Optimizing lung transplant immunosuppression: Beyond calcineurin inhibition

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It is often difficult, or even impossible, for surgeons to answer some important questions in the context of a prospective, randomized clinical trial. Although the randomized trial is the gold standard for comparing two therapeutic interventions, a number of statistical, practical, and ethical considerations, combined with our own preconceived ideas, usually prevent the contemplation of a randomized trial. A common problem may be that limited patient numbers or small differences between treatments may prevent a large enough trial to provide a valid statistical power to detect legitimate treatment differences. In fact, the majority of randomized surgical trials are flawed by this very lack of a power calculation in the original trial design.¹

In other cases, a preponderance of phase II clinical trial data may make it appear unethical to assign patients to one of the arms of a randomized study. This is often difficult to disconnect from the separate problem of surgeon bias. However, these are distinct and should not be confused or intertwined. When a preponderance of the informed medical community believes that one treatment is better on the basis of clinical experience and scientific outcomes, random treatment assignment becomes unethical. However, if a given physician, or group of physicians, believes strongly in treatment efficacy, yet is balanced by an informed but skeptical group of physicians, this creates the setting of clinical equipoise, a condition of legitimate professional uncertainty about the optimal treatment. We as surgeons have been quick to accept simple case series, often from a single institution, as adequate proof of efficacy, limiting our ability or willingness to subject these questions to the more rigorous examination of a randomized clinical trial.¹

Dr Zuckermann and his colleagues² are to be commended for the discipline of the design of their study comparing cyclosporine A (CSA/MMF) to tacrolimus (TAC/MMF), both combined with mycophenolate mofetil and steroids. Their group identified a potential improved immunosuppressive drug regimen on the basis of knowledge of drug mechanism, combined with small pilot data published by other groups. By addressing this in a prospective randomized trial, they have provided us with much clearer outcomes than would have been accomplished by continued ad hoc immunosuppressive management. In this case, the trial did not display a treatment difference, but this should not be too surprising, despite a sincere effort of scientific objectivity. There are four limitations in trial design that may have prevented this trial from showing differences between the two treatment assignments.

First, a single institution study may not provide an adequate patient base to detect legitimate treatment differences. Ideally, one would estimate the degree of expected differences in the primary outcome and perform power calculations to determine the necessary cohort size. In a series like this, these calculations may have led the authors to abandon the study at the outset, rather than exert effort on a course of investigation that had no potential to reveal treatment differences. On the other hand, it may have led the authors to seek other institutions to collaborate with in order to achieve an adequate sample size. These considerations of statistical power are not revealed in the article, but it would require major differences in outcomes to be detected by 37 patients in each treatment group.

The second problem is closely related to the small sample size as well. The

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authors were very careful to stratify cytomegalovirus status and cystic fibrosis diagnosis between treatment groups. However, other potentially relevant variables, such as sex, diagnosis, or type of lung transplant procedure, were not equal between groups. Although none of these reached statistical significance, confounding variables like these could easily skew a small study and result in an inaccurate conclusion.

The third difficulty is in the analysis of treatment failure. The major benefit of a randomized trial is that it allows the most direct comparison between treatment groups—first by minimizing the impact of unsuspected variables and second by observing and analyzing the patients throughout the study, whether or not they were really able to complete their assigned treatment. This “intention to treat” analysis maintains the perspective of how the treatment will work in the real world. If we analyze only the patients whose assigned treatment was successfully completed, we will overestimate the benefit of that therapy, and in fact we may lose the most valuable data on what happens to the remaining patients. For this reason, censoring patients who drop out of a trial, or who cross over to the other arm of the trial, undermines the major strength of randomizing patients. In the case of the study by Zuckermann and associates, 4 patients from the cyclosporine group crossed over to tacrolimus because of recurrent rejection, at which time they were censored from further analysis. Indeed, examination of these patients and inclusion of them in the cyclosporine statistics, even after crossover, provide the most legitimate comparison of the true impact of each treatment assignment.

Despite these methodologic problems, probably the most important reason that this study did not display a treatment difference is due to the underlying scientific question. It is conceptually understandable why the CSA/MMF and TAC/MMF groups did not show significant outcome differences. Cyclosporine A binds to cyclophilin and tacrolimus to FK-binding protein. They have different potencies and are dosed to different adjusted trough levels. However, they have the *same basic mechanism of action*—inhibition of the calcium-calmodulin dependent phosphatase, calcineurin. Using different compounds to block the same step in the response to alloantigen would not be expected to show marked differences in outcome.

Azathioprine interferes with purine synthesis, as does mycophenylate mofetil. However, mycophenylate mofetil is considerably more potent. Mycophenylate mofetil blocks inosine monophosphate dehydrogenase, which is required for purine synthesis. Unlike other blood and parenchymal cells, T and B lymphocytes use the inosine monophosphate dehydrogenase pathway exclusively for purine synthesis. As a result, mycophenylate mofetil should produce less neutropenia at doses that are highly effective against T and B cell proliferation. The studies cited by the authors attest to

the advantages of tacrolimus and mycophenylate mofetil individually. However, it is likely that using mycophenylate mofetil in place of azathioprine would produce a benefit that could mask or at least confound any additional benefit of tacrolimus.

Each transplant center will likely continue to define its own preferences for calcineurin inhibitors and blockers of purine metabolism. However, notable advances in the fight against acute rejection and bronchiolitis obliterans will have to come from novel treatment strategies, not merely refinements of existing protocols. Sirolimus (rapamycin) is a product of a *Streptomyces* species that binds to the same intracellular protein as tacrolimus, the so-called FK-binding protein, yet it does not inhibit calcineurin activity. Its effects arise from blocking cell cycle progression and calcium-independent signaling pathways in T and B cells. It may also inhibit mesenchymal cell proliferation and thereby offer a theoretical advantage in the prophylaxis against the fibroproliferative response to transplantation (also known as obliterative bronchiolitis). Despite reports that support the use of rapamycin in rescue therapy, prospective randomized data attesting to the efficacy of rapamycin in clinical lung transplantation to date is not compelling.

Antithymocyte globulin was shown to reduce the incidence of acute rejection and potentially affect obliterative bronchiolitis in the study from Duke.³ Recent studies have suggested that OKT-3, antithymocyte globulin, and interleukin 2 receptor antagonists produce similar reductions in rejection.⁴ The safety of interleukin 2 receptor antagonists may indeed be slightly better. In general, cytolytic therapies have a basic flaw: *targeting common T-cell receptors required for rejection will also interfere with their ability to facilitate the development of tolerance.*

Minimizing inflammation associated with implantation will theoretically limit lymphocyte trafficking into the allografts. Efforts to eradicate ischemia-reperfusion injury can reduce major histocompatibility complex-II antigen expression and will likely have lasting beneficial effects on acute and chronic rejection.

Ultimately, long-term optimization of graft function and viability will depend on the development of tolerance. This appears to be possible through the establishment of chimerism. Early results with donor-specific bone marrow transfusion suggested decreased acute rejection and bronchiolitis obliterans syndrome.⁵ However, how best to prepare stem cells for engraftment, the requirements for any facilitating marrow-derived cells, and the optimal pro-tolerant immunosuppressive all have yet to be defined. This is an exciting and novel area of lung transplant research.

Clearly the study in this issue represents an effort to address a current area of relative controversy. The authors have conducted a thoughtful randomized trial, al-

though with flaws that may limit its interpretation. However, the real challenge for us to make significant improvements in long-term lung transplant outcome will require the investigation of novel immunosuppressive strategies and not merely refinements of conventional treatments.

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