Pharmaceutical care in transplantation: current challenges and future opportunities

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“Creation of new therapies or reformulation of existing therapy via nanotherapy has encouraging potential to increasing opportunity for localized drug delivery, expand drug solubility and minimize toxicities”

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The United Network for Organ Sharing, the nonprofit organization that contracts with the government to manage the country's organ transplantation system, reports that over 750,000 transplants have been performed in the USA since 1988 [1]. Over 113,000 individuals are currently listed as candidates on waiting lists for organs [1]. In 2016, just over 23,000 referrals to organ procurement organizations met criteria for eligible or imminent death per the Organ Procurement and Transplantation Network definition, leading to almost 10,000 deceased donors for transplant [2].

Although a variety of clinical factors among the donors and recipients affect the success of individual solid organ transplants, outcomes and developments across the field continue to broadly improve over time. Kidney, liver and heart transplants collectively account for approximately 90% of procedures [1]. For kidney transplantation, all-cause graft failure at 6 months for deceased donor recipients declined 36% from 2005 to 2015 [3]. For living donor kidneys, nearly 82% of those transplanted in 2006 were still functioning 10 years post-transplant (censored for death) [3]. Over the past decade, graft survival has steadily improved for liver recipients, with 1-year failure rates in 2015 estimated at 10.5 and 5.1% for deceased and living donor transplants, respectively [4]. 1- and 5-year survival for heart transplants in 2009–2011 was 90.1 and 78.3%, with overall rates of death post-transplant decreased since 2005 [5]. Most recently, the field has seen expansion and preliminary successes in vascularized composite allotransplantation (VCA), or the transplantation of multiple tissues as a functional unit [6,7].

Despite considerable progress since the advent of transplantation in the 1950s, there does remain room for improvement. For instance, long-term patient survival for lung transplantation remains low and unchanged over time, estimated at 55.6% at 5 years [8]. Wide geographic variability is seen among liver transplantation, with significant differences in the average Model for End-Stage Liver Disease score at the time of transplant that has caused a fair degree of controversy across the literature [4,9]. Disparities are evident within kidney transplantation, where living donor transplants occur in 65.1% of white recipients, but only 12.3% of black recipients, despite almost equal racial distribution on the waiting list [3]. Furthermore, more than 10% of individuals on the waiting list for kidney transplants are for repeat procedures [10], signaling opportunities for improvement in the prevention of acute and chronic rejection.

Advances are particularly needed in the immunosuppressive regimens that are essential in the peri- and post-transplant periods across all organs [11]. As lifelong therapies, these medicines are essential in the prevention of rejection and long-term graft success, yet considerably increase the risk for infection and other toxicities. This article will review the major pharmacotherapy strategies used in solid organ transplant, along with some of the difficulties in using these therapies, and a look forward toward new opportunities and development.

The landscape of immunosuppression

Complex pharmacotherapy regimens for immunosuppression remain necessary for graft survival in the solid organ transplant recipient. While treatment may simplify if time, graft function and rejection potential permit, many patients remain on lifelong dual, triple or quadruple immunosuppression. In one cross-sectional analysis using the Medication Regimen Complexity Index, scores for kidney (17.9 ± 8.1) and liver (18.1 ± 8.8) transplant recipients were significantly higher for other chronic diseases, which broadly ranged from 3 to 7 on the Medication Regimen Complexity Index [12]. These therapies are additionally accompanied by narrow therapeutic indices, significant interpatient variability and high potential for drug interactions through both pharmacokinetic and pharmacodynamic processes. The major metabolic/transport pathways for immunosuppressant agents (e.g., CYP450 isoenzyme 3A4 and 3A5, UDP-GT and P-gp) play important roles for a host of other medications, heightening risk through induction, inhibition and substrate interactions. Most recipients also have pre-existing co-morbid conditions beyond their transplant needs (each often requiring their own pharmacotherapy), and further complications can arise with iatrogenic co-morbidities emerging from the use of immunosuppressive therapy itself, such as hypertension and diabetes mellitus. Each immunosuppressive class or agent targets unique molecular receptors to suppress lymphocyte activation and proliferation. In addition to temporary or lifelong corticosteroids, major options for maintenance immunosuppression include calcineurin inhibitors (CNI), antimetabolites and mammalian (mechanistic) target of rapamycin (mTOR) inhibitors.

Calcineurin inhibitors

After the discovery and clinical use of key CNI compounds cyclosporine and tacrolimus, graft survival in solid organ transplant increased significantly [13,14]. Despite their narrow therapeutic index, they remain a cornerstone of solid organ transplant pharmacotherapy through inhibition of T-lymphocyte activation and impairment of lymphocyte function and replication [15]. Frequent (i.e., weekly or even twice weekly) laboratory monitoring is often required to adjust total daily dosing to achieve goal trough concentrations, since elevated trough concentrations are associated with significant toxicities, while area under the curve concentrations below target are associated with acute and chronic cellular rejection [16].

Despite their efficacy successes, the adverse effects associated with CNIs can be substantial, including abnormal hair growth (e.g., alopecia with tacrolimus and hirsutism with cyclosporine), electrolyte and metabolic abnormalities (e.g., glucose intolerance, hyperlipidemia, hyperkalemia), gastrointestinal disturbances (e.g., anorexia, nausea, vomiting), hypertension, neurotoxicities (e.g., tremor, headache, visual abnormalities) and nephrotoxicity [17]. Due to their major absorption and metabolism through P-gp and CYP450 isoenzymes 3A4 and 3A5, significant dosing variability is required when considering individual patient's enzyme phenotype as well as numerous drug–drug interactions that affect the function of P-gp and CYP enzyme system. In fact, it has been demonstrated that CYP3A5 polymorphisms associated with normal or intermediate function are associated with higher total daily doses, which may increase risk of chronic CNI toxicities [18–20].

Pharmacogenomic tools that help predict metabolism and serum concentrations are often used in addition to clinical expertise to initiate and maintain CNI dosing. In fact, some centers require CYP3A5 genotype testing be completed during work-up for transplant listing, particularly in at-risk populations (e.g., African-Americans). If a normal or intermediate function CYP3A5 polymorphism is identified, clinicians may decide to employ one of more of the following strategies: more aggressive CNI dosing; purposeful addition of CYP3A4 and 3A5 isoenzyme inhibitors to decrease CNI metabolism; or utilization of more aggressive induction strategies in the absence of contraindications. Despite the known limitations with CNI dosing, it is unclear that pharmacogenomic testing actually improves outcomes. In a 2010 study, 280 patients receiving kidney transplants were prospectively assigned to receive tacrolimus using a standard regimen, or one adapted based on patient-specific CYP3A5 genotype [21]. Patients in the latter group receiving pharmacogenetic-informed dosing were more likely to achieve target drug concentrations 3 days post-initiation (43.2 vs 29.1%; $p = 0.03$), were able to reach their targets more rapidly and were less likely to require dose changes, compared with standard concentration-informed dosing [21]. However, no changes in clinical end points (e.g., graft survival, acute rejection, delayed graft function, drug toxicity) were seen in this study [21], nor in a longer-term follow-up of the patients [22]. A second randomized controlled trial published in 2016 evaluating a similar comparison among 240 kidney transplant patients failed to identify a difference in target concentration achievement on day 3 (37.4 vs 35.6%; $p = 0.079$ for standard and adapted dosing, respectively) or across any clinical outcomes [23]. Accordingly, it is likely that the pharmacokinetics of

tacrolimus are influenced importantly by polymorphisms beyond CYP3A5, and that transplant outcomes are similarly varied in their influences.

From a formulation perspective, more recently, the US FDA approved a novel extended-release formulation of tacrolimus (Envarsus XR®), which includes a proprietary technology (MeltDose) that frames tacrolimus particles into a 'solid solution', permitting a slower, more consistent absorption throughout the entire small bowel [24,25]. Since earlier achievement of therapeutic levels of a CNI provides greater protection against acute rejection [26], the technology is particularly hopeful. However, similar rates of toxicities still exist compared with conventional tacrolimus and access for all solid organ transplant patients is limited, since the product is only FDA-approved in kidney recipients [27,28].

Antimetabolites

Antimetabolites are also frequently used in solid organ transplant, including the two available agents azathioprine and mycophenolate mofetil (MMF), which both lead to impairment in T- and B-lymphocytes. Azathioprine is metabolized to 6-mercaptopurine and eventually to 6-ionsine monophosphate, which inhibits purine synthesis [29]. MMF is converted to mycophenolic acid, a noncompetitive inhibitor of IMPDH [29].

MMF is often preferred over azathioprine in kidney transplantation due to a preferable adverse effect profile and reduced risk of graft rejection [30], as well as improved graft survival in lung and heart recipients [31]. However, some considerations in its use are necessary. MMF is metabolized by CYP3A4/5 and UDP-GT pathways, increasing the potential for drug–drug interactions. It is contraindicated among women of child-bearing age due to teratogenicity [32], and associated with dose-related hematological effects, particularly leukopenia and gastrointestinal effects (e.g., diarrhea, nausea and vomiting). Recent animal data suggest that MMF-associated diarrhea may be spared in recipients who do not have an intact gut microbiome [33]. Whether strategies to alter a patient's microbiome can improve tolerance to MMF are yet to be evaluated. Therefore, patients with intolerable gastrointestinal side effects are often converted to azathioprine with some success. Hematological toxicities are also associated with azathioprine, and are more likely to occur in patients with deficiency in TPMT and/or NUDT15 activity. Therefore, clinicians often perform genotyping in patients to determine risk and/or cause of adverse effects. Finally, because azathioprine is converted to its metabolite via xanthine oxidase, inhibitors such as allopurinol can result in significant toxicities.

mTOR inhibitors

mTOR inhibitors bind to the FK-binding protein to inhibit the activity of mTOR and block the response of T- and B-lymphocytes to cytokines [34]. Their original development was aimed as a replacement or minimization strategy for CNIs (due to associated nephrotoxicity), but this was never fully realized due to less than optimal performance in comparative clinical trials [35,36]. Nevertheless, mTOR inhibitors are frequently used for renal sparing effects in organ transplant recipients. In addition, mTOR inhibitors are associated with low incidence and possible slowing of chronic rejection in thoracic transplant recipients [37]. Today, although not used in *de novo* solid organ transplant, mTOR inhibitors sirolimus (rapamycin) and everolimus may be utilized in later stages of maintenance immunosuppression in place of a component, or in augmentation with a patient's initial regimen [38].

Like CNIs, mTOR inhibitors possess a narrow therapeutic index, and must have trough concentrations monitored during initiation, after dose adjustment or after drug–drug interaction changes. In addition, due to their complex mechanism, there is an urgent need for efficient biomarkers to predict efficacy of mTOR-based therapy and avoid toxicity in patients who will indeed not benefit. Adverse effects with the class are not insignificant, and include hematological effects, metabolic and gastrointestinal abnormalities like the CNIs, impaired wound healing and interstitial pneumonitis, among others [39].

Sirolimus interestingly has been associated with increased mortality (hazard ratio: 1.43; 95% CI: 1.21–1.71) after kidney transplant in a meta-analysis of 21 randomized controlled trials, primarily associated with cardiovascular and infective complications [40]. This effect was seen in both *de novo* and conversion uses of the drug [40]. Similar increased risk of fatal adverse events (relative risk: 3.24; 95% CI: 1.21–8.67) has been noted in a different meta-analysis of 12 trials among patients with malignancy taking everolimus or temsirolimus (an mTOR inhibitor not utilized for transplant) [41], suggesting that this may be a class effect. The exact cause and significance of this effect is unknown, particularly as it has not been associated with allograft loss [42], but it adds further difficulty to the utility of mTOR inhibitors in solid organ transplantation.

Economic & humanistic impacts

Barriers in the provision of effective immunosuppression are not confined to the clinical aspects of these therapies. Financial concerns remain paramount for patients undergoing transplantation. Estimated billed charges per transplant in 2017 ranged from \$347,000 for pancreas, \$414,800 for kidney and as high as \$1.38 million for heart transplantation [43]. Although hospital admissions for the procedures themselves compose the bulk of the cost, outpatient medications for immunosuppressants and other necessary therapies are also significant, generally reaching over \$20,000 per patient in the first 180 days after discharge [43]. The Medicare program covers transplant procedures under Part A and B for patients with end-stage renal disease receiving dialysis, but outpatient coverage for immunosuppressive therapies post-transplant generally ends 36-month post-transplant if the beneficiary is not Medicare-eligible as a function of age or disability. Instances like this cause considerable difficulty in maintaining graft success long-term, particularly for recipients without significant financial resources. In a 2010 survey of all kidney transplant programs across the USA, more than 70% of programs reported problems with patients affording their medications, and 68% could directly attribute patient deaths and graft loss as a result of medication nonadherence associated with affordability [44].

The humanistic impacts of currently available immunosuppressive therapies are also relevant, particularly for patients who experience adverse effects. A prospective multicenter study before and up to 2 years post-liver transplantation suggested that persistent anxiety and depression were common (23 and 29%, respectively) [45]. Furthermore, these symptoms were associated with adverse effects stemming from immunosuppressive therapy, as well as decreases in adherence and health-related quality of life (QoL) [45]. Among adults more than 12 months post-kidney transplant, nonadherence with immunosuppressive therapy (seen in 33.6% of patients) was associated with lower mental health-related QoL and lower perceived social support [46]. And yet, despite knowing the importance of measures such as QoL, a recent systematic review of randomized controlled trials of maintenance immunosuppression after kidney transplantation found infrequent inclusion of patient-reported measures of well being [47].

Encouraging developments in immunosuppression

One area that may provide promising potential to overcome the barriers of traditional immunosuppression is nanotherapy. Creation of new therapies or reformulation of existing therapy via nanotherapy has encouraging potential to increasing opportunity for localized drug delivery, expand drug solubility and minimize toxicities [48,49]. There is also opportunity in nanoparticle-based delivery to be useful in a larger transplant arena, utilized as pretreatment of organs prior to transplantation for endothelial cell protection, incorporated in strategies to expand donor organs through use of allografts previously thought to be unsuitable, or in efforts to protect from ischemia-reperfusion injury [50]. Preliminary scientific work in the advancement of immunosuppression post-transplant has shown encouraging results. One example is the development and initial results of a rapamycin nanoimmunotherapy in a mouse model for heart transplantation, which demonstrated high encapsulation efficacy and cellular specificity [51,52]. Use of this strategy (in concert with a second nanotherapy augmentor) resulted in allograft survival rates greater than 70% at 100 days post-transplant, compared with 100% rejection before day 10 among mice receiving placebo [52].

Other nanoparticle delivery strategies are also being explored to optimize existing therapies. Nanoparticle delivery formulations of CNI, antimetabolite or mTOR inhibitors are in their infancy, with limited evaluations in animal models as a means to overcome the current variability and toxicity with standard systemic therapy. In one example, researchers trialed a hydrogel formulation of tacrolimus with controlled release triggered alongside inflammatory proteolytic enzymes; in a rat hind-leg VCA model, median graft survival for the hydrogel-treated rat extended past 100 days, in comparison to 11 days for control groups [53]. Another study utilized a topical formulation of mycophenolic acid (overcoming the barriers of skin esterases on MMF) to assess permeation and pharmacokinetic characterization in rats and identified higher tissue and lower systemic drug levels that may be useful for future application in VCA [54]. In another animal model, inhaled nanoparticle tacrolimus demonstrated similar efficacy to systemic tacrolimus with significantly lower serum concentrations, a strategy that could minimize current toxicity rates if studied in humans [55]. Finally, a number of reports have demonstrated improvement in lung function with the off-label use of nebulized CNI in patients with chronic lung allograft dysfunction [56–60]. In fact, the most recent randomized placebo-controlled trial demonstrated a survival advantage with the addition of the nebulized cyclosporine [58]. The benefits of such therapy in chronic lung allograft dysfunction may be related to drug dispersion in areas of the lung which remain well ventilated. Despite these preliminary findings, an FDA-approved formulation

of aerosolized cyclosporine or tacrolimus is not available. Further research is also needed to confirm whether there is a role for nebulized CNI beyond salvage therapy in lung recipients.

Future directions for transplantation

Despite the advent of modern immunosuppression, the morbidity associated with nonspecific lymphocyte inhibition is significant. Chronic diseases and infections, even with prophylaxis augmentation, are prevalent in solid organ transplant recipients. Current and future strategies to reduce exposure to immunosuppression include proactively identifying patients who may be more likely to experience rejection or who are acutely experiencing rejection.

Allomap[®] and Allosure[®] are noninvasive blood tests available for kidney and heart transplant recipients that can either detect genetic components of rejection or donor-derived cell-free DNA. With Allomap, heart transplant recipients can be risk-stratified for rejection using gene expression profiling [61–65]. With Allosure, kidney transplant recipients can be tested for elevations in fragments of donor-derived cell-free DNA, a biomarker signifying tissue injury [66–68] and acute rejection. There is growing interest in using these same assays in other solid organ transplant recipients, particularly lung transplant [69], which carries a high rate of acute and chronic rejection.

On the opposing spectrum, there is focus on detecting patients at the highest risk of opportunistic infections. Solid organ transplant recipients are at risk of cytomegalovirus (CMV) infection activation or reactivation while on maintenance immunosuppression. There is growing evidence that assays specific for CMV T-cells can be performed and may be useful in part of a risk-stratification algorithm in pre-transplant candidates, or even predicting patients experiencing early CMV disease [70–72]. Furthermore, therapeutic adoptive T-cell therapy has been used as salvage, therapy for severe CMV disease [73] and is being studied for more routine clinical use in the era T-cell infusions for leukemias.

Ultimately, many are excited at the prospect of transplant methods that would significantly reduce or potentially avoid the need for immunosuppression altogether. Simultaneous stem cell and solid organ transplant is sometimes indicated, and often results in a reduced need for immunosuppression after transplant. In addition, donor-specific T-regulatory cell therapy is being evaluated for kidney transplant recipients [74] and could likely be used in other solid organ transplants. Regeneration of organs through stem cells is another area of fast-moving research [75], and may someday be possible.

Conclusion

Solid organ transplantation has seen significant successes over the past several decades through the use of highly effective immunosuppression. Despite availability of a variety of therapies, adverse effects from complex drug regimens and graft failure continue to be prevalent, alongside significant financial and humanistic impacts for patients receiving these therapies. The need for individualized therapy to optimize outcomes is clear, and encouraging scientific developments in the pipeline are focused on the optimization of existing formulations through strategies such as nanoimmunotherapy. With continued scientific advancement in the field of transplantation, there will be significant opportunity to improve the length and QoL for patients worldwide.

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