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Unraveling the puzzle of liver transplantation for cholangicarcinoma

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- SENIOR PROFESSIONALS
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ACLF - Acute-on-Chronic Liver Failure
AIDPIT - Artificial Insulin Delivery, Pancreas and Islet Transplantation
EDTCO - The European Donation and Transplant Coordination Organisation
ELITA - The European Liver and Intestine Transplant Associationi
ELPAT - The European Platform on Ethical, Legal and Psychosocial Aspects of Organ Transplantation
EPITA - The European Pancreas and Islet Transplant Association
ESOT - European Society for Organ Transplantation
ITS - International Transplant Science

ABSTRACT SUBMISSION

ELITA
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The European
Liver and Intestine
Transplant Association

EF CLIF
EUROPEAN FOUNDATION
FOR THE STUDY OF
CHRONIC LIVER FAILURE

ELITA 30th Anniversary Meeting & ELITA-EF CLIF Monothematic Conference about ACLF, Alcohol and Liver Transplantation

9–11 March 2023, Madrid, Spain

#ESOT_ELITA

30th
ANNIVERSARY

EPITA
ESOT

The European
Pancreas and Islet
Transplant Association

12th EPITA Symposium & 41st AIDPIT Workshop

22-24 January 2023,
Innsbruck-Igls, Austria

#ESOT_EPITA

TRANSPLANT TRIAL WATCH
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Transplant Trial Watch

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Keywords: living kidney donation, systematic review, pregnancy, lung transplantation, bronchiolitis obliterans syndrome

To keep the transplantation community informed about recently published level 1 evidence in organ transplantation ESOT and the Centre for Evidence in Transplantation have developed the Transplant Trial Watch. The Transplant Trial Watch is a monthly overview of 10 new randomised controlled trials (RCTs) and systematic reviews. This page of Transplant International offers commentaries on methodological issues and clinical implications on two articles of particular interest from the CET Transplant Trial Watch monthly selection. For all high quality evidence in solid organ transplantation, visit the Transplant Library: www.transplantlibrary.com.

RANDOMISED CONTROLLED TRIAL 1

Pregnancy After Living Kidney Donation, A Systematic Review of the Available Evidence and a Review of the Current Guidance.

by Pippias, M., et al. American Journal of Transplantation [online ahead of print].

Aims

The aim of this study was to identify all available evidence investigating pregnancy complications post-living kidney donation, and to compare the quality and consistency of guidelines focusing on pregnancy in living kidney donors.

Interventions

A literature search was conducted on Embase, PubMed, MEDLINE, society webpages and guideline registries. Three independent reviewers performed the initial screening of study titles and abstracts. Eligibility assessment of full-text articles and data extraction were carried out by two independent reviewers. The methodological quality of the included studies were assessed using the Risk Of Bias In Non-randomized Studies of Interventions (ROBINS-I) tool.

Participants

16 studies were included in the review.

Outcomes

The main outcomes of interest were post-donation pregnancy complications, and the risk of adverse maternal, fetal and neonatal outcomes.

Follow-Up

Not applicable.

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CET Conclusion
This systematic review summarises the literature and guidelines relating to pregnancy following living kidney donation. The authors identified 16 studies reporting on 1399 post-donation pregnancies. Whilst the risk of pre-eclampsia increased post-donation, it is in keeping with an unselected general population. No difference was found in risk of other pregnancy or foetal complications. Guidelines were found to be generally consistent in advice. Methodology appears good, with well-described searches across a number of databases and screening by 3 reviewers. Risk of bias was assessed with the Robins-I tool and found to be low-moderate in most studies. Of note, studies were published over a long period (35 years) so it is perhaps not clear how relevant results of early studies are to today's practice. Overall, the authors graded the certainty of evidence in risk of hypertension and pre-eclampsia as "low" and for other foetal outcomes as "very low," reflecting the quality and size of the underlying evidence. This paper provides a very good summary of the evidence (and limitations thereof) regarding post-donation pregnancy.

Funding Source
Non-industry funded.

RANDOMISED CONTROLLED TRIAL 2

Impact of Lung Function Decline on Mortality in Lung Transplant Recipients: Long-Term Results From the L-CsA-i Study for the Prevention of Bronchiolitis Obliterans Syndrome.

by Kneidinger, N., et al. Frontiers in Medicine 2022; 9: 897581.

Aims
This study aimed to determine the association between forced expiratory volume in one second (FEV1) and risk of mortality in patients following lung transplantation, using the 10-year follow up data from the PARI Study No. 12011.201.

Interventions
Participants in the original trial were randomised to receive either liposomal Cyclosporine A inhalation (L-CsA-i) or placebo.

Participants
130 lung transplant recipients.

Outcomes
The main outcomes of interest were the association between the course of post-transplant FEV1 over time and the risk of mortality, time to progression to allograft dysfunction and survival.

Follow-Up
10 years.

CET Conclusion
This paper presents post hoc analyses from a previously published RCT. The original RCT investigated inhaled liposomal ciclosporin-A in the prevention of Bronchiolitis Obliterans Syndrome (BOS) after lung transplantation. 10-year follow up is now available for all 130 of the included patients. A strong association was found between baseline FEV1 and mortality risk and each 1% drop from baseline FEV1 was associated with 3.5% increased risk for mortality. The individual trajectories in lung function were highly variable between patients, however it seems that post-transplant FEV1 is a valid predictor of mortality and could be used to institute pre-emptive treatment.

Trial Registration
ClinicalTrials.gov—NCT01334892.

Funding Source
Industry funded.

CLINICAL IMPACT SUMMARY

This paper presents some long-term follow up from a previously published RCT of inhaled liposomal cyclosporine A in lung transplantation. The original study closed prior to reaching the target patient inclusion due to very slow accumulation of cases (1).

The paper by Kneidinger et al presents post hoc analyses from the RCT. The authors used the collected data to explore the relationship between decline in FEV1 and mortality in patients with single and double lung transplant.

Whilst patients were included in the trial they had FEV1 measurements every 2 months, and for this analysis they were requested every 6 months up to 10 years from inclusion. Complete data was retrieved for 91% of included patients, and reduced data for the remaining patients, censored at the last study visit. Mean follow up was 61 months.

On average, FEV1 deteriorated over time but the trajectory for showed a great deal of diversity between patients. A highly significant correlation was found between the relative drop in FEV1 compared to baseline and mortality. In broad terms a 1% reduction in FEV1 compared to baseline, related to 3.4% higher mortality risk. In cox regression analysis, type of transplant was the only significant independent predictor of mortality; with recipients of single lung transplants having increased risk of progression.

A significant amount of lung function must be lost before chronic lung allograft dysfunction can be diagnosed. Understanding the decline in FEV1 might allow early intervention and improvement in patient care through modification of the underlying process. Decline in FEV1 from baseline could also be used as a reliable surrogate outcome for mortality in clinical trials.

AUTHOR CONTRIBUTIONS

The author confirms being the sole contributor of this work and has approved it for publication.

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.

REFERENCE

1. Neurohr C, Kneidinger N, Ghiani A, Monforte V, Knoop C, Jaksch P, et al. A Randomized Controlled Trial of Liposomal Cyclosporine A for Inhalation in the Prevention of Bronchiolitis Obliterans Syndrome Following Lung Transplantation. *Am J Transpl* (2022) 22(1):222–9. doi:10.1111/ajt.16858

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NEWS AND VIEWS
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Eliminating Race From eGFR Calculations: Impact on Living Donor Programs

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Keywords: kidney transplantation, end stage renal disease, race, ethnicity, living kidney donation, transplant access, inequity

The recent decision to remove race-based calculations of kidney function for candidates on the national waitlist approved by the OPTN Board has set the tone towards a more equitable assessment of prospective transplant and donor candidates (1). The change will take effect by the 27th of July in the USA and will allow hospitals to use only race neutral equations (without the black race coefficient) (2). This policy change alone will not likely address all the existing disparities in kidney transplantation (3, 4), but a reappraisal of the elimination of race from eGFR calculations is needed in view of its potential impact on living donor kidney transplantation (LDKT), the best treatment option for patients affected by end stage renal disease (ESRD), both from the donor's and the recipient's perspective.

In greater detail, there remains a disparity in providing equitable access to racial minorities (5), especially in areas where social-related status often limits access to care, as in the USA, where private insurance affects to the likelihood of treatment exposure and transplant referral: a recent analysis showed in fact that African American candidates have a lower incidence of LDKT than candidates of other races, regardless of primary payer (6). Furthermore, in Low- and Middle-Income countries, where deceased organ donation programs are not well-established, LDKT is the only curative treatment alternative to dialysis or death (7).

Evidence is lacking regarding ethnicity and organ donation in Europe. In fact, data collection is not generally undertaken and standardized, based mainly on self-identification or recorded country of birth. Furthermore, the discrepancy between national methodologies limits access to data for various minority groups, which in turn renders not only national, but also gathered European data collection less reliable than and less comparable to what happens in the USA (6). Additionally, in many countries, "race" data are simply not collected, primarily because it is felt that it could amount to racial discrimination; the flipside is that since the data are not there, it is not possible to fully assess the extent of racial discrimination in many ways.

In the UK, non-white ethnic minorities, comprise 11% of the population, 7% of organ donors, 35% of people awaiting a kidney transplant and 21% of people who died on the waiting list (7). In other European countries, the situation is similar to or worse than that described in the UK, and in Norway, one of the countries with the highest LDKT rates, living organ donation appears to be rare amongst migrant and ethnic minority groups, who then rely upon organs from deceased donors (8), with mitigation for the disparity in access to kidney care between ethnic groups being advocated worldwide (9).

Demographic characteristics of donors (10), recipients (11), and the interaction between these two (12), are increasingly considered in the establishment of research protocols and healthcare

Abbreviations: CKD, chronic kidney disease; eGFR, estimated glomerular filtration rates; LDKT, living donor kidney transplantation; ESRD, end stage renal disease.

News & Views

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policies. To achieve better outcomes, and in consideration of the known discrepancy in life expectancy and morbidity between different ethnicities, it is therefore of utmost importance to consider comprehensively the interrelation between donor and recipient races on the respective health outcomes, to provide equitable access to individuals of different socio-racial backgrounds, yet without a further exacerbation of the already existing inequalities.

Race is a variable often considered in eGFR calculations, with the potentiality to overestimate renal function in Black patients, causing about 16% misclassification of kidney disease stage (2), and thus exacerbating health inequalities by the miscalculation of kidney function in minority groups. The equations most in use today include serum creatinine, age, sex, and race, and adjust the final calculation based on a presumed higher muscle mass in Black individuals; this applies specifically for the commonest methods in use among adults, namely the Modification of Diet in Renal Disease (MDRD) and the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) (13). Yet, there are additional social determinants of health in relation to income, education and general lifestyle conditions that could significantly affect the final eGFR calculation. Furthermore, most of the eGFR equations were originally developed considering a relatively small sample size and with limited demographic characteristics (i.e., White men), therefore their transferability to other backgrounds could be argued in view of the lack of inclusion of other specific demographic characteristics for the calculation of the equation itself and for its original validation, in contradiction with the principles of diversity and inclusion.

As a result of health inequality, Black and Asian minority people in need of a kidney transplant wait for longer in comparison to their Caucasian counterparts (14). This has also been proven for Hispanic ethnicity and female gender (15), where lack of formal education and minority race are negatively associated with referral to a transplant center (14). The extended time on the waiting-list unfortunately often leads to a deterioration of the general health conditions to a grade at which the underlying comorbidities of these candidates cause their ineligibility to undergo kidney transplantation, mostly because of the limited organ donor pool, with the sad result of death for many.

To possibly meet the organ donor offer, LDKT not only represents the best opportunity of success in terms of definitive renal replacement therapy, but it also allows pre-emptive treatment of kidney failure. Since LDKT is unfortunately a precious resource not available for everyone, educational campaigns aiming to expand living organ donation should target these minority backgrounds, and content related to risks for the altruistic act of donation by Black and Asian candidates should cover topics related to the effects of donor and recipient races on the respective health outcomes.

What is then the available evidence on the effect of race on living kidney donors, and the impact on recipients' outcomes? As previously stated, data on post-donation eGFR might be affected by the formulas used in the calculation, so they remain heterogenous and inconclusive, therefore a more accurate analysis could focus on the percentage change in eGFR or slope eGFR in longitudinal observations (11) or in a comprehensive assessment evaluating biological data, socioeconomic status, and eventual complementary data affecting the health-related status of an individual.

In greater detail, we previously demonstrated that race, *per se*, should not be a barrier to increase the living donor kidney pool: on average, 88% of the entire living donor pool of this international cohort are Caucasian, but with the help of the previous mentioned educational campaigns, up to 40% of Black and Asian minorities have proven to be a realistic target to contribute to the living organ donor pool (16).

If we look at the incidence of proteinuria, another important parameter to assess the parenchymal damage secondary to the compensation hyperfiltration of the remnant kidney, there seems to be no difference among Africans or Caucasians (8) 1-year post-donation, thus confirming that living donation is an option for all the races to increase chances and access to transplantation.

Besides, there is no difference in incidence of ESRD between the Caucasian and Asian or Hispanic/Latin ethnic backgrounds (8), thus providing further support to the hypothesis that in addition to just genetic conditions, there are factors such as socioeconomic deprivation and racial discrimination to be considered for the long-term outcomes.

To this regard, an analysis from the OPTN/UNOS database found significantly higher rates of ESRD in African donors compared to Caucasians: Lentine et al., adjusted HR 2.32 (1.48–3.62) $p < 0.001$ (17). There has also been higher incidence of ESRD reported in both Caucasian and African donors, in comparison to their healthy counterparts in the general population (10); however, more than three times higher ESRD rates in the general population are registered in African adults, 8%, compared to Caucasians, 2%–3% respectively, leading ultimately to a further disadvantage of African donors and creating a vicious cycle. Therefore, it is compelling to protect those who come forward for a generous act of self-giving, without additional harm secondary to a racial demographic.

Finally, if we look at what happens to Black kidney transplant recipients, in a recent meta-analysis we demonstrated no significant difference between the 1-year mortality in comparison to Caucasians (11), as well as with regards to the data on acute rejection, concluding that recipient's race is not related to patient and graft survivals (11).

In conclusion, Black deceased donors are more likely to experience CKD compared to Caucasians, mainly in view of the trends present in the general population.

This should not be considered a barrier to the expansion of the living donor pool and the possibility to offer LDKT to candidates of Black and Asian minorities should instead be concrete and actively incentivized.

The new proposed OPTN/UNOS race-neutral eGFR calculations (13) might be considered sufficiently accurate for clinical practice in many circumstances but may lead to systematic differences in accuracy of eGFR between race groups, with implications for individual patients and public health. There have also been some concerns that the elimination of the black coefficient would decrease the eGFR

and reduce the eligibility of potential black living donors, although this concern is not valid because most if not all centers do not use eGFR in the workup for living donors (4), but more reliable tests or 24 h urine clearance.

We believe that future studies need to focus on how to overcome this barrier in consideration of the current organ donor shortage, to minimize the effect of race in kidney function and provide equitable access to individuals of different socio-racial backgrounds. We also strongly support the omission of adjustment for ethnicity in the eGFR formulas, in agreement with current research looking at new endogenous filtration markers and interventions to eliminate racial and ethnic disparities, supporting consideration in health outcome differences due to health inequalities rather than race.

Transplant and Nephrology Societies should favor this new policy change to intervene on the long overdue negative impact of race on eGFR, with the aim to reduce delayed referrals for transplant and delays in qualifying for waiting time and for donor's eligibility. Equity in health means "equal opportunity" (18) and thus patients should all start from equal assessment to be offered equal treatment options.

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.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

CONFLICT OF INTEREST

PM is the chair of the Minority Affairs Committee of UNOS/OPTN.

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.

REFERENCES

1. OPTN. OPTN Board Approves Elimination of Race-Based Calculation for Transplant Candidate Listing - OPTN (2022). Available from: https://optn.transplant.hrsa.gov/news/optn-board-approves-elimination-of-race-based-calculation-for-transplant-candidate-listing/ (Accessed July 20, 2022).
2. Diao JA, Inker LA, Levey AS, Tighiouart H, Powe NR, Manrai AK. In Search of a Better Equation - Performance and Equity in Estimates of Kidney Function. *N Engl J Med* (2021) 384:396–9. doi:10.1056/NEJMp2028243
3. Martins PN, Pavlakis M, Diez A. New Equations for Estimating the GFR without Race. Reply. *N Engl J Med* (2022) 386:1672–3. doi:10.1056/NEJMc2119761
4. Doshi MD, Schaubel DE, Xu Y, Rao PS, Sung RS. Clinical Utility in Adopting Race-free Kidney Donor Risk Index. *Transpl Direct* (2022) 8:e1343. doi:10.1097/TXD.0000000000001343
5. Kim IK, Martins PN, Pavlakis M, Eneanya ND, Patzer RE. Past and Present Policy Efforts in Achieving Racial Equity in Kidney Transplantation. *Curr Transpl Rep* (2022) 9:114–8. doi:10.1007/s40472-022-00369-y
6. European Commission. Analysis and Comparative Review of equality Data Collection Practices in the European Union (2022). Available from: https://ec.europa.eu/info/sites/default/files/data_collection_in_the_field_of_ethnicity.pdf (Accessed July 20, 2022).
7. ODT Clinical. Annual Activity Report (2021). Available from: https://www.odt.nhs.uk/statistics-and-reports/annual-activity-report/ (Accessed July 20, 2022).
8. Husain SA, King KL, Adler JT, Mohan S. Racial Disparities in Living Donor Kidney Transplantation in the United States. *Clin Transpl* (2022) 36:e14547. doi:10.1111/ctr.14547
9. Vedadi A, Bansal A, Yung P, Famure O, Mitchell M, Waterman AD, et al. Ethnic Background Is Associated with No Live Kidney Donor Identified at the Time of First Transplant Assessment-An Opportunity Missed? A Single-center Retrospective Cohort Study. *Transpl Int* (2019) 32:1030–43. doi:10.1111/tri.13476
10. Bellini MI, Nozdrin M, Pengel L, Knight S, Papalois V. Risks for Donors Associated with Living Kidney Donation: Meta-Analysis. *Br J Surg* (2022) 109:671–8. doi:10.1093/bjs/znac114
11. Bellini MI, Nozdrin M, Pengel L, Knight S, Papalois V. The Impact of Recipient Demographics on Outcomes from Living Donor Kidneys: Systematic Review and Meta-Analysis. *J Clin Med* (2021) 10:5556. doi:10.3390/jcm10235556
12. Bellini MI, Nozdrin M, Pengel L, Knight S, Papalois V. How Good Is a Living Donor? Systematic Review and Meta-Analysis of the Effect of Donor Demographics on post Kidney Transplant Outcomes. *J Nephrol* (2022) 35:807–20. doi:10.1007/s40620-021-01231-7
13. 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
14. Park C, Jones MM, Kaplan S, Koller FL, Wilder JM, Boulware LE, et al. A Scoping Review of Inequities in Access to Organ Transplant in the United States. *Int J Equity Health* (2022) 21:22. doi:10.1186/s12939-021-01616-x
15. Cozzi E, Alvarez M, Carmona M, Mahillo B, Forsythe J, Lomero M, et al. An Analysis by the European Committee on Organ Transplantation of the Council of Europe Outlining the International Landscape of Donors and Recipients Sex in Solid Organ Transplantation. *Transpl Int* (2022) 35:10322. doi:10.3389/ti.2022.10322
16. Bellini MI, Charalampidis S, Stratigos I, Dor FJMF, Papalois V. The Effect of Donors' Demographic Characteristics in Renal Function Post-Living Kidney Donation. Analysis of a UK Single Centre Cohort. *J Clin Med* (2019) 8(6):883. doi:10.3390/jcm8060883
17. Lentine KL, Schnitzler MA, Garg AX, Xiao H, Axelrod D, Tuttle-Newhall JE, et al. Race, Relationship and Renal Diagnoses after Living Kidney Donation. *Transplantation* (2015) 99:1723–9. doi:10.1097/TP.0000000000000733
18. Braveman P, Gruskin S. Defining Equity in Health. *J Epidemiol Community Health* (2003) 57:254–8. doi:10.1136/jech.57.4.254

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POINT OF VIEW
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Interventions After First Post-Transplant Cutaneous Squamous Cell Carcinoma: A Proposed Decision Framework

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Cutaneous squamous cell carcinoma (CSCC) is a major cause of morbidity and mortality after organ transplant. Many patients subsequently develop multiple CSCC following a first CSCC, and the risk of metastasis and death is significantly increased compared to the general population. Post-transplant CSCC represents a disease at the interface of dermatology and transplant medicine. Both systemic chemoprevention and modulation of immunosuppression are frequently employed in patients with multiple CSCC, yet there is little consensus on their use after first CSCC to reduce risk of subsequent tumors. While relatively few controlled trials have been undertaken, extrapolation of observational data suggests the most effective interventions may be at the time of first CSCC. We review the need for intervention after a first post-transplant CSCC and evidence for use of various approaches as secondary prevention, before discussing barriers preventing engagement with this approach and finally highlight areas for future research. Close collaboration between specialties to ensure prompt deployment of these interventions after a first CSCC may improve patient outcomes.

Keywords: cancer, outcomes, transplant, skin cancer, management

Point of View

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A CLINICAL CASE

A 60 year old white male presents for kidney transplant follow-up, 21 years after a deceased donor transplant. Despite an early cellular rejection episode, he has maintained excellent allograft function (baseline creatinine 107 μmol/L) without humoral sensitization on a dual regimen of cyclosporine and azathioprine. He has a history of photodamage but no history of skin cancer or solid-organ malignancy. He has recently had a 1 cm tender keratotic nodule excised from his shin, confirmed histologically as invasive cutaneous squamous cell carcinoma (CSCC). The patient asks whether anything can be done to decrease his risk of cancer recurrence without putting their allograft at undue risk.

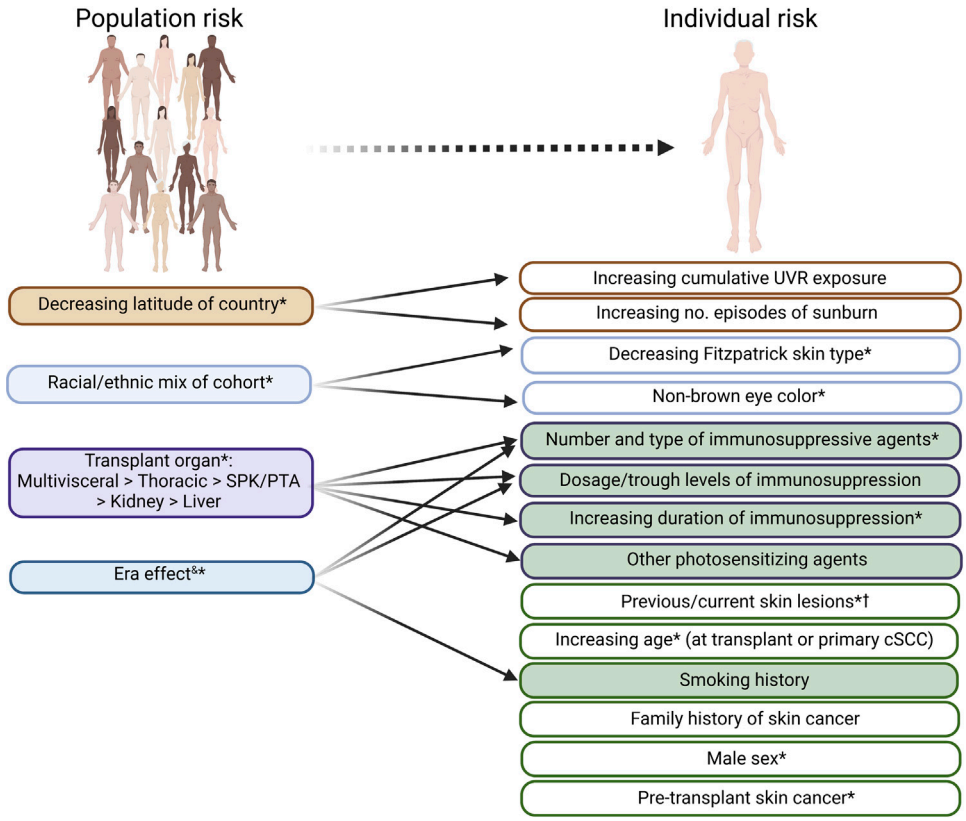

FIGURE 1 | Clinical risk factors for (further) cSCC development, which may be useful in risk stratifying organ transplant recipients. Factors predictive of cSCC risk at a population level are indicated on the left. Factors relevant at an individual level (which are often interrelated—demonstrated by arrows) are shown on the right, with potentially modifiable factors at time of first cSCC shown in green. *indicates risk factors shown to be independently predictive of development of further KC or cSCC lesions in at least one study. cSCC, cutaneous squamous cell carcinoma. UVR; UV radiation; SPK, simultaneous kidney-pancreas; PTA, pancreas transplant alone. &time period during which cohort data is collected: generally more historical cohorts demonstrate greater cSCC risk. † including human papillomavirus (HPV)-related viral warts or dysplasia and UVR-related photodamage and pre-malignant lesions.

TABLE 1 | Definition of stages of CSCC prevention used in this paper.

Prevention stage	Definition	Example(s) relevant to post-transplant CSCC
Primordial and primary	Prevent disease onset in susceptible individuals (i.e., with one or more risk factors)	Education regarding UV exposure, promoting use of photoprotection (such as sunscreen)
Secondary	Identify patients with early disease and prevent progression	Skin cancer screening, topical or systemic chemoprevention (including management of premalignant lesions) or modulation of immunosuppression in patient with first CSCC to prevent further CSCC.
Tertiary	Decrease morbidity and mortality of individuals with advanced disease	Surgery or radiotherapy to locally advanced lesions to prevent metastatic spread; immunotherapy for treatment of metastatic lesions
Quaternary	Protect individuals from medical interventions that may cause more harm than good	Avoiding sensitization and rejection resulting from immunosuppression modulation

Staging of disease prevention differs in post-transplant skin cancer compared to other diseases, where progression does not solely represent growth and metastasis of a single malignancy, but also the development of further asynchronous primary lesions. Summarized from references (20, 21, 31).

INTRODUCTION

Skin is the commonest site for post-transplant malignancy, with up to 200-fold increased incidence of keratinocyte carcinoma (KC) compared to immunocompetent populations (ICP) (1). CSCC accounts for 80% of KC in organ transplant recipients (OTR) (2). Half of OTR develop another CSCC within 3 years of their first (2–5). Metastatic risk from CSCC is doubled in OTR and those who develop multiple (>10) CSCC have up to 26% risk of metastasis (6, 7), with a 3 year median survival (8). CSCC represents a leading cause of cancer-related mortality for some OTR (2,8,9,10) and may be associated with increased risk of

internal malignancies (11, 12), consistent with findings in ICP that are not fully explained by known cancer risk factors (13,14,15) and presumably relate to common susceptibility mechanisms. Treatment and surveillance for post-transplant CSCC creates significant economic burden for healthcare providers and patients (16). Interventions to reduce risk are desirable to improve OTR wellbeing, healthcare resource usage, and future cancer-related mortality.

At a population level, cumulative incidence of CSCC amongst OTR is dependent upon several factors, the most important being immunosuppression intensity and geographic latitude (reflecting cumulative ultraviolet radiation (UVR) exposure) (2). 25% of white European OTR may ultimately develop CSCC, rising to 75% with significant UVR exposure (such as Australasia) (2). Pre-transplant CSCC is a major risk factor for post-transplant CSCC and consensus recommendations regarding the management of such patients have been published elsewhere (17). Individual risk factors are summarized in **Figure 1**. While used to guide cohort surveillance strategies (4, 18), prognostication using these factors [recently reviewed (19)], particularly for prediction of recurrence, lacks resolution to guide individual patient management.

We summarize staging of disease prevention for post-transplant skin cancer in **Table 1** (20, 21). Primary and secondary prevention strategies for CSCC in OTR include patient education, photoprotection, clinical skin surveillance and topical and oral chemoprevention (22), though data in transplant cohorts are limited with recommendations extrapolated from relatively small studies (23–25), expert opinion (26, 27), or studies in ICP (28–30).

Uncertainty about optimal timing of these interventions led to formulation of expert consensus-based recommendations for management, including a recent international Delphi panel of transplant dermatologists (26). While consensus was reached regarding topical and systemic agents in primary and secondary prevention of CSCC, consensus was not reached for optimal interventions after a first low-risk CSCC (LRCSCC; defined in this study, and this paper, as Brigham and Women's Hospital Stage T1 or T2a, or American Joint Committee on Cancer T1 or T2). Retrospective data suggest there is similar equipoise about optimal timing and nature of immunosuppressive regimen modification amongst transplant practitioners, particularly after first CSCC (3).

In the absence of definitive evidence, we provide an overview of potential interventions for secondary CSCC prevention after the first CSCC and suggest this timepoint as an optimal opportunity to consider initiation of such measures. We consider dermatology, transplant medicine and patient perspectives relevant to decision making and consider the current barriers to adoption of this practice. Finally, we propose a decision framework to guide management of after a first post-transplant CSCC.

DERMATOLOGICAL STRATEGIES

There is scant evidence to guide transplant dermatologists in predicting CSCC risk and employing secondary prevention measures in OTRs after their first LRCSCC. OTR with a history of CSCC should be counselled on skin self-examination and photoprotection and undergoing regular skin cancer surveillance (4, 18), though screening interval recommendations are not consistent across international guidelines. There is randomized controlled trial (RCT) evidence that regular use of sunscreen reduces the risk of first CSCC in ICP, but data for benefit in OTR are limited to case-control studies (32).

Actinic keratoses (AK) are clinically apparent hyperkeratotic papules and plaques representing epidermal dysplasia arising on sun-damaged skin; a small proportion proceed to invasive CSCC (0.01%–0.65% in ICP) (33). CSCC *in situ* (CSCCIS, Bowen disease) represents full-thickness epidermal dysplasia with a higher rate of transformation to CSCC (3%–5% in ICP) (34). AK and CSCCIS may become confluent in areas of 'field cancerization', with subclinical disease present in contiguous clinically normal photo-exposed skin. Management of premalignancy is an essential component of secondary prevention. Destructive therapies such as cryotherapy or surgical curettage and cautery tend to be favored for discrete lesions (24). In confluent areas of AK, topical "field directed" treatments are added (35). 5% 5-fluorouracil (5-FU) cream has demonstrated superiority in blinded trials over alternatives in ICP and has also been demonstrated to prevent CSCC (22, 35), with evidence of superiority in OTR limited but growing (29, 36, 37).

Dermatologists may consider oral chemoprevention for patients at high risk of subsequent CSCC, with options including oral retinoids (acitretin) or nicotinamide (26). Acitretin is effective with up to 42% reduction in rates of CSCC in kidney transplant recipients in RCTs (23, 25). However, reported rates of discontinuation due to side effects range from 19%–39% in RCTs of OTR, most commonly due to xerosis and alopecia (23, 25). "Rebound" CSCC formation 3–4 months after drug cessation is frequent, meaning acitretin should be regarded as a long-term strategy (38). These factors may account for part of the documented reluctance of dermatologists to start acitretin after a first CSCC, typically waiting until multiple/high-risk CSCC formation is evident (26). In Australian ICP with a history of multiple KC, oral nicotinamide (active vitamin B3) 500 mg twice daily was well tolerated and resulted in a 30% reduction in CSCC compared to placebo over 12 months, but also showed rebound effects upon discontinuation (24). Nicotinamide has been studied in two insufficiently powered RCTs in kidney transplant recipients (39), but concerns regarding lack of positive data has limited its broader use by dermatologists in OTR (26). Results from a larger Australian RCT are forthcoming. Neither nicotinamide nor acitretin have been associated with significant changes in kidney allograft function or risk of allosensitization.

MODIFICATION OF IMMUNOSUPPRESSION

There are two immunosuppression-based secondary prevention strategies that may reduce risk of subsequent CSCC after a first

CSCC: change of immunosuppressive agent or reduction in immunosuppressive intensity.

Change of Agent

Switch to Newer Agents

The direct carcinogenicity of various immunosuppressive agents is well established, particularly with those used prior to the mid-2000s. Azathioprine promotes UVA absorption by DNA, leading to UVA photosensitivity, mutagenicity and a unique mutational signature within CSCC (40, 41). Whilst azathioprine use is largely historical, it is still used in cases of mycophenolate intolerance and in recipients planning pregnancy: furthermore, Furthermore, the lag effect of CSCC development after transplant means many OTR who develop CSCC are still on this agent. Previous studies suggest up to 10% of Australian and US kidney transplant recipients, and up to 69% of Spanish heart transplant recipients, are receiving azathioprine (42). Mycophenolate does not promote UVA sensitivity, though may inhibit DNA repair mechanisms (43). Cyclosporine, but not tacrolimus, impairs UVR-induced DNA damage repair and apoptotic mechanisms and promotes tumor growth in pre-clinical models (41, 44). A large retrospective analysis of OTR found increased skin cancer risk with both cyclosporine and azathioprine compared to tacrolimus and mycophenolate, respectively (45). More recent regimens of tacrolimus and mycophenolate may be associated with a significant reduction in skin cancer risk compared to historical regimens and transition from azathioprine to mycophenolate appears to reduce first CSCC risk (45, 46). A major limitation to evidence for efficacy of this approach for secondary prevention is that the previous studies have been observational only. Belatacept may be an alternative or adjunct to calcineurin inhibitors (CNI) in certain kidney transplant recipients. The impact of belatacept on skin cancer is still emerging with a small single-center study showing lower risk of additional skin cancers after conversion from CNI to belatacept maintenance (47).

Switch to mTOR Inhibitor

Mammalian target of rapamycin inhibitors (mTORi) are associated with anti-malignant effects through multiple pathways *in vitro* (41). Several small studies alongside two large multicenter randomized trials assessed the effect of switching from CNI to sirolimus for CSCC secondary prevention in kidney transplant recipients (48, 49). A 25%–40% reduction in further CSCC risk over 2-year was seen in those converted to sirolimus, though only one study achieved significance across the cohort, and this was seen only after the first but not subsequent CSCC (48). A single episode of borderline rejection was seen across both studies and 5-year follow-up suggested similar patient and graft survival, arguing immunosuppression transition is safe (50). However, sirolimus was generally poorly tolerated with discontinuation and crossover in around a third of recipients due to adverse effects and a CSCC rebound effect was observed. Adverse effects include significant proteinuria, pneumonitis, oedema, impaired wound healing, teratogenicity and hyperlipidaemia. A meta-analysis of 21 trials found mTORi therapy was associated with a significant 60% reduction in KC risk, but also an increased risk of mortality due to infection and cardiovascular disease, though this may be partly due to higher intensity mTORi regimens used in earlier studies (51). For these reasons, sirolimus has not become a mainstay of therapy for CSCC primary or secondary prevention. Recent data have suggested that an alternative mTORi, everolimus, may demonstrate comparable transplant outcomes in low and moderate-risk patients when used alongside low-dose calcineurin inhibition compared to standard immunosuppression (52), and this may reignite interest in the use of mTORi as an immunosuppressant. Analysis of long-term outcomes from earlier studies suggest everolimus is broadly similar to sirolimus in efficacy in reducing KC burden, though tolerability remains a concern (53, 54).

Reduction in Immunosuppression Intensity

When considering reduction in immunosuppression intensity, the transplant practitioner may consider factors including graft function, pre-existing sensitization and history of rejection episodes, and perceived balance between rejection and future malignancy risk (**Figures 2**, **3**). A major limitation is the lack of methods to determine 'optimal' immunosuppression intensity at an individual level. Novel markers to stratify rejection risk are currently being developed, including circulating/urinary transcriptomics, HLA eplet mismatch profiling and donor-derived cell-free DNA [recently reviewed in (55)], but are not in widespread use and require validation regarding utility in guiding immunosuppression reduction.

Immunosuppression intensity is often related to clinical circumstances, including organ transplant type, and is correlated with first CSCC risk: for example, recipients on dual immunosuppression or with lower CNI trough levels exhibit reduced skin cancer risk compared to counterparts on triple immunosuppression or with greater trough levels (56, 57). Immunosuppression reduction or cessation (following graft failure) is associated with reduced risk and improved outcomes for virus-associated post-transplant malignancy such as lymphoma and Kaposi sarcoma (58), presumably by allowing greater immune control of cancer-associated viruses (59). However, data to support this approach for secondary prevention of CSCC is limited to retrospective cohort analyses, usually for advanced disease (3, 56). Immunosuppression modulation could synergize with chemopreventative approaches by permitting enhanced immune responses, but a combined approach has not been explored in either observational or trial settings.

TIMING OF INTERVENTIONS

In theory, the earlier the interventions are undertaken, the slower the accumulation of mutations developing, reducing risk of CSCC development.

A landmark trial showed reduction in CNI intensity at 1-year post-transplant was associated with reduced rates of malignancy over the following 5 years, of which two-thirds were skin cancer (57). While associated with an increased rate of acute rejection, this did not appear to compromise graft survival, possibly due to a relatively low event rate, and relatively high trough concentrations (by current

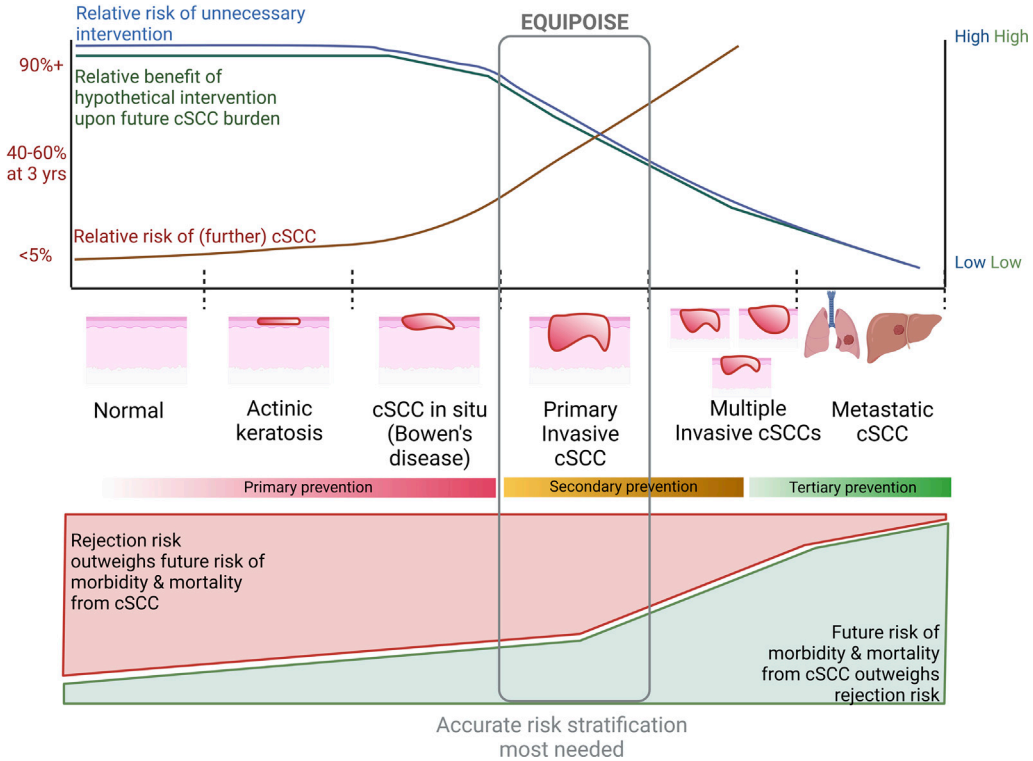

FIGURE 2 | Considerations in utilizing a hypothetical intervention for reduction of cSCC risk at various stages of disease. Top graph indicates relative risk of future cSCC at a patient level (approximate figures given on Y-axis) and unnecessary intervention, as well as relative benefit upon future malignancy risk from intervention at each stage of squamous carcinogenesis. This graph represents an extrapolation of trial and observational data. Bottom graph represents relative risk of morbidity and mortality from future cSCC and rejection with immunosuppression modulation. We postulate equipoise is greatest at time of first cSCC for most OTR, by which time the risk of further cSCC is high enough that more accurate methods of risk stratification are needed to delineate whether rejection upon immunosuppression modulation or future malignancy are more likely.

standards) of cyclosporine in the intervention arm. Rates of *de novo* donor-specific antibodies, a marker of allosensitization that reflects under-immunosuppression, or of further CSCC were not assessed. Intensity of cyclosporine therapy in the intervention (low dose) arm was roughly equivalent to that currently used and so whether even further reduction would benefit CSCC risk without compromising graft outcomes is uncertain as is the benefit of reduced doses of tacrolimus.

The most effective intervention timepoint may be before the first CSCC and when premalignant lesions are diagnosed. However, the risk of destabilizing graft function or introducing side-effects with immunosuppression modulation is likely greater than the potential benefit and in most cases quaternary prevention is more relevant (**Table 1**; **Figure 2**). Specifically, refractory cellular rejection through excessive immunosuppression reduction may require use of lymphocyte depleting monoclonal antibodies; the use of these at time of transplant as induction therapy is associated with increased risk of subsequent malignancy and it is reasonable to assume the same untoward shift in risk when used as rescue therapy in rejection, though increased CSCC risk has not been demonstrated directly (60).

In contrast, OTRs with a first CSCC are at high risk of further CSCC, representing the optimal time to modulate immunosuppression in most cases. This benefit may extend beyond the skin by impacting common underlying mechanisms responsible for both CSCC and solid organ malignancy (11–15). However, the risks of immunosuppression modulation based upon skin malignancy should be weighed against the 'number needed to treat' to prevent future skin and internal malignancy (**Figure 2**).

As indicated above, RCTs investigating CNI to sirolimus transition demonstrated that OTR with a single CSCC versus multiple CSCC at randomization gained the greatest benefit from a switch to sirolimus, with a striking 90% reduction in CSCC risk over the following 2 years (48–50).

These data indirectly suggest that immunosuppression modulation could be the most effective secondary prevention strategy, if implemented in a timely fashion. We suggest that after a first SCC, OTRs should be considered for transition off older agents, particularly azathioprine. Reduction of CNI target levels may also be appropriate. Sirolimus may be an option for those perceived to be at high risk of multiple subsequent CSCC, but tolerance is a major barrier.

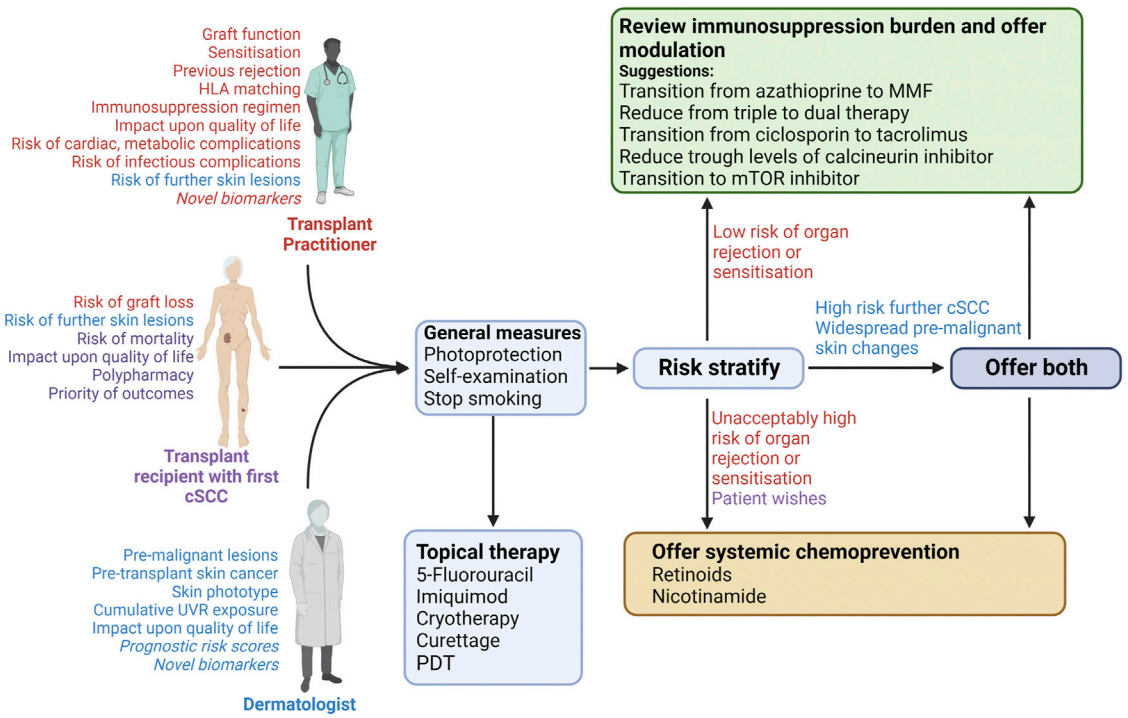

FIGURE 3 | Approach to risk stratification and interventions after primary cSCC in an organ transplant recipient. Free-text indicates the important considerations by each member of the discussion (indicated by colour coding: red will be mostly guided by transplant practitioner, purple by the patient, and blue by the dermatologist. Those considerations in italics are not in widespread use but may become relevant in the future). Discussion between the recipient, dermatologist and transplant practitioner should lead to lifestyles changes and the treating dermatologist should offer topical therapy for other lesions, irrespective of perceived cSCC risk. The respective clinicians should subsequently consider cSCC risk alongside perceived risk of allograft rejection/sensitisation. The relative risks of these will guide the offer of immunosuppression modulation and/or systemic chemoprevention. Final discussion between the dermatologist and transplant practitioner will guide on the final interventions offered to the patient. cSCC; cutaneous squamous cell carcinoma; CNI, calcineurin inhibitor; PDT, photodynamic therapy; UVR, UV radiation.

CONSIDERATIONS OF THE PATIENT

While the patient will rely on the dermatologist and transplant physician to counsel regarding relative risks, it is important to consider the patient's perspective.

The median time to first CSCC is typically many years after transplant, unless they have a pre-transplant history of CSCC (2); therefore, any intervention will generally be undertaken in the context of relatively stable graft function. Many OTR harbor an ongoing fear of rejection (61). Studies have found differences in prioritization of graft survival above other outcomes, including cancer and death (61–63), indicating outcomes of importance vary at a patient level. Many of the prevention tools available from a dermatology perspective do not incur risk for rejection but do warrant counselling on side effects and rebound CSCC upon drug cessation. Changes in immunosuppression may pose a rejection risk. While treatment of acute cellular rejection has good outcomes if detected rapidly, under-immunosuppression leading to humoral allosensitization is associated with significantly poorer graft survival and there is no consensus regarding effective treatment (64). Transplant recipients may be reluctant to change immunosuppression without individualized counselling balancing risk and benefits of this approach (61). Such counselling is difficult at present without more accurate CSCC risk stratification tools. Where immunosuppression modulation could be helpful, patients should be counselled regarding the uncertainty of individually predicting future CSCC risk, whilst emphasizing that a first CSCC is frequently associated with development of further lesions. Immunosuppression modulation at this timepoint may represent the optimal time to intervene and may also reduce the risk for other cancers, albeit with limited data to support this. Immunosuppression adjustment should be cautious and stepwise with close monitoring for graft function and sensitization.

HOW DO WE OVERCOME EQUIPOISE?

Two barriers contribute to clinical equipoise regarding secondary prevention: the need for risk stratification and evidence to guide sequencing of preventative strategies.

Perhaps most important is the need for accurate risk stratification, both for further CSCC and rejection. Cohort studies demonstrate that the majority of OTRs with CSCC will form multiple tumors over a 10-year period (4, 6, 7). Risk stratification is critical for formulating secondary prevention interventions, especially as these must be balanced against allograft function. One approach would be to develop more

accurate clinical prediction tools based on algorithms to prioritize skin cancer screening and interval surveillance following transplantation (4, 18). Increased intensity of dermatology follow-up in highest-risk cohorts would allow for earlier lesion detection but also an opportunity to initiate intervention with effective field therapies and discussion of chemoprevention agents.

Development of novel biomarkers to facilitate more accurate risk stratification after first CSCC as a complementary approach would serve two purposes: identification of those most likely to benefit from interventions and enrichment of trials with those at greatest risk. A full review of potential biomarkers is beyond the scope of this article. However, circulating immunological markers have been of interest as neoantigens that may drive immunological responses are common (especially in premalignancy) due to the high mutational burden in CSCC and the possible association with HPV (65). Other markers, including polygenic risk scores (66, 67), polymorphisms identified through genome-wide association studies (67–71), circulating (and tumoral) microRNA (72) and tumoral gene expression (73, 74) have been investigated for prognostic value in either OTR or ICP. Only a subset have been validated externally and/or for stratification of further CSCC risk (66, 67, 75, 76, 77). Synchronous stratification for rejection risk would reassure both practitioners and patients regarding immunosuppression reduction.

A second barrier is the lack of clarity regarding relative effectiveness of interventions to reduce secondary CSCC risk and how these should be sequenced. Several dermatological approaches are available to mitigate risk of second CSCC, but studies are limited. For immunosuppression, a single center retrospective study identified 24 different immunosuppression minimization strategies undertaken after first CSCC in kidney and heart transplant recipients (3). Since the sirolimus studies in the 2000s, interventional trials of immunosuppression modification for secondary CSCC risk reduction have been absent. What trial designs might address this? The "Randomised Evaluation of COVID-19 therapy (RECOVERY)' trial offers some inspiration: utilizing a simple design, central randomization with broad inclusion criteria and an adaptive trial platform design facilitated rapid, multi-center enrolment with a hard (mortality) endpoint to compare a series of possible treatments with established best care (78). A similar approach could facilitate a coordinated platform study of dermatological interventions after a first CSCC alongside immunosuppression modulation with the endpoint of subsequent CSCC (or locoregional recurrence/distant metastasis) development. The majority of subsequent CSCC development and poor outcomes are within the first 3 years of the first (2, 4), allowing for a medium-term follow-up period. The historical variety of immunosuppressive regimens have reduced over the last 20 years, coalescing around the use of tacrolimus, mycophenolic acid and/or corticosteroids, reducing the number of combinations to consider, though novel agents such as belatacept, proteosome inhibitors, IL-6 blockade and others may lead to future diversification of regimens.

CONCLUSION

In summary, while CSCC management is often considered complete after excision, we propose that the first CSCC diagnosis should be regarded as a "red flag" heralding an increased risk of further skin cancers and possibly internal malignancies. It therefore represents a key opportunity to proactively consider secondary preventive strategies, although as optimal preventative interventions and their sequencing remain unclear, further research is needed.

As summarized in **Figure 3**, based on existing evidence, we recommend that dermatologists should routinely communicate with the transplant team after diagnosis of a first post-transplant CSCC. This event should spark a discussion regarding risk of further lesions, with review of immunosuppression burden and use of chemopreventative therapies. This dialogue between dermatologists, transplant practitioners and patients should be viewed as part of an ongoing shared decision-making process, with the ultimate aim of reducing skin cancer risk, ensuring optimal allograft function and ultimately improving survival and quality of life.

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

No original data from studies involving human participants was included in this manuscript. The clinical case described is loosely based upon a real patient but details have been changed for teaching purposes and to ensure anonymity. Ethical approval was therefore not required for this manuscript.

AUTHOR CONTRIBUTIONS

Manuscript devised by MB, PM, AJ-P, and CH, and initial draft written by MB and PM. All other authors contributed to discussions regarding content, and draft editing.

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CONFLICT OF INTEREST

MB has previously received speaker's fees and an educational grant from Astellas. KB has received honoraria from Sanofi-Genzyme. AJ-P has previously received consulting fees from Regeneron Pharmaceuticals.

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.

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REFERENCES

1. Garrett GL, Blanc PD, Boscardin J, Lloyd AA, Ahmed RL, Anthony T, et al. Incidence of and Risk Factors for Skin Cancer in Organ Transplant Recipients in the United States. *JAMA Dermatol* (2017) 153(3):296–303. doi:10.1001/jamadermatol.2016.4920
2. Madeleine MM, Patel NS, Plasmeijer EI, Engels EA, Bouwes Bavinck JN, Toland AE, et al. Epidemiology of Keratinocyte Carcinomas after Organ Transplantation. *Br J Dermatol* (2017) 177(5):1208–16. doi:10.1111/bjd.15931
3. Euvrard S, Kanitakis J, Decullier E, Butnaru AC, Lefrancois N, Boissonnat P, et al. Subsequent Skin Cancers in Kidney and Heart Transplant Recipients after the First Squamous Cell Carcinoma. *Transplantation* (2006) 81(8):1093–100. doi:10.1097/01.tp.0000209921.60305.d9
4. Harwood CA, Mesher D, McGregor JM, Mitchell L, Leedham-Green M, Raftery M, et al. A Surveillance Model for Skin Cancer in Organ Transplant Recipients: a 22-year Prospective Study in an Ethnically Diverse Population. *Am J Transpl* (2013) 13(1):119–29. doi:10.1111/j.1600-6143.2012.04292.x
5. Wehner MR, Niu J, Wheless L, Baker LX, Cohen OG, Margolis DJ, et al. Risks of Multiple Skin Cancers in Organ Transplant Recipients: A Cohort Study in 2 Administrative Data Sets. *JAMA Dermatol* (2021) 157(12):1447–55. doi:10.1001/jamadermatol.2021.4148
6. Levine DE, Karia PS, Schmults CD. Outcomes of Patients with Multiple Cutaneous Squamous Cell Carcinomas: A 10-Year Single-Institution Cohort Study. *JAMA Dermatol* (2015) 151(11):1220–5. doi:10.1001/jamadermatol.2015.1702
7. Gonzalez JL, Reddy ND, Cunningham K, Silverman R, Madan E, Nguyen BM. Multiple Cutaneous Squamous Cell Carcinoma in Immunosuppressed vs Immunocompetent Patients. *JAMA Dermatol* (2019) 155(5):625–7. doi:10.1001/jamadermatol.2018.5595
8. Martinez JC, Otley CC, Stasko T, Euvrard S, Brown C, Schanbacher CF, et al. Defining the Clinical Course of Metastatic Skin Cancer in Organ Transplant Recipients: a Multicenter Collaborative Study. *Arch Dermatol* (2003) 139(3):301–6. doi:10.1001/archderm.139.3.301
9. Venables ZC, Autier P, Nijsten T, Wong KF, Langan SM, Rous B, et al. Nationwide Incidence of Metastatic Cutaneous Squamous Cell Carcinoma in England. *JAMA Dermatol* (2019) 155(3):298–306. doi:10.1001/jamadermatol.2018.4219
10. Garrett GL, Lowenstein SE, Singer JP, He SY, Arron ST. Trends of Skin Cancer Mortality after Transplantation in the United States: 1987 to 2013. *J Am Acad Dermatol* (2016) 75(1):106–12. doi:10.1016/j.jaad.2016.02.1155
11. Zamoiski RD, Yanik E, Gibson TM, Cahoon EK, Madeleine MM, Lynch CF, et al. Risk of Second Malignancies in Solid Organ Transplant Recipients Who Develop Keratinocyte Cancers. *Cancer Res* (2017) 77(15):4196–203. doi:10.1158/0008-5472.CAN-16-3291
12. Wisgerhof HC, Wolterbeek R, De Fijter JW, Willemze R, Bouwes Bavinck JN. Kidney Transplant Recipients with Cutaneous Squamous Cell Carcinoma Have an Increased Risk of Internal Malignancy. *J Invest Dermatol* (2012) 132(9):2176–83. doi:10.1038/jid.2012.132
13. Zheng G, Sundquist K, Sundquist J, Forsti A, Hemminki A, Hemminki K. Incidence Differences between First Primary Cancers and Second Primary Cancers Following Skin Squamous Cell Carcinoma as Etiological Clues. *Clin Epidemiol* (2020) 12:857–64. doi:10.2147/CLEP.S256662
14. Wheless L, Black J, Alberg AJ. Nonmelanoma Skin Cancer and the Risk of Second Primary Cancers: a Systematic Review. *Cancer Epidemiol Biomarkers Prev* (2010) 19(7):1686–95. doi:10.1158/1055-9965.EPI-10-0243
15. Rees JR, Zens MS, Gui J, Celaya MO, Riddle BL, Karagas MR. Non Melanoma Skin Cancer and Subsequent Cancer Risk. *PLoS ONE* (2014) 9(6):e99674. doi:10.1371/journal.pone.0099674
16. Gordon LG, Rodriguez-Acevedo AJ, Papier K, Khosrotehrani K, Isbel N, Campbell S, et al. The Effects of a Multidisciplinary High-throughput Skin Clinic on Healthcare Costs of Organ Transplant Recipients. *J Eur Acad Dermatol Venereol* (2019) 33(7):1290–6. doi:10.1111/jdv.15458
17. Zwald F, Leitenberger J, Zeitouni N, Soon S, Brewer J, Arron S, et al. Recommendations for Solid Organ Transplantation for Transplant Candidates with a Pretransplant Diagnosis of Cutaneous Squamous Cell Carcinoma, Merkel Cell Carcinoma and Melanoma: A Consensus Opinion from the International Transplant Skin Cancer Collaborative (ITSCC). *Am J Transpl* (2016) 16(2):407–13. doi:10.1111/ajt.13593
18. Jambusaria-Pahlajani A, Crow LD, Lowenstein S, Garrett GL, Melcher ML, Chan AW, et al. Predicting Skin Cancer in Organ Transplant Recipients: Development of the SUNTRAC Screening Tool Using Data from a Multicenter Cohort Study. *Transpl Int* (2019) 32(12):1259–67. doi:10.1111/tri.13493
19. Lowenstein SE, Garrett G, Toland AE, Jambusaria-Pahlajani A, Asgari MM, Green A, et al. Risk Prediction Tools for Keratinocyte Carcinoma after Solid Organ Transplantation: a Review of the Literature. *Br J Dermatol* (2017) 177(5):1202–7. doi:10.1111/bjd.15889
20. Perez M, Abisaad JA, Rojas KD, Marchetti MA, Jaimes N. Skin Cancer: Primary, Secondary, and Tertiary Prevention. Part I. *J Am Acad Dermatol* (2022) 87(2):255–68. doi:10.1016/j.jaad.2021.12.066
21. Rojas KD, Perez ME, Marchetti MA, Nichols AJ, Penedo FJ, Jaimes N. Skin Cancer: Primary, Secondary, and Tertiary Prevention. Part II. *J Am Acad Dermatol* (2022) 87(2):271–88. doi:10.1016/j.jaad.2022.01.053
22. Chung EYM, Palmer SC, Strippoli GFM. Interventions to Prevent Nonmelanoma Skin Cancers in Recipients of a Solid Organ Transplant: Systematic Review of Randomized Controlled Trials. *Transplantation* (2019) 103(6):1206–15. doi:10.1097/TP.0000000000002641
23. Bavinck JN, Tieben LM, Van der Woude FJ, Tegzess AM, Hermans J, ter Schegget J, et al. Prevention of Skin Cancer and Reduction of Keratotic Skin Lesions during Acitretin Therapy in Renal Transplant Recipients: a Double-Blind, Placebo-Controlled Study. *J Clin Oncol* (1995) 13(8):1933–8. doi:10.1200/JCO.1995.13.8.1933
24. Chen AC, Martin AJ, Choy B, Fernandez-Penas P, Dalziell RA, McKenzie CA, et al. A Phase 3 Randomized Trial of Nicotinamide for Skin-Cancer Chemoprevention. *N Engl J Med* (2015) 373(17):1618–26. doi:10.1056/NEJMoa1506197
25. George R, Weightman W, Russ GR, Bannister KM, Mathew TH. Acitretin for Chemoprevention of Non-melanoma Skin Cancers in Renal Transplant Recipients. *Australas J Dermatol* (2002) 43(4):269–73. doi:10.1046/j.1440-0960.2002.00613.x
26. Massey PR, Schmults CD, Li SJ, Arron ST, Asgari MM, Bouwes Bavinck JN, et al. Consensus-Based Recommendations on the Prevention of Squamous Cell Carcinoma in Solid Organ Transplant Recipients: A Delphi Consensus Statement. *JAMA Dermatol* (2021) 157:1219–26. doi:10.1001/jamadermatol.2021.3180
27. Stasko T, Brown MD, Carucci JA, Euvrard S, Johnson TM, Sengelmann RD, et al. Guidelines for the Management of Squamous Cell Carcinoma in Organ Transplant Recipients. *Dermatol Surg* (2004) 30(4 2):642–50. doi:10.1111/j.1524-4725.2004.30150.x
28. Lopez AT, Carvajal RD, Geskin L. Secondary Prevention Strategies for Nonmelanoma Skin Cancer. *Oncology (Williston Park)* (2018) 32(4):195–200.
29. Jansen MHE, Kessels J, Nelemans PJ, Kouloubis N, Arits A, van Pelt HPA, et al. Randomized Trial of Four Treatment Approaches for Actinic Keratosis. *N Engl J Med* (2019) 380(10):935–46. doi:10.1056/NEJMoa1811850
30. Weinstock MA, Thwin SS, Siegel JA, Marcolivio K, Means AD, Leader NF, et al. Chemoprevention of Basal and Squamous Cell Carcinoma with a Single Course of Fluorouracil, 5%, Cream: A Randomized Clinical Trial. *JAMA Dermatol* (2018) 154(2):167–74. doi:10.1001/jamadermatol.2017.3631
31. Kisling LA. *Prevention Strategies*. Orlando, FL: StatPearls (2022).
32. Ulrich C, Jurgensen JS, Degen A, Hackethal M, Ulrich M, Patel MJ, et al. Prevention of Non-melanoma Skin Cancer in Organ Transplant Patients by

Regular Use of a Sunscreen: a 24 Months, Prospective, Case-Control Study. *Br J Dermatol* (2009) 161(3):78–84. doi:10.1111/j.1365-2133.2009.09453.x
33. Werner RN, Sammain A, Erdmann R, Hartmann V, Stockfleth E, Nast A, et al. The Natural History of Actinic Keratosis: a Systematic Review. *Br J Dermatol* (2013) 169(3):502–18. doi:10.1111/bjd.12420
34. Tokez S, Wakkee M, Louwman M, Noels E, Nijsten T, Hollestein L. Assessment of Cutaneous Squamous Cell Carcinoma (cSCC) *In Situ* Incidence and the Risk of Developing Invasive cSCC in Patients with Prior cSCC *In Situ* vs the General Population in the Netherlands, 1989-2017. *JAMA Dermatol* (2020) 156(9):973–81. doi:10.1001/jamadermatol.2020.1988
35. Nemer KM, Council ML. Topical and Systemic Modalities for Chemoprevention of Nonmelanoma Skin Cancer. *Dermatol Clin* (2019) 37(3):287–95. doi:10.1016/j.det.2019.02.004
36. Heppt MV, Steeb T, Niesert AC, Zacher M, Leiter U, Garbe C, et al. Local Interventions for Actinic Keratosis in Organ Transplant Recipients: a Systematic Review. *Br J Dermatol* (2019) 180(1):43–50. doi:10.1111/bjd.17148
37. Hasan ZU, Ahmed I, Matin RN, Homer V, Lear JT, Ismail F, et al. Topical Treatment of Actinic Keratoses in Organ Transplant Recipients: a Feasibility Study for SPOT (Squamous Cell Carcinoma Prevention in Organ Transplant Recipients Using Topical Treatments). *Br J Dermatol* (2022) 187:324–37. doi:10.1111/bjd.20974
38. Harwood CA, Leedham-Green M, Leigh IM, Proby CM. Low-dose Retinoids in the Prevention of Cutaneous Squamous Cell Carcinomas in Organ Transplant Recipients: a 16-year Retrospective Study. *Arch Dermatol* (2005) 141(4):456–64. doi:10.1001/archderm.141.4.456
39. Giacalone S, Spigariolo CB, Bortoluzzi P, Nazzaro G. Oral Nicotinamide: The Role in Skin Cancer Chemoprevention. *Dermatol Ther* (2021) 34(3):e14892. doi:10.1111/dth.14892
40. Inman GJ, Wang J, Nagano A, Alexandrov LB, Purdie KJ, Taylor RG, et al. The Genomic Landscape of Cutaneous SCC Reveals Drivers and a Novel Azathioprine Associated Mutational Signature. *Nat Commun* (2018) 9(1):3667. doi:10.1038/s41467-018-06027-1
41. Corchado-Cobos R, García-Sancha N, González-Sarmiento R, Pérez-Losada J, Cañueto J. Cutaneous Squamous Cell Carcinoma: From Biology to Therapy. *Int J Mol Sci* (2020) 21(8):2956. doi:10.3390/ijms21082956
42. Jiyad Z, Olsen CM, Burke MT, Isbel NM, Green AC. Azathioprine and Risk of Skin Cancer in Organ Transplant Recipients: Systematic Review and Meta-Analysis. *Am J Transpl* (2016) 16(12):3490–503. doi:10.1111/ajt.13863
43. Ming M, Zhao B, Qiang L, He Y-Y. Effect of Immunosuppressants Tacrolimus and Mycophenolate Mofetil on the Keratinocyte UVB Response. *Photochem Photobiol* (2015) 91(1):242–7. doi:10.1111/php.12318
44. Mittal A, Colegio OR. Skin Cancers in Organ Transplant Recipients. *Am J Transpl* (2017) 17(10):2509–30. doi:10.1111/ajt.14382
45. Gibson JAG, Cordaro A, Dobbs TD, Griffiths R, Akbari A, Whitaker S, et al. The Association between Immunosuppression and Skin Cancer in Solid Organ Transplant Recipients: a Control-Matched Cohort Study of 2, 852 Patients. *Eur J Dermatol* (2021) 31(6):712–21. doi:10.1684/ejd.2021.4108
46. Vos M, Plasmeijer E, van Bemmel B, van der Bij W, Klaver N, Erasmus M, et al. Azathioprine to Mycophenolate Mofetil Transition and Risk of Squamous Cell Carcinoma after Lung Transplantation. *J Heart Lung Transpl* (2018) 37(7):853–9. doi:10.1016/j.healun.2018.03.012
47. Wang M, Mittal A, Colegio OR. Belatacept Reduces Skin Cancer Risk in Kidney Transplant Recipients. *J Am Acad Dermatol* (2020) 82(4):996–8. doi:10.1016/j.jaad.2019.09.070
48. Euvrard S, Morelon E, Rostaing L, Goffin E, Brocard A, Tromme I, et al. Sirolimus and Secondary Skin-Cancer Prevention in Kidney Transplantation. *N Engl J Med* (2012) 367(4):329–39. doi:10.1056/NEJMoa1204166
49. Hoogendijk-van den Akker JM, Harden PN, Hoitsma AJ, Proby CM, Wolterbeek R, Bouwes Bavinck JN, et al. Two-year Randomized Controlled Prospective Trial Converting Treatment of Stable Renal Transplant Recipients with Cutaneous Invasive Squamous Cell Carcinomas to Sirolimus. *J Clin Oncol* (2013) 31(10):1317–23. doi:10.1200/JCO.2012.45.6376
50. Dantal J, Morelon E, Rostaing L, Goffin E, Brocard A, Tromme I, et al. Sirolimus for Secondary Prevention of Skin Cancer in Kidney Transplant Recipients: 5-Year Results. *J Clin Oncol* (2018) 36(25):2612–20. doi:10.1200/JCO.2017.76.6691
51. Knoll GA, Kokolo MB, Mallick R, Beck A, Buenaventura CD, Ducharme R, et al. Effect of Sirolimus on Malignancy and Survival after Kidney Transplantation: Systematic Review and Meta-Analysis of Individual Patient Data. *BMJ : Br Med J* (2014) 349:g6679. doi:10.1136/bmj.g6679
52. Berger SP, Sommerer C, Witzke O, Tedesco H, Chadban S, Mulgaonkar S, et al. Two-year Outcomes in De Novo Renal Transplant Recipients Receiving Everolimus-Facilitated Calcineurin Inhibitor Reduction Regimen from the TRANSFORM Study. *Am J Transpl* (2019) 19(11):3018–34. doi:10.1111/ajt.15480
53. Preterre J, Visentin J, Saint Cricq M, Kaminski H, Del Bello A, Prezelin-Reydit M, et al. Comparison of Two Strategies Based on Mammalian Target of Rapamycin Inhibitors in Secondary Prevention of Non-melanoma Skin Cancer after Kidney Transplantation, a Pilot Study. *Clin Transpl* (2021) 35(3):e14207. doi:10.1111/ctr.14207
54. Lim WH, Russ GR, Wong G, Pilmore H, Kanellis J, Chadban SJ. The Risk of Cancer in Kidney Transplant Recipients May Be Reduced in Those Maintained on Everolimus and Reduced Cyclosporine. *Kidney Int* (2017) 91(4):954–63. doi:10.1016/j.kint.2016.11.008
55. Bestard O, Thaunat O, Bellini MI, Bohmig GA, Budde K, Claas F, et al. Alloimmune Risk Stratification for Kidney Transplant Rejection. *Transpl Int* (2022) 35:10138. doi:10.3389/ti.2022.10138
56. Otley CC, Maragh SLH. Reduction of Immunosuppression for Transplant-Associated Skin Cancer: Rationale and Evidence of Efficacy. *Dermatol Surg* (2006) 31(2):163–8. doi:10.1111/j.1524-4725.2005.31038
57. Dantal J, Hourmant M, Cantarovich D, Giral M, Blancho G, Dreno B, et al. Effect of Long-Term Immunosuppression in Kidney-Graft Recipients on Cancer Incidence: Randomised Comparison of Two Cyclosporin Regimens. *Lancet* (1998) 351(9103):623–8. doi:10.1016/S0140-6736(97)08496-1
58. Van Leeuwen MT, Webster AC, McCredie MRE, Stewart JH, McDonald SP, Amin J, et al. Effect of Reduced Immunosuppression after Kidney Transplant Failure on Risk of Cancer: Population Based Retrospective Cohort Study. *BMJ* (2010) 340(11 2):c570–c. doi:10.1136/bmj.c570
59. Barozzi P, Bonini C, Potenza L, Masetti M, Cappelli G, Gruarin P, et al. Changes in the Immune Responses against Human Herpesvirus-8 in the Disease Course of Posttransplant Kaposi Sarcoma. *Transplantation* (2008) 86(5):738–44. doi:10.1097/TP.0b013e318184112c
60. Hall EC, Engels EA, Pfeiffer RM, Segev DL. Association of Antibody Induction Immunosuppression with Cancer after Kidney Transplantation. *Transplantation* (2015) 99(5):1051–7. doi:10.1097/TP.0000000000000449
61. De Pasquale C, Veroux M, Indelicato L, Sinagra N, Giaquinta A, Fornaro M, et al. Psychopathological Aspects of Kidney Transplantation: Efficacy of a Multidisciplinary Team. *World J Transpl* (2014) 4(4):267–75. doi:10.5500/wjt.v4.i4.267
62. Howell M, Tong A, Wong G, Craig JC, Howard K. Important Outcomes for Kidney Transplant Recipients: a Nominal Group and Qualitative Study. *Am J Kidney Dis* (2012) 60(2):186–96. doi:10.1053/j.ajkd.2012.02.339
63. Howell M, Wong G, Rose J, Tong A, Craig JC, Howard K. Patient Preferences for Outcomes after Kidney Transplantation: A Best-Worst Scaling Survey. *Transplantation* (2017) 101(11):2765–73. doi:10.1097/TP.0000000000001793
64. Nickerson PW. What Have We Learned about How to Prevent and Treat Antibody-mediated Rejection in Kidney Transplantation? *Am J Transpl* (2020) 20(S4):12–22. doi:10.1111/ajt.15859
65. Borden ES, Kang P, Natri HM, Phung TN, Wilson MA, Buetow KH, et al. Neoantigen Fitness Model Predicts Lower Immune Recognition of Cutaneous Squamous Cell Carcinomas Than Actinic Keratoses. *Front Immunol* (2019) 10:2799. doi:10.3389/fimmu.2019.02799
66. Stapleton CP, Chang BL, Keating BJ, Conlon PJ, Cavalleri GL. Polygenic Risk Score of Non-melanoma Skin Cancer Predicts post-transplant Skin Cancer across Multiple Organ Types. *Clin Transpl* (2020) 34(8):e13904. doi:10.1111/ctr.13904
67. Seviiri M, Law MH, Ong JS, Gharahkhani P, Nyholt DR, Hopkins P, et al. Polygenic Risk Scores Stratify Keratinocyte Cancer Risk Among Solid Organ Transplant Recipients with Chronic Immunosuppression in a High Ultraviolet Radiation Environment. *J Invest Dermatol* (2021) 141(12):2866–75.e2. doi:10.1016/j.jid.2021.03.034
68. Chahal HS, Lin Y, Ransohoff KJ, Hinds DA, Wu W, Dai HJ, et al. Genome-wide Association Study Identifies Novel Susceptibility Loci for Cutaneous

Squamous Cell Carcinoma. *Nat Commun* (2016) 7:12048. doi:10.1038/ncomms12048
69. Siiskonen SJ, Zhang M, Li WQ, Liang L, Kraft P, Nijsten T, et al. A Genome-wide Association Study of Cutaneous Squamous Cell Carcinoma Among European Descendants. *Cancer Epidemiol Biomarkers Prev* (2016) 25(4): 714–20. doi:10.1158/1055-9965.EPI-15-1070
70. Sarin KY, Lin Y, Daneshjou R, Ziyatdinov A, Thorleifsson G, Rubin A, et al. Genome-wide Meta-Analysis Identifies Eight New Susceptibility Loci for Cutaneous Squamous Cell Carcinoma. *Nat Commun* (2020) 11(1):820. doi:10.1038/s41467-020-14594-5
71. Asgari MM, Wang W, Ioannidis NM, Itnyre J, Hoffmann T, Jorgenson E, et al. Identification of Susceptibility Loci for Cutaneous Squamous Cell Carcinoma. *J Invest Dermatol* (2016) 136(5):930–7. doi:10.1016/j.jid.2016.01.013
72. Geusau A, Borik-Heil L, Skalicky S, Mildner M, Grillari J, Hackl M, et al. Dysregulation of Tissue and Serum microRNAs in Organ Transplant Recipients with Cutaneous Squamous Cell Carcinomas. *Health Sci Rep* (2020) 3(4):e205. doi:10.1002/hsr2.205
73. Blue ED, Freeman SC, Lobl MB, Clarey DD, Fredrick RL, Wysong A, et al. Cutaneous Squamous Cell Carcinoma Arising in Immunosuppressed Patients: A Systematic Review of Tumor Profiling Studies. *JID Innov* (2022) 2(4): 100126. doi:10.1016/j.xjidi.2022.100126
74. Wysong A, Newman JG, Covington KR, Kurley SJ, Ibrahim SF, Farberg AS, et al. Validation of a 40-gene Expression Profile Test to Predict Metastatic Risk in Localized High-Risk Cutaneous Squamous Cell Carcinoma. *J Am Acad Dermatol* (2021) 84(2):361–9. doi:10.1016/j.jaad.2020.04.088
75. Bottomley MJ, Harden PN, Wood KJ. CD8+ Immunosenescence Predicts Post-Transplant Cutaneous Squamous Cell Carcinoma in High-Risk Patients. *J Am Soc Nephrol* (2016) 27(5):1505–15. doi:10.1681/ASN.2015030250
76. Carroll RP, Segundo DS, Hollowood K, Marafioti T, Clark TG, Harden PN, et al. Immune Phenotype Predicts Risk for Posttransplantation Squamous Cell Carcinoma. *J Am Soc Nephrol* (2010) 21(4):713–22. doi:10.1681/ASN.2009060669
77. Hope CM, Grace BS, Pilkington KR, Coates PT, Bergmann IP, Carroll RP. The Immune Phenotype May Relate to Cancer Development in Kidney Transplant Recipients. *Kidney Int* (2014) 86(1):175–83. doi:10.1038/ki.2013.538
78. Normand S-LT. The RECOVERY Platform. *N Engl J Med* (2021) 384(8):757–8. doi:10.1056/NEJMe2025674

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Outcomes After Liver Transplantation With Incidental Cholangiocarcinoma

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Cholangiocarcinoma (CCA) is currently a contraindication to liver transplantation (LT) in the United Kingdom (UK). Incidental CCA occurs rarely in some patients undergoing LT. We report on retrospective outcomes of patients with incidental CCA from six UK LT centres. Cases were identified from pathology records. Data regarding tumour characteristics and post-transplant survival were collected. CCA was classified by TNM staging and anatomical location. 95 patients who underwent LT between 1988–2020 were identified. Median follow-up after LT was 2.1 years (14 days-18.6 years). Most patients were male (68.4%), median age at LT was 53 (IQR 46-62), and the majority had underlying PSC (61%). Overall median survival after LT was 4.4 years. Survival differed by tumour site: 1-, 3-, and 5-year estimated survival was 82.1%, 68.7%, and 57.1%, respectively, in intrahepatic CCA (n = 40) and 58.5%, 42.6%, and 30.2% in perihilar CCA (n = 42; p = 0.06). 1-, 3-, and 5-year estimated survival was 95.8%, 86.5%, and 80.6%, respectively, in pT1 tumours (28.2% of cohort), and 65.8%, 44.7%, and 31.1%, respectively, in pT2-4 (p = 0.018). Survival after LT for recipients with incidental CCA is inferior compared to usual outcomes for LT in the United Kingdom. LT for earlier stage CCA has similar survival to LT for hepatocellular cancer, and intrahepatic CCAs have better survival compared to perihilar CCAs. These observations may support LT for CCA in selected cases.

Keywords: survival, tumor, intrahepatic, hilar, indication, transplant, distal, hepatocellular

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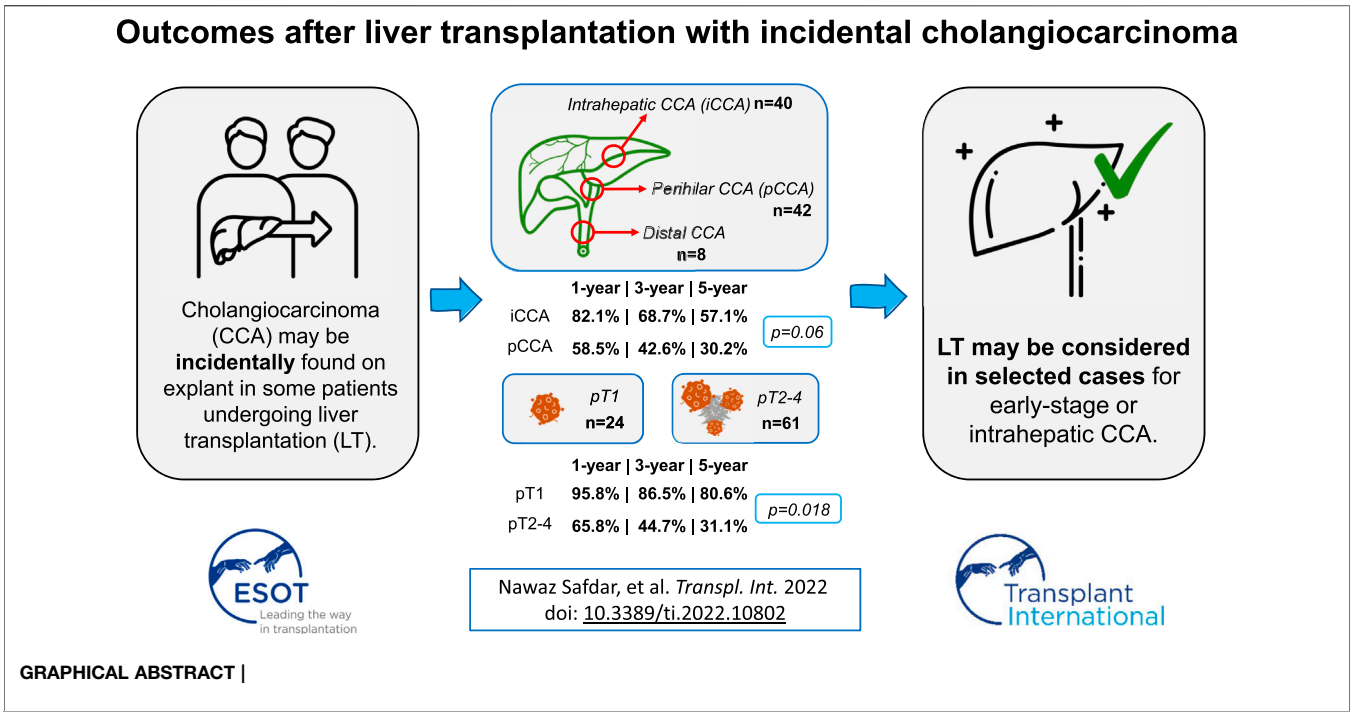
Abbreviations: CCA, cholangiocarcinoma; CT, computed tomography; dCCA, distal cholangiocarcinoma; ELTR, European Liver Transplant Registry; HCC, hepatocellular carcinoma; iCCA, intrahepatic cholangiocarcinoma; LT, liver transplant; MRI, magnetic resonance imaging; NACRT, neoadjuvant chemoradiation therapy; pCCA, perihilar cholangiocarcinoma; PSC, primary sclerosing cholangitis; pTNM, tumour-node-metastasis.



GRAPHICAL ABSTRACT |

INTRODUCTION

Cholangiocarcinoma (CCA) is the second most common primary hepatic malignancy, with increasing age-standardised incidence rate in England from 2.7 in 2001 to 4.3 per 100,000 in 2017 (1). Despite improvement in diagnostic tools, chemotherapeutic agents, and surgical techniques, the age-standardised mortality rate has increased from 2.6 in 2001 to 4.7 per 100,000 in 2017 (2, 3). Primary sclerosing cholangitis (PSC) is a known risk factor for the development of CCA (4): individuals diagnosed with PSC have a 15%–20% lifetime risk of developing CCA (5). A meta-analysis of 11 studies found that cirrhosis, hepatitis B, hepatitis C, alcohol, diabetes and obesity are also risk factors for development of CCA (6).

Diagnosis of CCA can be extremely challenging, as a significant proportion of patients do not present with definite mass lesions. Although intrahepatic CCA (iCCA) can be visualised as mass-forming lesions (7), perihilar CCA (pCCA) and distal CCA (dCCA) can be tricky to detect due to their infiltrative nature (8). Difficulties with diagnostic imaging suggest that previously undetected CCA may be found in patients undergoing liver transplant (LT) for other indications.

Early experience with LT for unselected CCAs was disappointing with 5-year survival rates ranging from 18% to 25% (9). Recent data suggest that carefully selected patients may do well after transplantation (10). Using the Mayo protocol of aggressive neoadjuvant chemotherapy for small (<3 cm), non-metastatic pCCA, Heimbach et al. found a 5-year survival of 69% in highly selected patients with pCCA, on a background of PSC (11). A subsequent multicentre study involving 12 US LT centres demonstrated a recurrence-free survival of 78%, 65% and 59% at 2-, 5- and 10-years, respectively, using the Mayo protocol (12). A recent Dutch retrospective study looking at 732 consecutive patients with pCCAs (13) identified that only 5% of them were potentially eligible for LT using the same protocol. Recent evidence for iCCA suggests that LT might be beneficial for tumours smaller than 2 cm, when compared to surgical resection. An international multicentre study demonstrated that very early (<2 cm) iCCAs had a 5-year survival of 65% following LT (10).

The purpose of this multicentre study was to retrospectively describe the outcomes after LT in recipients that were diagnosed with CCA incidentally on explant.

METHODS AND MATERIALS

Study Design

LT recipients with previously undetected CCA, occasionally on a background of hepatocellular carcinoma (HCC), subsequently found on explant were identified retrospectively from pathology databases across six LT centres in the United Kingdom. A standardised data proforma was completed at each centre including patient age, gender, pre-LT factors (aetiology for liver transplant, pre-transplant imaging, intervention details, biochemical data), LT factors (date of transplant, biochemical markers, explant histopathology data) and post-LT factors (adjuvant chemotherapy data and survival outcomes). Date of last follow-up or death was used to calculate survival times, including those with short follow-up or death during inpatient stay for LT. There was an absence of a pre-determined minimum follow-up period and therefore we have reported estimated survival rates. CCA was classified by tumour-node-metastasis (pTNM) stage using the American Joint Committee on Cancer

TABLE 1 | Summary of patient demographics and aetiology of disease (N = 95).

Gender	
Male	65 (68.4%)
Female	30 (31.6%)
Median age (IQR) years	53 (46–62)
Aetiology of liver disease (*n* = 95; %)	
Primary sclerosing cholangitis (PSC)	58 (61%)
Hepatitis C (HCV)	13 (13.7%)
Alcoholic liver disease (ALD)	9 (9.5%)
Cryptogenic liver disease	5 (5.3%)
Non-alcoholic fatty liver disease (NAFLD)	3 (3.2%)
Haemochromatosis	3 (3.2%)
Primary Biliary Cholangitis (PBC)	1 (1.1%)
Secondary Biliary Cirrhosis (SBC)	1 (1.1%)
Auto-immune Hepatitis (AIH)	1 (1.1%)
Recurrent cholangitis	1 (1.1%)
Presence of hepatocellular carcinoma	
Yes	28/95
No	35/95
Unknown	32/95

TABLE 2 | Tumour characteristics including site and staging (N = 95).

Site	
iCCA	40 (42.1%)
pCCA	42 (44.2%)
dCCA	8 (8.4%)
Unknown	5 (5.3%)
Stage	
pT1	24 (25.3%)
pT2	41 (43.1%)
pT3	11 (11.6%)
pT4	9 (9.5%)
Unknown	10 (1.1%)
Size	
>2 cm	10/95
<2 cm	9/95
Unknown	76/95
Lymphatic invasion	
Yes	9/95
No	16/95
Unknown	70/95
Vascular invasion	
Yes	7/95
No	19/95
Unknown	69/95

and Union for International Cancer Control 8th edition (14) and by three anatomical locations iCCA, pCCA and dCCA. CCAs identified near or within the gallbladder were included in the dCCA group. For iCCA, a further sub-classification of T1a and T1b existed which focused on tumour size. We classed these tumours as T1 since pCCA classification did not include tumour size. All patients had undergone pre-operative cross-sectional imaging with at least one modality: Magnetic resonance imaging (MRI) and/or Computed tomography (CT). No ethical approval was sought as only anonymised, routinely collected clinical data was used and no additional procedures were performed.

Statistical Analysis

Parametric data were described using mean and range, and non-parametric data were described using median and interquartile range (IQR). *p*-values below 0.05 were considered statistically significant. Survival was analysed with Kaplan-Meier analysis using the Log-rank test to compare groups. Fisher's exact test was used to compare association between categorical variables. STATA 16 MP (StataCorp. 2019. Stata Statistical Software: Release 16. College Station, TX: StataCorp LLC.) was used for statistical analyses, and Origin Pro 2020 (Origin (Pro), Version 2020. Origin Lab Corporation, Northampton, MA, United States) was used as graphing software.

RESULTS

Overall Cohort

97 patients with incidental CCA on explant were identified from six UK LT centres between January 1988 and August 2020. Two patients were excluded due to lack of survival data with the remaining 95 patients included in the final analysis: Birmingham (*n* = 30), Cambridge (*n* = 10), Edinburgh (*n* = 22), Leeds (*n* = 9), Newcastle (*n* = 6), and Royal Free (*n* = 18). LT were performed between January 1988 and August 2020. Median follow-up after LT was 2.1 years (range 14 days–18.6 years). Most patients were male (68.4%), median age was 53 (IQR 46–62), and PSC (61%) was the most common underlying liver disease (**Table 1**). Few patients had findings on pre-operative imaging that indicated duct dilatation (45.0%) and duct thickening (19.4%). Tumour characteristics including site and stage are summarised in **Table 2**. Data on adjuvant chemotherapy was only available for 19 patients, summarised in **Table 3**.

Overall Survival

Overall median survival was 4.4 years (IQR: 0.9–8.4) (**Figure 1**). At the date of last follow-up (1 August 21), 36 (38%) patients were still alive. The 1-, 3-, and 5-year estimated survival rates were 71.9% (95% CI: 61.5%–79.9%), 55.5% (95% CI: 44.5%–65.4%), and 43.6% (95% CI: 32.0%–54.6%), respectively.

Survival by Site

Survival was further analysed by site of tumour, including 90 patients with relevant data available. The median survival was 42.7 months (IQR 18.4–122.6), 68.5 months (25.3–109.7), and 23.8 months (7.4–75.2) for dCCA, iCCA and pCCA, respectively (**Figure 2**). Survival was lowest in patients with pCCA. Both iCCA (82.1% and 68.7%) and dCCA (87.5% and 62.5%) had similar estimated survival at 1- and 3-years, however, overall 5-year estimated survival was highest in the iCCA cohort (57.1%). There was no statistical difference in survival between the 3 groups (log-rank test

TABLE 3 | A summary of chemotherapy for patients where data was available.

Regimen	Cycles	Stage	Site	Dead/Alive	Survival (days)
5-FU	1	—	pCCA	Dead	328
Gemcitabine/Oxaliplatin	6	pT3	pCCA	Dead	40
Gemcitabine	—	pT2	pCCA	Dead	785
Capecitabine	8	pT2	pCCA	Dead	987
Gemcitabine/Oxaliplatin	5	—	—	—	—
Capecitabine	6	pT3	iCCA	Alive	—
Gemcitabine/Cisplatin	5	pT2	pCCA	Dead	356
Doxorubicin (chemoembolization)	—	pT1	iCCA	Dead	2084
Doxorubicin	—	pT1	iCCA	Alive	—
Doxorubicin	—	pT1	iCCA	Alive	—
Doxorubicin	—	pT1	iCCA	Alive	—
Doxorubicin	—	pT1	iCCA	Alive	—
Doxorubicin	—	pT2	iCCA	Dead	328
Doxorubicin	—	pT2	iCCA	Alive	—
Doxorubicin	—	pT2	iCCA	Alive	—
Doxorubicin	—	pT2	iCCA	Alive	—
Doxorubicin	—	pT2	iCCA	Alive	—
Capecitabine	4	pT3	pCCA	Alive	—
Capecitabine	—	pT3	pCCA	Alive	—

TABLE 4 | Distribution of patients across the sites and stages stratified by aetiology.

	PSC (n = 58)	Non-PSC (n = 37)
Site		
iCCA (n = 40)	12	28
pCCA (n = 42)	37	5
dCCA (n = 8)	6	2
Unknown (n = 5)	3	2
Stage		
pT1 (n = 24)	13	11
pT2 (n = 41)	24	21
pT3 (n = 11)	9	2
pT4 (n = 9)	7	2
Unknown (10)	5	5

p = 0.12). After excluding the small group of patients with dCCA, a second log-rank test between iCCA and pCCA was also statistically non-significant (p = 0.06).

Survival by Staging

Survival analysis was carried out on patients stratified by pTNM staging. This cohort included 85 patients with pTNM staging information. For pT1 (n = 24), pT2 (n = 41), pT3 (n = 11), and pT4 (n = 9), median survival rates were 99.2 months (IQR 69.5–111.8), 31.3 months (8.2–149.5), 23.2 months (1.7–52.6), and 46.6 months (9.7–75.2), respectively (**Figure 3A**). The 1-, 3-, and 5-year estimated survival rates were highest in the pT1 group. Based on differing survival, pT1 demonstrated relatively superior survival compared to pT2-4 ("other") staged disease (p = 0.018; **Figure 3B**).

Survival by Aetiology

Survival analysis was completed by stratifying by aetiology of liver disease. All 95 patients were considered for this section of the analysis. The cohort was split into two groups: 1) Patients with a diagnosis of PSC (n = 58), and 2) patients with any other diagnosis (n = 37). Median survival was 26.2 months (IQR 8.2–99.2) for patients with PSC and 69.5 months (42.7–109.7) months for alternate aetiologies (p = 0.073) (**Figure 4A**). Patients with PSC had 1-, 3-, and 5-year estimated survival rates of 66.4% (95% CI: 52.5%–77.1%), 43.5% (95% CI: 30.1%–56.2%), and 34.4% (95% CI: 21.7%–47.5%), respectively, and the non-PSC group had 1-, 3-, and 5-year estimated survival rates of 80.6% (95% CI: 63.5%–90.2%), 76.5% (95% CI: 58.1%–87.7%), and 59.1% (95% CI: 35.9%–76.4), respectively. The stage and site of disease by aetiology can be found in **Table 4**.

The cohort was further stratified by tumour site within the PSC cohort (iCCA or pCCA) (**Figure 4B**). Patients with pCCA and PSC (n = 37) had 1-, 3-, and 5-year estimated survival rates of 58.3% (95% CI: 40.7%–72.4%), 40.0% (95% CI: 23.8%–55.7%), and 28.8% (95% CI: 14.3%–45.1%), respectively, and patients with iCCA and PSC (n = 12) had 1-, 3-, and 5-year estimated survival rates of 82.6% (95% CI: 46.5%–95.3%), 54.4% (95% CI: 22.4%–78.0%), and 42.3% (95% CI: 13.2%–69.4%), respectively (p = 0.62).

Finally, patients with PSC and early pCCA (pT1-2; n = 22) had 1-, 3-, and 5-year estimated survival rates of 63.6% (95% CI: 40.3%–79.9%), 39.1% (95% CI: 18.9%–58.8%), and 33.1% (95% CI: 14.2%–53.4%), respectively.

DISCUSSION

In this study of patients undergoing liver transplant who were found to have a previously undetected CCA, we found that small tumours with no vascular or lymphatic invasion (pT1) had much better 5-year survival than larger tumours (pT2, pT3, pT4). pCCAs also had poorer 5-year survival when compared to iCCA, however this was not statistically significant. Within the limits of the size of the cohort, patients with CCA on a

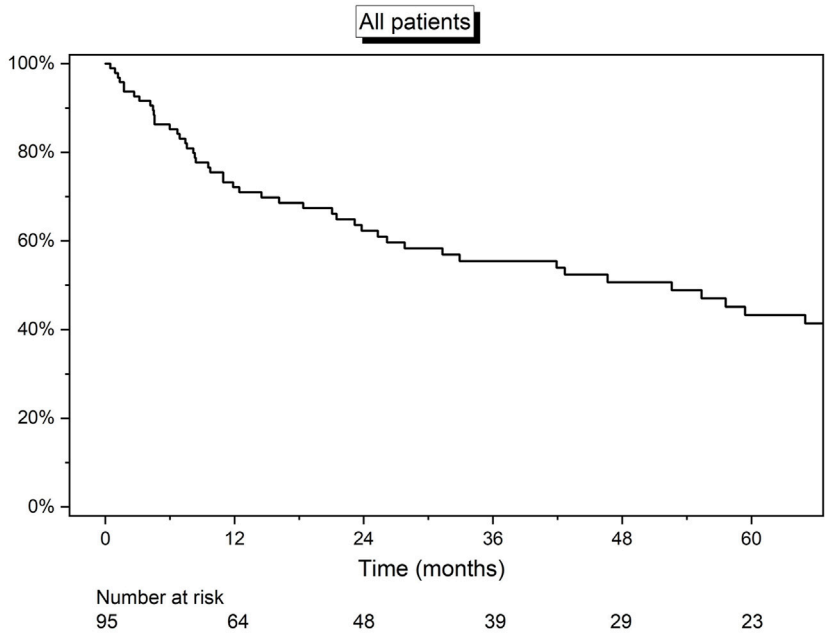


FIGURE 1 | Kaplan-Meier curve detailing the overall survival of all 95 patients.

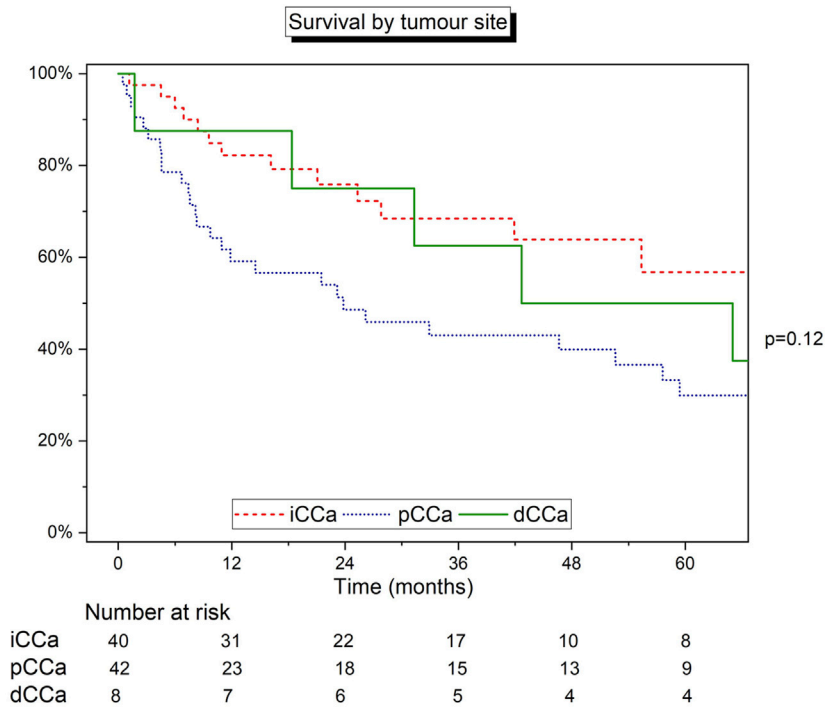


FIGURE 2 | Kaplan-Meier curve detailing the survival of patients grouped by site of tumour. Log-rank test.

background of PSC, tended to have worse medium-term survival when compared to other indications, although this group had more advanced disease.

Cholestatic liver disease is a common indication for liver transplantation with patient and graft survival comparable to other indications. Previously, 5-year survival of patients with PSC

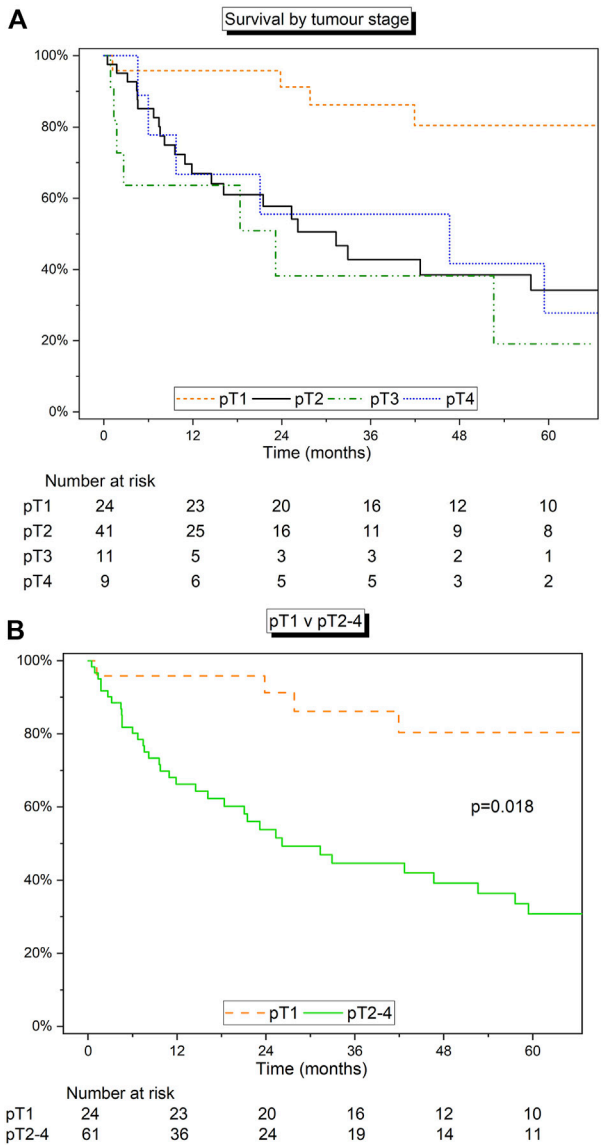


FIGURE 3 | (A) Kaplan-Meier curve detailing the survival of patients grouped by size of tumour using pTNM staging. **(B)** Kaplan-Meier curve detailing the survival of pT1 vs. pT2-4. Log-rank test.

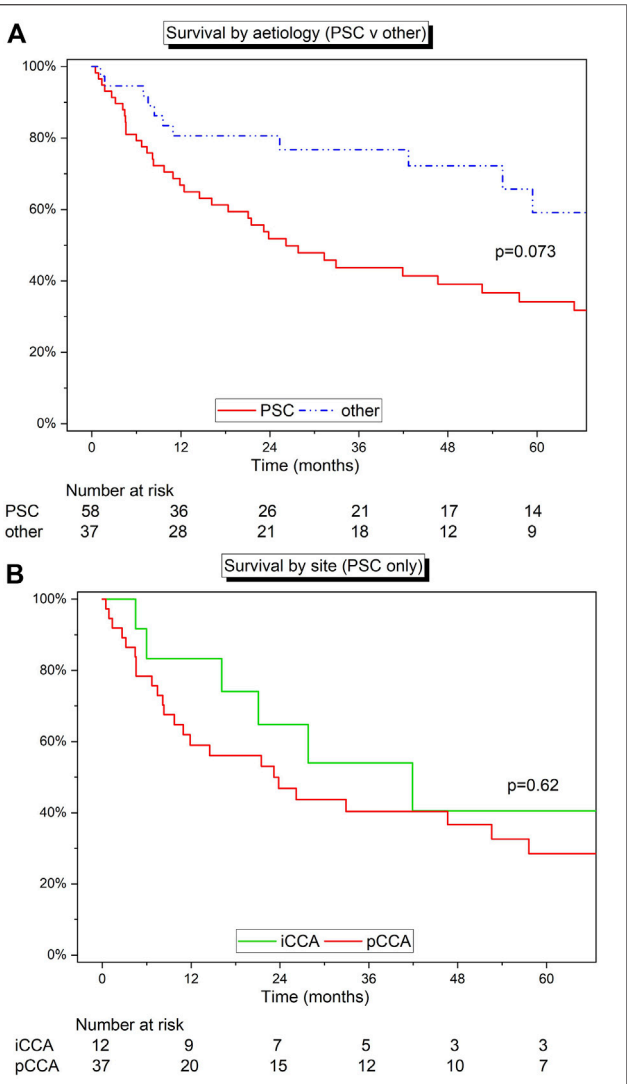


FIGURE 4 | (A) Kaplan-Meier curve detailing the survival of patients with PSC *versus* all the other indications for LT. **(B)** Kaplan-Meier curve detailing the survival of patients with PSC grouped by site (iCCA v pCCA). Log-rank test.

undergoing LT has been shown to be up to 83% (15). This contrasts with our cohort with cancer on explant, which demonstrated a much lower median survival of 34.4%. This disparity may exist due to the severity of disease observed in the PSC cohort relative to the non-PSC aetiologies: 30.2% advanced disease (pT3/4) with PSC compared to 12.5% with non-PSC (Fisher's exact test: p = 0.071) and highlights the challenge of detection of even advanced CCA in patients with PSC at the time of LT.

Results from our study indicate that early "low risk" stages of CCA have favourable medium-term i.e., 5-year survival. A Scandinavian study has previously shown that CCAs with a pT2 stage or below had a 5-year survival of 48% (16). We narrowed this group further to include pT1 only and demonstrated better survival at 5-years, which is similar to survival rates of people receiving transplants for HCCs. This finding confirms the need for improved protocols for earlier detection of CCA before LT, particularly in PSC patients, and a UK service evaluation offering LT as definitive treatment for a select group of patients diagnosed with early stages of iCCA and pCCA. It also offers multidisciplinary teams additional information with which to counsel patients currently receiving treatment for hepatobiliary disease.

Accurate diagnosis of CCA type is important since it can dictate whether a patient is selected for surgical (resection or transplant) or conservative (chem (radio)otherapy) management. For example, inclusion into the Mayo protocol for LT in pCCA requires the diagnosis of CCA. However, diagnosing these tumours, both in PSC and non-PSC patients, is often

challenging. Pathological confirmation of CCA before therapy was obtained in only 52% (45 out of 87) of PSC patients in the Mayo cohort (11). Pre-treatment pathological confirmation was associated with significantly inferior 5-year survival after start of therapy (50% vs 80%; $p = 0.001$) and after transplantation (66% vs. 92%; $p = 0.01$) in the PSC cohort, when compared with no pathological confirmation (17). From these findings, we could imply that pathological confirmation is more likely in larger, more advanced tumours and that half of the PSC patients from the Mayo cohort may have had low-risk tumours and hence better long-term survival, which is also observed in our cohort with pT1 tumours. A subgroup analysis of early stage (pT1-2) pCCA patients with PSC ($n = 22$) showed 1-, 3-, and 5- year estimated survivals of 63.6%, 39.1%, and 33.1%, respectively, in our cohort. Interestingly, in a retrospective review (18) of European Liver Transplant Registry (ELTR) data from 21 centres between 1990–2010, only 28 (19%) patients out of 249 met the strict selection criteria of the Mayo clinic, and only 5% in a Dutch study of 732 pCCA patients from two centres (13).

Our cohort with pCCAs demonstrated a 5-year overall survival of 30.2%. This was in-line with a recent meta-analysis by Cambridge et al. (19) which reported a 5-year survival of 31.6% in patients not receiving neoadjuvant chemoradio-therapy (NACRT). In this series, 5-year survival increased to 65.1% in patients receiving NACRT. In contrast, in the ELTR series, the 5-year survival without NACRT was 58%, which is comparable to the group that received NACRT. These seemingly conflicting data highlight the need for a multi-centre study to definitively address outcomes for highly selected cases of unresectable pCCA.

When comparing by site of disease, we found a significant difference in 5-year survival between the three sites (iCCA, pCCA and dCCA). After excluding the small dCCA cohort, the difference was not significant, but this might be explained by the underpowered nature of our study. iCCAs trended towards a better survival than pCCAs and due to the limited nature of our data, no causal conclusions can be drawn.

Various chemotherapy regimens were utilised in our cohort, however, the decision to provide adjuvant therapy was a difficult one due to the incidental nature of these tumours on explant. Nonetheless, Gemcitabine with or without platinum based alkylating agents (Cis- or Oxi-platin), 5-fluorouracil (5-FU) and Capecitabine were used in a handful of patients. Due to our small numbers and heterogeneity of the data, we were unable to compare survival across the groups. A recent review of adjuvant therapy by Nara et al. found that Capecitabine (BILCAP trial) had a significant difference in survival compared to controls (20) and has since been adopted in various international treatment protocols. A limitation of the BILCAP trial was the failure to find a difference in survival during intention-to-treat analysis. Furthermore, in a separate group of patients with iCCA, chemoembolization using doxorubicin was performed. Previous studies have commented on the acceptable disease control afforded by this therapy and its efficacy as palliative, rather than curative, therapy (21–23).

This study has limitations. It is a retrospective study and due to the long follow-up period, there existed some variation in the data that was collected. These factors limited the scope of detailed analysis involving the entire cohort. There was a lack of central review of pre-transplant imaging and explant histology, and therefore it was difficult to comment on the presence of concomitant HCC alongside cholangiocarcinoma- survival analysis stratified by HCC was excluded as a result. Furthermore, our data is unable to comment on the rate of misdiagnosed HCC on pre-transplant evaluation that was later diagnosed as cholangiocarcinoma on explant. Due to the evolution of staging criteria, patients with earlier transplants had missing staging characteristics and therefore were unable to be included in stratified analysis. Criteria for staging also differed across different sites of CCA- iCCA classification includes measurements of tumour size whereas pCCA classification is based on spread past the bile duct. Additionally, our study was unable to comment on adjuvant chemotherapy or pre-operative ablation performed on patients, and data regarding imaging and imaging characteristics was incomplete due to inconsistent records across hospital systems. Specific parameters like size of tumour, which have previously been used as grouping criteria in the literature, were missing from some cases (10). As a result of non-standardised follow-up protocol across centres, we were unable to comment on the recurrence and recurrence-free survival of CCA. Nevertheless, our cohort represents the largest group of patients with incidental CCA at explant reported in the literature.

In conclusion, our UK multicentre series of patients undergoing liver transplantation, who were found to have CCA on explant, showed improved survival in earlier stage disease (pT1) and in those with iCCA. The late stage of detection and adverse outcomes in the pCCA patients, particularly those with PSC, highlights the need for improved methods of detecting CCA at the time of transplant assessment and monitoring on the waiting list to avoid undertaking transplants with an anticipated poor outcome. However, these data encouragingly support a planned UK prospective service evaluation of liver transplantation in selected cases of early stage CCA.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

NS: writing and critical review of manuscript, analysis and critical review of data. AH: critical review of data analysis, writing and

critical review of manuscript. RF, FJ, LM, SM, JP, IR, SS, RJ, HS, NH, JB, and DT: data collection, critical review and contribution to manuscript. RjP: critical review and contribution to manuscript. RcP: writing and critical review of manuscript, critical review of data analysis, research design.

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

1. Genus T, Tataru D, Morement H, Toledano M, Khan S. (late Submission Poster) Incidence and Mortality Rates of Cholangiocarcinoma in England. *Ann Oncol* (2019) 30:iv155. doi:10.1093/annonc/mdz183.007
2. Burton A, Tataru D, Driver RJ, Bird TG, Huws D, Wallace D, et al. Primary Liver Cancer in the UK: Incidence, Incidence-Based Mortality, and Survival by Subtype, Sex, and Nation. *JHEP Rep* (2021) 3(2):100232. doi:10.1016/j.jhepr.2021.100232
3. Banales JM, Marin JJG, Lamarca A, Rodrigues PM, Khan SA, Roberts LR, et al. Cholangiocarcinoma 2020: the Next Horizon in Mechanisms and Management. *Nat Rev Gastroenterol Hepatol* (2020) 17(9):557–88. doi:10.1038/s41575-020-0310-z
4. Liu R, Cox K, Guthery SL, Book L, Witt B, Chadwick B, et al. Cholangiocarcinoma and High-Grade Dysplasia in Young Patients with Primary Sclerosing Cholangitis. *Dig Dis Sci* (2014) 59(9):2320–4. doi:10.1007/s10620-014-3152-0
5. Morris-Stiff G, Bhati C, Olliff S, Hübscher S, Gunson B, Mayer D, et al. Cholangiocarcinoma Complicating Primary Sclerosing Cholangitis: a 24-year Experience. *Dig Surg* (2008) 25(2):126–32. doi:10.1159/000128169
6. Palmer WC, Patel T. Are Common Factors Involved in the Pathogenesis of Primary Liver Cancers? A Meta-Analysis of Risk Factors for Intrahepatic Cholangiocarcinoma. *J Hepatol* (2012) 57(1):69–76. doi:10.1016/j.jhep.2012.02.022
7. Van Beers BE. Diagnosis of Cholangiocarcinoma. *HPB (Oxford)* (2008) 10(2):87–93. doi:10.1080/13651820801992716
8. Hann LE, Greatrex KV, Bach AM, Fong Y, Blumgart LH. Cholangiocarcinoma at the Hepatic Hilus: Sonographic Findings. *AJR Am J Roentgenol* (1997) 168(4):985–9. doi:10.2214/ajr.168.4.9124155
9. Meyer CG, Penn I, James L. Liver Transplantation for Cholangiocarcinoma: Results in 207 Patients. *Transplantation* (2000) 69(8):1633–7. doi:10.1097/00007890-200004270-00019
10. Sapisochin G, Facciuto M, Rubbia-Brandt L, Marti J, Mehta N, Yao FY, et al. Liver Transplantation for "Very Early" Intrahepatic Cholangiocarcinoma: International Retrospective Study Supporting a Prospective Assessment. *Hepatology* (2016) 64(4):1178–88. doi:10.1002/hep.28744
11. Heimbach JK, Gores GJ, Haddock MG, Alberts SR, Nyberg SL, Ishitani MB, et al. Liver Transplantation for Unresectable Perihilar Cholangiocarcinoma. *Semin Liver Dis* (2004) 24(2):201–7. doi:10.1055/s-2004-828896
12. Darwish Murad S, Kim WR, Harnois DM, Douglas DD, Burton J, Kulik LM, et al. Efficacy of Neoadjuvant Chemoradiation Followed by Liver Transplantation for Perihilar Cholangiocarcinoma at 12 US Centers. *Gastroenterology* (2012) 143:88. doi:10.1053/j.gastro.2012.04.008
13. Vugts JJA, Gaspersz MP, Roos E, Franken LC, Olthof PB, Coelen RJS, et al. Eligibility for Liver Transplantation in Patients with Perihilar Cholangiocarcinoma. *Ann Surg Oncol* (2021) 28(3):1483–92. doi:10.1245/s10434-020-09001-8
14. Amin MB, Greene FL, Edge SB, Compton CC, Gershenwald JE, Brookland RK, et al. The Eighth Edition AJCC Cancer Staging Manual: Continuing to Build a Bridge from a Population-Based to a More "Personalized" Approach to Cancer Staging. *CA Cancer J Clin* (2017) 67(2):93–9. doi:10.3322/caac.21388
15. Adam R, Karam V, Delvart V, O'Grady J, Mirza D, Klempnauer J, et al. Evolution of Indications and Results of Liver Transplantation in Europe. A Report from the European Liver Transplant Registry (ELTR). *J Hepatol* (2012) 57(3):675–88. doi:10.1016/j.jhep.2012.04.015
16. Friman S, Foss A, Isoniemi H, Olausson M, Höckerstedt K, Yamamoto S, et al. Liver Transplantation for Cholangiocarcinoma: Selection Is Essential for Acceptable Results. *Scand J Gastroenterol* (2011) 46(3):370–5. doi:10.3109/00365521.2010.533384
17. Rosen CB, Darwish Murad S, Heimbach JK, Nyberg SL, Nagorney DM, Gores GJ. Neoadjuvant Therapy and Liver Transplantation for Hilar Cholangiocarcinoma: Is Pretreatment Pathological Confirmation of Diagnosis Necessary? *J Am Coll Surg* (2012) 215(1):31–8. doi:10.1016/j.jamcollsurg.2012.03.014
18. Mantel HTJ, Westerkamp AC, Adam R, Bennet WF, Seehofer D, Settmacher U, et al. Strict Selection Alone of Patients Undergoing Liver Transplantation for Hilar Cholangiocarcinoma Is Associated with Improved Survival. *PLOS ONE* (2016) 11(6):e0156127. doi:10.1371/journal.pone.0156127
19. Cambridge WA, Fairfield C, Powell JJ, Harrison EM, Søreide K, Wigmore SJ, et al. Meta-analysis and Meta-Regression of Survival after Liver Transplantation for Unresectable Perihilar Cholangiocarcinoma. *Ann Surg* (2020) 273:240–50. doi:10.1097/SLA.0000000000003801
20. Nara S, Esaki M, Ban D, Takamoto T, Shimada K, Ioka T, et al. Adjuvant and Neoadjuvant Therapy for Biliary Tract Cancer: a Review of Clinical Trials. *Jpn J Clin Oncol* (2020) 50(12):1353–63. doi:10.1093/jjco/hyaa170
21. Aliberti C, Benea G, Tilli M, Fiorentini G. Chemoembolization (TACE) of Unresectable Intrahepatic Cholangiocarcinoma with Slow-Release Doxorubicin-Eluting Beads: Preliminary Results. *Cardiovasc Intervent Radiol* (2008) 31(5):883–8. doi:10.1007/s00270-008-9336-2
22. Kiefer MV, Albert M, McNally M, Robertson M, Sun W, Fraker D, et al. Chemoembolization of Intrahepatic Cholangiocarcinoma with Cisplatinum, Doxorubicin, Mitomycin C, Ethiodol, and Polyvinyl Alcohol: a 2-center Study. *Cancer* (2011) 117(7):1498–505. doi:10.1002/cncr.25625
23. Aliberti C, Carandina R, Sarti D, Pizzirani E, Ramondo G, Mulazzani L, et al. Chemoembolization with Drug-Eluting Microspheres Loaded with Doxorubicin for the Treatment of Cholangiocarcinoma. *Anticancer Res* (2017) 37(4):1859–63. doi:10.21873/anticanres.11522

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SYSTEMATIC REVIEW AND META-ANALYSIS
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Early-Phase Clinical Trials of Bio-Artificial Organ Technology: A Systematic Review of Ethical Issues

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Regenerative medicine has emerged as a novel alternative solution to organ failure which circumvents the issue of organ shortage. In preclinical research settings bio-artificial organs are being developed. It is anticipated that eventually it will be possible to launch first-in-human transplantation trials to test safety and efficacy in human recipients. In early-phase transplantation trials, however, research participants could be exposed to serious risks, such as toxicity, infections and tumorigenesis. So far, there is no ethical guidance for the safe and responsible design and conduct of early-phase clinical trials of bio-artificial organs. Therefore, research ethics review committees will need to look to related adjacent fields of research, including for example cell-based therapy, for guidance. In this systematic review, we examined the literature on early-phase clinical trials in these adjacent fields and undertook a thematic analysis of relevant ethical points to consider for early-phase clinical trials of transplantable bio-artificial organs. Six themes were identified: cell source, risk-benefit assessment, patient selection, trial design, informed consent, and oversight and accountability. Further empirical research is needed to provide insight in patient perspectives, as this may serve as valuable input in determining the conditions for ethically responsible and acceptable early clinical development of bio-artificial organs.

Keywords: ethics, regenerative medicine, bioengineering, research ethics, first-in-human clinical trials, bio-artificial organs, clinical trials, early-phase clinical trials

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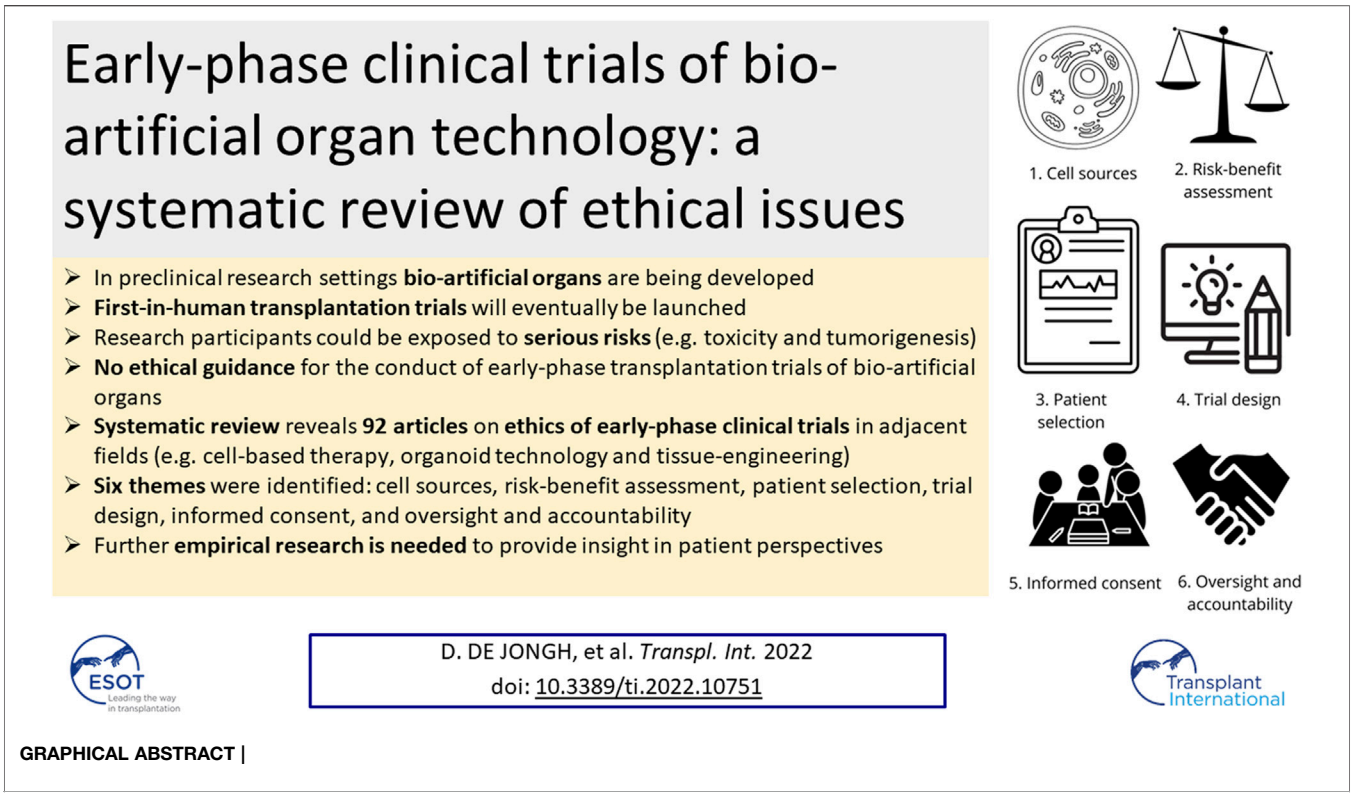
INTRODUCTION

For patients with end-stage organ failure, having an organ transplant is often the best and only cure. Advances in surgical techniques and immunosuppressive medication means that organ transplantation is now widely and successfully used. However, there are still important challenges to overcome, notably the shortage of donor organs and the short and long-term side effects of taking lifelong immunosuppressive medication.

In the last decade, the multi-disciplinary field of regenerative medicine has emerged. Regenerative medicine uses technologies such as tissue engineering and 3D bioprinting to (re)generate, repair or replace damaged tissues and organs. Regenerative medicine and tissue engineering are terms often used interchangeably in the scientific literature. In this article however we use the term regenerative medicine to refer to the aim of the intervention (to regenerate), and tissue engineering to refer to the



GRAPHICAL ABSTRACT |

method for creating regenerative products. Regenerative medicine could, by way of illustration, combine patient-derived cells (e.g., in the form of organoids made from induced pluripotent stem cells) with cutting-edge technologies such as tissue engineering, to develop transplantable personalized bio-artificial organs. For example, the European Commission-funded VANGUARD project aims to engineer a vascularized and immune-protected bio-artificial pancreas for transplantation into patients with Type I Diabetes. The ambition of the VANGUARD project[1] is for the transplanted bio-artificial pancreas to produce insulin and treat the underlying diabetic disease without requiring the patient to take lifelong immunosuppressive medication. Similarly, in other disease areas, first steps are being taken towards the generation of transplantable bio-artificial organs, including livers (1), bladders (2), kidneys (3), hearts (4), small intestines (5) and lungs (6, 7). These bio-artificial organs are currently still at the preclinical stage and are being tested in laboratory settings or animal studies.

It is likely that researchers will reach a point at which sufficient preclinical evidence has been collected to suggest that bio-artificial organs might be beneficial and safe for humans. At that point, early-phase clinical trials will be initiated to test the safety and efficacy of these products in humans. In early-phase clinical trials, human research participants could be exposed to serious risks, such as toxicity, infections and tumorigenesis. This is especially so in regenerative medicine trials requiring invasive and non-reversible procedures, resulting in permanent alterations of participants' bodies (8).

It is not clear to what extent existing ethics oversight and guidance for the conduct of clinical trials is applicable to or sufficient for the clinical translation of bio-artificial organs. First, drug authorities, including the US Food and Drug Administration (FDA) and the European Medicines Agency (EMA), were originally set up to decide on marketing authorisation of *pharmaceutical agents*, not complex cell-based products. In Europe, bio-artificial organs are likely to be classified as Advanced Therapy Medicinal Products (ATMPs) (9), just like cell-based therapies. However, this classification may not completely cover the bio-artificial organ as, unlike most pharmaceutical agents, it is not a substance that can be injected or infused, but a complex product—more like a (cell-based) device—to be used in transplantation, which involves a (innovative) surgical intervention. Second, while there are internationally recognised guidelines for the ethical conduct of research involving human subjects, issued for instance by the Council for international Organization of Medical Science (CIOMS) (10) and the World Medical Association (WMA) (11), these guidelines should be expanded in order to make them applicable to the clinical translation of bio-artificial organs. The ethics guidelines of the International Society for Stem Cell Research (ISSCR) have been developed specifically for human stem cell research and clinical translation of cell-based interventions (12), but do not discuss applications of regenerative medicine in *organ transplantation*. Without the relevant guidance,

[1]VANGUARD. New generation cell therapy: bioartificial pancreas to cure type 1 diabetes. https://vanguard-project.eu/ (Accessed 1 July 2022).

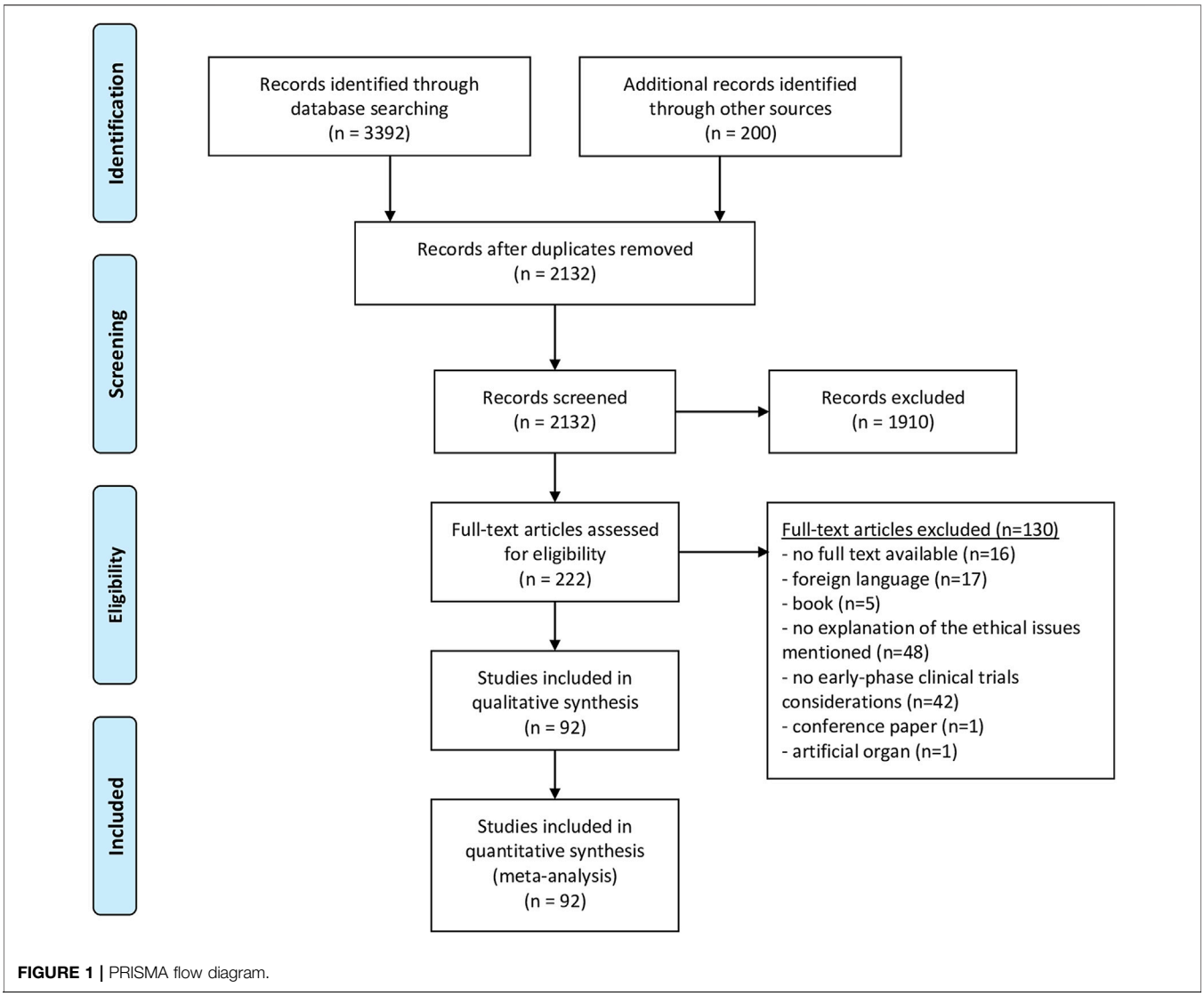


FIGURE 1 | PRISMA flow diagram.

it would be difficult for research ethics review committees (RECs) to evaluate the ethical acceptability of early-phase clinical trials of bio-artificial organs. Therefore, guidance on the safe and responsible design and conduct of early-phase clinical trials of transplantable bio-artificial organs should be developed.

In this systematic review we examined the published literature on early-phase clinical trials in the adjacent fields of regenerative medicine, including tissue-engineering, 3D bioprinting, cell-based therapy, organoid technology and synthetic biology. We undertook a thematic analysis of relevant ethical points to consider for early-phase clinical trials of transplantable bio-artificial organs. The results of our systematic review and thematic analysis will be valuable for researchers, research ethics review boards, policy makers and clinicians with an interest in regenerative medicine and involved in the translation of bio-artificial organs for clinical transplantation. However, above we hope our analysis will contribute to the preparation of robust guidelines and recommendations in this highly complex and evolving field.

TABLE 1 | Inclusion and exclusion criteria.

Inclusion criteria	Exclusion criteria
Articles in the fields of regenerative medicine, tissue-engineering, 3D printing, cell-based therapy, organoid technology, synthetic biology and bio-artificial organs describing ethical points to consider (issues, questions, or challenges) for early-phase clinical trials	Letters to the editor Editorials Opinion articles Non-biological medical devices Engineering a specific tissue only for research purpose Describing ethical issues associated with pre-clinical research only

METHODS

We performed a systematic review of the literature, following the PRISMA statement, as far as applicable (see **Supplementary Materials**). The review protocol has not been published or registered. The authors (DJ, EB and EM) developed the search strategy in consultation with a university librarian. We conducted

TABLE 2 | Included articles.

Author	Title	Year	Journal	Research field
Aalto-Setälä et al.	Obtaining consent for future research with induced pluripotent cells: opportunities and challenges	2009	PLoS Biology	Cell-Based Therapy
Afshar et al.	Ethics of research on stem cells and regenerative medicine: ethical guidelines in the Islamic Republic of Iran	2020	Stem Cell Research & Therapy	Regenerative Medicine
No Author	European Medicines Agency, CAR Secretariat and US Food and Drug Administration	2011	Regenerative Medicine	Cell-Based Therapy
Apatoff et al.	Autologous stem cell therapy for inherited and acquired retinal disease	2017	Regenerative Medicine	Cell-Based Therapy
Attico et al.	Approaches for effective clinical application of stem cell transplantation	2018	Current Transplantation Reports	Cell-Based Therapy
Baker et al.	Ethical considerations in Tissue Engineering Research: case Studies in Translation	2016	Methods	Tissue Engineering
Bhangra et al.	Using Stem Cells to Grow Artificial Tissue for Peripheral Nerve Repair	2016	Stem Cells International	Cell-Based Therapy
Bliss et al.	Optimizing the Success of Cell Transplantation Therapy for stroke	2010	Neurobiology of Disease	Cell-Based Therapy
Bobba et al.	The current state of stem cell therapy for ocular disease	2018	Experimental Eye Research	Cell-Based Therapy
Bredenoord et al.	Human tissues in a dish: The research and ethical implication of organoid technology	2017	Science	Organoid Transplantation
Brignier et al.	Embryonic and adult stem cell therapy	2010	Journal of Allergy and Clinical Immunology	Cell-Based Therapy
Chan.	Current and emerging global themes in the bioethics of regenerative medicine: the tangled web of stem cell translation	2017	Regenerative Medicine	Cell-Based Therapy
Chan.	Research Translation and Emerging Health Technologies: Synthetic Biology and Beyond	2018	Health Care Anal	Synthetic Biology
Chung	Stem-cell-based Therapy in the field of urology: a review of stem cell basic science, clinical application and future directions in the treatment of various sexual and urinary conditions	2015	Expert Opinion in Biological Therapy	Cell-Based Therapy
Coombe et al.	Current approaches in regenerative medicine for the treatment of diabetes: introducing CRISPR/CAS9 technology and the case for non-embryonic stem cell therapy	2018	American Journal Stem Cells	Cell-Based Therapy
Court et al.	Bioartificial liver support devices: historical perspectives	2003	ANZ Journal of Surgery	Bioengineered Organs
Daley et al.	Setting Global Standards for Stem Cell Research and Clinical Translation: The 2016 ISSCR Guidelines	2016	Stem Cell Reports	Cell-Based Therapy
Davis et al.	The role of Stem Cells for Reconstructing the Lower Urinary Tracts	2018	Current Stem cell Research & Therapy	Cell-Based Therapy
Davidson.	Brave Pioneers or Clinical Cowboys?	2010	Cell Stem Cell	Cell-Based Therapy
De Vries et al.	Ethical Aspects of Tissue Engineering: A Review	2008	Tissue engineering	Tissue Engineering
De Windt et al.	Ethics in musculoskeletal regenerative medicine; guidance in choosing the appropriate comparator in clinical trials	2019	Osteoarthritis and Cartilage	Regenerative Medicine
Fears et al.	Inclusivity and diversity: Integrating international perspectives on stem cell challenges and potential	2021	Stem Cell Reports	Cell-Based Therapy
Fung et al.	Responsible Translation of Stem Cell Research: An Assessment of Clinical Trial Registration and Publications	2017	Stem Cell Reports	Cell-Based Therapy
Garg et al.	Stem Cell Therapies in Retinal Disorders	2017	Cells	Cell-Based Therapy
Genske et al.	Rethinking risk assessment for emerging technology first-in-human trials	2016	Medicine, Health Care and Philosophy	Synthetic Biology
Giancola et al.	Cell therapy: cGMP Facilities and manufacturing	2012	Muscles, Ligaments and Tendons Journal	Cell-Based Therapy
Gilbert et al.	Print Me an Organ? Ethical and Regulatory Issues Emerging from 3D Bioprinting in Medicine	2018	Science and Engineering Ethics	3D Bioprinting
Goula et al.	Advanced Therapy Medicinal Products Challenges and Perspectives in Regenerative Medicine	2020	Journal of Clinical Medicine Research	Regenerative Medicine
Haake et al.	Concise Review: Towards the Clinical Translation of Induced Pluripotent Stem Cell-Derived Blood Cells- *Ready for Take-Off*	2019	Stem Cells Translational Medicine	Cell-Based Therapy
Habets et al.	The inherent ethical challenge of first-in-human pluripotent stem cell trials	2014	Regenerative Medicine	Cell-Based Therapy
Hara et al.	New Governmental Regulatory System for Stem Cell-Based Therapies in Japan	2014	Therapeutic Innovation & Regulatory Science	Cell-Based Therapy
Hayakawa et al.	A study on ensuring the quality and safety of pharmaceuticals and medical devices derived from the processing of allogeneic human somatic stem cells	2015	Regenerative Therapy	Cell-Based Therapy
Hildebrandt	Horses for courses: an approach to the qualification of clinical trial sites and investigators in ATMPs	2020	Drug Discovery Today	Cell-Based Therapy
Hug	Understanding voluntariness of consent in first-in-human cell therapy trials	2020	Regenerative Medicine	Cell-Based Therapy
Hyun	Allowing innovative Stem Cell-Based Therapies Outside of Clinical Trials: Ethical and Policy Challenges	2010	Journal of Law, Medicine and Ethics	Cell-Based Therapy

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TABLE 2 | (*Continued*) Included articles.

Author	Title	Year	Journal	Research field
Hyun et al.	New ISSCR Guidelines Underscore Major Principles for Responsible Translational Stem Cell Research	2008	Cell Stem Cell	Cell-Based Therapy
Kim et al.	Report of the International Stem Cell Banking Initiative Workshop Activity: Current Hurdles and Progress in Seed-Stock Banking of Human Pluripotent Stem cells	2017	Stem Cells Translational Medicine	Cell-Based Therapy
King et al.	Ethical issues in stem cell research and therapy	2014	Stem Cell Research & Therapy	Cell-Based Therapy
Kleiderman et al.	Overcoming barriers to facilitate the regulation of multi-centre regenerative medicine clinical trials	2018	Stem Cell Research & Therapy	Regenerative Medicine
Knoepfler	From Bench to FDA to Bedside: US Regulatory Trends for New Stem Cell Therapies	2015	Advanced Drug Delivery Reviews	Cell-Based Therapy
Kusunose et al.	Informed consent in clinical trials using stem cells: Suggestions and points of attention from informed consent training workshops in Japan	2015	South African Journal of Bioethics and Law	Cell-Based Therapy
Lederer et al.	Neural stem cells: mechanisms of fate specification and nuclear reprogramming in regenerative medicine	2008	Biotechnology Journal	Cell-Based Therapy
Lee et al.	Conditional approvals for autologous stem cell-based interventions	2018	Perspectives in Biology and Medicine	Cell-Based Therapy
Levin et al.	Special Commentary: early Clinical Development of Cell Replacement Therapy: Considerations for the National Eye Institute Audacious Goals Initiative	2017	Ophthalmology	Cell-Based Therapy
Lim et al.	Whole Organ and Tissue Reconstruction in Thoracic Regenerative Surgery	2013	Mayo clinic Proceedings	Tissue Engineering
Liras	Future research and therapeutic applications of human stem cells: general, regulatory, and bioethical aspects	2010	Journal of translational Medicine	Cell-Based Therapy
Liu et al.	Advances in Pluripotent Stem Cells: History, Mechanisms, Technologies, And Applications§	2020	Stem Cell Reviews and Reports	Cell-Based Therapy
Lomax et al.	Return of results in translational iPS cell research: considerations for donor informed consent	2013	Stem Cell Research & Therapy	Cell-Based Therapy
Lomax et al.	Regulated, reliable and reputable: Protect patients with uniform standards for stem cell treatments	2020	Stem Cells Translational Medicine	Cell-Based Therapy
Lowenthal et al.	Specimen Collection for Induced Pluripotent Stem Cell Research: Harmonizing the Approach to Informed Consent	2012	Stem Cells Translational Medicine	Cell-Based Therapy
Lowenthal et al.	Ethics and Policy Issues for Stem Cell Research and Pulmonary Medicine	2014	Chest	Cell-Based Therapy
Lu et al.	Tissue Engineered Constructs: Perspectives on Clinical Translation	2015	Annals of Biomedical Engineering	Tissue Engineering
Madariaga et al.	Bioengineering Kidneys for Transplantation	2014	Seminars in Nephrology	Bioengineered Organs
Maekawa et al.	Development of Novel Advanced Cell and Gene Therapy and GMP-Controlled Cell Processing	2005	Japan Medical Association journal	Cell-Based Therapy
Main et al.	Managing the potential and pitfalls during clinical translation of emerging stem cell therapies	2014	Clinical and Translational Medicine	Cell-Based Therapy
Masuda et al.	New Challenges for Intervertebral Disc Treatment Using Regenerative Medicine	2010	Tissue engineering	Regenerative Medicine
Moradi et al.	Research and therapy with induced pluripotent stem cells (iPSCs): Social, legal and ethical considerations	2019	Stem Cell Research & Therapy	Cell-Based Therapy
Nagamura	The Importance of Recruiting a Diverse Population for Stem Cell Clinical Trials	2016	Current Stem Cell Reports	Cell-Based Therapy
Naghieh et al.	Biofabrication Strategies for Musculoskeletal Disorders: Evolution towards Clinical Application	2021	Bioengineering	3D Bioprinting
Nagpal et al.	PERSPECTIVES: Stroke survivors' views on the design of an early-phase cell therapy trial for patients with chronic ischaemic stroke	2019	Health Expectations	Cell-Based Therapy
Neri	Genetic Stability of Mesenchymal Stromal Cells for Regenerative Medicine Applications: A Fundamental Biosafety Aspect	2019	International Journal of Molecular Sciences	Cell-Based Therapy
Niemansburg et al.	Participant selection for preventive Regenerative Medicine trials: ethical challenges of selecting individuals at risk	2015	Journal of Medical ethics	Regenerative Medicine
Niemansburg et al.	Regenerative medicine interventions for orthopedic disorders: ethical issues in the translation into patient	2013	Regenerative Medicine	Regenerative Medicine
Niemansburg et al.	Ethical implications of regenerative medicine in orthopedics: an empirical study with surgeons and scientists in the field	2014	The spine Journal	Regenerative Medicine
O'Donnell et al.	Beyond the Present Constraints That Prevent a Wide Spread of Tissue Engineering and Regenerative Medicine Approaches	2019	Frontiers Bioengineering and Biotechnology	Regenerative Medicine
Oerlemans et al.	Regenerative Urology Clinical Trials: An Ethical Assessment of Road Blocks and Solution	2013	Tissue engineering	Tissue Engineering
Oerlemans et al.	Towards a Richer Debate on Tissue Engineering: A Consideration on the Basis of NEST-Ethics	2012	Science Engineering Ethics	Tissue Engineering

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TABLE 2 | (*Continued*) Included articles.

Author	Title	Year	Journal	Research field
O'Keefe	American Society for Bone and Mineral Research- Orthopaedic Research Society Joint Task Force Report on Cell-Based Therapies	2020	Journal of Bone and Mineral Research	Cell-Based Therapy
Otto et al.	Ethical considerations in the translation of biofabrication technologies into clinic and society	2016	Biofabrication	3D Bioprinting
Parent et al.	The ethics of testing and research of manufactured organs on brain-dead/recently deceased subjects	2019	Journal of Medical Ethics	Bioengineered Organs
Patuzzo et al.	3D bioprinting Technology: Scientific Aspects and Ethical Issues	2018	Science and Engineering Ethics	3D Bioprinting
Schneemann et al.	Ethical challenges for pediatric liver organoid transplantation	2020	Science Translational Medicine	Organoid Transplantation
Scopetti et al.	Mesenchymal stem cells in neurodegenerative diseases: Opinion review on ethical dilemmas	2020	World Journal of Stem Cells	Cell-Based Therapy
Sekar et al.	Current standards and ethical landscape of engineered issues—3D bioprinting perspective	2021	Journal of Tissue Engineering	3D Bioprinting
Seok et al.	A Personalized 3D-Printed Model for Obtaining Informed Consent Process for Thyroid Surgery: A Randomized Clinical Study Using a Deep Learning Approach with Mesh-Type 3D Modeling	2021	Journal of Personalized Medicine	3D Bioprinting
Shineha et al.	A Comparative Analysis of Attitudes on Communication Toward Stem Cell Research and Regenerative Medicine Between the Public and the Scientific Community	2018	Stem Cells Translational Medicine	Regenerative Medicine
Sievert et al.	Tissue Engineering for the Lower Urinary Tract: A Review of a State of the Art Approach	2007	European Urology	Tissue Engineering
Smith et al.	Challenging misinformation and engaging patients: characterizing a regenerative medicine consult service	2020	Regenerative Medicine	Regenerative Medicine
Sniecinski et al.	Emerging stem cell based strategies for treatment of childhood disease	2018	Transfusion and Apheresis Science	Cell-Based Therapy
Stegemann et al.	Cell therapy for bone repair: narrowing the gap between vision and practice	2014	European Cells and Materials	Cell-based therapy
Sugarman and Bredenoord	Real-time ethics engagement in biomedical research	2020	EMBO reports	Organoid transplantation
Sutherland and Mayer	Ethical and Regulatory Issues Concerning Engineered Tissues for Congenital Heart Repair	2003	Thoracic and Cardiovascular Surgery	Tissue Engineering
Takashima et al.	Lessons for reviewing clinical trials using induced pluripotent stem cells: examining the case of a first-in-human trial for age-related macular degeneration	2018	Regenerative Medicine	Cell-Based Therapy
Taylor et al.	Ethics of bioengineering organs and tissues	2014	Expert Opinion on Biological Therapy	Tissue Engineering
Trommelmans et al.	Ethical reflections on clinical trials with human tissue engineered products	2008	Journal of Medical Ethics	Tissue Engineering
Trommelmans et al.	Informing participants in clinical trials with *ex vivo* human tissue-engineered products: what to tell and how to tell it?	2008	Journal Tissue Engineering Regenerative Medicine	Tissue Engineering
Trommelmans et al.	An Exploratory Survey on the Views of European Tissue Engineers Concerning the Ethical Issues of Tissue Engineering Research	2009	Tissue Engineering	Tissue Engineering
Trommelmans et al.	Is tissue engineering a new paradigm in medicine? Consequences for the ethical evaluation of tissue engineering research	2009	Medical Health Care and Philosophy	Tissue Engineering
Tsang	Legal and ethical status of stem cells as medicinal products	2005	Advanced Drug Delivery	Cell-Based Therapy
Vijayavenkataraman et al.	3D bioprinting - An Ethical, Legal and Social Aspects (ELSA) framework	2016	Bioprinting	3D Bioprinting
Zamborsky et al.	Regenerative Medicine in Orthopaedics and Trauma: Challenges, Regulation and Ethical Issues	2018	Orthopaedics and Trauma	Cell-Based Therapy
Zocchi et al.	Regulatory, ethical, and technical considerations on regenerative technologies and adipose-derived mesenchymal stem cells	2019	European Journal of Plastic Surgery	Regenerative Medicine

[a]*Author name stated in **bold**: ethical considerations for early-phase regenerative trials are elaborately discussed in the paper.*

the literature search in September 2021, using seven scientific databases: PubMed, EMBASE, Medline, Web of Science Core Collection, Cochrane Central Register of Controlled Trials and PsycINFO. An additional systematic search of the grey literature (i.e., relevant literature published outside of commercial or academic publishing) was conducted in Google Scholar. Search strings were constructed by keywords and their truncation, and relevant database-specific subjects headings [MeSH terms] (see **Supplementary Materials**). Due to language barriers, only articles in English or Dutch were considered for full-text analysis. We screened all titles and abstracts until September 2021 with no restriction for date of publication. Only outdated research guidelines that have subsequently been updated were not included. Based on title and abstract, articles that fulfilled the inclusion criteria were selected. Two researches independently carried out the selection (DJ and EB). Articles were discussed in case of differences between DJ and EB in the selection to come to a consensus. Full-texts were screened by DJ. The articles that did

FIGURE 2 | Summary of the content of the article.

not meet the inclusion criteria during full-text screening, were excluded. Finally, the reference lists of the articles selected for full-text screening were checked for scientific articles or other documents that may be relevant and included if inclusion criteria were fulfilled (by DJ) (see **Figure 1**).

Inclusion and Exclusion Criteria

The inclusion criteria of this systematic review were as follows: articles in the adjacent fields of regenerative medicine, tissue-engineering, 3D bioprinting, cell-based therapy, organoid technology, synthetic biology, and bio-artificial organs describing ethical points to consider (issues, questions or challenges) for early-phase clinical trials. Letters to the editor, editorials and opinion articles were included as non-research manuscripts. Articles that only discussed pre-clinical research were excluded from our sample. For reasons of feasibility, articles discussing transplantation of non-biological medical devices instead of biological materials (e.g., pacemakers, blood glucose monitors, insulin pumps, or cardioverter defibrators) and articles discussing engineering of specific tissues for purposes other than organ transplantation (e.g., engineering of brains and reproductive organs for research purposes) were excluded. Finally, conference abstracts and articles were excluded (**Table 1**).

Analyses and Syntheses

The method of qualitative content analysis was employed (13). Qualitative content analysis is an inductive (bottom-up) approach to categorize ethical considerations and to develop themes within a coding frame. One researcher (DJ) conducted the analyses. Firstly, codes were assigned to all the considerations mentioned in each publication. Secondly, themes (e.g., patient selection) were created out of these codes by DJ. Thirdly, DJ, EM and EB discussed whether the created words describing the themes were representative of the codes until agreement was reached. Finally, a coding framework was built out of the identified themes. The coding framework was used to systematically keep track of ethical considerations mentioned per article.

Qualitative Content Analysis

We did not conduct a quality appraisal procedure, as there are no suitable criteria for appraisal of the quality of the literature included. This is a well-documented limitation of systematic reviews of (bio) ethical literature (14, 15).

RESULTS

The selection procedure is presented in a PRISMA Flow diagram (**Figure 1**). The search produced 2132 hits, of which 222 were deemed eligible on the basis of title and abstract, and 92 articles were included after reference checking and full-text screening. The publication dates ranged from January 2003 to March 2021 (**Table 2**).

Themes

Six themes were identified: cell source, risk-benefit assessment, patient selection, trial design, informed consent, and oversight and accountability. The content of the article referring to the six identified ethical themes is summarized in **Figure 2**.

Research Fields

These six themes were found in seven different research fields (**Table 2**). The largest body of literature focusses on ethical considerations around early-phase trials in the field of cell-based therapy; 55 articles are published in this field, and the authoritative ISSCR guidelines are widely used (12, 16–26). There

TABLE 3 | Points to consider in relation to cell sources.

Cell source	Risks and benefits	Points to consider
Xenogeneic cells or tissue	Medical risks: Risk of zoonoses Individuals could object to use cells derived from animals on religious or socio-cultural grounds	- The use of animal cells should be minimized - Components of animal origin should be replaced with human or chemically defined components whenever possible - The use of viral transcription factor genes, retroviruses or pathogenic agents should be minimized - Quality control systems, standard operating procedures (SOPs) and Good Manufacturing Practice (GMP) should be used
Autologous cells	Medical benefits: No immunological rejection	- It may not be possible to harvest sufficient numbers of patients' cells - The production cost could be high - The timeframe for cell harvest could be insufficient for timely treatment - Extra surgical interventions for participants could be necessary - Quality control systems, SOPs and GMP should be used
Allogeneic donor cells	Medical risks: Immunological rejection and disease transmission Relational issues: Ownership and privacy issues Some donors may not want their cells to become an integral, growing part of another person.	- Adequate donor consent should be obtained in a process that includes discussion of: aim of the research, return of research results, incidental findings, possibilities for withdrawal of consent, potential future research - Additional safeguards should be adopted to protect personal data - A policy should be developed on whether and how incidental findings of donor cell (genetic) screening should be returned to the cell donors and/or their relatives - Records on medical and family history of the donor of the cells should be obtained periodically - Quality control systems, SOPs and GMP should be used
Highly manipulated and/or genetic modified cells	Medical risks: Unexpected behavior of cells or tissue (e.g., tumor formation, epigenetic or genetic instability)	- Strong pre-clinical data (of the safety and functions of the cells and or tissues) should be provided - The use of manipulated cells should be minimized - Participants should be monitored for a long time - Researchers should adhere to cell processing and manufacturing protocols - Quality control systems, SOPs and GMP should be used

is less literature on ethical aspects of early-phase clinical trials in the field of 3D bioprinting, and organoid transplantation; seven articles were published on 3D bioprinting, three articles on bio-artificial organs, and two on organoid transplantation. Six empirical studies using questionnaires and interviews to investigate patients' and professionals' views on ethical considerations in early-phase clinical trials, were included. Seven papers were published in surgical journals.

Theme 1: Cell Sources

53 out of 92 articles mention ethical considerations related to the sources of cells used to generate complex tissue-engineered products such as bio-artificial or 3D bio-printed organs for transplantation into humans (9, 12, 16–24, 26–68). There are four types of cell sources: 1) xenogeneic cells, 2) autologous, 3) allogeneic donor, and 4) highly manipulated or/and genetically modified cells in humans, each with their own sets of ethical considerations (**Table 3**).

Firstly, xenogeneic cells are associated with a risk of zoonosis (17, 20, 38, 47–49). For instance, issues related to the transmission of the infectious porcine retrovirus (PERV) from pig to human (69). Potential future patients could also reject the use of these cells to generate bio-artificial organs on religious grounds or for socio-cultural reasons (e.g., to protect animal rights/welfare) (33, 38, 48, 50, 52), even if their religious leaders take a more moderate stance (33). According to the literature, using these cells for transplantation into humans should be minimized as much as possible (12, 17, 38).

Secondly, the use of autologous cells (cells taken from the patient, who is both the donor and recipient) will make immunosuppressive therapy unnecessary (9, 16, 27–29, 33, 38–45, 68), and is perceived to carry fewer risks than the use of other cell types (33). However, challenges include the high production costs (29, 57, 70), extra surgical interventions for participants (50), the time required for their production (29, 40, 50, 57, 70), and the difficulty of standardizing manufacturing procedures (4057, 70).

Thirdly, besides the medical risks of transplanting allogeneic donor cell (cells taken from another human being), for example developing immunological problems, use of these cells also raises relational issues (20, 27, 30, 38, 41, 43, 63, 71, 72). Relational issues include questions such as: Who is the owner of the human cells once it is separated from the body (30,38,41,43)?; Can cells from the human body be subjected to laws regarding property rights (38,43)?, and; To what extent can the donor's privacy and confidentiality be ensured by adopting additional measures (e.g., pseudonymisation) (20, 27, 30, 38, 41, 43, 63, 71, 72). Removing the donor's personal information is

often not desirable, because subsequent research may necessitate ongoing access to the information about the cell donor's health status requiring personal data of the donor (e.g., their name and/or address) (20,52). Further, some donors may not want their cells to become an integral, growing part of another person (12, 20, 32, 52, 73). In addition, in the course of donor cell (genetic) screening, researchers should develop a policy on whether and how incidental findings (e.g., genetic risk) will be returned to the donors and/or their relatives (12, 20, 52, 63). Donors might consider their privacy violated if scientists know their future susceptibility to genetic disorders (52). Researchers should obtain an adequate informed consent from donors to respect their autonomy (12, 20, 22, 27, 28, 34, 38, 43, 45, 52, 57, 63, 67, 72–76), and give them some degree of insight and perhaps control over the use of donated materials by informing them about the types of incidental findings they wish to receive, future commercial applications, individualized research and therapeutic uses (12, 20, 27, 38, 43, 52, 72, 76), for instance by maintaining an ongoing dialogue with the donors (76). Moreover, to safeguard the health of the recipient over the years, it may be necessary to periodically obtain records on the medical and family history of the cell donor to monitor potential health risks, such as long-term immunological or tumorigenic reactions (12, 19, 20, 22, 27, 28, 32, 34, 35, 39, 41, 49, 51–53).

Lastly, the use of highly manipulated cells (i.e., cells of which the biological nature or structural function has been altered during the manufacturing process) and/or genetically modified cells raises safety concerns, and requires more quality controls to avoid undesired events (9, 12, 18, 20–23, 27, 28, 33, 35, 40, 50, 61, 63). For instance, these cells could have an increased risk of being tumorigenic, genetically unstable or toxic (12, 18, 35). Therefore, some authors recommend avoiding the use of manipulated cells whenever possible (e.g., tumor formation, epigenetic or genetic instability) (9, 12, 18, 20, 22). However, cell manipulation and/or genetic modification might be useful and even necessary for the generation of a bio-artificial organ (e.g., to repair disease-causing mutations) (20). Cells used in tissue-engineered products are often differentiated *in vitro* prior to being combined with a scaffolding material, for example collagen, to form artificial tissue, therefore tissue-engineered products are mostly classified as more than minimally manipulated (18).

Theme 2: Risk-Benefit Assessment

One of the conditions for ethically responsible clinical research is a favorable risk-benefit ratio (**Table 4**). This means that the risks and burdens of trial participation must be outweighed by the expected scientific or social value and the (potential) benefits

TABLE 4 | Points to consider in relation to risk-benefit assessment.

Points to consider in relation to risk-benefit assessment
- Researchers should provide robust pre-clinical data (i.e. safety and efficacy of the product should be rigorously demonstrated in laboratory tests and animal models)
- Personalization of the bio-artificial organ makes the product variable; therefore, the quality control and safety requirements of mass manufacturing do not apply
- Researchers should monitor and follow up participants for a long time after the study
- Efforts should be made not only to minimize the risks, but also to maximize the scientific and social value of a trial, in order to improve the risk-benefit ratio
- Clinical teams who conduct clinical trials of bioartificial organs should have experience with regenerative medicine technologies and with post-trial follow-up care

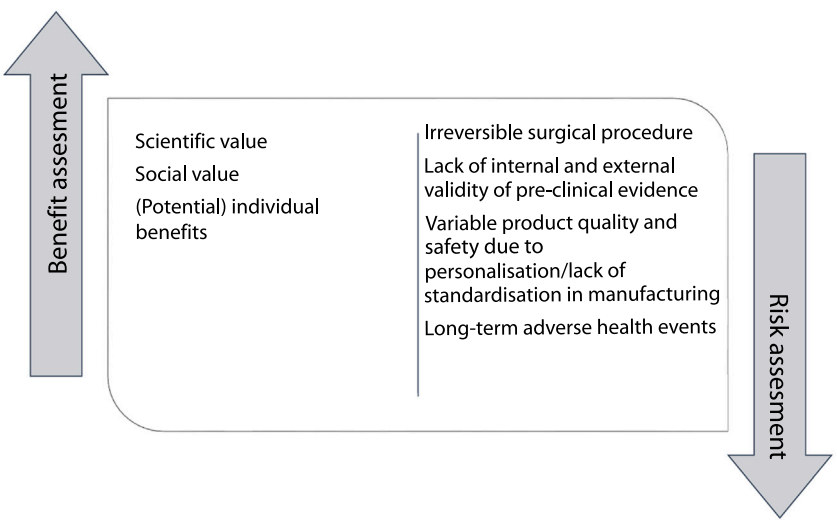

FIGURE 3 | Risk-benefit assessment.

for individual participants (12, 16, 21, 23, 24, 28, 29, 32, 34, 37, 45, 50, 53, 57, 64, 66–69, 77, 78) (**Figure 3**). The requirement of a favorable risk-benefit is difficult to meet in early-phase research, because the potential direct benefits to individual research participants in these trials are limited and uncertain (69). In the absence of direct medical benefit, justification of exposing individual research participants to potential harms in early-phase clinical trials is sought in expected scientific and/or social value (24, 30, 50, 66, 79). These include the benefits gained for science and society: generalizable knowledge and health gains for future patients (50). Knowledge of the working mechanism and the interaction of a regenerative medicine technology with the body, gathered in early-phase clinical trials, is necessary to move these technologies to the next clinical phase of clinical development (24, 30, 50, 66, 69). The anticipated social value of bio-artificial organs is potentially high, as they are intended as cures for patients with end-stage organ failure and might be more cost-effective than existing organ replacement therapies (66). At this stage, however, the social value is highly uncertain.

Transplanting regenerative medicine into human recipients requires an irreversible (innovative) surgical procedure, which is associated with risks of harms and complications. Once the regenerative product is implanted in the body, it may not be possible to completely remove it (50). For instance, surgical removal of the product will be impractical or associated with greater risks [i.e., infections or complications of anesthesia (33)], and there will be some irreversible changes, such as scarring (50, 70). In addition, unlike non-biological medical devices, the regenerative product will most likely interact and integrate with the rest of the body, which may have uncertain, possibly unforeseeable long-term adverse health events for the recipient (16, 18, 21, 23, 24, 27, 28, 31–34, 37–40, 48, 50, 58, 62, 66–70, 72, 73, 77, 79–86).

When researchers are dealing with uncertain but potentially high risks, they are advised, before undertaking an early-phase clinical trial, to provide preclinical evidence of high internal validity (e.g., through replication) and external validity (e.g. through careful study design) (12, 16, 23, 27–29, 31, 34–37, 43, 46, 49–51, 53, 57, 59, 61, 62, 64–69, 77, 79–81, 84, 85, 87–90). Some argue that large animals should be used, because these animals can better imitate the human anatomy and/or pathology than small animals (1281). Others recommend to involve unbiased third parties to repeat some of the research (69). Even if robust preclinical evidence is available using these strategies, some unexpected risk will inevitably remain, such as unforeseeable long-term adverse health events for the recipient. Researchers should be aware that preclinical evidence from animal models may not correctly predict the duration, function and interaction that occur in a human body (16, 24, 27, 31, 34, 37, 39, 50, 65, 68, 79–82). In addition, the personalization of regenerative medicine makes the product variable, therefore, the quality control and safety requirements of mass manufacturing for external validity do not apply (32, 34, 35, 48). A major benefit of personalization, however, is that it may take away or reduce the need for the use of life-long immunosuppressive therapy for recipients, and avoid well-known side effects such as infections and nephropathy (45, 69).

To detect health risks associated with potential long-term adverse events, such as genetic instability, undirected or uncontrolled cell growth, research participants must be carefully monitored (16, 19, 21, 23, 24, 28, 29, 32, 34, 42, 46, 50, 58, 64, 67–70, 81–83, 85), with long-term follow-up (12, 19, 21, 23, 27–29, 32, 34, 35, 37, 38, 40, 46, 50, 51, 53, 62, 66–70, 73, 79, 81, 85, 87, 91, 92). On the one hand, intensive monitoring may be perceived as reassuring or beneficial by research participants (50, 83, 93). On the other hand, possible life-long follow-up could also be burdensome for participants (50). Given the complexity of tissue-engineered products, clinical teams conducting these studies should have experience with other regenerative medicine therapies (e.g., cell-based therapy) and with post-trial follow-up care (81).

Theme 3: Patient Selection

In the patient selection procedure, a new kind of trade-off has to be made: against enormous benefits stand potentially large risks (e.g., tumour formation). Selection of patients in early-phase clinical trials is a major ethical theme in the literature (12, 27, 31–34, 37, 42, 43, 45, 48, 50, 66, 67, 69, 70, 77, 81, 82, 94). Potential target groups can be divided into 5 categories: healthy individuals, individuals at risk, children, patient with early-stage disease and patients with end-stage disease (**Table 5**). First, it is considered unacceptable to ask 1) healthy individuals for clinical studies of regenerative medicine applications, especially of tissue-engineered products which are designed to function in the body of the recipient, given the high risks (34) and lack of benefit (32,34). Also, when regenerative applications are personalized (i.e., composed, in part, of patient-derived material), the only eligible recipient will likely be the patient themselves (48). Second, the scholarly literature contains arguments in favour of the selection of 2) individuals at risks, with 3) early-stage disease (31, 37, 48, 50, 69, 77, 81, 94), and 4) children (37, 38, 48, 78). These individuals are relatively healthy, if a regenerative medicine application is used into one of these groups, it may help 1) to achieve more health benefit, and 2) to prevent (long-term) severe complications (31, 37, 48, 50, 69, 77, 81, 94). On the other hand, it is uncertain whether these individuals, who may not have developed or will develop symptoms at all, will indeed come to suffer from end-stage organ failure at all and be in need for a transplant. At the same time, as the procedure is novel, risky and invasive, their current physical condition could worsen significantly (50). Lastly, based on the literature, the most eligible patients for early-phase clinical trials are patients who have reached the 5) end-stage of their disease (12, 27, 31, 33, 34, 42, 43, 45, 48, 66, 69, 70, 81, 82, 94). These patients no (or no longer) have effective or suitable treatment options at the time of enrolment and may be facing limited life expectancy (12, 27, 31, 33, 34, 42, 43, 45, 48, 66, 69, 70, 81, 82, 94). When serious complications occur, they may have less to lose than healthy individuals or patients with stable disease (, 12, 32–34, 48, 50, 66, 67, 77, 94). Also, for patients who have reached the end-stage of their disease, a bio-artificial organ could potentially be associated with greater medical benefits.

TABLE 5 | Points to consider in relation to patient selection.

Suggested research participants for early-phase clinical trials	Reasons for and against selection
Healthy individuals	For - Healthy individuals are most resilient to physical harms (thus, harms are minimized) Against - No clinical value for the participant - Risks are too high
Individuals at risk - No symptoms - Risk factors for disease	For - Less damage to the body from disease or disease-related complications, which could lead to better health outcomes compared to more advanced disease stages - Disease can be prevented Against - Risks could be too high - Unnecessary treatment (participants may not develop the disease)
Early-stage patients - Mild to moderate disease - Medically controlled disease	For - Less damage to the body from disease or disease-related complications, which could lead to better health outcomes compared to more advanced disease stages Against - Risks are too high - Alternative treatment options may be available - Treatment could worsen the disease
Children Diagnosed with the disease	For - Less damage to the body - Serious complications can be prevented - Benefit can be enjoyed the longest Against - Risks may be too high - Alternative treatment options may be available - The disease may not proceed to advanced stages - Long-term follow-up may be burdensome for the participants - Children are unable to provide informed consent
Advanced-stage/end-stage patients - Severe disease - Unstable disease - No or no longer a suitable treatment option available	For - There is an unmet medical need, as effective treatment options are not or no longer available - Potential for medical benefit from participation in the trial - Less to lose when serious complications occur Against - The body is already damaged; this damage might be irreparable - Treatment could worsen the disease

Theme 4: Trial Design

Intervention

Six articles in our sample argued that the traditional model for clinical translation—phases I to phases II, III and IV, in which toxicity and/or efficacy of new drugs are tested—may not be suitable for clinical trials of transplantable applications of regenerative medicine in humans (17, 24, 37, 38, 62, 81). Schneemann et al. proposed that early-phase transplantation trials should combine safety and efficacy outcomes in their trial design to maximise participants' chances at obtaining medical benefit (37). Schneemann et al. suggested participants should be given a "dose" (in the context of bio-artificial organs: a certain quantity of engineered tissue) that is expected to be therapeutic, and efficacy should be added as an outcome measure (37). Combined safety and efficacy trials are associated with lower risks and costs than traditional studies, which could have positive effects on the likelihood of successful clinical development and help prevent promising interventions from failing (17, 81).

Outcomes

In the literature, relevant outcome measures for regenerative medicine clinical trials are discussed in 18 papers (12, 16, 19, 21, 24, 32, 34, 37, 43, 50, 61, 64, 69, 77, 80, 81, 87, 94). Both clinical outcome measures (e.g., survival rate or functional status) and patient-reported outcome measures (PROMs) (e.g., quality of life or experienced symptoms) are considered important (12, 21, 34, 43, 69, 77, 81, 87, 94). In later stages of clinical development and implementation, registries should be set up so that real-world outcome data can be collected to facilitate fair evaluation of the benefits of this technology. In addition, in later stages researchers should not only measure clinical outcome measures, but also PROMs, in order to ensure that new technologies not only affect biological parameters favourably, but also improve patients' lives (37, 69, 94). By giving potential participants the opportunity to define outcome measures, they become active stakeholders in the trial design (37, 69, 78, 94). Further, asking patients to define outcomes could help increase the enrolment of participants in the trial (21, 37, 69, 94).

TABLE 6 | points to consider in relation to trial design.

Trial design	Points to consider
Intervention Outcomes 1. Patient-reported (e.g., quality of life, treatment satisfaction and experienced symptoms) 2. Professional defined (survival rate, functional status and biological parameters)	- Researchers should set up combined efficacy and safety trials - Patients should be actively involved in research design as stakeholders - PROMs should be developed for later-phase clinical trials and adopted in trial design
Skills and materials	- Learning curves of surgeons should be corrected for - The effects of the risks associated with surgical procedures on the outcomes of trials should be corrected

Skills and Materials

Authors also suggest to involve surgeons early on in the trial design, since they know what surgical skills and materials are needed to perform surgical trials safely (43, 37, 35, 12, 87). Clinical translation of bio-artificial organs in transplantation may require surgeons to learn new techniques and develop new instruments, therefore minimizing the number of surgeons involved is suggested (**Table 6**). Additionally, different surgeons may learn and refine surgical techniques in different ways, which may (temporarily) affect the outcomes of trials (34, 68, 95). Therefore, it is advised to account for a learning curve and for variability in experience between surgeons (32, 68, 66, 77, 96).

Theme 5: Informed Consent

The ethical requirements of clear informed consent is mentioned frequently in the literature (12, 16, 17, 20–25, 27, 29, 31–34, 37, 38, 43, 45, 50–52, 59, 60, 64–69, 75–77, 79, 81, 83, 85, 89, 90, 92, 93, 97, 98). Valid informed consent requires that participants must be adequately informed about relevant aspects of research participation, including the aim of the procedure, duration of the study, their right to withdraw, and the risks and benefits implications of the trial (**Table 7**). Less often mentioned as an essential component in informed consent is information on the specific composition of the regenerative medicine application, although some authors find it important (33, 81, 83). One survey showed that participants want to be especially informed about issues that could directly affect their health status, such as foreseeable risks, impact on quality of life and safety measures (83). Participants are worried about the risks associated with genetic manipulation of transplantable tissue and about commercialization of cells (33, 83).

Given the lack of evidence on the risks, however, it could be difficult for researchers to provide full disclosure. Rather, participants should be made aware of the uncertainties surrounding the risks and benefits of investigational regenerative medicine technologies (20, 21, 23, 24, 32–34, 65, 72, 81, 98). Participants should be given the opportunity to consult an independent expert (33, 98), and can be offered psychological support (81), or consult a patient advocates (81), to assist them in the decision-making process (33, 60, 81, 83, 84, 98). To minimize "the therapeutic misconception," the (sometimes) mistaken belief among research participants that they will benefit from trial participation, measures should be taken to ensure that research participants are aware of the fact that research is conducted not with the goal of providing them medical treatment, but of obtaining generalizable information (12, 16, 17, 21, 24, 25, 29, 31, 33, 37, 50, 57, 60, 64, 67, 69, 81, 93, 97, 98). Researchers should avoid presenting the potential of the product in an overly optimistic light, overestimating the possible benefits, or giving unrealistic timelines for it to reach the clinic (30). Also, to strengthen comprehension, researchers are advised to present information about the trial not only in writing but also visually (33, 60, 68, 79), encourage patients to ask questions, and avoid scientific jargon by using only simple words or easily understood terminology during the informed consent process (20–22, 29, 31, 57, 69, 93, 98). Researchers may use the teach-back method (98) or even an "exam" or questionnaire (33) to ensure that participants understand the information and make an informed choice (33, 34, 81, 98, 99). Participants must also be aware that

TABLE 7 | points to consider in relation to informed consent.

Procedural	Substantial
- Informed consent from participants with decisional capacity or their legally authorized representative should be obtained - Relevant information about the trial, should also be presented visually - Patients should be encouraged to ask questions - Scientific jargon should be avoided by using only simple words or easily understood terminology - The teach-back method, exams or questionnaires could be used to ensure that participants understand the relevant information - Participants should be encouraged to ask independent experts/patient advocates for advice or assistance in the decision-making process - Participants need to be informed that the intervention is not likely to provide direct medical benefits	- Potential risks, benefits and uncertainties - Composition of the product - The irreversible nature of the intervention - How adverse events will be dealt with - The right and practical difficulty to withdraw - How life-long follow up will be organized - The possibility to consent for partial or complete autopsy in the event of death

participating in a trial might diminish their chances of getting access to future treatment opportunities (21,48,50).

A widely endorsed norm in research ethics is that participants should always have the right to withdraw their consent without negative consequences for the health care they receive. However, for participants in early-phase clinical trials of regenerative medicine technologies, withdrawal may be complicated (34). While it may be possible to withdraw from follow-up, removal of bio-artificial organs (in their entirety) may not be possible. For this reason, the opportunities for withdrawal or lack thereof, and the implications of trial participation for the future health and safety of participants must be discussed beforehand, as part of the informed consent process (34). In particular, research participants should be aware of the need for a long-term follow-up and the possibility of (long-term) adverse events (32, 34, 81). Lastly, some authors suggest informing and asking participants to provide consent for a partial or complete autopsy after their death. Obtaining this information will improve the scientific value of the study and contribute to the safety of future research participants (12).

Theme 6: Oversight and Accountability

The literature suggests that researchers should be especially careful when communicating with patients, physicians, other stakeholders, and the general public about regenerative medicine applications, as overly optimistic expectations might easily arise (17, 21, 22, 25, 29, 46, 52, 57, 62, 64, 67, 69, 78, 80, 81, 86, 90, 93, 94, 100) (**Table 8**). The ways in which research is represented in the media affects societal perspectives and frames policy debates (17, 67, 86, 100). In frontier science, of which research on bio-artificial organ transplantation is an example, researchers might wish or feel compelled to attract media attention to obtain financial support (17). However, they should refrain from inaccurate or incomplete representation of research, as this could ultimately have negative consequences for the advancement of the field and the integrity. For instance, researchers should avoid sharing findings with the press before peer review (17, 62) or could follow the ISSCR guidelines with regard to the conduct, public engagement and accountability of clinical trials (12, 16). In addition, researchers should be open to (international) collaboration between scientists, ethicists and clinicians (18, 22, 23, 25, 28, 35, 36, 38, 39, 41, 45, 50, 54, 57, 63–65, 73, 77, 81, 84–86, 89, 96, 100–102) and the conduct of interdisciplinary dialogues, involving scientists, such as engineers and biologists, but also patients, clinicians, policy makers, industry partners, ethicists, and the general public (17, 24, 29, 35, 37, 38, 46, 55, 64, 73, 80, 81, 84, 86, 90, 93) to encourage responsible innovation, and build and maintain long-term trust in research and the development of regenerative medicine applications. Adopting a similar strategy around bio-artificial organ technologies is highly desirable.

All research involving clinical applications of regenerative medicine must be subjected to independent RECs for approval. The main task of these oversight bodies is to ensure ethical conduct of clinical research and to protect human research participants. However, it is uncertain whether existing RECs have sufficient specific technical and clinical expertise in the fields of both organ transplantation and regenerative medicine to be able to evaluate the risks associated with bio-artificial organ transplantation trials. Multiple authors have proposed to set up specialized RECs or advisory boards with experts from various backgrounds for the evaluation of clinical trials of regenerative medicine technologies (9, 16, 19, 20, 22, 24, 28, 29, 32, 45, 46, 62–65, 67, 69, 77, 78, 80, 85, 92). These experts could assist RECs in assessing the scientific underpinnings of the clinical trial protocols and the risks of abnormal product function and proliferation (16). According to some, such specialized RECs should ideally also include lay people (21, 80). Moreover, authors recommend providing education opportunities for surgeons, researchers, nurses and ethicist in training, on the ethical aspects related to ATMPs (9, 20–22, 29, 36, 40, 45, 64, 65, 69, 70, 73, 77, 87, 92, 93).

Researchers should pre-register clinical trials and publish understandable and complete data on each step along the research pathway regardless of whether the data is positive, negative or inconclusive (12, 16, 24, 28, 29, 69, 80, 81). Being transparent about data could also inspire other researchers to go into new research directions (69).

TABLE 8 | points to consider in relation to oversight and accountability.

Oversight and accountability	Points to consider
Public awareness and patient engagement	- The information should be publicly available - Interdisciplinary dialogues between scientists, ethicists, patients, policy-makers, clinicians, industry partners, and the general public should be stimulated - Dissemination of non-peer-reviewed research results should be avoided - Participants should be referred to patient advocacy groups - Participants should have an active role in research (e.g., as active stakeholders)
Strengthening of RECs	- RECs should be expanded with experts in regenerative medicine/organ transplantation or set up advisory boards or specialized working groups to support RECs - Patient representatives should be invited to participate in RECs - Educational activities should be organized for RECs
Stimulate (data) transparency, minimize publication bias and diminish selective reporting to create long-term trust in research	- Preclinical researchers should publish negative, positive and inconclusive results - Researchers should pre-register clinical trials - Data monitoring plans should be put in place - Researchers, clinicians and regulators should be stimulated to collaborate - Guidance should be periodically revised

DISCUSSION

In the rapidly evolving field of regenerative medicine, it is important that early-phase clinical trials are performed in a responsible and ethically acceptable way. Such trials can lead to unforeseeable serious harm for research participants, as, for instance, has occurred during early-phase clinical trials of gene therapies in the 1990s, in which research participants have died (103). Yet clinical translation of bio-artificial organ technologies has the potential to make available life-saving therapeutic products to patients suffering from end-stage organ failure and to remove the need of (life-long) immunosuppressive therapy, which has hitherto been a serious disadvantage of organ transplantation.

To our knowledge, this is the first systematic review of the literature on early-phase clinical trials in regenerative medicine, tissue engineering, cell-based therapy, bio-engineered organs, organoid transplantation, synthetic biology, and 3D bioprinting, which summarizes relevant ethical points to consider in early-phase research on transplantable bio-artificial organs. Our review reveals that a significant body of literature exists on ethical considerations around early-phase trials in the field of cell-based therapy. However, there is strikingly little literature on ethical aspects of early-phase clinical trials in the field of 3D bioprinting, and organoid transplantation. There is also little attention for ethical aspects of early-phase regenerative medicine trials in surgery; only seven papers were published in surgical journals. A further noticeable finding in this review was the paucity of empirical ethics research in the scientific fields that were included in the review: only six empirical studies were found (21, 77, 83, 93, 94, 98), three of which focussed on the perceived ethical challenges of regenerative medicine among professionals in the field (21, 77, 83), and three of which focussed on patients' perspectives (93, 94, 98) on ethical considerations for early-phase clinical regenerative trials. Yet insight in patients' perspectives is essential to assessing the social value of new technologies and to determining the conditions under which it should be offered to patients.

In total, six themes were identified in the literature: cell source, risk-benefit assessment, patient selection, trial design, informed consent, and oversight and accountability. We found that ethical considerations around cell sources were mentioned most often, which is consistent with an earlier review of the ethical aspects of tissue engineering by de Vries et al (38). For each of the six themes, we have distilled and discussed ethical points to consider, which can be valuable for research groups and RECs who will be setting up or evaluating early-phase clinical transplantation trials of bio-artificial organs in the future, and for health care professionals working in the field of organ transplantation with an interest in innovative technologies. Below, we would like to reflect on important points made on two themes: trial design and informed consent. These themes are underrepresented in the literature, and need specific attention before early-phase bio-artificial organ transplantation trials can be initiated, and evaluated by RECs.

First, when designing clinical trials, researchers should not focus exclusively on gathering data on clinical outcomes, but also on understanding research participants' perspectives. Qualitative studies of patients' perspectives can help elucidate their needs and preferences with regard to the set-up and conduct of clinical trials, the use of outcome measures, the design and performance characteristics of the product that is being developed, the type of follow-up care that will be offered, etc., so that the process of clinical development and the resulting bio-artificial organ technologies are optimally aligned with patients' perspectives, to improve their quality of life. Also, trials should be designed such that data on long-term clinical outcomes of transplantable bioartificial organ technologies can be gathered. An exploratory survey among European tissue-engineers by Trommelmans et al. found that the majority of respondents insisted on long-term follow-up (83). Given the irreversibility of transplantation of bio-artificial organs and its potential for adverse events emerging only after a long time, long-term follow-up procedures may be essential in trials of bio-artificial organs. This requires long-term—possibly even lifelong—commitment of participants (34), and long-term trust relationships between researchers and patients. Barriers to long-term follow-up studies frequently reported include outdated contact information, lack of financial reimbursement for follow-up services, and direct and indirect costs charged to participants (104,105). Researchers in regenerative medicine could learn from prior experiences in overcoming these barriers. One such strategy is to discuss the long-term follow-up planning with participants during the informed consent procedure (106). Additional research is needed to identify barriers specific to long-term follow-up of bio-artificial organ transplantation trials, and to develop strategies for overcoming them.

Second, during the informed procedure, researchers should communicate reasonably foreseeable risks and benefits associated with participation in clinical trials. However, little guidance exists on how researchers should communicate such risk and benefits in cutting edge early-phase research (107, 108), in which there is a high degree of uncertainty surrounding these risks and benefits due to limited knowledge. There are concerns that researchers might overestimate and exaggerate the benefits in early-phase clinical trials, which is a potential source of "therapeutic misconception" (109, 110). For instance, Kimmelman et al. (110) analysed patient information and informed consent documents on risky, novel, experimental early-phase gene-transfer trials for seriously ill patients, and concluded that these were often inappropriately optimistic about the direct benefits for individual participants. The results of this study are relevant, because early-phase bio-artificial organs will also be risky and experimental. To prevent therapeutic misconception, researchers should provide realistic information to participants about the individual medical benefits and uncertainties of participation in early-phase clinical trials.

We consider it remarkable that it is often recommended, in various research fields, to use questionnaires, or extraordinarily written or oral exams, to check whether research participants have understood relevant information about clinical trial participation (16, 21, 33, 108, 110–112). It is believed that the exam approach will leave more time for the researcher, during a subsequent informed consent discussion, to focus on the aspects about which the participant's knowledge is not yet sufficient, and tailor the process to the participant's individual informational needs (113). However, it is unclear whether this focus on formally "testing" participants' knowledge of (the science underlying) the trial will lead to better informed, more autonomous decisions about research

participation. It may also place more responsibility or liability on research participants when—deciding about—participating in novel, possibly risky trials. Further research will be needed to understand and improve communication about risks and benefits of participation in early-phase clinical trials of bio-artificial organs.

We did not limit this review to one specific bio-artificial organ type. Instead, we developed a general list of ethical points to consider for all bio-artificial organ technologies. However, these points to consider may play out differently in specific bio-artificial organ technologies, and may vary with organ type; for instance, to a greater extent than for hearts, lungs, and livers, there are alternative (organ replacement) therapies available for pancreases or kidneys. This difference may affect risk-benefit assessment and patient selection of a clinical trial, which needs to be taken into account.

In conclusion, there is no specific ethical guidance for the safe and responsible design and conduct of early-phase clinical trials of transplantable bio-artificial organs. However, we have shown that ethical considerations from adjacent research fields may be useful for early-phase transplantable bio-artificial organs trials. In particular, the irreversibility, uncertainty of outcomes, the ethical considerations around the cell sources used to generate the product (e.g., donor cells), and the need for life-long follow-up studies makes clinical translation of bio-artificial organ technologies ethically contentious. Ethical themes that researchers and RECs should consider when designing or evaluating studies include cell source, risk-benefit assessment, patient selection, trial design, informed consent, and oversight and accountability. Patient engagement and empirical studies of patients' perspectives on (organ-) specific bio-artificial organ technologies will be essential to realizing the social value of research and clinical translation of bio-artificial organs, and to ensuring adequate informed consent for research participation.

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CONFLICT OF INTEREST

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SUPPLEMENTARY MATERIAL

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Supplementary Data Sheet S1 | PRISMA checklist.

Supplementary Data Sheet S2 | Capsule sentence summary.

Supplementary Data Sheet S3 | Search term.

REFERENCES

1. Mazza G, Rombouts K, Rennie Hall A, Urbani L, Vinh Luong T, Al-Akkad W, et al. Decellularized Human Liver as a Natural 3D-Scaffold for Liver Bioengineering and Transplantation. *Sci Rep* (2015) 5:13079–15. doi:10.1038/srep13079
2. Atala A, Bauer SB, Soker S, Yoo JJ, Retik AB. Tissue-engineered Autologous Bladders for Patients Needing Cystoplasty. *Lancet* (2006) 367:1241–6. doi:10.1016/S0140-6736(06)68438-9
3. Jeremy JS, Jacques G, Sarah G, Gabriel G, Joseph PV, Harald CO. Regeneration and Experimental Orthotopic Transplantation of a Bioengineered Kidney. *Nat Med* (2013) 19:646–51. doi:10.1038/nm.3154

4. Guyette JP, Charest JM, Mills RW, Jank BJ, Moser PT, Gilpin SE, et al. Bioengineering Human Myocardium on Native Extracellular Matrix. *Circ Res* (2016) 118:56–72. doi:10.1161/CIRCRESAHA.115.306874
5. Kitano K, Schwartz DM, Zhou H, Gilpin SE, Wojtkiewicz GR, Ren X, et al. Bioengineering of Functional Human Induced Pluripotent Stem Cell-Derived Intestinal Grafts. *Nat Commun* (2017) 8:765. doi:10.1038/s41467-017-00779-y
6. Wilkinson DC, Alva-Ornelas JA, Sucre JMS, Vijayaraj P, Durra A, Richardson W, et al. Development of a Three-Dimensional Bioengineering Technology to Generate Lung Tissue for Personalized Disease Modeling. *Stem Cell Transl Med* (2017) 6:622–33. doi:10.5966/sctm.2016-0192
7. Ohata K, Ott HC. Human-scale Lung Regeneration Based on Decellularized Matrix Scaffolds as a Biologic Platform. *Surg Today* (2020) 50:633–43. doi:10.1007/s00595-020-02000-y
8. Kimmelman J. Ethics, Ambiguity Aversion, and the Review of Complex Translational Clinical Trials. *Bioethics* (2012) 26:242–50. doi:10.1111/j.1467-8519.2010.01856.x
9. Knoepfler PS. From Bench to FDA to Bedside: US Regulatory Trends for New Stem Cell Therapies. *Adv Drug Deliv Rev* (2015) 82:192–6. doi:10.1016/j.addr.2014.12.001
10. Ballantyne A, Eriksson S. Research Ethics Revised: The New CIOMS Guidelines and the World Medical Association Declaration of Helsinki in Context. *Bioethics* (2019) 33:310–1. doi:10.1111/bioe.12581
11. World Medical Association. World Medical Association Declaration of Helsinki: Ethical Principles for Medical Research Involving Human Subjects. *JAMA - J Am Med Assoc* (2013) 310:2191–4. doi:10.1001/jama.2013.281053
12. Daley GQQ, Hyun I, Apperley JFF, Barker RAA, Benvenisty N, Bredenoord ALL, et al. Setting Global Standards for Stem Cell Research and Clinical Translation: The 2016 ISSCR Guidelines. *Stem Cel Rep* (2016) 6:787–97. doi:10.1016/J.STEMCR.2016.05.001
13. Schreier M. Qualitative Content Analysis. In: *The SAGE Handbook of Qualitative Data Analysis*. London: SAGE Publications (2014). p. 170–83. doi:10.4135/9781446282243.n12
14. Mertz M. How to Tackle the Conundrum of Quality Appraisal in Systematic Reviews of Normative Literature/information? Analysing the Problems of Three Possible Strategies (Translation of a German Paper). *BMC Med Ethics* (2019) 20:81–12. doi:10.1186/s12910-019-0423-5
15. Mertz M, Strech D, Kahrass H. What Methods Do Reviews of Normative Ethics Literature Use for Search, Selection, Analysis, and Synthesis? In-Depth Results from a Systematic Review of Reviews. *Syst Rev* (2017) 261. doi:10.1186/s13643-017-0661-x
16. Hyun I, Lindvall O, Ährlund-Richter L, Cattaneo E, Cavazzana-Calvo M, Cossu G, et al. New ISSCR Guidelines Underscore Major Principles for Responsible Translational Stem Cell Research. *Cell Stem Cell* (2008) 3:607–9. doi:10.1016/j.stem.2008.11.009
17. Bredenoord AL, Clevers H, Knoblich JA. Human Tissues in a Dish: The Research and Ethical Implications of Organoid Technology. *Science* (2017) 80:eaaf9414–7. doi:10.1126/science.aaf9414
18. Bhangra KS, Busuttil F, Phillips JB, Rahim AA. Using Stem Cells to Grow Artificial Tissue for Peripheral Nerve Repair. *Stem Cell Int* (2016) 2016:7502178. doi:10.1155/2016/7502178
19. Neri S. Genetic Stability of Mesenchymal Stromal Cells for Regenerative Medicine Applications: A Fundamental Biosafety Aspect. *Int J Mol Sci* (2019) E2406. doi:10.3390/ijms20102406
20. Moradi S, Mahdizadeh H, Šarić T, Kim J, Harati J, Shahsavarani H, et al. Research and Therapy with Induced Pluripotent Stem Cells (iPSCs): Social, Legal, and Ethical Considerations. *Stem Cel Res Ther* (2019) 10:341–13. doi:10.1186/S13287-019-1455-Y
21. Kusunose M, Nagamura F, Muto K. Informed Consent in Clinical Trials Using Stem Cells: Suggestions and Points of Attention from Informed Consent Training Workshops in Japan. *S Afr J Bioeth L* (2015) 8:49. doi:10.7196/SAJBL.8016
22. Kleiderman E, Boily A, Hasilo C, Knoppers BM. Overcoming Barriers to Facilitate the Regulation of Multi-centre Regenerative Medicine Clinical Trials. *Stem Cel Res Ther* (2018) 9:307. doi:10.1186/s13287-018-1055-2
23. Hyun I. Allowing Innovative Stem Cell-Based Therapies outside of Clinical Trials: Ethical and Policy Challenges. *J L Med Ethics* (2010) 38:277–85. doi:10.1111/J.1748-720X.2010.00488.X
24. Habets MGJL, Van Delden JJM, Bredenoord AL. The Inherent Ethical challenge of First-In-Human Pluripotent Stem Cell Trials. *Regen Med* (2014) 9:1–3. doi:10.2217/rme.13.83
25. Fung M, Yuan Y, Atkins H, Shi Q, Bubela T. Responsible Translation of Stem Cell Research: An Assessment of Clinical Trial Registration and Publications. *Stem Cel Rep* (2017) 8:1190–201. doi:10.1016/j.stemcr.2017.03.013
26. Brignier AC, Gewirtz AM. Embryonic and Adult Stem Cell Therapy. *J Allergy Clin Immunol* (2010) 125:S336–44. doi:10.1016/j.jaci.2009.09.032
27. Hayakawa T, Aoi T, Umezawa A, Ozawa K, Sato Y, Sawa Y, et al. A Study on Ensuring the Quality and Safety of Pharmaceuticals and Medical Devices Derived from the Processing of Allogeneic Human Somatic Stem Cells. *Regen Ther* (2015) 2:70–80. doi:10.1016/J.RETH.2015.05.001
28. Liras A. Future Research and Therapeutic Applications of Human Stem Cells: General, Regulatory, and Bioethical Aspects. *J Transl Med* (2010) 8:131. doi:10.1186/1479-5876-8-131
29. Main H, Munsie M, O'Connor MD. Managing the Potential and Pitfalls during Clinical Translation of Emerging Stem Cell Therapies. *Clin Transl Med* (2014) 3:10. doi:10.1186/2001-1326-3-10
30. Otto IA, Breugem CC, Malda J, Bredenoord AL. Ethical Considerations in the Translation of Regenerative Biofabrication Technologies into Clinic and Society. *Biofabrication* (2016) 8. doi:10.1088/1758-5090/8/4/042001
31. Vijayavenkataraman S, Lu WF, Fuh JYH. 3D Bioprinting – an Ethical, Legal and Social Aspects (ELSA) Framework. *Bioprinting* (2016) 1:11–21. doi:10.1016/j.bprint.2016.08.001
32. Trommelmans L, Selling J, Dierickx K. Is Tissue Engineering a New Paradigm in Medicine? Consequences for the Ethical Evaluation of Tissue Engineering Research. *Med Health Care Philos* (2009) 12:459–67. doi:10.1007/s11019-009-9192-0
33. Trommelmans L, Selling J, Dierickx K. Informing Participants in Clinical Trials with *Ex Vivo* Human Tissue-Engineered Products: what to Tell and How to Tell it? *J Tissue Eng Regen Med* (2008) 2:236–41. doi:10.1002/TERM.82
34. Trommelmans L, Selling J, Dierickx K. Ethical Reflections on Clinical Trials with Human Tissue Engineered Products. *J Med Ethics* (2008) e1. doi:10.1136/jme.2007.022913
35. Sutherland FWH, Mayer JE. Ethical and Regulatory Issues Concerning Engineered Tissues for Congenital Heart Repair. *Semin Thorac Cardiovasc Surg Pediatr Card Surg Annu* (2003) 6:152–63. doi:10.1053/PCSU.2003.50012
36. Stegemann JP, Verrier S, Gebhard F, Laschke MW, Martin I, Simpson H, et al. Cell Therapy for Bone Repair: Narrowing the gap between Vision and Practice. *Eur Cel Mater* (2014) 27:1–4. doi:10.22203/ECM.V027SA01
37. Schneemann SA, Boers SN, van Delden JjmNieuwenhuis EES, Fuchs SA, Bredenoord AL. Ethical Challenges for Pediatric Liver Organoid Transplantation. *Sci Transl Med* (2020) 12:8471. doi:10.1126/scitranslmed.aau8471
38. De Vries RBM, Oerlemans A, Trommelmans L, Dierickx K, Gordijn B. Ethical Aspects of Tissue Engineering: A Review. *Tissue Eng B Rev* (2008) 14:367–75. doi:10.1089/ten.teb.2008.0199
39. Giancola R, Bonfini T, Iacone A. Cell Therapy: cGMP Facilities and Manufacturing. *Muscles Ligaments Tendons J* (2012) 2:243–7.
40. Madariaga MLL, Ott HC. Bioengineering Kidneys for Transplantation. *Semin Nephrol* (2014) 34:384–93. doi:10.1016/J.SEMNEPHROL.2014.06.005
41. Naghieh S, Lindberg G, Tamaddon M, Liu C. Biofabrication Strategies for Musculoskeletal Disorders: Evolution towards Clinical Applications. *Bioengineering* (2021) 8. doi:10.3390/bioengineering8090123
42. Levin LA, Miller JW, Zack DJ, Friedlander M, Smith LEH. Special Commentary: Early Clinical Development of Cell Replacement Therapy: Considerations for the National Eye Institute Audacious Goals Initiative. *Ophthalmology* (2017) 124:926–34. doi:10.1016/J.OPHTHA.2017.02.017
43. O'Donnell BT, Ives CJ, Mohiuddin OA, Bunnell BA. Beyond the Present Constraints that Prevent a Wide Spread of Tissue Engineering and Regenerative Medicine Approaches. *Front Bioeng Biotechnol* (2019) 7:95. doi:10.3389/FBIOE.2019.00095
44. O'Keefe RJ, Tuan RS, Lane NE, Awad HA, Barry F, Bunnell BA, et al. American Society for Bone and Mineral Research-Orthopaedic Research Society Joint Task Force Report on Cell-Based Therapies. *J Bone Miner Res* (2020) 35:3–17. doi:10.1002/JBMR.3839

45. Sekar MP, Budharaju H, Zennifer A, Sethuraman S, Vermeulen N, Sundaramurthi D, et al. Current Standards and Ethical Landscape of Engineered tissues—3D Bioprinting Perspective. *J Tissue Eng* (2021) 6: 20417314211027677–33. doi:10.1177/20417314211027677
46. Scopetti M, Santurro A, Gatto V, Russa RLA, Manetti F, Errico SD, et al. Mesenchymal Stem Cells in Neurodegenerative Diseases: Opinion Review on Ethical Dilemmas. *World J Stem Cell* (2020) 12:168–77. doi:10.4252/WJSC.V12.I3.168
47. Court FG, Wemyss-Holden SA, Dennison AR, Maddern GJ. Bioartificial Liver Support Devices: Historical Perspectives. *ANZ J Surg* (2003) 73:739–48. doi:10.1046/j.1445-2197.2003.02741.x
48. Gilbert F, O'Connell CD, Mladenovska T, Dodds S. Print Me an Organ? Ethical and Regulatory Issues Emerging from 3D Bioprinting in Medicine. *Sci Eng Ethics* (2018) 24:73–91. doi:10.1007/S11948-017-9874-6
49. Liu G, David BT, Trawczynski M, Fessler RG. Advances in Pluripotent Stem Cells: History, Mechanisms, Technologies, and Applications. *Stem Cel Rev Rep* (2020) 16:3–32. doi:10.1007/S12015-019-09935-X
50. Niemansburg SL, Van Delden JJM, Dhert WJA, Bredenoord AL. Regenerative Medicine Interventions for Orthopedic Disorders: Ethical Issues in the Translation into Patients. *Regen Med* (2013) 8:65–73. doi:10.2217/RME.12.112
51. Bobba S, Di Girolamo N, Munsie M, Chen F, Pébay A, Harkin D, et al. The Current State of Stem Cell Therapy for Ocular Disease. *Exp Eye Res* (2018) 177:65–75. doi:10.1016/j.exer.2018.07.019
52. Aalto-Setälä K, Conklin BR, Lo B. Obtaining Consent for Future Research with Induced Pluripotent cells:Opportunities and Challenges. *Plos Biol* (2009) 7:e1000042. doi:10.1371/journal.pbio.1000042
53. Tsang L. Legal and Ethical Status of Stem Cells as Medicinal Products. *Adv Drug Deliv Rev* (2005) 57:1970–80. doi:10.1016/j.addr.2005.08.005
54. Chung E. Stem-cell-based Therapy in the Field of Urology: A Review of Stem Cell Basic Science, Clinical Applications and Future Directions in the Treatment of Various Sexual and Urinary Conditions. *Expert Opin Biol Ther* (2015) 15:1623–32. doi:10.1517/14712598.2015.1075504
55. Coombe L, Kadri A, Martinez JF, Tatachar V, Gallicano GI. Current Approaches in Regenerative Medicine for the Treatment of Diabetes: Introducing CRISPR/CAS9 Technology and the Case for Non-embryonic Stem Cell Therapy. *Am J Stem Cell* (2018) 7:104–13.
56. Garg A, Yang J, Lee W, Tsang S. Stem Cell Therapies in Retinal Disorders. *Cells* (2017) 6:4. doi:10.3390/CELLS6010004
57. King NMP, Perrin J. Ethical Issues in Stem Cell Research and Therapy. *Stem Cel Res Ther* (2014) 5:85. doi:10.1186/SCRT474
58. Lederer CW, Santama N. Neural Stem Cells: Mechanisms of Fate Specification and Nuclear Reprogramming in Regenerative Medicine. *Biotechnol J* (2008) 3:1521–38. doi:10.1002/BIOT.200800193
59. Zamborsky R, Kilian M, Csobonyeiova M, Danisovic L. Regenerative Medicine in Orthopaedics and Trauma: Challenges, Regulation and Ethical Issues. *Ortop Traumatol Rehabil* (2018) 20:173–80. doi:10.5604/01.3001.0012.0764
60. Takashima K, Inoue Y, Tashiro S, Muto K. Lessons for Reviewing Clinical Trials Using Induced Pluripotent Stem Cells: Examining the Case of a First-In-Human Trial for Age-Related Macular Degeneration. *Regen Med* (2018) 13:123–8. doi:10.2217/rme-2017-0130
61. Sievert KD, Amend B, Stenzl A. Tissue Engineering for the Lower Urinary Tract: A Review of a State of the Art Approach. *Eur Urol* (2007) 52:1580–9. doi:10.1016/J.EURURO.2007.08.051
62. Hara A, Sato D, Sahara Y. New Governmental Regulatory System for Stem Cell-Based Therapies in Japan. *Ther Innov Regul Sci* (2014) 48:681–8. doi:10.1177/2168479014526877
63. Kim JH, Kurtz A, Yuan BZ, Zeng F, Lomax G, Loring JF, et al. Report of the International Stem Cell Banking Initiative Workshop Activity: Current Hurdles and Progress in Seed-Stock Banking of Human Pluripotent Stem Cells. *Stem Cell Transl Med* (2017) 6:1956–62. doi:10.1002/SCTM.17-0144
64. Fears R, Akutsu H, Alentajan-Aleta LT, Caicedo A, Campos de Carvalho AC, Čolić M, et al. Inclusivity and Diversity: Integrating International Perspectives on Stem Cell Challenges and Potential. *Stem Cel Rep* (2021) 16:1847–52. doi:10.1016/J.STEMCR.2021.07.003
65. Hildebrandt M. Horses for Courses: an Approach to the Qualification of Clinical Trial Sites and Investigators in ATMPs. *Drug Discov Today* (2020) 25:265–8. doi:10.1016/J.DRUDIS.2019.10.003
66. de Windt TS, Niemansburg SL, Vonk LA, van Delden JM, Roes KCB, Dhert WJA, et al. Ethics in Musculoskeletal Regenerative Medicine; Guidance in Choosing the Appropriate Comparator in Clinical Trials. *Osteoarthritis Cartilage* (2019) 27:34–40. doi:10.1016/j.joca.2018.08.022
67. Lowenthal J, Sugarman J. Ethics and Policy Issues for Stem Cell Research and Pulmonary Medicine. *Chest* (2015) 147:824–34. doi:10.1378/CHEST.14-1696
68. Oerlemans AJM, Feitz WFJ, Van Leeuwen E, Dekkers WJM. Regenerative Urology Clinical Trials: An Ethical Assessment of Road Blocks and Solutions. *Tissue Eng Part B Rev* (2013) 19:41–7. doi:10.1089/TEN.TEB.2012.0136
69. Baker HB, McQuilling JP, King NMP. Ethical Considerations in Tissue Engineering Research: Case Studies in Translation. *Methods* (2017) 99: 135–44. doi:10.1016/J.YMETH.2015.08.010
70. Lee TL, Lysaght T. Conditional Approvals for Autologous Stem Cell-Based Interventions: Conflicting Norms and Institutional Legitimacy. *Perspect Biol Med* (2018) 61:59–75. doi:10.1353/PBM.2018.0027
71. Oerlemans AJM, Van Den Berg PP, Van Leeuwen E, Dekkers WJM. Ethical Issues Regarding the Donation and Source of Cells for Tissue Engineering: a European Focus Group Study. *Tissue Eng Part B Rev* (2011) 17:229–34. doi:10.1089/TEN.TEB.2010.0683
72. Oerlemans AJM, van Hoek MEC, van Leeuwen E, van der Burg S, Dekkers WJM. Towards a Richer Debate on Tissue Engineering: a Consideration on the Basis of NEST-Ethics. *Sci Eng Ethics* (2013) 19:963–81. doi:10.1007/S11948-012-9419-Y
73. Goula A, Gkioka V, Michalopoulos E, Katsimpoulas M, Noutsias M, Sarri EF, et al. Advanced Therapy Medicinal Products Challenges and Perspectives in Regenerative Medicine. *J Clin Med Res* (2020) 12:780–6. doi:10.14740/JOCMR3964
74. Haake K, Ackermann M, Lachmann N. Concise Review: Towards the Clinical Translation of Induced Pluripotent Stem Cell-Derived Blood Cells—Ready for Take-Off. *Stem Cell Transl Med* (2019) 8:332–9. doi:10.1002/SCTM.18-0134
75. Lomax GP, Shepard KA. Return of Results in Translational iPS Cell Research: Considerations for Donor Informed Consent. *Stem Cel Res Ther* (2013) 4:6. doi:10.1186/SCRT154
76. Lowenthal J, Lipnick S, Rao M, Hull SC. Specimen Collection for Induced Pluripotent Stem Cell Research: Harmonizing the Approach to Informed Consent. *Stem Cell Transl Med* (2012) 1:409–21. doi:10.5966/sctm.2012-0029
77. Niemansburg SL, Van Delden JJM, Öner FC, Dhert WJA, Bredenoord AL. Ethical Implications of Regenerative Medicine in Orthopedics: An Empirical Study with Surgeons and Scientists in the Field. *Spine J* (2014) 14:1029–35. doi:10.1016/j.spinee.2013.10.016
78. Chan S. Research Translation and Emerging Health Technologies: Synthetic Biology and beyond. *Health Care Anal* (2018) 26:310–25. doi:10.1007/s10728-016-0334-2
79. Niemansburg SL, Habets MGJL, Dhert WJA, Delden Jjm vanBredenoord AL. Participant Selection for Preventive Regenerative Medicine Trials: Ethical Challenges of Selecting Individuals at Risk. *J Med Ethics* (2015) 41:914–6. doi:10.1136/MEDETHICS-2014-102625
80. Genske A, Engel-Glatter S. Rethinking Risk Assessment for Emerging Technology First-In-Human Trials. *Med Health Care Philos* (2016) 19: 125–39. doi:10.1007/s11019-015-9660-7
81. Taylor DA, Caplan AL, Macchiarini P. Ethics of Bioengineering Organs and Tissues. *Expert Opin Biol Ther* (2014) 14:879–82. doi:10.1517/14712598.2014.915308
82. Afshar L, Aghayan HR, Sadighi J, Arjmand B, Hashemi SM, Basiri M, et al. Ethics of Research on Stem Cells and Regenerative Medicine: Ethical Guidelines in the Islamic Republic of Iran. *Stem Cel Res Ther* (2020) 11: 396. doi:10.1186/s13287-020-01916-z
83. Trommelmans L, Selling J, Dierickx K. An Exploratory Survey on the Views of European Tissue Engineers Concerning the Ethical Issues of Tissue Engineering Research. *Tissue Eng Part B Rev* (2009) 15:241–7. doi:10.1089/TEN.TEB.2008.0647
84. Sugarman J, Bredenoord AL. Real-time Ethics Engagement in Biomedical Research: Ethics from Bench to Bedside. *EMBO Rep* (2020) 21:e49919–4. doi:10.15252/embr.201949919

85. Seok J, Yoon S, Ryu CH, Kim SK, Ryu J, Jung YS. A Personalized 3D-Printed Model for Obtaining Informed Consent Process for Thyroid Surgery: A Randomized Clinical Study Using a Deep Learning Approach with Mesh-type 3D Modeling. *J Pers Med* (2021) 574. doi:10.3390/JPM11060574
86. Davison AC. Brave Pioneers or Clinical Cowboys? *Cell Stem Cell* (2010) 6: 504–5. doi:10.1016/j.stem.2010.05.011
87. Attico E, Sceberras V, Pellegrini G. Approaches for Effective Clinical Application of Stem Cell Transplantation. *Curr Transpl Rep* (2018) 5: 244–50. doi:10.1007/s40472-018-0202-0
88. Apatoff MBL, Sengillo JD, White EC, Bakhoum MF, Bassuk AG, Mahajan VB, et al. Autologous Stem Cell Therapy for Inherited and Acquired Retinal Disease. *Regen Med* (2018) 13:89–96. doi:10.2217/rme-2017-0089
89. Lu L, Arbit HM, Herrick JL, Segovis SG, Maran A, Yaszemski MJ. Tissue Engineered Constructs: Perspectives on Clinical Translation. *Ann Biomed Eng* (2015) 43:796–804. doi:10.1007/S10439-015-1280-0
90. Patuzzo S, Goracci G, Gasperini L, Ciliberti R. 3D Bioprinting Technology: Scientific Aspects and Ethical Issues. *Sci Eng Ethics* (2018) 24:335–48. doi:10.1007/S11948-017-9918-Y
91. Nagamura F. The Importance of Recruiting a Diverse Population for Stem Cell Clinical Trials. *Curr Stem Cel Rep* (2016) 2:321–7. doi:10.1007/s40778-016-0062-4
92. Lomax GP, Torres A, Millan MT. Regulated, Reliable, and Reputable: Protect Patients with Uniform Standards for Stem Cell Treatments. *Stem Cell Transl Med* (2020) 9:547–53. doi:10.1002/SCTM.19-0377
93. Shineha R, Inoue Y, Ikka T, Kishimoto A, Yashiro Y. A Comparative Analysis of Attitudes on Communication toward Stem Cell Research and Regenerative Medicine between the Public and the Scientific Community. *Stem Cell Transl Med* (2018) 7:251. doi:10.1002/SCTM.17-0184
94. Nagpal A, Hillier S, Milton AG, Hamilton-Bruce MA, Koblar SA. PERSPECTIVES: Stroke Survivors' Views on the Design of an Early-phase Cell Therapy Trial for Patients with Chronic Ischaemic Stroke. *Health Expect* (2019) 22:1069–77. doi:10.1111/hex.12932
95. Zocchi ML, Vindigni V, Pagani A, Pirro O, Conti G, Sbarbati A, et al. Regulatory, Ethical, and Technical Considerations on Regenerative Technologies and Adipose-Derived Mesenchymal Stem Cells. *Eur J Plast Surg* (2019) 426(42):531–48. doi:10.1007/S00238-019-01571-5
96. Masuda K, Lotz JC. New Challenges for Intervertebral Disc Treatment Using Regenerative Medicine. *Tissue Eng Part B Rev* (2010) 16:147–58. doi:10.1089/TEN.TEB.2009.0451
97. Lim ML, Jungebluth P, Ajalloueian F, Friedrich LH, Gilevich I, Grinnemo KH, et al. Whole Organ and Tissue Reconstruction in Thoracic Regenerative Surgery. *Mayo Clin Proc* (2013) 88:1151–66. doi:10.1016/J.MAYOCP.2013.03.011
98. Smith C, Martin-Lillie C, Higano JD, Turner L, Phu S, Arthurs J, et al. Challenging Misinformation and Engaging Patients: Characterizing a Regenerative Medicine Consult Service. *Regen Med* (2020) 15:1427–40. doi:10.2217/rme-2020-0018
99. Hug K. Understanding Voluntariness of Consent in First-In-Human Cell Therapy Trials. *Regen Med* (2020) 15:1647–60. doi:10.2217/RME-2019-0126
100. Davis NF, Cunnane EM, Mulvihill JJ, Quinlan MR, Bolton DM, Walsh MT, et al. The Role of Stem Cells for Reconstructing the Lower Urinary Tracts. *Curr Stem Cel Res Ther* (2018) 13:458–65. doi:10.2174/1574888x13666180426113907
101. Maekawa T, Kimura S, Kasai Y. Development of Novel Advanced Cell and Gene Therapy and GMP-Controlled Cell Processing. *Jpn Med Assoc J* (2005) 48:81–4.
102. Sniecinski I, Seghatchian J. Emerging Stem Cell Based Strategies for Treatment of Childhood Diseases. *Transfus Apher Sci* (2018) 57:311–5. doi:10.1016/J.TRANSCI.2018.05.011
103. Lehrman S. Virus Treatment Questioned after Gene Therapy Death. *Nature* (1999) 401:517–8. doi:10.1038/43977
104. Waterman AD, Dew MA, Davis CL, McCabe M, Wainright JL, Forland CL, et al. Living-donor Follow-Up Attitudes and Practices in U.S. Kidney and Liver Donor Programs. *Transplantation* (2013) 95:883–8. doi:10.1097/TP.0B013E31828279FD
105. Henderson ML, Thomas AG, Shaffer A, Massie AB, Luo X, Holscher CM, et al. The National Landscape of Living Kidney Donor Follow-Up in the United States. *Am J Transpl* (2017) 17:3131–40. doi:10.1111/AJT.14356
106. Keshvani N, Feurer ID, Rumbaugh E, Dreher A, Zavala E, Stanley M, et al. Evaluating the Impact of Performance Improvement Initiatives on Transplant Center Reporting Compliance and Patient Follow-Up after Living Kidney Donation. *Am J Transpl* (2015) 15:2126–35. doi:10.1111/ajt.13265
107. Kahrass H, Bossert S, Schürmann C, Strech D. Details of Risk–Benefit Communication in Informed Consent Documents for Phase I/II Trials. *Clin Trials* (2021) 18:71–80. doi:10.1177/1740774520971770
108. Benjaminy S, Kowal SP, MacDonald IM, Bubela T. Communicating the Promise for Ocular Gene Therapies: Challenges and Recommendations. *Am J Ophthalmol* (2015) 160:408–15. doi:10.1016/j.ajo.2015.05.026
109. Henderson GE, Davis AM, King NMP, Easter MM, Zimmer CR, Rothschild BB, et al. Uncertain Benefit: Investigators' Views and Communications in Early Phase Gene Transfer Trials. *Mol Ther* (2004) 10:225–31. doi:10.1016/J.YMTHE.2004.05.013
110. Kimmelman J, Palmour N. Therapeutic Optimism in the Consent Forms of Phase 1 Gene Transfer Trials: an Empirical Analysis. *J Med Ethics* (2005) 31: 209–14. doi:10.1136/jme.2003.006247
111. Beck S, Berner AM, Bignell G, Bond M, Callanan MJ, Chervova O, et al. Personal Genome Project UK (PGP-UK): A Research and Citizen Science Hybrid Project in Support of Personalized Medicine. *BMC Med Genomics* (2018) 108. doi:10.1186/s12920-018-0423-1
112. Gordon EJ, Daud A, Caicedo JC, Cameron KA, Jay C, Fryer J, et al. Informed Consent and Decision-Making about Adult-To-Adult Living Donor Liver Transplantation: a Systematic Review of Empirical Research. *Transplantation* (2011) 92:1285–96. doi:10.1097/TP.0B013E31823817D5
113. Spoon EQW, Kortram K, Ismail SY, Nieboer D, D'ancona FCH, Christiaans MHL, et al. Living Kidney Donor Knowledge of provided Information and Informed Consent: The PRINCE Study. *J Clin Med* (2022) 11:698. doi:10.3390/jcm11030698

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Public Perceptions and Information Needs of VCA Transplantation and Donation: A Mixed Methods Study

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Vascularized Composite Allotransplantation (VCA) involves transplantation of multiple tissues from a donor to a recipient (e.g., skin, muscle, bone). Little is known about the US public's perceptions of and attitudes toward VCA organ donation. This multi-site, cross-sectional, mixed methods study involved focus groups and surveys to assess members of the general public's attitudes about VCA, and willingness and barriers to donate VCA organs. Qualitative data were analyzed by thematic analysis; quantitative data were analyzed by descriptive statistics. In focus groups (n = 6, 42 participants), most participants were female (57%) and Black (62%) with mean age of 42.6 years. Three main themes emerged: 1) awareness and perceptions of VCA, 2) purpose of VCA donation, 3) and barriers to VCA donation. Participants had heard little about VCA and sought information about VCA donation. Participants perceived VCA as challenging their concepts of "normality" and voiced concerns that VCA would create "Frankenstein[s]." Barriers to VCA donation included disruptions to end-of-life arrangements and information gaps regarding the donation process. Participants reported moderate to high willingness to donate their hands (69%) and face (50%) Public education efforts should address the specific needs and concerns of the public to facilitate VCA donation and family authorization.

Keywords: vascularized composite allotransplantation, donation, perceptions, public education, focus group, qualitative, information needs

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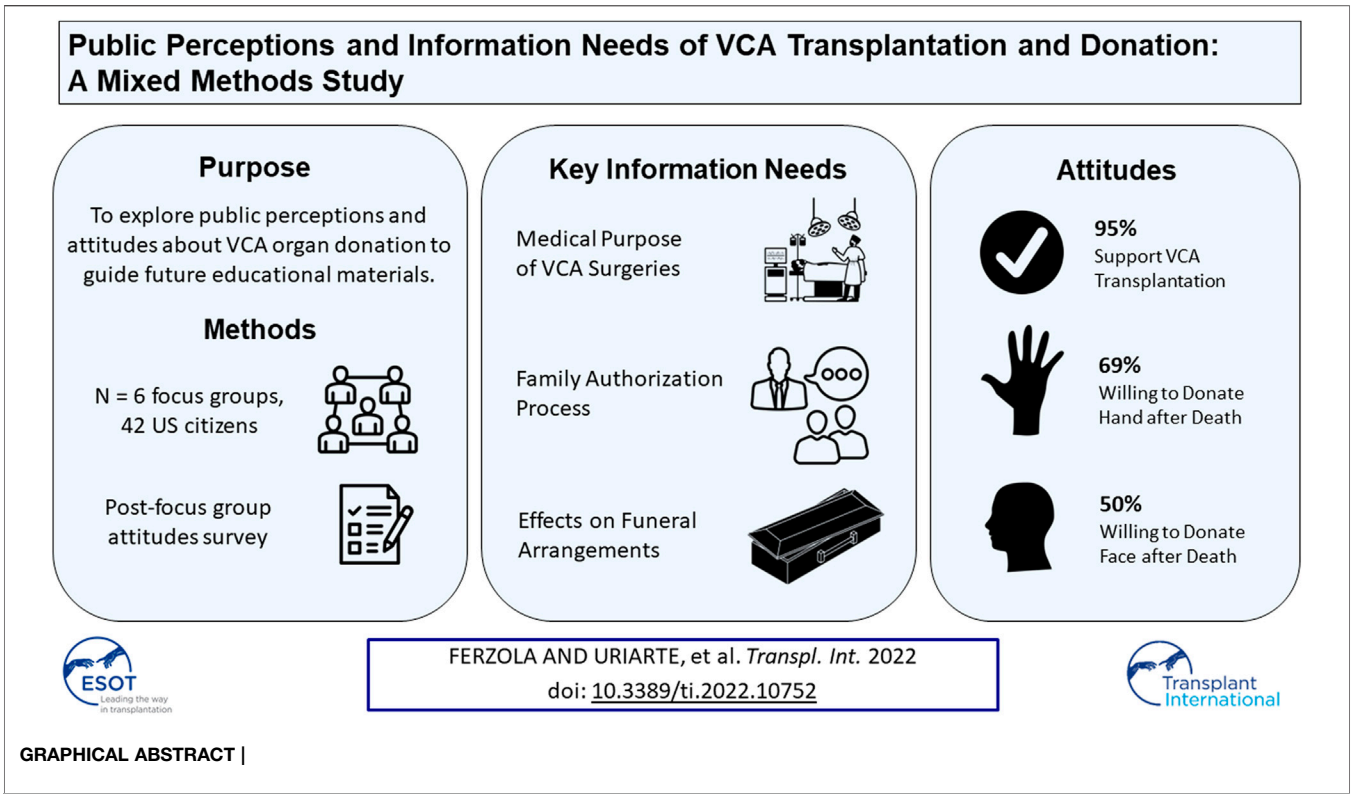
Abbreviations: DMV, department of motor vehicles; JHU, Johns Hopkins University; NU, Northwestern University; OPTN, organ procurement and transplantation network; US, United States; VCA, vascularized composite allotransplantation; VCAs, vascularized composite allografts.



GRAPHICAL ABSTRACT |

INTRODUCTION

Vascularized Composite Allotransplantation (VCA) involves the transplantation of intact vascularized body parts, such as the hand, face, abdominal wall, and uterus, from a donor to a recipient (1, 2). VCA can potentially improve the quality of life for individuals who have suffered catastrophic traumatic injury, infection, and/or congenital anomalies (3). VCAs include the hand, upper extremity, face, uterus, penis, abdominal wall, and larynx. In 2014, the United States (US) Organ Procurement and Transplantation Network (OPTN) defined VCAs as organs, thereby applying the same regulatory status for policy development and allocation as solid organs within the country. There have been more than 100 VCAs performed in the United States since 1998, and over 165 VCAs have been performed worldwide (4, 5). Despite advances in the field, VCA authorization and subsequent donation rates in the US remain low, and information needs of the public regarding VCA transplantation and donation are little examined, which may help explain low prevalence of VCA (6).

Prior research reports little public awareness of VCA in the US and in other countries, but suggests a promising willingness to donate VCA organs once the public is minimally informed about VCA (7–9). Although the US media has featured several VCA-related human-interest pieces, including news stories about face transplants, VCA information in the public sphere has been limited, and more comprehensive educational materials about VCA transplantation and donation are needed (10). Due to the lack of educational materials and the prominence of popular culture ideals surrounding the purpose of VCA, the public may misunderstand or hold misconceptions about VCA. For example, public opinion surveys about face transplantation in the US and worldwide have reported a common belief in VCA's purpose being primarily for cosmesis and psychological wellbeing rather than for functional use and survival benefits (7, 8, 11). Survey studies have found that public attitudes towards VCA are generally favorable, but may differ depending on the organ type (e.g., 53.8% willingness to donate a hand vs 39.0% willingness to donate a face) (7, 8, 12, 13).

Specific reasons for and insights into public willingness to donate VCA organs and barriers to VCA donation have been little examined, apart from perceived psychosocial benefits and risks regarding face transplantation (8, 9, 11). In addition, prior research on public attitudes about VCA has been based largely on surveys, and no research has qualitatively assessed the public's perceptions and attitudes to gain in-depth insights into potential facilitators and barriers to VCA donation. Qualitative research is well-suited for examining group perceptions and elaborating on reported attitudes as well as identifying knowledge gaps in not well known topics, such as VCA.

Understanding public perceptions of and attitudes towards VCA can help identify knowledge gaps and concerns to address in order to foster public understanding and trust with VCA authorization and donation (14). Identifying knowledge gaps in the public's understanding of VCA can reveal specific topics on which to provide information, common misconceptions to dispel, and barriers for donation to address. This paper assessed

the public's information needs, perceptions, and concerns about VCA to inform the development of educational materials to increase awareness of VCA donation.

MATERIALS AND METHODS

Study Design

We conducted a multi-site, cross-sectional, mixed-methods study involving focus groups and surveys to assess the general public's knowledge, perceptions, and willingness to donate or authorize VCA organs (15). A qualitative approach is useful for obtaining new, first-hand knowledge and descriptions about a phenomenon (16). Qualitative methods and results are reported in accordance with the Consolidated Criteria for Reporting Qualitative studies (17). Mixed methods enabled the elaboration and clarification of findings and increased validity of results (18).

Setting and Participant Selection

The study was conducted at Johns Hopkins University (JHU) and Northwestern University (NU). Individuals were eligible for inclusion if they were English-speaking adults (>18 years) and US residents. Participants were recruited outside of Departments of Motor Vehicles (DMVs) in Baltimore, MD (n = 1 location) and Chicago, IL (n = 5 locations) between June and August 2019. DMVs offer excellent access to the general population for broad representation of the public. Research staff recruited interested individuals in-person by handing out flyers outside of DMVs and obtained their contact information for follow-up calls to schedule focus groups. Data collection occurred from June 2019 to December 2019. The Institutional Review Boards JHU (IRB00179535) and NU (STU00207605) granted approval. Participants provided written informed consent.

Data Collection

We conducted n = 6 in-person focus groups (n = 3 focus groups at JHU in Baltimore, n = 3 focus groups at NU in Chicago), based on *a priori* goals for reaching thematic saturation (19, 20). Focus groups and surveys were conducted to assess public attitudes about VCA and inform subsequent development of VCA educational materials. A team of qualitative researchers and VCA experts developed the focus group moderator's guide based on a prior content analysis of available public educational resources about VCA (10). The moderator's guide was not pilot tested, but was reviewed by social scientists and clinical VCA experts to enhance face and content validity. Focus group questions assessed public perceptions, knowledge, and willingness to donate VCA organs. Focus groups were conducted by expert or trained focus group moderators (EJG, HCS, AF). Moderators used standardized guides to ask open-ended questions and encourage group participation (**Supplementary File S1**). Research assistants took hand-written field notes about the discussion and participant interactions. Before each focus group, the research team presented minimal information about the definition of VCA, types of VCA organs, and the definition of deceased donor to facilitate discussion. The research team answered participants' questions related to relevant VCA discussion topics. Focus groups lasted approximately 60–120 min and were audio-recorded. Immediately following the focus groups, participants completed the paper attitudes survey in-person. The attitude items were adapted from a survey investigating attitudes toward VCA in metropolitan populations (8). The survey included closed-ended questions assessing support, willingness, and distaste for VCA using a 5-point Likert scale, and demographics (e.g., gender, age, race, education, marital status, employment, household income, health insurance, and prior experience with organ transplantation; **Supplementary File S2**, survey questionnaire). Participants were compensated $35 and $50 at NU and JHU, respectively, for their time.

Qualitative Analysis

Audio recordings of focus groups were de-identified and transcribed verbatim. We analyzed transcripts using thematic analysis with both deductive and inductive coding (21, 22). Deductive codes were developed based on the questions asked during the focus groups. Inductive codes emerged for new topics during the focus groups (23). Transcripts were coded by four researchers (AF, HCS, NA, JU) trained in qualitative research methods by EJG, who has qualitative research expertise. Two researchers coded each transcript. Multiple rounds of coding with different coder pairs were conducted to establish inter-rater reliability (kappa ≥0.80). Differences in coding were reconciled by group consensus (24). After coding, we developed themes through writing code summaries to analyze common and disparate thematic concepts within each code segment across all focus groups and then compared thematic concepts across all codes. We used NVivo (12. lnk, QSR International Inc., Burlington, MA) for qualitative analysis.

Quantitative Analysis

Descriptive statistics were performed on the post-focus group survey items assessing participants' attitudes toward VCA. We calculated frequencies, means, and standard deviations (SDs) and compared attitudes by study site using Chi-squared and t-tests (p-value ≥ 0.05 was considered significant). We used Stata 17.0/MP for Linux (College Station, Texas).

RESULTS

Demographics

Forty-two individuals (JHU: n = 15, NU: n = 27) participated in the focus groups (participation rate: 17%). Focus groups included, on average, 7 participants (range: 3–11). Most participants were female (57%), African American (62%), and had no prior experience with organ transplantation (69%). Participant demographic characteristics are detailed in **Table 1**. Sites differed demographically in terms of race/ethnicity, education level, employment status, and primary health insurance.

Focus Group Themes

Three main themes, or unifying concepts about subjects or meanings within the data (24), emerged from the focus

TABLE 1 | Participants' sociodemographic characteristics.

Variable	Total (N = 42) N (%)	JHU (*n* = 15) n (%)	NU (*n* = 27) n (%)	*p*-value
Age, mean [SD] (range)[a]	42.6 [14.2] (20–72)	45.1 [13.3] (24–62)	41.3 [14.5] (20–72)	0.33
Gender				
Female	24 (57.1)	6 (40.0)	18 (66.7)	0.12
Male	18 (42.9)	9 (60.0)	9 (33.3)	
Race/Ethnicity				
African American/Black	26 (61.9)	13 (86.7)	13 (48.1)	0.02[b]
White	9 (21.4)	2 (13.3)	7 (25.9)	0.45
Hispanic	5 (11.9)	0 (0.0)	5 (18.5)	0.14
Asian	4 (9.5)	0 (0.0)	4 (14.8)	0.28
Other	1 (3.7)	0 (0.0)	1 (3.7)	0.36
Marital Status				
Never married/single	19 (45.2)	6 (40.0)	13 (48.1)	0.31
Married/Domestic partner/Civil union	14 (33.3)	7 (46.7)	7 (25.9)	
Separated or Divorced	5 (11.9)	0 (0.0)	5 (18.5)	
Widowed	4 (9.5)	2 (13.3)	2 (7.4)	
Education				
Less than high school graduate	2 (4.8)	1 (6.7)	1 (3.7)	0.010
High school graduate	13 (31.0)	8 (53.3)	5 (18.5)	
Some college	14 (33.3)	6 (40.0)	8 (29.6)	
College graduate	8 (19.0)	0 (0.0)	8 (29.6)	
Post graduate degree	5 (11.9)	0 (0.0)	5 (18.5)	
Health Literacy (Help Needed for Reading Health Materials)[c]				
Adequate	37 (88.1)	15 (100)	22 (81.5)	0.18
Inadequate	5 (11.9)	0 (0.0)	5 (18.5)	
Employment Status				
Employed full-time	17 (40.5)	3 (20.0)	14 (51.9)	0.012
Not employed	12 (28.6)	8 (53.3)	4 (14.8)	
Retired	5 (11.9)	2 (13.3)	3 (11.1)	
Employed part-time	4 (9.5)	0 (0.0)	4 (14.8)	
Disabled	2 (4.8)	2 (13.3)	0 (0.0)	
Homemaker	1 (2.4)	0 (0.0)	1 (3.7)	
Student	1 (2.4)	0 (0.0)	1 (3.7)	
Income[d]				
<$15,000	13 (31.7)	6 (40.0)	7 (26.9)	0.21
$15,000-$34,999	12 (29.3)	6 (40.0)	6 (23.1)	
$35,000-$54,999	11 (26.8)	2 (13.3)	9 (34.6)	
$55,000-$74,999	3 (7.3)	0 (0.0)	3 (11.5)	
$75,000-$94,999	1 (2.4)	1 (6.7)	0 (0.0)	
$95,000+	1 (2.4)	0 (0.0)	1 (3.8)	
Primary health insurance				
Private	19 (46.3)	2 (13.3)	17 (65.4)	<0.001
Medicaid/Medicare	16 (39.0)	12 (80.0)	4 (15.4)	
None	4 (9.8)	1 (6.7)	3 (11.5)	
Other	2 (4.9)	0 (0.0)	2 (7.7)	
Registered Donor[e]				
Yes	21 (52.5)	8 (53.3)	13 (52.0)	1.00
No	19 (47.5)	7 (46.6)	12 (48.0)	
Experience with organ transplant				
Neither me nor anyone in my family has received a transplant or been on a transplant list	29 (70.7)	9 (60.0)	20 (76.9)	0.19
Not sure	5 (12.2)	4 (26.7)	1 (3.8)	
Someone in my family has received a transplant or been on a transplant list	4 (9.8)	0 (0.0)	4 (15.4)	
I have received a transplant or been on a transplant list	3 (7.3)	2 (13.3)	1 (3.8)	
Hours on the internet in a week				
I did not use the computer	3 (7.1)	1 (6.7)	2 (7.4)	0.95
Less than 5 h	6 (14.3)	3 (20.0)	3 (11.1)	
5–10 h	8 (19.0)	3 (20.0)	5 (18.5)	
10–15 h	8 (19.0)	2 (13.3)	6 (22.2)	
15–20 h	1 (2.4)	0 (0.0)	1 (3.7)	
More than 20 h	16 (38.1)	6 (40.0)	10 (37.0)	

[a]*JHU* n = *1 not reported.*

[b]p-*values measured across each race relative to each other.*

[c]*Participants with responses "never," and "rarely" were considered to have adequate health literacy. Responses of "sometimes," "often," and "always," were considered to have inadequate health literacy.*

[d]*NU* n = *1 not reported.*

[e]*NU* n = *2 not reported.*

groups: 1) awareness and perceptions of VCA, 2) VCA donation, 3) and barriers to donate VCA organs. Each theme comprised 3 or 4 sub-themes. Themes and corresponding representative excerpts can be found in **Table 2**.

Awareness and Perceptions of VCA

Most participants reported being unfamiliar with VCA. Participants discussed their initial perceptions of VCA, compared VCA to solid organ donation, and asked questions about a variety of VCA topics.

Initial Perceptions of VCA

Most participants across all focus groups had never heard of VCA prior to study recruitment. While participants had not heard of the term "VCA", some participants recalled hearing about face and hand transplants through major news outlets and newspaper articles. Participants associated VCA, particularly face transplants, with popular culture references including the television show "Game of Thrones" and the movie "Face Off." Participants perceived VCA as a procedure from fantasy or science fiction and commented about the potential of VCA to create "cyborgs," "clones," or "Frankenstein[s]." Accordingly, they expressed concerns that as VCA evolved, it may push the boundaries of "normality." Furthermore, participants perceived VCA as "weird" or strange to imagine "your face on someone else's [face/body]."

Perceptions of VCA in Relation to Other Solid Organ Transplantation

As VCA was an unfamiliar topic, participants used their knowledge of the more familiar solid organ transplants (e.g., liver, kidney, and heart transplantation) to ask about or note similarities and differences compared to VCA. Participants described solid organs as "internal," while they classified VCA organs as "external" because people can visualize it or "see how it looks." When discussing "external" organs such as hands or faces, discussions focused on the appearance of the donated VCA organ on its recipient after surgery.

Participants viewed "internal" organ transplantation as vital or lifesaving, but questioned the medical "purpose" or necessity of VCA, specifically VCAs such as uterus and penis. They also questioned if the potential benefits of VCA would outweigh the risks to its recipients (e.g., side effects, medical complications, immunosuppression drugs).

Questions About VCA

Overall, participants asked 208 questions about VCA during focus group discussions, reflecting their information needs. Participants asked about numerous topics including the history of VCA, potential VCA recipients, outcomes of VCA recipients, and the processes for donating and for receiving VCAs. Regarding the relationship between VCA donors and recipients, participants desired clarification on how donors and recipients are matched for skin color and size, if recipients would appear exactly like their donor, and if the recipient's new appearance would create legal identification issues (e.g., identification photos, fingerprints). A comprehensive list of participant questions can be found in **Table 3**.

VCA Donation

Across focus groups, participants discussed reasons that they were more or less willing to donate or authorize VCA organs. Some participants expressed that they were willing to donate all VCA organs, while others provided reasons for being unwilling to donate specific VCA organs. Participants sought clarification on the VCA donation and authorization processes.

In the post-focus group surveys, nearly all participants (95%) reported that they "strongly agreed" or "agreed" that they were in support of VCA transplantation (**Table 4**). Furthermore, only a few individuals (4%) reported that VCA transplantation was distasteful to them.

Reasons to Donate VCA Organs

Participants willing to donate any VCA organ noted their perceived benefits of VCA were to "help a lot of people" and to improve the quality of life of its recipients. Participants proposed that burn victims, people from the military, and people with "defects" could potentially benefit from being recipients of VCA. Participants reported that they would feel comfortable donating VCA organs to a recipient who had undergone "something traumatic" and who would "use it wisely," but they would not donate to someone who only wanted to pursue VCA for plastic surgery. Some participants reported being amenable to donating VCA organs because they perceived VCA donation to be a similar concept to solid organ donation. Other participants expressed that they would be willing to donate VCA organs "for the name of science," or in order to advance the field.

Willingness to Donate Hands and Face

Participant's comments suggested mixed opinions and hesitation or concern about donating hands and faces. Compared to donating solid organs, participants perceived hand or face to be "weird" and "emotional," as the hands and face are more closely related to appearance and personal identity. Participants did not want to donate their own hands and/or face because they did not want family members to feel uncomfortable during funerals. Participants were also concerned that family members could experience emotional "trauma" from seeing their loved one's organs on the recipient's body.

Despite expressed concerns about hand and face donation, participants reported moderate to high willingness to donate their hands (69%) and face (50%). Participants were more willing to receive hands (76%) or a face (61%) than to donate these organs (**Table 4**).

Willingness to Donate Penis and Uterus

Participants expressed strong views about uterus and penis transplants. They reported not being willing to donate a uterus or penis if prospective recipients wanted to have "a sex change" because these motivations went against participants' religious and personal beliefs. Comments about changing bodies and genders

TABLE 2 | Representative excerpts by theme.

1: Awareness and perceptions of VCA	
1.1: Initial Perceptions of VCA	"It's like the way things look has so much more of an impact on people even though people do not always say it does it does. And so I think that's why there's like this weird . . . it's just like a little awkward because I do not even know if I could -- it would be just as weird to imagine your hand on someone else's or like your face on someone else's." [Site 1, FG 2, Woman C]
1.2: Perceptions of VCA in Relation to Other Solid Organ Transplantation	"What would pop in my head if somebody told me that they had a [VCA] transplant? I do not know, I guess I would just look at them and say, 'It looks good.' Or maybe, 'They messed you up.'" [Site 2, FG 2, Man A] "Is [VCA] important? I mean, I can see an internal organ. I mean, you'll die. But if you live with one hand, you will not potentially die, and that's what we're hoping for, that nobody dies." [Site 1, FG 1, Man A] "You can live without a uterus and you can live without a penis. So what's the medical reason for somebody to have to get somebody else's uterus or penis other than them wanting it? Is that what you were saying? . . . What's the purpose of giving it to somebody?" [Site 2, FG 3, Woman A]
1.3: Questions about VCA	A comprehensive list of participant questions can be found in **Table 3**
2: VCA Donation	
2.1: Reasons to Donate VCA Organs	"I personally do not have any negative emotions toward [VCA] at all . . . It is [a] positive thing because I think it's cool after you die, where you have one last thing to help however many people . . . and you can help that many people regardless of whether its extending life or just improving quality of life. Like that would be important to me, and I think that would be important to my family too." [Site 1, FG 1, Woman C] "I'd probably just do it for the name of science. Just for the future, not necessary to save people, but just in the name of science, so they can further study and perfect it on how to do this with people in the future." [Site 1, FG 3, Man A]
2.2: Willingness to Donate Hands and Face	"If I was a donor, I would donate my hand no problem, but not my face. Because that would be weird for my children. You know, they're going to want to have a little funeral for me and even though I will not be here any longer, I just think that's weird. The thing [s] that you gotta think [about face donation], and I will not donate. I do not care if I were getting cremated, I will not do it." [Site 1, FG 2, Woman A] "I think I would be more okay with an organ than with a hand. I do not know, it does not matter but it just feels weird . . . But now, when I think hand or face, I feel different than internal organ. And I think it's emotional . . . [Site 2, FG 1, Woman D]
2.3: Willingness to Donate Penis and Uterus	"Like, if I died, I would not want nobody getting my uterus . . . If it was just that they just wanted to have some children I do not agree with that." [Site 2, FG 3, Woman A] "What if somebody comes in and just be like, 'I want a sex change?'. . . Because I feel as though, like myself, if I'm donating my body to help somebody, I do not want it to go to somebody that just wants their chemicals changed." [Site 2, FG 2, Man D]
2.4: VCA Authorization	"And that person's family, it do not matter if it resonate. If that person says what they want to do, it should be done." [Site 1, FG 1, Male B] "I think that's [VCA authorization] pointless because, if you already signed up for it when you were alive, and then somebody got to reauthorize it for when you dead or you're about to die, . . . then it would be an issue." [Site 2, FG 3, Female A] "Female F: They want it right away. They kept calling about my mother when she passed, like they want it right then and then. Like its no, you cannot . . . they cannot grieve Female D: Grieve Female F: They cannot wait that long. It has to be right away. So you have to make your mind up immediately Female D: That's why she was saying, they all have to do that before they pass. You know, then it's their decisions, your loved ones." [Site 1, FG 3, Females D and F] "I think I would have to tell them, 'When I go, you might see somebody that might look like me, might get my face, might get my hand, they might touch you and feel my --. . . I think that would be right, something that you can discuss with your family and your loved ones. It's still your hand, your face, that's a part of you, so if I've been around you for 70 years then I'm going to know your hands, I'm going to know your face. If I had to give this to somebody else to live, I would want somebody to expect that it might come up they might visualize me when I'm gone and they . . . they may have a trauma." [Site 2, FG 1, Male A]
3: Barriers to Donate	
3.1: Religious and Cultural Beliefs	"Yeah. I see some difficulties when it comes to religion. And donating and different things because families have difficulties even dealing with whether their loved one want to be cremated or not or go the traditional route. That's based on some religious beliefs. And, yeah, if religion is going to play a big part in whether the family or if the donor has not specified what they want to do other than being an organ donor, that will play a big part in whether families are willing to do that." [Site 2, FG 3, Male 1]
3.2: Fear of Death	"Ok, well I think there's going to be people rational or irrational that are going to have fears about well what's really going to happen to my body parts? there's just a lot of fear out there that is maybe unfounded that still rattles around and keeps people from donating." [Site 1, FG 1, Female A]

(Continued on following page)

TABLE 2 | (*Continued*) Representative excerpts by theme.

3: Barriers to Donate	
3.3: Need to Improve Public Awareness of VCA	"Information is key, you know, every community if they're not properly informed, their mind's going to run wild with the idea of what could happen, what could be, so that's what I think it comes down to is properly educating people." [Site 2, FG 1, Man B] "I think the biggest issue is a lack of education, and a lack of awareness. And that people do not know that much about it. If they just knew just as much about [VCA] as they did about a heart transplant." [Site 1, FG 2, Woman C]
3.4: Suggestions to Increase VCA Awareness	"The important information -- my opinion is it should be about saving lives . . . It should be mainly about the quality of their life and how donating these different parts of the body would or could affect someone else's quality of life. They could live a little better or a little longer. I think that that should be stated a lot that would help [Site 1, FG 3, Man A] "Write about real life experiences. People that have gone through the process, received a hand or hands and face and how their life was improved." [Site 2, FG 1, Man A]

sparked heated discussion between participants as they disagreed whether there should be "stipulations" or non-medically related eligibility criteria to receive VCA.

VCA Authorization

Participants expressed confusion about the authorization process for VCA donation in the US because it requires a next-of-kin or family authorization after the registered donor's death, which is not required for solid organ donation. Some participants who were registered donors believed that they had already authorized VCA donation, and thus were confused about family authorization. After clarifying the VCA authorization process, some participants stated that there was "no point in signing up" as a VCA organ donor because the family and/or next of kin "will still have to agree" to the donation decision. Participants discussed the importance of interested potential VCA donors to speak with their families about their desire to become VCA donors and agreed that the family or next of kin should "respect" and concede to the individual's "wishes" to donate VCA organs.

Participants expressed several concerns for their families in making VCA authorization decisions. Participants discussed the burden placed on families who would have to make authorization decisions quickly to ensure VCA organs remain viable for transplantation. Participants feared that family members would not have ample time to "grieve" the death of their loved ones. Participants agreed that families should have discussions about VCA donation wishes to prepare for the burden of decision making and seeing their loved one's VCA organs on another individual.

Barriers to Donate

Across focus groups, participants discussed potential barriers to VCA donation which included: religious and/or cultural beliefs, fear arising from thoughts about death, and lack of information and awareness of VCA donation. Further, participants made suggestions on how to increase public knowledge and awareness of VCA donation to address potential barriers.

Religious and Cultural Beliefs

Participants commented that VCA donation might violate religious and cultural beliefs and interfere with the donor's plans "to have an open casket" funeral, especially after face donation. Participants recognized that individuals from various religious and cultural backgrounds may want to keep their bodies intact after death. Some participants commented that the organ procurement process might inhibit family member's ability to "grieve" for their loved one before an organ procurement agent approaches them to make an authorization decision.

Fear of Death

Participants discussed the visceral or "irrational" fear that the public may experience when they first hear about VCA donation. Participants stated that fear could arise from associating VCA donation with death and imagining their body parts, including their limbs and faces, being removed. Furthermore, participants recognized that people may fear VCA because of its relative newness compared to solid organ transplantation and the lack of knowledge and awareness about VCA donation among the public.

Need to Improve Public Awareness of VCA

Focus group participants stated that the lack of information and awareness about VCA would prevent the public from donating VCA organs. Participants acknowledged that people may be misinformed and possess "incorrect ideas" about VCA donation and its purpose. Participants suggested that the lack of awareness surrounding VCA could be addressed through education.

Suggestions to Increase VCA Awareness

Participants recognized the importance of educating the public about VCA to increase awareness. Participants recommended including a description and purpose of VCA in educational materials, such as clarifying that VCA is for medical rather than cosmetic reasons to improve a person's quality of life. Because many participants were learning about VCA for the first time, they suggested explaining the acronym "VCA" and making the term VCA understandable. Participants agreed that public educational materials should be comprehensive and describe risks, side effects, and outcomes. Participants explained that providing clear information about the pros and cons of VCA could help

TABLE 3 | Representative participant questions.

Success and outcomes of VCA (*n* = 6 focus groups)
All of [VCA] surgeries been a success?
What's the percentages of the [VCA] completely working? What's the percentages of the failure?
Has there been a time you have attached the hand and had to remove it because it just did not work?
Will you function normally [after VCA]?
Suppose the hands or the upper limbs, they get done and everything but they're not successful. Do they redo it and try to connect it and find the problem? Or that's it for you?
What was the success rate?
How did the [recipients] accept it? You know, how's their mental state?
Another concern like going back to psychological aspects, how—a person's face is almost integral to who they are as a person. How much does the face transplant affect their appearance to the point where they become indistinguishable from who they used to be? Like how successful is that?
Are they still working the same for them? How my hands working when I had, how do they actually feel you know. What type of joy would it bring to them after they have received it?
VCA Surgery Process (*n* = 6 focus groups)
When you do the kidneys or when you do the blood transfusion, with the hand or the face, do you still have to have that same blood type?
Or is [VCA] like a graft again where they take a part and try to grow it or--?
With the face transplants is it the full face or do they just get parts of their face transplanted?
With these transplants right here, like the uterus and the penis, so when they transplant, do they transplant the full uterus, and the full penis? Or is it partial?
History of VCA (*n* = 5 focus groups)
Where was the first VCA performed?
Are they doing it in the States?
Now how long have they been doing this procedure, the VCAs?
Is this something being done now or are you talking futuristic?
Timeline of VCA Process (*n* = 5 focus groups)
How long does the process take for the surgeries and everything? You said you've got to match and do the blood and all that. Like, how long would we be waiting?
How long is the recovery?
How lengthy is the process, like donor as well as recipient, to have to fill out complete paperwork? Is it hard or is it easy?
Like the process of rehabilitation, do you have to go through the same process with that transplanted arm or limb, just like if you were to rehabilitate yourself? Would it be like the same process you have to work that hand out or limbs out the same way?
Appearance of VCA Organs (*n* = 5 focus groups)
When they say face transplant it's like you completely change it?
How would the face and everything, how would they get you to look close to that skin or something like that?
Will [the donated face] be the exact same look as me?
Does there have to be some kind of compatibility? Like, small versus large, women versus men?
Now do they match color and color?
Cost of VCA (*n* = 5 focus groups)
Well who will pay for that, insurance would not pay for that, right?
Okay say if it was me and I needed one of these VCA transplants, I would not even be able to afford it because I looked it up and they cost four, five million dollars for some of these so how would that work out for me?
Would insurance cover it or you got to pay for it in cash?
Potential VCA Recipients (*n* = 4 focus groups)
So [VCA] would only be just for soldiers and veterans?
Was it just a regular person that got it done?
Would [VCA] just be for the other people that can afford it?
With this transplant, does age have anything to do with it? Do you have to be 18 and over or 21 and over to be qualified to do transplant? Or can it be a child?
So can it be used for people who have been severely burned, third degree burns?
Becoming VCA Donors (*n* = 4 focus groups)
So you can pick [which organs to donate], you can be like, "Okay, you can take their hand or their foot"?
VCA is going to be added to the Motor Vehicles if people want to donate this . . . ?
Will it become like a part of like a contract where you know when you go to the hospital and you sign the waiver about being treated and everything, will you start putting that into the form, too, like if you want if something happens, would you want your body donated? Would that start becoming inside of that contract?
The question on our driver's license, it's just are you a donor, yes or no . . . did it always imply every part of your body?
You know how you sign up to be an organ donor? You've got to actually go and sign up to be a VCA donor? Or is it all composited into one?
Family Authorization (*n* = 3 focus groups)
Say if somebody is an organ donor and they got one of those "do not resuscitate" orders do they still ask their family for their organs?
So even though you signed off to be an organ donor your family still got to agree with it at the end?
Even if you do not sign off on it, your family will still have to agree at the end anyway, right?
Would you ask [the family] right away when they die, or would you wait? You know, cause some people are grieving, and they get angry, and they'll be like, "No!" Would you ask right away, or would you wait?
Religious/Cultural Concerns (*n* = 3 focus groups)
Have you surveyed any other religions, and know which ones would be the ones that say no?
Do they include religion to it as a factor when they go to pick [VCA organs]?
They just do not consider religion and lifestyle? If it's the same blood type then you're getting it. That's how it goes pretty much?

(Continued on following page)

TABLE 3 | (*Continued*) Representative participant questions.

Funeral Concerns (*n* = 3 focus groups)
When do they take off the face and the hand? After the funeral?
How soon after the person like dies would you take their hands and face?
Will you still be able to have a funeral?
Association of VCA with other medically-related procedures (*n* = 2 focus groups)
Is it like I can just call and say, "Hey, I want to do this" like plastic surgery?
What if somebody comes in and just be like, "I want a sex change?"
If this is at all possible, then we're talking about possibly clones. Are we going that far?

TABLE 4 | Participant attitudes about VCA.

Factor		N[a]	Strongly disagree n (%)	Disagree n (%)	Neutral/ Unsure n (%)	Agree n (%)	Strongly agree n (%)	*p*-value[b]
I support VCA transplantation	Total	42	0 (0)	0 (0)	2 (5)	16 (38)	24 (57)	0.10
	Northwestern	27	0 (0)	0 (0)	2 (7)	7 (26)	18 (67)	
	Johns Hopkins	15	0 (0)	0 (0)	0 (0)	9 (60)	6 (40)	
I would be willing to donate my hand upon death	Total	42	0 (0)	2 (5)	11 (26)	12 (29)	17 (40)	<0.001
	Northwestern	27	0 (0)	1 (4)	9 (33)	2 (7)	15 (56)	
	Johns Hopkins	15	0 (0)	1 (7)	2 (13)	10 (67)	2 (13)	
I would be willing to donate my face upon death	Total	40	2 (5)	5 (12)	13 (32)	6 (15)	14 (35)	0.09
	Northwestern	26	2 (8)	2 (8)	8 (31)	2 (8)	12 (46)	
	Johns Hopkins	14	0 (0)	3 (21)	5 (36)	4 (29)	2 (14)	
I would be willing to receive a hand transplant after a severely deforming accident	Total	42	0 (0)	0 (0)	10 (24)	21 (50)	11 (26)	0.70
	Northwestern	27	0 (0)	0 (0)	7 (26)	12 (44)	8 (30)	
	Johns Hopkins	15	0 (0)	0 (0)	3 (20)	9 (60)	3 (20)	
I would be willing to receive a face transplant after a severely deforming accident	Total	41	1 (2)	1 (2)	14 (34)	18 (44)	7 (17)	0.30
	Northwestern	26	1 (4)	0 (0)	7 (27)	12 (46)	6 (23)	
	Johns Hopkins	15	0 (0)	1 (7)	7 (47)	6 (40)	1 (7)	
VCA is distasteful to me	Total	42	17 (40)	16 (38)	7 (17)	1 (2)	1 (2)	0.20
	Northwestern	27	12 (44)	7 (26)	6 (22)	1 (4)	1 (4)	
	Johns Hopkins	15	5 (33)	9 (60)	1 (7)	0 (0)	0 (0)	

For understanding participant attitudes from the post-focus group survey, agree and strongly agree were combined for general favorability.

[a]*N refers to number of respondents to each question.*

[b]*p-value measured for differences between research sites; missing data was treated as a separate variable.*

potential recipients and donors make informed decisions. Further, participants suggested including VCA success stories and recipient testimonials to make VCA more relatable or appealing to the general public.

Participants also recommended different types of informational modalities to educate the public. Participants suggested the use of social media and advised sharing educational materials in public locations that typically engage large numbers of people (e.g., train stations and bus stops). Participants mentioned targeting education campaigns to potential audiences who could benefit most from VCA educational materials, specifically students and healthcare workers. Overall, participants recommended making information accessible, comprehensive, and relatable to the public.

DISCUSSION

Our qualitative study of public attitudes about VCA in the US found that while participants were generally unaware of VCA, they may be willing to donate certain VCA organs after being informed about VCA and they may possess certain religious/cultural beliefs that prevent them from donating. Participants' information needs about and barriers to VCA donation should be addressed through educational materials to help increase awareness and accurate knowledge of VCA, its purpose, and the authorization and donation process.

Participants' impressions of VCA pertained to misrepresentations and/or misconceptions about VCA likely due to a lack of awareness about the procedure and the information presented in the public sphere through popular

television shows and movies. Media and popular culture influences the daily lives of the public, which affects how and what people think about themselves and others, including personal and social issues (25). In addition, prior media coverage of VCA and organ transplantation in general has tended to promote stories that are "sensational" rather than strictly for educational purposes (10). Thus, raising awareness and properly educating the public about VCA may help to address misinformation spread through media and to foster understanding about the purpose of VCA transplantation and donation.

Participants, through focus groups and surveys, reported varying levels of comfort in supporting specific VCA organs. Our focus group and survey findings corroborate previous survey studies from around the world that reported less willingness to donate VCA organs than solid organs (kidney, liver, heart, lungs) and greater willingness to donate hands than the face, penis, or uterus (8, 12, 13). In addition, qualitative insights from focus groups corroborate reasons for lower willingness to donate the face, including not wanting to donate in order to retain one's identity and bodily integrity after death and to allow for their family's grieving (8, 11). Moreover, our focus group study found that participants in the two US metropolitan areas sampled from might be unwilling to donate a uterus or penis to a recipient who desired to alter their sex, which contrasts with a US survey study reporting 69.3% public willingness to donate a uterus or penis to an individual of a different sex (13). To our knowledge, no VCAs have been performed for the purpose of transgender sex changes to date.

Participants expressed confusion about the authorization process to become a VCA donor in the US and how this differs from solid organ donation. Participants viewed donation as complicated mostly because individuals were unaware of the proper procedure(s) of becoming a VCA donor. Many participants believed that once an individual becomes a registered donor through the Department of Motor Vehicles, they are authorizing VCA donation in addition to authorizing donation for other solid organs. Moreover, because VCA authorization occurs quickly after the death of the potential donor and is provided by the next of kin, VCA authorization may become a burden for family members dealing with grief and funeral planning. VCA educational materials should address confusion regarding VCA authorization by explaining the steps needed to become a VCA donor. Such information will educate and better enable individuals to engage in conversations with family members to express their desire to become a VCA donor and help family members prepare for next-of-kin authorization.

Participants recommended making information accessible, comprehensible, and relatable to increase public knowledge and awareness of VCA. Our prior content analysis of existing educational materials for VCA, including materials from OPOs, transplant centers, OPTN, and the Department of Defense, revealed that most materials referenced a specific story (75%), some materials described potential benefits (15%), and few mentioned the appearance of a transplanted VCA organ (1%) (10). While pre-existing materials were relatable by describing specific case studies of individuals who the public can see and feel empathy for, materials did not address topics such as the difference between VCA and other solid organs, VCA authorization and donation processes, and culturally specific burial customs which were topics of discussion in focus groups. By addressing these information gaps and concerns, educational materials may increase the public's awareness and understanding of VCA and help ameliorate concerns about VCA donation.

Educational materials should address participants' most prevalent information needs, such as describing VCA outcomes transparently, understanding the VCA evaluation and surgical process, information on the state of VCA, and dispelling misconceptions such as appearance modification after transplant. Through educational materials, we may also begin to address concerns that individuals hold about family donation and the cultural and/or religious barriers to donation.

Our study has several strengths. We conducted focus groups at multiple sites located in large, geographically distinct US cities, with participants representing diverse backgrounds, which increases the transferability and generalizability of our findings. A limitation of this study is that participant statements and attitudes towards VCA may not reflect actual behaviors. We recruited from urban and suburban DMVs, and thus findings may not be generalizable to rural populations (26). With the results of this study outlining the major barriers and concerns about VCA and VCA donation, future research should leverage study findings to inform the development of educational materials, then assess whether implementation of educational interventions with a culturally competent focus can contribute to an increase in positive public perceptions of VCA and VCA donation rates.

CONCLUSION

Our study assessed the public's knowledge, perceptions, and willingness to donate VCA organs to inform the development of educational materials to increase awareness of VCA donation. Study findings revealed that although the general public may have concerns and information needs about VCA donation, willingness to donate VCA organs is generally favorable. Public education should address the specific information needs and concerns outlined by members of the public in order to better prepare the public to become VCA donors and/or authorize VCA donation.

DATA AVAILABILITY STATEMENT

The datasets presented in this article are not readily available because the raw data are proprietary and might be used to author future publications. Requests to access the datasets should be directed to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Johns Hopkins University Institutional Review Board and the Northwestern University Institutional Review Board. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

ML conceived and designed the research study, oversaw the study, and participated in performing the research. EJG contributed to research design, oversaw the study and participated in performing the research and data analysis. GB, IJ, DS, CC, and JS contributed to research design. AF and JU contributed to coordination of the study, subject recruitment, data collection, data analysis, and writing of the paper. HS, NA, and WL contributed to subject recruitment, data collection, data analysis, and writing of the paper. MD, CS, SK, LY, KV, and SV contributed to writing of the paper.

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AUTHOR DISCLAIMER

The analyses described here are the responsibility of the authors alone and do not necessarily reflect the views or policies of the US Department of Health and Human Services, the US Department of Defense, nor does mention of trade names, commercial products or organizations imply endorsement by the US Government.

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.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontierspartnerships.org/articles/10.3389/ti.2022.10752/full#supplementary-material

REFERENCES

1. Caplan AL, Parent B, Kahn J, Dean W, Kimberly LL, Lee WPA, et al. Emerging Ethical Challenges Raised by the Evolution of Vascularized Composite Allotransplantation. *Transplantation* (2019) 103(6):1240–6. doi:10.1097/TP.0000000000002478
2. Dean W, Randolph B. Vascularized Composite Allotransplantation: Military Interest for Wounded Service Members. *Curr Transpl Rep* (2015) 2:290–6. doi:10.1007/s40472-015-0072-7
3. Cherikh WS, Cendales LC, Wholley CL, Wainright J, Gorantla VS, Klassen DK, et al. Vascularized Composite Allotransplantation in the United States: A Descriptive Analysis of the Organ Procurement and Transplantation Network Data. *Am J Transpl* (2019) 19(3):865–75. doi:10.1111/ajt.15062
4. Lewis HC, Cendales LC. Vascularized Composite Allotransplantation in the United States: A Retrospective Analysis of the Organ Procurement and Transplantation Network Data after 5 Years of the Final Rule. *Am J Transpl* (2021) 21(1):291–6. doi:10.1111/ajt.16086
5. Jacoby A, Cohen O, Gelb BE, Ceradini DJ, Rodriguez ED. Vascularized Composite Allotransplantation and Immunobiology: The Next Frontier. *Plast Reconstr Surg* (2021) 147(6):1092e–1093e. doi:10.1097/PRS.0000000000007954
6. Vece GR, Robinson A, Rosendale J, Cherikh W, Curran C, Wholley C, et al. Maximizing Donors' Gifts: A Comparison of Actual and Expected Solid Organ Yield Among VCA Donors. *Am J Transpl* (2021) 21(3):1263–8. doi:10.1111/ajt.16319
7. Denadai R, Araujo KC, Pinho AS, Denadai R, Araujo GH, Raposo-Amaral CE. Public's Knowledge and Attitudes toward Vascularized Composite Allotransplantation and Donation. *Aesthet Plast Surg* (2016) 40(2):301–8. doi:10.1007/s00266-016-0623-z
8. Sarwer DB, Ritter S, Reiser K, Spitzer JC, Baumann BM, Patel SN, et al. Attitudes toward Vascularized Composite Allotransplantation of the Hands and Face in an Urban Population. *Vascularized Compos Allotransplantation* (2014) 1(1-2):22–30. doi:10.4161/23723505.2014.975021
9. Agbenorku P, Agbenorku M, Agamah G. Awareness and Attitudes towards Face and Organ Transplant in Kumasi, Ghana. *Ghana Med J* (2013) 47(1):30–4.
10. Van Pilsum Rasmussen SE, Uriarte J, Anderson N, Doby B, Ferzola A, Sung H, et al. Public Education Materials about Vascular Composite Allotransplantation and Donation in the United States: Current Scope and Limitations. *Clin Transpl* (2020) 34(11):e14066. doi:10.1111/ctr.14066
11. Murphy DC, Hoyle V, Saleh D, Rees J, Bound Alberti F. Central Importance of Emotional and Quality-Of-Life Outcomes in the Public's Perception of Face Transplantation. *Br J Surg* (2021) 108(10):1145–8. doi:10.1093/bjs/znab120
12. Rodrigue JR, Tomich D, Fleishman A, Glazier AK. Vascularized Composite Allograft Donation and Transplantation: A Survey of Public Attitudes in the United States. *Am J Transpl* (2017) 17(10):2687–95. doi:10.1111/ajt.14302
13. Mills EC, Alfonso AR, Wolfe EM, Park JJ, Sweeney GN, Hoffman AF, et al. Public Perceptions of Cross-Sex Vascularized Composite Allotransplantation. *Ann Plast Surg* (2020) 85(6):685–90. doi:10.1097/SAP.0000000000002472
14. Riley K, Evans MM, Hupcey J, Sellers PD, Machluf K. Impact of an Educational Intervention on Organ Donation Attitudes in College-Aged Students. *Omega (Westport)* (2021) 84(1):116–25. doi:10.1177/0030222819880708
15. R. Krueger, M. Casey, (editors). *Focus Groups: A Practical Guide for Applied Research*. 5th ed. Thousand Oaks, CA: Sage (2015).
16. Patton M. *Qualitative Research & Evaluation Methods*. 4th ed. Los Angeles, CA: Sage Publications (2015).
17. Tong A, Sainsbury P, Craig J. Consolidated Criteria for Reporting Qualitative Research (COREQ): a 32-item Checklist for Interviews and Focus Groups. *Int J Qual Health Care* (2007) 19(6):349–57. doi:10.1093/intqhc/mzm042
18. Creswell J, Plano Clark VL. *Designing and Conducting Mixed Methods Research*. 3rd ed. Thousand Oaks, CA: Sage Publications (2007).
19. Guest G, Namey E, McKenna K. How Many Focus Groups Are Enough? Building an Evidence Base for Nonprobability Sample Sizes. *Field Methods* (2017) 29(1):3–22. doi:10.1177/1525822X16639015
20. Coenen M, Stamm TA, Stucki G, Cieza A. Individual Interviews and Focus Groups in Patients with Rheumatoid Arthritis: a Comparison of Two Qualitative Methods. *Qual Life Res* (2012) 21(2):359–70. doi:10.1007/s11136-011-9943-2

21. Nowell LS, Norris JM, White DE, Moules NJ. Thematic Analysis: Striving to Meet the Trustworthiness Criteria. *Int J Qual Methods* (2017) 16: 160940691773384. doi:10.1177/1609406917733847
22. Gibbs GR. Analyzing Qualitative Data. *Analyzing Qual Data* (2007) 703: 38–56. doi:10.4135/9781849208574
23. Bradley EH, Curry LA, Devers KJ. Qualitative Data Analysis for Health Services Research: Developing Taxonomy, Themes, and Theory. *Health Serv Res* (2007) 42(4):1758–72. doi:10.1111/j.1475-6773.2006.00684.x
24. Van Pilsum Rasmussen SE, Warsame F, Eno AK, Ying H, Covarrubias K, Haugen CE, et al. Perceptions, Barriers, and Experiences with Successful Aging before and after Kidney Transplantation: A Focus Group Study. *Transplantation* (2020) 104(3):603–12. doi:10.1097/TP.0000000000002848
25. Tisdell EJ. Critical Media Literacy and Transformative Learning:Drawing on Pop Culture and Entertainment Media in Teaching for Diversity in Adult Higher Education. *J Transformative Educ* (2008) 6(1):48–67. doi:10.1177/1541344608318970
26. Reynolds-Tylus T, Quick BL, King AJ, Moore M. Illinois Department of Motor Vehicle Customers' Reasons for (Not) Registering as an Organ Donor. *Prog Transpl* (2019) 29(2):157–63. doi:10.1177/1526924819835838

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ORIGINAL RESEARCH
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Entering the Third Decade After Kidney Transplantation: Excellent Graft Function Refers to Superior Graft but Not Patient Survival

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Kidney transplant recipients (KTRs) with ultralong-term survival represent a growing, yet insufficiently studied patient cohort. In this single-center retrospective study, we analyzed 248 ultralong-term survivors (≥20 years). KTRs were classified into those with superior graft function (defined as eGFR ≥45 ml/min + proteinuria ≤300 mg/day + eGFR-slope ≤ 2 ml/min/1.73 m^2/year) and inferior graft function regarding the risk of CKD progression. 20 years post-transplant, median eGFR was 54 ml/min (11–114), proteinuria 200 mg/24 h (0–7,620), eGFR decline 0.45 ml/min/1.73 m^2/year (11.7 6.5) and DSA had been detected in 19.7% of KTRs. We identified 96 KTRs (38.7%) with superior (group 1) and 152 KTRs (61.3%) with inferior graft function (group 2). Donation after cardiac death, female sex, glomerulonephritis as primary disease, and early TCMR were independently associated with inferior graft function. Graft survival was significantly better in group 1 compared to group 2 (LogRank, $p < 0.001$). Besides group affiliation (HR 20.515, $p = 0.003$), multivariable analysis identified DSA development (HR 3.081, $p = 0.023$) and donor age (HR 1.032, $p = 0.024$) as independent factors. Interestingly, there was no significant difference in patient survival (LogRank, $p = 0.350$). In ultralong-term survivors, excellent graft function refers to superior graft survival but does not extend ultimate patient survival. DSA-formation should be taken seriously even in the ultralong-term.

Keywords: kidney transplantation, TCMR, *de novo* DSA, kidney allograft function, survival

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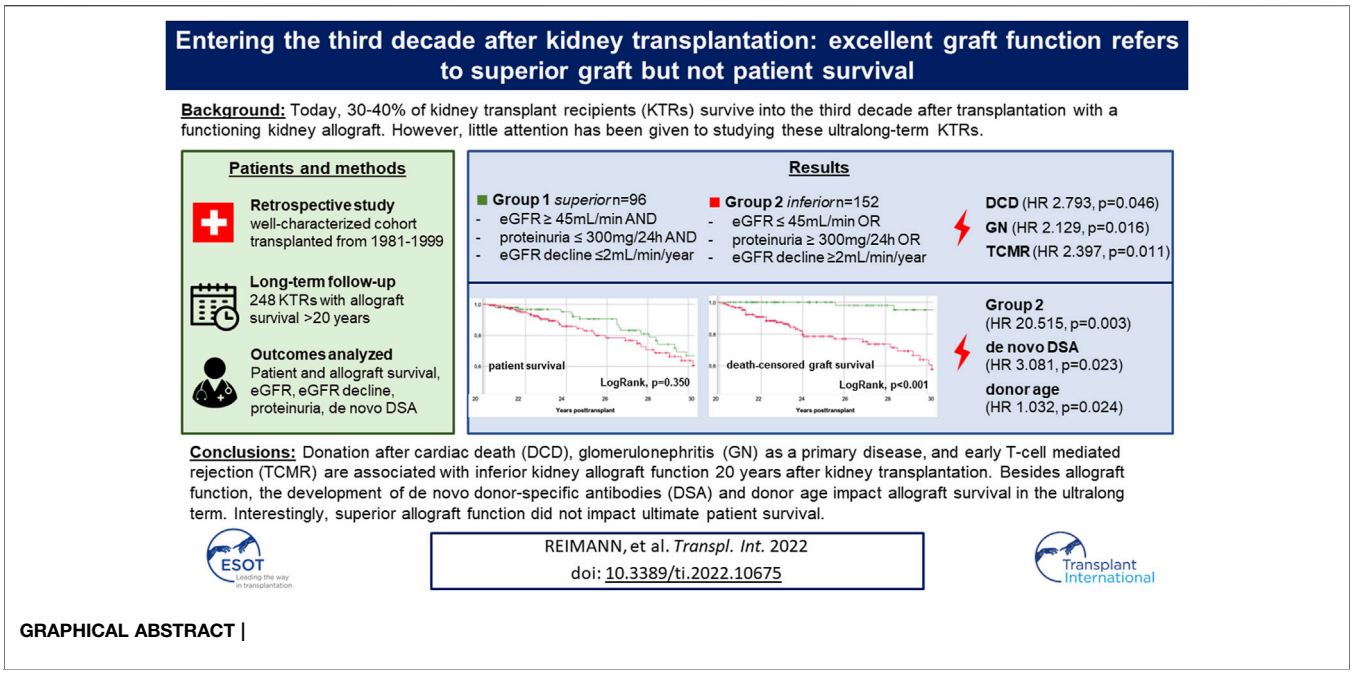
Abbreviations: ABMR, Antibody-mediated rejection; BMI, Body mass index; BSA, Body surface area; CI, Confidence interval; CKD, Chronic kidney disease; CKD-EPI, Chronic kidney disease epidemiology collaboration; CNI, Calcineurin inhibitor; CTS, Collaborative Transplant Study; DCD, Donation after cardiac death; DGF, Delayed graft function; DSA, Donor specific antibody; dnDSA, *De novo* donor specific antibody; DC-GF, Death-censored graft failure; DWFG, Death with functioning graft; eGFR, Estimated glomerular filtration rate; ESKD, End stage kidney disease; HLA, Human leukocyte antigen; KTR, Kidney transplant recipient; MDRD, Modification of diet in renal disease; MFI, Mean fluorescence intensity; PCR, Protein-to-creatinine ratio; ULS, Ultralong-term survivors.



GRAPHICAL ABSTRACT |

INTRODUCTION

Kidney transplantation has become standard procedure in care of patients with end stage kidney disease (ESKD) and by today, is the preferred treatment for most of them (1). Over the past decades, short- and long-term graft survival have improved remarkably (2,3,4,5). For Europe, the Collaborative Transplant Study (CTS) reports an estimated 20-year death-uncensored graft survival rate of 41% for first deceased donor kidney transplant recipients (KTRs) from 1990–2020 and 16.8 years death-uncensored graft half-life (6). According to Coemans et al., who performed a comprehensive analysis of CTS data, death-censored 20-year graft survival rate even exceeded 50% for the transplant decade 1996–2005 (2). However, the authors reported survival data beyond 20 years to be sparse (2). The latest registry report from Australia and New Zealand (ANZDATA) reveals 30% 20-year death-uncensored graft survival for first deceased donor KTRs (3). Other comprehensive registry reports limit their analysis to a maximum of 10-year death-uncensored graft survival (for deceased donors 49.5% in the US (4), 58.5% in Canada (5).

Hence there is a growing population of KTRs who have lived with a functioning graft for several decades (7,8,9,10,11,12,13,14,15). Considering this development, surprisingly little attention has been given to the study of ultralong-term survivors (ULS) (7,8,9,10,11,12,13,14,15). Knowledge about their clinical characteristics, graft function, and alloimmunization is extremely limited and outcome as well as causes of graft losses in ULS have rarely been reported (7,8,9,10,11,12,13,14,15).

To optimize ultralong-term aftercare and to overcome the still important challenge of further improving long-term outcome (16), it is crucial to learn more about this particular patient group (9). To address these needs, we studied a large cohort of KTRs who have lived with a functioning graft for ≥20 years and aimed to investigate the following questions:

(1) What graft function (estimated glomerular filtration rate (eGFR), proteinuria, eGFR decline) do KTRs display 20 years post-transplant?
(2) What factors influence graft function 20 years post-transplant?
(3) What is the incidence of donor specific antibody (DSA)-formation in ULS?
(4) What is the outcome regarding graft and patient survival beyond 20 years post-transplant?
(5) What factors influence ultimate graft and patient survival of ULS?

METHODS

KTRs and Data Collection

This single-center retrospective study was approved by the local Ethics committee of Zurich, Switzerland (Basec Number: 2019–02082) without informed consent requirement and performed in adherence to the declaration of Helsinki.

We considered all adult (age ≥16 years at the date of transplantation) KTRs transplanted at University Hospital Zurich between 1 January 1981 and 31 December 1999. Among a total of 1,180 single-kidney transplantations performed at our institution during this era, we identified 304 KTRs with documented graft survival ≥20 years. 22 KTRs who had denied consent had to be excluded, further 34 KTRs due to insufficient data. This led to a total study cohort of 248 KTRs (**Figure 1**).

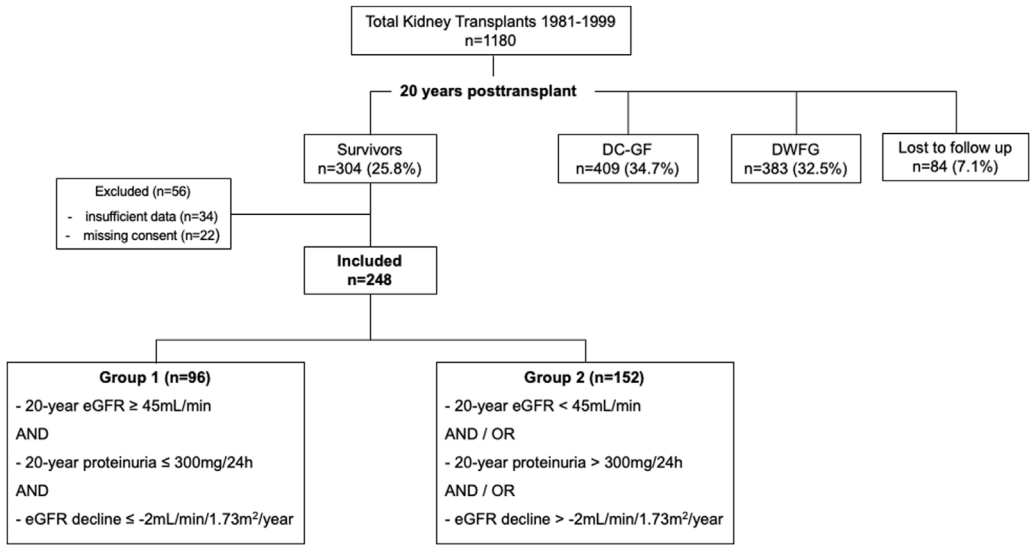


FIGURE 1 | Flow chart all kidney transplants 1981–1999. DC-GF, death-censored graft failure. DWFG, death with functioning graft. Grouping criteria: 20-year eGFR: BSA-deindexed CKD-EPI at the 20-year *post-transplant visit*, 20-year proteinuria: Protein-to-creatinine ratio (mg/mmol) multiplied by 10 at the 20-year *post-transplant visit*. eGFR decline: eGFR CKD-EPI slope 15–20 years post-transplant.

At our center, follow-up care after the first post-transplant year is generally performed quarterly in our outpatient clinic or, in stable conditions, by local nephrologists, complemented by an annual visit at our center. For data collection, we reviewed medical records from the electronic database of the hospital registry. End of follow-up and data collection was 31 January 2021. To evaluate characteristics 20 years post-transplant, we identified the 20-year post-transplant *visit* for each KTR, defined as the closest and most complete visit to the date of transplantation plus 20 years. For all KTRs, median time from transplantation to the 20-year post-transplant visit was 240 months (range 228–248 months). If the 20-year post-transplant visit did not reveal full data, we checked medical records from 19–21 years post-transplant for completion. Cases with insufficient documentation between 19–21 years post-transplant were excluded, as stated above.

Graft Function

From serum-creatinine at the 1-year and 20-year post-transplant visit, we calculated baseline 1-year and 20-year eGFR, using the following formulas: Modification of Diet in Renal Disease (MDRD) (17), Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) (18), and Cockcroft-Gault (19). We did not include the race coefficient for MDRD and CKD-EPI (20). For MDRD and CKD-EPI, we additionally calculated body surface area (BSA)-deindexed eGFR, multiplying eGFR by KTR's individual BSA (21), divided by 1.73 m^2 (22,23). To indicate stability or decline of graft function, respectively, eGFR (CKD-EPI) slopes (24) were calculated for the last 5 years of the 20-year period, i.e., 15–20 years post-transplant. Baseline 20-year proteinuria was assessed by multiplying urine-to-creatinine ratio (PCR) (mg/mmol) from spot urine at the 20-year post-transplant visit by 10 (25). For 31 KTRs (12.5%), PCR was calculated from 24-hour collection urine, as before 2005, measurement of proteinuria was obtained by 24-hour collection urine at our center. PCR below detection limit was included in the analysis with a value of zero.

Maintenance Immunosuppression and DSA-Screening

All donors and recipients were typed for human leukocyte antigen (HLA)-A, -B and -DR. Since approximately 2009, annual HLA antibody-monitoring using Luminex based assay (One Lambda, Canoga Park, CA, United States) became standard procedure in KTR-care at our center. In case of worsening graft function or progression of proteinuria, screening may have been performed more often. If Luminex mix assay was positive and/or clinical suspicion was high, an additional Luminex single antigen bead assay was performed to test for DSA. We classified KTRs with ≥1 DSA-positive Luminex single antigen bead assay up to 21 years post-transplant as DSA-positive, irrespective of the level of mean fluorescence intensity (MFI). KTRs were classified as DSA-negative in case HLA antibody-monitoring (Luminex mix assay only or both, Luminex mix and single bead assay) did not show DSA up to 21 years post-transplant or if the very first screening was performed beyond 21 years post-transplant and negative for DSA. KTRs were excluded from this sub-analysis in case of missing HLA antibody-screening during the observation period (n = 36, 14.5%) or if the very first screening was performed beyond 21 years post-transplant and DSA-positive, thus the date of DSA-occurrence was indeterminable (n = 14, 5.6%).

Group Categorization

According to the KDIGO 2012 Clinical Practice Guideline for the risk of CKD progression, KTRs were stratified into two groups

TABLE 1 | Basic recipient and donor characteristics.

20-year survivors	Total (*n* = 248)		Group 1 (*n* = 96)		Group 2 (*n* = 152)		*p*-value
		n		n		n	
First transplant	248	227 (91.5%)	96	92 (95.8%)	152	135 (88%)	
Second transplant		21 (8.5%)		4 (4.2%)		17 (11.2%)	0.089
Female KTR	248	92 (37.1%)	96	26 (27.1%)	152	66 (43.4%)	**0.014***
KTR age (years)[a]	248	39.9 (17.3–68.8)	96	38.1 (17.6–67.3)	152	40.3 (17.3–68.8)	0.663
Cause of ESKD[b]	248		96		152		
GN[c]		89 (35.9%)		28 (29.2%)		61 (40.1%)	0.106
Uropathy[d]		45 (18.1%)		16 (16.7%)		29 (19.1%)	0.756
Diabetes mellitus		5 (2.0%)		2 (2.1%)		3 (2.0%)	1.0
Hypertension		4 (1.6%)		1 (1.0%)		3 (2.0%)	1.0
ADPKD[e]		30 (12.1%)		13 (13.5%)		17 (11.2%)	0.723
Alport syndrome		9 (3.6%)		6 (6.3%)		3 (2.0%)	0.093
Other		28 (11.3%)		12 (12.5%)		16 (10.5%)	0.785
Unknown		38 (15.3%)		18 (18.8%)		20 (13.2%)	0.313
Pretransplant dialysis	248		96		152		
preemptive transplantation		6 (2.4%)		2 (2.1%)		4 (2.6%)	1.0
HD[f] (only HD or PD/HD)		193 (77.8%)		77 (80.2%)		116 (76.3%)	
only PD[g]		49 (19.8%)		17 (17.7%)		32 (21.1%)	0.615
pretransplant dialysis (months)[h]	221	25 (2–164)	90	22 (2–120)	131	28 (3–164)	**0.047***
Total HLA Mismatch (A, B, DR)	248	3 (0–6)	96	3 (1–6)	152	3 (0–6)	0.343
Total PIRCHE-II (A, B, DR)		38.23 (0–111.63)		38.99 (14.07–97.72)		37.92 (0–111.63)	0.663
0–2 HLA Mismatches		48 (19.4%)		15 (15.6%)		33 (21.7%)	
3–6 HLA Mismatches		200 (80.6%)		81 (84.4%)		119 (78.3%)	0.309
HLA A Mismatch		1 (0–2)		1 (0–2)		1 (0–2)	0.07
HLA B Mismatch		1 (0–2)		1 (0–2)		1 (0–2)	0.712
PIRCHE-II (A, B)		26.35 (0–85.74)		28.13 (4.31–79.97)		25.24 (0–85.74)	0.665
HLA DR Mismatch		1 (0–2)		1 (0–2)		1 (0–2)	0.813
PIRCHE-II (DR)		8.06 (0–58.58)		6.94 (0–38.70)		9.44 (0–58.58)	0.221
Donor characteristics							
Living donor transplant	248	14 (5.6%)	96	3 (3.1%)	152	11 (7.2%)	0.278
Donation after cardiac death	248	28 (11.3%)	96	6 (6.3%)	152	22 (14.5%)	0.063
Donor age (years)	247	32 (3–72)	96	25 (3–63)	151	37 (3–72)	**0.001****
Male donor	245	167 (68.2%)	94	65 (69.1%)	151	102 (67.5%)	0.904
CIT (hours)[i]	242	14 (1–34)	96	14.25 (1.5–34)	146	13.5 (1–32.5)	0.307
Era of transplantation	248		96		152		
1981–1989		92 (37.1%)		41 (42.7%)		51 (33.6%)	
1990–1999		156 (62.9%)		55 (57.3%)		101 (66.4%)	0.187

[a]*At the date of transplantation.*
[b]*End stage kidney disease.*
[c]*Glomerulonephritis, incl. vasculitis, systemic lupus erythematosus, and suspected chronic GN.*
[d]*Incl. congenital anomalies of the kidney and urinary tract, CAKUT.*
[e]*Autosomal dominant polycystic kidney disease.*
[f]*Hemodialysis (only HD or both, PD and HD).*
[g]*Peritoneal dialysis.*
[h]*Only KTRs with the first transplant.*
[i]*Cold ischemia time.*

based on graft function 20 years post-transplant. (50) Criteria for superior graft function (Group 1) were: 1) 20-year eGFR ≥45 ml/min (BSA-deindexed CKD-EPI), 2) 20-year proteinuria ≤300 mg/24 h, and 3) eGFR (CKD-EPI) decline ≤-2 ml/min/1.73 m^2/year 15–20 years post-transplant. Subjects in group 1 had to meet all 3 criteria. KTRs who did not pass ≥1 criteria were assigned to group 2. Two cases with missing data on proteinuria that fulfilled the other criteria for group 1 were classified as insufficient data and excluded, consequently. Two cases were categorized according to BSA-indexed CKD-EPI, because of unknown BSA (missing documentation of patient's height).

Survival

We separately studied patient survival (treating graft loss as a censored event), death-uncensored and death-censored (treating death as a censored event) graft survival, calculated from the date of transplantation to KTR's death

TABLE 2 | Post-transplant complications and 1-year kidney allograft function.

8-year survivors	Total (*n* = 248)		Group 1 (*n* = 96)		Group 2 (*n* = 152)		*p*-value
	n		n		n		
Delayed graft function (DGF)	248	37 (14.9%)	96	10 (10.4%)	152	27 (17.8%)	0.143
Rejection	248	80 (32.3%)	96	20 (20.8%)	152	60 (39.5%)	**0.002***
Early TCMR (<12 months)	248	53 (21.4%)	96	17 (17.8%)	152	46 (30.3%)	**0.036***
Late TCMR (>12 months)	248	3 (1.2%)	96	0 (0.0%)	152	3 (2.0%)	0.285
Late ABMR (>12 months)	248	14 (5.6%)	96	3 (1.2%)	152	11 (7.2%)	0.259
Early CMV infection (<12 months)	248	25 (10.1%)	96	8 (8.3%)	152	17 (11.2%)	0.523
Post-transplant parathyreoidectomy	248	20 (8.1%)	96	5 (5.2%)	152	15 (9.9%)	0.235
Graft function at 1 year post-transplantation							
Serum-creatinine µmol/L	215	96 (48–145)	86	98 (48–138)	139	95 (58–145)	0.558
eGFR CKD-EPI[a]	215	70 (43–117)	86	70 (43–115)	139	72 (45–117)	0.498

[a]*ml/min/1.73 m²*.

TABLE 3 | Cox Regression analysis to assess group classification of KTRs 20 years post-transplantation.

Multivariate Cox regression analysis	HR	95% CI	*p*-Value
Number of transplants (second)	1.385	0.287–6.691	0.685
Recipient sex (female)	2.473	1.329–4.604	**0.004***
Cause of ESKD (GN)	2.129	1.152–3.934	**0.016***
Pretransplant dialysis (months)	1.015	1.001–1.030	**0.041***
Donation after cardiac death (DCD)	2.793	1.017–7.667	**0.046***
Donor age (years)	1.037	1.017–1.058	**<0.001***
Early TCMR (<12 months)	2.397	1.222–4.700	**0.011***

Multivariable Cox regression models for group classification at 20 years post-transplantation. Reference category in parentheses. HR, hazard ratio; CI, confidence interval.

or graft loss (return to permanent dialysis or re-transplantation), whatever came first. If there was no event, survival dates were censored at the date of last follow-up or end of data collection (31 January 2021).

Specific causes of graft loss and results of indication biopsy were evaluated for KTRs with death-censored graft loss in group 1.

Calculation of Predicted Indirectly ReCognizable HLA-Epitopes Scores

The HLA-derived mismatched peptide epitopes presented by KTRs HLA-molecules were calculated using the PIRCHE-II algorithm. Presentation of both HLA class I (HLA-A, B) and HLA class II derived peptides (HLA-DR, DQ) were calculated for each HLA locus. Detection of HLA antigens was performed by DNA-based HLA-typing technology using blood samples. Either sequence-specific oligonucleotide (SSO) or sequence-specific primer (SSP) technologies were used to generate low-resolution HLA typing results. The imputation of probable allele resolution results needed for the PIRCHE-II calculation was achieved by the use of the imputation algorithm included in the PIRCHE-II calculation. The PIRCHE-II algorithm is available online (https://www.PIRCHE-II.org).

Statistical Analysis

Statistical analysis was performed using SPSS (Version 26, IBM, Armonk, NY, United States). Continuous variables are expressed as median (range minimum-maximum) and compared using Mann Whitney-U Test. Categorical data are expressed as number (%) and compared using Chi (2) test, corrected for Yates in 2x2 tables. If expected cell count was ≤5, we used Fisher's Exact test instead. Missing values were not imputed. Survival was analysed using the Kaplan-Meier method and compared with LogRank test. Univariable and multivariable Cox proportional hazards models with enter method were used to investigate factors associated with survival. Variables with a *p*-value ≤0.05 in the univariable analysis were included in the multivariable model. For categorical variables in the multivariable model, assumption of proportional hazards was assessed visually by Kaplan Meier curves (26). For all tests, statistical significance was assumed for a two-tailed *p*-value <0.05.

RESULTS

Table 1 shows basic characteristics, **Table 2** post-transplant complications and 1-year graft function, and **Table 3** multivariable Cox regression analysis for group categorization of the 248 KTRs included in this study. Median KTR-age at the date of transplantation was 39.9 years, 92/248 (37.1%) KTRs were female.

Graft Function

Table 4 shows detailed information on graft function 20 years post-transplant. Median serum-creatinine was 124 µmol/L, median eGFR 54 ml/min (BSA-deindexed CKD-EPI), median proteinuria 200 mg/24 h, and median eGFR decline −0.45 ml/min/1.73 m^2/year. CKD-related laboratory findings are shown in the **Supplementary Table S1**.

Immunosuppression and DSA-formation

Maintenance immunosuppression is shown in **Table 5**, results of HLA antibody-screenings in **Table 6**. Within the

TABLE 4 | Characteristics and graft function 20 years posttransplant.

20-year survivors	Total (*n* = 248)		Group 1 (n = 96)		Group 2 (*n* = 152)		*p*-value
	N		n		n		
KTR age (years)[a]	248	59.9 (37.1–89.1)	96	58.2 (37.9–87.4)	152	60.4 (37.1–89.1)	0.643
BMI (kg/m^2)[b]	246	25.2 (14–40.8)	95	25.5 (18.1–40.8)	151	24.7 (14–38.9)	0.291
BMI <18.5 kg/m^2		4 (1.6%)		1 (1.1%)		3 (2.0%)	
BMI 18.5–24.9 kg/m^2		116 (47.2%)		41(43.2%)		75 (49.7%)	
BMI 25–29.9 kg/m^2		86 (35.0%)		37 (38.9%)		49 (32.5%)	
BMI 30–34.9 kg/m^2		29 (11.8%)		12 (12.6%)		17 (11.3%)	
BMI ≥35 kg/m^2		11 (4.5%)		4 (4.2%)		7 (4.6%)	0.814
Graft function	248		96		152		
Serum-creatinine µmol/L		124 (54–496)		101 (54–170)		142 (60–496)	<**0.001*****
eGFR CKD-EPI[c]		51 (11–102)		63 (40–98)		41 (11–102)	<**0.001*****
eGFR deindexed CKD-EPI[d]		54 (11–114)		65 (45–114)		43 (11–111)	<**0.001*****
eGFR MDRD[c]		48 (11–97)		59 (38–97)		39 (11–92)	<**0.001*****
eGFR deindexed MDRD[d]		51 (12–104)		62 (43–104)		41 (12–103)	<**0.001*****
eGFR Cockcroft Gault[d]		55 (11–140)		67 (34–117)		45 (11–140)	<**0.001*****
CKD stage[e]	248		96		152		
G1 (eGFR ≥90 ml/min)		13 (5.2%)		7 (7.3%)		6 (3.9%)	
G2 (eGFR 60–89 ml/min)		77 (31.0%)		55 (57.3%)		22 (14.5%)	
G3a (eGFR 45–59 ml/min)		70 (28.2%)		34 (35.4%)		36 (23.7%)	
G3b (eGFR 30–44 ml/min)		52 (21.0%)		0(0.0%)		52 (34.2%)	
G4 (eGFR 15–29 ml/min)		33 (13.3%)		0 (0.0%)		33 (21.7%)	
G5 (eGFR <15 ml/min)		3 (1.2%)		0 (0.0%)		3 (2.0%)	<**0.001*****
Proteinuria[f]	246	200 (0–7,620)	96	98 (0–300)	150	400 (0–7,620)	<**0.001*****
0–300 mg/24 h		152 (61.8%)		96 (100%)		56 (37.3%)	
301–1,000 mg/24 h		67 (27.2%)		0 (0.0%)		67 (44.7%)	
1,001–3,500 mg/24 h		21 (8.5%)		0 (0.0%)		21 (14.0%)	
>3,500 mg/24 h		6 (2.4%)		0 (0.0%)		6 (4.0%)	<**0.001*****
eGFR decline[g]	246	−0.45 (−11.7–6.5)	96	0.45(−2.0–6.5)	150	−1.25 (−11.7–6.3)	<**0.001*****
≤ −2 ml/min/1.73 m^2/year		189 (76.8%)		96 (100%)		93 (62.0%)	
> −2 ml/min/1.73 m^2/year		57 (23.2%)		0 (0.0%)		57 (38.0%)	<**0.001*****

[a]*At the* 20-year posttransplant visit.
[b]*Body mass index.*
[c]*ml/min/1.73 m^2.*
[d]*ml/min.*
[e]*KDIGO chronic kidney disease classification[25], according to BSA-deindexed CKD-EPI.*
[f]*Protein-to-creatinine ratio (mg/mmol), multiplied by 10.*
[g]*According to CKD-EPI, mL/min/1.73 m^2/year, 15–20 years posttransplant.*

first two post-transplant decades, 39/198 (19.7%) KTRs had developed ≥1 DSA, predominantly (29/39, 74.4%) against HLA Class II. Total PIRCHE-II scores (median 42.61 (range: 10.00–111.63) vs. median 33.46 (range: 0.00–99.22)) and PIRCHE-II scores for HLA-class II (HLA-DR; median 11.27 (range: 00.00–58.58) vs. median 4.29 (range: 0.00–28.70)) were significantly higher among KTRs developing DSA compared to KTRs not developing DSA (p = 0.021, p = 0.020). No differences were observed for PIRCHE-II scores for HLA-class I (HLA-A, -B; median 29.69 (range: 2.07–85.74) vs. median 23.26 (range: 0.00–76.93) between KTRs developing and not developing DSA (p = 0.116). Group 1 and group 2 did neither significantly differ in amount of DSA-positive KTRs nor in number, category, or maximal MFI of detected DSA (all p > 0.05). No differences were observed for the total PIRCHE-II scores (A, B, DR) and the PIRCHE-II scores per locus between group 1 and group 2 (p > 0.05).

KTR-Categorization and Group Comparison

Subdivision of the cohort is shown in **Figure 1**. 96/248 (38.7%) KTRs fulfilled the criteria for superior graft function (group 1). The remaining 152/248 (61.3%) KTRs were classified to group 2. **Figure 2** displays distribution of all KTRs according to baseline 20-year eGFR and proteinuria, group subdivision is marked by color.

Multivariable Cox regression analysis is shown in **Table 3**. The strongest impact on group affiliation was observed for donation after cardiac death (DCD; HR 2.793, 95% CI 1.017–7.667, p = 0.041), female sex (HR 2.473, 95% CI 1.329–4.604, p = 0.004), early TCMR (HR 2.397, 95% CI 1.222–4.700, p = 0.011), and glomerulonephritis as primary disease (HR 2.129, 95% CI 1.152–3.934, p = 0.016). While 17 of 152 KTRs (11.2%) of group 2 developed recurrence of primary disease, only 1 of 96 KTRs (1.0%) of group 1 did (p < 0.001). A minor impact was observed for donor age (HR 1.037, 95%

TABLE 5 | Maintenance immunosuppression 20 years posttransplant.

8-year survivors	Total (*n* = 248)	Group 1 (n = 96)	Group 2 (*n* = 152)	*p*-value
	n	n	n	
	248	96	152	
CNI-based IS	210 (84.7%)	77 (80.2%)	133 (87.5%)	0.170
Ciclosporin-based IS	177 (71.4%)	71 (74.0%)	106 (69.7%)	0.567
CsA/MPA	75 (30.2%)	34 (35.4%)	41 (27.0%)	
CsA/MPA/Steroid	22 (8.9%)	5 (5.2%)	17 (11.2%)	
CsA/Aza	56 (22.6%)	23 (24.0%)	33 (21.7%)	
CsA/Aza/Steroid	14 (5.6%)	5 (5.2%)	9 (5.9%)	
CsA/Steroid	2 (0.8%)	0 (0.0%)	2 (1.3%)	
CsA only	7 (2.8%)	3 (3.1%)	4 (2.6%)	
CsA/mTORi	1 (0.4%)	1 (1.0%)	0 (0.0%)	
Tacrolimus-based IS	33 (13.3%)	6 (6.3%)	27 (17.8%)	**0.016***
Tac/MPA	17 (6.9%)	4 (4.2%)	13 (8.6%)	
Tac/MPA/Steroid	9 (3.6%)	1 (1.0%)	8 (5.3%)	
Tac/Aza	5 (2.0%)	1 (1.0%)	4 (2.6%)	
Tac/Aza/Steroid	2 (0.8%)	0 (0.0%)	2 (1.3%)	
mTOR-Inhibitor-based IS (CNI-free)	12 (4.8%)	4 (4.2%)	8 (5.3%)	0.771
mTORi/MPA	7 (2.8%)	2 (2.1%)	5 (3.3%)	
mTORi/MPA/Steroid	3 (1.2%)	0 (0.0%)	3 (2.0%)	
mTORi/Aza	1 (0.4%)	1 (1.0%)	0 (0.0%)	
mTORi/Aza/Steroid	1 (0.4%)	1 (1.0%)	0 (0.0%)	
Other	26 (10.5%)	15 (15.6%)	11 (7.2%)	0.059
Aza/Steroid	19 (7.7%)	11 (11.5%)	8 (5.3%)	
Aza only	1 (0.4%)	1 (1.0%)	0 (0.0%)	
MPA/Steroid	5 (2.0%)	2 (2.1%)	3 (2.0%)	
MPA only	1 (0.4%)	1 (1.0%)	0 (0.0%)	
Overall Steroid-containing IS	77 (31.0%)	25 (26.0%)	52 (34.2%)	0.225

IS, Immunosuppression; CNI, calcineurin inhibitor, CsA, Cyclosporine A; MPA, mycophenolic acid, incl. Mycophenolate mofetil, Aza, Azathioprine; mTORi, Mammalian target of rapamycin inhibitor; Tac, Tacrolimus.

CI 1.017–1.058, $p < 0.001$) and length of pretransplant dialysis (HR 1.015, 95% CI 1.001–1.030, $p = 0.041$).

Survival

Survival analyses are shown in **Figures 3A–C**, **4A–C**. 93/248 (37.5%) graft losses were recorded during follow-up: 53/248 (21.4%) KTRs died with a functioning graft (death with functioning graft, DWFG), 40/248 (16.1%) KTRs lost their graft while still alive (death-censored graft failure, DC-GF). Median death-uncensored graft survival was 29.9 years (95% Confidence Interval (CI) 28.4–31.4 years). For death-censored graft survival and for patient survival Kaplan Meier curves did not reach 50%.

In group 1, 26/96 (27.1%) grafts failed during follow-up: 23/26 (88.5%) due to DWFG, 3/26 (11.5%) due to DC-GF. These latter 3 KTRs were analyzed more closely: 1 KTR was DSA-negative 20 years post-transplant but developed *de novo* DSA (dnDSA) during the third post-transplant decade. Graft loss resulted from biopsy-proven chronic antibody mediated rejection (ABMR). The other two KTRs both had their first HLA antibody-screening performed during the 28th year post-transplant and were DSA-positive by then (which, due to indeterminable date of DSA-development, led to exclusion from DSA-sub-analysis, as stated above). Indication biopsy showed glomerulopathy and low level glomerulitis in one, and glomerulopathy and vasculopathy with signs of *de novo* IgA nephropathy in the other case. In group 2, 67/152 (44.1%) grafts failed during follow-up: 30/67 (44.8%) due to DWFG, 37/67 (55.2%) due to DC-GF. Death-censored and death-uncensored graft survival was significantly superior in group 1 (LogRank, both $p < 0.001$, **Figures 4A,C**). In contrast, there was no significant difference in patient survival (LogRank, $p = 0.35$, **Figure 4B**).

Univariable and multivariable Cox regression analysis are shown in **Tables 7,8**. For DC-GF (**Table 7**), we found a significant impact of group affiliation (HR 20.515, 95%CI 2.730–154.143, $p = 0.003$), overall DSA-development (HR 3.081, 95% CI 1.165–8.146, $p = 0.023$), donor age (HR 1.032, 95% CI 1.004–1.061, $p = 0.024$). For patient survival (**Table 8**), only KTR-age (HR 1.082, 95% CI 1.051–1.113, $p < 0.001$) and CsA-based immunosuppression (HR 0.297, 95% CI 0.149–0.593, $p < 0.001$) were significantly associated with outcome.

DISCUSSION

ULS represent a growing, yet insufficiently studied patient population (8,9). To address this new challenge in transplant long-term aftercare (7,9), we herein analyzed 248 KTRs with a functioning graft ≥20 years. In line with earlier ULS-reports (7,8,9,11,12,14,15) graft function was remarkably good:

TABLE 6 | DSA screening within the first two posttransplant decades.

20-year survivors	**Total (*n* = 248)**	**Group 1 (*n* = 96)**	**Group 2 (*n* = 152)**
Excluded	50 (20.2%)	21 (21.9%)	29 (19.1%)
No Screening[a]	36 (14.5%)	15 (15.6%)	21 (13.8%)
unknown DSA-onset[b]	14 (5.6%)	6 (6.3%)	8 (5.3%)

20-year survivors	**Total (*n* = 198)**		**Group 1 (*n* = 75)**		**Group 2 (*n* = 123)**		***p*-value**
		n		n		n	
Overall DSA	198	39 (19.7%)	75	11 (14.7%)	123	28 (22.8%)	0.228
HLA-Class I		15 (7.6%)		3 (4.0%)		17 (13.8%)	0.029*
HLA-Class II		29 (14.6%)		8 (10.7%)		26 (21.1%)	0.079
Number of DSA[c]	198	0 (0–6)	75	0 (0–2)	123	0 (0–6)	0.119
Number of DSA[d]	39	1 (1–6)	11	1 (1–2)	28	1 (1–6)	0.062
0	198	159 (80.3%)	75	64 (85.3%)	123	95 (77.2%)	
1	198	27 (13.6%)	75	10 (13.3%)	123	17 (13.8%)	
2	198	8 (4.0%)	75	1 (1.3%)	123	7 (5.7%)	
3	198	1 (0.5%)	75	0 (0.0%)	123	1 (0.8%)	
4	198	2 (1.0%)	75	0 (0.0%)	123	2 (1.6%)	
6	198	1 (0.5%)	75	0 (0.0%)	123	1 (0.8%)	0.512
Number of DSA Class I[c]	198	0 (0–3)	75	0 (0–1)	123	0 (0–3)	0.130
Number of DSA Class I[d]	39	0 (0–3)	11	0 (0–1)	28	0 (0–3)	0.278
0	198	183 (92.4%)	75	72 (96.0%)	123	111 (90.2%)	
1	198	11 (5.6%)	75	3 (4.0%)	123	8 (6.5%)	
2	198	2 (1.0%)	75	0 (0.0%)	123	2 (1.6%)	
3	198	2 (1.0%)	75	0 (0.0%)	123	2 (1.6%)	0.542
Number of DSA Class II[c]	198	0 (0–3)	75	0 (0–2)	123	0 (0–3)	0.191
Number of DSA Class II[d]	39	1 (0–3)	11	1 (0–2)	28	1 (0–3)	0.449
0	198	169 (85.4%)	75	67 (89.3%)	123	102 (82.9%)	
1	198	21 (10.6%)	75	7 (9.3%)	123	14 (11.4%)	
2	198	6 (3.0%)	75	1 (1.3%)	123	5 (4.1%)	
3	198	2 (1.0%)	75	0 (0.0%)	123	2 (1.6%)	0.544
MFI[e]							
Max MFI all DSA	39	5202 (552 21′896)	11	5840 (552–17′203)	28	4765 (681–21′896)	0.618
Max MFI DSA Class I	15	1,146 (552–7,577)	3	653 (552–7,577)	12	1,175 (720–6,278)	0.448
Max MFI DSA Class II	29	6,605 (502–21′896)	8	6,851 (558–17′203)	21	6,605 (502–21′896)	0.981
PIRCHE-II scores							
Total PIRCHE-II score (A, B, DR)	39	42.61 (10.00–111.63)	11	40.80 (14.07–97.72)	28	42.79 (10.00–111.63)	0.852
PIRCHE-II score (A, B)	39	29.69 (2.07–85.74)	11	30.99 (4.31–79.97)	28	28.85 (2.07–85.74)	0.311
PIRCHE-II score (DR)	39	11.27 (0–28.70)	11	11.00 (0–28.70)	28	13.43 (0–58.58)	0.598
DSA-onset (months)[f]	39	211 (0–250)	11	213 (0–250)	28	208 (148–249)	0.492

[a]*No HLA-antibody screening during the observation period.*
[b]*First HLA-antibody screening performed beyond 21 years posttransplant and DSA-positive.*
[c]*All KTRs.*
[d]*DSA-positive KTRs only.*
[e]*MFI, mean fluorescence intensity. Highest value measured up to 21 years posttransplant.*
[f]*Time from transplantation to first DSA-detection in months.*

20 years post-transplant, the majority (64.5%) of the KTRs was in stage 1-3a of CKD classification (25). 38.7% fulfilled the criteria for superior graft function, i.e., had high and stable 20-year eGFR and low proteinuria (group 1).

Group comparison revealed a significant difference in DCD, early TCMR, recipient gender, and glomerulonephritis as primary disease. For the first, previous studies suggest comparable survival rates for kidneys from DCD. (27) For the second, although in general associated with reduced graft survival, the impact of successfully treated early TCMR on ultralong-term survival has not been well studied. (51) However, our data suggest that initial acute kidney injury and associated nephron loss either due to DCD or early TCMR may have an impact in the ultralong-term, and predispose these KTRs to decreased graft function and proteinuria through chronic hyperfiltration and chronic histologic lesions of interstitial fibrosis/tubular atrophy. Regarding the effect of KTR-gender on ultralong-term survival, results are conflicting (8,9,10,13). However, the predominance of men in group 1 surprises, given their higher risk of chronic graft failure (28). Our finding could result from potential underestimation of GFR in women by the applied equations and stresses the need for further, gender-specific studies (29). Although the time of onset and severity of recurrence of the underlying disease vary widely, our data suggest that glomerulonephritis recurrence strongly influences the risk of impaired renal function in the ultralong-term. However, in our analysis, no factor had an independent impact on further survival.

Data on long-term maintenance immunosuppression is extremely limited (30). In our center, standard

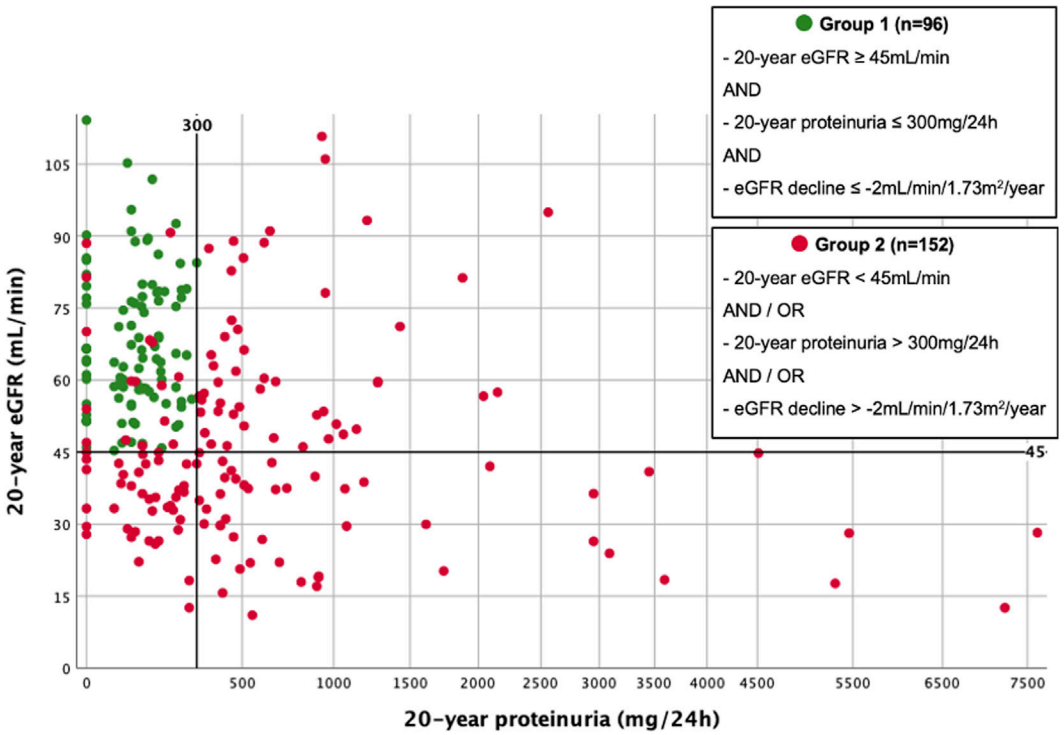


FIGURE 2 | Scatterplot illustrating group subdivision. Scatterplot of all 20-year survivors, according to 20-year eGFR (BSA-deindexed CKD-EPI) and 20-year proteinuria. Group subdivision is marked by color.

immunosuppression during the respected period was composed of Ciclosporin, Azathioprine and Corticosteroids. 20 years post-transplant, 71% of the ULS were still under Ciclosporin-based maintenance therapy (no significant group difference). However, group 2 contained significantly more KTRs with Tacrolimus-based immunosuppression. Changes in immunosuppressive therapy over time could not be analyzed in this study, but we presume that this difference results from conversion from Ciclosporin to Tacrolimus in response to supposed immune-related injury and the development of DSA by intensifying maintenance immunosuppression (30). The deleterious effect of calcineurin inhibitors (CNI) on long-term graft outcome has become an increasing matter of debate (31,32). Regarding ULS, data is scarce and inconsistent: Bererhi et al. only found 3% of ULS with CNI-based maintenance immunosuppression and therefore hypothesized that avoiding CNI could favor ultralong-term graft survival (7). In contrast, Traynor et al. reported 40% and Kettler et al. even 68% of ULS with CNI-based therapy (8,12). Given the prolonged exposure to immunosuppression, determining optimal long-term immunosuppression is especially important for ULS (9). But this urgent question still remains unanswered (33).

To target therapeutic interventions and optimize ultralong-term aftercare, we need to improve our understanding of late graft loss (16,31), which includes patient's death (DWFG) and loss of graft function while still alive (DC-GF) (16). In this study, we drew a detailed picture of graft and patient survival of 248 ULS. While overall graft survival was already remarkably good (median death-uncensored graft survival 29.9 years), for KTRs with superior graft function, it was outstanding. In fact, group 1 only involved 3 events of DC-GF. In contrast, graft survival in group 2 was clearly inferior. Corresponding with the fact that graft failure is preceded by graft dysfunction (16,34), 92.5% of all events of DC-GF in this study occurred in group 2.

In their comprehensive study of 177 ULS, McCaughan et al. observed that DC-GF after 20 years is uncommon (9). Our study shows that this is particularly true for ULS with preserved graft function, while in group 2, DC-GF accounted for the majority (55.2%) of graft losses. A multivariable Cox regression model for DC-GF confirmed a strong influence of group affiliation.

Donor age profoundly impacts graft quality (35) and is an important risk factor in graft outcome (2,35). In this study, comparable to earlier ULS-reports (7,8,9,10,12,13), donors were young (median 32 years), a clear difference to more recently transplanted KTRs (2). Very interestingly, donors were significantly younger in group 1, and donor age had significant impact on DC-GF beyond 20 years post-transplant. This phenomenon might be attributed to the loss of functional nephrons with aging and consecutive decreased functional reserve (36) and increased vulnerability to transplant-related injury (37,38). In previous studies, univariable analyses revealed significant association of donor age with ultralong-term survival, however, in multivariable models the effect showed only a trend and missed statistical significance (9,10).

Late DC-GF is profoundly driven by alloimmune mechanisms (31,39,51). DSA are associated with increased risk of late graft

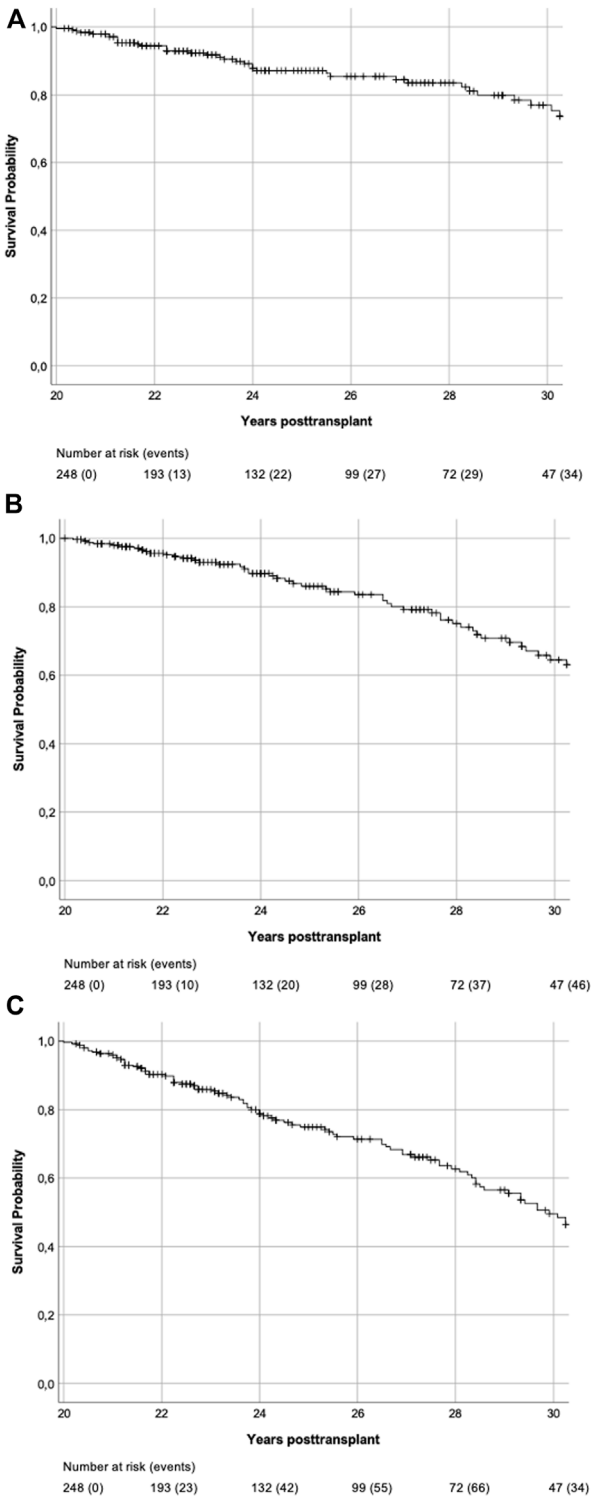


FIGURE 3 | (A) Death-censored graft survival. Kaplan-Meier Plot of death-censored graft survival of all 20-year survivors. **(B)** Patient survival. Kaplan-Meier Plot of patient survival of all 20-year survivors. **(C)** Death-uncensored graft survival. Kaplan-Meier Plot of death-uncensored graft survival of all 20-year survivors. Median death-uncensored graft survival was 29.9 years (95% CI 28.4–31.4 years).

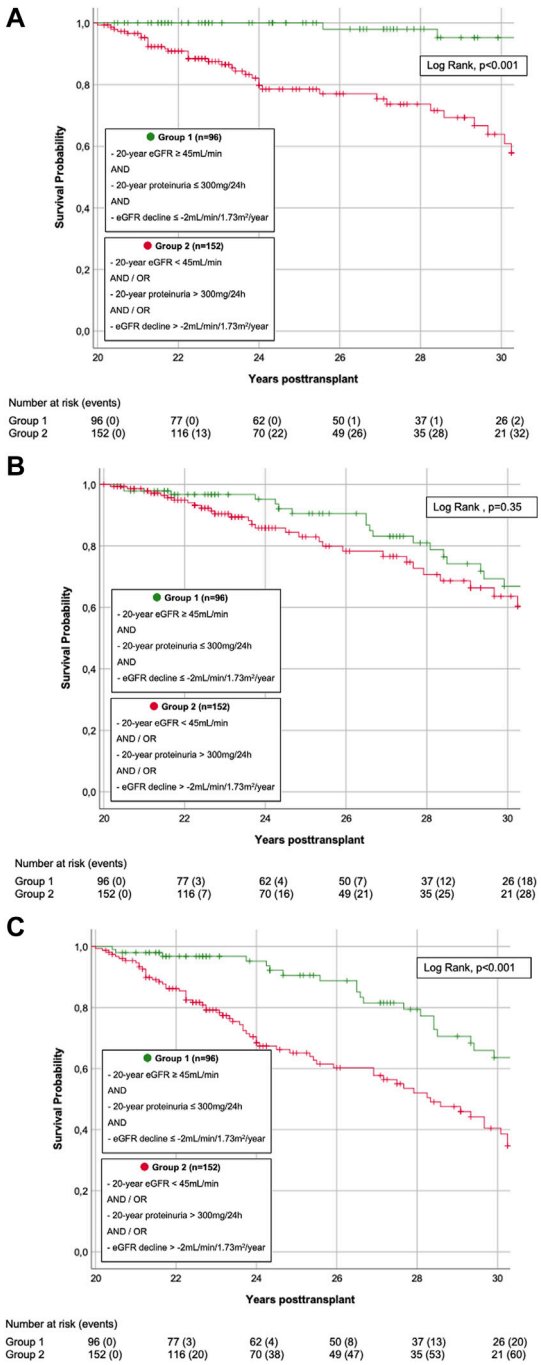


FIGURE 4 | (A) Death-censored graft survival. Kaplan-Meier Plot of death-censored graft survival. Death-censored graft survival was significantly superior in KTRs s with superior graft function (group 1) compared to KTRs with inferior graft function (group 2) (LogRank, $p < 0.001$). **(B)** Patient survival. Kaplan-Meier Plot of patient survival. Patient survival did not significantly differ in KTRs with superior graft function (group 1) compared to those with inferior graft function (group 2) (LogRank, $p = 0.350$). **(C)** Death-uncensored graft survival. Kaplan-Meier Plot of death-uncensored graft survival. Death-uncensored graft survival was significantly superior in KTRs s with superior graft function (group 1) compared to KTRs with inferior graft function (group 2) (LogRank, $p < 0.001$).

TABLE 7 | Cox Regression analysis to assess the risk of kidney allograft loss in KTRs 20 years post-transplantation.

Univariate Cox regression	HR	95% CI	*p*-Value
Group (Group 2)	11.533	3.533–37.650	< **0.001**
20-year eGFR (BSA-deindexed CKD-EPI)	0.926	0.906–0.947)	< **0.001**
20-year proteinuria	1.001	1.000–1.001	< **0.001**
eGFR (CKD-EPI) decline	0.786	0.703–0.879	< **0.001**
Time on pretransplant dialysis (per month)	0.995	0.979–1.012	0.581
Pretransplant dialysis[a] (PD)	0.664	0.311–1.419	0.291
HLA Mismatch (per mismatch)	0.907	0.686–1.199	0.492
DSA (DSA-positive)	2.719	1.081–6.841	**0.034**
Donation after cardiac death (DCD)	1.684	0.484–5.882	0.413
Donor sex (male)	1.790	0.936–3.424	0.078
Donor Age (per year)	1.043	1.021–1.065	< **0.001**
Retransplantation (retransplant)	2.094	0.926–4.738	0.076
GN[b] as the cause of ESKD (all other)	1.463	0.785–2.725	0.231
Transplant era (1981–1989)	0.836	0.414–1.691	0.619
BMI 20 years post-transplant	0.973	0.904–1.047	0.458
HbA1c 20 years post-transplant	1.246	0.770–2.016	0.37
CSA-based immunosuppression (CsA-free)	0.794	0.394–1.598	0.518
steroid-containing immunosuppression (steroids)	2.572	1.382–4.787	**0.003**
Early TCMR (<12 months)	1.847	0.905–3.771	0.092
Multivariate Cox Regression			
Group (Group 2)	20.515	2.730–154.143	**0.003**
DSA (DSA-positive)	3.081	1.165–8.146	**0.023**
Donor age (per year)	1.032	1.004–1.061	**0.024**
Steroid-containing immunosuppression (steroids)	2.844	1.295–6.246	**0.009**

[a]*Only HD vs. PD/HD.*

[b]*Glomerulonephritis, incl. vasculitis, systemic lupus erythematosus, and suspected chronic GN.*

Univariable and multivariable Cox regression models for death-censored graft failure. Reference category in parentheses. HR, hazard ratio; CI, confidence interval.

failure (40,41) and provide a well-established biomarker predicting ABMR and graft loss (40,42). However, little is known about the role of DSA in the context of ultralong-term survival (12,43). Analysis of DSA-screenings revealed several interesting findings: First, in our cohort of ULS only, cumulative incidence of DSA-formation during the first two post-transplant decades was 19.7% and thus within the range reported from general KTR-population (42,44). Secondly, we could not find any significant group difference in cumulative incidence of DSA-positive KTRs, duration to first DSA-detection, HLA-class, HLA-mismatches, PIRCHE-II socres, MFI, and number of detected DSA. These results surprise, as KTRs who develop dnDSA have been shown to have higher rates of eGFR decline (41). Thirdly, however, DSA-formation was identified as an independent risk factor for DC-GF. The association of steroid use with DC-GF must be interpreted here with the restart of steroids after the onset of DSA. In our cohort, DSA were detected surprisingly late (median 211 months post-transplant), a finding probably biased by transplant era and available techniques. However, it is known that DSA-formation can appear anytime, even several years post-transplant (42,43,44) and that time from dnDSA-onset to graft dysfunction ranges from months to years (44). So nevertheless, it is suggestive that ultralong-term survival of our cohort was favored by substantially late DSA-development and that their deleterious impact on graft survival manifested not until the third post-transplant decade. Given the close relationship between dnDSA, ABMR and ultimate graft loss, this result points towards a potential target of intervention in order to further improve long-term graft survival (45). However, further studies are needed to address this question (45).

Hence, DC-GF is predominantly seen in ULS with inferior graft function. However, despite the known link of declining graft function with increased mortality (46), there was no significant group difference in patient survival. The risk of DWFG increases with time since transplantation (47) and in ULS, it represents the leading cause of graft loss (8,9,14,15). Our results correspond with the findings from Gaston et al. who stated that mortality risk is largely independent of graft function (16). Beyond 20 years post-transplant, leading causes of death are cardiovascular disease and malignancy (8,9), both highly prevalent in ULS (7,8,9,14,15). For example, McCaughan et al. reported cancer in 37% and cardiovascular disease in 27% of 20-year survivors and therefore stated that, in management of ULS, focus should shift on prevention and optimal therapy of these comorbidities (9).

Our results allow us to specify this statement and lead to further clinical implications. Indeed, in case of good, stable graft function up to 20 years post-transplant, risk of ultimate DC-GF is very low, and focus should be on controlling the medical comorbidities (9). In contrast, in KTRs with inferior ultralong-term graft function, risk of DC-GF may not be neglected, and aftercare should equally concentrate on preventing ultimate loss of graft function. Given that DSA and ABMR, respectively, are potentially treatable conditions (45). Our findings argue for continuing DSA-monitoring even in the setting of ultralong-term survival. Additionally, KTRs with inferior ultralong-term graft function might be considered for biopsy, not only to

TABLE 8 | Cox Regression analysis to assess the mortality risk of KTRs 20 years post-transplantation.

Univariate Cox regression analysis	HR	95% CI	*p*-Value
Group (Group 2)	1.301	0.749–2.261	0.350
20-year eGFR (BSA deindexed CKD-EPI)	0.995	0.981–1.009	0.469
20-year proteinuria	1.000	1.000–1.001	< **0.001**
eGFR (CKD)-EPI) decline	1.071	0.949–1.208	0.266
Recipinet sex (male)	1.742	1.013–2.995	**0.045**
Recipient age (per year)	1.084	1.054–1.115	< **0.001**
Time on pretransplant dialysis (per month)	1.003	0.992–1.014	0.616
Pretransplant dialysis[a] (PD)	0.645	0.335–1.241	0.189
Retransplantation (retransplant)	1.044	0.416–2.625	0.926
GN[2] as the cause of ESKD (all other)	0.456	0.244–0.854	**0.014**
Transplant era (1981–1989)	1.136	0.613–2.105	0.685
BMI 20 years post-transplant	0.989	0.929–1.053	0.730
HbA1c 20 years post-transplant	1.126	0.727–1.745	0.595
CsA-based immunosuppression (CsA-free)	0.372	0.214–0.645	< **0.001**
containing steroid-containing immunosuppression (steroid-free)	1.936	1.126–3.327	**0.017**
Multivariate Cox regression analysis			
Recipient sex (male)	1.829	1.021–3.276	**0.042**
Recipient age (per year)	1.094	1.062–1.126	< **0.001**
CSA-based immunosuppression (CsA-free)	0.297	0.149–0.593	< **0.001**
containing steroid-containing immunosuppression (steroid-free)	1.701	0.866–3.340	0.123
GN[b] as Cause for ESKD (all other)	0.827	0.429–1.595	0.571

[a]*Only HD vs. PD/HD.*

[b]*Glomerulonephritis, incl. vasculitis, systemic lupus erythematosus, and suspected chronic GN.*

Univariable and multivariable Cox regression models for patient survival. Reference category in parentheses. HR, hazard ratio; CI, confidence interval.

evaluate immune injury, but also to detect evidence of CNI-toxicity and to adjust immunosuppression, accordingly (48,49).

Our study has several limitations. First, it is a retrospective and single-center analysis with the intrinsic limitations and potential biases. Secondly, our cohort differs in several aspects from more recently transplanted KTRs. Thirdly, DSA-subanalysis is limited by transplant era and available techniques: it affected time of first and frequency of subsequent screenings, 50 cases with missing or insufficient data had to be excluded, and KTRs were mostly not typed for HLA-DP and -DQ.

However, most of these limitations are inevitably associated with the retrospective design of research on ULS and the according necessity of lengthy follow-up (8). In addition, our study cohort is not dominated by living donor transplantations, which would suggest better organ quality and possibly better HLA matching, so our results translate well to the general transplant cohort. The reason for this is that only about 50 living donations were performed in the observation period from 1981 to 1999 at our center. Our study provides an important contribution in improving understanding of this unique, increasingly important patient population (7,8,9). Comprehensive follow-up enables us to give extensive overview of ultralong-term graft function, alloimmunization, and ultimate outcome beyond 20 years post-transplant and to identify corresponding risk factors and potential therapeutic targets required to improve ULS-aftercare.

CONCLUSION

Overall, KTRs with ultralong-term survival ≥20 years do extremely well. Particularly KTRs with stable and high eGFR and low proteinuria likely keep their graft function and ultimately die of medical comorbidities. The risk of graft failure is predominantly seen in KTRs with inferior graft function. This graft function-related risk profile could augment long-term monitoring and treatment.

DATA AVAILABILITY STATEMENT

The data underlying this article will be shared on reasonable request to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Cantonal Ethics Committee Zurich. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

AR participated in data collection, data analysis, and writing of the paper. JN participated in data collection and writing of the paper. RW participated in writing of the paper. TM participated in research design and writing of the paper. TS participated in research design, data collection, data analysis and writing of the paper.

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.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontierspartnerships.org/articles/10.3389/ti.2022.10675/full#supplementary-material

REFERENCES

1. Chadban SJ, Ahn C, Axelrod DA, Foster BJ, Kasiske BL, Kher V, et al. Summary of the Kidney Disease: Improving Global Outcomes (KDIGO) Clinical Practice Guideline on the Evaluation and Management of Candidates for Kidney Transplantation. *Transplantation* (2020) 104(4): 708–14. doi:10.1097/TP.0000000000003137
2. 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
3. ANZDATA Registry. *Chapter 7: Kidney Transplantation, Australia and New Zealand Dialysis and Transplant Registry*. 43rd Report. Adelaide, Australia (2020). Available at: http://www.anzdata.org.au (Accessed December 14, 2021).
4. United States Renal Data System. *USRDS Annual Data Report: Epidemiology of Kidney Disease in the United States*. Bethesda, MD: National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases (2021).
5. Canadian Institute for Health Information. *Treatment of End-Stage Organ Failure in Canada, Canadian Organ Replacement Register, 2011 to 2020: End-Stage Kidney Disease and Kidney Transplants – Data Tables*. Ottawa, ON: CIHI (2021).
6. Collaborative Transplant Study. CTS Outcome Graphs (2022). *Graph K-15103E-0222*. Available at: https://www.ctstransplant.org/public/graphics/archive.shtml (Accessed December 14, 2021).
7. Bererhi L, Pallet N, Zuber J, Anglicheau D, Kreis H, Legendre C, et al. Clinical and Immunological Features of Very Long-Term Survivors with a Single Renal Transplant: Renal Transplant Patients after 30 Years. *Transpl Int* (2012) 25(5): 545–54. doi:10.1111/j.1432-2277.2012.01451.x
8. Traynor C, Jenkinson A, Williams Y, O'Kelley P, Hickey D, Denton M, et al. Twenty-Year Survivors of Kidney Transplantation. *Am J Transpl* (2012) 12(12):3289–95. doi:10.1111/j.1600-6143.2012.04236.x
9. McCaughan JA, Courtney AE. The Clinical Course of Kidney Transplant Recipients after 20 Years of Graft Function: Outcome after 20 Years of Transplant Function. *Am J Transpl* (2015) 15(3):734–40. doi:10.1111/ajt.13041
10. Manna GL, Capelli I, Gasperoni L, Comai G, Ravaioli M, Marchetti A, et al. Long Term Outcomes of Kidney Transplant: Characteristics of Recipients with 20 or More Years of Graft Survival. *J Med Surg Pathol* (2016) 01(02). doi:10.4172/2472-4971.1000109
11. Parajuli S, Mandelbrot DA, Aziz F, Garg N, Muth B, Mohamed M, et al. Characteristics and Outcomes of Kidney Transplant Recipients with a Functioning Graft for More Than 25 Years. *Kidney Dis* (2018) 4(4):255–61. doi:10.1159/000491575
12. Kettler B, Scheffner I, Bräsen JH, Hallensleben M, Richter N, Heiringhoff KH, et al. Kidney Graft Survival of >25 Years: a Single center Report Including Associated Graft Biopsy Results. *Transpl Int* (2019) 32(12):1277–85. doi:10.1111/tri.13469
13. El-Agroudy AE, Bakr MA, Hassan NA, Ismail AM, Ali-El-Dein B, Shehab El-Dein AB, et al. Characteristics of Long-Term Live-Donor Renal Allograft Survivors. *Am J Nephrol* (2003) 23(3):165–71. doi:10.1159/000070333
14. Peddi V, Whiting J, Weiskittel P, Alexander J, First M. Characteristics of Long-Term Renal Transplant Survivors. *Am J Kidney Dis* (1998) 32(1):101–6. doi:10.1053/ajkd.1998.v32.pm9669430
15. Rao VK. Long Term Results and Complications of Renal Transplantation: Observations in the Third Decade. *Indian J Nephrol* (2001) 11:155–9.
16. Gaston RS, Fieberg A, Helgeson ES, Eversull J, Hunsicker L, Kasiske BL, et al. Late Graft Loss after Kidney Transplantation: Is "Death with Function" Really Death with a Functioning Allograft? *Transplantation* (2020) 104(7):1483–90. doi:10.1097/TP.0000000000002961
17. Levey AS, Bosch JP, Lewis JB, Greene T, Rogers N, Roth D. A More Accurate Method to Estimate Glomerular Filtration Rate from Serum Creatinine: a New Prediction Equation. Modification of Diet in Renal Disease Study Group. *Ann Intern Med* (1999) 130(6):461–70. doi:10.7326/0003-4819-130-6-199903160-00002
18. Levey AS, Stevens LA, Schmid CH, Zhang YL, Castro AF, Feldman HI, et al. CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration): a New Equation to Estimate Glomerular Filtration Rate. *Ann Intern Med* (2009) 150:604–12. doi:10.7326/0003-4819-150-9-200905050-00006
19. Cockcroft DW, Gault MH. Prediction of Creatinine Clearance from Serum Creatinine. *Nephron* (1976) 16:31–41. doi:10.1159/000180580
20. Zelnick LR, Leca N, Young B, Bansal N. Association of the Estimated Glomerular Filtration Rate with vs without a Coefficient for Race with Time to Eligibility for Kidney Transplant. *JAMA Netw Open* (2021) 4(1): e2034004. doi:10.1001/jamanetworkopen.2020.34004
21. DuBois D, DuBois E. A Formula to Estimate the Approximate Surface Area if Height and Weight Be Known. *Arch Intern Med* (1916) 17:863–71. doi:10.1001/archinte.1916.00080130010002
22. Delanaye P, Krzesinski JM. Indexing of Renal Function Parameters by Body Surface Area: Intelligence or Folly. *Nephron Clin Pract* (2011) 119(4):c289–92. doi:10.1159/000330276
23. Vlasschaert C, Thibodeau S, Parmar MS. De-indexed Estimated Glomerular Filtration Rates: A Simple Step towards Improving Accuracy of Drug Dosing of Renally Excreted Medications in Moderate to Severe Obesity. *Nephrology* (2020) 25(1):29–31. doi:10.1111/nep.13621
24. Mitch W, Walser M, Buffington G, Lemann J. A Simple Method of Estimating Progression of Chronic Renal Failure. *Lancet* (1976) 2:1326–8. doi:10.1016/s0140-6736(76)91974-7
25. Kidney Disease: Improving Global Outcomes (KDIGO) CKD Work Group. KDIGO 2012 Clinical Practice Guideline for the Evaluation and Management of Chronic Kidney Disease. *Kidney inter., Suppl* (2013) 3:1–150.
26. Zwiener I, Blettner M, Hommel G. Survival Analysis— Part 15 of a Series on Evaluation of Scientific Publications. *Dtsch Arztebl Int* (2011) 108(10):163–9. doi:10.3238/arztebl.2010.0163
27. Summers DM, Watson CJ, Pettigrew GJ, Johnson RJ, Collett D, Neuberger JM, et al. Kidney Donation after Circulatory Death (DCD): State of the Art. *Kidney Int* (2015) 88(2):241–9. doi:10.1038/ki.2015.88
28. Meier-Kriesche HU, Ojo AO, Leavey SF, Hanson JA, Leichtman AB, Magee JC, et al. Gender Differences in the Risk for Chronic Renal Allograft Failure. *Transplantation* (2001) 71(3):429–32. doi:10.1097/00007890-200102150-00016
29. Carrero JJ, Hecking M, Chesnaye NC, Jager KJ. Sex and Gender Disparities in the Epidemiology and Outcomes of Chronic Kidney Disease. *Nat Rev Nephrol* (2018) 14(3):151–64. doi:10.1038/nrneph.2017.181
30. Heemann U, Abramowicz D, Spasovski G, Vanholder R. Endorsement of the Kidney Disease Improving Global Outcomes (KDIGO) Guidelines on Kidney Transplantation: a European Renal Best Practice (ERBP) Position Statement. *Nephrol Dial Transpl* (2011) 26(7):2099–106. doi:10.1093/ndt/gfr169
31. El-Zoghby ZM, Stegall MD, Lager DJ, Kremers WK, Amer H, Gloor JM, et al. Identifying Specific Causes of Kidney Allograft Loss. *Am J Transpl* (2009) 9(3): 527–35. doi:10.1111/j.1600-6143.2008.02519.x
32. Matas AJ. Chronic Progressive Calcineurin Nephrotoxicity: an Overstated Concept. *Am J Transpl* (2011) 11(4):687–92. doi:10.1111/j.1600-6143.2011.03505.x
33. Wojciechowski D, Wiseman A. Long-Term Immunosuppression Management: Opportunities and Uncertainties. *Clin J Am Soc Nephrol* (2021) 16(8):1264–71. doi:10.2215/CJN.15040920
34. Gourishankar S, Leduc R, Connett J, Cecka JM, Cosio F, Fieberg A, et al. Pathological and Clinical Characterization of the 'Troubled Transplant': Data

from the DeKAF Study. *Am J Transpl* (2010) 10(2):324–30. doi:10.1111/j.1600-6143.2009.02954.x
35. Rao PS, Schaubel DE, Guidinger MK, Andreoni KA, Wolfe RA, Merion RM, et al. A Comprehensive Risk Quantification Score for Deceased Donor Kidneys: The Kidney Donor Risk Index. *Transplantation* (2009) 88(2): 231–6. doi:10.1097/TP.0b013e3181ac620b
36. Denic A, Glassock RJ, Rule AD. Structural and Functional Changes with the Aging Kidney. *Adv Chronic Kidney Dis* (2016) 23(1):19–28. doi:10.1053/j.ackd.2015.08.004
37. Summers DM, Johnson RJ, Hudson A, Collett D, Watson CJ, Bradley JA. Effect of Donor Age and Cold Storage Time on Outcome in Recipients of Kidneys Donated after Circulatory Death in the UK: a Cohort Study. *Lancet* (2013) 381(9868):727–34. doi:10.1016/S0140-6736(12)61685-7
38. Moreso F, Seron D, Gil-Vernet S, Riera L, Fulladosa X, Ramos R, et al. Donor Age and Delayed Graft Function as Predictors of Renal Allograft Survival in Rejection-free Patients. *Nephrol Dial Transpl* (1999) 14(4):930–5. doi:10.1093/ndt/14.4.930
39. Einecke G, Sis B, Reeve J, Mengel M, Campbell PM, Hidalgo LG, et al. Antibody-Mediated Microcirculation Injury Is the Major Cause of Late Kidney Transplant Failure. *Am J Transpl* (2009) 9(11):2520–31. doi:10.1111/j.1600-6143.2009.02799.x
40. Lachmann N, Terasaki PI, Budde K, Liefeldt L, Kahl A, Reinke P, et al. Anti-Human Leukocyte Antigen and Donor-specific Antibodies Detected by Luminex Post-transplant Serve as Biomarkers for Chronic Rejection of Renal Allografts. *Transplantation* (2009) 87(10):1505–13. doi:10.1097/TP.0b013e3181a44206
41. Wiebe C, Gibson IW, Blydt-Hansen TD, Pochinco D, Birk PE, Ho J, et al. Rates and Determinants of Progression to Graft Failure in Kidney Allograft Recipients with *De Novo* Donor-specific Antibody: Post- *Dn* DSA Clinical Histologic Progression. *Am J Transpl* (2015) 15(11):2921–30. doi:10.1111/ajt.13347
42. Zhang R. Donor-Specific Antibodies in Kidney Transplant Recipients. *Clin J Am Soc Nephrol* (2018) 13(1):182–92. doi:10.2215/CJN.00700117
43. Calp-Inal S, Ajaimy M, Melamed ML, Savchik C, Masiakos P, Colovai A, et al. The Prevalence and Clinical Significance of C1q-Binding Donor-specific Anti-HLA Antibodies Early and Late after Kidney Transplantation. *Kidney Int* (2016) 89(1):209–16. doi:10.1038/ki.2015.275
44. Konvalinka A, Tinckam K. Utility of HLA Antibody Testing in Kidney Transplantation. *J Am Soc Nephrol* (2015) 26(7):1489–502. doi:10.1681/ASN.2014080837
45. Schinstock CA, Mannon RB, Budde K, Chong AS, Haas M, Knechtle S, et al. Recommended Treatment for Antibody-Mediated Rejection after Kidney Transplantation: The 2019 Expert Consensus from the Transplantion Society Working Group. *Transplantation* (2020) 104(5):911–22. doi:10.1097/TP.0000000000003095
46. Lorenz EC, El-Zoghby ZM, Amer H, Dean PG, Hathcock MA, Kremers WK, et al. Kidney Allograft Function and Histology in Recipients Dying with a Functioning Graft: Kidney Function in DWF. *Am J Transpl* (2014) 14(7): 1612–8. doi:10.1111/ajt.12732
47. Ojo AO, Hanson JA, Wolfe RA, Leichtman AB, Agodoa LY, Port FK. Long-term Survival in Renal Transplant Recipients with Graft Function. *Kidney Int* (2000) 57(1):307–13. doi:10.1046/j.1523-1755.2000.00816.x
48. Kasiske BL, Zeier MG, Chapman JR, Craig JC, Ekberg H, Garvey CA, et al. KDIGO Clinical Practice Guideline for the Care of Kidney Transplant Recipients: a Summary. *Kidney Int* (2010) 77(4):299–311. doi:10.1038/ki.2009.377
49. Baker RJ, Mark PB, Patel RK, Stevens KK, Palmer N. Renal Association Clinical Practice Guideline in post-operative Care in the Kidney Transplant Recipient. *BMC Nephrol* (2017) 18(1):174. doi:10.1186/s12882-017-0553-2
50. Levin A, Stevens PE. Summary of KDIGO 2012 CKD Guideline: behind the Scenes, Need for Guidance, and a Framework for Moving Forward. *Kidney Int* (2014) 85(1):49–61. doi:10.1038/ki.2013.444
51. Sellarés J, de Freitas DG, Mengel M, Reeve J, Einecke G, Sis B, et al. Understanding the Causes of Kidney Transplant Failure: the Dominant Role of Antibody-Mediated Rejection and Nonadherence. *Am J Transpl* (2012) 12(2):388–99. doi:10.1111/j.1600-6143.2011.03840.x

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Influence of Graft Ureter Length, a Donor-Related Factor, on Urinary Tract Infections After Living-Donor Kidney Transplantation: A Single-Center Analysis of 211 Cases

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Urinary tract infection (UTI) occurs in 25% of recipients of living-donor kidney transplantation (LDKT). Female sex, age, and anatomical abnormalities have been reported as recipient-related risk factors for UTI after LDKT; few studies have reported donor-related factors. We retrospectively examined UTI occurrence within 5 years of transplantation in recipients (n = 211) who underwent LDKT at our hospital between April 2011 and April 2021. All nephrectomies were performed using a retroperitoneal pure laparoscopic approach. The ureter was dissected at the lower level of the common iliac artery and trimmed to the shortest length, enough to reach the bladder using extra vesicular ureterocystoneostomy with a 3 cm submucosal tunnel. Twenty-nine recipients (13.7%) developed UTI within 5 years, and the median time to onset was 40.0 days. After adjusting for the well-known factors, including recipient sex, graft ureter length was an independent factor for UTI occurrence (HR 1.25, 95% CI 1.02~1.53, p = 0.028) in the multivariate Cox regression analysis. The long ureter is usually trimmed, and the widest part is used for anastomosis, which may increase the possibility of reflux from the bladder to the ureter in the standard technique. The ureter length may be associated with the incidence of UTI after LDKT.

Keywords: kidney transplantation, donor-related factors, ureter length, urinary tract infection, ureter diameter

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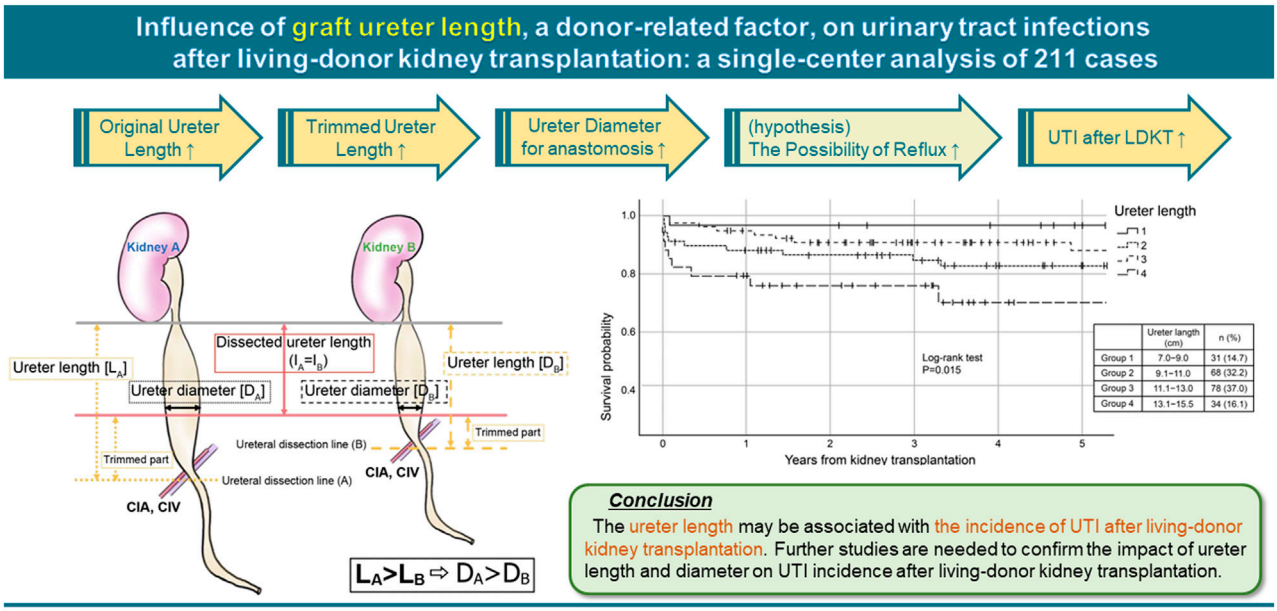
Abbreviations: ADPKD, autosomal dominant polycystic kidney disease; AMR, antibody-mediated rejection; AUC, area under the curve; BMI, body mass index; BSA, body surface area; BW, body weight; CFU, colony-forming unit; CI, confidence interval; CIA, common iliac artery; CIV, common iliac vein; CSA, cyclosporine; DSA, donor specific antibody; HLA, human leukocyte antigen; HR, hazard ratio; LDKT, living-donor kidney transplantation; MMF, mycophenolate mofetil; OR, operation room; TAC, tacrolimus; UTI, urinary tract infection; VUR, vesicoureteral reflex.



GRAPHICAL ABSTRACT |

INTRODUCTION

Urinary tract infection (UTI) is one of the most common infections after kidney transplantation. UTI has been reported to occur in 25% of recipients within the first year after living-donor kidney transplantation (1–3). It is associated with increased risks of acute rejection, allograft dysfunction, graft loss, increased duration of hospitalization, and mortality (1, 3–6). Furthermore, recurrent UTI, which occurs in 7% of the patients after kidney transplantation, is one of the leading causes of allograft loss and death (7). Therefore, prediction, early detection, and prevention of UTI is essential.

Recipient-related factors, such as older age, female sex, recurrent UTI before kidney transplantation, number of days with indwelling urinary catheter, congenital urinary tract malformations, vesicoureteral reflux (VUR), history of UTI 1 month before kidney transplantation, and autosomal dominant polycystic kidney disease (ADPKD), are known to increase the incidence of UTI (8, 9). Although there have been many studies regarding the influence of recipient-related risk factors on UTI after kidney transplantation, only a few studies have reported donor-related factors other than deceased donor kidneys (3, 10, 11).

We hypothesized that donor-related factors could also affect the incidence of UTI. We retrospectively examined the association between the incidence of UTI after living-donor kidney transplantation and donor-related factors.

MATERIALS AND METHODS

We performed a retrospective analysis of the factors related to UTI occurrence within 5 years of living-donor kidney transplantation. Consecutive 211 recipients who underwent living-donor kidney transplantation at our hospital from April 2011 to April 2021 were included.

A list of recipient and donor characteristics was made according to the previous reports (3, 8–11), and the corresponding information was collected from the electronic medical records. The following recipient characteristics were collected: age, sex, body weight (BW, kg), body mass index (BMI, kg/m^2), body surface area (BSA, m^2), presence of diabetes, history of dialysis and duration of dialysis (months), pre-transplant bladder volume (ml). The following donor characteristics were collected: age, sex, weight (kg), BMI (kg/m^2), BSA (m^2), graft weight (g), graft volume (cm^3), graft major axis (mm), graft density (g/cm^3), graft ureter length (cm), side of the graft (left or right). The ureter length was defined as the length from the lower pole of the kidney to the stump of the ureter. Graft volume and graft density were calculated as follows: graft volume (cm^3) = Long diameter (mm) x short diameter (mm) x thickness (mm) x 4/3 x π x 1000; graft density (g/cm^3) = graft weight (g)/graft volume (cm^3). The eGFR slope (ΔeGFR/year) was calculated as follows: eGFR slope = (the latest eGFR—eGFR at 1-year post-transplant)/(post-operative years of the latest eGFR—1).

Definition of UTI

We included all recipients with symptomatic uncomplicated and complicated UTI for the analysis. Urinalysis and urine cultures were performed if the recipient had a fever or complained of urinary symptoms.

The definitions of UTI related terms are as follows. Asymptomatic bacteriuria: positive urine culture (identified as $>10^5$ colony-forming unit [CFU]) without any symptoms. Uncomplicated UTI (simple cystitis): positive urine culture (identified as $>10^5$ CFU) with local urinary symptoms such as dysuria, frequency, and urgency without systemic symptoms such as fever and abdominal pain. Complicated UTI: positive urine culture (identified as $>10^5$ CFU) with the systemic manifestation of fever, graft pain, chills, malaise caused by the same bacteria in urine, or biopsy with findings consistent with pyelonephritis(12).

Immunosuppression Protocols

All patients were administered methylprednisolone (500 mg/body) immediately before graft reperfusion, and basiliximab (20 mg/body) on days 0 and 4. The standard protocol consisted of administration of tacrolimus (TAC), mycophenolate mofetil (MMF), and methylprednisolone. The dosages of TAC and MMF were adjusted to achieve optimal trough levels and area under the curve (AUC) of 0–4 levels as previously reported (13). MMF was started at a dose of 2,500 mg/day when TAC was used and 3,000 mg/day when cyclosporine (CSA) was used from day 1 to day 14; thereafter, MMF was administered at doses of 2,000 mg/day and 1,500 mg/day when TAC and CSA were used, respectively. Methylprednisolone doses were reduced gradually from 60 mg/day on day 0–10 mg/day on day 19 and maintained at 5 mg/day from 6 months after transplantation. Desensitization therapy consisted of rituximab (100~200 mg/body) twice on day 1 and day 14 or once on day 1, double filtration plasmapheresis four times before kidney transplantation, and MMF (1,000 mg/day) with prednisolone (10 mg/day) from day 14. The intensity of desensitization therapy was determined by the risk-stratified method but modified according to the patient background.

Operative Methods and Post-operative Managements

All nephrectomies were performed using a retroperitoneal pure laparoscopic approach. The surrounding tissue of the ureter was carefully preserved and dissected at the lower level of the common iliac artery. We measured the longest length of the ureter from the inferior pole of the graft kidney to the tip of the ureter while trailing the ureter down to the kidney after completing the back-table procedures. The graft kidney was placed on the right iliac fossa and the iliac vessels were used for the anastomoses of the artery and vein. The vena cava or aorta was not used for the anastomosis in this cohort. During the study period, the donor and recipient surgeries were performed by the same surgical team, and no technical changes were made. In this study, two primary surgeons were involved in the recipient surgeries, which were performed or supervised by at least one of these surgeons. One primary surgeon performed or supervised all donor surgeries. Several surgeons, mostly residents, accompanied each surgery.

Ureterocystoneostomy was performed using the extravesical anastomosis method as previously reported (14). Briefly, the ureter was trimmed to the shortest length that was enough to reach the bladder, and the tip of the ureter was spatulated to 7 mm. The ureteroneocystostomy was conducted by the Lich–Gregoir method using 5-0 polydioxanone monofilament continuous sutures (15, 16). A 3 cm long submucosal tunnel was created as an anti-reflux procedure, and a 5-French 14 cm gauge double-J ureteral stent was placed. Urine was collected using a urethral catheter immediately after induction of anesthesia and before performing the surgery; sample for a urine culture was collected at the time of the catheter placement. Bladder capacity was measured by the free-fall water-filling method (upper limit 400 ml). We used cefazoline for donor nephrectomy and recipient surgery. The urethral catheter was removed on postoperative day 5, and the double-J catheter was removed using a cystoscope on postoperative day 6, unless any adverse events occurred. The voided volume after the removal of the double-J catheter was determined based on the bladder capacity measured in the operating room.

This study conformed with the principles outlined in the Declaration of Helsinki of 1964 and the Declaration of Istanbul of 2018. The protocol was approved by the ethics committee at Japanese Red Cross Kumamoto Hospital (study approval number 490), and the requirement of written informed consent was waived considering the retrospective and non-invasive nature of this study. None of the transplant donors were from a vulnerable population and all the donors or next of kin provided freely given written informed consent.

Statistical Analysis

Baseline characteristics were evaluated for significant differences by Chi-square test for categorical variables, Shapiro–Wilk test of normality for quantitative variables, and t-test or Mann–Whitney U test for significant differences. Cox proportional hazard model was used to examine each factor that was considered to affect the incidence of UTI. A p-value <0.05 was considered to be significant. To identify independent predictors of outcomes, donor-related factors with significant differences were identified using univariate analyses, and multivariate analyses were performed with known factors, such as recipient sex, by using Cox proportional hazards models. Forward stepwise logistic regression was performed to identify the potential independent risk factors associated with the UTI within 5 years of transplantation. The analyses of the incidence of UTI within 5 years were performed using the Kaplan–Meier method, and statistical differences between curves were assessed using the log-rank test. Initial UTI events for recurrent cases were used for Kaplan–Meier and Cox analysis. Cases with missing data were not included in the study. All statistical analysis was performed using IBM SPSS Statistics for Windows, version 25 (IBM Corp., Armonk, NY, United States).

TABLE 1 | Baseline characteristics.

Characteristics (*n* = 211)	UTI(–) *n* = 182 (86.3%)	UTI(+) *n* = 29 (13.7%)	*p* value
Recipient			
Recipient age, median (years old, IQR)	47.0 (33.0~58.0)	52.0 (46.0~61.0)	0.063
Female recipient, n (%)	51 (28.0%)	17 (58.6%)	0.001
Body weight, median (kg, IQR)	60.6 (52.0~71.2)	59.0 (51.1~67.4)	0.378
BMI, median (kg/m^2, IQR)	22.2 (19.6~25.0)	22.5 (19.9~24.6)	0.863
BSA, median (m^2, IQR)	1.7 (1.5~1.8)	1.60 (1.5~1.8)	0.232
Diabetes mellitus, n (%)	60 (33.0%)	12 (41.4%)	0.375
Dialysis dependence, n (%)	114 (62.6%)	21 (72.4%)	0.308
Duration of hemodialysis, median (months, IQR)	19.5 (7.8~47.0)	26.0 (7.5~77.0)	0.464
Bladder volume, median (mL, IQR)	304.6 ± 132.3	286.7 ± 146.3	0.508
Neurogenic bladder, n (%)	4 (2.2%)	1 (3.4%)	0.526
Recurrent UTI before transplantation, n (%)	3 (1.6%)	1 (3.4%)	0.449
Donor			
Donor age, median (years old, IQR)	57.0 (50.0~64.3)	59.0 (53.5~65.5)	0.332
Female donor, n (%)	127 (69.8%)	15 (51.7%)	0.054
Body weight, median (kg, IQR)	57.7 (51.6~66.0)	61.0 (53.7~67.3)	0.190
BMI, median (kg/m^2, IQR)	22.7 (20.7~24.9)	23.2 (22.0~24.9)	0.388
BSA, median (m^2, IQR)	1.6 (1.5~1.7)	1.7 (1.5~1.8)	0.138
Graft weight, median (g, IQR)	156.5 (136.0~186.5)	168.0 (147.5~226.0)	0.020
Graft major axis, median (mm, IQR)	105.0 (100.0~110.0)	110.0 (100.0~115.0)	0.126
Graft density (g/mm^3, IQR)	1.2 (1.0~1.6)	1.4 (1.1~1.8)	0.286
Ureter length, median (cm, IQR)	11.5 (10.0~12.0)	11.5 (11.0~14.0)	0.080
Left kidney graft, n (%)	156 (85.7%)	29 (100.0%)	0.016
Recipient X Donor			
No. HLA mismatches (total), mean ± SD			
Class 1	2.0 ± 1.1	2.1 ± 1.0	0.538
Class 2	1.0 ± 0.6	1.1 ± 0.8	0.376
Total	3.0 ± 1.5	3.2 ± 1.6	0.378
Incompatible transplantation, n (%)	53 (29.1%)	13 (44.8%)	0.090

UTI, urinary tract infection; BMI, body mass index; BSA, body surface area; HLA, human leukocyte antigen.

TABLE 2 | Recipient outcomes.

Characteristics (*n* = 211)	UTI(–) *n* = 182 (86.3%)	UTI(+) *n* = 29 (13.7%)	*p* value
5-year patient survival	98.4%	100%	0.640
5-year graft survival	93.4%	100%	0.161
All cause graft failure, n (%)	18 (9.9%)	2 (6.9%)	0.460
Creatinine at 1 year, median (mg/dL, IQR)	1.27 (1.02~1.47)	1.11 (0.86~1.42)	0.094
Urinary protein at 1 year, median (mg/day, IQR)	137.0 (79.0~261.5)	88.0 (56.5~195.5)	0.028
eGFR at 1 year, median (ml/min/1.73 m^2, IQR)	47.4 (39.5~53.8)	45.3 (38.1~55.1)	0.947
ΔeGFR/year, median (ml/min/1.73 m^2, IQR)	–0.64 (–1.85~0.85)	–0.62 (–2.26~1.01)	0.988
BK virus infection, n (%)	7 (3.8%)	2 (6.9%)	0.357
Rejection, n (%)	17 (9.3%)	1 (3.4%)	0.257
Post-operative complications, n (%)	20 (11.0%)	2 (6.9%)	0.389
Re-intervention, n (%)	11 (6.0%)	1 (3.4%)	0.489
Double-J stent (≧7 days), n (%)	32 (17.9%)	7 (25.0%)	0.255
Double-J stent placement duration, median (days, IQR)	6.0 (6.0~6.0)	6.0 (6.0~9.75)	0.118
De novo DSA, n (%)	23 (12.6%)	1 (3.4%)	0.123

UTI, urinary tract infection; GFR, glomerular filtration rate; DSA, donor specific antibody.

RESULTS

Baseline Characteristics

Table 1 shows the baseline characteristics of the recipients and donors. The incidence of UTI within 5 years after transplantation was 13.7% ($n = 29$). Of these, 14 out of 29 recipients experienced recurrent UTIs (six UTIs: $n = 2$, five UTIs: $n = 1$, four UTIs: $n = 1$, three UTIs: n = 1, and two UTIs: $n = 9$). The median time of onset was 40.0 days after transplantation (IQR, 11.5~445.5 days). There were six symptomatic uncomplicated UTI patients and 23 symptomatic complicated UTI patients. The distribution of the ureter length was not significantly different between the groups. Complications such as including uretero-ureteral anastomosis was not observed.

TABLE 3 | Cox proportional hazard model.

Variable	Univariate analysis		Multivariate analysis	
	HR (95% CI)	*p value*	HR (95% CI)	*p value*
Recipient sex (ref. male)	3.16 (1.51~6.61)	0.002	3.05 (1.45~6.40)	0.003
Donor sex (ref. male)	0.53 (0.26~1.09)	0.085		
Graft weight (per 10 g)	1.12 (1.03~1.20)	0.004		
Ureter length (per 1 cm)	1.27 (1.04~1.55)	0.020	1.25 (1.02~1.53)	0.028

HR, hazard ratio; CI, confidence interval.

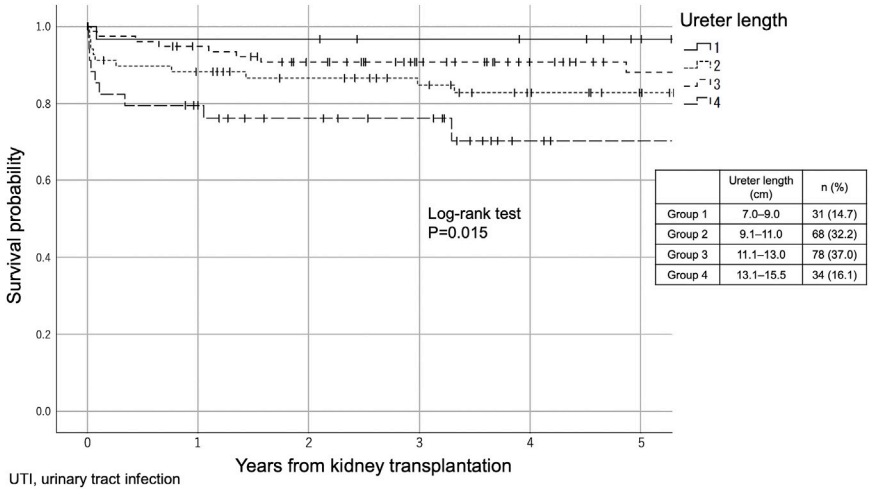


FIGURE 1 | The difference in UTI incidence between the four groups according to ureter length (Kaplan–Meier method). The 211 recipients were stratified into the following four groups according to the ureter length: Group 1: 7.0–9.0 cm (n = 31, 14.7%), Group 2: 9.1–11.0 cm (n = 68, 32.2%), Group 3: 11.1–13.0 cm (n = 78, 37.0%) and Group 4: 13.1–15.5 cm (n = 34, 16.1%).

For recipient-related factors, female sex (UTI vs. non-UTI: 58.6% vs. 28.0%, p = 0.001) was significantly different between the groups. There were no significant differences in age, weight, BMI, BSA, presence of diabetes mellitus, dialysis modality, duration of hemodialysis, and bladder capacity measured at the operating room. For donor-related factors, graft weight (UTI vs. non-UTI: 168.0 g [IQR: 147.5 g~226.0 g] vs. 156.5 g [IQR: 136.0 g~186.5 g], p = 0.020), and graft side (left, UTI vs. non-UTI: 100% vs. 85.7%, p = 0.016) were significantly different between the groups, while no significant differences were seen in age, sex, BW, BMI, BSA, major axis, and graft density. Other factors had no difference including the number of HLA mismatches and incompatible transplants. No patient had a history of catheterization before transplantation.

Recipient Outcomes

Table 2 shows the recipient outcomes after transplantation, such as the duration of double-J stent, BK virus infection, post-operative complications. Of the 211 recipients, 22 experiences postoperative complications (one case of urinary leak, two cases of ureteral stenosis, one case of ureteral hemorrhage, five cases of lymphocele, two cases of hyper-acute rejection, five cases of hemorrhage, two cases of hematoma, one case of deep vein thrombosis, one case of duodenal ulcer, one case of acute respiratory distress syndrome, and one case of premature ventricular contraction with suspected cytomegalovirus myocarditis), and reoperations were performed in 11 cases. One patient required re-transplantation. There were no differences regarding graft and patient survivals between the groups.

There were 39 recipients with long-term (more than 6 days) catheter placement, and there were no significant differences between the groups.

Cox Proportional Hazard Model

Table 3 shows the results of the univariate and multivariate analyses of each factor by Cox proportional hazard model. In the univariate analysis, recipient sex (HR 3.16, 95% CI 1.51~6.61, p = 0.002), graft weight (HR 1.12 per 10g, 95% CI 1.03~1.20, p = 0.004), and ureter length (HR 1.27 per 1 cm, 95% CI 1.04~1.55, p = 0.020) were significantly associated with UTI. Multivariate analysis that included donor sex revealed ureter length was an independent risk factor for UTI (HR 1.25 per 10mm, 95% CI 1.02~1.53, p = 0.028), even after adjusting for the recipient sex (HR 3.05, 95% CI 1.45~6.40, p = 0.003).

Ureter Length and the Incidence of UTI

We stratified 211 recipients into the following four groups according to the ureter length; Group 1: 7.0~9.0 cm (n = 31, 14.7%), Group 2: 9.1~11.0 cm (n = 68, 32.2%), Group 3:

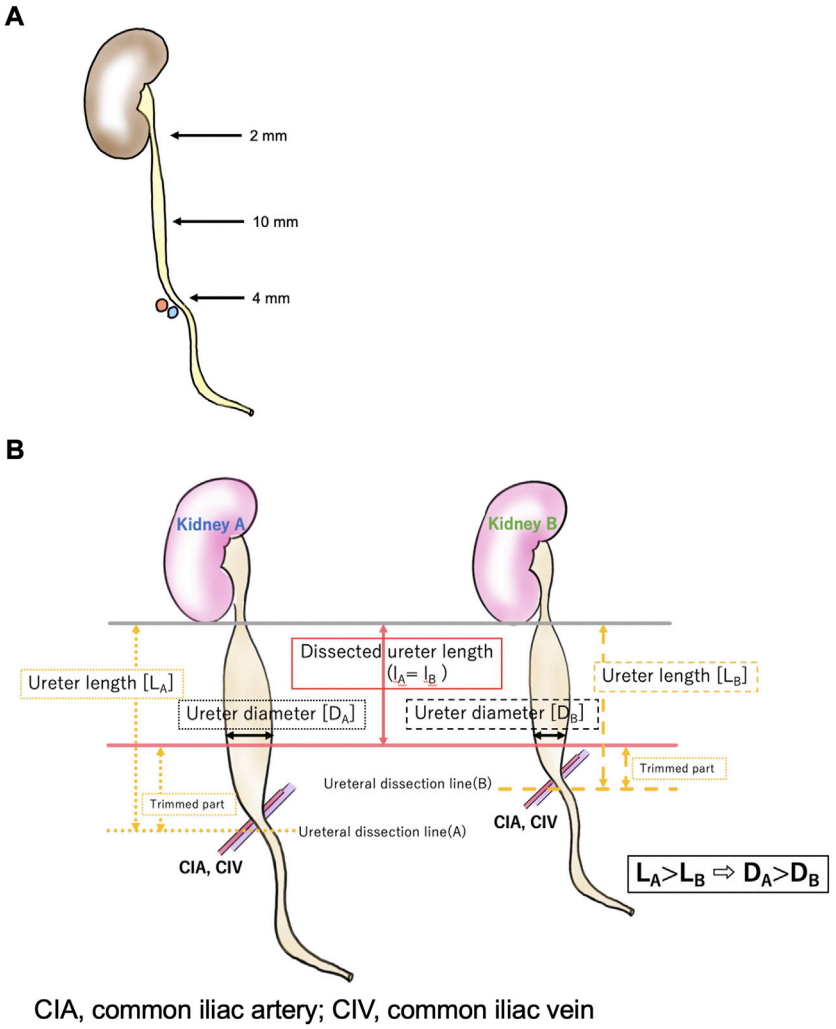


FIGURE 2 | The relationships between the dissected ureter length and ureter diameter.

11.1~13.0 cm (n = 78, 37.0%) and Group 4:13.1~15.5 cm (n = 34, 16.1%). UTI-free survivals in 5 years were significantly different between the groups (p = 0.015, Log-rank test), and significant differences were observed between Groups 1 and 3 (p = 0.008) and between Groups 3 and 4 (p = 0.010, **Figure 1**).

DISCUSSION

UTI is one of the most frequent infections after kidney transplantation and is associated with acute rejection, allograft dysfunction, graft loss, and increased mortality (1, 3–6). Previous studies reported that older age, female recipient sex, history of UTI 1 month before kidney transplantation, recurrent UTI, congenital urinary tract malformations, VUR, ADPKD, and the number of days with an indwelling urinary catheter after kidney transplantation increase the incidence of UTI (8, 9). Differences in the anatomy of the urinary tract (short urethra and proximity of the urethral opening to the vagina and anus) are considered the reason for high risk of UTI in for women compared with that in men (6). Regarding age, elderly recipients, especially those over 65 years old, are at a higher risk of UTI due to decreased mobility, poor hygiene in nursing homes, a higher incidence of urinary retention by prostatic hyperplasia and bladder atrophy, and a weakened immune system (2, 3, 8, 9, 10, 11). There are contradictory reports regarding the role of DM; some studies have reported that it is involved in the incidence of UTI and while others have reported that it is not involved (3, 8, 17). Meanwhile, reported donor-related factors are limited to kidneys from deceased donors (6, 18). Chuang et al. assumed that a kidney from a deceased donor due to graft injury caused by prolonged ischemia time, or intense immunosuppressive drugs used for the induction of deceased donor kidney transplantation (6). As the availability of detailed deceased donor data was limited for privacy reasons in Japan, we could not include those cases for the analysis in the present study.

To our knowledge, this is the first study to propose the graft ureter length as a risk factor affecting UTI incidence in the long-term after living-donor kidney transplantation, even after adjusting for the

well-known known factor, recipient female sex. Although the relation between graft ureter length and the incidence of UTI needs comprehensive discussion, we speculate that the length of the ureter is closely related to the diameter of the tip of the ureter trimmed for anastomosis. According to previous studies, the ureter tapers caudally from the pyeloureteral transition (5.67 ± 0.94 mm) to a small diameter (3.96 ± 0.65 mm) at the lower pole of the kidney. It then widens to its maximum diameter (5.11 ± 1.34 mm) at the abdominal ureter and retracts (3.59 ± 1.20 mm) at the pelvic margin across the common iliac artery(19, 20) (**Figure 2A**). The ureter was dissected at the lower level of the common iliac artery (median length of the removed ureter: 115.0 cm [95% CI, 100.0~125.0 cm]) by the same surgeon, similar to the present study. Then the ureter was trimmed to the shortest length, enough to reach the bladder, and ureterocystoneostomy was performed using the extravesical anastomosis method by the same surgeons in a similar manner. Short ureters at the time of nephrectomy (before trimming) were anastomosed at the narrower diameter; the short ureter would hardly cause UTI in the standard technique. The distance from the iliac fossa, where the kidney was placed, to the bladder was approximately the same, regardless of body size; additionally, the length of the ureter after implantation was approximately the same. Thus, the long ureter is usually trimmed long (L) and ends up being used at the widest part (r) for anastomosis, which may increase the possibility of reflux from the bladder to the ureter in the standard technique (**Figure 2B**), as the large ureteral diameter is known to be one of the risk factors for severe UTI in VUR (21, 22).

Conversely, the duration of dialysis and preoperative bladder capacity, which are well-established risk factors for post-transplant VUR (23–25) were not associated with the frequency of UTI in the present study. According to a Japanese single-center report by Inoue et al., graft VUR was observed in 29.7% of recipients 1 year after living-donor kidney transplantation (24). Although we did not evaluate the VUR incidence after transplantation in the present study, the incidence of VUR was assumed to be similar as we adopted the same extravesical ureterocystoneostomy procedure. Given that asymptomatic bacteriuria occurs in 19–31% of recipients after kidney transplantation (26) and VUR is associated with increased post-voiding residual urine volume (27, 28), this high incidence of VUR can evoke febrile upper UTI. Thus, we included afebrile UTI in the lower urinary tract in the analysis.

Besides these factors, the long duration of indwelling urethral catheter insertion, age, and DM have been reported as risk factors (8, 9); however, no correlation was observed in our study. Regarding the duration of catheter insertion, removing the urethral catheter on postoperative day 5 and the double-J ureteral stent on postoperative day 6 in almost all cases might have contributed to no significant differences. The difference in UTI incidences between the present study and the other studies might be due to the relatively homogenous population. The frequency of HLA alleles varies by race and ethnicity, and island countries, such as Japan, exhibit a special genetic phenomenon called linkage disequilibrium in which a limited number of alleles are conserved as haplotypes (29). This allows a rather lower intensity of baseline immunosuppression than the US or Europe, where thymoglobulin is mainly used for the induction therapy (30–32).

There are several limitations to the present study. This is a single-center, retrospective study. We did not measure the length of the sacrificed ureter and the diameter of the ureter at the site of anastomosis. Also, we did not routinely check the post-transplant VUR. VUR may be a confounding factor for ureter length and ureter length may be a predictor of VUR. This aspect needs further investigation.

CONCLUSION

The ureter length may be associated with the incidence of UTI after living-donor kidney transplantation. Further studies are needed to confirm the impact of ureter length and diameter on UTI incidence after living-donor kidney transplantation.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusion 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 Japanese Red Cross Kumamoto Hospital. The requirement of written informed consent was waived considering the retrospective and non-invasive nature of this study.

AUTHOR CONTRIBUTIONS

SK and SY designed the study, wrote the paper, and analyzed the data. SY and YH prepared the manuscript, collected data, and interpreted the results. MT collected and managed the preoperative data. All other authors critically reviewed the manuscript. All authors approved the final version of the manuscript.

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

1. Ariza-Heredia EJ, Beam EN, Lesnick TG, Cosio FG, Kremers WK, Razonable RR. Impact of Urinary Tract Infection on Allograft Function after Kidney Transplantation. *Clin Transpl* (2014) 28:683–90. doi:10.1111/ctr.12366
2. Ariza-Heredia EJ, Beam EN, Lesnick TG, Kremers WK, Cosio FG, Razonable RR. Urinary Tract Infections in Kidney Transplant Recipients: Role of Gender, Urologic Abnormalities, and Antimicrobial Prophylaxis. *Ann Transpl* (2013) 18:195–204. doi:10.12659/AOT.883901
3. Lee JR, Bang H, Dadhania D, Hartono C, Aull MJ, Satlin M, et al. Independent Risk Factors for Urinary Tract Infection and for Subsequent Bacteremia or Acute Cellular Rejection: a Single-center Report of 1166 Kidney Allograft Recipients. *Transplantation* (2013) 96:732–8. doi:10.1097/TP.0b013e3182a04997
4. Pellé G, Vimont S, Levy PP, Hertig A, Ouali N, Chassin C, et al. Acute Pyelonephritis Represents a Risk Factor Impairing Long-Term Kidney Graft Function. *Am J Transpl* (2007) 7:899–907. doi:10.1111/j.1600-6143.2006.01700.x
5. Abbott KC, Swanson SJ, Richter ER, Bohen EM, Agodoa LY, Peters TG, et al. Late Urinary Tract Infection after Renal Transplantation in the United States. *Am J Kidney Dis* (2004) 44:353–62. doi:10.1053/j.ajkd.2004.04.040
6. Chuang P, Parikh CR, Langone A. Urinary Tract Infections after Renal Transplantation: a Retrospective Review at Two US Transplant Centers. *Clin Transpl* (2005) 19:230–5. doi:10.1111/j.1399-0012.2005.00327.x
7. Britt NS, Hagopian JC, Brennan DC, Pottebaum AA, Santos CAQ, Gharabagi A, et al. Effects of Recurrent Urinary Tract Infections on Graft and Patient Outcomes after Kidney Transplantation. *Nephrol Dial Transpl* (2017) 32: 1758–66. doi:10.1093/ndt/gfx237
8. Sorto R, Irizar SS, Delgadillo G, Alberú J, Correa-Rotter R, Morales-Buenrostro LE. Risk Factors for Urinary Tract Infections during the First Year after Kidney Transplantation. *Transpl Proc* (2010) 42:280–1. doi:10.1016/j.transproceed.2009.11.029
9. Mitra S, Alangaden GJ. Recurrent Urinary Tract Infections in Kidney Transplant Recipients. *Curr Infect Dis Rep* (2011) 13:579–87. doi:10.1007/s11908-011-0210-z
10. Camargo LF, Esteves ABA, Ulisses LRS, Rivelli GG, Mazzali M. Urinary Tract Infection in Renal Transplant Recipients: Incidence, Risk Factors, and Impact on Graft Function. *Transpl Proc* (2014) 46:1757–9. doi:10.1016/j.transproceed.2014.05.006
11. Wu X, Dong Y, Liu Y, Li Y, Sun Y, Wang J, et al. The Prevalence and Predictive Factors of Urinary Tract Infection in Patients Undergoing Renal Transplantation: A Meta-Analysis. *Am J Infect Control* (2016) 44:1261–8. doi:10.1016/j.ajic.2016.04.222
12. Grabe M, Bjerklund-Johansen TE, Botto H, Çek M, Naber KG, Tenke P, et al. *Guidelines on Urological Infections*, 182. Arnhem: European association of urology (2015). p. 237–57.
13. Yamanaga S, Watarai Y, Yamamoto T, Tsujita M, Hiramitsu T, Nanmoku K, et al. Frequent Development of Subclinical Chronic Antibody-Mediated Rejection within 1 Year after Renal Transplantation with Pre-transplant Positive Donor-specific Antibodies and Negative CDC Crossmatches. *Hum Immunol* (2013) 74:1111–8. doi:10.1016/j.humimm.2013.06.022
14. Tanaka K, Hidaka Y, Yamanaga S, Kinoshita K, Kaba A, Toyoda M, et al. Living Donor Kidney Transplantation for a Recipient after 41 Years of Hemodialysis. *Transplantology* (2021) 3:1–5. doi:10.3390/transplantology3010001
15. Riedmiller H, Gerharz EW. Antireflux Surgery: Lich-Gregoir Extravesical Ureteric Tunnelling. *BJU Int* (2008) 101:1467–82. doi:10.1111/j.1464-410X.2008.07683.x
16. Gregoir W, Van Regemorter G. Le Reflux Vesico-Ureteral Congenital. *Urol Int* (1964) 18:122–36. doi:10.1159/000279233
17. Giessing M. Urinary Tract Infection in Renal Transplantation. *Arab J Urol* (2012) 10:162–8. doi:10.1016/j.aju.2012.01.005
18. Glazier DB, Jacobs MG, Lyman NW, Whang MIS, Manor E, Mulgaonkar SP. Urinary Tract Infection Associated with Ureteral Stents in Renal Transplantation. *Can J Urol* (1998) 5:462–6.
19. Kim H-H, Kim K-W, Choi YH, Lee SB, Baba Y. Numerical Analysis of Urine Flow with Multiple Sizes of Double-J Stents. *Appl Sci (Basel)* (2020) 10:4291. doi:10.3390/app10124291
20. Wong SL, Abdul Hamid H. Observation of Ureteric Diameter in Negative Intravenous Urogram in Hospital Universiti Kebangsaan malaysia. *Malays J Med Sci* (2010) 17:4–9.
21. Troesch VL, Wald M, Bonnett MA, Storm DW, Lockwood GM, Cooper CS. The Additive Impact of the Distal Ureteral Diameter Ratio in Predicting Early Breakthrough Urinary Tract Infections in Children with Vesicoureteral Reflux. *J Pediatr Urol* (2021) 17:208.e1–208.e5. doi:10.1016/j.jpurol.2021.01.003
22. Cooper CS, Birusingh KK, Austin JC, Knudson MJ, Brophy PD. Distal Ureteral Diameter Measurement Objectively Predicts Vesicoureteral Reflux Outcome. *J Pediatr Urol* (2013) 9:99–103. doi:10.1016/j.jpurol.2011.12.011
23. Hotta K, Miura M, Wada Y, Fukuzawa N, Iwami D, Sasaki H, et al. Atrophic Bladder in Long-Term Dialysis Patients Increases the Risk for Urological Complications after Kidney Transplantation. *Int J Urol* (2017) 24:314–9. doi:10.1111/iju.13297
24. Inoue T, Satoh S, Saito M, Numakura K, Tsuruta H, Obara T, et al. Correlations between Pretransplant Dialysis Duration, Bladder Capacity, and Prevalence of Vesicoureteral Reflux to the Graft. *Transplantation* (2011) 92(3):311–5. doi:10.1097/TP.0b013e318223d7d6
25. Jung GO, Chun JM, Park JB, Choi G-S, Kwon CHD, Joh JW, et al. Clinical Significance of Posttransplantation Vesicoureteral Reflux during Short-Term Period after Kidney Transplantation. *Transpl Proc* (2008) 40:2339–41. doi:10.1016/j.transproceed.2008.06.027
26. Coussement J, Scemla A, Abramowicz D, Nagler EV, Webster AC. Antibiotics for Asymptomatic Bacteriuria in Kidney Transplant Recipients. *Cochrane Database Syst Rev* (2018) 2:CD011357. doi:10.1002/14651858.CD011357.pub2
27. Chang S-J, Tsai L-P, Hsu C-K, Yang SS. Elevated Postvoid Residual Urine Volume Predicting Recurrence of Urinary Tract Infections in Toilet-Trained Children. *Pediatr Nephrol* (2015) 30:1131–7. doi:10.1007/s00467-014-3009-y
28. Tsai J-D, Chang S-J, Lin C-C, Yang SS-D. Incomplete Bladder Emptying Is Associated with Febrile Urinary Tract Infections in Infants. *J Pediatr Urol* (2014) 10:1222–6. doi:10.1016/j.jpurol.2014.06.013
29. Robinson J, Barker DJ, Georgiou X, Cooper MA, Flicek P, Marsh SGE. IPD-IMGT/HLA Database. *Nucleic Acids Res* (2020) 48:D948-D955–55. doi:10.1093/nar/gkz950
30. Hart A, Lentine KL, Smith JM, Miller JM, Skeans MA, Prentice M, et al. OPTN/SRTR 2020 Annual Data Report: Kidney. *Am J Transpl* (2021) 22(2): 21–136. doi:10.1111/ajt.16982
31. Lim MA, Kohli J, Bloom RD. Immunosuppression for Kidney Transplantation: Where Are We Now and where Are We Going? *Transpl Rev* (2017) 31:10–7. doi:10.1016/j.trre.2016.10.006
32. Japanese Society for Clinical Renal Transplantation. Annual Progress Report from the Japanese Renal Transplant Registry: Number of Renal Transplantations in 2020 and Follow-Up Survey. *Jap J Transpl* (2021) 56: 195–216.

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ORIGINAL RESEARCH
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Immune Profiling of Peripheral Blood Mononuclear Cells at Pancreas Acute Rejection Episodes in Kidney-Pancreas Transplant Recipients

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Profiling of circulating immune cells provides valuable insight to the pathophysiology of acute rejection in organ transplantation. Herein we characterized the peripheral blood mononuclear cells in simultaneous kidney-pancreas transplant recipients. We conducted a retrospective analysis in a biopsy-matched cohort (n = 67) and compared patients with biopsy proven acute rejection (BPAR; 41%) to those without rejection (No-AR). We observed that CD3+ T cells, both CD8+ and CD4+, as well as CD19+ B cells were increased in patients with BPAR, particularly in biopsies performed in the early post-transplant period (<3 months). During this period immune subsets presented a good discriminative ability (CD4+ AUC 0.79; CD8+ AUC 0.80; B cells AUC 0.86; $p < 0.05$) and outperformed lipase (AUC 0.62; $p = 0.12$) for the diagnosis of acute rejection. We further evaluated whether this could be explained by differences in frequencies prior to transplantation. Patients presenting with early post-transplant rejection (<3 months) had a significant increase in T-cell frequencies pre-transplant, both CD4+ T cells and CD8+ T cells ($p < 0.01$), which were associated with a significant inferior rejection-free graft survival. T cell frequencies in peripheral blood correlated with pancreas acute rejection episodes, and variations prior to transplantation were associated with pancreas early acute rejection.

Keywords: graft rejection, simultaneous pancreas kidney transplantation, immune profiling, peripheral blood mononuclear cells, T cells, B cells

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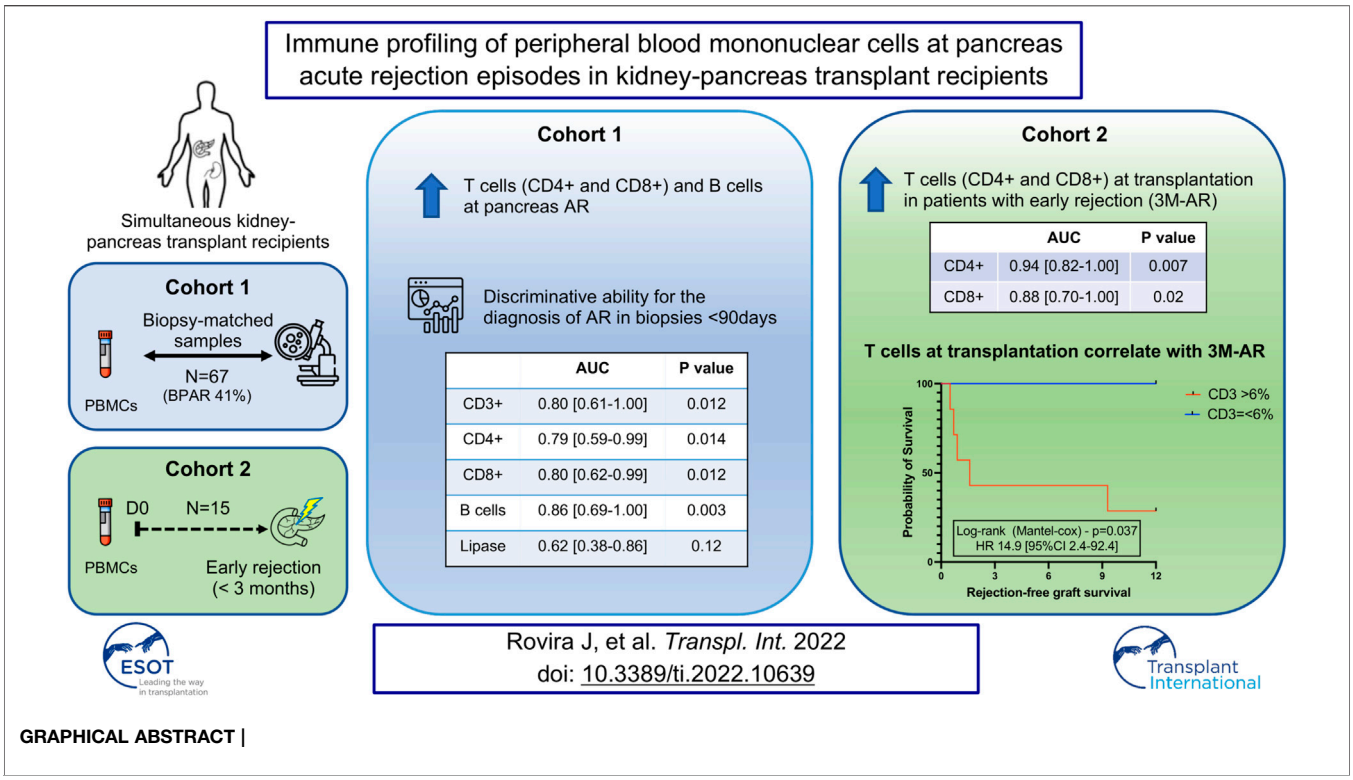

GRAPHICAL ABSTRACT |

INTRODUCTION

Immune profiling in solid organ transplant recipients has contributed to an increase in the understanding of the pathophysiology of acute rejection (1). This approach has also lead to novel insights in other solid organs transplants, such as kidney (2–5), liver (6), heart (7), and lung transplantation (8, 9). There have been several studies highlighting the relevance of subsets of T cells and B cells on the outcome of organ transplantation (4, 5, 10, 11). This understanding of transplant immunology has led to the development of strategies to mitigate immunosuppression side effects, by identification of donor-specific B and T cells prior to transplantation and adjusting immunosuppression accordingly (12), or through the treatment with regulatory cell therapies (13).

Immune profiling and functional characterization of peripheral blood mononuclear cells (PBMCs) in kidney transplant recipients (KTRs) provides relevant information regarding induced immunosuppressive status (14), and immunological risk (2, 15, 16, 17), and correlate with long-term graft survival (3). Despite the simplicity of PBMCs phenotyping compared to functional (12, 18), and genetic analysis (19, 20), their characterization have been crucial to unravel the pathophysiology of rejection and tolerance (21, 22). We now know that regulatory T (Tregs) (17) and B cells (Bregs) (23) are both increased in immune tolerant patients, and that T (17), B (19), and NK (24) cell subsets in peripheral blood correlate with graft acute rejection.

Simultaneous Pancreas Kidney transplantation (SPKTx) is the best treatment alternative for patients with insulin-dependent diabetes mellitus (DM) and end stage renal disease (ESRD) (25, 26). Pancreas graft rejection remains as the leading cause of graft failure after the first 90 days, with acute rejection incidences up to 21% in the first 12 months (27–31). Several risk factors for acute rejection have been identified, such as are donor age, pancreas cold ischemia time, donor cause of death (32), transplantation type (29, 30), and the presence of donor specific antibodies (DSA) (33–35). Despite a high incidence of acute rejection, peripheral blood immune profiling during acute rejection episodes is scarce.

Herein we present a study aiming at characterizing circulating leukocytes in recipients of simultaneous pancreas-kidney transplantation. The main objectives were to explore the differential expression of circulating leukocytes during episodes of pancreas acute rejection, and to explore the correlation between the pre-transplant rates of different leukocytes subsets and the development of acute rejection in the early post-transplant period.

MATERIALS AND METHODS

Study Design and Patient's Population

Peripheral blood mononuclear cells (PBMCs) were collected from pancreas transplant recipients admitted for pancreas transplantation and at the time of pancreas graft biopsies. Biopsies were performed either for-cause or for surveillance. Blood collection was performed prior to the transplant or the biopsy procedure. For this study we conducted a retrospective analysis using the stored patient samples. Collection and use of patient blood samples for the current study was approved by local

ethical IRB board (HCB_2016_0479) and was conducted in full adherence to the Declaration of Helsinki. All patients provided written informed consent to participate in the study.

Between January 2017 and December 2019 a total of 108 pancreas graft biopsies were performed in our center. We excluded all samples in which a biopsy-matched blood sample was not obtained (n = 17), and those in which graft biopsy could not be performed or sample was not suitable for histological diagnosis (n = 24). During the same period a total of 15 simultaneous pancreas-kidney transplants were performed, from which PBMCs were available both at 1) the day of transplantation; and 2) pancreas graft biopsy performed within the first 3 months (either for surveillance or for cause, whichever was first).

Pancreas Graft Biopsies and Blood Samples

For cause biopsies were indicated according to hospital´s protocol–1) >3xs increase in serum amylase or lipase; 2) hyperglycemia (fasting blood glucose >120 mg/dl); 3) *de novo* donor-specific antibodies (DSA); or 4) *de novo* anti-glutamic acid decarboxylase antibodies (GAD). Surveillance biopsies were performed according to center protocol at 3 weeks and at 12 months after transplantation, or as surveillance 4 weeks following the completion of the treatment for an acute rejection episode. Samples were obtained by ultrasound-guided percutaneous needle punch. Histological and immunohistochemical evaluation of pancreas graft biopsies was performed according to the 2011 Banff criteria (36, 37).

Blood samples were obtained contemporaneously to pancreas graft biopsy and used to measure glucose (mg/dl), amylase (U/L), lipase (U/L), creatinine (mg/dl), C-Peptide (ng/ml), HbA1C (%), and anti-GAD (U/ml). Serum samples at time of biopsy were screened for HLA class I and II antibodies using the Lifecodes LifeScreen Deluxe flow bead assay (Immucor, Stamford, CT, United States). Antibody specificities, including the presence of DSA, were determined using the Lifecodes Single Antigen bead assay (Immucor, Stamford, CT, United States) in patients with positive screening for HLA antibodies.

Characterization of Circulating Leukocytes

Blood samples were collected in two separate EDTA tubes and processed separately. In one red blood cells (RBCs) were removed from whole blood samples with RBC lysis buffer (Invitrogen™) and cells were resuspended at a concentration of 10^6/ml in complete MACS buffer to determine a broad spectrum of leukocytes. In the other, PBMCs were isolated from whole blood samples by standard Ficoll density gradient (Ficoll-Paque premium, GE healthcare Bio-Science AB) and were resuspended at a concentration of 10^6/ml in complete MACS buffer to determine T and B cell subsets. Six different panels were designed aiming at interrogating the immune cells for markers of cell activity, memory, and differentiation, with focus on T and B cells. The gating of T cell subsets and B cell subsets are defined in **Supplementary Figure S1**. Cell surface markers were stained with antibodies indicated in **Supplementary Table S1**, and used according to the instructions of the manufacturer. Except for the leukocyte panel, Aqua Live/Dead fixable dead cell kit (Thermo Fisher Scientific, Waltham, MA, United States) was used unambiguously to remove dead cells. Flow cytometry analysis was performed on a FACS Canto II (BD Biosciences, Heidelberg, Germany). Data were analyzed using FlowJo software (Tree Star, Ashland, OR, United States).

Immunosuppression Protocol

Induction therapy was used in all patients with rabbit anti-human lymphocytes polyclonal antibodies (Thymoglobulin 1.25 mg/kg/d for 4 consecutive days), and maintenance immunosuppression protocol with tacrolimus, mycophenolate, and prednisone. Prednisone withdrawal was attempted between months 3–6 in all patients with low immunological risk, absence of acute rejection episodes during the first 90 days, and good tolerance to mycophenolate treatment doses.

Statistical Analysis

Comparisons of median measurements were performed using Mann-Whitney U test and p value < 0.05 was considered statistically significant. Kaplan–Meier was used to estimate unadjusted patient, graft, and rejection-free survivals and compared using log-rank test. Binominal logistic regression was used to calculate odds ratio, and Cox proportional regression performed to estimate grafts' hazards. Statistical analysis was performed using SPSS (version 22, IBM, United States) software, with all tests 2-tailed and significance considered if $p < 0.05$.

RESULTS

Immune Profile in the Peripheral Blood at Pancreas Acute Rejection

A total of 67 biopsy-matched (biopsy-proven acute rejection [BPAR] n = 28; No rejection n = 39) PBMCs samples were performed during the period analyzed. Most biopsies were performed per protocol (52%) or surveillance following treatment of an acute rejection (AR) episode (23%), and performed for-cause in 18 cases (25%), with a median time from transplant to biopsy of 11.9 months [IQR 0.9–13.8]. At time of biopsy 18 patients (28%) presented DSA. Patients with a BPAR had higher lipase ($p < 0.001$) and glucose levels ($p = 0.02$), without differences in serum creatinine nor amylase, compared to those without AR. The demographics and immunological parameters at time of biopsy are described in detail in **Table 1**.

Phenotypic characterization of immune cells by flow cytometry showed a significant increase of T cells ($CD3^+CD19^-CD56^-$) ($p = 0.0187$) in BPAR compared to those without AR (**Figures 1A,B**), including $CD8^+$ ($CD3^+CD8^+CD4^-$; $p = 0.007$) and $CD4^+$ ($CD3^+CD4^+CD8^-$; $p = 0.0459$) T cell lineages (**Figures 1C,D**). The only T cell subsets significantly increased in patients with BPAR were $CD8^+$ naïve and central memory (**Figure 2A**), and $CD4^+$ naïve (**Figure 2B**). No other major differences within the $CD8^+$ or $CD4^+$ lineages were observed between groups, neither $TCR\alpha\beta^+$ nor $TCR\gamma\delta^+$ ($p >$

TABLE 1 | Patients' demographics in biopsy-related samples.

	Overall (*n* = 67)	No-AR (*n* = 39)	BPAR (*n* = 28)	P
Age at biopsy (years)	40.9 ± 9.7	40.5 ± 8.7	41.4 ± 11.1	0.75
Gender (male;%)	55%	51%	61%	0.30
Type of Transplant				0.27
SPK (%)	87%	85%	89%	
PAK (%)	13%	15%	11%	
Indication for biopsy (n[%])				0.053
For-cause	17 (25%)	10 (26%)	7 (25%)	
Surveillance post-rejection	15 (23%)	5 (13%)	10 (36%)	
Per protocol 3 weeks	18 (27%)	11 (28%)	7 (25%)	
Per protocol 12 months	17 (25%)	13(33%)	4 (14%)	
Time to biopsy (months)	11.9 [0.9–13.8]	12 [0.9–13.3]	11.5 [1.2–19.2]	0.37
cPRA (%)				
Class I	0 [0–16]	0 [0–11]		0.81
Class II	16 [0–51]	0 [0–46]		0.49
Total	45 [0–54]	22 [0–60]		0.99
DSA (yes; n [%])	19 (28%)	8 (21%)	11 (38%)	0.40
De novo (% of DSA+)	92%	75%	100%	
Amylase (U/L)	99 [73–145]	100 [73–140]	98 [73–166]	0.51
Lipase (U/L)	45 [30–82]	38 [24–63]	69 [49–144]	<0.001
Glucose (mg/ml)	89 [79–108]	85 [76–96]	93 [81–116]	0.023
HbA1C (%)	5.6 ± 1.6	5.7 ± 1.5	5.4 ± 1.7	0.90
C-Peptide (ng/ml)	3.3 [2.3–4.6]	3.5 [2.4–4.9]	2.8 [2.1–4.6]	0.26
Anti-GAD (U/mL)	0.3 [0.1–2.7]	0.3 [0.1–3.4]	0.2 [0.1–2.6]	0.86
sCreatinine (mg/dl)	1.29 ± 0.6	1.40 ± 0.7	1.14 ± 0.4	0.23
eGFR (ml/min/1.73 m^2)	70 ± 24	66 ± 27	76 ± 20	0.15
Immunosuppression				
Prednisone	94%	92%	97%	0.15
Tacrolimus	97%	97%	96%	0.64
Mycophenolate	94%	95%	93%	0.67
Sirolimus	8%	5%	11%	0.46
Banff Category				
No rejection	58%			
Indeterminate	10.4%		*n* = 7	
Acute Cellular grade 1	20.9%		*n* = 14	
Acute cellular grade 2	7.5%		*n* = 5	
Acute cellular grade 3	1.5%		*n* = 1	
Antibody mediated rejection	1.5%		*n* = 1	

0.05). The percentage of B cells ($CD19^+CD3^-$) was higher in BPAR group compared to biopsies without signs of rejection (p = 0.005) (**Figure 1E**). A deeper analysis of B cell subsets using anti-CD27 and anti-IgD antibodies revealed that both naive and classical memory B cells were increased in patients with BPAR (**Figure 2C**; **Supplementary Figure S1B**). In addition, the percentages of NK ($CD3^-CD56^+$), of NKT ($CD3^+CD56^+$) and monocytes ($CD14^+$) were not different between those with or without BPAR (**Figures 1F,G**).

$CD4^+$ and $CD8^+$ T Cells Discriminate Early Acute Rejection

Having identified that both $CD8^+$ and $CD4^+$ T cells, as well as B cells, were increased at the time of acute rejection, we explored their ability to classify between those with and without BPAR. In a receiver operating curve analysis, both $CD4^+$ (AUC 0.66 [95% CI 0.53–0.80]; p = 0.027), $CD8^+$ (AUC 0.68 [95% CI 0.54–0.82]; p = 0.017), and B cells (AUC 0.69 [95% CI 0.55–0.83]; p = 0.012) presented a poor discriminative capacity. Most relevant, lipase outperformed any of the cell markers (AUC 0.72 [95% CI 0.58–0.85]; p = 0.004). We then evaluated whether timing post-transplant could influence the correlation between immune cell subtypes and acute rejection episodes. We observed that in biopsies performed in the early post-transplant period (<90 days) $CD3^+$ T cells were increased in patients with T cell mediated rejection compared to those without rejection (**Figure 3A**), with a tendency towards an increase also in patients with indeterminate for rejection. Within this period T cells (AUC 0.80 [95% CI 0.61–1.0]; p = 0.012), both $CD4^+$ (AUC 0.79 [95% CI 0.59–0.99]; p = 0.014) and $CD8^+$ (AUC 0.80 [95% CI 0.62–0.99]; p = 0.012), and B cells (AUC 0.86 [95% CI 0.69–1.0]; p = 0.003) outperformed lipase for the diagnosis of acute rejection (AUC 0.62 [95% CI 0.38–0.86]; p = 0.12) (**Figure 3B**).

Concomitant Kidney Graft Rejection

Since patients included in the analysis were recipients of simultaneous kidney-pancreas transplantation, we assessed whether there were concomitant kidney graft rejections at the time points evaluated, either concordant with pancreas graft rejection in BPAR group, or discordant in the no rejection group. Since per hospital policy simultaneous biopsies to both organs are not performed, we observed that in only one case there

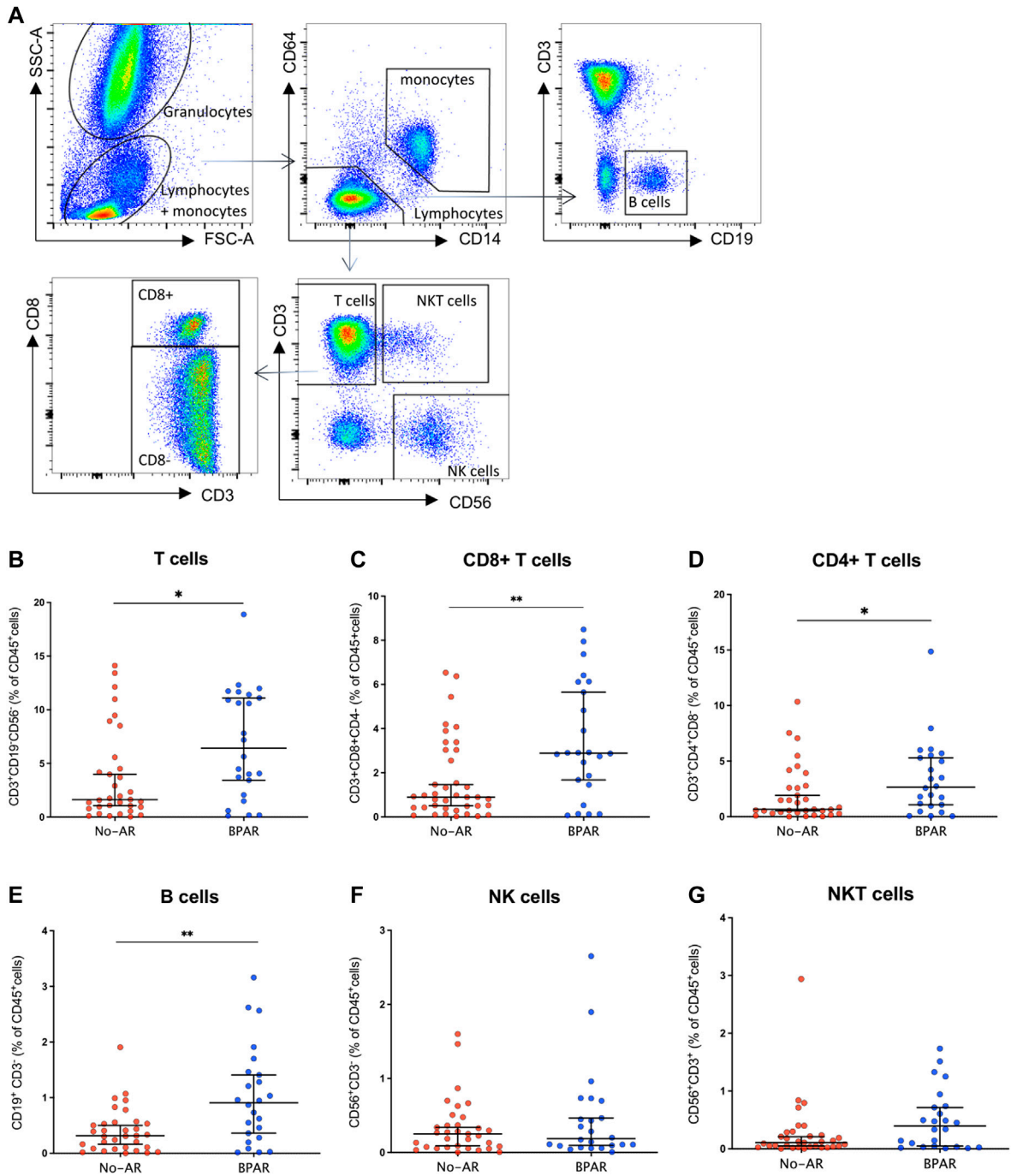

FIGURE 1 | Immune cell lineages during acute rejection episodes. Gating strategy for the characterization of circulating leukocytes **(A)**. $CD3^+$ **(B)** $CD8^+$ **(C)**, and $CD4^+$ **(D)** T cells; B cells **(E)**, NK cells **(F)**, and NKT cells **(G)** have been presented as percentages of total $CD45^+$ cells in $n = 28$ biopsy-matched blood samples with acute rejection and $n = 39$ without rejection. Box plots were calculated using unpaired Mann–Whitney U test. $^*p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$. Mean with SEM.

was a concomitant (on consecutive days) kidney graft biopsy, which was concordant with severe T cell mediated rejection - grade IIA in the kidney and grade 3 in the pancreas graft. Though serum creatinine is not a sensitive marker for kidney graft rejection, in particular subclinical rejection, we explored whether there were unperceived differences between groups. Both groups presented similar serum creatinine (BPAR 1.14 ± 0.71 vs. no AR 1.40 ± 0.37 mg/dl) at time of pancreas graft biopsy (**Table 1**). Moreover, no differences between groups were observed when patients were stratified by indication for and

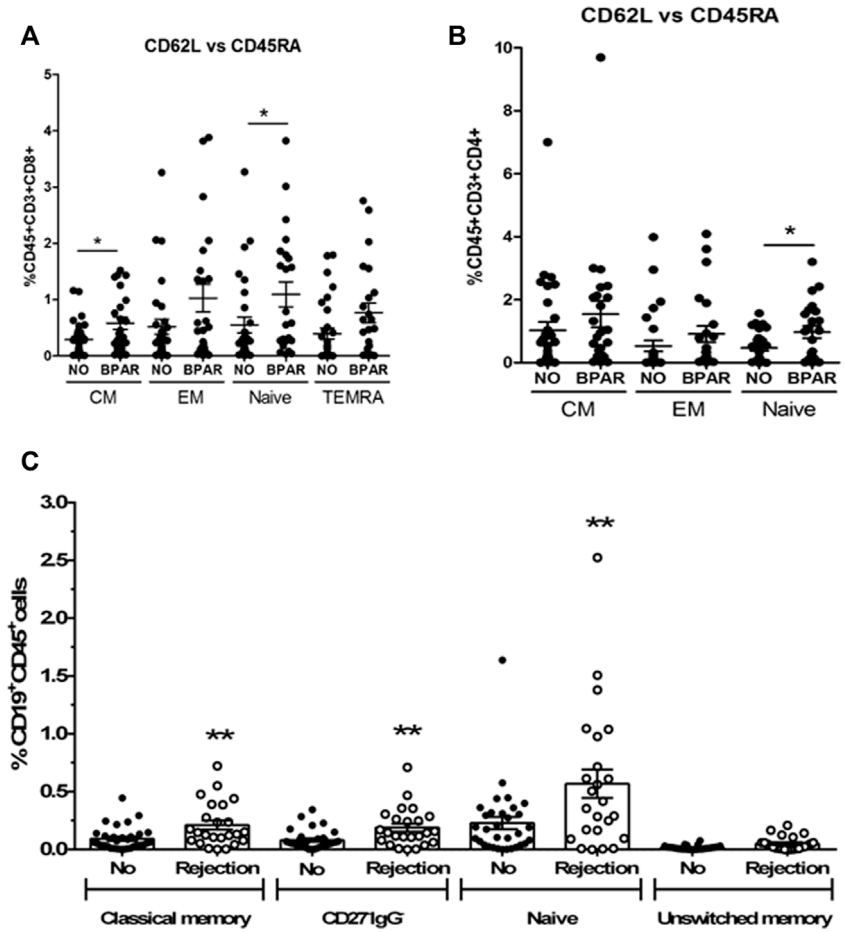

FIGURE 2 | T and B cell subsets during acute rejection episodes. Relative frequencies of $CD8^+$ T cell subsets **(A)**: CD8 Central memory (CM, $CD3^+CD8^+CD45RA^-CD62L^+$), CD8 Effector memory (EM, $CD3^+CD8^+CD45RA^-CD62L^-$), CD8 Naive ($CD3^+CD8^+CD45RA^+CD62L^+$), CD8 terminal differentiated effector memory (TEMRA, $CD3^+CD8^+CD45RA^+CD62L^-$). Relative frequencies of $CD4^+$ T cell subsets **(B)**: CD4 Central memory (CM, $CD3^+CD4^+CD45RA^-CD62L^+$), CD4 Effector memory (EM, $CD3^+CD4^+CD45RA^-CD62L^-$), CD4 Naive ($CD3^+CD4^+CD45RA^+CD62L^+$). Relative frequencies of B cell subsets **(C)**: naïve ($CD27^-IgD^+$), unswitched memory ($CD27^+IgD^+$), classic memory ($CD27^+IgD^-$) and double negative $CD27^-IgG^-$ cells. Biopsy-matched blood samples from patients with a biopsy proven acute rejection (BPAR) has been compared to those without rejection (No AR). Box plots were calculated using unpaired Mann–Whitney U test. $^*p < 0.05$; $^{**}p < 0.01$. Mean with SEM.

histological classification of pancreas graft biopsy (for cause vs. surveillance; BPAR vs. no rejection; $p > 0.05$).

Immune' Profiling at Pancreas Transplantation

Having identified that the discriminative ability of T cells in peripheral blood was particularly pronounced in the early post-transplant period, we explored whether these could be associated with an increased relative number prior to transplantation, or a resistance to T-cell depleting therapy. To do so we performed the immune profile from 15 patients who received a first simultaneous pancreas-kidney transplantation (SPK) and had a biopsy performed during the first 3 months after transplantation. Five had BPAR (3M-AR), whereas the remaining 10 had normal graft biopsies (No-AR).

At the time of transplantation, 3M-AR patients showed a significant increase of T cell populations ($CD3^+$, $CD8^+$ and $CD4^+$; $p < 0.005$) (**Figure 4A**). However, the percentage of B and NK cells were comparable in both groups (**Figure 4A**). More detailed analyzes on T and B cell subsets using surface markers revealed that 3M-AR patients had higher levels of $TCR\alpha\beta^+$ naive T cells, either $CD4^+$ and $CD8^+$ cells, and memory $CD4^+$ T cells compared to patients without rejection (**Figure 5**).

To evaluate the response to T-cell depleting therapy, thymoglobulin, we firstly analyzed the total lymphocyte depletion in peripheral blood during the first days after transplantation. The reduction and subsequent lymphocyte recovery was similar between both groups (**Figure 4B**) during the first 14 days after transplant. Nonetheless, at time of first biopsy (BPAR median 0.7 months, no rejection median 1.2 months; $p = 0.12$), the reduction in T cells, particularly in

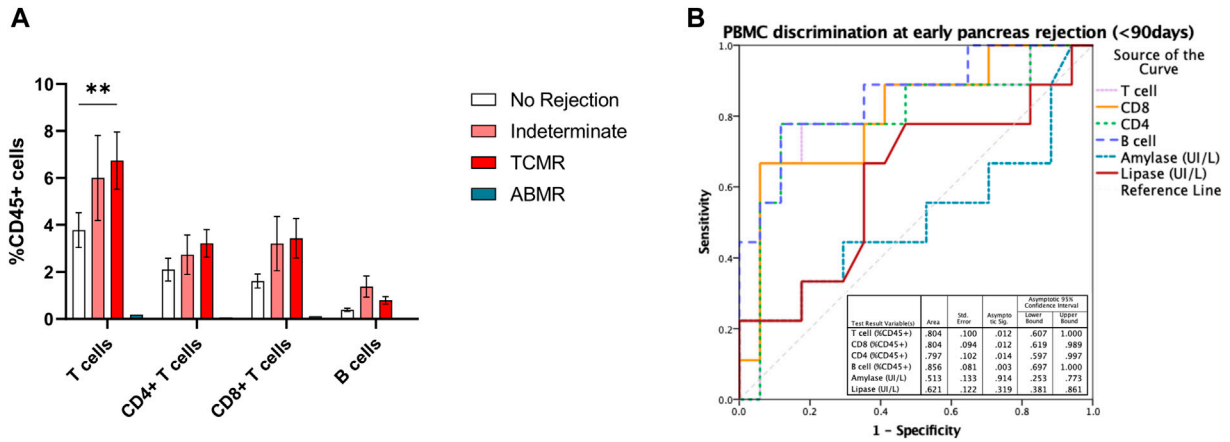

FIGURE 3 | T and B cell subsets in early pancreas graft rejection. Relative frequencies of $CD19^+$ B cells, $CD3^+$ T cells, and its subsets $CD4^+$ and $CD8^+$ T cells **(A)** in patients with biopsies performed during the first 3 months after pancreas transplantation, and correlation with histological classification. Receiver operating curve (ROC) performed to discriminate the ability of B and T cell subsets in peripheral blood to discriminate pancreas acute rejection in the first 3 months after pancreas transplantation **(B)**. Box plots were calculated using unpaired Mann–Whitney U test. $^*p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$. Mean with SEM.

$CD8^+$ T cells, was higher in those with BPAR compared to no rejection group (**Figures 4C,D**).

T Cells at Transplantation Correlate With Early Acute Rejection

We then explored whether immune profiling at pancreas transplantation (D0) could correlate with the risk of acute rejection early after transplantation. Having identified that in peripheral blood both $CD8^+$ and $CD4^+$ T cells, as well as B cells, were increased during early acute rejection episodes, we explored their ability to classify those at risk for early acute rejection prior to transplantation. In a receiver operating curve analysis, both $CD4^+$ (AUC 0.94 [95% CI 0.82–1.0]; $p = 0.007$) and $CD8^+$ (AUC 0.88 [95% CI 0.70–1.0]; $p = 0.020$) presented good discriminative capacity, whereas B cells failed (AUC 0.70 [95% CI 0.42–0.98]; $p = 0.221$) (**Figure 6A**). At cut-off of 6%, $CD3^+$ T cells presented a sensitivity of 100% and a specificity of 70% for the diagnosis of acute rejection. We then stratified patients according to $CD3^+$ T cells at time of transplantation. Those with $CD3^+$ T cells >6% presented an inferior rejection-free graft survival (at 3 months 43% vs. 100% in those with $CD3^+$ =<6%; Log-rank $p = 0.037$), and a 15 times superior risk for an acute rejection during the first year (HR 14.9 [95% CI 2.4–92.4]; $p = 0.04$) (**Figure 6B**).

DISCUSSION

The work herein presented aimed at exploring the immune profiling of peripheral blood mononuclear cells and their correlation with acute rejection episodes in kidney-pancreas transplant recipients. Though an exploratory analysis, we were able to identify that during acute rejection episodes, $CD4^+$ and $CD8^+$ T cells, as well as $CD19^+$ B cells, were increased compared to those without rejection. Moreover, we were able to identify that patients who developed an early acute rejection episode had higher T cells (either $CD3^+$, $CD4^+$, and $CD8^+$) at the time of transplantation compared to the rest.

The finding of a positive correlation between the presence of an increased number of T and B cells during pancreas acute rejection episodes was somewhat expected, since it translates a normal immune response during inflammatory conditions, increasing both T and B cell trafficking as a response to local cytokine release. This may explain the increase in $CD8^+$ naïve T cells, but not in other activation or differentiation subsets, such as effector memory T cells. This discordance between the activation and clonality of both T cells (20) and B cells (19) in peripheral blood when compared to those infiltrating the graft has been described in kidney transplantation, and may partially explain the limited ability of these cell subsets to discriminate between those with and without pancreas acute rejection, and halt its use as biomarker for acute rejection.

T-cell depleting agents have been widely used as induction therapy in pancreas transplantation in most centers worldwide (38) leading to a reduction in the incidence of acute rejection. Despite the use of thymoglobulin (a T-cell depleting polyclonal antibodies) as induction therapy, we identified that during early pancreas acute rejection episodes both T and B cell subsets presented a good capacity to discriminate between those with BPAR and those without rejection. There are some cell subsets that have been reported to be resistant depletion by T-cell depleting agents. Mouse anti-thymocyte globulin (mATG) preferential depletes naïve T cells, resulting in an increased ratio of regulatory and memory T cells within 1 day after mATG administration (39). In a mouse model of kidney transplant, ATG was effective in depleting T cells, but favored the expansion of T follicular helper cells following depletion. Treatment with ATG also increased germinal center B cells and lead to higher titers of antigen-specific antibodies compared to controls (40). Though a small percentage of those who receive a

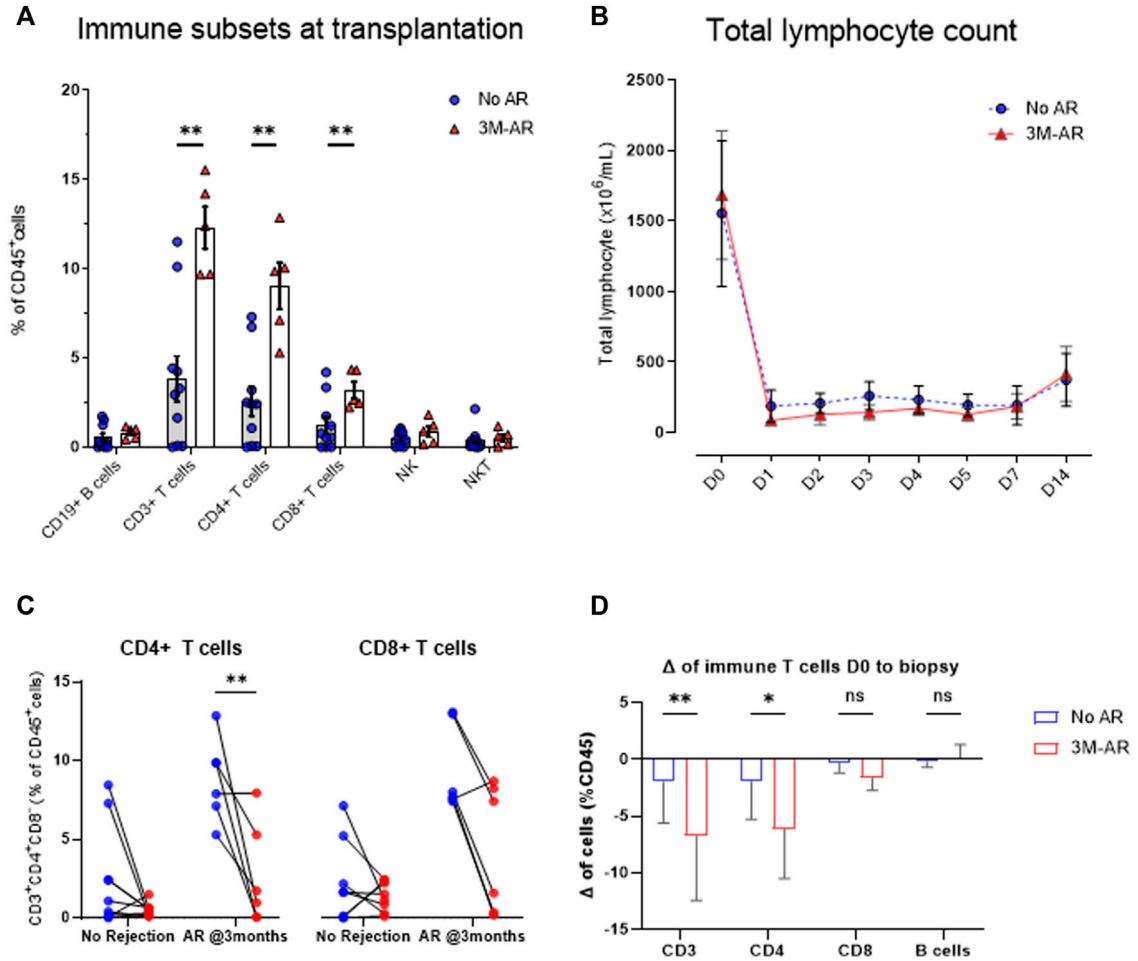

FIGURE 4 | Immune cell lineages at time of transplantation. At time of transplantation, patients who eventually developed an acute rejection episode during the first 3 months post-transplant (3M-AR) presented a higher rate of $CD3^+$, $CD8^+$, and $CD4^+$ T cells ($p < 0.01$) compared to those without rejection (No AR). No differences were observed on B cells, nor NK cells **(A)**. Significant decrease of total lymphocyte count in whole blood during the first days due to the use of T -cell depleting antibodies **(B)**, stratified by those who presented an acute rejection episode during the 3 months (3M-AR) compared to those without rejection (no AR) proven by biopsy. Delta in peripheral blood $CD4^+$ and $CD8^+$ T cell frequencies from the time of transplant to the day of first biopsy, stratified by presence (3M-AR) or absence (No AR) of acute rejection–individual patient data **(C)** and average of cohort **(D)**. Box plots were calculated using unpaired Mann–Whitney U test. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$. Mean with SEM.

de novo simultaneous kidney-pancreas transplant is sensitized (20%), pre-transplant work up is based only on humoral response (anti-HLA antibodies), and no functional cellular analysis was performed to determine the presence of donor-specific memory T cells at time of transplantation.

T cells subpopulations have been described to correlate with the risk of acute rejection after kidney transplantation (2). In our study we identified those patients with pre-transplant $CD3^+$ T cells >6% presented an increased the risk for pancreas acute rejection during the first year up to 15 times. The small sample size halts the extrapolation of these data to clinical practice. These results concur with a recent study from Chellappa et al, which also identified that patients' who develop acute rejection during the first year after transplantation present at the time of transplant an increase in activated $CD3^+$ T cells, both $CD4^+$ and $CD8^+$ (38). As postulated previously, this might be correlated to the presence of donor-specific memory T cells prior to transplantation. Moreover, it must be taken into consideration that most pancreas transplant recipients had type 1 diabetes mellitus (T1D). T1D is an autoimmune disease that may relapse after pancreas transplantation, and the presence of islet specific T cells identified in peripheral blood during relapse in the pancreas graft. Yet considered in pancreas transplantation, may be a potential role autoimmunity triggering an alloimmune response. Pancreatic beta cells express major histocompatibility type II (MHC-II) and during inflammatory conditions may behave as antigen presenting cells (APC) (41). In mouse models of islet transplantation, activation of auto-reactive T cells leads to rejection of the islet graft mediated by alloreactivity (42). Hence, to which extent the herein

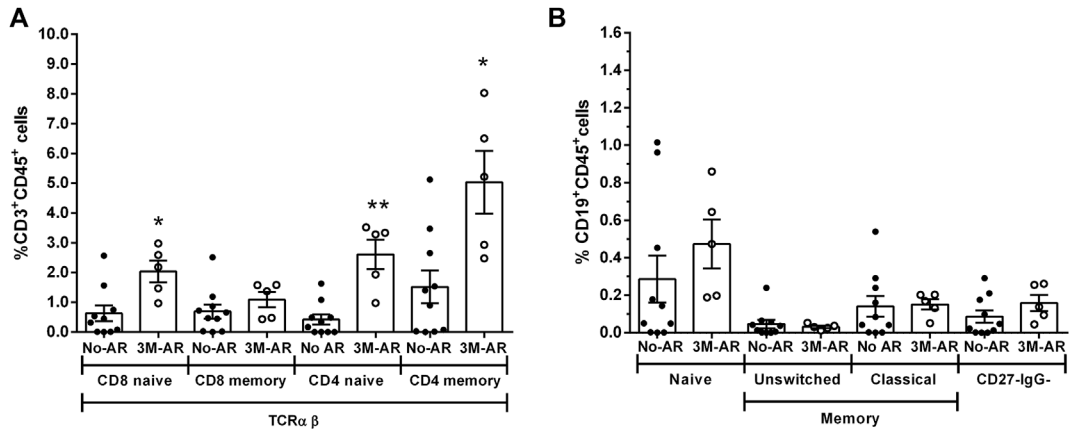

FIGURE 5 | T and B cell subsets at time of transplantation. Relative frequencies of T cell subsets in TCRαβ **(A)**: CD8 naïve ($CD3^+CD8^+CD45RA^+CDRO^-$), CD8 memory ($CD3^+CD8^+CD45RA^-CDRO^+$), CD4 naïve ($CD3^+CD8^+CD45RA^+CD45RO^-$), CD4 memory ($CD3^+CD8^+CD45RA^-CD45RO^+$) cells. Relative frequencies of B cell subsets **(B)**: naïve ($CD27^-IgD^+$), unswitched memory ($CD27^+IgD^+$), classic memory ($CD27^+IgD^-$) and double negative $CD27^-IgG^-$ cells. Blood samples from patients at time of transplantation who presented a biopsy proven acute rejection during the first 3 months (3M-AR) has been compared to those without rejection during the same period (No AR). Box plots were calculated using unpaired Mann–Whitney U test. *$p < 0.05$; **$p < 0.01$. Mean with SEM.

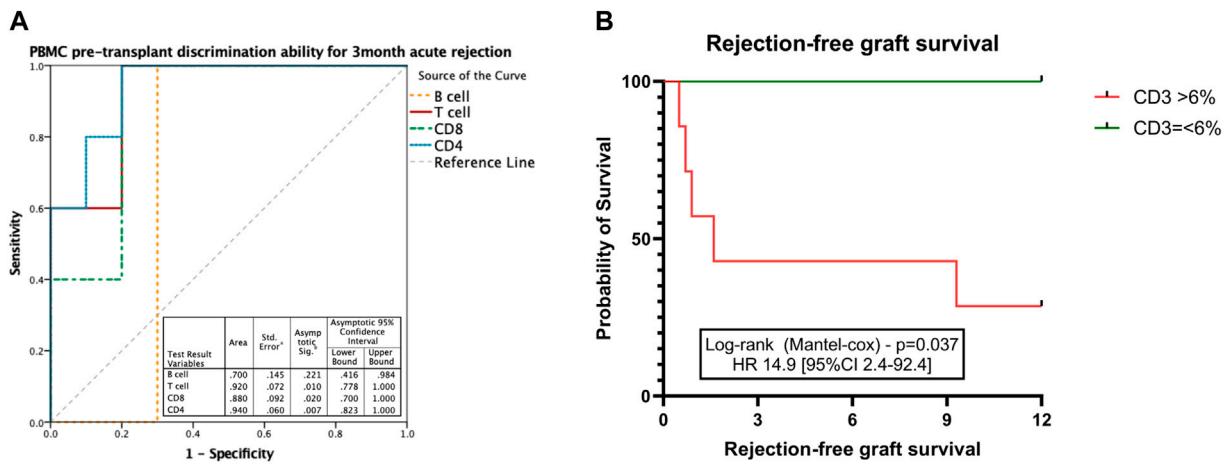

FIGURE 6 | Discriminative ability for early acute rejection of T cell subsets at time of transplantation. ROC curve performed to discriminate the ability of $CD3^+$ T cell count at transplantation to correlate with pancreas acute rejection at 3 months after transplantation **(A)**. Kaplan-Meier estimated pancreas rejection-free graft survival according to $CD3^+$ T cell frequencies at the time of transplantation **(B)**. Box plots were calculated using unpaired Mann–Whitney U test. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$. Mean with SEM.

identified increase in peripheral blood $CD4^+$ memory T cells at time of transplantation may translate an increase in auto-reactive T cells and subsequent graft rejection remains to be addressed.

Another relevant finding in our study was the increase in $CD19^+$ B cells in patients with acute rejection. The diagnosis of antibody-mediated rejection (ABMR) in pancreas transplantation, and according to the Banff classification (36), depends on the presence of characteristic histological lesions, presence of C4d staining, and circulating DSA. The later correlate not only with an increased risk for graft failure (33, 34), but their presence has also been associated with sub-clinical acute rejection episodes. In our study we have identified that during acute rejection episodes $CD19^+$ B cells were increased, despite having only one case of biopsy-proven acute rejection. Nonetheless, up to 38% had DSA at the time of biopsy. In a series of pancreas graft performed per protocol biopsies, Uva et al identified that 54% of the patients did not present signs of acute rejection despite having circulating DSA (43). These results correlate with another recent study in which, exploring a gene set to evaluate the expression of ABMR in pancreas graft biopsy, there was no correlation between the presence of DSA and ABMR gene expression (44). Finally, we have recently reported that donor-derived

cfDNA was increased in patients with DSA despite the absence of signs of ABMR in graft biopsy (45). Altogether, these studies highlight that histological ABMR may be underdiagnosed in pancreas transplantation, and is important to design larger studies in patients with pancreas ABMR aiming at exploring the molecular and genetic biomarkers, and more in depth functional analysis of peripheral blood mononuclear cells. NK cells, which were proportionally similar between those with and without pancreas acute rejection, have been described as key players in ABMR and chronic ABMR in kidney transplantation (46).

The authors would like to highlight some additional limitations to this study. The first and most relevant relies on the small single center cohort, which limits the validity of the results and their extrapolation to other populations. Despite the longitudinal design, pancreas transplantation is a minority procedure, with a median of 15 procedures/year performed at our center. This study was also not designed to perform a longitudinal evaluation of circulating leukocytes at different time-point of the post-transplant period, which may bias interpretation of subsets of populations in biopsies performed early after transplantation due to the use of induction therapy with T-cell depleting agent. Despite this limitation, on the longitudinal study only first SPK transplant recipients were included, and the observed correlation of T cells at transplantation with post-transplant outcomes is not biased by immunosuppression. Moreover, the high acute rejection rate observed in our population may be related to the fact that almost a third relied on clinical criteria, which may have led to an overdiagnosis and treatment. Finally, in biopsy-related samples, indication for biopsy was dependent on the attending physician criteria, which may differ from other centers practices.

In conclusion, to the authors' knowledge this is the first study aiming at exploring immune cell profiling in kidney-pancreas transplant recipients and their correlation with pancreas graft acute rejection. These results pave the way towards more in depth studies that may further characterize these cellular populations and ultimately lead to the individualization of immunosuppression.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusion 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 Research Ethics Committee from Hospital Clinic de Barcelona. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

PV-A, JR, MJR-B, AG-C, and MJR contributed to the conception and design of the study. PV-A, JR, MJR-B, EB-M, NH-G, and ML-R contributed to the acquisition and analysis of data for the work. PV-A, JR, MJR-B, GP, EM-M, DC, IR, MC, JC, MJR, FD, and AG-G contributed to the interpretation of data for the work. PV-A, JR, MJR-B, AG-C, and MJR wrote the first draft of the manuscript. All authors contributed to the manuscript revision, read, and approved the submitted version.

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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.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontierspartnerships.org/articles/10.3389/ti.2022.10639/full#supplementary-material

REFERENCES

1. Liu Y, Liu X, Zhou S, Xu R, Hu J, Liao G, et al. Single-Cell Profiling of Kidney Transplant Recipients with Immunosuppressive Treatment Reveals the Dynamic Immune Characteristics. *Front Immunol* (2021) 12:639942. doi:10.3389/fimmu.2021.639942
2. San Segundo D, Millan O, Munoz-Cacho P, Boix F, Paz-Artal E, Talayero P, et al. High Proportion of Pretransplantation Activated Regulatory T Cells (CD4+CD25highCD62L+CD45RO+) Predicts Acute Rejection in Kidney Transplantation: Results of a Multicenter Study. *Transplantation* (2014) 98(11):1213–8. doi:10.1097/TP.0000000000000202
3. San Segundo D, Fernandez-Fresnedo G, Rodrigo E, Ruiz JC, Gonzalez M, Gomez-Alamillo C, et al. High Regulatory T-Cell Levels at 1 Year Posttransplantation Predict Long-Term Graft Survival Among Kidney Transplant Recipients. *Transpl Proc* (2012) 44(9):2538–41. doi:10.1016/j.transproceed.2012.09.083
4. Veronese F, Rotman S, Smith RN, Pelle TD, Farrell ML, Kawai T, et al. Pathological and Clinical Correlates of FOXP3+ Cells in Renal Allografts during Acute Rejection. *Am J Transpl* (2007) 7(4):914–22. doi:10.1111/j.1600-6143.2006.01704.x
5. Kollins D, Stoelcker B, Hoffmann U, Bergler T, Reinhold S, Banas MC, et al. FOXP3+ Regulatory T-Cells in Renal Allografts: Correlation with Long-Term Graft Function and Acute Rejection. *Clin Nephrol* (2011) 75(2):91–100.
6. Toby TK, Abecassis M, Kim K, Thomas PM, Fellers RT, LeDuc RD, et al. Proteoforms in Peripheral Blood Mononuclear Cells as Novel Rejection Biomarkers in Liver Transplant Recipients. *Am J Transpl* (2017) 17(9):2458–67. doi:10.1111/ajt.14359
7. Starling RC, Stehlik J, Baran DA, Armstrong B, Stone JR, Ikle D, et al. Multicenter Analysis of Immune Biomarkers and Heart Transplant Outcomes: Results of the Clinical Trials in Organ Transplantation-05 Study. *Am J Transpl* (2016) 16(1):121–36. doi:10.1111/ajt.13422
8. Durand M, Lacoste P, Danger R, Jacquemont L, Brosseau C, Durand E, et al. High Circulating CD4(+)CD25(hi)FOXP3(+) T-Cell Sub-population Early after Lung Transplantation Is Associated with Development of Bronchiolitis Obliterans Syndrome. *J Heart Lung Transpl* (2018) 37(6):770–81. doi:10.1016/j.healun.2018.01.1306
9. Coiffard B, Pelardy M, Loundou AD, Nicolino-Brunet C, Thomas PA, Papazian L, et al. Effect of Immunosuppression on Target Blood Immune Cells within 1 Year after Lung Transplantation: Influence of Age on T Lymphocytes. *Ann Transpl* (2018) 23:11–24. doi:10.12659/aot.906372
10. Ibrahim EH, Aly M, Morath C, Sayed DM, Ekpoom N, Opelz G, et al. Relationship of Transitional Regulatory B and Regulatory T Cells and Immunosuppressive Drug Doses in Stable Renal Transplant Recipients. *Immun Inflamm Dis* (2021) 9(4):1252–71. doi:10.1002/iid3.473
11. Ibrahim EH, Aly MG, Opelz G, Morath C, Zeier M, Susal C, et al. Higher CD19+CD25(+) Bregs Are Independently Associated with Better Graft Function in Renal Transplant Recipients. *BMC Nephrol* (2021) 22(1):180. doi:10.1186/s12882-021-02374-2
12. Bestard O, Cruzado JM, Lucia M, Crespo E, Casis L, Sawitzki B, et al. Prospective Assessment of Antidonor 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
13. Sawitzki B, Harden PN, Reinke P, Moreau A, Hutchinson JA, Game DS, et al. Regulatory Cell Therapy in Kidney Transplantation (The ONE Study): a Harmonised Design and Analysis of Seven Non-randomised, Single-Arm, Phase 1/2A Trials. *Lancet* (2020) 395(10237):1627–39. doi:10.1016/S0140-6736(20)30167-7
14. Papp G, Boros P, Nakken B, Szodoray P, Zeher M. Regulatory Immune Cells and Functions in Autoimmunity and Transplantation Immunology. *Autoimmun Rev* (2017) 16(5):435–44. doi:10.1016/j.autrev.2017.03.011
15. Luque S, Lucia M, Melilli E, Lefaucheur C, Crespo M, Loupy A, et al. Value of Monitoring Circulating Donor-Reactive Memory B Cells to Characterize Antibody-Mediated Rejection after Kidney Transplantation. *Am J Transpl* (2019) 19(2):368–80. doi:10.1111/ajt.15055
16. Gandolfini I, Crespo E, Baweja M, Jarque M, Donadei C, Luque S, et al. Impact of Preformed T-Cell Alloreactivity by Means of Donor-specific and Panel of Reactive T Cells (PRT) ELISPOT in Kidney Transplantation. *PLoS One* (2018) 13(7):e0200696. doi:10.1371/journal.pone.0200696
17. 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
18. Udomkarnjananun S, Kerr SJ, Townamchai N, van Besouw NM, Hesselink DA, Baan CC. Donor-specific ELISPOT Assay for Predicting Acute Rejection and Allograft Function after Kidney Transplantation: A Systematic Review and Meta-Analysis. *Clin Biochem* (2021) 94:1–11. doi:10.1016/j.clinbiochem.2021.04.011
19. Pineda S, Sigdel TK, Liberto JM, Vincenti F, Sirota M, Sarwal MM. Characterizing Pre-transplant and post-transplant Kidney Rejection Risk by B Cell Immune Repertoire Sequencing. *Nat Commun* (2019) 10(1):1906. doi:10.1038/s41467-019-09930-3
20. Aschauer C, Jelencsics K, Hu K, Heinzel A, Gregorich MG, Vetter J, et al. Prospective Tracking of Donor-Reactive T-Cell Clones in the Circulation and Rejecting Human Kidney Allografts. *Front Immunol* (2021) 12:750005. doi:10.3389/fimmu.2021.750005
21. Baeten D, Louis S, Braud C, Braudeau C, Ballet C, Moizant F, et al. Phenotypically and Functionally Distinct CD8+ Lymphocyte Populations in Long-Term Drug-free Tolerance and Chronic Rejection in Human Kidney Graft Recipients. *J Am Soc Nephrol* (2006) 17(1):294–304. doi:10.1681/ASN.2005020178
22. Li Y, Koshiba T, Yoshizawa A, Yonekawa Y, Masuda K, Ito A, et al. Analyses of Peripheral Blood Mononuclear Cells in Operational Tolerance after Pediatric Living Donor Liver Transplantation. *Am J Transpl* (2004) 4(12):2118–25. doi:10.1111/j.1600-6143.2004.00611.x
23. Nova-Lamperti E, Chana P, Mobillo P, Runglall M, Kamra Y, McGregor R, et al. Increased CD40 Ligation and Reduced BCR Signalling Leads to Higher IL-10 Production in B Cells from Tolerant Kidney Transplant Patients. *Transplantation* (2017) 101(3):541–7. doi:10.1097/TP.0000000000001341
24. Xu X, Han Y, Huang H, Bi L, Kong X, Ma X, et al. Circulating NK Cell Subsets and NKTlike Cells in Renal Transplant Recipients with Acute Tcellmediated Renal Allograft Rejection. *Mol Med Rep* (2019) 19(5):4238–48. doi:10.3892/mmr.2019.10091
25. Ojo AO, Hanson JA, Wolfe RA, Leichtman AB, Agodoa LY, Port FK. Long-term Survival in Renal Transplant Recipients with Graft Function. *Kidney Int* (2000) 57(1):307–13. doi:10.1046/j.1523-1755.2000.00816.x
26. Gruessner RW, Sutherland DE, Gruessner AC. Mortality Assessment for Pancreas Transplants. *Am J Transpl* (2004) 4(12):2018–26. doi:10.1111/j.1600-6143.2004.00667.x
27. Uva PD, Papadimitriou JC, Drachenberg CB, Toniolo MF, Quevedo A, Dotta AC, et al. Graft Dysfunction in Simultaneous Pancreas Kidney Transplantation (SPK): Results of Concurrent Kidney and Pancreas Allograft Biopsies. *Am J Transpl* (2019) 19(2):466–74. doi:10.1111/ajt.15012
28. Dong M, Parsaik AK, Kremers W, Sun A, Dean P, Prieto M, et al. Acute Pancreas Allograft Rejection Is Associated with Increased Risk of Graft Failure in Pancreas Transplantation. *Am J Transpl* (2013) 13(4):1019–25. doi:10.1111/ajt.12167
29. Niederhaus SV, Leverson GE, Lorentzen DF, Robillard DJ, Sollinger HW, Pirsch JD, et al. Acute Cellular and Antibody-Mediated Rejection of the Pancreas Allograft: Incidence, Risk Factors and Outcomes. *Am J Transpl* (2013) 13(11):2945–55. doi:10.1111/ajt.12443
30. Ventura-Aguiar P, Ferrer J, Revuelta I, Paredes D, de Sousa-Amorim E, Rovira J, et al. Pancreas Outcomes between Living and Deceased Kidney Donor in Pancreas after Kidney Transplantation Patients. *Nephrol Dial Transpl* (2018) 33(11):2052–9. doi:10.1093/ndt/gfy133
31. Sollinger HW, Odorico JS, Becker YT, D'Alessandro AM, Pirsch JD. One Thousand Simultaneous Pancreas-Kidney Transplants at a Single center with 22-year Follow-Up. *Ann Surg* (2009) 250(4):618–30. doi:10.1097/SLA.0b013e3181b76d2b
32. Kopp WH, Verhagen MJ, Blok JJ, Huurman VA, de Fijter JW, de Koning EJ, et al. Thirty Years of Pancreas Transplantation at Leiden University Medical Center: Long-Term Follow-Up in a Large Eurotransplant Center. *Transplantation* (2015) 99(9):e145–51. doi:10.1097/TP.0000000000000604

33. Mittal S, Page SL, Friend PJ, Sharples EJ, Fuggle SV. De Novo donor-specific HLA Antibodies: Biomarkers of Pancreas Transplant Failure. *Am J Transpl* (2014) 14(7):1664–71. doi:10.1111/ajt.12750
34. Malheiro J, Martins LS, Tafulo S, Dias L, Fonseca I, Beirao I, et al. Impact of De Novo Donor-specific Anti-HLA Antibodies on Grafts Outcomes in Simultaneous Pancreas-Kidney Transplantation. *Transpl Int* (2016) 29(2): 173–83. doi:10.1111/tri.12687
35. Cantarovich D, De Amicis S, Akl A, Devys A, Vistoli F, Karam G, et al. Posttransplant Donor-specific Anti-HLA Antibodies Negatively Impact Pancreas Transplantation Outcome. *Am J Transpl* (2011) 11(12):2737–46. doi:10.1111/j.1600-6143.2011.03729.x
36. Drachenberg CB, Torrealba JR, Nankivell BJ, Rangel EB, Bajema IM, Kim DU, et al. Guidelines for the Diagnosis of Antibody-Mediated Rejection in Pancreas Allografts-Updated Banff Grading Schema. *Am J Transpl* (2011) 11(9): 1792–802. doi:10.1111/j.1600-6143.2011.03670.x
37. Kandaswamy R, Stock PG, Miller J, Skeans MA, White J, Wainright J, et al. OPTN/SRTR 2019 Annual Data Report: Pancreas. *Am J Transpl* (2021) 21(2): 138–207. doi:10.1111/ajt.16496
38. Chellappa S, Kushekhar K, Hagness M, Horneland R, Tasken K, Aandahl EM. The Presence of Activated T Cell Subsets Prior to Transplantation Is Associated with Increased Rejection Risk in Pancreas Transplant Recipients. *J Immunol* (2021) 207(10):2501–11. doi:10.4049/jimmunol.2001103
39. Ruzek MC, Neff KS, Luong M, Smith KA, Culm-Merdek K, Richards SM, et al. *In Vivo* characterization of Rabbit Anti-mouse Thymocyte Globulin: a Surrogate for Rabbit Anti-human Thymocyte Globulin. *Transplantation* (2009) 88:170–9. doi:10.1097/TP.0b013e3181abc061
40. Gassen RB, Borges TJ, Pérez-Sáez MJ, Zhang H, Al Jurdi A, Llinàs-Mallol L, et al. T Cell Depletion Increases Humoral Response Favoring T Folicular Helper Cells Expansion. *Am J Transpl* (2022) 22(7):1766–78.
41. Li Y, Sun F, Yue TT, Wang FX, Yang CL, Luo JH, et al. Revisiting the Antigen-Presenting Function of β Cells in T1D Pathogenesis. *Front Immunol* (2021) 12: 690783. doi:10.3389/fimmu.2021.690783
42. Burrack AL, Landry LG, Siebert J, Coulombe M, Gill RG, Nakayama M. Simultaneous Recognition of Allogeneic MHC and Cognate Autoantigen by Autoreactive T Cells in Transplant Rejection. *J Immunol* (2018) 200(4): 1504–12. doi:10.4049/jimmunol.1700856
43. Uva PD, Quevedo A, Roses J, Toniolo MF, Pilotti R, Chuluyan E, et al. Anti-Hla Donor-specific Antibody Monitoring in Pancreas Transplantation: Role of Protocol Biopsies. *Clin Transpl* (2020) 34(8):e13998. doi:10.1111/ctr.13998
44. Roufosse C, Drachenberg C, Renaudin K, Willicombe M, Toulza F, Dominy K, et al. Molecular Assessment of Antibody-Mediated Rejection in Human Pancreas Allograft Biopsies. *Clin Transpl* (2020) 34(11):e14065. doi:10.1111/ctr.14065
45. Ventura-Aguiar P, Ramirez-Bajo MJ, Rovira J, Banon-Maneus E, Hierro N, Lazo M, et al. Donor-derived Cell-free DNA Shows High Sensitivity for the Diagnosis of Pancreas Graft Rejection in Simultaneous Pancreas-Kidney Transplantation. *Transplantation* (2022) 106:1690–7. doi:10.1097/TP.0000000000004088
46. Koenig A, Chen CC, Marcais 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

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Neo-Adjuvant Use of Sorafenib for Hepatocellular Carcinoma Awaiting Liver Transplantation

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Data on efficacy and safety of sorafenib in a neoadjuvant setting for HCC awaiting liver transplantation (LT) are heterogeneous and scarce. We aimed to investigate the trajectory of patients treated with sorafenib while awaiting LT. All patients listed for HCC and treated with sorafenib were included in a monocentric observational study. A clinical and biological evaluation was performed every month. Radiological tumor response evaluation was realized every 3 months on the waiting list and every 6 months after LT. Among 327 patients listed for HCC, 62 (19%) were treated with Sorafenib. Sorafenib was initiated for HCC progression after loco-regional therapy (LRT) in 50% of cases and for impossibility of LRT in 50% of cases. The mean duration of treatment was 6 months. Thirty six patients (58%) dropped-out for tumor progression and 26 (42%) patients were transplanted. The 5-year overall and recurrent-free survival after LT was 77% and 48% respectively. Patients treated for impossibility of LRT had acceptable 5-year intention-to-treat overall and post-LT survivals. Conversely, patients treated for HCC progression presented high dropout rate and low intention-to-treat survival. Our results suggest that it is very questionable in terms of utility that patients treated for HCC progression should even be kept listed once the tumor progression has been observed.

Keywords: liver transplantation, cancer, hepatocellular carcinoma (HCC), cancer-therapeutics, sorafenib, neoadjuvant therapy, tyrosine kinase inhibitor

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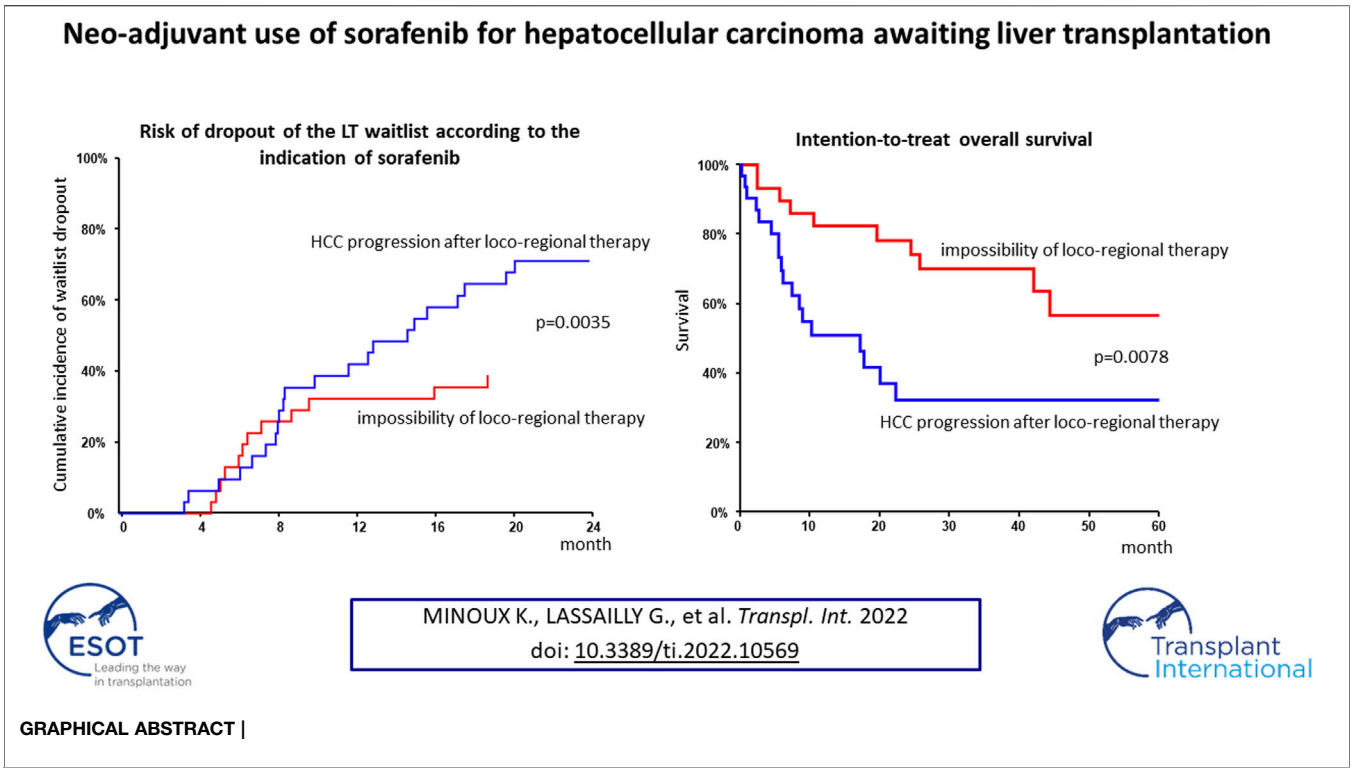
Abbreviations: AE, Adverse event; AFP, Alpha-fetoprotein; CMV, Cytomegalovirus; CI, Confidence interval; CR, Complete response; CT, Computerized tomography; HAT, Hepatic artery thrombosis; HCC, Hepatocellular carcinoma; HFS, Hand-foot syndrome; LRT, Loco-regional therapy; LT, Liver transplantation; MELD, Model for End-stage Liver Disease; MRI, Magnetic Imaging Resonance; OS, Overall survival; PBC, Primary biliary cholangitis, PD, Progressive disease; PR, Partial response; RFA, Radiofrequency ablation; SCH, Subcapsular hematoma; SD, Stable disease/Standard deviation; SMV, Superior mesenteric vein; TACE, Transarterial chemoembolization; TARE, Transarterial radioembolization.



GRAPHICAL ABSTRACT |

INTRODUCTION

Liver Transplantation (LT) is the only therapy that, unlike other curative treatments (ablative therapies, surgical resection), simultaneously cures hepatocellular carcinoma (HCC) and the underlying liver disease. However, very few patients are eligible for LT because of their condition (age, comorbidities), behavior (observance, abstinence in alcohol consumption) and tumor biology and spread. The eligibility of LT in our country is based on the alpha-fetoprotein (AFP) score which includes the number of nodules, their size, and the AFP level (1). According to the French agency for organ allocation (Agence de la Biomédecine), HCC is currently the leading indication for LT in France, accounting for 30% of registrations on the waiting list. The dropout nor shortage imposes a waiting time before LT which may lead to tumor progression beyond accepted criteria.

Strategies to minimize or avoid waitlist dropout related to tumor progression include loco-regional therapy (LRT). Indeed, transarterial modalities (transarterial chemoembolization—TACE, transarterial radioembolization—TARE) and percutaneous thermal ablative strategies (radio frequency ablation—RFA, microwave ablation) have been widely adopted by transplant programs to bridge HCC candidates before LT. A consensus statement for LT for HCC has recommended LRT if the anticipated waiting time for an organ to become available exceeds 6 months (2). By limiting the risk of progression on the waiting list, LRT also reduces the risk of recurrence after LT, especially when a partial or complete response according to mRECIST is achieved before LT (3–5). Other prognostic factors such as low AFP level, low number of tumor nodules and small total tumor diameter at baseline, extended post-interventional tumor necrosis, well differentiated tumor grade and lack of microvascular invasion have been shown to reduce post-LT HCC recurrence (6). Tumor recurrence is the main cause of mortality after LT for HCC with a 5-year survival of 22% in case of recurrence (7). It is therefore crucial to optimize management of patients awaiting LT to improve their long-term prognosis.

Sorafenib is a multikinase inhibitor with activity against both the tumor cell directly (inhibition of cell proliferation, notably through the Raf signaling pathway) and the endothelial cells of blood vessels (inhibition of angiogenesis through the VEGF and PDGF signaling pathway) (8). It was the first systemic therapy to prolong survival in patients with advanced HCC, suggesting that its use in the neoadjuvant setting may be beneficial (9). However, there remains a concern that sorafenib's anti-angiogenic effect may interfere with tissue repair-healing and thus lead to increased post-LT complications. Data on efficacy and safety of sorafenib in this setting are heterogeneous and scarce so far (10–17).

We sought to analyze in a large cohort of patients treated with sorafenib as neoadjuvant therapy for HCC: 1) Trajectories of patients awaiting LT treated with sorafenib (Intention-to-treat survival, dropout rate, tolerance, radiological response to treatment), 2) peri-operative morbidity and 3) overall (OS) and recurrence-free survival after LT.

PATIENTS AND METHODS

Study Characteristics and Population

This is a single-center, non-randomized and observational study. We included all candidate to LT for HCC patients

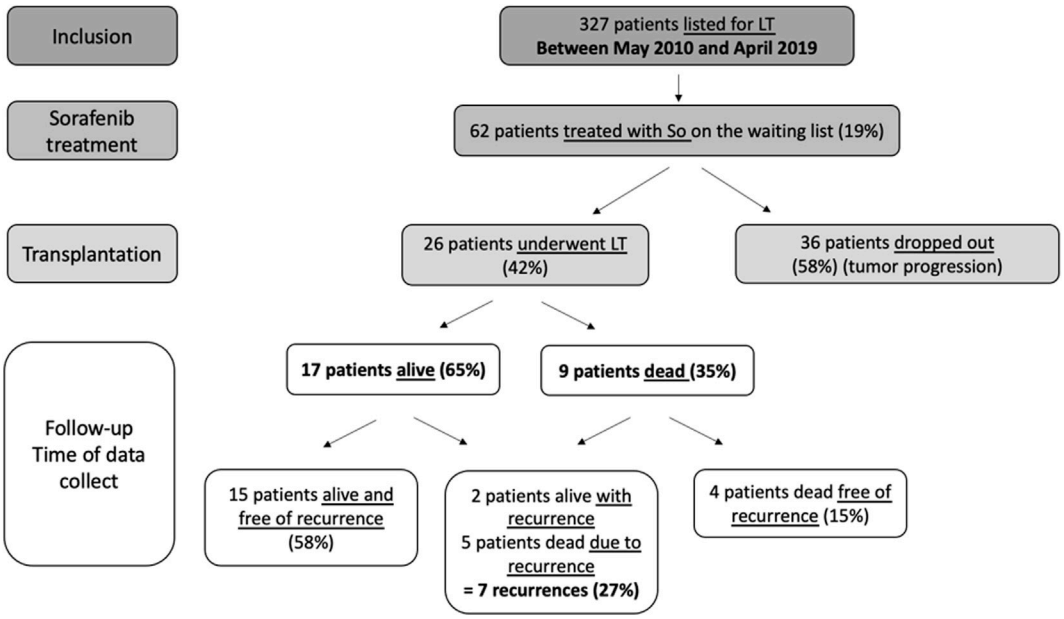


FIGURE 1 | Flow chart.

listed between May 2010 and April 2019 and treated with sorafenib for at least 1 day on the waiting list. Patients were identified thanks to the nationwide CRISTAL registry. Diagnosis of HCC was established by pathological analysis of directed biopsies or according to the non-invasive criteria of the European Association for the Study of the Liver (EASL) guidelines (18, 19). Each indication of LT was submitted to validation of a multidisciplinary liver committee, which included at least a liver surgeon, a hepatologist, an oncologist and a radiologist specialized in HCC and LT.

All patients had measurable disease parameters that had been classified according to mRECIST (modified Response Evaluation Criteria in Solid Tumours) with no evidence of radiologically definable major vascular invasion or extrahepatic metastases. Study flow chart is presented in **Figure 1**. In accordance with French law, all patients were informed that their medical information could be used for non-interventional research purposes (according to the Jardé law).

Indication and Management of Sorafenib

Sorafenib was used on-label after validation by multidisciplinary liver conference. It was initiated in two different cases: in case of tumor progression after failure of all types of LRT, or in case of impossibility of another LRT (multifocal tumor or technical impossibility). The technical impossibility and/or contraindication of another LRT has always been retained during a multidisciplinary committee considering all the available therapeutic alternatives. Main contraindication of TACE were arterio-portal shunt and portal vein thrombosis. In some cases low hypervascularity of HCC and/or multifocal small tumors (diameter < 2 cm) were the main drawbacks. Main contraindications of percutaneous thermal ablative strategies were the presence of ascites on imagery and some location such as hepatic dome.

Sorafenib was mainly introduced to prevent dropout but could also be introduced in few cases ($n = 4$) to try tumor down-staging by reducing tumor burden for patients initially outside eligibility criteria (AFP score > 2). In terms of trajectory, such patients who had been putt on the waiting list and treated with sorafenib had to present partial/complete response and/or a decrease in AFP level to allow being transplanted.

Patients started treatment either at 400 mg twice a day (full dose) or at 200 mg twice a day with escalation at full dose in case of good liver function and absence of side effects.

Follow-Up Awaiting Liver Transplantation

Liver transplant waiting list time was defined as the number of days from the time of activation on the liver transplant waiting list until the day of transplantation. Physical examination, adverse events and laboratory monitoring including biochemical and hematological parameters were carried out every month. Laboratory-based MELD and AFP score were calculated at each visit. Dose modifications, temporary treatment pauses, and symptomatic treatments were prescribed depending on side effects which were graded using the National Cancer Institute's Common Terminology Criteria for adverse events. In case of a grade 2 adverse event, treatment was reduced to half dose and the patient was reassessed on day 15. In case of a grade 3 side effect, treatment was discontinued. Treatment was continued until the day of transplantation or until tumor progression.

Contrast-enhanced CT-scan or MRI was performed at baseline and repeated every 3 months. Radiological tumor response during treatment with sorafenib was assessed

according to mRECIST (1). Complete response (CR) was defined as the absence of arterially enhanced areas in all target lesions; partial response (PR) and progressive disease (PD) as a greater than 30% decrease and a greater than 20% increase, respectively, in the sum of the longest diameters of arterial enhanced areas in all target lesions; and stable disease (SD) as neither PR nor PD. Radiological assessment of tumor characteristics (number of nodules, maximum nodule diameter and sum of all diameters) was collected retrospectively on last imaging preceding sorafenib introduction and on final pretransplant or prior to dropout imaging.

Explant Histopathology Examination

All liver explants were examined by an experienced hepato-pathologist. Tumor characteristics, gross appearance (nodular or infiltrative), extent of tumor necrosis, vascular invasion, cell differentiation and presence of satellite nodules were analyzed.

Peri-Operative Morbidity and Follow-Up

Peri-operative complications including incidences of surgical revision, sepsis, hemorrhage, vascular thrombosis, overall bile duct complication and bile duct stenosis, asymptomatic CMV infection, pathologically confirmed acute cellular rejection and re-transplantation were reported. Blood loss until the first month after LT and length of patient's hospital stay were collected. Occurrences of HCC tumor recurrence after LT and OS were also identified.

Post-transplant monitoring was adapted to date of LT and included 6-monthly contrast-enhanced CT-scan or MRI imaging coupled with AFP measurements during the first 5 years of follow-up, then annually during 5 additional years. The database was fixed on March 2021 for the last news.

Statistical Analysis

Demographic (age, gender), clinical (underlying liver disease, type of LRT preceding listing, waiting list time), carcinologic (AFP score), laboratory (MELD-score, AFP level and AFP score at listing), explant tumor characteristics and radiologic variables (tumor characteristics, Milan criteria) were registered. HCC recurrence free survival events were censored at the date of death or HCC recurrence. Continuous variables were summarized as means and standard deviation (SD) or medians and interquartile range (IQR). Comparisons of categorical and continuous variables were performed using the Chi-square test and the Mann–Whitney U-test, respectively. OS and recurrence-free survival rates were determined according to the Kaplan-Meier method. Patient survival in different groups was compared using the log-rank test. Survivals were expressed as percentage and 95% confidence interval (CI). A univariate linear regression comparison has been performed to identify predictors of HCC recurrence. A p value of 0.05 or less was considered statistically significant. Cumulative incidences of waitlist dropout with LT as competing risk event and HCC recurrence after LT with death without recurrence as competing event have been performed. All statistical analyses were performed using NCSS version 9.

RESULTS

Patient Characteristics at Listing

During the period of May 2010 to April 2019, 327 HCC candidates were listed for LT. Of these patients, 62 (19%) were treated with sorafenib awaiting LT, among them 26 (42%) underwent LT and 36 (58%) dropped-out from the waiting list for tumor progression (**Figure 1**). Patient main characteristics are presented in **Table 1**. The majority of patients were middle-aged men and had compensated alcohol-related cirrhosis. There were no significant differences in demographic characteristics or therapeutic management prior to listing among the 2 groups, transplanted group (LT) and dropout group.

HCC Characteristics at Listing

HCC characteristics are presented in **Table 2**. Approximately one third of patients had one nodule, one third had two nodules and one third had at least three nodules. Patients who dropped-out of the waiting list tended to have a larger maximum tumor diameter than transplanted patients (29.5 vs. 22.9 mm, $p = 0.08$). Mean AFP-level was 47.4 ± 123 UI/L.

Patient Management on Waiting List

Treatment indication is presented in **Table 3**. Half of the total cohort started sorafenib for tumor progression and the other half started sorafenib because of impossibility of LRT. There was a significant difference between the two groups in terms of treatment indication. Most transplanted patients who dropped-out initiated treatment because of tumor progression. Mean and median waiting time were respectively 13 ± 4.5 and 12.5 months (IQR: 11–14.7) from listing to LT, and respectively 10.4 ± 5.4 and 8.3 months (IQR: 6.2–15) from listing to dropout or death.

Sorafenib was discontinued in 71% of all patients, mainly for hepatic decompensation in the LT group and mainly for tumor progression in the dropout group. Sixty-nine % of the transplanted patients had continued sorafenib until LT. In the total cohort, sorafenib was initiated at a median dose of 400 mg (IQR: 400–800) and continued for a mean duration of 6 months, with no significant differences between the LT and the dropout group. Gastrointestinal disorders (mainly diarrhea) tended to be more frequent in the LT group than in the dropout group ($p = 0.07$).

Radiologic Assessment Prior to Liver Transplantation or Dropout

Maximum mean and median tumor diameter prior to LT or dropout was significantly higher in the dropout group than in the LT group ($p = 0.002$). Last mRECIST radiological response prior to LT or dropout is detailed in **Table 4**. Of the total cohort, 48.4% achieved disease control and 11.3% achieved objective response. Disease control was achieved in 73% in the LT group and 30.6% in dropout group ($p = 0.001$).

TABLE 1 | Patient characteristics at listing.

	Total cohort[a] *n* = 62	LT *n* = 26	Dropout *n* = 36	*p*
Age (years)				
Mean ± SD	59 ± 7.9	57 ± 9.7	60.5 ± 5.9	0.2
Median (IQR range)	61.2 (57.3–63.3)	61 (54.8–62.3)	61.5 (57.7–64.4)	
Gender, M/F, n (%)	51 (82.3%)/11 (17.7%)	20 (76.9%)/6 (23.1%)	31 (86.1%)/5(13.9%)	0.3
Etiology of cirrhosis, n (%)				
Alcohol	50 (80.7%)	20 (76.9%)	30 (83.3%)	0.2
Viral	5 (8.1%)	1 (3.9%)	4 (11.1%)	
Metabolic	3 (4.8%)	2 (7.7%)	1 (%)	
Hemochromatosis	1 (1.6%)	1 (3.8%)	0	
PBC	1 (1.6%)	1 (3.8%)	0	
Non cirrhotic liver	2 (3.2%)	1 (3.8%)	1 (2.8%)	
MELD				
Mean ± SD	10 ± 3.9	9.9 ± 3.2	10.1 ± 4.4	0.7
Median (IQR range)	9 (7–12.25)	9 (7–13)	8.5 (6–12)	
Treatment before listing, n (%)				
None	14 (22.6%)	8 (30.8%)	6 (16.7%)	0.3
TACE alone	22 (35.5%)	8 (30.8%)	14 (38.9%)	
Surgery alone	8 (12.9%)	4 (15.4%)	4 (11.1%)	
RFA alone	6 (9.7%)	1 (3.9%)	5 (13.9%)	
Combinations				
2 procedures				0.6
3 procedures	8 (12.9%)	3 (11.5%)	5 (13.9%)	
4 procedures	3 (4.8%)	1 (3.9%)	2 (5.6%)	
	1 (1.7%)	1 (3.9%)	0	

[a]*No missing data.*

TABLE 2 | HCC characteristics at listing.

	Total cohort[a] *n* = 62	LT *n* = 26	Dropout *n* = 36	*p*
Tumor number				
Mean ± SD	2.2 ± 1.3	2.5 ± 1.5	2 ± 1	
Median (IQR range)	2 (1–3)	2 (1–3)	2 (1–2)	0.1
Maximum tumor diameter				
Mean ± SD (mm)	26.7 ± 16.5	22.9 ± 8.2	29.5 ± 20.2	0.08
Total tumor diameter				
Mean ± SD (mm)	46.4 ± 27.3	45.1 ± 22.1	47.4 ± 30.8	0.8
Number of nodules, n (%)				
1 nodule	21 (33.9%)	8 (30.8%)	13 (36.1%)	0.07
2 nodules	23 (37.1%)	6 (23.1%)	17 (47.2%)	
3 nodules	9 (14.5%)	7 (26.9%)	2 (5.6%)	
>3 nodules	9 (14.5%)	5 (19.2%)	4 (11.1%)	
Largest nodule, n (%)				
<30 mm	44 (71%)	20 (76.9%)	24 (66.7%)	0.3
≥30 mm	18 (29%)	6 (23.1%)	12 (33.3%)	
Unique tumor, n (%)				
≤30 mm	18 (29%)	6 (23.1%)	12 (33.3%)	0.4
>30 mm	3 (4.8%)	2 (7.7%)	1 (2.8%)	
AFP-level (UI/L):				
Mean ± SD	47.4 ± 123.7	50.7 ± 126.1	45.1 ± 123.7	0.1
Median (IQR range)	8 (4–25.5)	6 (4–14)	11 (5–30)	
Milan criteria fulfilled, n (%)				
Yes/No	43 (69.4%)/19(30.7%)	18 (69.2%)/8 (30.8%)	25 (69.4%)/11 (30.6%)	0.9
AFP score, n (%)				
0	38 (61.3%)	14 (53.9%)	24 (66.7%)	0.4
1	8 (12.9%)	3 (11.5%)	5 (13.9%)	
2	12 (19.4%)	8 (30.8%)	4 (11.1%)	
3	3 (4.8%)	1 (3.9%)	2 (5.6%)	
4	1 (1.6%)	0	1 (2.8%)	

[a]*No missing data.*

TABLE 3 | Tolerance and treatment management of sorafenib.

	N	Total cohort	LT	Dropout	*p*
Treatment indication, n (%)					
Tumor progression	62	31 (50%)	8 (30.8%)	23 (63.Z%)	**0.01**
Impossibility of LRT		31 (50%)	18 (69.2%)	13 (36.1%)	
Treatment withdrawal, n (%)	61	42 (71%)	8 (30.8%)	34 (97.1%)	**<0.0001**
Reason for withdrawal, n (%)	42				
Intolerance		5 (11.9%)	0	5 (14.7%)	**0.009**
Tumor progression		22 (52.4%)	1 (12.5%)	21 (61.8%)	
Hepatic decompensation		13 (31%)	6 (75%)	7 (20.6%)	
Fatigue		2 (4.8%)	1 (12.5%)	1 (%)	
Sorafenib treatment duration (months)	62				
Mean ± SD		6 ± 7	8 ± 10	4.6 ± 3	0.4
Median (IQR range)		4.5 (2.25–7)	4.9 (1.1–10.9)	4.15 (2.3–6.2)	
Median start dose (IQR range)	61	400 (400–800)	800 (400–800)	400 (400–800)	0.4
Dose reduction, n (%)	61	25 (41%)	11 (44%)	14 (38.9%)	0.7
Aggravation at 1 month after introduction n (%)	62	13 (21%)	5 (19.2%)	8 (22.2%)	0.8
Adverse events, n (%)	62				
HFS/skin injury		26 (41.9%)	13 (50%)	13 (36.1%)	0.3
Fatigue		13 (21%)	5 (19.2%)	8 (22.2%)	0.8
		2 (3.2%)	1 (3.9%)	1 (2.8%)	0.8
Hematological toxicity		10 (16.1%)	5 (19.2%)	5 (13.9%)	0.6
Liver decompensation		23 (37.1%)	13 (50%)	10 (27.8%)	0.07
Gastrointestinal disorders		4 (6.5%)	1 (3.9%)	3 (8.3%)	0.5
Digestive bleeding		2 (3.2%)	2 (7.7%)	0	0.09
Hypertension		1 (1.6%)	0	1 (2.8%)	0.4
Neuropathy					
Sorafenib at time of LT, n (%)	26	—	18 (69.2%)	—	NA

TABLE 4 | Tumor characteristics and last radiological tumor response prior to LT or dropout.

	N	Total cohort	LT	Dropout	*p*
Sum of largest diameters (LD) (mm)	58				
Mean ± SD		65 ± 43	52 ± 28	75 ± 50	0.1
Median (IQR range)		56 (33.5–92.5)	50 (30.5–70)	60 (35–127)	
Maximum tumor diameter (mm):	58				
Mean ± DS		32.7 ± 25	22.1 ± 11	41.3 ± 29	**0.002**
Median (IQR range)		25 (18–37)	20 (17–27)	35 (20–53)	
Last mRECIST radiological response, n (%)	62				
CR		1 (1.6%)	1 (3.9%)	0	**0.001**
PR		6 (9.7%)	6 (23.1%)	0	
SD		23 (37.1%)	12 (46.2%)	11 (30.6%)	
PD		32 (51.6%)	7 (26.9%)	25 (69.4%)	

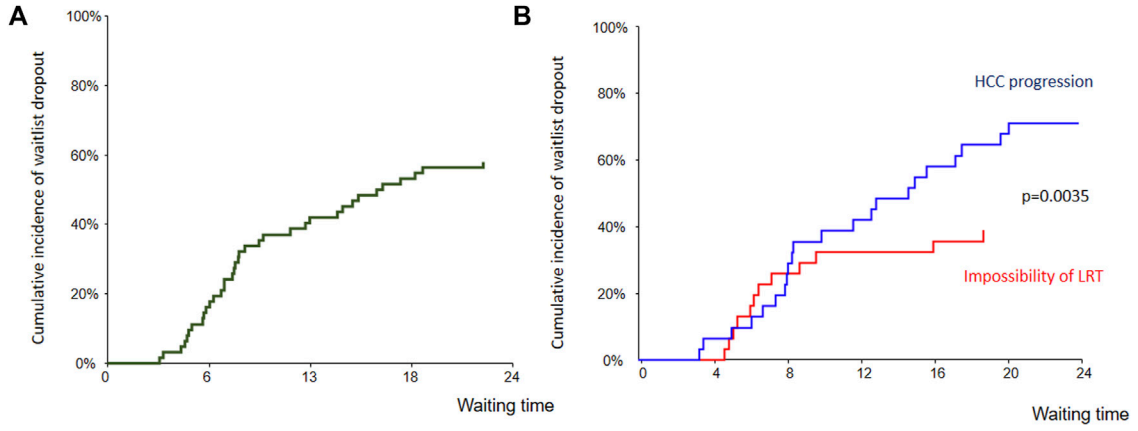
Intention-to-Treat Survival and Incidence Rate of Dropout

Cumulative incidence of waitlist dropout for the whole cohort is presented in **Figure 2A**. One- and 2-years dropout rates were 39% (95% CI: 28%–53%) and 56% (95% CI: 45%–70%). Patients treated with sorafenib for HCC progression had higher risk of dropout as compared with those treated for impossibility of LRT (p = 0.0035). At 1 year cumulative incidence rates of dropout were 32% (95% CI: 19%–53%) for impossibility of LRT and 42% for tumor progression (95% CI: 28%–63%) (**Figure 2B**). Among the four patients who were listed beyond eligibility criteria and treated with sorafenib in order to achieve tumor down-staging, only one have been transplanted.

Intention-to-treat overall survival (OS) of the whole cohort is presented **Figure 3A**. Briefly, OS at years 1, 3 and 5 was 66% (95% CI: 54–79), 51.5% (95% CI: 38–65) and 44% (95% CI: 29%–59%), respectively. Patients treated with sorafenib for HCC progression had lower survival as compared with those treated for impossibility of LRT (p = 0.0078) (**Figure 3B**).

Predictors of Dropout

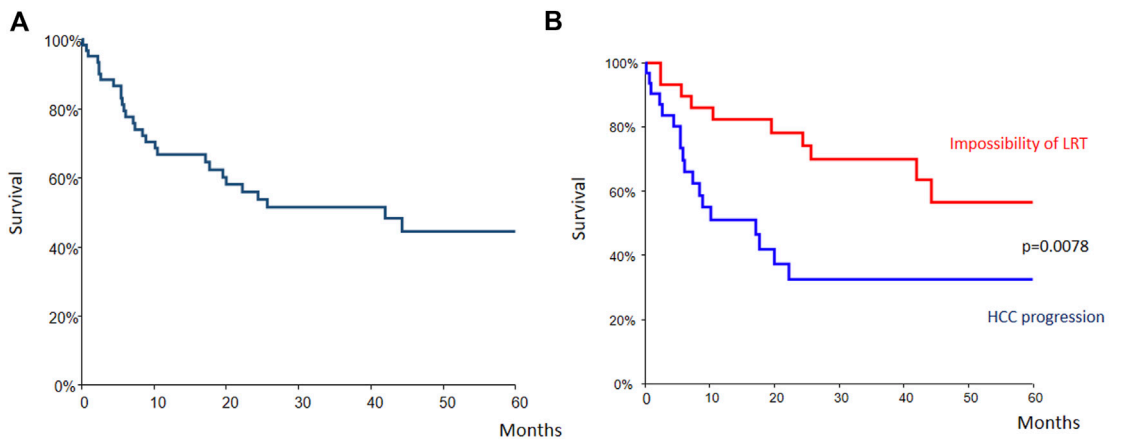
We included discriminant factors associated with dropout in a logistic regression multivariable analysis. These factors were number of HCC at listing, maximal tumor diameter at listing, sorafenib's indication and maximal tumor diameter at last radiological evaluation. Among them, sorafenib's indication for tumor progression (Odds ratio 0.2, coefficient regression −1.5, p =



Pts at risk	12 mo	24 mo
n	28	2

Pts at risk	12 mo	24 mo
HCC progression	16	2
Impossibility of LRT	12	0

FIGURE 2 | Cumulative incidence of waitlist dropout [**(A)**: whole cohort; **(B)**: according to sorafenib's indication].



Pts at risk	12 mo	24 mo	36 mo	48 mo	60 mo
n	36	27	20	11	9

Pts at risk	12 mo	24 mo	36 mo	48 mo	60 mo
HCC progression	13	8	5	4	4
Impossibility of LRT	23	19	15	7	5

FIGURE 3 | Intention-to-treat overall survival [**(A)**: whole cohort; **(B)**: according to sorafenib's indication].

0.03) and maximal tumor diameter at last radiological evaluation (Odds ratio 1.08, coefficient regression 0.08, $p = 0.006$) were independent predictors of dropout.

Explant Histopathology Analysis

Pathological examination exposed in **Table 5** showed that most explants had ≥ 4 nodules (76%) which contained minimal necrosis (56.3%), no satellite nodules (75%) and no microvascular (80%) or macrovascular (96%) invasion. Most tumors were well-differentiated (64%) and not infiltrative (92%).

Post-Liver Transplantation Morbidity

Post-transplant complications are presented in **Table 6**. Median length of hospital stay was 19.5 days (IQR: 15.75–29.5). Eight patients underwent revision surgery (30%), of which four were related to bleeding episodes, two to bowel dehiscence, one to bile leakage and one to wall abscess. Seven bleeding episodes occurred (27%), of which four were graft hematomas, one wall hematoma, one digestive ulcer and one hemoperitoneum. Bile duct stenosis concerned three patients (11%), of which two were treated endoscopically and one required no specific management because of the absence of biological repercussions. Two

TABLE 5 | Explant pathologic characteristics.

	n	LT
Largest diameter, mean (mm) ± SD	25	24.9 ± 11
Sum of diameter, mean (mm) ± SD	23	61.3 ± 32.5
Tumor number, n (%)	25	
1 nodule		3 (12%)
2 or 3 nodules		3 (12%)
≥4 nodules		19 (76%)
Extent of tumor necrosis, n (%)	16	
Complete (no viable tumor) (100%)		1 (6.3%)
Subtotal necrosis (≥90%)		1 (6.3%)
Partial necrosis (≥50% and <90%)		3 (18.8%)
Minimal necrosis (<50%)		9 (56.3%)
No necrosis (0%)		2 (12.5%)
Differentiation grade, n (%)	25	
Well differentiated		16 (64%)
Moderately and poorly differentiated		8 (32%)
Not applicable (complete necrosis)		1 (4%)
Infiltrative HCC, n (%)	25	2 (8%)
Satellite nodules, n (%)	16	4 (25%)
Microvascular invasion, n (%)	25	5 (20%)
Macrovascular invasion, n (%)	25	1 (4%)

TABLE 6 | Post-transplant complications (no missing data).

	LT
Length of hospital stay (days)	
Mean ± SD	26.5 ± 17.6
Median (IQR range)	19.5 (15.75–29.5)
Revision surgery, n (%)	8 (30.8%)
Bleeding	4
Bowel dehiscence	2
Bile leakage	1
Wall abscess	1
Bleeding, n (%)	7 (26.9%)
Graft hematoma (SCH/subhepatic)	4 (1/3)
Wall hematoma	1
Digestive ulcer	1
Hemoperitoneum	1
Number of peri-operative packed red blood cells	
Mean ± SD	4.8 ± 6.5
Median (IQR range)	3 (1–5.5)
Bile duct stenosis, n (%)	3 (11.5%)
Thrombosis, n (%)	7 (26.9%)
Hepatic artery thrombosis	3 (11.5%)
Pulmonary embolism	1 (3.8%)
Portal/SMV thrombosis	2 (7.7%)
Renal vein thrombosis	1 (3.8%)
Asymptomatic CMV infection, n (%)	10 (38.5%)
Re-transplantation, n (%)	1 (3.9%)
Acute rejection, n (%)	4 (15.4%)
Sepsis, n (%)	9 (34.6%)

patients presented with bile leakage. Vascular thrombosis occurred in seven patients (27%) and are detailed in **Table 6**. One patient underwent re-transplantation for severe ischemic cholangitis related to hepatic artery thrombosis. Acute rejection occurred in four patients. Rejection episodes were moderate for three patients and severe for one patient.

One patient had a severe complication. After declamping, the patient presented hemodynamic instability requiring the introduction of noradrenaline. At wound closure, the patient presented a hypertensive peak with tachycardia, followed by severe hypotension and cardiac arrest. Post-arrest (no flow 0, low flow 3 min), cardiac echocardiography showed biventricular failure. Thoracic CT scan showed a sub-segmental pulmonary embolism which did not explain the severity of the clinical condition. Brain scan and coronary angiography did not show any lesion. Due to the persistence of the cardiac failure, ECMO was implemented. The episode was resolutive and no other cardiovascular complications were noted.

HCC Recurrence and Survival

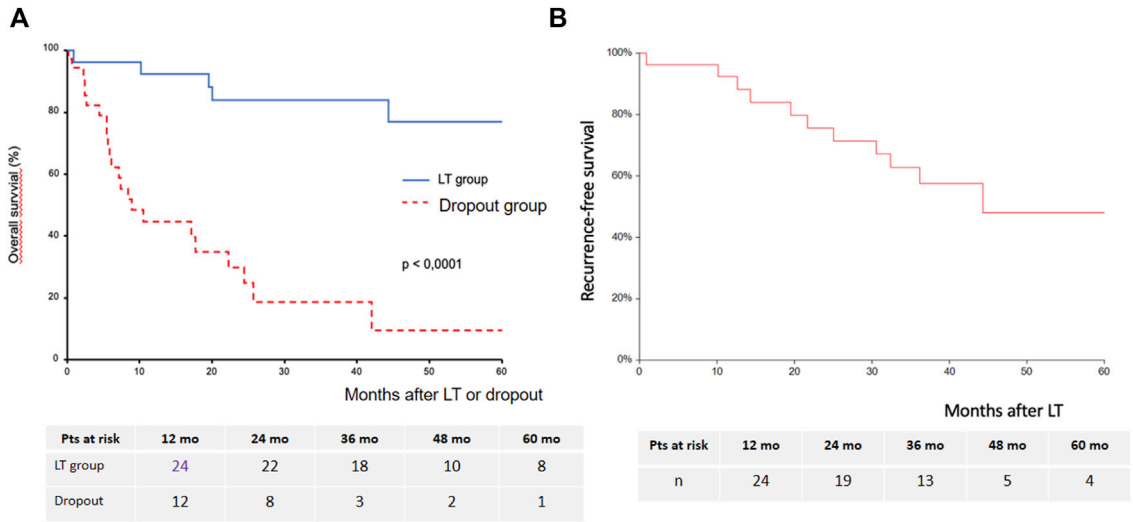
Mean and median follow-up time were 44.3 ± 24 and 43 months (IQR 28.3–64.9). In the LT group, OS at years 1, 3 and 5 was 96.2%, 83.9% and 76.9%, respectively. In the dropout group, OS at years 1, 3 and 5 was 48.4%, 18.6% and 9%, respectively (**Figure 4A**). There was a significant difference in OS between the LT group and the dropout group ($p < 0.0001$). The 5-year recurrence-free survival among the transplanted patients was 48% (95% CI: 24%–72%) (**Figure 4B**). Sorafenib's indication did not significantly impact OS after LT (data not shown).

Seven transplanted patients (27% of the LT group) experienced HCC recurrence, which was intrahepatic only for one patient, intrahepatic and extrahepatic for one patient, and extrahepatic for five patients. Extrahepatic tumor recurrence occurred as lung metastases in four patients and lymph nodes metastases in two patients. The mean time to recurrence was 24.7 ± 9 (13–36) months. The 3-year cumulative incidence of HCC recurrence was 32% (95% CI: 17%–59%) (**Figure 5A**). Sorafenib's indication was not a predictor of HCC recurrence (**Figure 5B**).

Demographic, clinical, radiological and explant features were analyzed using linear regression model to identify factors predicting HCC recurrence after LT and are summarized in **Table 7**. The solely identified factor was the number of HCC within the native liver (HR 1.15, $p = 0.03$). A linear regression multivariable was not performed because of the low number of transplanted patients ($n = 26$) and low number of recurrence ($n = 7$).

DISCUSSION

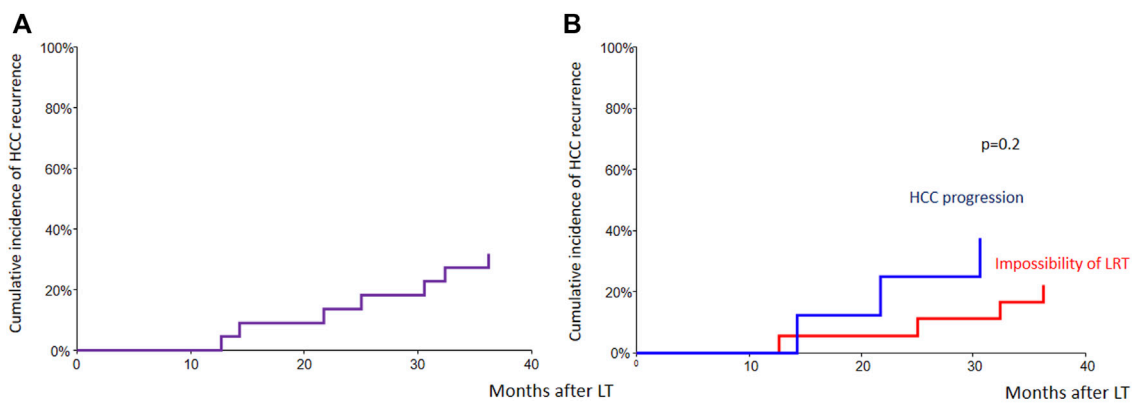
In the present study, we aimed to analyze natural history and trajectories of patients awaiting LT treated with sorafenib as neoadjuvant therapy, peri-operative morbidity and overall and recurrence-free survival after LT. Twenty-six patients treated with sorafenib (42% of the cohort) underwent LT. Thus, dropout from the waiting list remains a major issue as 58% of our cohort experienced it for tumor progression. Among these patients, half dropped-out after around 8 months (monthly rate of dropout at 3.25% the first year), exceeding the expected average dropout rate of 20% at 12 months according to the Agence de Biomédecine data. In the literature dropout depends on multiple factors, including wait list time, HCC characteristics (solitary tumor greater than 3 cm, two or three tumor nodules), elevated baseline AFP level (≥100 ng/ml), increased AFP concentration,



Pts at risk	12 mo	24 mo	36 mo	48 mo	60 mo
LT group	24	22	18	10	8
Dropout	12	8	3	2	1

Pts at risk	12 mo	24 mo	36 mo	48 mo	60 mo
n	24	19	13	5	4

FIGURE 4 | Estimated overall survival after LT or after dropout **(A)** and recurrence-free survival after LT **(B)**.



Pts at risk	12 mo	24 mo	36 mo
n	25	19	13

Pts at risk	12 mo	24 mo	36 mo
HCC progression	7	5	3
Impossibility of LRT	18	14	10

FIGURE 5 | Cumulative incidence of HCC recurrence [**(A)**: whole cohort; **(B)**: according to sorafenib's indication].

Child-Pugh status, MELD score at listing, use of bridge therapy and response to bridge therapy (20–24). Median waiting time of 12.5 months before LT in our study was consistent with the 12 months median waiting time according to the Agence de Biomédecine data. In our study, there was no significant difference in tumor burden, AFP level or MELD score at listing between the LT and the dropout group which could explain an increase in the dropout rate. Other factors such as tumor biology, genetic signature and escape mechanisms may explain differences in terms of progression on the waiting list. Investigations of the mechanisms underlying the acquired resistance to sorafenib have been led in many studies. One of these mechanisms implicates overexpression of hepatocyte growth factor receptor (HGFR) product of the MET gene which leads to the activation of the Akt and ERK (extracellular signaling-regulated kinase) pathway (25).

Sorafenib failed more frequently to prevent dropout as compared with other studies in a neoadjuvant setting. Truesdale et al. reported that there were no dropout for HCC progression among 10 patients in the sorafenib group of their study (11). Kulik et al. reported the occurrence of disease progression during the trial in only one patient under sorafenib and radioembolization and one patient of the control group (15). Frenette et al. recorded a 20% rate of dropout for tumor progression in their study (12). One explanation for our higher dropout rate may lie in sorafenib

TABLE 7 | Risk factors for HCC recurrence after LT.

	N	HR	95% CI	*p*
Age (years)	26	1	0.95–1.05	0.8
Gender male	26	0.83	0.32–2.1	0.7
Etiology of cirrhosis (alcohol vs. others)	26	1	0.36–2.7	0.9
Indication of sorafenib	26	1.5	0.6–3.5	0.3
MELD	26	1	0.9–1.2	0.4
HCC number at LT	26	1.07	0.8–1.3	0.5
Total HCC diameter at LT	26	1	0.98–1.01	0.7
Unique HCC, ≤30 mm	26	2.3	0.8–6.3	0.1
AFP-level (UI/L) at listing	26	1	0.99–1	0.2
AFP score at listing	26	1.26	0.8–1.9	0.3
Milan criteria fulfilled at listing	26	0.96	0.4–2.2	0.9
Mean sorafenib start dose	25	1	0.9–1	0.3
AFP score prior to LT	26	2.5	0.5–12	0.2
Last mRECIST radiological response prior to LT	26	0.9	0.55–1.6	0.8
Waiting time from listing to LT	26	1.11	0.9–1.3	0.09
Tumor number on explant	25	1.15	1–1.3	**0.003**
Differentiation grade	25	0.6	0.25–1.5	0.2
Satellite nodules	17	0.9	0.28–2.9	0.8
Microvascular invasion	26	1.4	0.5–3.8	0.5
Re-LT	26	4.47	0.52–38.6	0.25

treatment indication, which influenced significantly dropout rate. Indeed, patients treated with sorafenib after tumor progression (50% of our cohort) had a significantly higher dropout rate than patients treated with sorafenib because of impossibility of another LRT (multifocal tumor or technical impossibility) (p = 0.01). These findings corroborate those of Cuchetti et al. who showed that patients with no response to bridge therapy had the highest dropout rates (23). Our results suggested two different trajectories of natural history which was confirmed by the intention-to-treat survival analysis showing a better survival in patients treated for impossibility of LRT compared to those treated for HCC progression.

The most frequent treatment-related AEs related to sorafenib were dermatological disorders (41.9%), gastrointestinal disorders (37.1%) and fatigue (21%). These results are consistent with the most common events reported in major clinical trials (9, 26). However, these events occurred less frequently in comparison to the safety reports from previous sorafenib monotherapy trials (12, 13). Approximatively half of our cohort started sorafenib at full dose (400 mg twice daily) whereas in other neoadjuvant sorafenib studies, it was initiated at full dose in almost all patients. As a result, we reported fewer dose reductions in our study (41%) than in the other studies. In addition, mean sorafenib treatment time was 6 months, which is higher than findings in other neoadjuvant sorafenib studies where treatment duration ranged from 2.9 to 5.2 months (11–16).

In our cohort, the disease control rate (CR, PR and SD) was 73.2% in transplanted patients. Published series on mRECIST tumor response to TACE prior to LT showed similar rates ranging from 75% to 88% (27–29). Only one study assessed mRECIST tumor response to sorafenib, in combination with TACE (13). This study recorded a disease control rate of 69.5% prior to LT or dropout. One additional point of interest of our study is the well-known underestimation of tumor burden by radiological assessment, compared to histological findings, which is illustrated by the difference in sum of diameter between both evaluations. This notion has been well described in the literature, with rates of tumor under-staging by preoperative imaging ranging between 20% and 40% in most centers (28–31).

Interaction of sorafenib with the transplantation setting is of particular interest for transplant surgeons. High post-LT complication rates have been reported in patients receiving sorafenib before LT (11, 15), but no firm conclusions can be drawn due to the small sample sizes, and other reports showed no increased complication rate (12–14). In our study, the incidence of bile duct stenosis was 11.5% and that of bile leakage was 3.8%. Kulik et al. and Truesdale et al. described both a potentially increased risk for biliary complications of respectively 62.5% and 67% in a sorafenib neoadjuvant setting (11, 15). Our results were in parity with the estimated average rates of the systematic review conducted by Akamatsu in a total of 14,359 liver transplantations, which were of 12% for biliary stricture and 7.8% for biliary leakage (32). Concerning thrombosis, incidence of hepatic artery thrombosis (HAT) was of 3.9% and of 1% for portal vein thrombosis in Duffy et al.'s cohort of 4234 LT recipients (33). In our study, we reported an unexpected higher rate of HAT of 11.5% and of portal vein thrombosis of 7.7%. Among all five (19%) patients who experienced HAT or portal vein thrombosis in our study, three (12%) patients had stopped sorafenib at least 6 months before LT, which makes the impact of sorafenib in the occurrence of thrombosis questionable. Finally, post-operative bleeding was observed in seven (27%) patients, of which four (15%) had continued sorafenib until LT and three (12%) had stopped treatment at least 2 months before LT. When considering only patients having continued sorafenib until LT, these results are below the 20% rate of bleeding leading to revision surgery reported by Schrem and al (34). No pseudo-aneurysm of the hepatic artery were noted in our study, whereas Eilard et al. and

Truesdale et al. both recorded respectively a 16.7% and 11.1% rate of pseudo-aneurysm of the hepatic artery. Thus, our study suggests that sorafenib use prior to LT with discontinuation only on the day of transplantation appeared to be safe without increased risk of surgical or transplant-related complications. A case control study could be useful to accurately respond to the question of higher post-LT morbidity in transplanted patients treated with sorafenib.

The rationale for using sorafenib during waiting-list time relies also in its potential to prevent recurrence. In our country, use of AFP score allow to select candidates with a 70% probability of overall survival at 5 years and allows to transplant patients with at low risk of recurrence beyond Milan criteria. Currently, we observe and consider as acceptable a recurrence rate around 15% 5 years following LT. Results of recurrence rates in previous neoadjuvant sorafenib studies were heterogeneous, ranging from 0 to 42%, and impacted by limited sample size (11–15). In our cohort of 26 transplanted patients, seven patients (27%) experienced HCC recurrence, and 15 patients (58%) were alive and free of recurrence at the end of follow-up. However, recurrence free survival close to 50% is questionable in terms of "utility" to transplant such patients, even if new treatments have emerged and give huge benefit in terms of post-recurrence survival. It is important to notice that four patients who had presented HCC recurrence at month 21, 25, 30 and 36 have died more than 5 years after LT (month 64, 66, 83 and 97) which suggest an improvement in the management of HCC recurrence.

This study weakness is the non-randomized design of the study and it is difficult to perceive what would have been the access to LT of patients without sorafenib in the absence of control group. To our knowledge, this is the largest cohort reported to date of use of sorafenib in a neoadjuvant setting. We also recognize that our strategy may appear conflicting with recent guidelines of HCC treatment but neoadjuvant immunotherapy approaches could be associated with significant risks of allograft rejection and such strategy need to be very cautiously explored in dedicated studies.

In conclusion, sorafenib as neoadjuvant treatment provided access to LT for 42% of patients while one- and two-years dropout rates were 39% and 56% (monthly rate of dropout at 3.25% the first year). However, we probably have to separate two different situations of use. Indeed, sorafenib as neoadjuvant treatment can certainly play an important role for patients with impossibility of LRT, as it provides acceptable 5-years intention-to-treat overall and post-LT survivals. Conversely, patients treated for HCC progression presented high dropout rate and low intention-to-treat survival. Thus, it is very questionable in terms of utility with a scarce donor pool, if they should even be considered for still kept listed once the tumor progression has been observed.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

SD designed the study, performed the data analysis, and drafted and edited the manuscript. KM, GL, and MN assisted in data analysis, and edited the manuscript. HL, MA, VC, ST, PM, AL, GL, OG, EN-K, and EB assisted in data collection and edited the manuscript.

CONFLICT OF INTEREST

SD reports receiving lecture fees from Novartis, Sandroz, Intercept, Astellas, Roche, Ipsen and Abbvie and serving as a board member of Novartis and Biotest.

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.

REFERENCES

1. Duvoux C, Roudropoutt-Thoraval F, Decaens T, Pessione F, Badran H, Piardi T, et al. Liver Transplantation for Hepatocellular Carcinoma: a Model Including α-fetoprotein Improves the Performance of Milan Criteria. *Gastroenterology* (2012) 143(4):986–94. doi:10.1053/j.gastro.2012.05.052
2. Bruix J, Sherman M. Management of Hepatocellular Carcinoma. *Hepatology* (2005) 42(5):1208–36. doi:10.1002/hep.20933
3. Otto G, Herber S, Heise M, Lohse AW, Mönch C, Bittinger F, et al. Response to Transarterial Chemoembolization as a Biological Selection Criterion for Liver Transplantation in Hepatocellular Carcinoma. *Liver Transpl* (2006) 12(8): 1260–7. doi:10.1002/lt.20837
4. Pommergaard HC, Rostved AA, Adam R, Thygesen LC, Salizzoni M, Gómez Bravo MA, et al. Locoregional Treatments before Liver Transplantation for Hepatocellular Carcinoma: a Study from the European Liver Transplant Registry. *Transpl Int* (2018)) 31(5):531–9. doi:10.1111/tri.13123
5. Agopian VG, Morshedi MM, McWilliams J, Harlander-Locke MP, Markovic D, Zarrinpar A, et al. Complete Pathologic Response to Pretransplant Locoregional Therapy for Hepatocellular Carcinoma Defines Cancer Cure after Liver Transplantation: Analysis of 501 Consecutively Treated Patients. *Ann Surg* (2015) 262(3):536–45. doi:10.1097/SLA.0000000000001384
6. Kornberg A, Witt U, Matevossian E, Küpper B, Assfalg V, Drzezga A, et al. Extended Postinterventional Tumor Necrosis-Implication for Outcome in Liver Transplant Patients with Advanced HCC. *PLoS One* (2013) 8(1): e53960. doi:10.1371/journal.pone.0053960
7. Roayaie S, Schwartz JD, Sung MW, Emre SH, Miller CM, Gondropoutlesi GE, et al. Recurrence of Hepatocellular Carcinoma after Liver Transplant: Patterns and Prognosis. *Liver Transpl* (2004) 10(4):534–40. doi:10.1002/lt.20128

8. Liu L, Cao Y, Chen C, Zhang X, McNabola A, Wilkie D, et al. Sorafenib Blocks the RAF/MEK/ERK Pathway, Inhibits Tumor Angiogenesis, and Induces Tumor Cell Apoptosis in Hepatocellular Carcinoma Model PLC/PRF/5. *Cancer Res* (2006) 66(24):11851–8. doi:10.1158/0008-5472.CAN-06-1377
9. Llovet JM, Ricci S, Mazzaferro V, Hilgard P, Gane E, Blanc JF, et al. Sorafenib in Advanced Hepatocellular Carcinoma. *N Engl J Med* (2008) 359(4):378–90. doi:10.1056/NEJMoa0708857
10. Vitale A, Volk ML, Pastorelli D, Lonardi S, Farinati F, Burra P, et al. Use of Sorafenib in Patients with Hepatocellular Carcinoma before Liver Transplantation: a Cost-Benefit Analysis while Awaiting Data on Sorafenib Safety. *Hepatology* (2010) 51(1):165–73. doi:10.1002/hep.23260
11. Truesdale AE, Caldwell SH, Shah NL, Argo CK, Al-Osaimi AM, Schmitt TM, et al. Sorafenib Therapy for Hepatocellular Carcinoma Prior to Liver Transplant Is Associated with Increased Complications after Transplant. *Transpl Int* (2011) 24(10):991–8. doi:10.1111/j.1432-2277.2011.01299.x
12. Frenette CT, Boktour M, Burroughs SG, Kaseb A, Aloia TA, Galati J, et al. Pre-transplant Utilization of Sorafenib Is Not Associated with Increased Complications after Liver Transplantation. *Transpl Int* (2013) 26(7):734–9. doi:10.1111/tri.12117
13. Hoffmann K, Ganten T, Gotthardtp D, Radeleff B, Settmacher U, Kollmar O, et al. Impact of Neo-Adjuvant Sorafenib Treatment on Liver Transplantation in HCC Patients - a Prospective, Randomized, Double-Blind, Phase III Trial. *BMC Cancer* (2015) 11(15):392. doi:10.1186/s12885-015-1373-z
14. Eilard MS, Andersson M, Naredi P, Geronymakis C, Lindnér P, Cahlin C, et al. Prospective Randomized Pilot Study of Y90+/-Sorafenib as Bridge to Transplantation in Hepatocellular Carcinoma. *BMC Cancer* (2019) 19(1): 568. doi:10.1186/s12885-019-5760-8
15. Kulik L, Vouche M, Koppe S, Lewandropoutwski RJ, Mulcahy MF, Ganger D, et al. Prospective Randropoutmized Pilot Study of Y90+/-Sorafenib as Bridge to Transplantation in Hepatocellular Carcinoma. *J Hepatol* (2014) 61(2): 309–17. doi:10.1016/j.jhep.2014.03.023
16. Golse N, Radenne S, Rode A, Ducerf C, Mabrut JY, Merle P. Liver Transplantation after Neoadjuvant Sorafenib Therapy: Preliminary Experience and Literature Review. *Exp Clin Transpl* (2018) 16(2):227–36. doi:10.6002/ect.2015.0299
17. Saidi RF, Shah SA, Rawson AP, Grossman S, Piperdi B, Bozorgzadeh A. Treating Hepatocellular Carcinoma with Sorafenib in Liver Transplant Patients: an Initial Experience. *Transpl Proc* (2010) 42(10):4582–4. doi:10.1016/j.transproceed.2010.09.147
18. European Association For The Study Of The Liver, European Organisation For Research And Treatment Of Cancer. EASL-EORTC Clinical Practice Guidelines: Management of Hepatocellular Carcinoma. *J Hepatol* (2012) 56(4):908–43. doi:10.1016/j.jhep.2011.12.001
19. Lencioni R, Llovet J. Modified RECIST (mRECIST) Assessment for Hepatocellular Carcinoma. *Semin Liver Dis* (2010) 30(01):52–60. doi:10.1055/s-0030-1247132
20. Llovet JM, Fuster J, Bruix J. Intention-to-treat Analysis of Surgical Treatment for Early Hepatocellular Carcinoma: Resection versus Transplantation. *Hepatology* (1999) 30(6):1434–40. doi:10.1002/hep.510300629
21. Yao FY, Bass NM, Nikolai B, Davern TJ, Kerlan R, Wu V, et al. Liver Transplantation for Hepatocellular Carcinoma: Analysis of Survival According to the Intention-To-Treat Principle and Dropout from the Waiting List. *Liver Transpl* (2002) 8(10):873–83. doi:10.1053/jlts.2002.34923
22. Yamashiki N, Gaynor JJ, Kato T, Reddy KR, Sobhonslidsuk A, Levi D, et al. Competing Risks Analysis of Predictors of Delisting Owing to Tumor Progression in Liver Transplant Candidates with Hepatocellular Carcinoma. *Am J Transpl* (2004) 4(5):774–81. doi:10.1111/j.1600-6143.2004.00412.x
23. Cucchetti A, Cescon M, Bigonzi E, Piscaglia F, Golfieri R, Ercolani G, et al. Priority of Candidates with Hepatocellular Carcinoma Awaiting Liver Transplantation Can Be Reduced after Successful Bridge Therapy: Response to Bridge Therapy and HCC Priority. *Liver Transpl* (2011) 17(11):1344–54. doi:10.1002/lt.22397
24. Mehta N, Dropoutdge JL, Roberts JP, Yao FY. A Novel Waitlist Dropout Score for Hepatocellular Carcinoma – Identifying a Threshold that Predicts Worse post-transplant Survival. *J Hepatol* (2021) 74(4):829–37. doi:10.1016/j.jhep.2020.10.033
25. Xiang QF, Zhan MX, Li Y, Liang H, Hu C, Huang YM, et al. Activation of MET Promotes Resistance to Sorafenib in Hepatocellular Carcinoma Cells via the AKT/ERK1/2-EGR1 Pathway. *Artif Cell Nanomed Biotechnol* (2019) 47(1): 83–9. doi:10.1080/21691401.2018.1543195
26. Cheng AL, Kang YK, Chen Z, Tsao CJ, Qin S, Kim JS, et al. Efficacy and Safety of Sorafenib in Patients in the Asia-Pacific Region with Advanced Hepatocellular Carcinoma: a Phase III Randomised, Double-Blind, Placebo-Controlled Trial. *Lancet Oncol* (2009) 10(1):25–34. doi:10.1016/S1470-2045(08)70285-7
27. Millonig G, Graziadei IW, Freund MC, Jaschke W, Stadlmann S, Ladurner R, et al. Response to Preoperative Chemoembolization Correlates with Outcome after Liver Transplantation in Patients with Hepatocellular Carcinoma. *Liver Transpl* (2007) 13(2):272–9. doi:10.1002/lt.21033
28. Seehofer D, Nebrig M, Denecke T, Kroencke T, Weichert W, Stockmann M, et al. Impact of Neoadjuvant Transarterial Chemoembolization on Tumor Recurrence and Patient Survival after Liver Transplantation for Hepatocellular Carcinoma: a Retrospective Analysis. *Clin Transpl* (2012) 26(5):764–74. doi:10.1111/j.1399-0012.2012.01609.x
29. Kim DJ, Clark PJ, Heimbach J, Rosen C, Sanchez W, Watt K, et al. Recurrence of Hepatocellular Carcinoma: Importance of mRECIST Response to Chemoembolization and Tumor Size. *Am J Transpl* (2014) 14(6):1383–90. doi:10.1111/ajt.12684
30. Reddy MS, Manas DM. Accuracy of Staging as a Predictor for Recurrence after Liver Transplantation for Hepatocellular Carcinoma. *Transplantation* (2007) 83(3):367–8. doi:10.1097/01.tp.0000250657.24081.47
31. Chen JW, Kow L, Verran DJ, McCall JL, Munn S, Balderson GA, et al. Poorer Survival in Patients Whose Explanted Hepatocellular Carcinoma (HCC) Exceeds Milan or UCSF Criteria. An Analysis of Liver Transplantation in HCC in Australia and New Zealand. *HPB* (2009) 11(1):81–9. doi:10.1111/j.1477-2574.2009.00022.x
32. Akamatsu N, Sugawara Y, Hashimoto D. Biliary Reconstruction, its Complications and Management of Biliary Complications after Adult Liver Transplantation: a Systematic Review of the Incidence, Risk Factors and Outcome. *Transpl Int* (2011) 24(4):379–92. doi:10.1111/j.1432-2277.2010.01202.x
33. Duffy JP, Hong JC, Farmer DG, Ghobrial RM, Yersiz H, Hiatt JR, et al. Vascular Complications of Orthotopic Liver Transplantation: Experience in More Than 4, 200 Patients. *J Am Coll Surg* (2009) 208(5):896–903. doi:10.1016/j.jamcollsurg.2008.12.032
34. Schrem H, Klußmann A, Focken M, Emmanouilidis N, Oldhafer F, Klempnauer J, et al. Post-operative Hemorrhage after Liver Transplantation: Risk Factors and Long-Term Outcome. *Ann Transpl* (2016) 21:46–55. doi:10.12659/aot.895605

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BRIEF RESEARCH REPORT
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Autologous Mesenchymal Stem Cells for Treatment of Chronic Active Antibody-Mediated Kidney Graft Rejection: Report of the Phase I/II Clinical Trial Case Series

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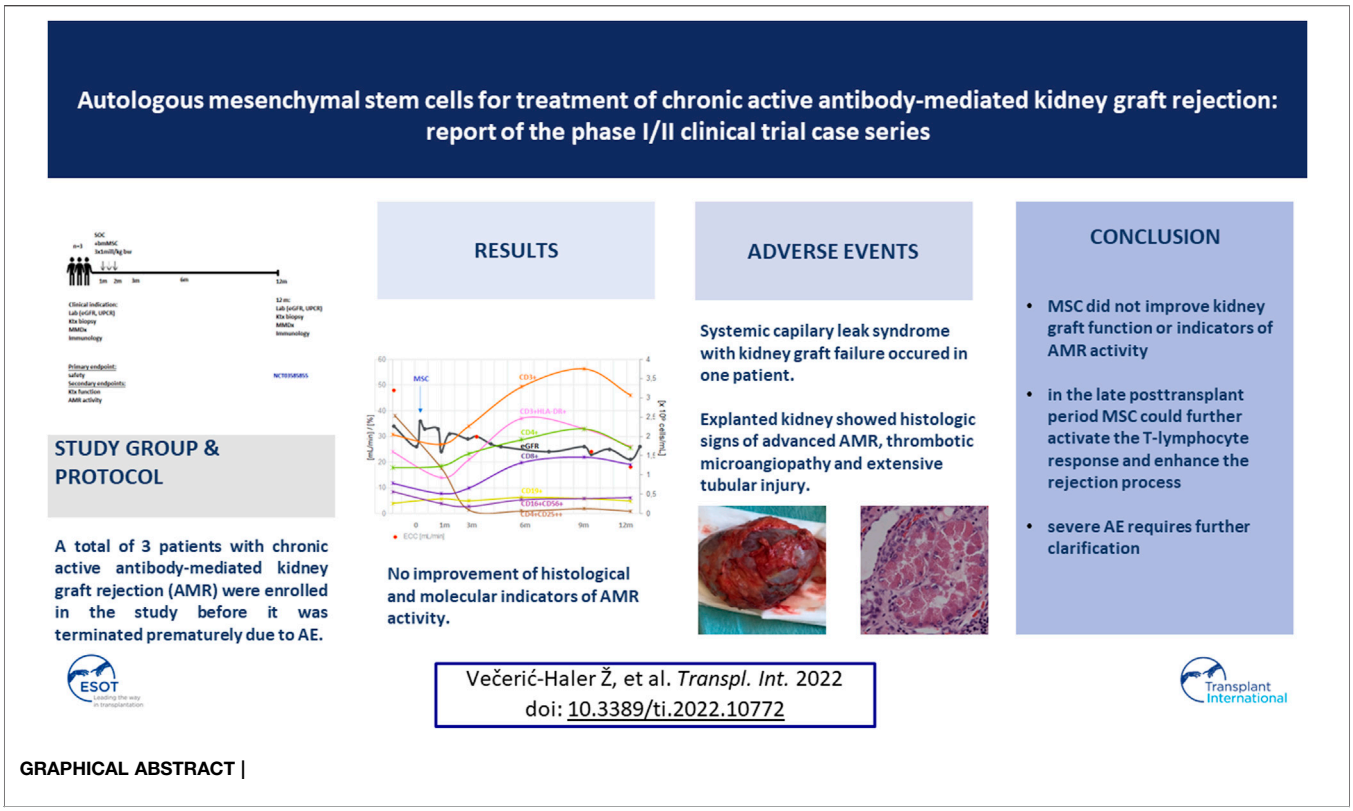
Mesenchymal stem cell (MSCs) therapy has already been studied in kidney transplant recipients (KTRs), and the available data showed that it is safe and well tolerated. The aim of this study was to evaluate the safety and efficacy of autologous MSCs in combination with standard therapy in KTRs with biopsy-proven chronic active antibody-mediated rejection (AMR). Patients with biopsy-proven chronic active AMR received treatment with autologous bone marrow-derived MSCs (3×10^6 cells/kg iv) after completion of standard therapy and were followed for up to 12 months. The primary endpoints were safety by assessment of adverse events. Secondary endpoints included assessment of kidney graft function, immunological and histological changes related to AMR activity and chronicity assessed by conventional microscopy and molecular transcripts. A total of 3 patients were enrolled in the study before it was terminated prematurely because of adverse events. We found that AMR did not improve in any of the patients after treatment with MSCs. In addition, serious adverse events were observed in one case when autologous MSCs therapy was administered in the late phase after kidney transplantation, which requires further elucidation.

Keywords: kidney transplant, mesenchymal stem cells, stem cells, antibody mediated rejection, kidney allograft



GRAPHICAL ABSTRACT |

INTRODUCTION

Chronic antibody-mediated rejection (AMR) is a major challenge to long-term graft survival in kidney transplant recipients (KTRs) (1). New technologies, including genomic studies to improve the specificity and sensitivity of renal biopsies such as Molecular Microscope Diagnostic System (MMDx) (2) and assays to detect donor-specific antibodies (DSAs), have provided important insights into the pathophysiology and diagnosis of chronic AMR. Unfortunately, these advances have not yet translated into improved outcomes because, in the absence of therapies that can suppress the formation of antibodies by plasma cells, available therapies can only slow the progression of graft injury.

Mesenchymal stem cells (MSCs) have attracted much interest due to their immunomodulatory properties (3). In kidney transplantation, MSCs have been used in a number of small and two large studies to induce immune tolerance, treat and prevent T-cell rejection, reduce interstitial fibrosis/tubular atrophy, minimize nephrotoxic immunosuppressants (4–13), and more recently to target chronic AMR resistant to conventional therapies (**Supplementary Table S1**) (14–16). With the exception of few studies using third-party MSCs (4, 11, 14–16), autologous or kidney donor-derived cells were used to avoid alloimmunization. In the recent pilot study by (14) who were the first to report the use of allogeneic bone marrow-derived MSCs (bmMSCs) in two KTRs with chronic active AMR refractory to rituximab and intravenous immunoglobulin, no improvement in graft function was observed. In contrast (15), recently demonstrated the efficacy of allogeneic bmMSCs in 23 KTRs in improving graft function and survival compared with matched controls after 2 years of follow-up. No association between MSC therapy and serious complications was observed in these studies (17, 18).

The therapeutic mechanism of MSCs has not been fully elucidated. With regard to organ transplantation, MSCs have been shown to induce long-term graft acceptance by *in vivo* generation of regulatory T cells and suppression of T cell proliferation in response to autoantigens and alloantigens in a non-MHC-linked manner (19). In the context of humoral response, preclinical studies have shown that MSCs can reduce circulating allospecific antibodies and allospecific IgG deposition in the graft, with these effects being mediated by regulatory T cells (20–22).

Here, we report the safety and efficacy of a 12-month follow-up of a case series of patients with chronic active AMR who received autologous bmMSCs in combination with standard of care (SOC) therapy at a late stage after kidney transplantation. Patients were enrolled in the study protocol (ClinicalTrials.gov, number NCT03585855), which was discontinued due to serious adverse events, including kidney graft loss in one patient (published elsewhere) (23).

MATERIALS AND METHODS

Study Design

This was a prospective, investigator-initiated, interventional, single-center clinical study. The study was approved by the National Medical Ethics Committee of the Republic of Slovenia (permit number 0120-215/2018-4) and conducted in

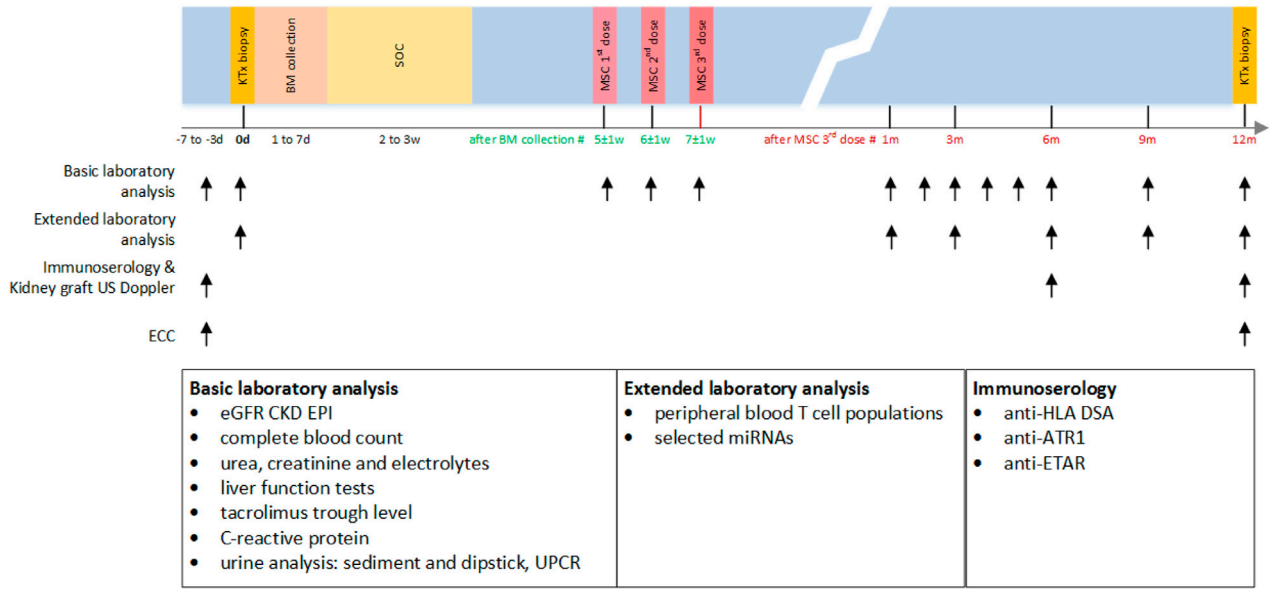


FIGURE 1 | Study design. BM, bone marrow; w, week; d, day; KTx, kidney transplant; SOC, standard of care treatment (including plasmapheresis 7x, intravenous immunoglobulins 100 mg/kg 7x, and corticosteroids); MSC, mesenchymal stem cell therapy; eGFR CKD EPI, estimated glomerular filtration rate according to the Chronic Kidney Disease Epidemiology Collaboration formula; ECC, excreted creatinine clearance; US, ultrasound; UPCR, urinary protein:creatinine ratio; HLA, human leukocyte antigen; DSA, donor specific antibodies; ATR1, antibodies against angiotensin receptor type 1; ETAR, antibodies against endothelin-1 type A receptor; miRNA, microRNA expression.

accordance with the Declaration of Helsinki. Written informed consent was obtained from all participants. The inclusion and exclusion criteria are presented in the **Supplementary Material**. The trial is registered with ClinicalTrials.gov, NCT03585855.

Procedures

The study design is shown in **Figure 1**. All participants received SOC immunosuppression for chronic active AMR (including plasmapheresis 7x, intravenous immunoglobulins (IVIg) 100 mg/kg 7x, and corticosteroids) followed by 3 infusions of autologous bmMSCs at a single dose of 1×10^6 cells/kg 1 week apart (total 3×10^6 cells/kg). The protocol was developed based on data from studies in experimental animal models, clinical data on previously experimental MSC treatment of renal pathologies, and treatment results of graft-versus-host disease in allogeneic stem cell transplant setting. Our center's extensive experience with various experimental stem cell treatments also influenced the development of study design. During the follow-up period of up to 12 months, patients were monitored for adverse events according to CTCAE 5.0; estimated glomerular filtration rate (eGFR) was determined according to the Chronic Kidney Disease Epidemiology Collaboration formula (CKD-EPI) monthly for the first 6 months and once every 3 months thereafter; excreted creatinine clearance (ECC) before and after 12 months; kidney transplant Doppler ultrasound at 0, 6, and 12 months; anti-HLA DSAs, antibodies to angiotensin receptor type 1 (anti-ATR1), and anti-endothelin 1 type A receptor antibodies (anti-ETAR) at 0, 6, and 12 months; immunophenotyping of peripheral blood T-cell populations and selected miRNA expression at 0, 1, 3, 6, 9, and 12 months; kidney biopsies including analysis of molecular transcripts by MMDx before and 12 months after MSCs application.

Mesenchymal Stem Cell Preparation and Culture Protocol

MSCs were prepared and cultured as described in the **Supplementary Material**, where data on viability and phenotypic characteristics of MSC therapy are also listed.

Kidney Graft Function

Kidney function was assessed by eGFR, calculated using the CKD-EPI study formula with serum creatinine (s-Cr), and by 24-hour urine collection and measurement of ECC.

Conventional and Molecular Kidney Graft Biopsy Assessment

Scoring of kidney biopsies and histological diagnosis of AMR were performed in a blinded fashion by a renal pathologist, using the 2019 Banff classification (24). Immunohistochemical staining, including CD44 (dilution 1:200; Cell Marque, Rocklin, United States) and CD105 (dilution 1:100; Epitomics, Burlingame, United States) was performed on kidney graft biopsies 12 months after MSCs application. Precision molecular assessment of kidney transplant biopsies was performed with MMDx using the reported protocol (24).

TABLE 1 | Baseline and end of follow-up characteristics of patients treated with MSCs.

	Patient #1			Patient #2			Patient #3	
Cause of end stage kidney disease	Autosomal dominant polycystic kidney disease			Reflux nephropaty			IgA nephropathy	
Time after Tx (years)	9			9			5	
Age	53			56			26	
Sex	Male			Male			Male	
Maintenance IS	cyclosporine, MMF			cyclosporine, MMF			tacrolimus, MMF, steroid	
Basic kidney graft function and proteinuria prior to and 12 months after MSCs								
s-Cr (μmol/L)	189	240		246	347		240	NA-dialysis dependant
ECC (ml/min)	48	18		24	18		24	NA
eGFR (ml/min/ 1.73m^2)	34	21		20	17		28	NA
Proteinuria (g/day)	0.75	1.5		1.3	1.75		3.4	NA
Immune monitoring prior to, 6 and 12 months after MSCs								
HLA DSA specifity (MFI) prior, 6, and 12 months after MSCs	DQA1 (530)	DQA1 (140)	DQA1 (190)	DQA1 (1940) DQB1 (460)	DQA1 (1830), DQB1 (240)	DQA1 (1390), DQB1 (210)	DQB1 (3390), DQA1 (2520)	NA
ATR1 (U/ml) antibodies prior to, 6, and 12 months after MSCs	5.5	4.6	6.3	45.6 (positive)	59.8 (positive)	63.2 (positive)	5.9	NA
ETAR (U/ml) antibodies prior to, 6, and 12 months after MSCs	8.6	3.3	6.0	48.2 (positive)	45.0 (positive)	57.8 (positive)	4.9	NA
Banff score in renal tansplant biopsies prior to and 12 months after MSCs administratin								
Bannf score	t0,i1, ti1, v0, ptc2 cv2, g2, cg3, mm1, ci1, ct1, ah2, i-IFTA2, C4d0, t-IFTA0, ptcml1, pvl0	t0,i1, ti2, ptc2, v0, cv2, g2, cg3, mm1, ci2, ct2, ah2, i-IFTA2, C4d0, t-IFTA0, ptcml2, pvl0		t0, i1, ti2, v0, ptc3 cv2, g3, cg3, mm1, ci1, ct1, ah3, i-IFTA1, C4d0, t-IFTA0, ptcml3, pvl0	t0, i1, ti2, v0, ptc3 cv2, g2, cg3, mm1, ci2, ct2, ah3, i-IFTA2, C4d0, t-IFTA0, ptcml3, pvl0		t0, i2, ti2, v1, ptc3 cv2, g3, cg3, mm1, ci2, ct2, ah2, i-IFTA3, C4d0, t-IFTA1, ptcml3, pvl0	t3, i3, ti3, v3, ptc3 cv3, g3, cg3, mm3, ci3, ct3, ah2, i-IFTA3, C4d0, t-IFTA2, ptcml3, pvl0, thrombotic microangiopathy, severe tubular damage

Tx-transplantation; IS-immunosuppression; MMF-mycophenolate mofetil; s-Cr-serum creatinine; ECC-excreted creatinine clearence; eGFR-estimated glomerular filtration rate; HLA-human leukocyte antigen; DSA-donor specific antibodies; ATR1- antibodies against angiotensin receptor type 1; ETAR-antibodies against endothelin-1 type A receptor; t-tubulitis; i-inflammation in non-scarred cortex; ti-total cortical inflammation; v- endarteritis; ptc-peritubular capillaritis; cv-arterial intimal fibrosi;, g-glomerulitis; cg-transplant glomerulopathy; mm-mesangial matrix expansion; ci-interstitial fibrosis in cortex; ct;tubular atrophy, ah-artriolar hyalinosis; i-IFTA inflammation in scarred cortex; C4d; linear staining in ptc or medullary vasa recta by immunofluorescence, t-IFTA- tubulitis in tubules within scarred cortex; ptcml-peritubulr capillary basement membrane multilayering; pvl- intrarenal polyomavirus load level. For details regarding Banff scoring schemes, see Loupy et al, Am J Transplant, 2020;20:2318-2331.

RNA Isolation and miRNA Quantification

Expression of selected miRNAs was determined by Quantitative real-time polymerase chain reaction (qPCR). The details are provided in the **Supplementary Material**.

Immunological Monitoring

Details of HLA, anti-ETAR, and anti-ATR1 antibodies, peripheral blood lymphocyte populations, and serum cytokine analyses are provided in the **Supplementary Material**.

Outcomes

Primary outcome measures were safety of MSC therapy assessed by adverse events at 12 months. Secondary outcome measures included changes in kidney graft function, histology, MMDx scores, and miRNA expression during the 12-month follow-up.

Statistical Analysis

All the analyses in the study were descriptive and all graphs were created using Microsoft Excel 2021.

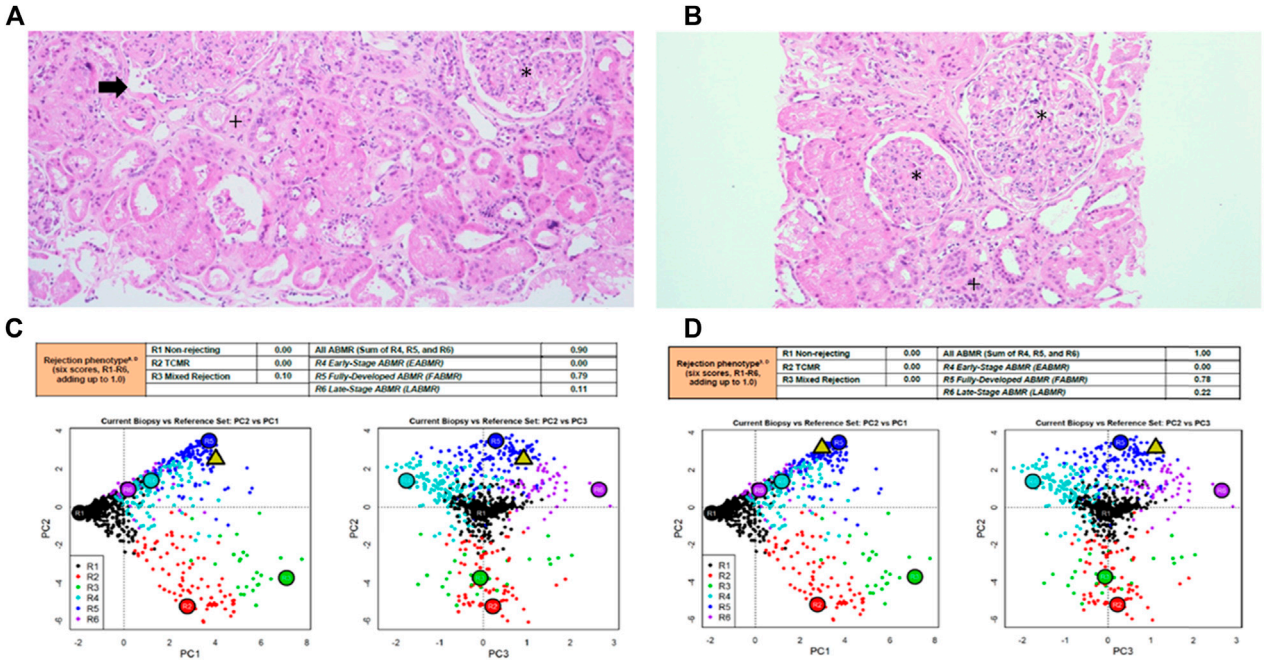


FIGURE 2 | Kidney graft histopathology and molecular microscope diagnostic report reports in patient #1 and patient #2. Kidney graft histopathology 12 months after application of MSCs in patient #1 **(A)** and patient #2 **(B)**, hematoxylin eosin, x100. Molecular microscope diagnostic report (MMDx) for kidney transplant biopsy in patient #1 **(C)** and in patient #2 **(D)** 12 months after application of MSCs showing fully-developed antibody-mediated rejection with severe microvascular inflammation and molecular features of extensive interstitial fibrosis/tubular atrophy. *glomerulitis with transplant glomerulopathy (double contour formation), +interstitial fibrosis and tubular atrophy, → peritubular capillaritis.

RESULTS

Baseline and end of follow-up characteristics of patients are presented in **Table 1**. Kidney graft histopathology and MMDx reports are shown in **Table 1** and **Figure 2**, kidney graft function, peripheral blood lymphocyte populations and serum concentration of cytokines are presented in **Figure 3**, with additional details provided in the **Supplementary Material**.

Patient #1

Patient #1 was a 53-year-old man with end stage kidney disease due to ADPKD who received the first deceased donor kidney transplant 9 years before enrollment in the study. After histological diagnosis of chronic active AMR, patient received SOC therapy, followed by MSCs 3×10^6/kg at 1-week intervals. He reported no adverse events. Histologic assesment before therapy revealed focal glomerulitis and 30% moderate peritubular capillaritis without tubulitis, double contour formation (transplant glomerulopathy) in 10 of 21 glomeruli, 20% interstitial fibrosis/tubular atrophy, and a lymphocytic interstitial infiltrate in areas of interstitial fibrosis (i-IFTA2). A very sparse lymphocytic interstitial infiltrate consisting of CD3+T lymphocytes, scarce $CD68^+$ macrophages, and few CD79a+B lymphocytes was present in another 20% of the preserved renal cortex. Immunofluorescence for C4d in the peritubular capillaries was negative. Electron microscopy showed peritubular capillary basement membrane multilayering (ptcml1), see **Table 1**.

During follow-up, patient experienced a continuous decrease in kidney function and an increase in proteinuria. The MFI values of DSAs decreased after standard and MSC therapy and remained stable thereafter. Surveillance kidney biopsy at 12 months showed similar histologic features to the previous biopsy, with a decrease in peritubular capilaritis (from 30% to 15%) and an increase in chronicity (30% interstitial fibrosis/tubular atrophy and ptcml2). The number of $CD3^+$ lymphocytes, CD79a+ lymphocytes, and $CD68^+$ macrophages was similar to the biopsy before MSCs administration. MMDx analysis 12 months after MSCs therapy showed persistence of fully developed AMR with molecular classifiers of inflammation and fibrosis in the range of highly elevated values.

After MSCs infusion, the concentration of helper $CD3^+CD4^+$ and cytotoxic $CD3^+CD8^+$ T lymphocytes increased. The proportion of activated $CD3^+HLA\text{-}DR^+$ lymphocytes increased, whereas the absolute number and proportion of $CD4^+CD25^{++}$ T lymphocytes within the total $CD4^+$ population decreased. $CD4^+CD25^{++}$ T lymphocytes were still suppressed 12 months after MSCs infusion. NK ($CD16^+CD56^+$) and B lymphocyte ($CD19^+$) concentrations were consistent with patient age and stable throughout the follow-up period. MSC therapy had no effect on memory T cells. Evaluation of timely expression of miRNA associated with AMR showed no significant changes in expression profiles during the observation period (**Supplementary Figure S1**).

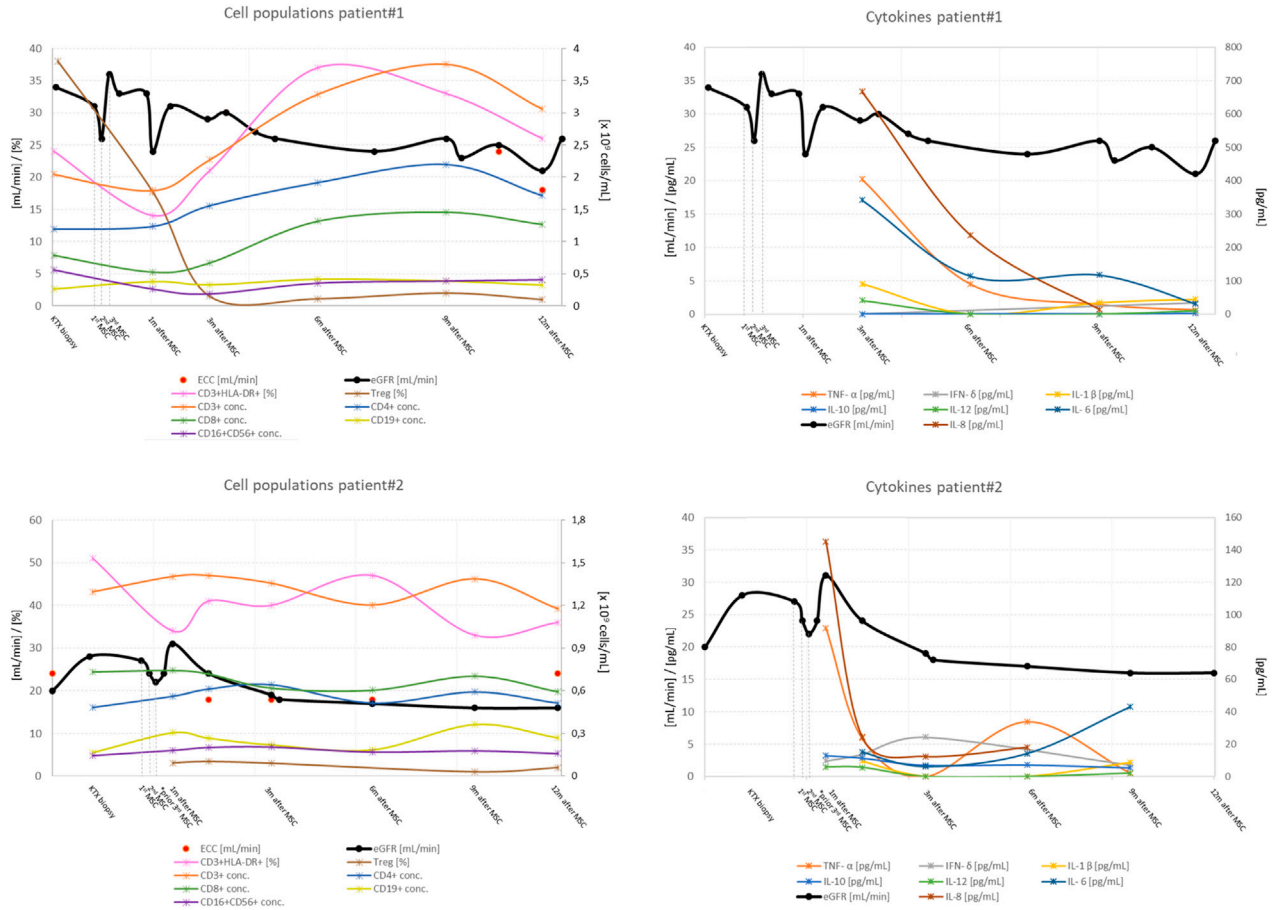


FIGURE 3 | Kidney graft function, peripheral blood lymphocyte populations and serum concentration of cytokines before and after application of MSCs in patient #1 and patient #2. Legend: eGFR, estimated glomerular filtration rate; conc, concentration; Treg, $CD4^{+}CD25^{++}$ T cells (this subset contains a proportion of Tregs cells). Cytokine concentrations after mesenchymal stem cells application (in U/ml for soluble interleukin 2 receptor and in pg/ml for other cytokines); TNF, tumor necrosis factor; IL, interleukin; s-IL-2-R, soluble interleukin 2 receptor; IFN, interferon, *patient#2 received only two doses of MSCs.

Patient #2

Patient #2 was a 56-year-old man with end stage kidney disease due to reflux nephropathy. He received his second deceased donor kidney 9 years before participation in the study and had mixed acute AMR and T-cell rejection 5 years after transplantation, which was treated with steroid pulses, plasmapheresis, IVIg, and additional therapy with rituximab and bortezomib. Kidney graft function remained stable for 4 years but steadily deteriorated in the last 2 months before enrollment.

After graft biopsy that revealed chronic active AMR with anti-HLA DSAs, anti-ATR1 and anti-ETAR antibodies, he received SOC treatment, followed by 2×10^{6}/kg MSCs at 1-week intervals. After the second dose, we observed an increase in s-Cr and noninfectious diarrhea, and because of the observed side effects, the third dose of MSCs was withheld. The potential side effects resolved spontaneously.

During follow-up, kidney function decreased and proteinuria increased. Although the concentration of T lymphocytes remained unchanged, the proportion of activated T lymphocytes increased, while at the same time the proportion and absolute number of $CD4^{+}CD25^{++}$ T lymphocytes continued to decrease after treatment with MSCs. The concentration of memory T lymphocytes was not affected by treatment. Surveillance kidney biopsy 12 months after MSC treatment revealed persistent chronic active AMR, with anti-HLA DSAs, anti-ETAR, and anti-ATR1 antibodies still present. Histologically, there was global glomerulitis with transplant glomerulopathy, diffuse peritubular capillaritis, and an increase in chronic Banff scores: intersitital fibrosis (from 15% to 35%), tubular atrophy (from 10% to 30%), and peritubular capillary basement membrane multilayering (from ptcml 1 to ptcml3), see **Table 1**. The amount of $CD3^{+}$ lymphocytes, CD79a+ lymphocytes, and $CD68^{+}$ macrophages were similar to those obtained by biopsy before MSCs were administered. MMDx 12 months after MSC treatment showed molecular classifiers indicative of microvascular inflammation and fibrosis associated with persistent chronic active AMR. Expression of miRNA associated with AMR also showed no

significant changes in expression profiles during the observation period (**Supplementary Figure S1**).

Patient #3

Patient #3 was a 26-year-old man with end stage kidney disease due to IgA nephropathy. He received a deceased kidney transplant 4 years before participation in the study. Two years after transplantation, he was diagnosed with mixed T-cell rejection (Banff 4/IB) and AMR treated with high-dose steroids, plasmapheresis, antithymocyte globulin, and rituximab. After 3 years of stable kidney function, s-Cr and proteinuria increased in the last months before enrollment. Graft biopsy revealed chronic active AMR. Because he had a history of childhood acute lymphoblastic leukemia, we performed a bone marrow aspiration before entering the study, which showed mild reactive changes. After completion of SOC therapy, he received MSCs (3×10^6 cells/kg) according to the study protocol.

Severe systemic side effects occurred after the third administration of MSCs, including acute noninfectious gastroenteritis, ascites, splenomegaly, resistant hypertension, hemolytic anemia, pancytopenia, and nephrotic range proteinuria. After the third administration of MSCs, his kidney function deteriorated (s-Cr 390 μmol/L, eGFR 10 ml/min/1.73 m^2) and kidney graft explantation had to be performed 2 months after the MSCs administration. The full course and temporal evolution of the adverse reaction including histopathological changes have been described in detail previously (23).

DISCUSSION

Here we present the results of the first phase I/II case series of KTRs with chronic active AMR treated with autologous bmMSCs in combination with SOC treatment in the late period after kidney transplantation.

In our centre, the standard treatment protocol for chronic active AMR consists of corticosteroids, plasmapheresis, and IVIg. In patients who do not respond to the SOC therapy, rituximab and bortezomib have been used in the past. However, this did not improve graft function and survival, while the risk of such potentiated therapies for life-threatening side effects increased (25, 26). Because of the disappointing treatment results, we developed a study protocol to investigate the safety and efficacy of therapy with autologous bmMSCs superimposed on standard therapy. Autologous MSCs were chosen instead of third-party MSCs to prevent alloimmunization. Unfortunately, due to premature study termination, only three patients could be enrolled, two of whom are presented in detail here (patient #1 and #2), while the course of patient #3, who experienced serious adverse events in the form of systemic capillary leak syndrome requiring discontinuation of the study protocol, has been described elsewhere (23).

The clinical trials of MSCs in kidney transplantation published through December 2021 (**Supplementary Table S1**) are mainly phase I or early phase II studies in which MSCs were administered before, at or early after transplantation against a background of regular immunosuppression to induce immunologic tolerance. With the exception of four reported studies (4, 11, 14, 15), the MSCs used were of autologous origin. Only two studies reported the administration of MSCs in the late period after kidney transplantation (14, 15). These studies have shown that treatment with MSCs is safe and feasible.

While patient #1 experienced no adverse events after MSCs administration, patient #2 experienced worsening graft function and grade 1 diarrhea immediately after the second administration of MSCs. Because patient #3 had already experienced serious adverse events during this period, which also started with noninfectious diarrhea after the second MSCs administration and increased to fully developed systemic capillary leak syndrome, we decided not to continue the third MSCs administration in patient #2. Diarrhea gradually resolved, and graft function stabilized. Noninfectious diarrhea in a KTR who had received allogeneic MSCs was recently described in a study by (14).

There appears to have been a transient MSC-mediated impairment of graft function in the period up to 1 month after MSC infusion, which returned to baseline in patient #1 and patient #2. This observation may be related to the timing of MSCs infusion in relation to the timing of transplantation. When used after kidney transplantation, transient graft dysfunction occurred, which was not observed when the infusion was applied before transplantation (7, 8). This observation is consistent with previous experimental models in which rodents developed kidney dysfunction, presumably as a consequence of preferential homing of the infused cells at the site of tissue injury, which releases chemotactic signals such as hyaluronic acid (27) or complement components (28). In the absence of chemotactic signals, such as during stem cell infusion before allografting or when experimentally antagonized by complement inhibitors, MSCs preferentially recruit to lymphoid organs without graft dysfunction, increasing numbers of T regulatory cells (Tregs) and inducing long-term graft acceptance (29).

After returning to baseline, the function of the transplanted kidney slowly deteriorated over a 12-month period in patient #1 and patient#2. End-stage kidney graft failure occurred 3 and 2 years after AMR treatment, respectively, which is consistent with treatment outcomes in our historical cohorts of patients with chronic active AMR, in whom the 1-year survival rate of a transplanted kidney was 56% and the 3-year survival rate was 41% (25, 26, 30). Anti-HLA DSAs were present in all three patients before treatment, and their MFI levels decreased after treatment with standard therapy in combination with MSCs. Histopathological findings before and 12 months after MSC treatment in patient #1 and patient #2 showed comparable chronic changes in all parts of the nephron. No $CD105^+CD44^+$ (markers co-expressed by MSCs) or ectopic tissue infiltrates, which would indicate transdifferentiation of infused MSCs, were found in the

biopsy specimens. Molecular analysis of the kidney biopsy before and after treatment showed that the classifiers of fully developed severe AMR, including g-, cg-, and ptc-related molecular features, persisted. Previously, we identified miRNAs (*miR-29c, miR-126, miR-146a, miR-150, miR-155, and miR-223*) which are typically expressed in patients with AMR (31). Selected miRNAs analysis in MSC-treated patients after MSCs application did not show any significant visible changes in their expression.

After MSC therapy, the percentage of activated T lymphocytes increased. Analysis of T-cell differentiation showed an increased Th1/Th2 ratio with decreasing numbers and ratios of $CD4^{+}CD25^{++}$ T lymphocytes (i.e., $CD4^{+}CD25^{high}$ cells that express a high level of CD25 and may contain a proportion of Tregs) during the observation period. MSC therapy had no effect on the number of NK cells and B lymphocytes. Despite an increased percentage of activated T lymphocytes in the peripheral blood, we observed no increase in interstitial inflammation, peritubular capillaritis, or other signs of activity in the renal transplant biopsies compared with the biopsies before MSCs were administered in patient#1 and #2. However, in patient# 3, severe glomerular and tubular damage with endarteritis and thrombotic microangiopathy were noted, as we reported previously. The results suggest that MSC therapy does not alleviate rejection by enhancing the regulatory immune cell component. Rather, it may be responsible for a transient activation of the T-lymphocyte response, which in some cases may enhance the rejection process. The results of our immune monitoring do not coincide with the general knowledge regarding MSCs function both *in vivo* and *in vitro*. For example, Carrion et al (32) and Casiraghi et al (28) showed that MSCs suppress the proliferation, activation, and differentiation of Th1 and Th17 cells and increase the proportion of regulatory T cells when added at the beginning of the polarization process. In addition, MSCs can also suppress proliferation and activation of differentiated Th1 and Th17 cells. Such conflicting results are difficult to interpret and could be related to the quality of MSC products. On the other hand, they could reflect the functional plasticity of MSCs in a specific clinical setting. For MSCs to fully develop their immunosuppressive potential *in vivo*, they first need to undergo proper licensing by the inflammatory environment (33). In this manner, MSCs therapy was successful in a graft-versus-host disease setting with an extensive inflammatory microenvironment (34), whereas its use was detrimental in a heart transplant model where recipients were pretretated with MSCs in the absence of inflammatory stimuli (35). Furthermore, certain microenvironment factors (such as toll-like receptor ligands) have been shown to induce a pro-inflammatory MSC type, that can support T cell activation (36).

Although we currently have limited data related to the results of AMR treatment with MSCs, the largest research to date[15] has shown that allogeneic MSCs in combination with immunosuppressive drugs are effective in terms of delaying the deterioration of graft function, probably by decreasing anti-HLA DSAs levels and reducing DSA-induced injury. Unfortunately, our case series results could not confirm this. This discrepancy may be due to the use of autologous MSCs with potentially poor quality and immunomodulatory efficacy of bmMSCs obtained from patients with advanced graft failure and long-term treatment with bone marrow immunosuppressants. Prior exposure of bone marrow to chemotherapeutic agents may lead to alterations in the expansion capacity, phenotype, and DNA injury of MSCs, resulting in genetic instability and therapy-related malignancy (37, 38). MSCs obtained from patients with advanced kidney failure have been shown to be of lower quality (39). Similarly, MSCs in our cases exhibited altered morphology with more flattened cells than would have been expected for early culture **(Supplementary Material)**. The impact of above-mentioned factors on outcome in our patients is difficult to assess, but given the data from preclinical studies in similar cases (i.e., uremic milieu, distorted stem cell niche, use of immunosuppressants), decreased MSC function might be expected.

CONCLUSION

Taken together, the administration of autologous MSCs in the three patients with chronic active AMR did not improve kidney graft function and had no protective effect on histological and molecular indicators of AMR activity. From an immunological perspective, treatment with autologous MSCs, when given in the late posttransplant period, could further activate the T-lymphocyte response, which may enhance the rejection process. The safety of MSC treatment in patients after solid organ transplantation should be closely monitored for the occurrence of as-yet unexplained adverse reactions. Further studies with prolonged follow-up are needed before continuing MSCs administration to patients in the late period after transplantation.

STUDY LIMITATIONS

The main limitation of the study is the small sample size, as the study was terminated prematurely due to serious adverse events in one of the patients. As a result, the originally planned comparison cohort of patients treated with SOC alone was not included. Another shortcoming that may have affected the treatment outcome is that we used a slightly modified protocol for bone marrow isolation and MSCs preparation in patient#2 to ensure a less invasive bone marrow collection.

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 National Medical Ethics Committee of the Republic of Slovenia (permit number 0120-215/2018-4). The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

ŽV-H participated in research design and performance, data acquisition, data analysis, and manuscript writing; MA participated in research design, manuscript writing and critical overview; PFH, NK, and EB participated in sample and data analysis, manuscript writing (histology, MMDx, miRNA); MS and SZ participated in research design, sample aquisition and preparation (bone marrow procedures); PP, UŠ, MK, LG, KH, and AB participated in sample acquisition and preparation, sample and data analysis, manuscript draft corrections (MSC production); MO and JP participated in sample acquisition and preparation (kidney graft procedures); GM, MO, AAR participated in research performance, data acquisition.

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CONFLICT OF INTEREST

Authors MK, AB, and LG were employed by the company Educell d.o.o Cell Therapy Service.

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.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontierspartnerships.org/articles/10.3389/ti.2022.10772/full#supplementary-material

REFERENCES

1. Nickerson PW. What Have We Learned about How to Prevent and Treat Antibody-Mediated Rejection in Kidney Transplantation? *Am J Transpl* (2020) 20(4):12–22. doi:10.1111/ajt.15859
2. Halloran PF, Venner JM, Madill-Thomsen KS, Einecke G, Parkes MD, Hidalgo LG, et al. Review: The Transcripts Associated with Organ Allograft Rejection. *Am J Transpl* (2018) 18(4):785–95. doi:10.1111/ajt.14600
3. Shi Y, Wang Y, Li Q, Liu K, Hou J, Shao C, et al. Immunoregulatory Mechanisms of Mesenchymal Stem and Stromal Cells in Inflammatory Diseases. *Nat Rev Nephrol* (2018) 14(8):493–507. doi:10.1038/s41581-018-0023-5
4. Erpicum P, Weekers L, Detry O, Bonvoisin C, Delbouille MH, Gregoire C, et al. Infusion of Third-Party Mesenchymal Stromal Cells After Kidney Transplantation: A Phase I-II, Open-Label, Clinical Study. *Kidney Int* (2019) 95(3):693–707. doi:10.1016/j.kint.2018.08.046
5. Pan GH, Chen Z, Xu L, Zhu JH, Xiang P, Ma JJ, et al. Low-dose Tacrolimus Combined with Donor-Derived Mesenchymal Stem Cells after Renal Transplantation: A Prospective, Non-randomized Study. *Oncotarget* (2016) 7(11):12089–101. doi:10.18632/oncotarget.7725
6. Peng Y, Ke M, Xu L, Liu L, Chen X, Xia W, et al. Donor-derived Mesenchymal Stem Cells Combined with Low-Dose Tacrolimus Prevent Acute Rejection after Renal Transplantation: A Clinical Pilot Study. *Transplantation* (2013) 95(1):161–8. doi:10.1097/TP.0b013e3182754c53
7. Perico N, Casiraghi F, Gotti E, Introna M, Todeschini M, Cavinato RA, et al. Mesenchymal Stromal Cells and Kidney Transplantation: Pretransplant Infusion Protects from Graft Dysfunction while Fostering Immunoregulation. *Transpl Int* (2013) 26(9):867–78. doi:10.1111/tri.12132
8. Perico N, Casiraghi F, Introna M, Gotti E, Todeschini M, Cavinato RA, et al. Autologous Mesenchymal Stromal Cells and Kidney Transplantation: A Pilot Study of Safety and Clinical Feasibility. *Clin J Am Soc Nephrol* (2011) 6(2):412–22. doi:10.2215/CJN.04950610
9. Reinders ME, Roemeling-van Rhijn M, Khairoun M, Lievers E, de Vries DK, Schaapherder AFM, et al. Bone Marrow-Derived Mesenchymal Stromal Cells from Patients with End-Stage Renal Disease Are Suitable for Autologous Therapy. *Cytotherapy* (2013) 15(6):663–72. doi:10.1016/j.jcyt.2013.01.010
10. Reinders MEJ, Groeneweg KE, Hendriks SH, Bank JR, Dreyer GJ, de Vries APJ, et al. Autologous Bone Marrow-Derived Mesenchymal Stromal Cell Therapy with Early Tacrolimus Withdrawal: The Randomized Prospective, Single-Center, Open-Label TRITON Study. *Am J Transpl* (2021) 21:3055–65. doi:10.1111/ajt.16528
11. Sun Q, Huang Z, Han F, Zhao M, Cao R, Zhao D, et al. Allogeneic Mesenchymal Stem Cells as Induction Therapy Are Safe and Feasible in Renal Allografts: Pilot Results of a Multicenter Randomized Controlled Trial. *J Transl Med* (2018) 16(1):52. doi:10.1186/s12967-018-1422-x
12. Tan J, Wu W, Xu X, Liao L, Zheng F, Messinger S, et al. Induction Therapy with Autologous Mesenchymal Stem Cells in Living-Related Kidney Transplants: A Randomized Controlled Trial. *JAMA* (2012) 307(11):1169–77. doi:10.1001/jama.2012.316
13. Vanikar AV, Trivedi HL, Kumar A, Gopal SC, Patel HV, Gumber MR, et al. Co-infusion of Donor Adipose Tissue-Derived Mesenchymal and Hematopoietic Stem Cells Helps Safe Minimization of Immunosuppression in Renal Transplantation - Single center Experience. *Ren Fail* (2014) 36(9):1376–84. doi:10.3109/0886022X.2014.950931
14. Ban TH, Lee S, Kim HD, Ko EJ, Kim BM, Kim KW, et al. Clinical Trial of Allogeneic Mesenchymal Stem Cell Therapy for Chronic Active Antibody-Mediated Rejection in Kidney Transplant Recipients Unresponsive to Rituximab and Intravenous Immunoglobulin. *Stem Cell Int* (2021) 2021:6672644. doi:10.1155/2021/6672644
15. Wei Y, Chen X, Zhang H, Su Q, Peng Y, Fu Q, et al. Efficacy and Safety of Bone Marrow-Derived Mesenchymal Stem Cells for Chronic Antibody-Mediated Rejection after Kidney Transplantation- A Single-Arm, Two-Dosing-Regimen, Phase I/II Study. *Front Immunol* (2021) 12:662441. doi:10.3389/fimmu.2021.662441
16. Dreyer GJ, Groeneweg KE, Heidt S, Roelen DL, van Pel M, Roelofs H, et al. Human Leukocyte Antigen Selected Allogeneic Mesenchymal Stromal Cell Therapy in Renal Transplantation: The Neptune Study, a Phase I Single-center Study. *Am J Transpl* (2020) 20(10):2905–15. doi:10.1111/ajt.15910
17. Zhao L, Hu C, Han F, Cai F, Wang J, Chen J. Induction Therapy with Mesenchymal Stromal Cells in Kidney Transplantation: A Meta-Analysis. *Stem Cell Res Ther* (2021) 12(1):197. doi:10.1186/s13287-020-01721-8

18. Willekens B, Wens I, Wouters K, Cras P, Cools N. Safety and Immunological Proof-Of-Concept Following Treatment with Tolerance-Inducing Cell Products in Patients with Autoimmune Diseases or Receiving Organ Transplantation: A Systematic Review and Meta-Analysis of Clinical Trials. *Autoimmun Rev* (2021) 20(8):102873. doi:10.1016/j.autrev.2021.102873
19. Im KI, Park MJ, Kim N, Lim JY, Park HS, Lee SH, et al. Induction of Mixed Chimerism Using Combinatory Cell-Based Immune Modulation with Mesenchymal Stem Cells and Regulatory T Cells for Solid-Organ Transplant Tolerance. *Stem Cell Dev* (2014) 23(19):2364–76. doi:10.1089/scd.2013.0617
20. Casiraghi F, Perico N, Cortinovis M, Remuzzi G. Mesenchymal Stromal Cells in Renal Transplantation: Opportunities and Challenges. *Nat Rev Nephrol* (2016) 12(4):241–53. doi:10.1038/nrneph.2016.7
21. Engela AU, Baan CC, Dor FJ, Weimar W, Hoogduijn MJ. On the Interactions Between Mesenchymal Stem Cells and Regulatory T Cells for Immunomodulation in Transplantation. *Front Immunol* (2012) 3:126. doi:10.3389/fimmu.2012.00126
22. Weiss ARR, Dahlke MH. Immunomodulation by Mesenchymal Stem Cells (MSCs): Mechanisms of Action of Living, Apoptotic, and Dead MSCs. *Front Immunol* (2019) 10:1191. doi:10.3389/fimmu.2019.01191
23. Veceric-Haler Z, Kojc N, Sever M, Zver S, Svajger U, Pozenel P, et al. Case Report: Capillary Leak Syndrome with Kidney Transplant Failure Following Autologous Mesenchymal Stem Cell Therapy. *Front Med* (2021) 8:708744. doi:10.3389/fmed.2021.708744
24. 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
25. Gubensek J, Buturovic-Ponikvar J, Kandus A, Arnol M, Lindic J, Kovac D, et al. Treatment of Antibody-Mediated Rejection after Kidney Transplantation - 10 Years' Experience with Apheresis at a Single Center. *Ther Apher Dial* (2016) 20(3):240–5. doi:10.1111/1744-9987.12430
26. Oblak T, Lindic J, Gubensek J, Kveder R, Ales Rigler A, Skoberne A, et al. Treatment of Antibody-Mediated Rejection of Kidney Grafts with Bortezomib And/or Rituximab Compared to Standard Regimen: Experience of Slovene National Center. *Clin Nephrol* (2017) 88(13):91–6. doi:10.5414/CNP88FX21
27. Herrera MB, Bussolati B, Bruno S, Morando L, Mauriello-Romanazzi G, Sanavio F, et al. Exogenous Mesenchymal Stem Cells Localize to the Kidney by Means of CD44 Following Acute Tubular Injury. *Kidney Int* (2007) 72(4):430–41. doi:10.1038/sj.ki.5002334
28. Casiraghi F, Todeschini M, Azzollini N, Cravedi P, Cassis P, Solini S, et al. Effect of Timing and Complement Receptor Antagonism on Intragraft Recruitment and Protolerogenic Effects of Mesenchymal Stromal Cells in Murine Kidney Transplantation. *Transplantation* (2019) 103(6):1121–30. doi:10.1097/TP.0000000000002611
29. Casiraghi F, Azzollini N, Todeschini M, Cavinato RA, Cassis P, Solini S, et al. Localization of Mesenchymal Stromal Cells Dictates Their Immune or Proinflammatory Effects in Kidney Transplantation. *Am J Transpl* (2012) 12(9):2373–83. doi:10.1111/j.1600-6143.2012.04115.x
30. Gubensek J, Buturovic-Ponikvar J, Kandus A, Kovac J, Marn-PernAt A. Plasma Exchange and Intravenous Immunoglobulin in the Treatment of Antibody-Mediated Rejection after Kidney Transplantation: a Single-center Historic Cohort Study. *Transpl Proc* (2013) 45(4):1524–7. doi:10.1016/j.transproceed.2012.09.123
31. Borstnar S, Veceric-Haler Z, Bostjancic E, Pipan Tkalec Z, Kovac D, Lindic J, et al. Uromodulin and microRNAs in Kidney Transplantation-Association with Kidney Graft Function. *Int J Mol Sci* (2020) 21(16):E5592. doi:10.3390/ijms21165592
32. Carrion F, Nova E, Luz P, Apablaza F, Figueroa F. Opposing Effect of Mesenchymal Stem Cells on Th1 and Th17 Cell Polarization According to the State of CD4+ T Cell Activation. *Immunol Lett* (2011) 135(1-2):10–6. doi:10.1016/j.imlet.2010.09.006
33. Wang Y, Chen X, Cao W, Shi Y. Plasticity of Mesenchymal Stem Cells in Immunomodulation: Pathological and Therapeutic Implications. *Nat Immunol* (2014) 15(11):1009–16. doi:10.1038/ni.3002
34. Ren G, Zhang L, Zhao X, Xu G, Zhang Y, Roberts AI, et al. Mesenchymal Stem Cell-Mediated Immunosuppression Occurs via Concerted Action of Chemokines and Nitric Oxide. *Cell Stem Cell* (2008) 2(2):141–50. doi:10.1016/j.stem.2007.11.014
35. Renner P, Eggenhofer E, Rosenauer A, Steinmann JF, Slowik P. Mesenchymal Stem Cells Require a Sufficient, Ongoing Immune Response to Exert Their Immunosuppressive Function. *Transpl Proc* (2009) 41(6):2607–11. doi:10.1016/j.transproceed.2009.06.119
36. Waterman RS, Tomchuck SL, Henkle SL, Betancourt AM. A New Mesenchymal Stem Cell (MSC) Paradigm: Polarization into a Pro-Inflammatory MSC1 or an Immunosuppressive MSC2 Phenotype. *PLoS One* (2010) 5(4):e10088. doi:10.1371/journal.pone.0010088
37. El-Sayed M, El-Feky MA, El-Amir MI, Hasan AS, Tag-Adeen M, Urata Y, et al. Immunomodulatory Effect of Mesenchymal Stem Cells: Cell Origin and Cell Quality Variations. *Mol Biol Rep* (2019) 46(1):1157–65. doi:10.1007/s11033-018-04582-w
38. Mueller LP, Luetzkendorf J, Mueller T, Reichelt K, Simon H, Schmoll HJ. Presence of Mesenchymal Stem Cells in Human Bone Marrow after Exposure to Chemotherapy: Evidence of Resistance to Apoptosis Induction. *Stem Cells* (2006) 24(12):2753–65. doi:10.1634/stemcells.2006-0108
39. Klinkhammer BM, Kramann R, Mallau M, Makowska A, van Roeyen CR, Rong S, et al. Mesenchymal Stem Cells from Rats with Chronic Kidney Disease Exhibit Premature Senescence and Loss of Regenerative Potential. *Plos One* (2014) 9(3):e92115. doi:10.1371/journal.pone.0092115

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