

Recent advances in kidney transplantation: a viewpoint from the Descartes advisory board*

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ABSTRACT

Transplantation medicine is a rapidly evolving field. Keeping afloat of the published literature to offer the best clinical care to our patients is a daunting task. As part of its educational mission, the Descartes advisory board identified seven topics in kidney transplantation where there has been substantial progresses over the last years: kidney allocation within Eurotransplant; kidney exchange strategies; kidney machine perfusion strategies; the changing landscape of anti-human leukocyte antigen (HLA) antibodies; the new immunosuppressive drugs in the pipeline; strategies for immunosuppression minimization; and the continuous enigma of focal segmental glomerular sclerosis recurrence after transplantation. Here, we have summarized the main knowledge and the main challenges of these seven topics with the aim to provide transplant professionals at large with key bullet points to successfully understand these new concepts.

Keywords: allocation, HLA antibodies, immunosuppression, machine perfusion, recurrence of FSGS

INTRODUCTION

The first human kidney transplant was performed more than 60 years ago. Since then, transplantation has become a highly complex trans-disciplinary field that involves immunology,

genetics, surgery, nephrology, intensive care, infectious diseases, pathology, psychology and pharmacology. Transplantation is constantly evolving at a pace that reflects the many specialties it encompasses. With more than 2500 articles already published in 2017, keeping abreast with renal transplantation science in order to provide patients with the best clinical care is no easy task.

The Descartes advisory board is the Transplantation working group of European Renal Association - European Dialysis and Transplant Association (ERA-EDTA) (<http://www.era-edta.workinggroups.org/en-US/group/descartes>) and is a pan-European expert panel of transplant physicians and surgeons. The main missions of the Descartes group are to educate professionals and to undertake research reflecting all aspects of kidney transplantation. As part of this process, it is important to provide up-to-date reviews of the most rapid developments in the field. While it is not always easy to select all areas with significant developments, herein we identify seven topics in kidney transplantation where there has been substantial progress over the last years: kidney allocation: towards a new scheme within Eurotransplant; kidney exchange strategies; kidney machine perfusion strategies; the changing landscape of anti-HLA antibodies (Abs); the new immunosuppressive drugs in the pipeline; strategies for immunosuppression minimization; and the continuous enigma of focal segmental glomerular sclerosis (FSGS) recurrence after transplantation. This review summarizes the current knowledge as well as the challenges and

opportunities with the aim to provide transplant professionals with key messages to better grasp this fast-moving field.

KIDNEY ALLOCATION: TOWARDS A NEW SCHEME WITHIN EUROTRANSPLANT

Given the scarcity of donors, allocation is at the forefront of ethical and clinical challenges. Although most allocation systems are based on societal consensus as well as scientific evidence, there are substantial differences between various approaches and each and every one of them can be justified within the settings of individual countries. On a conceptual level, many questions are not agreed upon: is it justified to give priority to children? If utility is the major goal of an allocation policy, should we not offer the best donor organs to the best recipients, or should we even consider allocating organs to patients even if their life will not be prolonged by the transplant?

Eurotransplant traditionally focused on the best possible histocompatible HLA match between donor and recipient [1]. However, over the last decade donor rates in the participating countries evolved differently. While they remained high in Belgium, they dropped remarkably in Germany. Overall, donor rates within Eurotransplant went down, while the demand for organs continued to increase. As a result of this discrepancy, waiting time increased.

Nowadays waiting time in some countries is so long that it outweighs any benefit of matching in many circumstances, particularly in younger recipients. A major innovation was the initiation in 1999 of the Eurotransplant Senior Programme (ESP), where kidneys from donors older than 65 years were preferentially allocated to recipients older than 65 years [2]. The idea was that elderly patients have a shorter life expectancy and, thus, would benefit from organs with a reduced graft survival, which would not be suitable for younger recipients. The ESP allowed elderly patients to get a faster access to organs. However, even in this special programme, waiting times have become longer and longer [3].

As a result, new strategies need to be developed. Currently, a complicated algorithm allocates the kidney to the patient with the highest score, which is a sum of points for HLA matching, waiting time, ischaemia factor (distance between the retrieval centre and the recipient centre) and other parameters. In the future, a minimal match should be defined for every patient. This concept is based on the fact that HLA A/B mismatches (MMs) trigger anti-HLA sensitization, thereby limiting the possibility of re-transplantation—a major issue in young, but not in old recipients. On the other hand, HLA DR MMs increase the risk for acute cellular rejection for all patients. As a result, a minimal match allocation in younger patients should aim to avoid HLA DR MMs with a maximum of two HLA A/B MMs, while for elderly patients a full DR matching would be sufficient. In the case of two patients with the same HLA match, the length of waiting time should be the deciding factor.

With this somewhat hierarchical approach, a number of obstacles can be tackled. Firstly, matching will not increase waiting time the same way as before, as patients have to wait until the pre-specified minimal number of HLA A/B and DR matches are achieved. This will allow for a better match without

an adverse effect on the waiting times, mainly in older recipients. On the other hand, as no patient will be transplanted based on waiting time alone, taking HLA matching into account should increase graft survival. Currently some patients have little chance of ever getting an organ because they harbour rare HLA antigens for which there are very few HLA-compatible donors. Any future allocation should allow these patients to enter a special programme for preferential allocation (the acceptable MM programme), which will be based not only on the percentage of preformed anti-HLA Abs but also on the likelihood to be transplanted, which should also reduce extreme waiting times.

Secondly, donated organs should be differentiated into categories based on donor age (although most sophisticated donor risk indexes have been proposed). Younger organs will be allocated to patients matched for HLA A/B and HLA DR, while organs from older donors will be allocated preferentially based on HLA DR alone. This allows for a longevity matching, providing longer functioning organs to patients with an anticipated better survival.

The new allocation will certainly not increase the number of transplanted organs but will hopefully make a better use of the available organs.

Bullet points:

- (i) Due to the lack of donors and the increasing demands for organs, waiting time is getting longer in most countries.
- (ii) In Eurotransplant, waiting time has a greater weight than histocompatibility in the allocation algorithm.
- (iii) New allocation rules have to be developed that define a minimal match in order to improve matching without a detrimental effect on the waiting times.

KIDNEY EXCHANGE STRATEGIES (THE EUROPEAN PAIRED EXCHANGE PROGRAMME)

Strategies for desensitization of HLA or blood groups A, B, and O (ABO) incompatible live donor allograft recipients have been available for some time and offer a survival benefit compared with remaining on the waiting list for a deceased donor transplant [4]. Nevertheless, desensitization protocols and maintenance immunosuppression are associated with higher costs compared with ABO and potentially HLA compatible kidney transplantation [5]. Furthermore, the outcome for HLA incompatible transplantation is still inferior when compared with compatible live donor transplants [6].

An elegant way to improve blood group and histocompatibility matching is to form pairs or chains of donor–recipient combinations, where the recipients are incompatible with their own live donor but compatible with the swapped organ from other live donors. The most straightforward illustration of this concept is the direct exchange of donor organs between two pairs of incompatible donor–recipient (because of either ABO incompatibility or a positive cross-match towards the intended donor). Increasingly, sophisticated algorithms exist in large networks of participants that match and lead to histocompatible chains of several dozen donor–recipient pairs [7–9]. The longest

kidney exchange chain in which 30 donor–recipient pairs were included was reported in 2012 (<http://www.nytimes.com/2012/02/19/health/lives-forever-linked-through-kidney-transplant-chain-124.html>). These chains have recently been enhanced by the addition of non-directed altruistic donors, which unlock further incompatible pairs. Programmes of this magnitude face many challenges and raise some concerns (will the swapped living donor show up when its recipient has already received the kidney from the exchanged living donor?) that need to be addressed with the greatest possible care in order to achieve a successful outcome for all those involved [10].

If one thinks further and considers in an exchange algorithm not just blood group and histocompatibility matching, but also other variables predicting outcome, such as ‘age incompatibility’ or viral infections (e.g. Human Immunodeficiency Virus (HIV)), an even better matching could be achieved. In fact, these factors are already considered in the allocation of single deceased donor kidneys. Recently, United Network for Organ Sharing (UNOS) has introduced a system of allocation based on the biological age of the kidney, whereby the 20% of the best deceased donor kidneys according to the Kidney Donor Performance Index are allocated to the 20% recipients with the longest estimated post-transplant survival to avoid futility [11]. HIV-positive deceased donors are allocated to HIV-positive recipients, an approach recently expanded to live donor transplantation between HIV-positive donor recipient pairs [12, 13]. In order to further improve matching by enlarging the donor pool, developing a mixed exchange model involving deceased and live donors could be very promising. The largest exchange programmes exist in the USA because the US-wide legislation facilitates the regulatory framework of donor exchange networks.

The legal situation in Europe is quite different because each European Union (EU) member state has its own legislation and regulatory frameworks. This makes cross-border exchanges challenging and sometimes impossible. While Germany, the country with the largest population of all European states, does not support donor exchanges due to the unclear legal situation, neighbouring countries have a more liberal policy. Recently, the first cross-border live donor kidney exchange transplants took place between the Czech Republic and Austria [14]. The recent (2016) European Collaboration in Science and Technology (EU COST) action initiative (CA15210) is a collaborative effort among many EU member states to develop a framework for a ‘European Network for Collaboration on Kidney Exchange Programmes’.

At the same time, Eurotransplant started an initiative to bundle the current national paired kidney exchange programmes and is working on a framework that should ultimately lead to living donor transplants across national borders in a single large exchange programme. Legal restrictions alluded to above may preclude some Eurotransplant member nations from participating, but the establishment of such a programme would be a tremendous success, benefiting many patients. It is important to note that Eurotransplant covers only eight EU member states with roughly 135 million inhabitants. Therefore, the next logical step would be to explore the options to partner with large non-Eurotransplant countries with well-established

exchange programmes such as Italy, Spain, France or even the UK. It remains to be seen if these admirable initiatives (Eurotransplant and COST) will be successful and eventually could be merged into a single programme. Given the current political difficulties in Europe, precise forecasting is certainly difficult, if not impossible.

Bullet points:

- (i) Paired donations allow to overcome ABO- and HLA-incompatible transplantation by swapping organ donors.
- (ii) These paired donations programmes could include other parameters that influence graft outcome such as viral infections or age disparity.
- (iii) Initiatives to enlarge paired donation programmes across Europe are presently developed by Eurotransplant and the EU.

KIDNEY MACHINE PERFUSION STRATEGIES

The rapid increase in the age of the deceased donor population has led to a resurgence in machine perfusion developments. It is becoming clear that static cold storage is inadequate for the preservation of extended criteria donors (ECDs) and, more importantly, will not allow for any expansion of acceptance criteria.

Although machine perfusion was attempted by the pioneers of transplantation, technology was at that time the limiting factor in the development of perfusion devices. Driven initially by a need to lengthen preservation, machine perfusion is now being explored as a strategy to minimize the reperfusion injury, assess organ function [15] and as a vector for delivering therapies to improve the quality of the organ [16].

Conceptually, machine perfusion can be undertaken at hypothermic temperatures (with or without oxygen delivery) or under normothermic conditions. Furthermore, it can be delivered as a continuous preservation strategy or at certain time points (such as pre-implantation) as an assessment and repair strategy.

The early clinical trials reported mixed results for hypothermic (non-oxygenated) machine perfusion (HMP) with a reduction in the rate of delayed graft function (DGF) in donation after brain death (DBD) [17] and better graft survival for ECD kidneys [18]. However, none of these effects was confirmed in donors after circulatory death (DCD) [18, 19]. The addition of oxygen to the cold perfusion appears to have a beneficial effect by restoring the adenosine tri-phosphate (ATP) content [20] and modulating the inflammatory response [21]. The Consortium for Organ Preservation in Europe is currently exploring the benefits of oxygenated cold perfusion in two clinical trials due to report next year (ISRCTN63852508 and ISRCTN32967929). One of the key issues with HMP is the lack of a reliable assessment method. Perfusion parameters (flow and resistance) and injury markers [neutrophil gelatinase-associated lipocalin, interleukin 18 (IL-18), liver-type fatty acid-binding protein] as measured in the perfusate had a modest correlation with clinical outcomes such as estimated glomerular filtration rate (eGFR) at 6 months post-transplant [22].

Although normothermia is associated with a higher metabolic rate and cooling might be more appropriate, normothermic machine perfusion (NMP) delivered for 1 h pre-implantation

appears to lead to a significant reduction in DGF when compared with static cold storage (4% versus 36%) in a small clinical series [23] and is currently being explored in a large randomized control trial in the UK (ISRCTN15821205). The understanding of the protective mechanisms of NMP is evolving and in addition to restoring ATP, upregulation of heat shock protein 70 and restoration of near physiological pressure circulation appear to have a beneficial effect. Further research is needed to determine the role of acellular perfusates, the optimal oxygen concentration and delivery as well as the feasibility of extended NMP preservation. The major advantage of NMP is the ability to provide a better organ assessment. Hosgood *et al.* proposed a composite index of renal function including renal flow, macroscopic appearance and urine production while on the pump [15] and are currently evaluating the role of this score in a study of kidneys declined for transplantation in the UK. Another potential advantage of NMP is the ability to deliver targeted therapies such as gene-silencing to promote cell survival or mesenchymal stem cells in a fully functioning kidney.

These developments in kidney perfusion are challenging the traditional transplant model with organ recovery followed by static cold storage and subsequent transplantation. These approaches could be used alone or in combination, leading to individualized perfusion strategies, tailored to the quality of the donated kidneys and applied throughout the entire period of preservation or as a period of *ex situ* reconditioning and repair in the transplant centre or in a purpose-build organ reconditioning facility. While many questions surrounding the use of machine perfusion remain unanswered, it is clear that the days of static cold storage are rapidly coming to an end [24].

Bullet points:

- (i) Static cold storage is inadequate for the preservation of ECD and will not allow for an expansion of organ acceptance criteria.
- (ii) HMP has shown conflicting results for DBD and DCD donors.
- (iii) Normothermic *ex situ* perfusion allows a better organ assessment pre-implantation and appears to lead to a significant reduction in DGF.

THE CHANGING LANDSCAPE OF ANTI-HLA ABS

The complement-dependent cytotoxicity (CDC) assay has been the main test used to detect anti-HLA Abs for decades. Cytotoxic Abs cause hyperacute rejection and their detection by a positive cross-match using donor cells has been an absolute contra-indication for transplantation. However, patients can also develop anti-HLA Abs that do not necessarily cause a positive CDC cross-match and (hyper)acute rejection [1]. This was revealed by the recent development of more sensitive solid-phase techniques for the detection of anti-HLA Abs, such as the LUMINEX technology. This technology uses HLA antigen-coated microbeads [25] rather than donor cells, is fast and is more sensitive than CDC.

Damage from HLA donor-specific antibodies (DSAs) may be mediated through the activation of the classic complement

pathway. Alternatively, DSAs can cause endothelium graft injury independently of complement either directly by activating the endothelial cells or indirectly by recruiting myeloid cells such as monocytes or cytopathic innate immune effectors such as natural killer (NK) cells, which may produce antibody-dependent cell cytotoxicity [26].

Since antibody-mediated rejection (AMR) is now widely considered as the leading cause for renal graft loss, HLA DSAs have emerged as promising biomarkers and risk predictors for AMR. However, it rapidly became obvious that not all DSAs are equally detrimental, while some are even 'benign,' i.e. without any clinically relevant consequence on graft outcome. Two important determinants of DSA pathogenicity are their strength (or titre) and their complement-binding properties [26].

The DSA strength is usually expressed as the mean fluorescence intensity (MFI) determined by LUMINEX, which approximates Ab titres. While there is a significant positive correlation between pre-existing or *de novo* DSA MFI and the occurrence/severity of AMR as well as the subsequent risk of graft failure, this correlation is far from perfect. In fact, several technical limitations of solid-phase bead assays are known to alter the ability of MFI to capture the real 'amount' of circulating Abs. For instance, single antigen beads can display on their surface denatured Class I HLA molecules (expressing only the heavy chains) that can interact with Abs *in vitro*. However, these Abs do not recognize cell-bound HLA alleles *in vivo* and are thus not deleterious. This phenomenon may result in falsely elevated DSA MFI [27]. Conversely, the prozone effect is now largely described as a frequent artifact that falsely lowers the MFI of DSAs in LUMINEX assays. This phenomenon, which preferentially affects the analysis of samples containing high levels of HLA Abs, is a major confounder of the relationship between DSA MFI and the risk of AMR/graft loss. This prozone effect should be systematically accounted for and corrected by appropriate actions (serum dilution or serum pre-treatment with dithiothreitol or EDTA) [28].

The capacity of post-transplant DSAs to bind certain components of the complement cascade (mainly C1q and as more recently demonstrated, C3d) can be evaluated with the LUMINEX assay evaluation and is associated with the occurrence, the severity and outcome of AMR [29]. This leads to the inevitable question of whether the C1q- and/or C3d-binding ability of DSA should be tested routinely in clinical practice. Although there are compelling data to support the pathogenicity of C1q-binding DSAs, it is questionable whether they are an independent risk factor for AMR and graft failure beyond a certain MFI value corrected for the prozone effect [28, 30]. As a matter of fact, *in vitro* C1q fixation is directly determined by the density of Abs bound to the antigenic bead and may not always reflect the inherent property of a given DSA to activate complement. Furthermore, C1q non-binding DSAs cannot be considered safe since their persistence over time could also lead to AMR and lower graft survival [31, 32].

Therefore, at present, clinicians rely only on the level of MFI of anti-HLA Abs to decide whether a kidney bearing these alloantigens can be safely transplanted [25]. Although there is no definite consensus on MFI cutoffs to define unacceptable

antigens [33], many centres consider that transplantation across a DSA with MFI >2000–3000 should not be undertaken [34, 35]. Moreover, if a solid-phase anti-HLA antibody is detected against an HLA antigen present on a previously rejected graft, this HLA MM should be excluded irrespective of its MFI [36]. Clinicians are thus confronted with complex choices [1]. On one hand, lowering the MFI thresholds can lead to a large spectrum of unacceptable HLA MM, leading to a high value of ‘calculated’ or ‘virtual’ panel reactive antibodies (PRA) which will severely restrict the available donor pool and prolong the waiting time. On the other hand, accepting a higher MFI puts the patient at increased risk of rejection and graft loss. Given that the annual mortality on the Eurotransplant waiting list in 2015 was close to 6% [37], this is an important competing risk to consider when selecting unacceptable HLA antigens for a particular patient.

Bullet points:

- (i) Sensitive technology allows detection of anti-HLA Abs that do not lead to a positive cross-match and/or classical complement-mediated cytotoxicity.
- (ii) To date, these non-cytotoxic anti-HLA Abs are best characterized by their MFI (a rough measure of their titres).
- (iii) It is generally considered that transplantation across a DSA with MFI >2000–3000 is associated with a higher risk of AMR and graft loss.

NEW IMMUNOSUPPRESSIVE DRUGS IN THE PIPELINE

Since the early days of solid organ transplantation, the immunosuppressive agents have evolved continuously and led to less graft rejection and improved survival. Several new drugs approved in the 1990s are still the basis of current immunosuppression [38]. Despite excellent short-term results with current immunosuppressants, there remains an unmet medical need for new regimens to improve long-term graft survival with a lower side-effect burden [39, 40].

The approval of belatacept in 2011 demonstrated the potential of a biological non-nephrotoxic maintenance immunosuppression by blocking co-stimulatory signals [41, 42]. For the first time since the introduction of cyclosporine, a new immunosuppressant showed an improvement in long-term graft survival. Despite encouraging data, belatacept is rarely used today due to cost issues, limited availability, fear of rejection, limited safety data and lack of supporting comparative data with tacrolimus (the main immunosuppressive agent currently in use).

The approval of belatacept marked the end of a decade of failure for immunosuppressive drug development in transplantation, during which many promising new agents were shelved due to limited efficacy, and/or an unfavourable side-effect profile. Only new galenic formulations of proven immunosuppressive substances (such as enteric-coated mycophenolate sodium or tacrolimus once-daily formulations) were successfully approved, providing a marginal benefit to patients. The excellent short-term outcomes, the generic cost environment and the economic failure of belatacept, together with the complexities of

transplantation have dramatically changed the perspectives for new drug development in transplantation. The pharmaceutical industry has shifted its focus towards other highly profitable areas such as cancer, multiple sclerosis [e.g. fingolimod, a sphingosine-1-phosphate receptor modulator, which sequesters lymphocytes in lymph nodes and alemtuzumab, an anti-CD52 monoclonal antibody (mAb) that depletes lymphocytes] and rheumatology [e.g. tofacitinib (a Janus kinase inhibitor blocking cytokine signalling) and rituximab (an anti-CD20 mAb that depletes B cells)].

Today, only one new compound (CFZ533) is under development for kidney transplantation in a Phase II clinical trial (ClinicalTrials.gov: NCT02217410, NCT02089087, NCT02291029, NCT02565576). This CD40–CD154 co-stimulation pathway blockade prolongs renal allograft survival in experimental models [41, 42]. Contrary to another CD40 mAb (ASKP1240), which had limited efficacy in renal transplantation, CFZ533 is a fully human, Fc-silent, anti-CD40 mAb, not causing B-cell depletion [43, 44]. Preliminary data suggest that CFZ533 is well tolerated with complete peripheral CD40 receptor occupancy for 28 days. The first efficacy results and the further developmental plan are awaited in the second half of 2017. Again, it is important to note that CFZ533 is also undergoing investigation in rheumatoid arthritis and other autoimmune diseases.

The fact that co-stimulation involves multiple co-stimulatory signals and its blockade is effective in transplantation raises the hope that other molecules targeting co-stimulation such as anti-CD28 Abs (e.g. FR104, a non-agonistic, pegylated monovalent humanized Fab antibody) [41, 42] will be developed. Future regimens may also involve two co-stimulatory blockers, e.g. against CD40–CD154 and against CD28–CD80 pathway, a combination that was highly effective in animal models [41, 42].

Besides the classical development of new drugs, many groups worldwide are working on cell therapies, in order to spare or even replace conventional immunosuppression by using haematopoietic stem cells and/or various immunoregulatory cells [45–49]. Recent progress in immunology and stem cell research together with the ability to culture specific immune cells into larger quantities have enabled first trials in transplantation. Initial results demonstrate the feasibility and safety of such cell therapies, although efficacy has yet to be demonstrated in larger series.

While industry was shifting its primary focus into other fields, transplant professionals ‘borrowed’ drugs from other areas, given the many similarities of immune mechanisms across different disease processes. Drugs approved for other indications such as rituximab, obinutuzumab (a second generation anti-CD20 mAb), alemtuzumab, bortezomib (a proteasome inhibitor that inhibits plasma cell maturation and antibody production), tocilizumab (an anti-IL-6 mAb that inhibits the immune response), eculizumab (an anti-C5 mAb blocking the terminal activation of the complement pathway) or C1-esterase inhibitors (a proximal inhibitor of the complement cascade) were tested for the treatment of antibody-mediated rejection (AMR) and desensitization—two of the current unmet needs in transplantation [50–53]. Because industry was reluctant to support such endeavours, many reports are small investigator-initiated

studies, underpowered to thoroughly investigate efficacy and safety in the context of transplantation. Until now, none of the larger trials reported a major breakthrough in the treatment of ABMR or desensitization. Further studies in this area with novel complement inhibitors [TNT009 (ClinicalTrials.gov: NCT02502903) and C1-esterase inhibitors (ClinicalTrials.gov: NCT03221842 and NCT02547220), as well as tocilizumab (ClinicalTrials.gov: NCT02108600)] are underway.

Another innovative approach to combat the deleterious effects of HLA-Abs is the use of IdeS. IdeS is an IgG Endopeptidase from *Streptococcus pyogenes*, which effectively cleaves all human IgG subclasses at the hinge region of heavy chains into F(ab')₂ and Fc fragments. IdeS also cleaves the B-cell receptor off circulating B cells, inhibiting IgG memory B-cell responses. Three clinical trials to remove anti-HLA Abs using IdeS were reported recently demonstrating acceptable safety and a rapid and complete elimination of all IgG Abs (including HLA-Abs) ([54]; ClinicalTrials.gov identifiers: NCT02426684, NCT02475551, NCT02224820). However, Abs are only transiently eliminated and autoantibodies against the bacterial endopeptidase may develop. These data suggest that IdeS could be useful for desensitization and treatment of ABMR, but adequately powered studies are needed to fully investigate this promising compound.

Despite several obstacles and a rather empty pipeline for novel immunosuppressants, several new drugs, cell therapies and other interventions are in development. Until these novel therapeutic strategies are available, optimization of the current immunosuppressants regimens, tailored to the individual patient and biomarker-driven with optimized drug dosing and better time-adapted protocols, remains the only option to continue improving long-term results after renal transplantation.

Bullet points:

- (i) Currently, only one novel immunosuppressant, an anti-CD40 mAb (CFZ533) blocking the co-stimulatory CD40–CD154 pathway, is in clinical development for the prevention of acute rejection in renal transplantation.
- (ii) First small trials investigating the safety and efficacy of cell therapies are under way aiming to modulate the immune system or induce tolerance.
- (iii) Several immunomodulatory drugs are being tested in clinical trials for the treatment of AMR and desensitization.

STRATEGIES FOR IMMUNOSUPPRESSION MINIMIZATION

Despite the sharp decrease in the incidence of acute rejection, the long-term outcome of kidney transplantation remains unchanged. Malignancies, infections, diabetes and drug-related toxicities including chronic kidney disease (CKD) still occur as direct consequences of immunosuppression, while inadequate control of the alloimmune response triggers chronic rejection, the main reason for late allograft loss. Therefore, strategies to safely minimize immunosuppression in order to improve long-term outcomes and decrease costs after kidney transplantation remain a major challenge.

Over decades, strategies to eliminate steroids have been developed to improve patients' metabolic profile (blood pressure,

lipids, glucose metabolism). A recent updated Cochrane review [55] has shown that steroid avoidance and withdrawal after kidney transplantation increases the risk of acute rejection by 50–70%. The majority of these rejection episodes were T-cell-mediated and reversible, and therefore there was no difference in patient mortality or graft loss up to 5 years after transplantation. Of note, a meta-analysis of randomized controlled trials of steroid avoidance or withdrawal regimens in paediatric kidney transplantation revealed an improved growth, with no impact on acute rejection rates [56]. The data were too scarce to draw meaningful conclusions on graft and patient survival. In summary, to date the long-term (>10 years) consequences of steroid avoidance and withdrawal remain unclear. Of note, as observed in the OSAKA trial, steroid avoidance may have a greater negative impact on the kidneys from expanded-criteria donors than standard criteria donors, and therefore it is likely that only low-immunological risk recipients of standard-criteria donor kidneys may benefit from a steroid-avoidance regimen [57]. The large ADVANCE trial has recently shown that steroid avoidance compared with a 10-day steroid withdrawal is associated with a statistically significant 5% increase in the incidence of early rejection episodes among patients receiving basiliximab induction and tacrolimus/mycophenolic acid (MPA) immunosuppression [58]. A possible explanation for the beneficial role of early steroid therapy is their ability to decrease some of the Th1 transcripts despite the administration of tacrolimus, MPA and basiliximab induction [59].

Chronic calcineurin inhibitor (CNI) nephrotoxicity was previously considered a significant contributor to the chronic attrition of kidney grafts. Thus, CNI avoidance, withdrawal or minimization protocols have been conducted using MPA and/or mammalian target of rapamycin inhibitors. Despite promising short-term results, these approaches were associated with more acute rejection and the appearance of *de novo* donor-specific Abs [60, 61]. More recently, the administration of belatacept was associated with better long-term kidney function, as well as better graft and patient survival [62]. While attractive, the routine use of belatacept is prohibited by costs and as a result, a combination of a CNI, MPA and steroids, along with basiliximab induction, remains the most common immunosuppressive combination used in clinical practice today [63].

Therefore, the real challenge nowadays is to define proper biomarkers of alloreactivity or operational tolerance to allow biomarker-driven safe immunosuppression minimization. Within the Biodrim consortium (www.biodrim.eu), low-risk patients defined by the absence of pre-transplant allo-reactive T cells detected by ELISPOT technique [64] are randomized between tacrolimus monotherapy versus standard triple immunosuppression in the ongoing large prospective Cellimin trial.

Along the same line, the proof-of-concept trial of sequential double induction protocol based on alemtuzumab (which reduces the number of effector T cells) and the anti-TNF mAb infliximab (targeting some of donor-specific memory effector T cells) followed by tacrolimus monotherapy showed excellent 5-year outcomes, even in T-cell presensitized patients. Interestingly, patients in the tacrolimus monotherapy arm exhibited a specific B-cell signature along with tolerance-associated genes and

inhibition of inflammation-related genes, suggesting that this protocol may allow safe immunosuppression reduction in the majority of patients [65]. The ongoing RIMINI trial aims to confirm this concept in a larger group of patients.

Bullet points:

- (i) Steroid avoidance in kidney transplantation offers several metabolic advantages, but is associated with higher early acute rejection rate.
- (ii) CNI avoidance/withdrawal cannot be recommended today due to higher rejection rate and the inability to appropriately identify the low-risk patients who might benefit from this strategy.
- (iii) Biomarker-driven minimization of maintenance immunosuppression offers great promises and is being tested in ongoing clinical trials.

THE CONTINUOUS ENIGMA OF FSGS RECURRENCE AFTER TRANSPLANTATION

An immediate massive proteinuria due to the recurrence of the nephrotic syndrome after kidney transplantation remains one of the most frustrating events for the nephrologist and patient alike [66]. Although most of these patients have a history of steroid-resistant nephrotic syndrome (SRNS) prior to transplantation, there are several risk factors for post-transplantation recurrence. First, up to 30% of children and young adults with SRNS harbour monogenic mutations of genes encoding for proteins of either the podocyte, the slit diaphragm or the glomerular basement membrane. While 53 genes are known today to cause an SRNS, the most prevalent are *NPHS1* (nephrin), *NPHS2* (podocin) and *WT1* (Wilms tumour 1) gene abnormalities. Patients with SRNS who carry a homozygous recessive mutation, a compound heterozygous mutation or a pathogenic dominant mutation as well as those with familial diseases but with unknown genetic abnormalities, do not develop recurrence after transplant [67, 68]. However, concern about the recurrence risk has been raised for recessive heterozygous, recessive susceptibility polymorphisms and bi-allelic diseases where an immunological second hit is conceivable. Secondly, among paediatric patients with no familial history and/or mutation identified, a recent study found that the initial steroid sensitivity is highly predictive of post-transplant disease recurrence. Nevertheless, despite extensive investigations into the significance of pathological data, the clinical parameters of onset or progression rate, or ethnicity, to date no reliable predictors for post-transplant recurrence have been identified.

Since the earliest observations, the strikingly precocious occurrence of podocyte effacement and slit membrane disruption pointed towards the presence of a possible pre-formed circulating podocytotoxic substance coined 'permeability factor' [69, 70]. The label of this factor has shifted from cytokine, to a substance with affinity for galactose [71], and more recently, to the soluble receptor for urokinase-type plasminogen activator (suPAR) [72, 73], with origin attributed to some immune system cell. SuPARs are able to bind to the $\alpha V\beta 3$ integrin on podocytes, thereby inducing activation, contraction, migration, foot process effacement and proteinuria in experimental settings.

However, the consistent presence in advanced CKD as well as in multiple primary or secondary glomerular diseases and the inverse correlation with eGFR raised doubts that suPAR plays a pathophysiological role in human FSGS.

As a consequence of the unclear pathophysiology, and due to the relative rarity of this condition, the therapy of post-transplant FSGS recurrence remains largely anecdotal and based on case series rather than on randomized controlled trials. Thus, a combination of removal of the putative circulating factor through plasmapheresis/plasmafiltration or adsorption techniques and the suppression of its production with immunosuppressive regimens including either cyclophosphamide, intravenous cyclosporine, tacrolimus or the anti-CD20 mAb rituximab are often used [74].

Another unsolved question is the utilization of living-related donation for patients with genetic FSGS [75]. As discussed above, the risk of recurrence of genetic FSGS after transplantation in the recipient is distinctly rare [76]. However, the risk for the donor is unclear. There are case reports of individuals who have developed FSGS, proteinuria and kidney failure after donating a kidney to a sibling with kidney failure due to FSGS. Cases of adult-onset FSGS have been described due to compound heterozygosity of the R229Q variant with a pathogenic podocin mutation [76]. As nephrectomy may unravel FSGS in these patients, it seems prudent to perform genetic analyses including the R229Q variant before donation [77, 78].

Bullet points:

- (i) Up to 30% of children and young adults with steroid-resistant FSGS harbour mutations of genes encoding proteins of the podocyte, the slit diaphragm or the glomerular basement membrane.
- (ii) These genetic FSGS almost never recur after transplantation.
- (iii) The therapy of post-transplant FSGS recurrence includes a combination of removal of the putative circulating factor through plasmapheresis/plasmafiltration or adsorption techniques and the suppression of its production with immunosuppressive regimen including either cyclophosphamide, intravenous cyclosporine, tacrolimus or the anti-CD20 mAb rituximab.

Despite many recent developments, renal transplantation still faces many challenges. With a persistent demand for transplantation and changes in the demographics of the donor population, smarter ways to use the current supply are needed. As illustrated by the developments highlighted in this review, current efforts are successfully directed towards increasing utilization and sharing as well as reducing kidney wastage and long-term organ loss.

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CONFLICT OF INTEREST STATEMENT

None declared.

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