Targets of new immunosuppressants in renal transplantation

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Although current immunosuppression is highly effective in avoiding acute rejection, it is associated with nephrotoxicity, cardiovascular morbidity, infection, and cancer. Thus, new drugs dealing with new mechanisms, as well as minimizing comorbidities, are warranted in renal transplantation. Few novel drugs are currently under investigation in Phase I, II, or III clinical trials. Belatacept is a humanized antibody that inhibits T-cell co-stimulation and has shown encouraging results in Phase II and III trials. Moreover, two new small molecules are under clinical development: AEB071 or sotrastaurin (a protein kinase C inhibitor) and CP-690550 or tasocitinib (a Janus kinase inhibitor). Refinement in selecting the best combinations for the new and current immunosuppressive agents is probably the main challenge for the next few years.

Kidney transplantation is the treatment of choice for end-stage renal disease owing to the use of potent immunosuppressive therapy. Current immunosuppression is highly effective in avoiding acute rejection, but it is associated with nephrotoxicity, cardiovascular morbidity, infection, and cancer. Therefore, the improvement in short-term graft survival has not been reflected in improved long-term outcomes.¹ Current immunosuppression strategies are primarily based on the use of an induction regimen using a monoclonal or polyclonal antibody, followed by maintenance immunosuppression based on a calcineurin inhibitor (CNI), an anti-proliferative agent, and low-dose corticosteroids.² Several CNI minimization and withdrawal protocols have been attempted with varied results.³ The use of mammalian target of rapamycin inhibitors for CNI minimization or withdrawal has been hampered by their adverse side-effect profile.⁴,⁵ Therefore, it is accepted that CNIs currently remain the cornerstone of maintenance immunosuppression in renal transplantation.⁶ Undoubtedly, new drugs dealing with new mechanisms, as well as minimizing comorbidities, are warranted in renal transplantation. The present trend in drug development is focused on preservation of long-term function and minimization of the adverse events of immunosuppressive drugs.⁷ Several small molecules and biological agents are currently being studied. Belatacept is a humanized antibody that inhibits T-cell co-stimulation and has shown encouraging results in Phase II and III trials. Moreover, two new small molecules are under clinical development: AEB071 or sotrastaurin (a protein kinase C (PKC) inhibitor) and CP-690550 (a Janus kinase (JAK) inhibitor). All three drugs are excellent examples to show how difficult it is to develop new immunosuppressants to overcome the CNI toxicities. Refinement in selecting the best combinations for the new and some current immunosuppressive agents is probably the main challenge for next few years.

BELATACEPT

In the near future, belatacept would be the first biological agent for use in long-term maintenance regimen in organ transplantation. Its parent molecule was CTLA4-Ig (abatacept), and resulted from the fusion of the extracellular domain of CTLA4 with the constant region fragment of
Clinical development of belatacept

The first clinical trial on the use of belatacept in clinical renal transplantation was a Phase II non-inferiority trial comparing the efficacy of belatacept vs cyclosporine (CsA) for prevention of acute rejection at 6 months post-transplant. Belatacept was administered as less (LI) or more intensive (MI) schedule. All patients also received MMF and corticosteroids as maintenance immunosuppression, and induction with basiliximab. At 6 months, the incidence of acute rejection was similar in all three groups ranging from 6 to 8%. The LI group experienced a higher incidence of subclinical rejection and treated episodes of subclinical rejection compared with the MI and CsA groups. Glomerular filtration rate was significantly higher in the belatacept groups compared with the CsA arm. Protocol biopsies demonstrated a significant reduction in the incidence of chronic allograft nephropathy in the belatacept group. Cardiovascular profile improved in the belatacept groups as they had a statistically significant lower risk of developing diabetes, need for treatment of hyperlipidemia, and a lower incidence of hypertension.

Two Phase III studies have been recently published. The BENEFIT (Belatacept Evaluation of Nephroprotection and Efficacy as First-line Immunosuppression Trial) study is a 3-year Phase III clinical trial, which randomized patients to three groups as previously described. Patient and graft survival are similar in three groups. However, the incidence of acute rejection was greater in MI (22%) and LI (17%) belatacept compared with CsA (7%), although no apparent impact on graft survival was demonstrated. Most acute rejection episodes occurred within the first 3 months and severity was higher in belatacept compared with CsA-treated patients. At the end of 2 years, glomerular filtration rate continued to be significantly higher in the belatacept-treated patients. Belatacept-treated patients also had sustained benefits in their cardiovascular and metabolic risk profile. The BENEFIT-EXT (Belatacept Evaluation of Nephroprotection and Efficacy as First-line Immunosuppression Trial—EXTended criteria donors) study is a 3-year randomized Phase III study in patients receiving an extended-criteria-donor kidney allograft. Patient and graft survival were similar in all the three groups. Renal function was statistically superior in MI belatacept vs CsA but not in LI vs CsA group. The incidence of chronic allograft nephropathy was similar in the three groups, probably because of the presence of renal damage at baseline. Interestingly, cardiovascular risk factors were lower in the belatacept-treated patients. The 2-year results showed similar trend.

Vincenti et al. recently published the 5-year safety data of their initial Phase II study. Belatacept-treated patients did not have a higher frequency of serious infections or post-transplant lymphoproliferative disorder (PTLD) compared with CsA. Remarkably, major cardiac adverse events occurred more frequently with CsA (2% belatacept vs 12% CsA).

Of the three major trials described above, a total of 13 patients in the belatacept groups have been diagnosed with PTLD (1.4%) compared with only one CsA patient (0.2%). Of the 13 cases of PTLD identified, six developed PTLD of the central nervous system. This is a diagnosis to be concerned about considering that this involvement is seldom encountered in solid organ transplantation. The majority of patients who developed PTLD had known risk factors for PTLD, including pre-transplant EBV-seronegative recipients, those receiving lymphocyte-cell-depleting agents, and those having a primary EBV infection.

In these lines, a recent Phase II analysis that excluded transplant recipients who were EBV seronegative before transplant has yet to report any cases of PTLD. The absence of PTLD in this analysis may be evidence enough to avoid use of belatacept in those recipients who are EBV seronegative before transplant. A total of 89 patients were randomized to receive belatacept-MMF, belatacept-sirolimus, or tacrolimus-MMF. All patients received thymoglobulin induction. Renal function was better in the belatacept-treated groups. Acute rejection occurred in four, one, and one patient in the belatacept-MMF, belatacept-SR, and TAC-MMF groups, respectively. The authors concluded that the use of belatacept in renal transplantation may allow CNI and corticosteroid avoidance, with acceptable rates of acute rejection and improved glomerular filtration rate.
discontinuation \((n = 83)\), or continued CNI therapy \((n = 88)\). At month 12, the mean change from baseline in glomerular filtration rate was higher in the belatacept group compared with the CNI group. Six patients in the belatacept group had acute rejection episodes, all within the first 6 months, and all resolved with no allograft loss. The overall safety profile was similar in each treatment group.

In summary, some concerns appear with the use of belatacept. It was originally anticipated that co-stimulation blockade would be successful in achieving immunological allograft tolerance; however, based on higher acute rejection rates, this does not appear to be the case, probably because of its inhibitory effect on regulatory T-cell expansion by MI belatacept regime in combination with basiliximab. Another limitation of this medication is that administration requires an intravenous infusion. As there is increased PTLD risk in EBV-seronegative patients, belatacept should be prescribed for EBV-seropositive patients only. Nevertheless, clinical trials suggest that the use of belatacept can lead to better renal function along with a lower incidence of diabetes and cardiovascular risk factors. Currently, on the basis of the manufacturer’s recommendation, the Food and Drug Administration is reviewing the LI-dosing regimen of belatacept as an immunosuppressive regimen in kidney transplant recipients. This LI belatacept protocol is associated with low acute rejection rates, but maintains a renal and cardiovascular favorable profile.

**JANUS KINASE INHIBITORS (CP-690550 OR TASOCITINIB)**

Janus kinases are cytoplasmic tyrosine kinases involved in cell proliferation, growth, and survival by integrating extracellular signaling induced by cytokines.\(^ {22}\) For instance, after co-stimulation, type I cytokines (interleukin (IL)-2, IL-4, IL-7, IL-9, IL-15, IL-2) bind to cell surface receptor members of the cytokine receptor common gamma (γc) chain family and activate JAKs. Nevertheless, JAKs participate in the signaling of many cytokine receptors in several cell types. Activation of JAKs induces cytokine receptor phosphorylation, as well as recruitment of signal transducers and activators of transcription, and catalyzes phosphorylation of signal transducers and activators of transcription that facilitates its dimerization and transportation to the nucleus where they regulate gene expression. There are four JAKs identified in mammals: JAK-1, 2, 3, and tyrosine kinase-2. JAK1 is activated by gp130 cytokines, type I interferon, interferon-γ, and β cytokines; its deficiency causes neurological defects and severe combined immunodeficiency. JAK2 is activated by erythropoietin, thrombopoietin, prolactin, growth hormone, γc cytokines, interferon-γ, and IL-12; its deficiency is lethal because of defective erythropoiesis. JAK3 is mainly expressed in hematopoietic cells and just activated by γc cytokines; its deficiency causes severe combined immunodeficiency. Tyrosine kinase-2 is activated by gp130 cytokines, type I interferon, IL-12, and IL-23; its deficiency induces minor effects.\(^ {29}\) Therefore, compared with other JAKs, which are ubiquitous and activated by several types of cytokines, its specificity makes JAK3 an interesting target for immunosuppression. However, there is high structural similarity between JAK2 and JAK3 that makes it difficult to synthesize compounds that are able to inhibit JAK3 without affecting JAK2. This is crucial as the safety profile depends on JAK3 inhibition selectivity.

CP-690550 or tasocitinib is a synthetic orally available inhibitor of JAK3 that maintains reasonable selectivity for JAK3. In vitro and animal studies demonstrated its potency and capacity to prevent rejection even in cynomolgus monkeys.\(^ {24}\) Therefore, CP-690550 is currently under clinical development.

A double-blinded, placebo-controlled Phase I trial assessing safety and tolerability of CP-690550 (5, 15, 30 mg b.i.d.) in renal transplant recipients reported that most adverse events were gastrointestinal or infectious.\(^ {25}\) In addition, high CP-690550 doses were associated with reduction of hemoglobin levels, demonstrating its inhibitory effect on JAK2. Further studies confirm that, although highly specific for JAK3, CP-690550 also inhibits JAK2 to some extent.\(^ {26}\) A 6-month Phase II trial and its extension to 12 months have been published.\(^ {27}\) In this trial, 61 adult renal transplant recipients were randomized to CP-690550 15 mg or 30 mg b.i.d., vs tacrolimus in combination with an IL-2 receptor antagonist, MMF, and steroids. In the high-dose arm, an increased incidence of BK virus nephropathy and cytomegalovirus infection required a protocol amendment, based on planned MMF withdrawal and rapid steroid taper. The consequence was 21.1% incidence of acute rejection in the high-dose arm. However, the low-dose arm provided excellent results that showed a 5.3% incidence of acute rejection and 76.9 ml/min glomerular filtration rate. These results were confirmed in the 12-month extension protocol in which CP-690550 was reduced to 15 mg b.i.d. In the CP-690550 arms, there was a trend toward more frequent anemia and neutropenia. Overall, the efficacy/safety profile of CP-690550 at 15 mg b.i.d. was comparable to tacrolimus, with the exception of a higher rate of viral infection. These results were used for designing ongoing protocols exploring the effects of a lower dose of CP-690550 in renal transplantation (5 and 10 mg b.i.d.). These preliminary data suggest that CP-690550 has the potential to improve current immunosuppression armamentarium. However, there still exist some concerns. Anemia is a common adverse event that has been reported in 30% of patients enrolled in the Phase II trial; lower doses and new combination strategies should be explored and, finally, new molecules with high JAK3 selectivity warranted.

**SOTRASTAURIN (AEB071)**

Protein kinase C has an important role in the immune response. It is well known that T-cell receptor activation with co-stimulation signaling leads to PKC activation and IL-2 production.\(^ {28}–^{30}\) On the basis of cofactor requirements, there are at least 10 PKC isoforms that can be divided into three categories: classical or conventional, novel, and atypical. The
z, β, and θ isoforms appear to have clear roles in either T- or B-cell signaling, thus suggesting that inhibition of several isoforms are needed to achieve full immunosuppression. The best characterized is PKCθ, which is mostly restricted to T lymphocytes and mediates activation of the transcription factors activator protein-1 and nuclear factor κB, leading to IL-2 production. In fact, knockout of PKCθ impairs T-cell activation in mice.31

Sotrastaurin is a small molecule that inhibits PKC activity, including classical (z, β) and novel (δ, ε, η, θ) isoforms. Similar to CNIs, sotrastaurin principally inhibits PKCθ acting on IL-2 gene promoters. Nevertheless, it has insignificant effect on downstream targets of calcineurin, such as nuclear factor of activated T cells.32,33 This feature led investigators to hypothesize that sotrastaurin can be as potent as CNIs without displaying nephrotoxicity. Non-human primate and healthy human volunteer studies have endorsed those in vitro sotrastaurin characteristics. Sotrastaurin, in monotherapy or in combination with other immunosuppressants, prolongs allograft survival in rats and cynomolgus monkeys.34,35

Preclinical and early clinical safety data demonstrated no signs of nephrotoxicity or hepatotoxicity, and no metabolic or blood pressure effects at standard exposures.28,29 Gastrointestinal effects were the dose-limiting toxicities in all species tested preclinically. In vitro tests indicated a modest potential for QT prolongation. However, in healthy volunteer studies, QT effects could not be confirmed at therapeutic doses. A reversible increase in mean ventricular heart rate was observed at a single dose of 500 mg, with mean heart rates remaining within the normal range.36 Similar to CNIs and mammalian target of rapamycin inhibitors, compensatory reduction in the dose of sotrastaurin is warranted when strong CYP3A4 inhibitors are coadministered.37

In a proof-of-concept study in patients with psoriasis, clinical severity was reduced by 69% after a 2-week treatment with sotrastaurin. This effectiveness was dose dependent and achieved with good drug tolerability.38

Results in renal transplantation have not been as good as it was expected.36 In one trial, patients were initially placed on tacrolimus/sotrastaurin/steroids treatment and then underwent conversion from tacrolimus to sodium mycophenolate (MPA) at 3 months, which resulted in an increased incidence of the primary composite endpoint (acute rejection, graft loss, or death) in the sotrastaurin arm. In another trial, de novo CNI-free arm of sotrastaurin/MPA/steroids was compared with tacrolimus/MPA/steroids. Again, acute rejection rate was higher in the sotrastaurin arm. These studies were prematurely stopped and the results from the first sotrastaurin Phase II trial in renal transplantation have been recently published.36 A total of 216 patients were randomized, 74 were allocated to control-MPA + standard exposure tacrolimus, 76 to sotrastaurin 200 mg b.i.d. + standard exposure tacrolimus, and 66 to sotrastaurin 200 mg b.i.d. + reduced exposure tacrolimus. Sotrastaurin-treated recipients who met conversion criteria at month 3 were converted to a CNI-free regimen of sotrastaurin 200 mg b.i.d. + MPA 720 mg b.i.d. During the 3-month pre-conversion period, all regimens showed comparable efficacy to control for the composite endpoint (acute rejection, graft loss, or death). However, after conversion from tacrolimus to MPA, both sotrastaurin + MPA regimens were inferior to the control for the primary composite endpoint. Thus, composite efficacy failure rates were 7.8, 44.8, and 34.1% at study end in the control, sotrastaurin + standard exposure tacrolimus, and sotrastaurin + reduced exposure tacrolimus. The majority of biopsies in the sotrastaurin groups were Banff IA or IB. Therefore, the initial sotrastaurin-tacrolimus regimen was efficacious and well tolerated, but the post-conversion sotrastaurin-MPA regimen showed inadequate efficacy. With regard to adverse events, gastrointestinal toxicities were frequent, mild, and similar in all groups.36 Tachycardia occurred at a higher incidence in both sotrastaurin groups compared with control in the peritransplant period, but returned to baseline levels at 1 week. Interestingly, the incidence of new-onset diabetes in the control group (14.9%) was nearly twice that in the sotrastaurin groups (6.7 and 7.7%). The sotrastaurin-tacrolimus combination seems to be as effective as tacrolimus-TPA, at least in the short term. This may be the rationale for designing studies of sotrastaurin with CNI minimization.28 In these lines, it has been recently reported that tacrolimus does not alter the pharmacokinetics of sotrastaurin; however, sotrastaurin increases tacrolimus area under the concentration-time curve by twofold.39

Given the lack of efficacy of the sotrastaurin + MPA regimen, attention has turned to explore sotrastaurin in combination with everolimus in a new Phase II trial. This trial is currently underway in Europe. There is some pharmacokinetic interaction between both drugs. Sotrastaurin exposure does not seem to be altered by everolimus, but sotrastaurin increases everolimus exposure by 20%.40

CONCLUSION

This review clearly illustrates the difficulty faced in developing new immunosuppressants to overcome the CNI toxicities. On one hand, belatacept given in combination with basiliximab, steroids, and MMF is associated with both PTLD in EBV-negative recipients and more rejection. On the other hand, tasocitinib is highly effective in preventing rejection, but is associated with some over-immunosuppression complications. Finally, sotrastaurin shows lack of efficacy in combination with an antiproliferative agent. Altogether, these findings suggest that refinement in selecting the best combinations for the new and some current immunosuppressive agents is probably the main challenge for the next years.

DISCLOSURE

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