-stage, is associated with lymphopenia and a decrease in $CD4^+$ T lymphocytes, perhaps with a disruption in the ratio between them. Moreover, successful kidney transplantation was found to restore total lymphocyte counts, as well as $CD4^+$ to normal levels especially in the long term, with a gradual increase of $CD8^+$ T lymphocyte proportion, up to the first-year post-transplantation. However, in the setting of lKTx no improvement in $CD28^-$ T cell proportion was found, but rather a great increase.

Changes in $CD4^+$ and $CD8^+$ T lymphocyte populations in CKD have been described in several studies, without their findings being consistent [4]. In a study published in 2004, an increase in total lymphocytes and $CD4^+$ T cell counts was described as early as 4 weeks after kidney transplantation [10]. However, the underlying mechanism is not well understood, as data on the recovery of thymic function after transplantation are insufficient [11, 12]. It is worth noting that in another more recent study, it was shown that $CD4^+$ T cell counts were lower compared to patients with CKD or healthy subjects, despite an increase in total T cell counts, 3 years after transplantation [13]. Nonetheless, it is not clear whether this numerical amelioration is accompanied by functional restoration. Chronic exposure to the uremic milieu may deeply affect lymphocytic function, a change that has not been proven to reverse after transplantation. It is interesting that old $CD4^+$ T cells have compromised metabolic pathways due to dysfunctional mitochondria accumulation and are not able to cover their energy demands after activation [14]. Moreover, their capacity to form immunological synapses with antigen-presenting cells is severely reduced [15].

The literature regarding alterations of Tregs after kidney transplantation remains very restricted. One study published in 2019 reports low levels of Tregs in the first 6 months after kidney transplantation, followed by a gradual recovery to near baseline levels during the first year [16]. In addition, the study by Wang et al. reported a further decrease in the number of Tregs after transplantation compared with CKD [13], but these findings were not confirmed by our study. It is likely that increased immunosuppression during the early period after transplantation plays a role in this phenomenon [17].

Reduced levels of Tregs likely have an important clinical role, as they have been associated with an increased risk of acute rejection and chronic graft dysfunction [18, 19]. Decreased proportions of Tregs have been reported in stable kidney graft recipients under immunosuppressive treatment [20]. However, the same study presents differences in the functional state of Tregs, highlighting the role of Foxp3 demethylation in these cells, which may be capable of inducing tolerance in neighboring effector T cells, reducing their reactivity [21]. This ability is impaired by the proinflammatory state in the setting of transplantation, potentially under the effect of interleukin (IL) 6 [21]. IL-6 can be secreted independently of $CD28$ costimulation, but in T cells, $CD28$ signaling acts as a

major amplifier of IL-6 production [22]. Moreover, experimental inhibition of IL-6 resulted in increase in Tregs frequency and function, and prolonged graft survival [21]. Whether loss of CD28 expression is associated with an improvement in Tregs number and function remains to be clarified, but is supported by our data (**Supplementary Figure S3**).

Data on NK changes in CKD and after kidney transplantation are even more limited. In a study published in 2016, a low frequency of NK was associated with immune risk phenotype (IRP) in transplant recipients both before and 1 year post-transplantation [23]. Although no specific immune phenotype was associated with opportunistic infection, the latter was more frequent in patients with IRP. The relationship between NK phenotype and IRP in dialysis patients was also reported by Dewolfe et al. The authors associated NK frequency and distinctive phenotypic characteristics of NK with IRP + status in dialysis patients [24]. Moreover, another small study found no difference in NK cell proportions or counts between healthy women and those who had undergone transplantation [25]. Adequate NK function may be of great interest in the context of transplantation. Besides immunosurveillance, NK have been shown to eliminate senescent T cells *in vitro* and *in vivo* [26], which secrete pro-inflammatory mediators, resulting in low-level chronic inflammation, associated with tissue dysfunction. It is worth noting that senescent T cells do not originate only from the recipient, but also senescent T cell from the graft may participate to increased inflammatory activity [26].

Despite age being a significant factor affecting T cell subsets in healthy individuals [27], data on the role of age in immune phenotype of CKD patients remain inconclusive [4, 7]. However, it has been reported that after transplantation, only younger individuals were capable of restoring, at least partially, the proportion of naïve T cells [28]. Moreover, expansion of the terminally differentiated T cell subset, expressing CD57 epitope, has been found to elderly kidney transplant recipients 1 year after transplantation and was associated with CMV infection [29]. This “memory expansion” of terminally differentiated cells occurs already during the first year after transplantation, and may not be related to clinical infection or viremia [30]. These cells are deemed senescent as they express several senescence features, among other telomere attrition [4, 29]. Our findings regarding age-dependent acceleration of senescence in recent transplant recipients are not discordant to these previous findings, as expression of CD57 interrelates to loss of CD28 expression, and the resulting cells are considered highly senescent [4, 31]. However, Schaenman et al. found no significant difference in CD28⁺ T cell percentage between young and elder kidney graft recipients [28].

One of the most interesting findings of our study was the prominent increase in CD28⁺ T cell subsets in kidney graft recipients after long-term transplantation, which was found to be independent of age. Similar results were reported in another study, in kidney grafts recipients, at least 4 years post-transplantation [32], with CD8⁺CD28⁺ T cells expressing innate-like receptors [9]. However, it is unclear whether CD28⁺ T cell subset expansion, due to other reasons, leads to increased graft survival, or transplantation *per se* results in accumulation of CD28⁺ T cells. We are inclined to

believe for the latter mechanism for several reasons. Firstly, CD28⁺ T cell proportion showed a downward tendency during the first year after transplantation, and in any case their proportion did not increase. Secondly, principal mechanism of CD28 loss is the repeated activation of the memory cell, leading to downregulation of transcription factor for CD28 [33], thus we can hypothesize that the longer the antigenic stimulation, the more prominent the CD28 loss [34]. Tolerance to the graft has been associated with the recipient age, with younger recipients having a greater risk to develop *de novo* Donor Specific Antibodies than elder patients [35]. Moreover, upregulation of CD28 expression in older individuals resulted in acceleration of graft rejection, in experimental animal models [36]. However, in our study, age was not found to be a confounding factor for CD8⁺CD28⁺ T cells proportion.

Transplant therapy regimen was found to affect immune phenotype in the rKTx group. ATG has been shown to induce apoptosis in CD4⁺CD28⁺ T cells, a T cell subset known for apoptosis deficits, rather than in CD4⁺CD28⁺ T cells, shortly after administration, in a small study published in 2012 [37]. However, levels of this T cell subset did not differ between patients with or without ATG administration, 1 year after transplantation. Of note, it has been postulated that one mechanism of increase of highly differentiated cell subsets, such as CD28⁺, is the rapid cell proliferation after cell elimination [38]. The same mechanism is also proposed by Lee et al. who associated ATG treatment with decreased CD4⁺ and increased CD28⁺ T cells, without reporting any correlation with patients' age. This latter study also suggests severe functional deficits in lymphocytes of ATG treated patients, that may last for up to 1 year after transplantation [39]. Nonetheless, according to our findings, CD28⁺ T cell expansion appears to be independent of treatment regimen in lKTx recipients. Similar results were reported by another study, for CMV responsive memory cells, which is a subset that tends to expand over time [30, 40].

Other immunosuppressive agents, widely used in transplantation, may have impact on CD28⁺ T cell subsets. It has been reported that kidney graft recipients have increased proportions of CD28⁺ T cells, even in the absence of CMV infection [41]. Even though these cells are deemed to be prone in apoptosis, their survival may increase in the presence of IL-6 and IL-15 [21, 42–44]. IL-15 may also promote CD28 expression loss *in vitro* [45]. Increased secretion of IL-6 may begin shortly after transplantation due to ischemia-reperfusion injury, innate immune system activation, and alloreactions against class II HLA molecules [21]. Whether the persistence of these reactions for years can lead to CD28⁺ T cell expansion is not clear. Moreover, CD28 signaling is key to IL-2 production, which is reported to be low in KTx patients [41, 46]. Treatment with tacrolimus has been associated with low IL-2 secretion in old CD4⁺ but not CD8⁺ T cells [47]. Moreover, cyclosporine A was reported to reduce telomerase activity, in T cell, *in vivo* [32]. Even though CD28⁺ T cells are characterized by reduced telomerase activity, it is not clear whether cyclosporine A could also decrease CD28 expression.

We acknowledge several limitations in our study. Even though the examined populations were age and sex matched, we could

not include comparable number of individuals in each patient group, and as a result some populations, such as lKTx patients, are under-represented. Moreover, this is a single center study and therefore, the generalizability of the results may be limited. The design of the study also reduces reliability of the results, as a longitudinal examination of the same patients at different timepoints would offer a better insight in the immune alterations. Despite the numerical changes in the NK cells found, no data on intensity of CD56 expression were available, as the reagent used was a combined CD16-CD56 antigen. Dim or bright expression of CD56 has been associated with functional differences. Finally, this study did not demonstrate any potential molecular mechanisms leading to these observations, nor associated the findings with clinical characteristics of the patients, such as rejection episodes or opportunistic infections. Especially for the lKTx cohort, CD28⁺ T cell expansion may predispose to cardiovascular and infectious complications [48].

Overall, our results indicate a significant fluctuation of the phenotype of cellular immunity components across the range of CKD. Receiving a kidney graft is capable of restoring some of these alterations shortly after transplantation, however the long-term antigenic stimulation in lKTx patients is associated with senescent T cell subsets expansion.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving humans were approved by Bioethics Committee of the Aristotle University of Thessaloniki. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

REFERENCES

1. Kramer A, Pippias M, Noordzij M, Stel VS, Andrushev AM, Aparicio-Madre MI, et al. The European Renal Association - European Dialysis and Transplant Association (ERA-EDTA) Registry Annual Report 2016: A Summary. *Clin Kidney J* (2019) 12(5):702–20. doi:10.1093/ckj/sfz011
2. Vogelzang JL, van Stralen KJ, Noordzij M, Diez JA, Carrero JJ, Couchoud C, et al. Mortality From Infections and Malignancies in Patients Treated With Renal Replacement Therapy: Data From the ERA-EDTA Registry. *Nephrol Dial Transpl* (2015) 30(6):1028–37. doi:10.1093/ndt/gfv007
3. Go AS, Chertow GM, Fan D, McCulloch CE, yuan HC. Chronic Kidney Disease and the Risks of Death, Cardiovascular Events, and Hospitalization. *New Engl J Med* (2004) 351(13):1296–305. doi:10.1056/NEJMoa041031
4. Lioulios G, Fylaktou A, Papagianni A, Stangou M. T Cell Markers Recount the Course of Immunosenescence in Healthy Individuals and Chronic Kidney Disease. *Clin Immunol* (2021) 225:108685. doi:10.1016/j.clim.2021.108685
5. Rudd CE. Upstream-Downstream: CD28 Cosignaling Pathways and T Cell Function. *Immunity* (1996) 4(6):527–34. doi:10.1016/s1074-7613(00)80479-3

AUTHOR CONTRIBUTIONS

GL and EM statistical analysis and manuscript drafting, EM and EK study design, MC and SS data collection, AX and AF methodology and lab supervision, MS and AF study supervision and manuscript final approval. All authors contributed to the article and approved the submitted version.

FUNDING

The author(s) declared that financial support was not received for this work and/or its publication.

CONFLICT OF INTEREST

The authors(s) declared that this work was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

GENERATIVE AI STATEMENT

The author(s) declared that generative AI was not used in the creation of this manuscript.

Any alternative text (alt text) provided alongside figures in this article has been generated by Frontiers with the support of artificial intelligence and reasonable efforts have been made to ensure accuracy, including review by the authors wherever possible. If you identify any issues, please contact us.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontierspartnerships.org/articles/10.3389/ti.2026.15622/full#supplementary-material>

6. Powell JD, Ragheb JA, Kitagawa-Sakakida S, Schwartz RH. Molecular Regulation of Interleukin-2 Expression by CD28 Co-Stimulation and Anergy. *Immunol Rev* (1998) 165:287–300.
7. Vagiots L, Lioulios G, Panteli M, Ouranos K, Xochelli A, Kasimatis E, et al. Kidney Transplantation and Cellular Immunity Dynamics: Immune Cell Alterations and Association With Clinical and Laboratory Parameters. *J Clin Med* (2024) 13(17):5093. doi:10.3390/jcm13175093
8. Poggio ED, Augustine JJ, Arrigain S, Brennan DC, Schold JD. Long-Term Kidney Transplant Graft Survival—Making Progress When Most Needed. *Am J Transplant* (2021) 21(8):2824–32. doi:10.1111/ajt.16463
9. Daniel L, Tassery M, Lateur C, Thierry A, Herbelin A, Gombert JM, et al. Allotransplantation Is Associated With Exacerbation of CD8 T-Cell Senescence: The Particular Place of the Innate CD8 T-Cell Component. *Front Immunol* (2021) 12:674016. doi:10.3389/fimmu.2021.674016
10. Satoh S, Tsuchiya N, Sato K, Ohtani H, Komatsuda A, Habuchi T, et al. Influence of Pretransplant Dialysis Modality on the Change of Lymphocyte Subset Populations and Acute Rejection Rates After Renal Transplantation. *Int J Urol* (2004) 11(10):825–30. doi:10.1111/j.1442-2042.2004.00915.x

11. Betjes MGH, Langerak AW, Klepper M, Litjens NHR. A Very Low Thymus Function Identifies Patients With Substantial Increased Risk for Long-Term Mortality After Kidney Transplantation. *Immun Ageing* (2020) 17:4. doi:10.1186/s12979-020-00175-z
12. Schaier M, Leick A, Uhlmann L, Kälble F, Morath C, Eckstein V, et al. End-Stage Renal Disease, Dialysis, Kidney Transplantation and Their Impact on CD4(+) T-Cell Differentiation. *Immunology* (2018) 155(2):211–24. doi:10.1111/imm.12947
13. Wang L, Rondaan C, de Joode AAE, Raveling-Eelsing E, Bos NA, Westra J. Changes in T and B Cell Subsets in End Stage Renal Disease Patients Before and After Kidney Transplantation. *Immun Ageing* (2021) 18(1):43. doi:10.1186/s12979-021-00254-9
14. Nian Y, Iske J, Maenosono R, Minami K, Heinbokel T, Quante M, et al. Targeting Age-specific Changes in CD4+ T Cell Metabolism Ameliorates Alloimmune Responses and Prolongs Graft Survival. *Aging Cell* (2021) 20(2):e13299. doi:10.1111/accel.13299
15. Garcia GG, Miller RA. Single-Cell Analyses Reveal Two Defects in Peptide-Specific Activation of Naive T Cells From Aged Mice. *The J Immunol* (2001) 166(5):3151–7. doi:10.4049/jimmunol.166.5.3151
16. Krajewska M, Kościelska-Kasprzak K, Kamińska D, Żabińska M, Myszkak-Kozłowska M, Gomułkiewicz A, et al. Kidney Transplant Outcome Is Associated With Regulatory T Cell Population and Gene Expression Early After Transplantation. *J Immunol Res* (2019) 2019:7452019. doi:10.1155/2019/7452019
17. Segundo DS, Ruiz JC, Fernández-Fresnedo G, Izquierdo M, Gómez-Alamillo C, Cacho E, et al. Calcineurin Inhibitors Affect Circulating Regulatory T Cells in Stable Renal Transplant Recipients. *Transpl Proc* (2006) 38(8):2391–3. doi:10.1016/j.transproceed.2006.08.081
18. Braza F, Durand M, Degauque N, Brouard S. Regulatory T Cells in Kidney Transplantation: New Directions? *Am J Transplant* (2015) 15:2288–300. doi:10.1111/ajt.13395
19. Bestard O, Cruzado JM, Lucia M, Crespo E, Casis L, Sawitzki B, et al. Prospective Assessment of Antidonator Cellular Alloreactivity Is a Tool for Guidance of Immunosuppression in Kidney Transplantation. *Kidney Int* (2013) 84(6):1226–36. doi:10.1038/ki.2013.236
20. Braza F, Dugast E, Panov I, Paul C, Vogt K, Pallier A, et al. Central Role of CD45RA- Foxp3hi Memory Regulatory T Cells in Clinical Kidney Transplantation Tolerance. *J Am Soc Nephrol* (2015) 26(8):1795–805. doi:10.1681/ASN.2014050480
21. Chandran S, Tang Q. Impact of interleukin-6 on T Cells in Kidney Transplant Recipients. *Am J Transplant* (2022) 22(S4):18–27. doi:10.1111/ajt.17209
22. Glinos DA, Soskic B, Williams C, Kennedy A, Jostins L, Sansom DM, et al. Genomic Profiling of T-Cell Activation Suggests Increased Sensitivity of Memory T Cells to CD28 Costimulation. *Genes Immun* (2020) 21(6–8):390–408. doi:10.1038/s41435-020-00118-0
23. Crepin T, Gaiffe E, Courivaud C, Roubiou C, Laheurte C, Moulin B, et al. Pre-Transplant End-Stage Renal Disease-Related Immune Risk Profile in Kidney Transplant Recipients Predicts Post-Transplant Infections. *Transpl Infect Dis* (2016) 18(3):415–22. doi:10.1111/tid.12534
24. Dewolfe D, Aid M, McGann K, Ghofrani J, Geiger E, Helzer C, et al. NK Cells Contribute to the Immune Risk Profile in Kidney Transplant Candidates. *Front Immunol* (2019) 10(AUG):1890. doi:10.3389/fimmu.2019.01890
25. Zhu L, Aly M, Wang H, Karakizlis H, Weimer R, Morath C, et al. Increased Natural Killer Cell Subsets With Inhibitory Cytokines and Inhibitory Surface Receptors in Patients With Recurrent Miscarriage and Decreased or Normal Subsets in Kidney Transplant Recipients Late Post-transplant. *Clin Exp Immunol* (2018) 193(2):241–54. doi:10.1111/cei.13142
26. Iske J, Dedeilia A, Xiao Y, Martin F, Emmert MY, Sage PT, et al. The Impact of T-Cell Aging on Alloimmunity and Inflammation. *Transplantation* (2024) 108:634–42. doi:10.1097/TP.00000000000004715
27. Weiskopf D, Weinberger B, Grubeck-Loebenstein B. The Aging of the Immune System. *Transpl Int* (2009) 22:1041–50. doi:10.1111/j.1432-2277.2009.00927.x
28. Schaanman JM, Rossetti M, Sidwell T, Groyberg V, Sunga G, Korin Y, et al. Increased T Cell Immunosenescence and Accelerated Maturation Phenotypes in Older Kidney Transplant Recipients. *Hum Immunol* (2018) 79(9):659–67. doi:10.1016/j.humimm.2018.06.006
29. Higdon LE, Gustafson CE, Ji X, Sahoo MK, Pinsky BA, Margulies KB, et al. Association of Premature Immune Aging and Cytomegalovirus After Solid Organ Transplant. *Front Immunol* (2021) 12:661551. doi:10.3389/fimmu.2021.661551
30. Higdon LE, Trofe-Clark J, Liu S, Margulies KB, Sahoo MK, Blumberg E, et al. Cytomegalovirus-Responsive CD8+ T Cells Expand After Solid Organ Transplantation in the Absence of CMV Disease. *Am J Transplant* (2017) 17(8):2045–54. doi:10.1111/ajt.14227
31. Lee GH, Lee JY, Jang J, Kang YJ, Choi SA, Kim HC, et al. Anti-Thymocyte Globulin-Mediated Immunosenescent Alterations of T Cells in Kidney Transplant Patients. *Clin Transl Immunol* (2022) 11(11):e1431. doi:10.1002/cti2.1431
32. Li P, Tian C, Ge N, Wang H, Liu L, Lou F, et al. Premature Senescence of T Cells in Long-Term Survivors of Renal Transplantation. *Biochem Biophys Res Commun* (2011) 407(3):599–604. doi:10.1016/j.bbrc.2011.03.079
33. Vallejo AN, Bryl E, Klarskov K, Naylor S, Weyand CM, Goronzy JJ. Molecular Basis for the Loss of CD28 Expression in Senescent T Cells. *J Biol Chem* (2002) 277(49):46940–9. doi:10.1074/jbc.M207352200
34. Heinbokel T, Elkhail A, Liu G, Edtinger K, Tullius SG. Immunosenescence and Organ Transplantation. *Transplant Rev* (2013) 27:65–75. doi:10.1016/j.trre.2013.03.001
35. Von Moos S, Schalk G, Mueller TF, Laube G. Age-Associated Decrease in De Novo Donor-Specific Antibodies in Renal Transplant Recipients Reflects Changing Humoral Immunity. *Immun Ageing* (2019) 16(1):9. doi:10.1186/s12979-019-0149-8
36. Heinbokel T, Quante M, Iske J, Nian Y, Maenosono R, Minami K, et al. CTLA4-Ig Prolongs Graft Survival Specifically in Young But Not Old Mice. *Am J Transplant* (2021) 21(2):488–502. doi:10.1111/ajt.16218
37. Duftner C, Dejaco C, Hengster P, Bijuklic K, Joannidis M, Margreiter R, et al. Apoptotic Effects of Antilymphocyte Globulins on Human Pro-Inflammatory CD4+CD28- T-Cells. *PLoS One* (2012) 7(3):e33939. doi:10.1371/journal.pone.0033939
38. Lee GH, Hong KT, Choi JY, Shin HY, Lee WW, Kang HJ. Immunosenescent Characteristics of T Cells in Young Patients Following Haploidentical Haematopoietic Stem Cell Transplantation From Parental Donors. *Clin Transl Immunol* (2020) 9(4):e1124. doi:10.1002/cti2.1124
39. Weimer R, Ettrich M, Renner F, Dietrich H, Süsal C, Deisz S, et al. ATG Induction in Renal Transplant Recipients: Long-Term Hazard of Severe Infection Is Associated With Long-Term Functional T Cell Impairment but Not the ATG-induced CD4 Cell Decline. *Hum Immunol* (2014) 75(6):561–9. doi:10.1016/j.humimm.2014.02.015
40. Lachmann R, Bajwa M, Vita S, Smith H, Cheek E, Akbar A, et al. Polyfunctional T Cells Accumulate in Large Human Cytomegalovirus-Specific T Cell Responses. *J Virol* (2012) 86(2):1001–9. doi:10.1128/JVI.00873-11
41. Welzl K, Weinberger B, Kronbichler A, Sturm G, Kern G, Mayer G, et al. How Immunosuppressive Therapy Affects T Cells From Kidney Transplanted Patients of Different Age: The Role of Latent Cytomegalovirus Infection. *Clin Exp Immunol* (2014) 176(1):112–9. doi:10.1111/cei.12205
42. Herndler-Brandstetter D, Landgraf K, Tzankov A, Jenewein B, Brunauer R, Laschober GT, et al. The Impact of Aging on Memory T Cell Phenotype and Function in the Human Bone Marrow. *J Leukoc Biol* (2011) 91(2):197–205. doi:10.1189/jlb.0611299
43. Lewis E, Weiler M, Chaimovitz C, Douvdevani A. Interleukin-15 Is the Main Mediator of Lymphocyte Proliferation in Cultures Mixed With Human Kidney Tubular Epithelial Cells. *Transplantation* (2001) 72(5):886–90. doi:10.1097/00007890-200109150-00025
44. Xie L, Zhang Z, Zhu P, Tian K, Liu Y, Yu Y. IL-21 Prevents Expansion of CD8+CD28- T Cells Stimulated by IL-15 and Changes Their Subset Distribution. *Transpl Proc* (2021) 53(7):2407–14. doi:10.1016/j.transproceed.2021.07.039
45. Borthwick NJ, Lowdell M, Salmon M, Akbar AN. Loss of CD28 Expression on CD8+ T Cells Is Induced by IL-2 Receptor γ Chain Signalling Cytokines and Type I IFN, and Increases Susceptibility to Activation-

- Induced Apoptosis. *Int Immunol* (2000) 12(7):1005–13. doi:10.1093/intimm/12.7.1005
46. Hombach A, Sent D, Schneider C, Heuser C, Koch D, Pohl C, et al. T-Cell Activation by Recombinant Receptors: CD28 Costimulation Is Required for Interleukin 2 Secretion and Receptor-Mediated T-Cell Proliferation But Does Not Affect Receptor-Mediated Target Cell Lysis. *Cancer Res* (2001) 61(5): 1976–82.
 47. Krenzien F, Quante M, Heinbokel T, Seyda M, Minami K, Uehara H, et al. Age-Dependent Metabolic and Immunosuppressive Effects of Tacrolimus. *Am J Transplant* (2017) 17(5):1242–54. doi:10.1111/ajt.14087
 48. Memmos E, Lioulios G, Antoniadis N, Fylaktou A, Stangou M. Immunosenescence in Kidney Transplant Recipients. *Transplantation* (2025) 109(12):e675–e681. doi:10.1097/TP.0000000000005484

Copyright © 2026 Lioulios, Moisidou, Christodoulou, Kasimatis, Xochelli, Memmos, Stai, Stangou and Fylaktou. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.



Association Between Cytomegalovirus Viremia Clearance and Post-Solid Organ Transplant Mortality in Patients With Refractory Cytomegalovirus Infection: SOLSTICE Post Hoc Analysis

Nassim Kamar^{1*}, Robin K. Avery², Tien Bo³, Joan Gu³, Deepali Kumar⁴ and Oliver Witzke⁵

¹Toulouse Rangueil University Hospital, INFINITY-Inserm U1291-CNRS U5051, University Paul Sabatier, Toulouse, France, ²School of Medicine, Johns Hopkins University, Baltimore, MD, United States, ³Takeda Development Center Americas, Inc., Cambridge, MA, United States, ⁴Ajmera Transplant Centre, University Health Network, Toronto, ON, Canada, ⁵Department of Infectious Diseases, West German Centre of Infectious Diseases, University Medicine Essen, University Duisburg-Essen, Essen, Germany

OPEN ACCESS

*Correspondence

Nassim Kamar,
✉ kamar.n@chu-toulouse.fr

Received: 28 July 2025

Revised: 16 October 2025

Accepted: 10 November 2025

Published: 26 November 2025

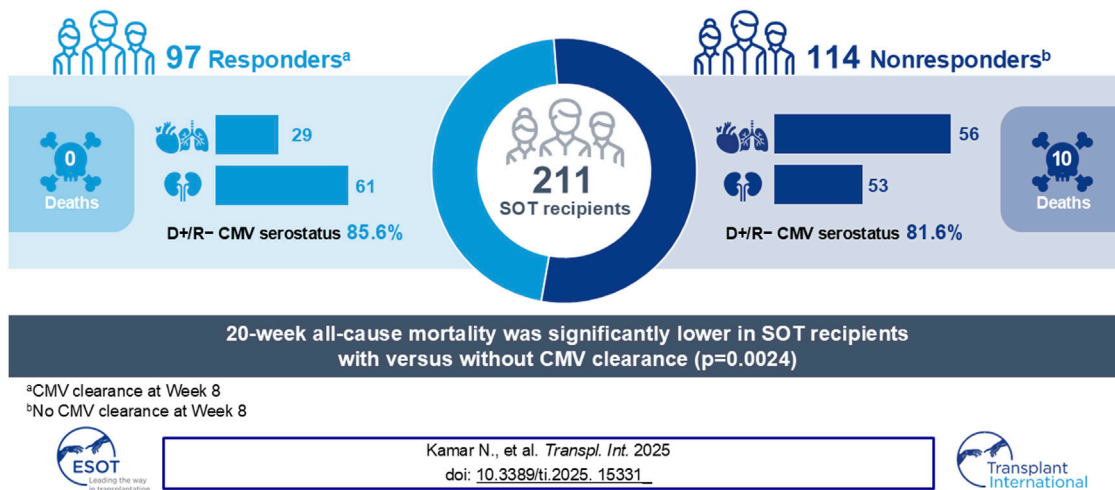
Citation:

Kamar N, Avery RK, Bo T, Gu J, Kumar D and Witzke O (2025) Association Between Cytomegalovirus Viremia Clearance and Post-Solid Organ Transplant Mortality in Patients With Refractory Cytomegalovirus Infection: SOLSTICE Post Hoc Analysis. *Transpl. Int.* 38:15331. doi: 10.3389/ti.2025.15331

Cytomegalovirus (CMV) infection following solid organ transplant (SOT) is associated with increased mortality risk. In the phase 3 SOLSTICE study (NCT02931539), more transplant recipients achieved CMV clearance after 8 weeks with maribavir than investigator-assigned therapy (IAT). In SOLSTICE, SOT recipients with refractory CMV infection were randomized 2:1 to receive maribavir or IAT for 8 weeks. This *post hoc* analysis assessed the impact of CMV clearance at Week 8 on mortality at Week 20. Patients who achieved CMV clearance at Week 8 were categorized as responders, and patients without CMV clearance, or who received maribavir rescue or alternative treatment, were categorized as nonresponders. All-cause mortality was assessed at Week 20 for responders and nonresponders using the Kaplan-Meier method with log-rank test. The analysis included 211 SOT recipients: 97 responders and 114 nonresponders. Week 20 all-cause mortality was significantly higher in nonresponders than responders ($p = 0.0024$). No deaths were reported in the responder group, and 10 deaths were reported in the nonresponder group (3 receiving IAT, 7 receiving maribavir). Median (range) time from treatment start to death was 30.5 (3–123) days. This analysis is consistent with other studies showing an increased risk of mortality with post-SOT CMV infection.

Keywords: SOT (solid organ transplant), survival, anti-CMV therapy, maribavir, antiviral

Association Between Cytomegalovirus Viremia Clearance and Post-Solid Organ Transplant Mortality in Patients With Refractory Cytomegalovirus Infection: SOLSTICE Post Hoc Analysis (NCT02931539)



GRAPHICAL ABSTRACT |

INTRODUCTION

Transplantation is a life-saving procedure with mean survival after solid organ transplant (SOT) estimated at 20–27 years for kidney and liver transplants, 15–16 years for heart transplants, and 9 years for lung transplants [1]. The most common causes of death in SOT recipients are cancer, graft failure, infection, and cardiovascular disease [2]. Studies of mortality in SOT recipients have identified several risk factors, including older age, male sex, acute rejection, low platelet count, and post-transplant infection [3–7].

Cytomegalovirus (CMV) is one of the most common pathogens associated with infection in SOT recipients [7, 8]. Data from a systematic review estimates the incidence of CMV infection between 5.2% and 76.4% dependent on the transplanted organ and treatment strategy [9]. Importantly, post-SOT CMV infection is associated with an increased risk of graft loss and mortality [7, 8, 10].

Established antiviral agents for management of post-SOT CMV are ganciclovir and its oral prodrug valganciclovir, despite the high incidence of myelosuppression with these agents [11–14]. Alternatively, in (val)ganciclovir resistant or refractory cases, intravenous foscarnet can be used. However, foscarnet is associated with nephrotoxicity and electrolyte depletion, thereby compromising patient safety [12, 14, 15].

First approved in 2021, maribavir is an oral treatment for post-transplant CMV infection/disease that is refractory (with or without resistance) to prior antiviral drugs or intolerant to treatment (in certain countries) [16–18]. The phase 3 SOLSTICE study compared maribavir with investigator-

assigned therapy (IAT; valganciclovir/ganciclovir, foscarnet, or cidofovir) in patients with refractory post-transplant CMV infection, with or without genotypically documented resistance [19]. The results showed that significantly more patients receiving maribavir achieved CMV clearance at the end of Week 8 compared with IAT (55.7% vs. 23.9%; adjusted difference [95% confidence interval (CI)]: 32.8% [22.80–42.74]; $p < 0.001$) and achieved CMV clearance and symptom control at the end of Week 8 that was maintained through Week 16 (18.7% vs. 10.3%; adjusted difference [95% CI]: 9.5% [2.02–16.88]; $p = 0.01$). Moreover, in the SOT population, a higher proportion of maribavir-treated patients achieved these endpoints across transplant organ types [20]. Maribavir-treated patients also had less neutropenia and less acute kidney injury compared with valganciclovir/ganciclovir- and foscarnet-treated patients, respectively [19]. Here we present data from a *post hoc* analysis of the SOLSTICE study designed to assess the impact of CMV viremia clearance at Week 8 on Week 20 mortality among SOT recipients with refractory CMV infection.

PATIENTS AND METHODS

Full details of the SOLSTICE study (ClinicalTrials.gov: NCT02931539) have been published previously [19]. In brief, SOLSTICE was a phase 3, randomized, open-label, multicenter study. Transplant recipients (SOT or hematopoietic cell transplant) were aged ≥ 12 years with CMV DNA ≥ 910 IU/mL at screening and were refractory (with or without resistance) to their most recent CMV treatment. Patients were randomized

TABLE 1 | Baseline characteristic of solid organ transplant recipients in the SOLSTICE study.

Characteristic	Responders (n = 97)	Nonresponders (n = 114)	p-value
Age, median (range), years	56 (25–77)	55 (19–79)	0.5701 ^a
Sex, n (%)			0.3195
Male	70 (72.2)	75 (65.8)	
Female	27 (27.8)	39 (34.2)	
Transplant type, n (%)			0.0170
Kidney	61 (62.9)	53 (46.5)	
Heart and/or lung	29 (29.9)	56 (49.1)	
Other	7 (7.2)	5 (4.4)	
Graft status at baseline, n (%)			0.1236
Functioning	85 (87.6)	103 (90.4)	
Functioning with complications	12 (12.4)	8 (7.0)	
Other	0	3 (2.6)	
Retransplant			0.0681
Yes	8 (8.2)	19 (16.7)	
No	89 (91.8)	95 (83.3)	
CMV serostatus, n (%)			
D+/R–	83 (85.6)	93 (81.6)	
D+/R+	7 (7.2)	12 (10.5)	
D–/R–	6 (6.2)	4 (3.5)	
D–/R+	1 (1.0)	3 (2.6)	
Missing	0	2 (1.8)	
CMV DNA levels at randomization, n (%)			0.3278
Low (<9,100 IU/mL)	45 (46.4)	42 (36.8)	
Intermediate (≥9,100 to <91,000 IU/mL)	39 (40.2)	51 (44.7)	
High (≥91,000 IU/mL)	13 (13.4)	21 (18.4)	
Cardiovascular disease			0.8529
Yes	7 (7.2)	9 (7.9)	
No	90 (92.8)	105 (92.1)	
Diabetes			0.2250
Yes	2 (2.1)	6 (5.3)	
No	95 (97.9)	108 (94.7)	
Anti-lymphocyte use			0.7220
Yes	32 (33.0)	35 (30.7)	
No	65 (67.0)	79 (69.3)	

^aAnalysis conducted for <65 vs. ≥65 years.

CMV, cytomegalovirus; D, donor; R, recipient.

2:1 to receive maribavir 400 mg twice daily or investigator's choice of monotherapy or combination therapy with ganciclovir/valganciclovir, foscarnet, or cidofovir for 8 weeks, with an additional 12 weeks of follow-up. The primary endpoint was confirmed CMV viremia clearance at the end of Week 8 [19].

In the present *post hoc* analysis, patients were categorized based on whether they achieved the primary endpoint. Responders were defined as patients with plasma CMV DNA below the lower limit of quantification (137 IU/mL) in 2 consecutive post-baseline samples, separated by ≥5 days at Week 8. Nonresponders were defined as patients who did not achieve CMV clearance at Week 8 (including those with missing virologic data) or who received maribavir rescue or alternative treatment before the end of Week 8.

All-cause mortality at Week 20 was plotted for responders and nonresponders (to maribavir or IAT) using the Kaplan-Meier method with log-rank test. Recipient characteristics were summarized using descriptive statistics and compared between responder and nonresponder groups using the chi-square test. Multivariate analyses were conducted using Cox proportional

hazards model for Week 20 mortality. Analyses were conducted using SAS® 9.4.

RESULTS

The 211 SOT recipients from the SOLSTICE study were included in the analysis, of whom 97 were categorized as responders and 114 as nonresponders (**Supplementary Figure S1**). Baseline characteristics were broadly similar; the only statistically significant difference in baseline characteristics between responders and nonresponders was transplanted organ ($p = 0.0170$) (**Table 1**). Most patients were kidney or lung recipients, and the majority had donor-positive/recipient-negative (D+/R–) CMV serostatus. More recipients in the nonresponder group had high CMV DNA levels (≥91,000 IU/mL) than responders ($n = 21$, 18.4% vs. $n = 13$, 13.4%).

In SOT recipients, 20-week all-cause mortality was significantly lower in responders than in nonresponders ($p = 0.0024$) (**Figure 1**). By Week 20, no deaths were reported in responders compared with 3 deaths in nonresponders who

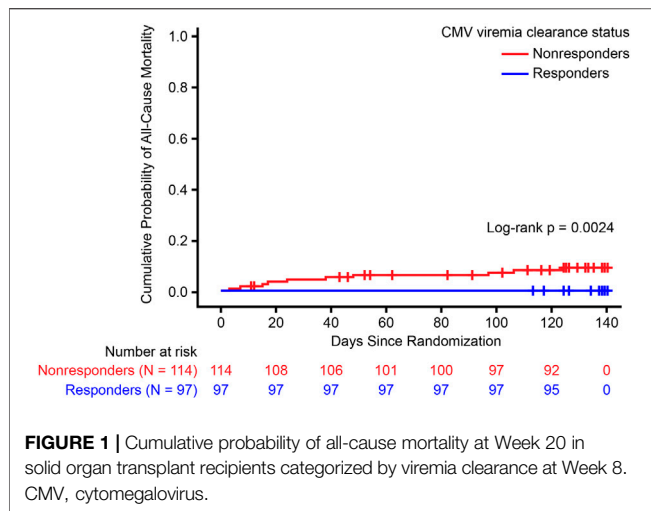


FIGURE 1 | Cumulative probability of all-cause mortality at Week 20 in solid organ transplant recipients categorized by viremia clearance at Week 8. CMV, cytomegalovirus.

received IAT and 7 deaths in nonresponders who received maribavir (Table 2). Cox regression analysis did not show any significant risk factors for death among baseline characteristics for viral load, cardiovascular disease, diabetes, induction therapy, or retransplant (Figure 2). Median (range) CMV viral load at the last measurement was 2,001.5 IU/mL (68.5–67,539.0) in nonresponders who died compared with 68.5 IU/mL (68.5–621,920.0) and 68.5 IU/mL (68.5–38,733.0) in nonresponders and responders, respectively, who survived (Table 3).

One of the deaths was considered related to maribavir by the investigator due to a drug interaction with posaconazole causing sudden cardiac death by arrhythmia. Other causes of death included respiratory failure ($n = 3$) and pulmonary embolism ($n = 2$). CMV infection contributed to cause of death in 4 participants. The characteristics of recipients who died and causes of death are shown in Table 4. Of the 10 recipients who died, 6 were male, and the median (range) age was 60.5 (28–77) years. Two recipients had received more than 1 transplant before

TABLE 2 | Mortality outcomes at Week 20 in SOT recipients categorized by viremia clearance at Week 8.

Cytomegalovirus viremia clearance status	Participants n/N	Events n (%) ^a	Time to event, median (range), days
Nonresponders			
Investigator-assigned treatment	51/69	3 (5.9)	38.0 (17.0–106.0)
Maribavir	63/142	7 (11.1)	24.0 (3.0–124.0)
Responders			
Investigator-assigned treatment	18/69	0	–
Maribavir	79/142	0	–

SOT, solid organ transplant.

^aDenominator is number of participants in each response category within each treatment group (n).

N, total number of SOT, recipients in each treatment group; n , number of participants in each response category within each treatment group.

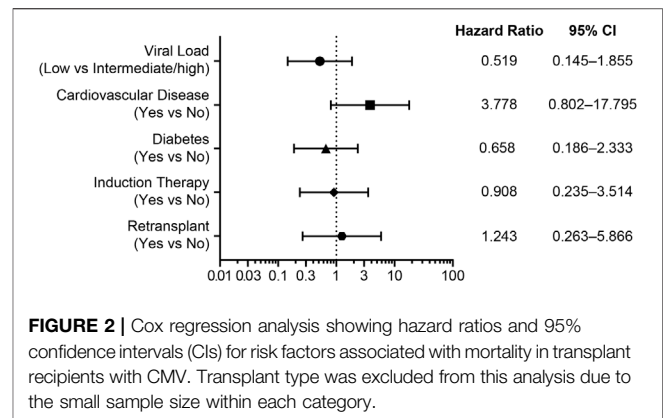


FIGURE 2 | Cox regression analysis showing hazard ratios and 95% confidence intervals (CIs) for risk factors associated with mortality in transplant recipients with CMV. Transplant type was excluded from this analysis due to the small sample size within each category.

study entry. Eight of the 10 recipients had cardiovascular comorbidities. No patients had a recent history of organ rejection, and all had functioning transplants at baseline. CMV serostatus was D+/R– in 8 of 10 (80%) patients who died, similar to the percentage of D+/R– in the cohort as a whole. The group who died included 1 patient who had received rescue therapy with maribavir after foscarnet. The median (range) time from SOT to treatment start was 253 (72–549) days, from treatment start to treatment end was 15 (2–58) days, from treatment start to death was 30.5 (3–123) days, and from treatment end to death was 9 (1–72) days. Only 2 of the patients who died completed the treatment course; of the 8 who discontinued, 5 were due to death.

DISCUSSION

This *post hoc* analysis of data from the phase 3, randomized SOLSTICE study showed that SOT recipients with refractory CMV infection who achieved CMV clearance at Week 8 had a significantly reduced probability of 20-week all-cause mortality than those without CMV clearance.

The present study adds to the body of evidence confirming that post-transplant CMV infection is a known risk factor for mortality in SOT recipients [4, 7, 22]. For example, a retrospective analysis of 1,454 renal transplant recipients showed that CMV disease was associated with a 2.5-fold increase in risk of death

TABLE 3 | CMV viral load (IU/mL) at last measurement.

Treatment	Responders		Nonresponders	
	Died	Survived	Died	Survived
Maribavir, n	0	79	7	56
Median	–	68.5	2,730.0	68.5
(range)	–	(68.5–3,071.0)	(68.5–67,539.0)	(68.5–27,871.0)
IAT, n	0	18	3	48
Median	–	68.5	1,882.0	68.5
(range)	–	(68.5–38,733.0)	(1,132.0–2,121.0)	(68.5–621,920.0)
Total, n	0	97	10	104
Median	–	68.5	2,001.5	68.5
(range)	–	(68.5–38,733.0)	(68.5–67,539.0)	(68.5–621,920.0)

TABLE 4 | Patient characteristics of nonresponders who died.

SOT type	CMV serostatus, D/R	CV comorbidities	CMV syndrome	Viral load category ^a	Lymphocyte count at baseline ^b	Study drug	Length of Tx, days	Time from SOT to Tx start, days	Time from Tx start to death, days	Time from Tx end to death, days	Cause of death
Kidney	+/-	Y	N	I	0.1	GCV	8	279	38	30	CMV-related pneumonia
Lung	+/-	Y	N	L	1.1	FSC	16	165	17	1	Respiratory failure
Kidney	+/+	Y	N	H	0.2	FSC	34	253	106	72	Unknown ^c
Heart	-/+	Y	Y	L	0.8 ^d	MBV	47	116	48	1	Cardiac arrest
Lung	+/-	N	Y	L	0.7	MBV	2	123	3	1	Deep vein thrombosis with probable progression to pulmonary embolus
Lung	+/-	Y	N	I	1.7	MBV	58	549	94	36	Respiratory failure
Kidney	+/-	N	Y	I	0.6	MBV	4	319	7	3	Drug-drug interaction with an outcome of sudden death ^e
Kidney	+/-	Y	N	I	0.1	MBV	8	253	23	15	Respiratory failure/CMV syndrome
Heart	+/-	Y	N	L	0.6	MBV	14	434	15	1	Massive pulmonary embolism
Heart	+/-	Y	N	I	0.9 ^f	MBV	56	72	123	67	Extensive venous thrombosis

CMV, cytomegalovirus; CV, cardiovascular; D/R, donor/recipient; FSC, foscarnet; GCV, ganciclovir; H, high; I, intermediate; L, low; MBV, maribavir; N, no; NA, not applicable; QTc, corrected QT, interval; Tx, treatment; Y, yes.

^aBaseline plasma CMV DNA, viral load was defined as low, <9,100 IU/mL; intermediate, ≥9,100 to <91,000 IU/mL; or high, ≥91,000 IU/mL.

^bBaseline measurement taken 1 day from the first dose of treatment.

^cFoscarnet-treated patient qualified for maribavir rescue (inadequate response and toxicity) [19] and developed CMV, encephalitis 2 days after maribavir initiation, patient died (cause unknown) 49 days after last dose of maribavir.

^dMeasurement taken 15 days from first dose of treatment.

^eThe investigator interpreted this event as sudden cardiac death due to arrhythmia and reported it as possible drug interaction. The patient had also received voriconazole, posaconazole, and domperidone. In studies of healthy adults, doses of maribavir up to 1,200 mg were not shown to prolong QTc [21].

^fMeasurement taken -1 day from the first dose of treatment.

($p < 0.001$) [4]. Similarly, a retrospective study of 88 lung transplant recipients showed that CMV disease was associated with a 4.2-fold increase in mortality ($p = 0.002$), whereas CMV infection and disease were associated with a 3.8-fold increase in mortality ($p = 0.001$) [7]. In a longitudinal study of CMV infection and viral load in 2,510 sequential kidney transplant recipients, CMV infection was associated with a significantly increased risk of death ($p = 0.0056$) [22].

Cox regression analysis did not identify any significant risk factors for mortality among the following baseline characteristics: viral load, cardiovascular disease, diabetes, induction therapy, and retransplant. As expected, median CMV viral load at latest measurement was higher in patients who died than in those who survived.

In the present analysis, 9 of the 10 recipients who died received transplants from CMV-positive donors. It is known

that CMV-positive donor status is associated with a higher risk of CMV infection [22] and mortality [23, 24]. A high proportion of the recipients who died in this analysis also had cardiovascular comorbidities, which are known risk factors for mortality following SOT [25, 26]. In this analysis, only 2 recipient deaths were caused by cardiac arrest, one of which was due to drug interactions. In this case, the investigator assigned the sudden cardiac death as possibly related to maribavir. However, doses of maribavir up to 1,200 mg in healthy adults have been shown not to prolong QTc [21]. The patient had also received posaconazole, voriconazole, and domperidone. Domperidone is associated with an increased risk of sudden cardiac death and arrhythmia and should not be taken with voriconazole [27, 28]. Half of the deaths in this analysis were due to respiratory failure and pulmonary embolism. Three patients receiving maribavir

experienced thrombosis or pulmonary embolism; however, none of these were deemed as related to maribavir. Baseline characteristics of nonresponders included higher proportions of lung or heart transplants and intermediate/high viral loads. Chi-square analysis showed a significant difference between responders and nonresponders for kidney, heart or lung, and other transplanted organs.

The association between CMV clearance and mortality in the present analysis does not necessarily mean that CMV clearance was the sole reason for reduced mortality. All-cause mortality is likely to be driven by multiple factors, and CMV clearance at 8 weeks of treatment may be a marker of the patient's general health, disease severity, or immune status. However, it does appear that failure to clear CMV identifies a group with an increased risk of death potentially due to other causes not directly related to CMV. Therefore, the nonresponder group may merit close follow-up and preventive care from the standpoint of cardiovascular issues and venous thromboembolism, as well as ongoing anti-CMV treatment.

Data from this *post hoc* analysis should be interpreted with caution, as mortality was not the primary endpoint of the SOLSTICE study. Moreover, the number of patients included in the analysis may not provide adequate power and may not reflect a thorough assessment of the underlying cause of mortality [29]. The potential for selection bias should also be taken into consideration.

In conclusion, achievement of viremia clearance at Week 8 was associated with a significantly reduced probability of mortality at Week 20. While many factors likely affect mortality in transplant recipients with refractory or resistant CMV infection, these findings suggest that achieving and maintaining CMV clearance in this complex patient population is a contributing factor to survival. Application of these findings to clinical practice is worthy of further study.

DATA AVAILABILITY STATEMENT

The datasets presented in this article are not readily available. The datasets, including the redacted study protocol, redacted statistical analysis plan, and individual participants data supporting the results reported in this article, will be made available within three months from initial request, to researchers who provide a methodologically sound proposal. The data will be provided after its deidentification, in compliance with applicable privacy laws, data protection, and requirements for consent and anonymization. Requests to access the datasets should be directed to TB, tien.bo@takeda.com.

ETHICS STATEMENT

The studies involving humans were approved by Advarra Inc., Alfred Hospital Ethics Committee, Chesapeake IRB, Columbia University Medical Center Institutional Review Board, Comité de Protection des Personnes Sud-Quest and Outre-Mer IV, Comité Etico de Investigación Clínica con Medicamentos del Hospital

Universitari Vall d'Hebron, Commissie Medische Etiek – UZ, Duke University Institutional Review Board, Ethikkommission an der Medizinischen Fakultät der Universität Leipzig, Hamilton Integrated Research Ethics Board, Henry Ford Health System Institutional Review Board, Institutional Review Board/Privacy Board Loyola University Chicago Health Sciences Division Institutional Review Board for the Protection of Human Research Subjects, Johns Hopkins Medicine Institutional Review Board, MUSC Institutional Review Board for Human Research, Northwestern University Institutional Review Board, Ochsner Clinic Foundation IRB, Partners Human Research Committee - Massachusetts General Hospital, Partners Human Research Committee, Schulman Associates IRB Inc., The Capital Region of Denmark, UCLA - Office of the Human Research Protection Program, University Health Network Research Ethics Board, University of Alberta Human Research Ethics Board, University of Chicago IRB, University of Cincinnati Institutional Review Board, University of Kentucky Office of Research Integrity Medical IRB, University of Maryland Baltimore Institutional Review Board, University of Minnesota Institutional Review Board, University of Pennsylvania Office of Regulatory Affairs, University of Utah Institutional Review Board, USC Health Sciences Institutional Review Board, UT Southwestern Institutional Review Board, Weill Cornell Medical College Institutional Review Board, West Midlands - Coventry & Warwickshire Research Ethics Committee, Western Institutional Review Board University of Alabama at Birmingham Institutional Review Board, Western Institutional Review Board. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

Study concept and design: TB and JG. Acquisition of data: NK, RA, DK, and OW. Data analysis and interpretation: NK, RA, TB, JG, DK, and OW. All authors contributed to the article and approved the submitted version.

FUNDING

The authors declare that financial support was received for the research and/or publication of this article.

CONFLICT OF INTEREST

NK: royalties: UpToDate; consulting: Astellas Pharma, Biotest, Chiesi, ExeViR, Hansa Biopharma, Merck Sharp & Dohme, and Takeda; honoraria: Astellas Pharma, AstraZeneca, Biotest, CSL Behring, Chiesi, ExeViR, GSK, Hansa Biopharma, Merck Sharp & Dohme, Novartis, Sanofi, Sandoz, and Takeda; travel support: Astellas Pharma, AstraZeneca, Biotest, CSL Behring, Chiesi, GSK, Hansa Biopharma, Merck Sharp & Dohme, Novartis, Sanofi, Sandoz, and Takeda. RKA: study grant support: AiCuris,

AstraZeneca, Merck, Regeneron, Takeda/Shire, and United Therapeutics. TB, JG: employees of Takeda and hold stock in Takeda. DK: research funding: GSK, Roche, and Takeda; consulting: GSK, Merck, and Takeda. OW: research grants for clinical studies, speaker's fees, honoraria, and travel expenses: Amgen, Alexion, Astellas Pharma, AstraZeneca, Basilea, Biotest, Bristol Myers Squibb, Chiesi, Correvio, Dr. F. Köhler Chemie, Gilead Sciences, GSK, Hexal Pharmaceuticals, Janssen, Moderna, MSD, Novartis, Pfizer, Roche, Sanofi, Shionogi, Takeda, Teva Pharmaceuticals, Tillotts Pharma, and UCB. Supported by an unrestricted grant: Rudolf-Ackermann-Stiftung (Stiftung für Klinische Infektiologie).

The authors declare that this study received funding from Takeda Development Center Americas, Inc., Cambridge, MA, United States.

The funder had the following involvement with the study: study design, analysis, interpretation of data, writing of the article, and decision to submit for publication.

GENERATIVE AI STATEMENT

The authors declare that no Generative AI was used in the creation of this manuscript.

REFERENCES

- Graham CN, Watson C, Barlev A, Stevenson M, Dharnidharka VR. Mean Lifetime Survival Estimates Following Solid Organ Transplantation in the US and UK. *J Med Econ* (2022) 25:230–7. doi:10.1080/13696998.2022.2033050
- Soborg A, Reekie J, Rasmussen A, Cunha-Bang CD, Gustafsson F, Rossing K, et al. Trends in Underlying Causes of Death in Solid Organ Transplant Recipients Between 2010 and 2020: Using the CLASS Method for Determining Specific Causes of Death. *PLoS One* (2022) 17:e0263210. doi:10.1371/journal.pone.0263210
- Gao S, Huang X, Zhou X, Dai X, Han J, Chen Y, et al. A Comprehensive Evaluation of Risk Factors for Mortality, Infection and Colonization Associated with CRGNB in Adult Solid Organ Transplant Recipients: A Systematic Review and Meta-Analysis. *Ann Med* (2024) 56:2314236. doi:10.1080/07853890.2024.2314236
- Pinto-Ramirez J, Garcia-Lopez A, Salcedo-Herrera S, Patino-Jaramillo N, Barbosa-Salinas J, et al. Risk Factors for Graft Loss and Death Among Kidney Transplant Recipients: A Competing Risk Analysis. *PLoS One* (2022) 17:e0269990. doi:10.1371/journal.pone.0269990
- Oriol I, Sabé N, Melilli E, Lladó L, González-Costello J, Soldevila L, et al. Factors Influencing Mortality in Solid Organ Transplant Recipients with Bloodstream Infection. *Clin Microbiol Infect* (2015) 21:1104.e9–14. doi:10.1016/j.cmi.2015.07.021
- Yoon J, Kim H, Choi D, Park B. Causes of Death and Associated Factors with Death After Liver Transplantation: A Nationwide Database Study. *HPB (Oxford)* (2024) 26:54–62. doi:10.1016/j.hpb.2023.09.011
- Beam E, Lesnick T, Kremers W, Kennedy CC, Razonable RR. Cytomegalovirus Disease Is Associated with Higher All-Cause Mortality After Lung Transplantation Despite Extended Antiviral Prophylaxis. *Clin Transpl* (2016) 30:270–8. doi:10.1111/ctr.12686
- Kotton CN, Kumar D, Caliendo AM, Huprikar S, Chou S, Danziger-Isakov L, et al. The Third International Consensus Guidelines on the Management of Cytomegalovirus in Solid-Organ Transplantation. *Transplantation* (2018) 102: 900–31. doi:10.1097/TP.0000000000002191
- Silva Junior HT, Tokat Y, Cai J, Singh I, Sandhu A, Demuth D, et al. Epidemiology, Management, and Burden of Cytomegalovirus in Solid Organ Transplant Recipients in Selected Countries Outside of Europe and North America: A Systematic Review. *Transpl Infect Dis* (2023) 25:e14070. doi:10.1111/tid.14070
- Stern M, Hirsch H, Cusini A, van Delden C, Manuel O, Meylan P, et al. Cytomegalovirus Serology and Replication Remain Associated with Solid Organ Graft Rejection and Graft Loss in the Era of Prophylactic Treatment. *Transplantation* (2014) 98:1013–8. doi:10.1097/TP.0000000000000160
- Hakki M, Aitken SL, Danziger-Isakov L, Michaels MG, Carpenter PA, Chemaly RF, et al. American Society for Transplantation and Cellular Therapy Series: #3-Prevention of Cytomegalovirus Infection and Disease After Hematopoietic Cell Transplantation. *Transpl Cell Ther* (2021) 27: 707–19. doi:10.1016/j.jtct.2021.05.001
- Allaw F, Haddad SF, Zakhour J, Kanj SS. Management of Cytomegalovirus Infection in Allogeneic Hematopoietic Stem Cell Transplants. *Int J Antimicrob Agents* (2023) 62:106860. doi:10.1016/j.ijantimicag.2023.106860
- Takahata M, Hashino S, Nishio M, Sugita J, Shigematsu A, Onozawa M, et al. Occurrence of Adverse Events Caused by Valganciclovir as Pre-Emptive Therapy for Cytomegalovirus Infection After Allogeneic Stem Cell Transplantation Is Reduced by Low-Dose Administration. *Transpl Infect Dis* (2015) 17:810–5. doi:10.1111/tid.12456
- Zavras P, Su Y, Fang J, Stern A, Gupta N, Tang Y, et al. Impact of Preemptive Therapy for Cytomegalovirus on Toxicities After Allogeneic Hematopoietic Cell Transplantation in Clinical Practice: A Retrospective Single-Center Cohort Study. *Biol Blood Marrow Transpl* (2020) 26:1482–91. doi:10.1016/j.bbmt.2020.03.019
- Avery RK, Arav-Boger R, Marr KA, Kraus E, Shoham S, Lees L, et al. Outcomes in Transplant Recipients Treated with Foscarnet for Ganciclovir-Resistant or Refractory Cytomegalovirus Infection. *Transplantation* (2016) 100:e74–80. doi:10.1097/TP.0000000000001418
- Takeda Pharmaceuticals America Inc. *Livtency*. Lexington, MA (2024).
- Takeda Pharmaceuticals Australia Pty Ltd. *Australian Product Information. LIVTENCITY® (Maribavir) Tablets* (2023).
- Takeda Pharmaceuticals International AG Ireland Branch. *Livtency. Summary of Product Characteristics* (2024).
- Avery RK, Alain S, Alexander BD, Blumberg EA, Chemaly RF, Cordonnier C, et al. Maribavir for Refractory Cytomegalovirus Infections with or Without

ACKNOWLEDGEMENTS

The authors would like to acknowledge Jess Auciello and Shun-Ping Quan for their contribution to data analysis. Under the direction of the authors, medical writing support for this publication was provided by Emma Green, PhD, employee of Envision Catalyst, an Envision Medical Communications agency, a part of Envision Pharma Group (Fairfield, CT, United States), and was funded by Takeda Development Center Americas, Inc., Cambridge, MA, United States.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontierspartnerships.org/articles/10.3389/ti.2025.15331/full#supplementary-material>

- Resistance Post-Transplant: Results from a Phase 3 Randomized Clinical Trial. *Clin Infect Dis* (2022) 75:690–701. doi:10.1093/cid/ciab988
20. Blumberg EA, Witzke O, Harber M, Ison MG, Saliba F, Kamar N, et al. Maribavir for Refractory Cytomegalovirus Infection (with or without Resistance) in Solid Organ Transplant Recipients: Subgroup Analysis of the Phase 3 Randomized SOLSTICE Study. *J Heart Lung Transpl* (2025) 44: 986–94. doi:10.1016/j.healun.2024.11.026
 21. Illic K, Song I, Wu J, Martin P. Evaluation of the Effect of Maribavir on Cardiac Repolarization in Healthy Participants: Thorough QT/QTc Study. *Clin Transl Sci* (2020) 13:1260–70. doi:10.1111/cts.12814
 22. Dobrer S, Sherwood KR, Hirji I, Lan J, Gill J, Matic N, et al. Viral Load Kinetics and the Clinical Consequences of Cytomegalovirus in Kidney Transplantation. *Front Immunol* (2023) 14:1302627. doi:10.3389/fimmu.2023.1302627
 23. Heim C, Müller PP, Tandler R, Cherikh WS, Toll AE, Stehlik J, et al. Cytomegalovirus Donor Seropositivity Negatively Affects Survival After Heart Transplantation. *Transplantation* (2022) 106:1243–52. doi:10.1097/TP.0000000000003961
 24. Vutien P, Perkins J, Biggins SW, Reyes J, Imlay H, Limaye AP. Association of Donor and Recipient Cytomegalovirus Serostatus on Graft and Patient Survival in Liver Transplant Recipients. *Liver Transpl* (2021) 27:1302–11. doi:10.1002/lt.26045
 25. Lentine KL, Costa SP, Weir MR, Robb JF, Fleisher LA, Kasiske BL, et al. Cardiac Disease Evaluation and Management Among Kidney and Liver Transplantation Candidates: A Scientific Statement from the American Heart Association and the American College of Cardiology Foundation. *J Am Coll Cardiol* (2012) 60: 434–80. doi:10.1016/j.jacc.2012.05.008
 26. Tovikkai C, Charman SC, Praseedom RK, Gimson AE, van der Meulen J. Time-Varying Impact of Comorbidities on Mortality After Liver Transplantation: A National Cohort Study Using Linked Clinical and Administrative Data. *BMJ Open* (2015) 5:e006971. doi:10.1136/bmjopen-2014-006971
 27. Ou LB, Moriello C, Douros A, Filion KB. Domperidone and the Risks of Sudden Cardiac Death and Ventricular Arrhythmia: A Systematic Review and Meta-Analysis of Observational Studies. *Br J Clin Pharmacol* (2021) 87: 3649–58. doi:10.1111/bcp.14737
 28. Zentiva Pharma UK Limited. Information for the User. *Domperidone 10 mg Film-Coated Tablets* (2024).
 29. Jiroutek MR, Turner JR. Why it is Nonsensical to Use Retrospective Power Analyses to Conduct a Postmortem on Your Study. *J Clin Hypertens (Greenwich)* (2018) 20:408–10. doi:10.1111/jch.13173

Copyright © 2025 Kamar, Avery, Bo, Gu, Kumar and Witzke. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.



Evaluating Post-Transplant Outcomes in Elderly Liver Recipients Over 70: A Propensity-Score Matching Analysis

Edoardo Prosperi¹, Enrico Prosperi^{1*}, Matteo Serenari^{1,2*}, Chiara Bonatti¹, Guido Fallani², Alberto Stocco¹, Giorgia Radi¹, Federica Mirici Cappa³, Cristiana Laici⁴, Antonio Siniscalchi⁴, Maria Cristina Morelli³, Matteo Ravaioli^{1,2} and Matteo Cescon^{1,2}

¹Department of Medical and Surgical Sciences, Alma Mater Studiorum, University of Bologna, Bologna, Italy, ²Hepatobiliary and Transplant Unit, IRCSS, Azienda Ospedaliero-Universitaria di Bologna, Bologna, Italy, ³Internal Medicine Unit for the Treatment of Severe Organ Failure, IRCCS Azienda Ospedaliero-Universitaria di Bologna, Bologna, Italy, ⁴Post-Surgical and Transplant Intensive Care Unit, Department of Digestive, Hepatic and Endocrine-Metabolic Diseases, IRCCS Azienda Ospedaliero-Universitaria di Bologna, Bologna, Italy

The rising prevalence of Metabolic Dysfunction-Associated Steatotic Liver Disease (MASLD) and Hepatocellular Carcinoma in the elderly population has increased the demand for liver transplantation (LT) in patients over 70 years. Advanced age, however, is still considered an independent risk factor. This study aims to evaluate post-transplant outcomes in patients aged over 70 years, traditionally viewed as an age limit for transplant. We retrospectively analyzed 584 LT recipients (36 aged ≥ 70 , 548 aged < 70). Viral cirrhosis was more frequent in the younger group (36.1% vs. 13.1%), while MASLD was more common in those over 70 (25% vs. 13.1%) ($p = 0.013$). Model for End-Stage Liver Disease (MELD) scores were lower in patients over 70 (13, IQR 9–17) compared to the younger group (15, IQR 10–23) ($p = 0.032$). Propensity score matching (3:1 ratio, without replacement) was performed based on MELD and cirrhosis etiology. After matching, no significant differences were found in postoperative outcomes, overall survival, or graft survival. Our findings demonstrate that carefully selected patients over 70 can achieve post-transplant outcomes comparable to younger patients. Advanced age alone should not be considered an absolute contraindication; instead, a comprehensive, multidimensional assessment is essential to identify suitable candidates.

OPEN ACCESS

*Correspondence

Enrico Prosperi,
✉ enrico.prosperi@studio.unibo.it
Matteo Serenari,
✉ matteo.serenari3@unibo.it

Received: 13 August 2025

Revised: 21 October 2025

Accepted: 10 November 2025

Published: 24 November 2025

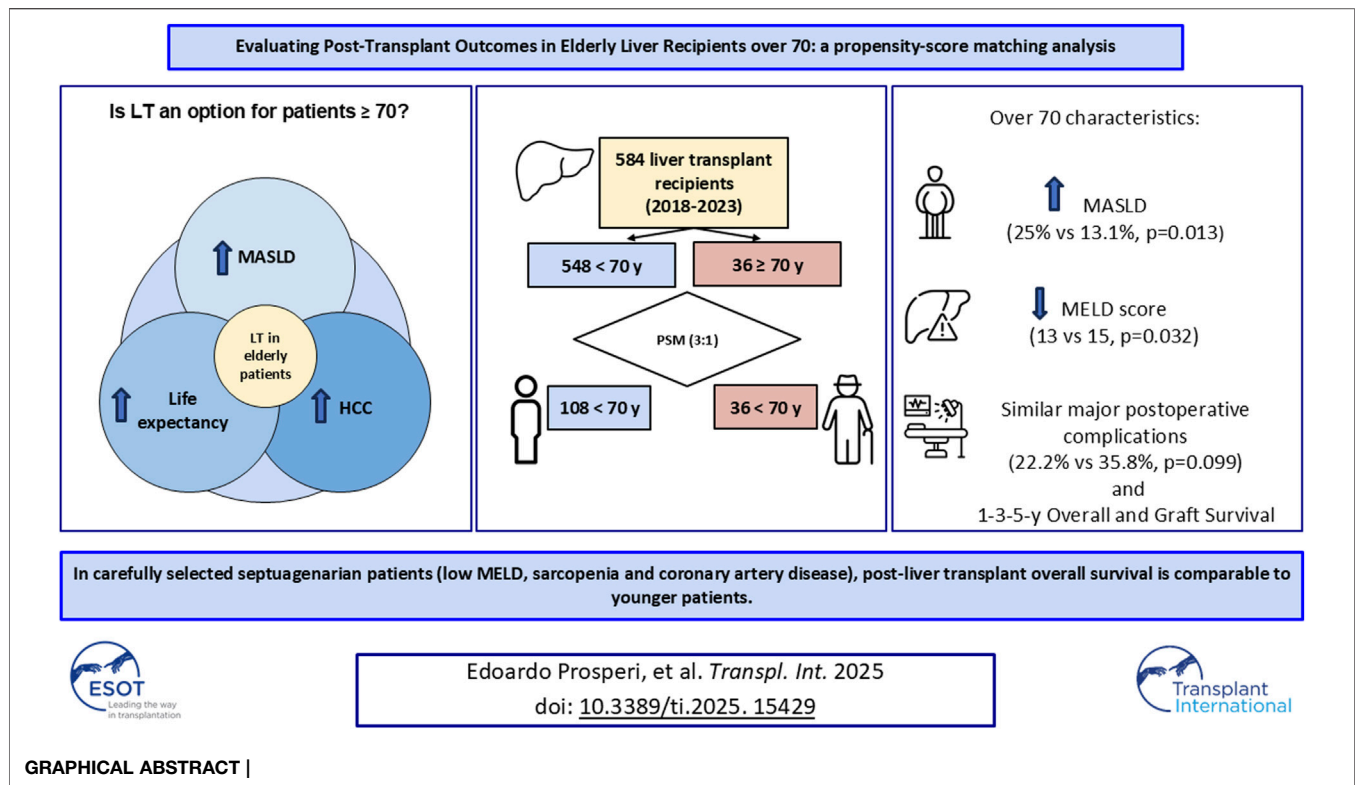
Citation:

Prosperi E, Prosperi E, Serenari M, Bonatti C, Fallani G, Stocco A, Radi G, Mirici Cappa F, Laici C, Siniscalchi A, Morelli MC, Ravaioli M and Cescon M (2025) Evaluating Post-Transplant Outcomes in Elderly Liver Recipients Over 70: A Propensity-Score Matching Analysis. *Transpl. Int.* 38:15429. doi: 10.3389/ti.2025.15429

Keywords: MASLD, propensity score matching, liver transplant recipients, elderly recipients, MELD score

INTRODUCTION

Liver transplant (LT) is the only curative treatment for end-stage liver disease (ESLD). Globally, along with a longer life expectancy, the prevalence of Metabolic Dysfunction-Associated Steatotic Liver Disease (MASLD) and Hepatocellular Carcinoma (HCC) is rising in patients older than 65 years old [1–3], resulting in an increased number of liver transplants performed in this group. This trend is observed both in the United States and in Europe [4–7]. Notably, older LT candidates often show multiple comorbidities, independently from liver disease, that negatively influence post-transplant outcomes [8, 9]. Despite the increasing amount of literature addressing this topic, findings remain inconclusive. Some studies have reported poorer outcomes associated with older age, including higher graft loss and reduced survival, in particular in patients with high Model For End-Stage Liver Disease (MELD) scores [10, 11]. Conversely, other works have proved that liver transplantation in selected patients aged 70 and older is



safe and feasible, showing graft survival and overall survival rates similar to younger cohorts [12–14] [15]. Importantly, the guidelines from American Association for the Study of Liver Diseases and the American Society of Transplantation do not consider age over 70 years old a contraindication to LT, in the absence of significant comorbidities [16].

This heterogeneity in published results probably reflects differences found in previous analyses in study design, patient selection and institutional practices.

Emerging evidence suggests that, rather than chronological age alone, the severity of comorbidities and liver disease and the general functional status of the patients represent more reliable prognostic indicators of post-transplant outcomes, underscoring the importance of careful selection in achieving favorable results.

We present our experience with LT recipients aged ≥ 70 years. This study aims to describe the characteristics of our cohort of recipients aged over 70 and evaluate their post-transplant outcomes in comparison to younger recipients.

MATERIALS AND METHODS

Study Design and Setting

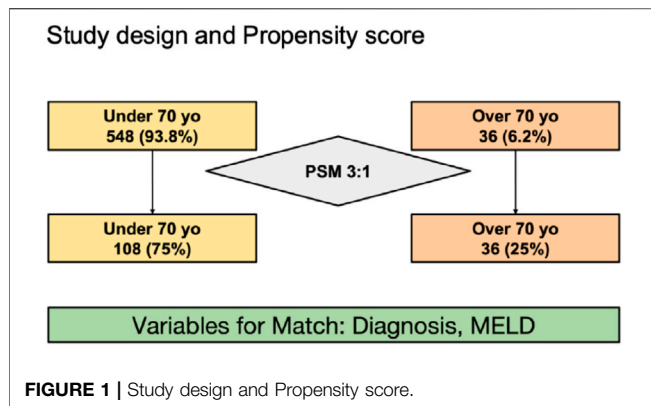
Between January 2018 and November 2023, all consecutive adult recipients who underwent cadaver-donor LT in our center were included. We divided the entire cohort in two groups based on age at LT: younger than 70 years old and aged 70 years old or older (Figure 1).

This study was approved by the Institutional Review Board of our hospital.

Data Collection and Clinical Variables

Data were collected prospectively and retrospectively analyzed. Recipient variables included demographics, MELD score at LT and urgent LT (defined, in our national practice, as MELD ≥ 28), indication for LT, comorbidities, and modified Charlson Comorbidity Index (high modified Charlson Comorbidity Index was defined as mCCI > 1) [17].

Donor variables included demographics, donation type, whole or split liver graft, and cold ischemia time (CIT). Postoperative outcomes included complications (defined as major if Clavien-Dindo grade ≥ 2 [18]), Early Allograft Dysfunction (EAD) [19], Comprehensive Complication Index (CCI), 90-day mortality, 90-day readmission, patient survival and graft survival. During the study period, orthotopic liver transplantation was preferably performed with preservation of the inferior vena cava, with few cases performed with vena cava replacement. Over the years, an increasing number of Extended Criteria Donor (ECD) grafts were treated with Hypothermic Oxygenated perfusion (HOPE), which is at present our standard preservation technique with ECDs [20]. Regarding the cardiological assessment, all LT candidates underwent a standardized preoperative cardiological evaluation, including transthoracic echocardiography and Doppler ultrasound of the supra-aortic vessels. Coronary computed tomography angiography (CCTA) was indicated in patients presenting with one major or two minor cardiovascular risk factors.



Major risk factors included: age >50 years, diabetes mellitus, history of angina, myocardial infarction, stroke, transient ischemic attack, heart failure, or chronic kidney disease.

Minor risk factors included: smoking, hypercholesterolemia, hypertension, chronic alcohol consumption, and male sex >40 years or female sex >45 years.

When CCTA findings were inconclusive or suspicious for significant coronary artery disease, invasive coronary angiography was performed. Coronary stent placement was undertaken in the presence of significant stenosis, provided that the patient could complete at least 6 months of dual antiplatelet therapy before transplantation.

Stress echocardiography and/or myocardial scintigraphy were performed in selected cases, depending on patient suitability for the test and the availability of nuclear medicine facilities.

CT scans at the time of LT in the group over 70 years old were evaluated for assessing sarcopenia. Psoas Muscle Area (PMA) (mm²) is defined as the cross-sectional area of the left and right psoas muscles at the level of the third lumbar vertebra (L3). We employed as PMA cut-offs to define sarcopenia values <1,561 mm² in men and <1464 mm² in women [21]. The Psoas Muscle Index (PMI) was obtained as PMA (cm²) divided by square height (m²). We employed as PMI cut-offs to define sarcopenia values <340 mm²/m² in men and <264 mm²/m² in women [22].

Multidisciplinary Board and Selection Process

Each candidate undergoes a final evaluation by a multidisciplinary board. Candidate assessment involves transplant surgeons, hepatologists, transplant anesthesiologists, radiologists, and psychologists. Additional evaluation by other specialists are requested in case of complex cases. Final eligibility is based on a comprehensive evaluation of the comorbidities, functional status, cognitive integrity, oncologic considerations (if applicable), and expected post-transplant benefit.

Objectives

The primary endpoint of the study was to evaluate post-transplant mortality, overall patient survival and graft survival comparing recipients aged 70 or older with those younger than 70.

The secondary endpoint was to evaluate postoperative complications.

Statistical Analysis

Categorical variables were reported as absolute numbers and percentages and compared using the Chi-square test or Fisher's exact test, as appropriate. Continuous variables were expressed as medians with interquartile ranges (IQR) and compared using the Mann-Whitney U test. A two-tailed p-value <0.05 was considered statistically significant. Statistical analyses were performed using STATA 18.

Patients of both groups were matched using the propensity score method (PSM) as described by Rubin and Rosenbaum [23]. The propensity score for an individual was calculated given the covariates of LT indication and MELD score, the two variables that differed significantly between the two groups. A 3:1 matching was performed without replacement to minimize conditional bias (Figure 1). For each recipient in the younger group, an elderly recipient with a minimum difference on the propensity score was matched.

RESULTS

Entire Cohort Characteristics

Within the cohort of 584 patients, the elderly group (n = 36, 6.2%) showed a median age of 71 (70–72 IQR), whereas the younger than 70 years group (n = 548, 93.8%) showed a median age of 58 (52–63 IQR) (Table 1).

Recipients' Characteristics

The majority of patients were male, with a higher percentage in the younger group (66.4% vs. 55.6%, p = 0.183) without a statistically significant difference. Median BMI was 25 in both groups (p = 0.363) (Table 1). The two groups differed significantly in cirrhosis etiology and median MELD score. Viral cirrhosis was the most frequent indication in the younger group (n = 198, 36.1% vs. n = 5, 13.9%, p = 0.013). MASLD was more frequent in the elderly group (n = 72, 13.1% vs. n = 9, 25%, p = 0.013). The elderly group had a significantly lower median MELD score (15 vs. 13, p = 0.017).

Clinically significant portal hypertension was present in 81.8% of the elderly group and in 83.9% of the younger group (p = 0.798). Diagnosis of Hepatocellular Carcinoma (HCC) did not differ between elderly and younger groups (36.1% vs. 40.9%, p = 0.573).

Complications of Cirrhosis and Laboratory Values

There were no statistically significant differences in terms of cirrhosis-related conditions between the two populations (Table 1). Ascites was the most frequent complication of cirrhosis in both groups (63.9% vs. 57.2%, p = 0.550). Encephalopathy, jaundice, and esophageal varices were slightly less frequent in the elderly population, but without reaching statistical significance (38.9% vs. 49.6%, p = 0.377; 40.9% vs. 49.8%, p = 0.418; 18.2% vs. 26.3%, p = 0.95, respectively).

TABLE 1 | Preoperative and donor Parameters before and after PSM.

Preoperative parameters	Before PSM				After PSM			
	Under 70 Yo	Over 70 Yo	Total	p-value	Under 70 Yo	Over 70 Yo	Total	p-value
	(N = 548)	(N = 36)	(N = 584)		(N = 108)	(N = 36)	(N = 144)	
Recipient age, years	58 (52–63)	71 (70–72)	58 (53–64)	<0.001	56 (48–63)	71 (70–72)	61 (52–70)	<0.001
Recipient gender male	364 (66.4%)	20 (55.6%)	384 (65.8%)	0.183	67 (62.0%)	20 (55.6%)	87 (60.4%)	0.491
BMI	25 (23–28)	25 (22–28)	25 (23–28)	0.363	26 (22.29)	25 (23–29)	26 (22–29)	0.458
mCCI >1	67 (12.2%)	7 (19%)	113 (26%)	0.207	5 (6.8%)	7 (19.4%)	12 (10.9%)	0.045
Liver transplant indication								
Viral	198 (36.1%)	5 (13.9%)	203 (34.8%)	0.013	22 (20.4%)	5 (13.9%)	27 (18.8%)	0.633
Alcohol-related liver disease	126 (23.0%)	7 (19.4%)	133 (22.8%)		17 (15.7%)	7 (19.4%)	24 (16.7%)	
MASLD	72 (13.1%)	9 (25.0%)	81 (13.9%)		19 (17.6%)	9 (25.0%)	28 (19.4%)	
Other	152 (27.7%)	15 (41.7%)	167 (28.6%)		50 (46.3%)	15 (41.7%)	65 (45.1%)	
Hepatocellular carcinoma	224 (40.9%)	13 (36.1%)	237 (40.6%)	0.573	44 (40.7%)	13 (36.1%)	57 (39.6%)	0.623
TIPS	33 (6.0%)	1 (2.8%)	34 (5.8%)	0.418	4 (3.7%)	1 (2.8%)	5 (3.5%)	0.623
Portal hypertension	354 (83.9%)	18 (81.8%)	372 (83.8%)	0.798	56 (71.8%)	18 (81.8%)	74 (74.0%)	0.344
Cirrhosis related complications								
Ascites	255 (57.2%)	14 (63.6%)	269 (57.5%)	0.550	52 (60.5%)	14 (63.6%)	66 (61.1%)	0.785
Hepatic encephalopathy	172 (49.6%)	7 (38.9%)	179 (49.0%)	0.377	33 (47.8%)	7 (38.9%)	40 (46.0%)	0.498
Jaundice	210 (49.8%)	9 (40.9%)	219 (49.3%)	0.418	27 (34.6%)	9 (40.9%)	36 (36.0%)	0.587
Coagulopathy	237 (56.8%)	13 (59.1%)	250 (56.9%)	0.835	38 (48.7%)	13 (59.1%)	51 (51.0%)	0.39
Esophageal varices	109 (26.3%)	4 (18.2%)	113 (25.9%)	0.395	18 (23.4%)	4 (18.2%)	22 (22.2%)	0.605
Comorbidities								
Respiratory	99 (23.5%)	8 (32.0%)	107 (24.0%)	0.334	20 (23.8%)	8 (32.0%)	28 (25.7%)	0.411
Renal	79 (18.5%)	4 (15.4%)	83 (18.3%)	0.694	7 (8.2%)	4 (15.4%)	11 (9.9%)	0.286
Cardiological	213 (47.8%)	12 (54.5%)	225 (48.1%)	0.534	41 (47.7%)	12 (54.5%)	53 (49.1%)	0.565
Coronarography	17 (3.1%)	2 (5.5%)	19 (3.2%)	0.422	4 (3.7%)	2 (5.5%)	6 (4.1%)	0.63
Diabetes	143 (31.2%)	13 (46.4%)	156 (32.0%)	0.093	29 (31.9%)	13 (46.4%)	42 (35.3%)	0.159
MELD at LT	15 (10–23)	13 (9–17)	15 (10–23)	0.032	12 (10–15)	13 (9–17)	12 (9–16)	0.149
Urgent LT	90 (16.4%)	4 (11.1%)	94 (16.1%)	0.401	5 (4.6%)	4 (11.1%)	9 (6.25%)	0.164
Platelets (10 ³ /mmc)	82.5 (49–138.5)	113 (65–149.5)	84 (50–139)	0.078	82.5 (49–138.5)	113 (65–149.5)	84 (50–139)	0.814
Sodium (mmol/L)	137 (134–139)	137.5 (134.5–139.5)	137 (134–139)	0.541	137 (134–139)	137.5 (134.5–139.5)	137 (134–139)	0.752
Albumin (g/dL)	3.5 (3–4)	3.7 (3.3–3.9)	3.5 (3–4)	0.2068	3.5 (3.1–4)	3.7 (3.3–3.9)	3.6 (3.2–4)	0.558
Previous abdominal surgery	334 (61.1%)	22 (61.1%)	356 (61.1%)	0.995	77 (72.0%)	22 (61.1%)	99 (69.2%)	0.222
Donor parameters								
Donor age, years	64 (49–76)	64.5 (56.5–75)	64 (50–76)	0.420	67 (48.5–78)	64.5 (56.5–75)	67 (52–78)	0.358
Donor gender male	307 (56.0%)	17 (47.2%)	324 (55.5%)	0.303	62 (57.4%)	17 (47.2%)	79 (54.9%)	0.288
Donor BMI	25 (23–28)	25 (23–28)	25 (23–28)	0.2	26 (23–29)	25 (23–29)	25 (23–29)	0.456
DCD	60 (10.9%)	5 (13.9%)	65 (11.1%)	0.587	22 (20.4%)	5 (13.9%)	27 (18.8%)	0.388
Extended criteria donor	465 (84.9%)	29 (80.6%)	494 (84.6%)	0.34	93 (86.1%)	29 (80.6%)	122 (84.7%)	0.422
Split donor graft	20 (3.65%)	0	20 (3.42%)	0.243	5 (4.6%)	0	5 (4.5%)	0.189
Donor bilirubin	0.78 (0.49–1.1)	0.49 (0.4–0.87)	0.75 (0.48–1.1)	0.242	0.7 (0.5–1.1)	0.5 (0.4–0.9)	0.7 (0.5–1.1)	0.22
Donor GOT (U/L)	31 (20–61)	28 (20–51)	31 (20–61)	0.693	37 (23–70)	28 (19–50)	34 (22–68)	0.436
Donor GPT (U/L)	25 (15–53)	22 (14–39)	24 (14–52)	0.699	34 (15–63)	21 (13–39)	30 (15–61)	0.401
Donor Sodium (mmol/L)	149 (144–156)	149 (144–161)	149 (144–156)	0.372	147 (142–155)	149 (144–161)	147 (142–156)	0.146
Cold ischemia time, minutes	390 (350–450)	372 (340–445)	390 (350–450)	0.203	380 (335–422)	372 (340–445)	375 (337–430)	0.818
HOPE	225 (41%)	18 (50%)	243 (41%)	0.292	38 (35.2%)	18 (50%)	56 (38.89%)	0.864

Continuous Values Expressed as Median (IQR), categorical values expressed as number (%). Abbreviations: mCCI, Modified Charlson Comorbidity Index; DCD, Donor after Cardiac Death; HOPE, Hypothermic Oxygenated Perfusion; GOT, Glutamic Oxaloacetic Transaminase; GPT, Glutamate Pyruvate Alanine Aminotransferase.

Presence of coagulopathy was similar between the two groups (59.1% vs. 56.8%, $p = 0.835$).

Sodium and albumin levels were comparable between the elderly and the younger groups [137.5 mmol/L IQR (134.5–139.5) vs. 137 mmol/L IQR (134–139), $p = 0.54$; 3.7 g/dL IQR (3.3–3.9) vs. 3.5 IQR (3–4), $p = 0.206$].

Comorbidities

Respiratory, renal and cardiological comorbidities, and diagnosis of diabetes were similar between the two groups (Table 1). Cardiological comorbidities were the most common in both groups and, although they did not show statistically significant difference, were slightly higher in the elderly group, as well as

TABLE 2 | Comorbidities and Sarcopenia scores of Elderly recipients.

Comorbidity	Frequency (%) or median
Hypertension	17 (47.2%)
Dyslipidemia	14 (40.0%)
Smoking	20 (55.5%)
Non-hepatic malignancy	2 (5.6%)
Chronic obstructive pulmonary disease (COPD)	9 (25.0%)
History of myocardial infarction (MI)	2 (5.6%)
History of percutaneous coronary intervention (PCI)	2 (5.6%)
Atrial fibrillation (AF)	2 (5.6%)
Coronary computed tomography angiography (CCTA)	21 (58.3%)
Coronary calcium score (agatston)	56 (0–989)
Psoas muscle area, (mm ²)	1,700 (1,300–2,390)
Male, (mm ²)	2,130 (1,600–2,500)
Female, (mm ²)	1,300 (1,000–1,600)
Psoas muscle index, mm ² /m ²	625 (445–801)
Male, mm ² /m ²	763 mm ² /m ² (555–849)
Female, mm ² /m ²	470 mm ² /m ² (410–625)
Sarcopenia (defined by PMA) (n,%)	13 (36.1%)
Male (n, %)	5 (22.73%)
Female (n, %)	8 (57.14%)
Sarcopenia (defined by PMI) (n,%)	0%

Continuous Values Expressed as Median (IQR), categorical values expressed as number (%).

diabetes and respiratory comorbidities (54.5% vs. 47.8%, $p = 0.534$; 46.4% vs. 31.2%, $p = 0.093$; 32% vs. 23.5%, $p = 0.334$, respectively). Pre-transplant coronarography rate was comparable between groups (5.5% vs. 3.1%, $p = 0.422$). The rate of previous abdominal surgery was equal (61.1% vs. 61.1%, $p = 0.995$). Before PSM analysis, mCCI >1 was slightly higher in the elderly group compared to the younger group, but without reaching statistical significance (19% vs. 12.2%, $p = 0.207$).

Specific Cardiological Comorbidities and Sarcopenia of the Elderly Group

Regarding specific history of cardiological comorbidities, 2 patients had history of myocardial infarction treated with PCI ($n = 2$, 5.6%) and 2 patients had history of atrial fibrillation. Coronary computed tomography angiography (CCTA) was performed in 21 patients, accounting for 58.3% of the group. Median Coronary Calcium Score (Agatston score) was 56 (IQR 0–989) (Table 2).

In the elderly cohort, median PMA was 1,710 mm² (IQR 1,300–2,390), lower in females than males (1,300 vs. 2,130 mm²). Median PMI was 6.25 mm²/m² (IQR 4.45–8.01), also lower in females than males (4.70 vs. 7.63 mm²/m²). Using PMA cut-offs, 13 patients (36.1%) were classified as sarcopenic (8 females, 5 males), whereas no patients met sarcopenia criteria using PMI cut-offs.

Donor Characteristics

There were no statistically significant differences between the elderly and the younger group regarding the donor's characteristics (Table 1). Median donor age was 64.5 years (IQR 56.5–75) in the elderly group vs. 64 years in the younger group (IQR 49–76) ($p = 0.420$), median donor BMI was 25 in both groups (IQR 23–28) ($p = 0.2$). Most of the grafts were classified as Extended criteria donors in both groups ($n = 29$, 80.6% vs. $n = 465$, 84.9%, $p = 0.489$). Rate of

DCD donors were comparable between the 2 groups (13.9% vs. 10.9%, $p = 0.587$). Median cold ischemia time was slightly lower in the elderly group, but without reaching statistical significance (372 min vs. 390 min, $p = 0.203$).

The use of Hypothermic oxygenated perfusion (HOPE) was comparable between the elderly and the younger group (50% vs. 41%, $p = 0.292$). The number of LT with split graft was 20 (3.65%) in the younger group, compared to 0 (0%) in the elderly group, without reaching statistical significance ($p = 0.243$).

Postoperative Outcomes, Graft and Overall Survival

There were no significant statistical differences between the elderly group and the younger group regarding postoperative parameters (Table 3). The rate of major complications was similar between the two groups (22.2% vs. 35.8%, respectively, $p = 0.099$), as was CCI (29.6, vs. 30.2, $p = 0.526$).

The rates of EAD, hepatic artery thrombosis, postoperative infection, biliary and cardiac complications, pneumonia and acute rejection were comparable between the two populations. The 90-day mortality rates were comparable between the two groups (5.6% vs. 5.11%, $p = 0.907$). Rates of re-transplantation within 90 days were similar across the elderly and the younger group (2.8% vs. 3.8%, $p = 0.748$).

The two patients who died within the first 90 postoperative days were female. Both had an mCCI >1, and neither had a history of coronary artery disease. However, they belonged to the lowest quartile for sarcopenia measurements, based on both PMA and PMI scores.

In the elderly group, 1-year overall survival was 94.4% and 3- and 5-year survival was 89.5%. Graft survival was 94.4% at 1 year, and 89.5% at 3 and 5 years. In the younger group, 1-, 3- and 5-year overall survival were 92.9%, 89.1% and 87.7% respectively, while 1-, 3- and 5-year graft survival were 89.5%, 85.5% and 84.4%, respectively (Figure 2; Table 4).

Adjusted Outcomes With Case-Matched Analysis

A case-matched analysis was performed using matched cohorts created from propensity scores. The 3:1 matching algorithm identified two matched cohorts ($n = 108$ in the under 70 group and 36 in the over 70 group) in which all differences between recipient characteristics were balanced (Table 1). This allowed for the direct comparison of outcomes between the study cohorts, which again demonstrated similar perioperative outcomes, overall survival and graft survival. However, mCCI >1, which did not reach statistical significance in the unadjusted analysis, after matching was significantly higher in the elderly group compared to the younger cohort (19.4% vs. 10.9%, $p = 0.045$) (Table 1).

DISCUSSION

Our study shows that carefully selected LT recipients aged 70 years or older can achieve outcomes comparable to those

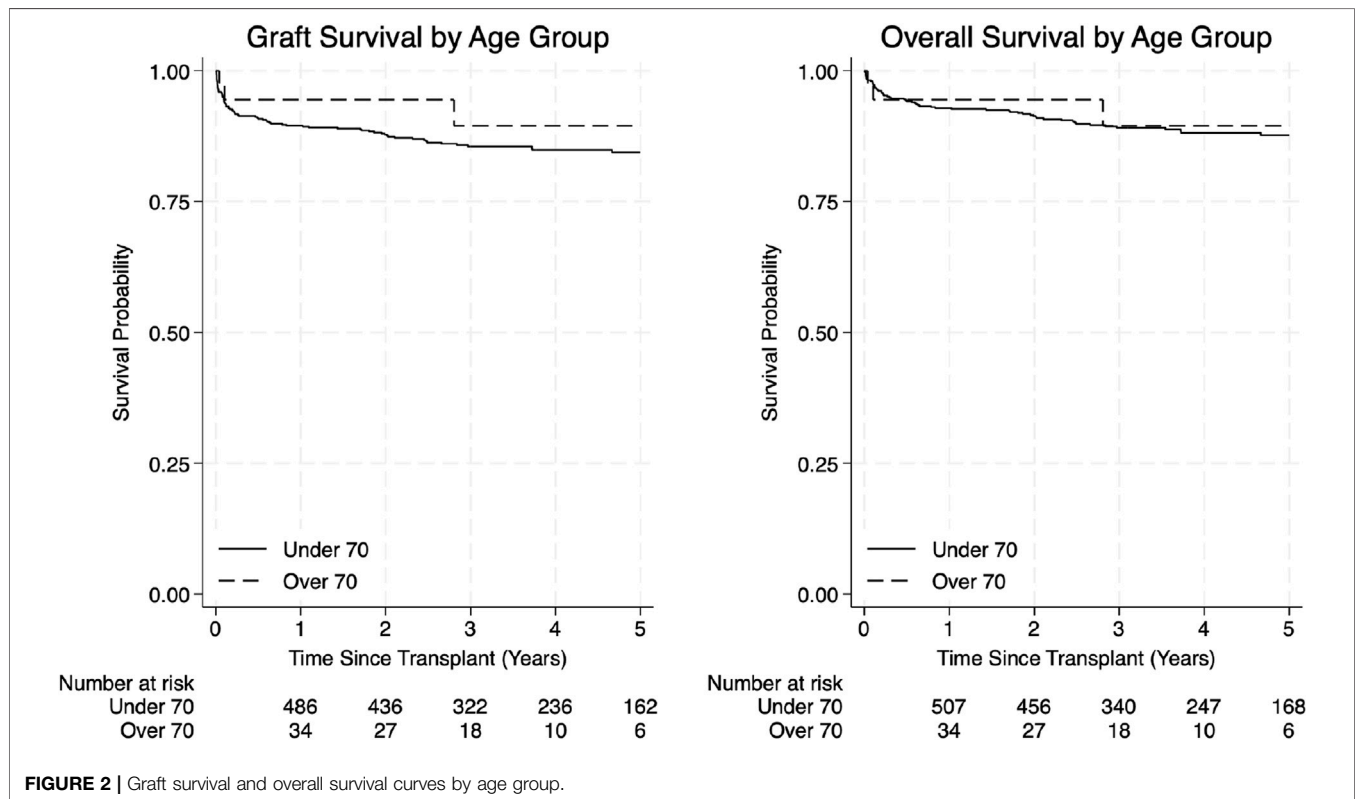
TABLE 3 | Postoperative parameters before and after PSM.

Post-operative parameters	Before PSM				After PSM			
	Under 70 Yo	Over 70 Yo	Total	p-value	Under 70 Yo	Over 70 Yo	Total	p-value
	(N = 548)	(N = 36)	(N = 584)		(N = 108)	(N = 36)	(N = 144)	
Early allograft dysfunction	154 (28.1%)	7 (19.4%)	161 (27.6%)	0.26	33 (30.6%)	7 (19.4%)	40 (27.8%)	0.197
Hepatic artery thrombosis	6 (1.1%)	0 (0.0%)	6 (1.1%)	0.528	1 (0.9%)	0 (0.0%)	1 (0.7%)	0.566
Re-transplantation ^a	21 (3.8%)	1 (2.8%)	22 (3.8%)	0.748	7 (6.5%)	1 (2.8%)	8 (5.6%)	0.401
Re-laparotomy	78 (14.7%)	4 (11.4%)	82 (14.5%)	0.84	11 (10.3%)	4 (11.4%)	15 (10.6%)	0.848
Biliary complication	55 (10.3%)	1 (2.9%)	56 (9.8%)	0.152	10 (9.3%)	1 (2.9%)	11 (7.7%)	0.213
Cardiac complication	50 (11.4%)	1 (4.5%)	51 (11.0%)	0.319	8 (9.4%)	1 (4.5%)	9 (8.4%)	0.464
Postoperative infection	225 (41.9%)	14 (40.0%)	239 (41.8%)	0.825	40 (38.1%)	14 (40.0%)	54 (38.6%)	0.841
Pneumonia	47 (10.5%)	4 (18.2%)	51 (10.9%)	0.261	7 (8.1%)	4 (18.2%)	11 (10.2%)	0.165
Acute cellular rejection	29 (5.4%)	0 (0.0%)	29 (5.1%)	0.158	7 (6.6%)	0 (0.0%)	7 (5.0%)	0.119
Readmission by 30 post-operative day	113 (26.0%)	7 (31.8%)	120 (26.3%)	0.548	23 (27.7%)	7 (31.8%)	30 (28.6%)	0.705
Clavien dindo >2	196 (35.8%)	8 (22.2%)	204 (34.9%)	0.099	35 (32.4%)	8 (22.2%)	43 (29.9%)	0.248
CCI	30.2 (20.9–48.3)	29.6 (20.9–38.35)	29.6 (20.9–47.6)	0.516	29.6 (20.9–39.6)	29.6 (20.9–38.4)	29.6 (20.9–39.5)	0.976
Failure to rescue	25 (4.5%)	1 (2.8%)	26 (4.5%)	0.615	5 (4.6%)	1 (2.8%)	6 (4.2%)	0.63
LOS (days)	16 (11–29)	14.5 (12–17)	16 (11–28)	0.289	15 (12–17)	14.5 (12–17)	15 (12–17)	0.738
Mortality ^b	28 (5.11%)	2 (5.6%)	30 (5.1%)	0.907	7 (6.5%)	2 (5.6%)	9 (6.2%)	0.842

Continuous Values Expressed as Median (IQR), categorical values expressed as number (%). Abbreviations: CCI, Comprehensive Complication Index; LOS, Length of Hospital Stay.

^aRe-Transplantation within 30 days post Liver Transplantation.

^bMortality Within 90 Days post Liver Transplantation.



of younger recipients, without significant differences in terms of postoperative complications, graft survival, and overall survival. The same results were obtained even after adjusted analysis eliminating preoperative differences, indicating that advanced

age alone should not be considered an absolute contraindication to LT.

In recent years, LT in elderly recipients has become a prominent and pressing issue. In particular, the rising

TABLE 4 | Overall survival and graft survival.

Graft survival				
Time (years)	Under 70	Over 70	Total	p-value
1	89.5 (86.6–91.8)	94.4 (79.6–98.6)	90.0 (87.2–92.2)	0.3484
3	85.5 (82.2–88.3)	89.5 (69.4–96.7)	86.0 (82.8–88.6)	
5	84.4 (80.9–87.4)	89.5 (69.4–96.7)	84.9 (81.5–87.8)	
Overall survival				
1	92.9 (90.4–94.7)	94.4 (79.6–98.6)	92.3 (90.6–94.8)	0.6947
3	89.1 (86.1–91.5)	89.5 (69.4–96.7)	89.2 (86.2–91.5)	
5	87.7 (84.3–90.4)	89.5 (69.4–96.7)	87.8 (84.6–90.4)	

Kaplan–Meier estimates of 1-, 3-, and 5-year graft survival, reported as Survival % (Lower CI – Upper CI). Comparison between groups was assessed using the Log-Rank test.

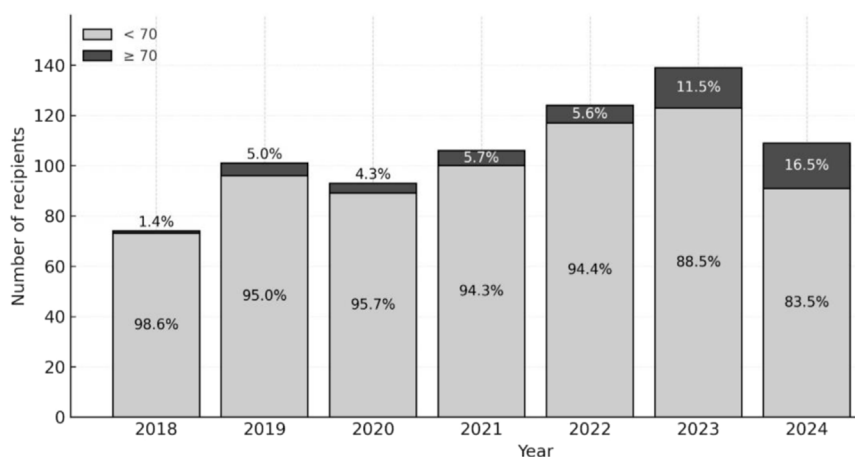


FIGURE 3 | Proportion of Liver Transplant recipients Aged ≥70 by year from 2018 to 2024.

prevalence of MASLD and HCC in patients older than 65 years old has led to an increased demand for LT in this age group, and it has been demonstrated that this population can achieve promising results, reaching a 5-year overall survival of 77% [1]. However, the studies focusing on LT performed in recipients aged 70 or older are limited in the literature.

Accordingly, in our center the proportion of LT recipients aged ≥70 has increased from 1.4% in 2018 to 16.5% in 2024 (Figure 3).

Our recipients aged ≥70 years demonstrated favorable outcomes with an overall survival of 94.4% at 1 year and a 3-year and 5-year survival of 89.5% and graft survival of 94.4% at 1 year, and a 3-year and 5-year survival of 89.5%, with no difference compared to the younger cohort.

These results are consistent with those reported by Wilson et al. [12] in a multicenter cohort of 312 LT recipients aged ≥70 years, from 2007 to 2011, with a median MELD score of 15. They found a 1-year overall survival of 84.9% and similar postoperative complications, with no differences in the adjusted analysis compared to a matched cohort of patients younger than 70 years old.

However, their mid- and long-term results were less favorable, with unadjusted survival rates in the elderly cohort of 74.0% at 3 years and 64.1% at 5 years, significantly lower compared to younger recipients.

Similarly, a recent single-center study by Toiv et al. [13], reported outcomes, from 2014 to 2023, of 43 liver transplant recipients aged ≥70 years in comparison to a cohort of 956 recipients aged <70 years. The elderly group had a lower median MELD score (21.4 vs. 24.9), a higher prevalence of MASLD (51.2% vs. 23.0%) higher rates of diabetes and hyperlipidemia compared to the younger group. The elderly group had significantly longer median hospital stay (25.5 vs. 14 days), but there were no differences in overall patient or graft survival compared to the younger cohort. In the elderly group overall survival was 93% at 1 year, and 78% at 3 and 5 years. The outcomes of this study are similar to ours; however, our older cohort had lower median MELD score (13 vs. 21.4), and shorter hospital stay (14.5 vs. 25.5 days), with no difference between groups, likely as effect of selection.

Earlier series have reported similar outcomes (Table 5). A study from Mayo Clinic analyzing data from 1998 to 2004 showed comparable 5-year survival rates and no significant differences in postoperative complications between LT recipients aged ≥70 years (73%) and <60 years (76%), in two matched cohorts of 42 patients in each group [15].

Similarly, Lipshutz et al., in a series from 1988 to 2005, demonstrated comparable graft and overall survival outcomes in patients aged ≥70 years (n = 62) compared to a younger group aged between 50 and 59 years (n = 864) [14]. Mousa et al.

TABLE 5 | Overview of liver transplant outcomes in patients ≥ 70 .

Author	Study population	No. of patients included	Compared groups	Outcomes
Lipshutz [14]	Single center, USA	926	≥ 70 years (n = 62) vs. 50–59 years (n = 864)	1-, 3-, 5- and 10-y OS: 73.3%, 65.8%, 47.1%, 39.7% (≥ 70 years); similar outcomes to younger patients in the unadjusted analysis
Aduen [15] Wilson [12]	Single center, USA SRTR and UHC databases, USA	84 12,445	≥ 70 (n = 42) vs. <60 years (n = 42) ≥ 70 years (n = 323) vs. 18–69 (n = 12,122) (1:1 matched)	Similar 5-year mortality risk and graft loss Unmatched analysis: similar GS, worse 1-, 3- and 5 years OS in the ≥ 70 years: 84.9, 74% and 64.1% Matched analysis: similar GS and OS
Sharpton [10]	UNOS registry	15,677	Stratified by age (18–59 (n = 11,966), 60–64 (n = 2,181), 65–69 (n = 1,177) and ≥ 70 years (n = 343) and MELD	≥ 70 years, MELD <20, 1 y GS: 85%, p = 0.01 ≥ 70 years, MELD 20–27, 1 y GS: 75%, p = 0.02 ≥ 70 years, MELD ≥ 28 , 1 y GS: 56%, p < 0.001 GS significantly lower than other groups, however attenuated for LT-MELD <28
Sharma [11]	UNOS registry	61,873 (HCC groups) 47,043 (HCV groups)	≥ 70 years vs. <70 years (HCC vs. non-HCC and HCV vs. non-HCV)	5 years OS: 59.9% vs. 68.6% (HCC, p < 0.01) and 61.2% vs. 74.2% (non-HCC, p < 0.001) 5 years OS: 60.7% vs. 69% (HCV, p < 0.01) and 62.6% vs. 78.5% (non-HCV, p < 0.001)
Gil [26]	KNHI, Korea	9,415	≥ 70 years (n = 84) vs. younger groups	Risk of death in ≥ 70 years four-fold higher (OR = 4.1, 95% CI 2.21–7.58; p < 0.001). 20% in-hospital mortality for ≥ 70 years
Toiv [13]	Single center, USA	999	≥ 70 n = (43) y vs. <70 years (n = 956)	Similar 1-, 3- and 5 years OS: 93, 78% and 78% (≥ 70 years) vs. 92, 86% and 82% (<70 years)

reported a 5- and 10-year overall survival after LT of 70.8% and 43.6% for a cohort of 162 patients over 70 years old [24]. As previously reported [3], HCC did not affect the outcomes of elderly patients after LT.

The first concern in elderly recipients is represented by early mortality.

In a nationwide Korean study from Gil et al. [25], who analyzed 9415 LT from 2007 to 2016 and included 84 recipients older than 70 years (47 Deceased Donor Liver Transplant and 37 Living Donor Liver Transplant), reported that in-hospital mortality increased significantly with age beyond 60 years old. Specifically, post-liver transplant in-hospital mortality of recipients over 70, adjusted for baseline liver disease, was 4.2 times higher than in patients aged between 51 and 55 years (20.2% vs. 4.8%), and almost 3 times higher after adjusting for perioperative complications. They also reported increasing costs with age [25].

Similarly, Gomez Gavara et al. [6], indicated age over 65 years as an independent prognostic factor for mortality and graft loss. They analyzed data of 10,172 liver recipients (1,062 older than 65 years old) from European Liver Transplant Registry (ELTR) and showed worse overall patient survival in recipients over 65 years (85% at 1 year, 71% at 3 years, 59% at 5 years and 55% at 10 years) and worse graft survival (84% at 1 year, 71% at 3 years, 59% at 5 years and 55% at 10 years), confirmed after PSM. However, in their subgroup analysis, they identified transplant indication as a major prognostic factor in elderly patients. In this group, while outcomes for HCC were similar to younger patients, alcohol-related cirrhosis showed worse 5 and 10-year survival of 47% and 33%, attributed to frailty and sarcopenia.

In our study, due to accurate selection, no increased mortality was observed in the elderly group.

MELD score has a prominent influence on postoperative outcomes and remains an important variable in assessing

candidacy for LT in patients over 70 years. Sharpton et al. [10] analyzed UNOS database that included all adult LT from 2005 to 2010, of which 343 were recipients older than 70 years old. They evaluated the combined effect of recipient's age and MELD at the time of LT and reported a poor 1-year graft survival of 56% for recipients ≥ 70 and MELD ≥ 28 .

However, in the same age group, MELD ≤ 20 and MELD between 20 and 27 showed better outcomes, with one-year graft survival rates of 85% and 75%, respectively, with no significant interaction between recipient age and MELD <28. Considering a lower median MELD in our older cohort, our results emphasize that, rather than age alone, liver disease severity should guide eligibility.

Moreover, cardiac risk is a critical prognostic factor in elderly recipients, as emerged by the series of Audet et al. [26]. In their study, recipients ≥ 65 years old (n = 34) showed higher mortality compared to younger controls. In the elderly group, 50% had a history of coronary artery disease (CAD), and the mortality rate due to cardiac causes was significantly higher. One and 3-year survival rates were 87% and 81.5%. As previously reported [14], cardiovascular events represent a major cause of mortality in older LT recipients and accurate cardiological screening is necessary to identify high-risk candidates.

In current literature, there are no strictly defined criteria for the exclusion of elderly patients from LT. Previous studies, as discussed above, have identified factors associated with increased post-transplant mortality risk: high MELD scores ≥ 28 , the presence of CAD and high comorbidity burden, expressed as mCCI >1 [17].

At our center the selection process for elderly recipients is based on a case-by-case evaluation, taking into account the benefits and risks associated with transplantation, the general health status, comorbidities, and perioperative risk. However, as reflected by our elderly cohort characteristics, selection is oriented to minimize these high-risk factors. Accordingly, in

our elderly cohort, only 2 patients had history of CAD and only 4 patients had MELD score ≥ 28 . Moreover, our elderly cohort showed a low comorbidity burden, with 81.6% of the elderly patients having mCCI < 2 .

Several limitations must be acknowledged to interpret our results appropriately. First, this is a retrospective single-center analysis with a relatively small number of elderly recipients. Given the design of our study, our purpose was not to draw definitive conclusions but to contribute to the ongoing research with additional data from our experience. Additionally, as recipient selection was based on a multidisciplinary board evaluation, the process did not rely on objective or reproducible selection criteria. Although our outcomes are quite favorable, they may be the result of careful local selection practices and may not be reproduced by other centers.

Second, this study includes only patients who received LT and therefore does not account for patients aged ≥ 70 years who were evaluated but ultimately excluded from liver transplantation. Because data on declined candidates were not systematically recorded during the study period, we were unable to analyze the characteristics in this group and the reasons for exclusion.

Third, frailty and sarcopenia were not consistently assessed in our cohort and were therefore not included in the comparative analysis. The modified Charlson Comorbidity Index (mCCI) was used as a surrogate measure of comorbidity burden, but this does not replace validated frailty metrics. Sarcopenia, as a surrogate marker of frailty, has been associated with unfavorable outcomes following liver transplantation [22]; however, as shown in our results, variability in cut-off thresholds across studies limits its reliability as a standalone clinical parameter.

Finally, long-term outcomes such as 10-year overall and graft survival could not be evaluated, as the follow-up period was limited to 5 years.

These limitations highlight the need for prospective, multicenter studies with standardized data collection and reproducible selection criteria for elderly candidates; future research should incorporate sarcopenia in combination with objective and functional frailty assessments, evaluate both accepted and declined candidates, and include quality of life assessment [7] to better define transplant candidacy in patients ≥ 70 years.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

REFERENCES

- Stepanova M, Kabbara K, Mohess D, Verma M, Roche-Green A, AlQahtani S, et al. Nonalcoholic Steatohepatitis Is the Most Common Indication for Liver Transplantation Among the Elderly: Data from the United States Scientific Registry of Transplant Recipients. *Hepatol Commun* (2022) 6:1506–15. doi:10.1002/hep4.1915
- Rich NE, Yopp AC, Singal AG, Murphy CC. Hepatocellular Carcinoma Incidence Is Decreasing Among Younger Adults in the

ETHICS STATEMENT

The studies involving humans were approved by the local Ethics Committee of IRCSS AOUBO, Comitato Etico Area Vasta Emilia Centro (CET AVEC). The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required from the participants or the participants' legal guardians/next of kin because of the observational nature of the study.

AUTHOR CONTRIBUTIONS

Conceptualization: EdP, EnP, MS, MC. Methodology: EdP, EnP, MS. Data curation: EdP, EnP, MS, CB, GF, AS, GR, FM, CL. Formal analysis: EnP, MS. Writing – original draft: EdP, EnP, CB. Writing – review and editing: MC, MS. Supervision: MC, AS, MM, MR.

FUNDING

The authors declare that no financial support was received for the research and/or publication of this article.

CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

GENERATIVE AI STATEMENT

The authors declare that Generative AI was used in the creation of this manuscript. AI was only employed in order to refine grammar and syntax. Scientific content and data interpretation belonged entirely to the authors.

Any alternative text (alt text) provided alongside figures in this article has been generated by Frontiers with the support of artificial intelligence and reasonable efforts have been made to ensure accuracy, including review by the authors wherever possible. If you identify any issues, please contact us.

United States. *Clin Gastroenterol Hepatol* (2020) 18:242–8.e5. doi:10.1016/j.cgh.2019.04.043

- Schwartz JJ, Pappas L, Thiesset HF, Vargas G, Sorensen JB, Kim RD, et al. Liver Transplantation in Septuagenarians Receiving Model for End-Stage Liver Disease Exception Points for Hepatocellular Carcinoma: The National Experience. *Liver Transplant* (2012) 18:423–33. doi:10.1002/lt.23385
- Guzzi J, Strand E, Ozturk B, Agarkov D, Deshpande R. Liver and Kidney Transplantation in Elderly Patients. *Transplantation* (2024) 5:98–109. doi:10.3390/transplantation5020010

5. Malinis MF, Chen S, Allore HG, Quagliarello VJ. Outcomes Among Older Adult Liver Transplantation Recipients in the Model of End Stage Liver Disease (Meld) Era. *Ann Transpl* (2014) 19:478–87. doi:10.12659/AOT.890934
6. Gómez-Gavara C, Lim C, Adam R, Zieniewicz K, Karam V, Mirza D, et al. The Impact of Advanced Patient Age in Liver Transplantation: A European Liver Transplant Registry Propensity-Score Matching Study. *HPB* (2022) 24:974–85. doi:10.1016/j.hpb.2021.11.007
7. Dolnikov S, Adam R, Cherqui D, Allard MA. Liver Transplantation in Elderly Patients: What Do We Know at the Beginning of 2020? *Surg Today* (2020) 50: 533–9. doi:10.1007/s00595-020-01996-7
8. Keswani RN, Ahmed A, Keeffe EB. Older Age and Liver Transplantation: A Review. *Liver Transplant* (2004) 10:957–67. doi:10.1002/lt.20155
9. Aloia TA, Knight R, Gaber AO, Ghobrial RM, Goss JA. Analysis of Liver Transplant Outcomes for United Network for Organ Sharing Recipients 60 Years Old or Older Identifies Multiple Model for End-Stage Liver Disease-Independent Prognostic Factors. *Liver Transplant* (2010) 16:950–9. doi:10.1002/lt.22098
10. Sharpton SR, Feng S, Hameed B, Yao F, Lai JC. Combined Effects of Recipient Age and Model for End-Stage Liver Disease Score on Liver Transplantation Outcomes. *Transplantation* (2014) 98:557–62. doi:10.1097/TP.000000000000090
11. Sharma M, Ahmed A, Wong RJ. Significantly Higher Mortality Following Liver Transplantation Among Patients Aged 70 Years and Older. *Prog Transplant* (2017) 27:225–31. doi:10.1177/1526924817715468
12. Wilson GC, Quillin RC, Wima K, Sutton JM, Hoehn RS, Hanseman DJ, et al. Is Liver Transplantation Safe and Effective in Elderly (≥70 Years) Recipients? A Case-Controlled Analysis. *HPB* (2014). 16. p. 1088–94. doi:10.1111/hpb.12312
13. Toiv A, Saleem A, Obri M, Jacobsen G, Varma A, Salgia R, et al. Retrospective Study of Liver Transplant Recipients over 69: Clinical Features and Outcomes of a Growing Group. *Transpl Proc* (2025) 57:1086–94. doi:10.1016/j.transproceed.2025.05.033
14. Lipshutz GS, Hiatt J, Mark Ghobrial R, Farmer DG, Martinez MM, Yersiz H, et al. *Outcome of Liver Transplantation in Septuagenarians A Single-Center Experience* (2025).
15. Aduen JF, Sujay B, Dickson RC, Heckman MG, Hewitt WR, Stapelfeldt WH, et al. Outcomes After Liver Transplant in Patients Aged 70 Years or Older Compared with Those Younger than 60 Years. *Mayo Clin Proc* (2009) 84: 973–8. doi:10.1016/S0025-6196(11)60667-8
16. Martin P, Dimartini A, Feng S, Brown R, Fallon M. Evaluation for Liver Transplantation in Adults: 2013 Practice Guideline by the American Association for the Study of Liver Diseases and the American Society of Transplantation. *Hepatology* (2014) 59:1144–65. doi:10.1002/hep.26972
17. Choi J, Choi EW, Choi YR, Hong S, Suh S, Hong K, et al. Modified Charlson Comorbidity Index as a Survival Prediction Tool for Older Patients After Liver Transplantation. *Ann Surg Treat Res* (2023) 104:358–63. doi:10.4174/ASTR.2023.104.6.358
18. Dindo D, Demartines N, Clavien PA. Classification of Surgical Complications: A New Proposal with Evaluation in a Cohort of 6336 Patients and Results of a Survey. *Ann Surg* (2004) 240:205–13. doi:10.1097/01.sla.0000133083.54934.ae
19. Olthoff KM, Kulik L, Samstein B, Kaminski M, Abecassis M, Emond J, et al. Validation of a Current Definition of Early Allograft Dysfunction in Liver Transplant Recipients and Analysis of Risk Factors. *Liver Transplant* (2010) 16:943–9. doi:10.1002/lt.22091
20. Ravaoli M, Germinario G, Dajti G, Sessa M, Vasuri F, Siniscalchi A, et al. Hypothermic Oxygenated Perfusion in Extended Criteria Donor Liver Transplantation—A Randomized Clinical Trial. *Am J Transplant* (2022) 22: 2401–8. doi:10.1111/ajt.17115
21. Golse N, Bucur PO, Ciacio O, Pittau G, Sa CA, Adam R, et al. A New Definition of Sarcopenia in Patients with Cirrhosis Undergoing Liver Transplantation. *Liver Transplant* (2017) 23:143–54. doi:10.1002/lt.24671
22. Kalafateli M, Mantzoukis K, Choi Yau Y, Mohammad AO, Arora S, Rodrigues S, et al. Malnutrition and Sarcopenia Predict Post-Liver Transplantation Outcomes Independently of the Model for End-Stage Liver Disease Score. *J Cachexia Sarcopenia Muscle* (2017) 8:113–21. doi:10.1002/jcsm.12095
23. Rosenbaum PR, Rubin DB. Constructing a Control Group Using Multivariate Matched Sampling Methods that Incorporate the Propensity Score. *Am Stat* (1985) 39:33–8. doi:10.1080/00031305.1985.10479383
24. Mousa OY, Nguyen JH, Ma Y, Rawal B, Musto KR, Dougherty MK, et al. Evolving Role of Liver Transplantation in Elderly Recipients. *Liver Transplant* (2019) 25:1363–74. doi:10.1002/lt.25589
25. Gil E, Kim JM, Jeon K, Park H, Kang D, Cho J, et al. Recipient Age and Mortality After Liver Transplantation: A Population-Based Cohort Study. *Transplantation* (2018) 102:2025–32. doi:10.1097/TP.0000000000002246
26. Audet M, Piardi T, Panaro F, Cag M, Ghislotti E, Habibeh H, et al. Liver Transplantation in Recipients over 65 Yr Old: A Single Center Experience. *Clin Transpl* (2010) 24:84–90. doi:10.1111/j.1399-0012.2009.00972.x

Copyright © 2025 Prosperi, Prosperi, Serenari, Bonatti, Fallani, Stocco, Radi, Mirici Cappa, Laici, Siniscalchi, Morelli, Ravaoli and Cescon. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.



Transplant International

Official journal of the European
Society for Organ Transplantation

Editorial Office

Avenue du Tribunal Fédéral 34
CH – 1005 Lausanne
Switzerland

Tel +41 (0)21 510 17 40
Fax +41 (0)21 510 17 01

tieditorialoffice@frontierspartnerships.org
frontierspartnerships.org/journals/transplant-international