Precision medicine in transplantation and hemodialysis

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ABSTRACT

In kidney transplantation, precision medicine has already entered clinical practice. Donor and recipient human leucocyte antigen (HLA) regions are genotyped in two class 1 and usually three class 2 loci, and the individual degree of sensitization against alloimmune antigens is evaluated by the detection of anti-HLA donor-specific antibodies. Recently, the contribution of non-HLA mismatches to outcomes such as acute T- and B-cell-mediated rejection and even long-term graft survival was described. Tracking of specific alloimmune T- and B-cell clones by next generation sequencing and refinement of the immunogenicity of allo-epitopes specifically in the interaction with HLA and T- and B-cell receptors may further support individualized therapy. Although the choices of maintenance immunosuppression are rather limited, individualization can be accomplished by adjustment of dosing based on these risk predictors. Finally, supplementing histopathology by a transcriptomics analysis allows for a biological interpretation of the histological findings and avoids interobserver variability of results. In contrast to transplantation, the prescription of hemodialysis therapy is far from precise. Guidelines do not consider modifications by age, diet or many comorbid conditions. Patients with residual kidney function routinely receive the same treatment as those without. A major barrier hitherto is the definition of 'adequate' treatment based on urea removal. Kt/V_{urea} and related parameters neither reflect the severity of uremic symptoms nor predict long-term outcomes. Urea is poorly representative for numerous other compounds that accumulate in the body when the kidneys fail, yet clinicians prescribe treatment based on its measurement. Modern technology has provided the means to identify other solutes responsible for specific features of uremic illness and their measurement will be a necessary step in moving beyond the standardized prescription of hemodialysis.

Keywords: genomics, hemodialysis, kidney transplantation, precision medicine, urea modeling

PRECISION MEDICINE IN KIDNEY TRANSPLANTATION

The aim of this mini-review is to evaluate the current contribution of 'precision medicine' to risk prediction, treatment plans and research directions in the field of end-stage renal disease. Although a clear definition of 'precision medicine' exists from the Food and Drug Administration, European Medicines Agency and other bodies, this short overview takes the liberty of a wider scope and includes also graft allocation and matching algorithms into this definition, because they can also be considered as therapeutic remedies. It is important to note that 'precision medicine' has become a buzz word in many articles in the life sciences field. We specifically want to point out that in this mini-review 'precision medicine' refers more to risk prediction and organ allocation than to conceptually different individual therapies in kidney transplantation. In the section on hemodialysis, it is clearly stated that many interesting questions such as the toxicity of uremic solutes can now finally be addressed by the available omics technologies, but the individual consequences on therapy prescriptions remain elusive.

THE COHORT APPROACH TO KIDNEY TRANSPLANTATION

The success of kidney transplantation depends on histocompatibility. Before solid-phase technologies became widely available to determine the degree and specificity of allosensitization, the selection of a suitable donor kidney was based on low resolution human leucocyte antigen (HLA) typing by serology. A negative cytotoxicity cross match before transplantation was mandatory to prevent acute humoral rejection by preformed donor-specific antibodies [1]. Other than that, no immunological risk stratification was possible, and the success rates thus were variable. Some transplant kidneys lasted for a long time whereas others failed rather quickly. Although the Banff biopsy grading system was established in 1991 and subsequently published in 1993 by Solez et al. [2] and updated every other year since then, no uniform and specific definitions of antibody-mediated rejections (ABMRs) were established before 2011 [3].

The discovery and wider utilization of calcineurin-inhibitor-based maintenance immunosuppression in the early 1990s led to a dramatic improvement in short-term outcomes, but long-term graft survival of patients beyond 1 year remained almost unchanged [4]. A key reason for these shortcomings was the

lack of individual immunological risk stratification and thus individualized maintenance immunosuppressive therapy. The clinical management after transplantation has been rather standardized with regular determination of estimated glomerular filtration rate (eGFR) and measurement of blood trough levels of maintenance immunosuppressive drugs such as calcineurin inhibitors. Tacrolimus was titrated to meet arbitrarily blood trough levels between 6 and 10 ng/mL [5]. Re-transplanted patients and those with a history of biopsy-confirmed rejection received higher tacrolimus doses. Surprisingly, with this crude cohort-based management algorithm and rather imprecise diagnostic tools, most patients nonetheless exhibited a median graft survival of 10 years. However, an annual graft attrition rate of 5% specifically for live donor kidneys in not acceptable [4].

In the last decade, great research efforts were undertaken to better understand alloimmunity and to determine a patient's individual rejection risk for a specific donor to recipient HLA match on the level of a high-resolution DNA sequencing. Transplantation is the prototypical example where in-depth multi-professional research allowed for a transition from a cohort-based approach to a more individualized risk prediction and guided therapy.

ADDING THE INDIVIDUALIZED PERSPECTIVE—THE PRESENCE AND NEAR FUTURE OF PRECISION MEDICINE

As the HLA system is the most polymorphic and genetically variable region in human, donor to recipient matching remains always a compromise between waiting time and the availability of a 'suitable' deceased or live donor kidney.

Tissue typing is done in most of the HLA laboratories of large transplant centers by DNA sequencing methods [6]. This high resolution of the genetic makeup of the polymorphic HLA regions of the donor and the recipient together with the identification of unacceptable antigens based on single beat donor-specific antibody (DSA) determination allows for a precise risk assessment before transplantation. Early graft failure due to preformed HLA antibodies must no longer happen. It is of note, however, that given the current graft half-life of about 10 years, many recipients will undergo re-transplantation, even multiple times if they are unfortunate enough to develop end-stage kidney disease early in life. These patients are usually highly sensitized and it may be necessary to transplant across a HLA barrier if other solutions are not available. Such solutions include live donor exchange either locally, regionally, internationally or even globally, or the enrolment in a deceased donor program for highly sensitized patients, that is, an acceptable mismatch program [7, 8]. On the other hand, the HLA proteins are encoded only on a short stretch of 4 million bases on chromosome 6 and there is particularly good evidence that genome-wide donor to recipient incompatibilities outside the HLA regions plays a critical role in 'chronic rejection' caused by indirect allorecognition of donor epitopes [9]. Recently, large consortia have been assembled to test the strength and consequences of the immune response according to the individual genetic makeup of the donor and recipients. Reindl-Schwaighofer *et al.* [10] showed that non-HLA
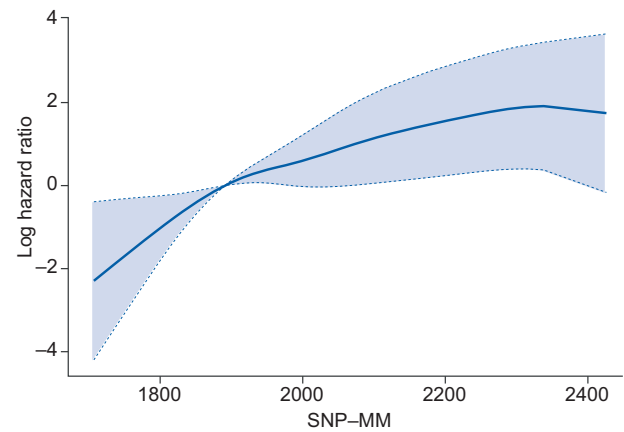

FIGURE 1: Risk of loss of graft function with increasing number of genome-wide non-HLA mismatches between donor and recipient. The analysis was adjusted for HLA eplet mismatch, donor age, donor gender and transplant center, as well as overall genome-wide single nucleotide polymorphism mismatch (SNP–MM). (Reprinted with permission from Ref. [10].)

incompatibilities of immune-accessible amino acid residues/peptides exhibit a similar threat to graft loss as HLA incompatibilities per unit increase of mismatches (Figure 1). Similarly, colleagues from the Columbia University published their findings on the risk of acute rejection and genetic mismatches in the *LIMS1* gene [11]. The authors have identified this gene as the strongest independent predictor of acute rejection among many full loss-of-function variants in the recipients who have received a kidney in which these proteins were expressed. The authors were able to detect LIMS1 expression in the kidney graft and found alloantibodies against this novel protein introduced with the grafted organ. These finding may explain HLA-DSA negative ABMRs as well as premature graft loss in well HLA-matched donor/recipient pairs.

Given the complexity of the alloimmune response and considerable uncertainty on specific epitope immunodominance, a realistic strategy to improve long-term outcomes will be tolerance induction through mixed chimerism without toxic conditioning (Figure 2) [4]. The protocols of the past of combined allogenic bone marrow and kidney transplant from the same donor were not applicable to clinical routine because of unacceptable high ratios of side effects to efficiency [12]. Novel methods utilize less toxic protocols that include inhibition of inflammation and a reduction of allogenic clones before transplantation. Recipients undergo leukapheresis and harvesting of regulatory T-cells (Tregs), which then are expanded *in vitro* and infused within the first days after the simultaneous donor bone marrow and kidney transplant (https://clinicaltrials.gov/ct2/show/NCT03867617). With such an approach, sufficient T-cell chimerism rates for tolerance induction are achievable in the first weeks after transplantation [13]. It is of note, however, that no absolute threshold of chimerism for tolerance has been established yet, and furthermore, rates may be different in other solid organ transplants [14]. Such an approach may be especially appealing for young recipients of live donor organs with an expected long lifetime. In these patients, the trade-off
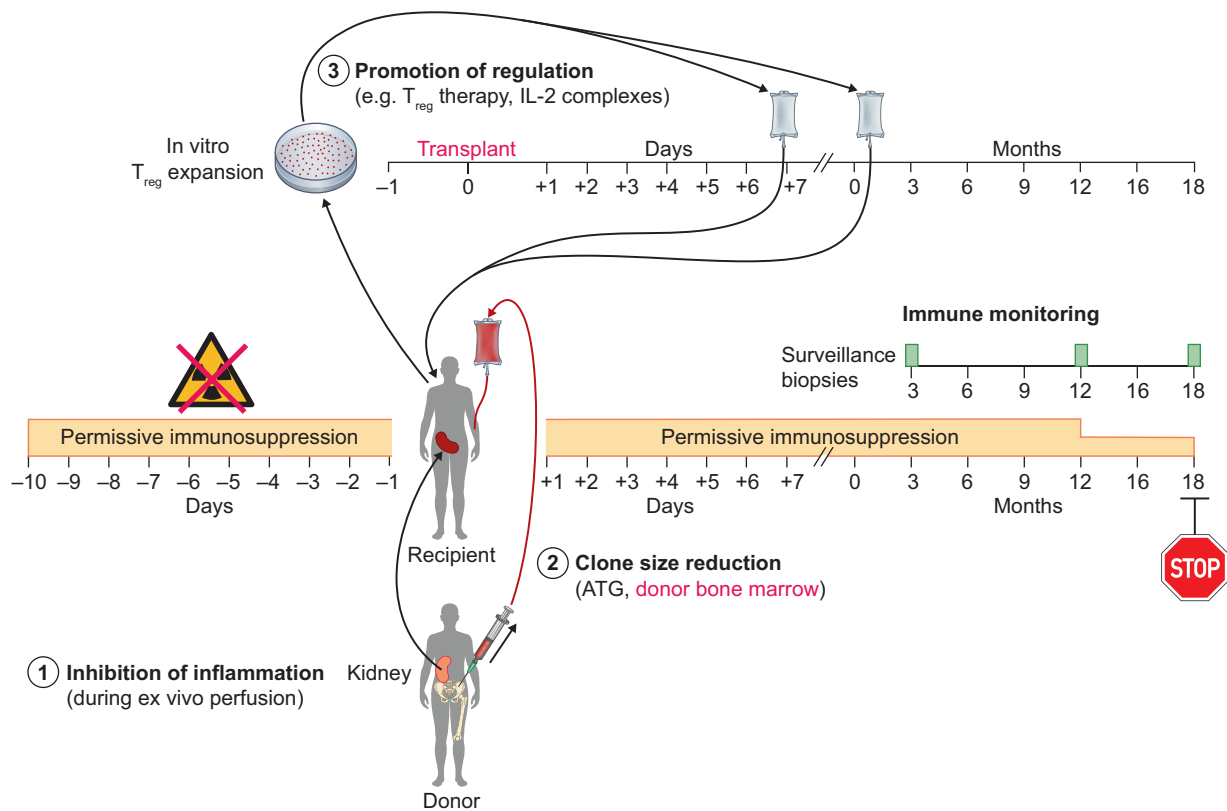
FIGURE 2: The concept of long-term allograft function trough tolerance induction by mixed chimerism. (Reprinted with permission from Ref. [4].)

between elevated peri-transplant risk but long-term patency with low or even without maintenance immunosuppression is an excellent alternative to standard kidney transplantation, but requires individual decision-making.

In order to estimate the risk of a clinically relevant alloimmune response after transplantation, tracking of alloreactive T cells determined by mixed lymphocyte reaction has been suggested previously [15]. Although the T-cell receptor repertoire is exceedingly complex with high inter-individual diversity, it is nowadays possible to determine the clonality and diversity by DNA sequencing usually of the complementary determining region 3 of the T-cell receptor beta chain [16]. Even more complex because of somatic hypermutation is the individual tracking of the B-cell alloimmune repertoire and network [17]. Recently, investigators showed that initially DSA-negative ABMRs may in fact be triggered by memory B cells, which after stimulation/transplantation exhibit clonal expansion and transformation to DSA-producing plasma cells [18]. Persistence of DSAs after treatment of ABMR is likely caused by memory B cells and thus pre-transplant risk stratification based on the existence of alloimmune memory B cell may be feasible [19].

Once the transplant has been performed, sequentially immune surveillance is performed in most transplant centers including DSA monitoring and management biopsies to guide individual immunosuppression. The histopathological examination and scoring according to international grading schemes will be supplemented in the near future by genome-wide molecular analysis of tissue transcripts. A clear molecular picture is mandatory to guide important treatment decisions. For example, almost half of the biopsies classified as 'clean' by pathologists show in fact molecular features of rejection (T-cell-mediated alloimmune response) and vice versa 50% of specimens classified as ABMR in histology were 'clean' molecularly [20]. In addition, the genome-wide analysis of transplant biopsy specimen studies on the utility of molecular profiling of blood or urine has been performed. However, none of the tests has reached sufficient characteristics yet to justify the routine use of these liquid biopsies.

Treatment of acute T-cell-mediated rejection is usually successfully performed by high doses steroids or anti-thymocyte immunoglobulins. Acute ABMR early after transplantation is also manageable but the treatment enigma persists for chronic ABMR. So far, no validated intervention exists and therefore management is very heterogeneous among the different transplant centers. Promising preliminary data suggest that anti-interleukin-6 antibody treatment might be a good option for certain individuals, but the final proof will need larger studies [21]. Based on these data, we conclude that precision diagnostics is already standard after kidney transplantation, but precision therapy is currently limited to risk-based pharmacodynamics of standard immunosuppressants.

PRECISION MEDICINE IN HEMODIALYSIS

Current shortcomings and barriers

While transplantation has advanced toward precision, hemodialysis has lagged far behind. This section will focus on our

failure to adjust the hemodialytic removal of uremic solutes to the condition of individual patients, but we also often fail to individualize hemodialysis treatment for control of calcium, potassium, acid–base and body fluid levels [24–26]. Uniform prescription for all patients began early in the history of hemodialysis. As described by Scribner [27], pioneers were initially able to start only a few patients on hemodialysis and a common prescription was identified, which kept them alive, if not well

> It soon became obvious that dialysis 8 to 10 hours three times weekly seemed to control all the major life-threatening complications. As a result, this became the usual dialysis schedule and we stopped our crude efforts to adjust the treatment schedule based on patient symptoms.

Treatments are now shorter because modern dialyzers remove solutes faster, but three times weekly treatment for a standard time without regard to patients' symptoms remains common to this day.

The 1970s saw an attempt at individualization of the hemodialysis prescription based on protein intake. Dietary protein restriction had been shown to ameliorate uremic symptoms before hemodialysis became available. Urea production provided a marker of net protein catabolism and a logically motivated effort was therefore made to determine whether hemodialysis should be adjusted to control the blood urea level. This effort, which culminated in the United Sates National Cooperative Hemodialysis Study (NCDS), ended strangely as analysis showed that the fraction of urea removed during each of 3 weekly treatments, as reflected by Kt/V_{urea}, predicted outcomes better than the blood urea level [28]. The NCDS included less than 200 patients studied over 1 year and urea was the only solute measured. The much larger Hemodialysis Study Group (HEMO) study performed 15 years later found that varying Kt/V_{urea} by approximately 30% had no distinguishable effect on patient outcomes [29]. Yet throughout the world today hemodialysis 'adequacy' is still commonly assessed by calculation of Kt/V_{urea} or a related urea kinetic parameter such as the equivalent renal urea clearance (EKR) or standard Kt/V_{urea}.

The weakness of prescribing hemodialysis to meet uniform urea-based guidelines is now widely recognized [30, 31]. So far, however, there has been little effort to develop chemical measures that better predict the effect of hemodialysis on either patients' symptoms or their long-term outcomes. The tendency has been rather to prescribe a little more treatment than is necessary to meet urea kinetic targets, hoping that this will do the patients good. This is also apparent in guidelines for treatment duration. The European Best Practice Guideline published in 2007 recommended that hemodialysis be prescribed at least three times per week for a total of at least 12 h [32]. The 2015 US KDOQI Guideline Update, citing associations of longer treatment times with better outcomes, recommended a 'bare minimum' treatment time of 3 h for patients with a residual urea clearance less than 2 mL/min on thrice weekly hemodialysis [33]. It is notable, however, that the recommendation for a 3-h minimum was rated '1 D', signifying a strong recommendation based on very low-quality evidence as carefully reviewed by Daugirdas et al. [34, 35]. The weakness of prescribing hemodialysis without regard to the differences among patients is even more clearly revealed in the treatment of patients with

residual kidney function, the presence of which should lessen the requirement for hemodialysis. To the extent that waste solutes are removed by the kidney, less hemodialysis is required to limit their accumulation in the body. A well-reasoned case has been made for reducing the intensity of hemodialysis in patients with residual function and particularly in those initiating hemodialysis [36–40], yet many of these patients are prescribed treatment thrice weekly for a standard minimum time, even though there is no evidence that this does them any good.

Toward more precise care in hemodialysis

A first step toward more precise care might be to stop enforcing guidelines that are not based on solid evidence. Such an approach has recently been advocated by the International Society of Peritoneal Dialysis [41]. Routine assessment of toxin removal is still recommended using urea and/or creatinine as surrogates but a peritoneal dialysis patient who is feeling well is not, however, obliged to increase the volume or frequency of exchanges to meet a numeric target. An analogous approach to hemodialysis would require continued routine measurement of Kt/V or EKR. Low values would suggest that symptoms such as fatigue and poor appetite were due to inadequate toxin removal and also alert providers to poor access function. In many patients, treatment time and frequency would still be determined by the need to remove fluid and inorganic ions and others might find by experimentation that they feel better with longer and/or more frequent treatment. However, patients who feel well and have adequate volume and inorganic ion control would not be obliged to spend more time on hemodialysis to achieve a urea kinetic target. It may be argued that solute removal beyond the level necessary to improve symptoms provides long-term benefit but trials conducted to date have, however, largely failed to show this. When the burden of more intense treatment is additional time on hemodialysis, the benefit should be better established before the treatment is imposed.

The amount of hemodialysis required to relieve uremic symptoms should thus now be left up to the patient. Treatment time would be limited only by cost. To go further we must measure uremic solutes other than urea. No single chemical compound can represent the behavior of the myriad solutes that accumulate in the plasma when the kidneys fail. However, urea turns out to have been a particularly unfortunate choice as an index solute for hemodialysis adequacy. It has the highest dialytic clearance of any known solute but in contrast the native kidney clears many solutes more rapidly than urea, which is in part reabsorbed in the proximal tubule. Low molecular weight proteins like β_2-microglobulin are cleared at rates close to the GFR and tubular secretion raises the clearances of many other solutes to levels much higher than the GFR [42–44]. As a result, levels of other solutes remain much higher relative to normal than urea levels in patients maintained on standard hemodialysis, as depicted in Figure 3. Measurement of urea has thus provided a misleading sense of how effectively conventional hemodialysis replaces normal renal function. Significant attention has indeed been paid to the removal of larger solutes by ultrafiltration [45]. Overall, however, the assessment of hemodialysis adequacy by urea removal has inhibited study of potential toxins that are less
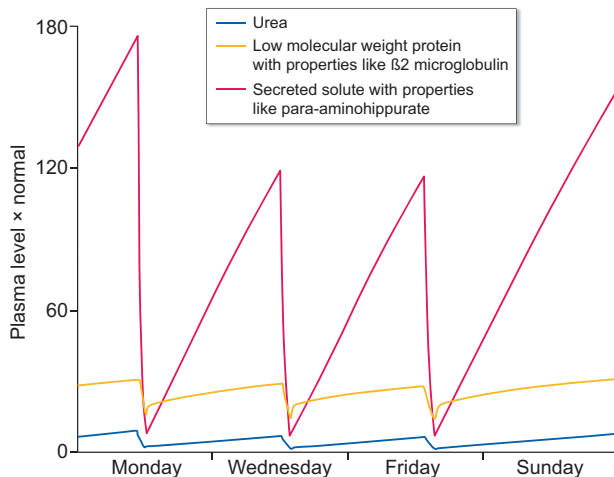
FIGURE 3: Plasma solute levels in a patient on conventional thrice weekly hemodialysis. Conventional hemodialysis largely fails to replicate control of solute levels achieved by the native kidney. Solute concentrations are presented as a multiple of normal throughout the weekly hemodialysis cycle for urea (blue line), a low molecular weight protein with properties like β_2-microglobulin (green line) and a secreted solute with properties like para-aminohippurate (red line). Model for a patient receiving thrice weekly treatment for 3.5 h with clearances for urea and β_2 microglobulin as observed in the HEMO study and a clearance for an unbound secreted solute based on that for phenylacetylglutamine proportional to urea as observed by Sirich *et al.* [22] with compartmental volumes and non-renal clearance for β_2-microglobulin as described by Ward *et al.* [23]. Levels of low molecular weight proteins, which did not have the 3 mL/min continuous non-renal clearance described for β_2-microglobulin, would rise much higher relative to normal in hemodialysis patients.

effectively cleared by hemodialysis, and discouraged development of new means to remove them.

Simultaneous determination of large numbers of solutes will be required to identify toxins responsible for various features of uremic illness. Metabolomic methods employing untargeted mass spectrometry have provided us with the capacity to make such measurements. Quantification of clinical endpoints associated with solute levels presents a more difficult problem. Several strategies can be envisioned. The first, which is now being pursued, is to analyze samples from large numbers of patients and rely on statistical methods to distinguish illness due to uremic solutes from the comorbid processes prevalent in the hemodialysis population. Solutes associated with symptoms might alternatively be identified by analysis of a smaller number of more carefully studied mostly younger patients, in whom disease was initially confined to the kidney. Uremic toxins derived from foods might be identified by manipulating the diet and genetic studies might reveal differences in gastrointestinal solute transport or hepatic metabolism, which keep solute levels low in those patients, who are relatively symptom free.

The studies proposed above would not take us directly to precision hemodialysis prescription. They would indeed reveal only associations between solute levels and clinical endpoints. It would then be necessary to test whether reducing the levels of specific solutes improved patients' health. Large studies would be required to distinguish the value of reducing solute levels in patients with different comorbidities, genetic backgrounds and life expectancies. Methods for reducing solute levels that extend beyond conventional hemodialysis will probably be required. Identification and measurement of important uremic toxins will, however, be a necessary first step in moving beyond the current practice of standardized hemodialysis prescription. It is of note, however, that so far no single 'uremic toxin' has been identified to be actually toxic.

CONCLUSIONS

In summary, we have highlighted the progress in therapy options toward the aim of truly individualized concepts of patients with end-stage renal failure. Kidney transplantation is undoubtably the best from of renal replacement therapy and great progress has been achieved in molecular risk prediction and subsequent individual pharmacodynamics of immunosuppression. Given the wide alloimmune response against HLA and non-HLA epitopes, we firmly believe that only tolerance induction, for example, trough mixed chimerism, has the potential to enhance graft longevity dramatically. Hemodialysis, on the other hand, has not seen many sophisticated interventions recently and very interesting research questions on the toxicity of uremic solutes derived from the gastrointestinal tract remain to be conducted.

CONFLICT OF INTEREST STATEMENT

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