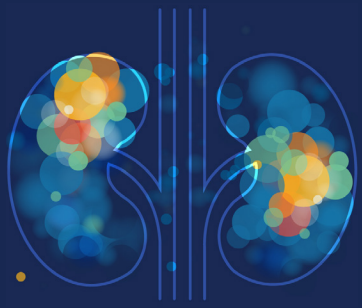
Whitepaper:

ANTIBODY MEDIATED REJECTION OF THE RENAL ALLOGRAFT: A BARRIER TO SUCCESSFUL TRANSPLANTATION



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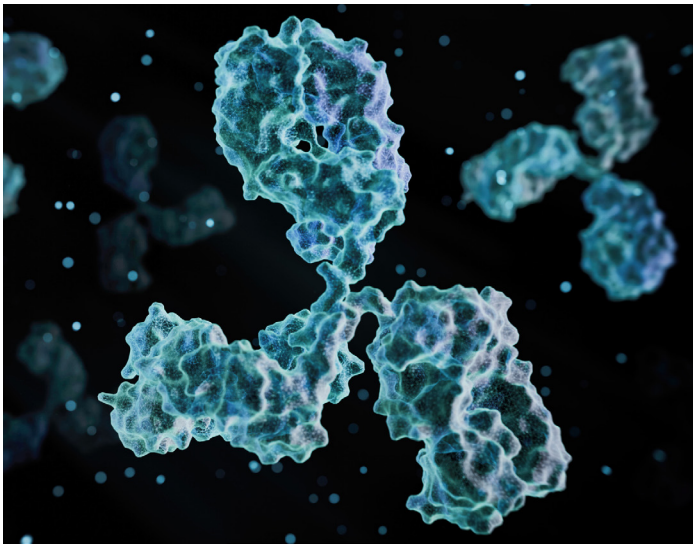
INTRODUCTION

Kidney disease is a rapidly increasing global healthcare and economic burden, and a major cause of mortality worldwide[1]. In the United Kingdom alone, approximately 7 million people are living with chronic kidney disease (CKD), and of these, it is estimated 5% will go on to develop end-stage kidney disease (ESKD) requiring kidney replacement therapy[2,3]. Renal transplantation is considered the treatment of choice for patients reaching ESKD, not only due to its cost-effectiveness compared to dialysis, but importantly for patients, it offers freedom from dialysis, an improved quality of life and a longer life expectancy[4,5]. Although short-term allograft survival has improved significantly over the past decades, registry data examining long-term allograft survival has not shown the same trajectory[6,7]. To address this discrepancy, a comprehensive understanding of the distinct causes of late graft loss is required. Insights from several cohort studies have consistently reported that after patient death with a functioning graft, alloimmune causes such as antibody-mediated rejection (AMR), are the leading cause of late allograft failure, accounting for between 35-64% of all cases[8-10] and are by far the most significant barrier to allograft survival. Both the formation of donor specific antibodies (DSAs) against the allograft and immune recognition of missing-self human leucocyte antigens (HLA) within the allograft are important mechanisms of pathogenicity in AMR.

PATHOGENESIS: BIOLOGY OF DONOR SPECIFIC ANTIBODIES

HLA molecules play a key role in the pathogenesis of organ rejection, as it is the HLA incompatibilities between the organ donor and recipient which leads to formation of antibodies against donor HLA antigens (non-self) in the recipient. These donor specific antibodies can be classified into two distinct categories: preformed and de-novo. Preformed DSAs are antibodies which are present prior to transplantation and occur due to previous exposure to non-self HLA, primarily allosensitization due to pregnancy, blood transfusion or prior transplantation. Patients sensitized with preformed antibodies are at higher risk of early rejection and allograft loss. In comparison, de-novo DSAs (dnDSA) develop after transplantation and are more commonly associated with late acute AMR and chronic AMR. The biology of dnDSA development can occur at any point post reperfusion of an allograft through both donor derived and recipient antigen presenting cell pathways. Commonly, allograft resident dendritic cells present donor antigens to recipient lymphocytes leading to the formation of alloantigen specific CD4+ T follicular helper cells (Tfh) and DSA secreting plasma cells (and memory B-cells). The reasons as to why a recipient might develop a dnDSA is multifactorial. A lack of immunosuppression, either through insufficient levels or a lack of adherence is linked to dnDSA development, while individual immunological risk factors, such as the number and specificity of HLA mismatch, also play a key role.

In addition, pro-inflammatory events, such as ischemic reperfusion injury (IRI), T-cell mediated rejection (TCMR) and recipient infections are also linked to dnDSA development, presumably due to exposure and upregulation of donor alloantigens alongside activation of recipient immune effector cells.



ANTIBODY MEDIATED REJECTION ALLOGRAFT INJURY

The donor vascular endothelial cells (ECs) are a single layer of cells lining the lumen of donor vasculature. These cells express multiple antigens and are the primary point of contact between the recipient's immune system and non-self. It is this interaction between the donor endothelium and recipient antibodies that account for much of the damage seen in allograft biopsies from patients with AMR. Allograft injury can occur through both direct and indirect antibody mediated mechanisms, alongside complement mediated injury.

The direct binding of DSA to donor ECs induces EC intracellular signaling and activation, leading to externalization of adhesion molecules with subsequent adhesion of leucocytes and platelets and production of transforming growth factor β (TGF-β), a critical component of maladaptive vascular remodeling and fibrosis seen in chronic allograft injury. Indirectly, DSAs bound to donor endothelium can regulate allograft inflammation through Fc receptor engagement on circulating immune cells; a process largely led by FcRγIIIa/CD16a expressing NK cells and monocytes. The cross-linking and activation of the Fc receptor leads to local production of pro-inflammatory chemokines and cytokines which serve to recruit further immune cells to the site while also enhancing the cytotoxicity of these newly recruited leucocytes. A common final effect of this pro-inflammatory cascade is the induction of additional EC donor antigen expression promoting increased DSA binding and further amplifying this cycle of inflammation. In addition, the binding and activation of the FcRγIIIa immune cells to the EC bound DSAs leads to cytotoxicity of the ECs through NK cell led granule and death receptor mediated pathways.

Complement mediated injury of the allograft occurs through activation of the classical complement cascade. DSAs bound to the graft endothelium bind the circulating C1q complex, with affinity of binding dependent on both the subtype and titer of DSA. The results of classical pathway activation is production of C3a and C5a, both potent inducers of inflammation which mediate recruitment and infiltration of leukocytes to the graft, and ultimately facilitate the formation of the membrane attack complex (MAC). The MAC, comprised of C5b-C9, is a pore forming molecule which inserts into the EC membrane where it causes a myriad of cellular activation, injury and proliferation.

NON-ANTIBODY MEDIATED DAMAGE: MISSING SELF-HYPOTHESIS

NK cells serve to distinguish self from non-self through their repertoire of killer Ig-like receptors (KIR). In the case of transplantation, the inhibitory KIR receptors on recipient NK cells are mismatched to the donor HLA which induces NK cell alloreactivity against the graft due to a lack of inhibitory KIR signaling[11]. Not all transplant recipients show evidence of EC injury, implying that even in a KIR/HLA mismatch scenario, a level of priming is likely required for NK cells to acquire their effector functions through this mechanism. This NK cell priming is thought to occur during some form of graft injury (e.g. ischemia/reperfusion) or viral infections, presumably through upregulation of stress/damaged induced cell ligands[12]. Once activated, these NK cells can exert cytotoxicity effector functions against donor cells either through the release of cytotoxic granules (perforin/granzymes) or via death receptor-mediated apoptosis **(Figure 1)**.

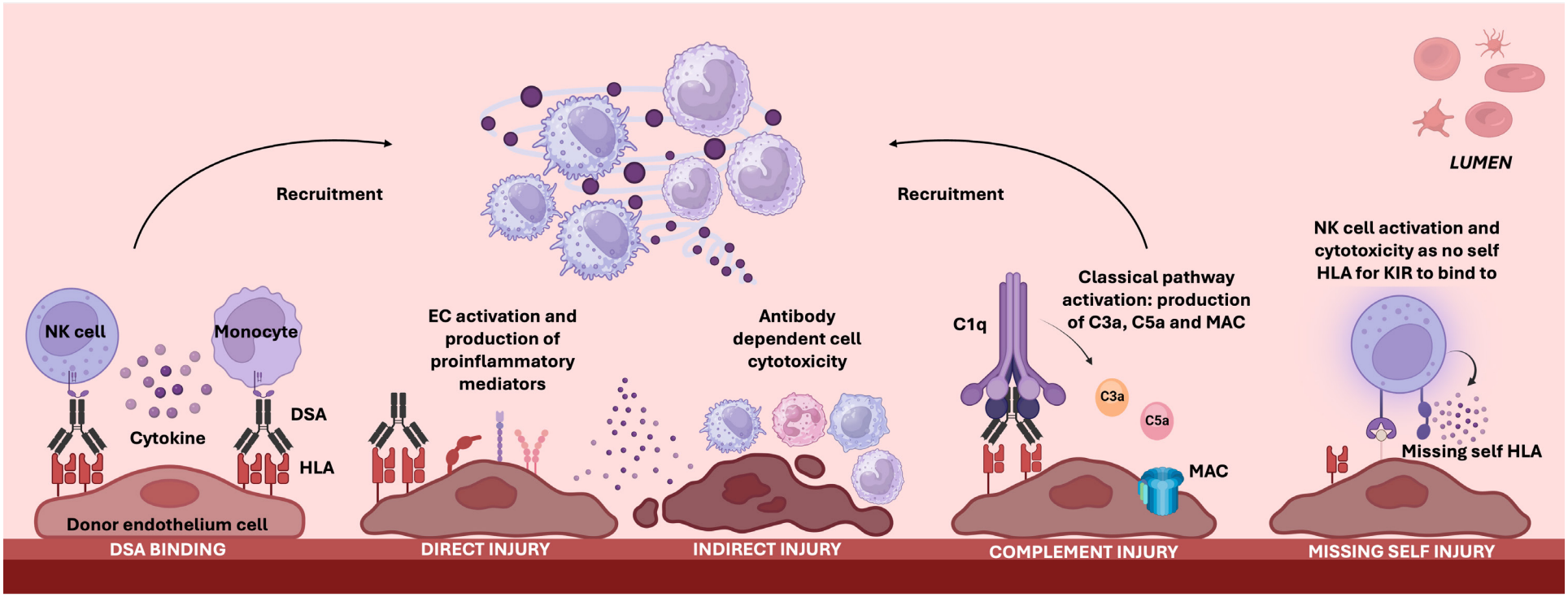


Figure 1: Immune response triggered by anti-HLA antibodies and missing-self mechanisms in AMR.

Recipient DSA binds to donor HLA antigens expressed on the allograft endothelium. The FcR on recipient NK and monocyte cells bind to the endothelium bound DSA, become activated and release effector cytokines which act on nearby immune cells to enhance cytotoxicity and directly cause endothelial cell damage, while chemokine production recruits additional immune cells to the site. Direct injury to EC occurs due to DSA binding triggering upregulation of adhesion molecules and pro-inflammatory transcriptional changes leading to cell proliferation and injury. Complement triggered injury is due to circulating C1q complex binding to EC bound DSA, leading to the production of potent serum complement factors (C3a/C5a) which act to recruit immune cells to the site, and cumulates in the formation of the MAC which induces EC activation and damage. Circulating NK cells continuously search for "self" through binding of their KIR receptors to self HLA expressed on cell surfaces. Successful binding of KIR and self HLA results in a deactivated NK cell phenotype. Within the allograft, recipient NK cells are activated in response to a lack of self HLA expressed on donor ECs, leading to NK cell activation and cytotoxicity. [HLA; human leucocyte antigen, FcR; Fc receptor, DSA; donor specific antibody, EC; endothelial cell, MAC; membrane attack complex, NK; natural killer, KIR; killer cell Ig-like receptor]

BIOMARKERS IN ANTIBODY MEDIATED REJECTION

A biomarker is an objective, quantifiable marker which can be measured and used to assess the status of a disease or response to treatment. Biomarkers play an important role in clinical drug development as they are often used as surrogate endpoints in clinical studies to improve clinical development efficiency. Biomarker development is particularly relevant to renal transplantation as there has been little improvement in long term allograft survival over the past two decades and the development of biomarkers able to predict allograft clinical outcomes would be beneficial both in a clinical trial setting and at a patient level.

Current biomarkers used in renal transplantation include standard renal functional measurements such as estimated glomerular filtration rate (eGFR) and levels of proteinuria, alongside histopathological assessment (an invasive biomarker) and anti-HLA antibody characterization and measurement. Each of these biomarkers have their limitations. When considering measurements of renal function (eGFR and proteinuria), by the time allograft dysfunction is present, significant tissue damage has occurred, so these are late markers of disease state. Histopathological assessment of biopsies (including transcript analysis) can detect subclinical AMR, but while some clinical units have protocolized surveillance biopsy programs, patient acceptance and cost can limit their implementation. Increasingly, more transplant units now have standardized anti-HLA antibody surveillance programs, which has the benefit of being non-invasive. However, antibody surveillance programs have their limitations too, with inconsistencies in positive cut off thresholds between units, and the high cost per test being common ones. In addition, most units will only screen for common anti-HLA antibodies, so patients with donor specific non-HLA antibodies will not be highlighted as high-risk for AMR. It is worth noting that not all patients with a detectable antibody will have AMR, therefore, it is important for clinicians to not just treat the antibody, but instead view it in combination with histology. The recent publication of the OuTSMART trial (investigating whether DSA screening and immunosuppression optimization improved allograft outcomes) did not show clinical benefit in those patients undergoing DSA screening and intervention, raising further questions around the practical utility of anti-HLA screening programs and their use as biomarkers[13].

Efforts have been dedicated to discovering and validating non-invasive markers which can identify the onset of rejection and act as early diagnostic biomarkers. Recently, there has been interest in using donor-derived cell-free DNA (dd-cfDNA) as a biomarker for graft rejection. The presence of dd-cfDNA in plasma of recipients arises from either apoptotic or necrotic allograft cells and can provide an early indication of allograft injury. The clinical validity of using dd-cfDNA has now been well studied in kidney transplant recipients, where it has shown to be elevated in patients with all types of rejection, alongside patients with de-novo DSA formation. Although dd-cfDNA correlates with clinical rejection, its release after allograft injury of any cause results in it lacking specificity, and at this time, it should be viewed as a predictive biomarker and used in combination with other standard biomarkers[14]. Leveraging non-invasive monitoring using predictive and diagnostic biomarkers will benefit transplant recipients and can provide valuable information around allograft injury, helping to risk-stratify patients and identify those who may benefit from invasive allograft biopsies.

ENDPOINT SELECTION FOR RENAL TRANSPLANT TRIALS

The selection of endpoints for clinical studies in renal transplant populations has historically been challenging. The traditional primary endpoints used in transplantation include recipient death, allograft failure, biopsy confirmed rejection and allograft dysfunction, which are all clearly clinically meaningful endpoints. However, difficulties arise as currently we are in an era where short-term allograft outcomes are often excellent, but this doesn't automatically translate to equally optimal long-term outcomes which remain sub-optimal. Central to the lack of improvements seen in longer-term allograft outcomes has been the paucity of clinical trials evaluating novel therapies in this field. A significant barrier to the successful execution of clinical trials in transplant populations has been the low frequency of those traditional primary endpoint rates such as allograft failure and recipient death which have historically necessitated unrealistically large sample sizes or follow up durations.

SINGLE MARKERS AS SURROGATE ENDPOINTS IN TRANSPLANTATION

The use of surrogate endpoints can often facilitate efficient trial designs which aim to evaluate long-term clinical outcomes, and in native renal conditions, conditional marketing authorizations have been granted by regulatory agencies based on proteinuria as a surrogate predictor of renal decline. Attempts to adopt the use of surrogate endpoints used in clinical studies in native renal disease to transplant studies have been made, however, due to the multifactorial nature of clinically relevant transplant related outcomes, and the unique situation in transplantation whereby both donor and recipient factors impact clinical outcomes, the use of such endpoints within transplantation require their own validation.

GLOMERULAR FILTRATION RATE (GFR), PROTEINURIA AND DONOR SPECIFIC ANTIBODIES

Consistent with findings observed in native renal populations, there is evidence that post-transplant estimated GFR (eGFR) at a given time point and eGFR percentage decline are both associated with death-censored allograft failure. Analyses of eGFR trajectory using the eGFR slope has been used in native renal disease populations to evaluate the efficacy of interventions on slowing disease progression, and although renal transplant populations have not historically been included in the large meta-analyses, eGFR slope has been used as a primary outcome measure in a small number of antibody mediated rejection clinical trials[15]. The use of proteinuria as a surrogate marker for the severity of glomerular injury in a myriad of native renal disease is well documented and accepted by regulatory authorities as a validated surrogate endpoint. Its use as a potential endpoint in renal transplantation is less clear. Proteinuria post transplantation has many causes, ranging from allograft rejection to reoccurrence of original disease, and although there is some evidence to suggest that elevations of proteinuria are associated with allograft outcomes, there are currently no studies in renal transplant recipients which show that modulation of proteinuria can slow progression of allograft failure. As both GFR and proteinuria are predictors of allograft outcome, combining both markers into a single functional endpoint is an attractive prospective, but further validation is necessary to demonstrate that any combined functional endpoint can predict long term allograft clinical outcomes in transplant populations. Similarly for DSAs, although they are widely recognized as a contributor to the development of AMR and allograft injury, their use as a single endpoint in clinical trials is not only restricted by the lack of standardized in their measurement and positivity thresholds across sites, but also post-hoc analyses of previous clinical studies (RITUX-ERA) have not shown convincing correlation between modulation of DSA levels and improved clinical outcomes[16]. The use of DSAs as a single endpoint does not adequately capture the full spectrum of rejection, considering that up to half of all cases of AMR are DSA-negative.

HISTOPATHOLOGICAL ENDPOINTS

Histopathological endpoints have played a central role in renal transplant studies with regulators accepting biopsy proven acute rejection (BPAR) as either a primary or composite endpoint for the prevention of rejection in clinical studies. Sequential updates to the Banff classification have improved diagnostic definitions for rejection types with borderline rejection, TCMR and AMR being distinct defined entities. In view of this, and the desire for study endpoints to be precisely defined, regulators are likely to stipulate that future trials within transplantation substitute BPAR for individual rejection types. In addition to the broad Banff diagnostic criteria, individual Banff lesion scores have also been used as endpoints in multiple clinical studies to assess the effect of investigational products on either improving active/chronic lesions or preventing the development of chronic lesions. Currently, the use of individual lesions on follow-up transplant biopsies has been restricted to secondary endpoint use or incorporated into composite scores for use as a surrogate endpoint[17], although moving forward, detailed scoring of all Banff lesions in trials will be valuable for future pooled or metanalyses.

One major limitation to the utilization of histology as formal endpoints into our transplant clinical trials have been repeated updates to the Banff classification system changing diagnostic thresholds and criteria for rejection alongside interoperator variability in reporting. The formation of the Banff endpoint working group have partnered with

regulatory authorities to overcome these challenges, with recommendations developed for the successful incorporation of histology endpoints into studies including; renal histopathologists participating in study and endpoint design, the inclusion of a panel of central pathologists who review whole slide digital images with sufficient clinical information such as DSA status (with appropriate adjudication mechanisms to address discordance), and centralized processing of ancillary testing such as immunohistochemistry stains which will help to standardize those technically challenging special stains required for full biopsy reporting[18].

THE FUTURE: COMPOSITE ENDPOINTS FOR TRANSPLANT STUDIES

Due to the multifactorial nature of risk factors associated with allograft loss, the lack of validation of any individual parameter for use as a surrogate endpoint, and a recognized need to help facilitate new immunosuppressive agents in transplant cohorts, a collaborative effort involving the pharmaceutical industry, academia and regulators combined forces in 2017 to form the Transplant Therapeutics Consortium (TTC). Largely driven by the TTC, a recent focus on developing a composite scoring system utilizing key biomarkers to accurately predict graft outcome has been successful: the iBox. The iBox is a risk prediction tool that can predict long-term death censored graft survival using multiple biomarkers that are known to be associated with allograft function and survival; eGFR, proteinuria, DSA, and histological scores. The accuracy of the iBox risk score to predict allograft survival has been confirmed in large post hoc analyses using trial-level data from interventional randomized controlled trials. As a result, in 2022, it gained approval from the European Medicines Agency (EMA) for use as a secondary efficacy endpoint in transplant recipients to support the evaluation of novel immunosuppressive therapies; predominantly to demonstrate superiority of an IMP against standard of care. In addition, at the end of 2024, it received acceptance of the Food and Drug Administration (FDA) Biomarker Qualification Plan as an efficacy endpoint for kidney transplant clinical trials and it is highly likely to be authorized as a reasonably likely surrogate endpoint used for accelerated approvals in the near future[19].

CONCLUSIONS

Antibody mediated rejection continues to be a significant risk to long term allograft survival in patients with a renal transplant. Although commendable progress has been made in our understanding of the pathophysiology behind this disease, this has not translated to clinical development within the field. The unique challenges to successful clinical study delivery in this area include not only recipient factors such as heterogeneity of the pathology, but also difficulties in designing and operationalizing efficient studies largely due to a lack of validated surrogate endpoints. The standardization of endpoints using validated tools such as the iBox will almost certainly help facilitate a more efficient drug development process within transplantation; both streamlining pooled analyses/meta-analyses of transplant populations and importantly supporting regulatory submissions, thus making this a more attractive therapeutic area for clinical development within the pharmaceutical industry.

FULL-SERVICE CLINICAL DEVELOPMENT

Medpace is a scientifically-driven, global, full-service clinical research organization (CRO) providing Phase I-IV clinical development services to the biotechnology, pharmaceutical and medical device industries. Medpace's mission is to accelerate the global development of safe and effective medical therapeutics through its high-science and disciplined operating approach that leverages local regulatory and deep therapeutic expertise across all major areas including oncology, cardiology, metabolic disease, endocrinology, nephrology, central nervous system and anti-viral and anti-infective.

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