Published in final edited form as:
Transplantation. 2014 July 27; 98(2): 117–121. doi:10.1097/TP.0000000000000260.

Tolerance - One Transplant for Life

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Abstract

A recent TTS workshop was convened to address the question: “What do we need to have in place to make tolerance induction protocols a “standard of care” for organ transplant recipients over the next decade?” In a productive two day meeting there was wide-ranging discussion on a broad series of topics resulting in five consensus recommendations: (1) Establish a registry of results for patients enrolled in tolerance trials; (2) Establish standardized protocols for sample collection and storage; (3) Establish standardized biomarkers and assays; (4) Include children aged 12 and older in protocols that have been validated in adults; (5) a task force to engage third party payers in discussions of how to fund tolerance trials. Future planned workshops will focus on progress in implementing these recommendations and identifying other steps that the community needs to take.

Introduction

In the over 60 years since the seminal demonstration of acquired transplantation tolerance in mice by Billingham, Brent, and Medawar, enormous efforts have been dedicated to translating that tantalizing possibility into a clinical reality. Careful and stepwise experiments in rodents and non-human primates led to the development of protocols that satisfied the risk-benefit analysis necessary for studies in humans, and although it took

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Report on the First International Workshop for Clinical Tolerance Boston, Massachusetts, USA March 14–15, 2014 Sponsored by The Transplantation Society

Conflict of Interest Declarations: JL is a co-founder of TRACT Therapeutics, Inc.
SS is a co-founder of SERC, Inc.
LAT has a family member employed by, and owns equity in, Novartis.

longer than everyone hoped, and many would have expected, operational tolerance to kidney and liver transplants has now been specifically induced in small numbers of patients. Not surprisingly, much of the early work was done in HLA-matched donor/recipient pairs, but increasing numbers of trials are now enrolling HLA-mismatched transplant recipients as well.

These are tremendous successes of which the field is justifiably proud, however they are just a start, and if we are to achieve a goal where tolerance is “routine” and not remarkable, more studies are needed. Unfortunately, it has proven challenging to leverage the initial promise of these early results into the next phase, i.e., new trials that involve more centers and larger numbers of patients. Recently, an international workshop of approximately 50 physicians and scientists was convened by The Transplantation Society in Boston to address the question: “What do we need to have in place to make tolerance induction protocols a “standard of care” for organ transplant recipients over the next decade?” This paper summarizes the proceedings of that meeting, and outlines consensus findings and recommendations for paths forward.

Overview of progress to date and the problems faced

Three centers in the US have enrolled renal transplant patients in tolerance protocols with successfully withdrawal from immunosuppression. The Stanford University protocol (1) uses post-transplant anti-thymocyte globulin (ATG) and total lymphoid irradiation (TLI). This is followed by the administration of a manipulated donor hematopoietic cell product controlling for the dose of CD34+ and CD3+ cells with weaning of immunosuppression (IS) based upon the presence of durable mixed chimerism. Overall, 16/22 HLA identical subjects have been taken off immunosuppressives. While the early experience in HLA mismatched transplantation did not succeed in achieving durable donor chimerism a new series with increased cell dosing is being employed and persistent chimerism has been achieved. No subjects have lost a graft.

Two trials are being conducted at Northwestern University. A total of 22 patients have been transplanted in a trial of mismatched and unrelated living donor kidneys using nonmyeloablative conditioning (fludarabine, cyclophosphamide, and low dose total body irradiation - TBI) combined with a manipulated stem cell product (2). Withdrawal of immunosuppression is conducted over a one year period and is based in part on the presence of durable donor chimerism. 12/19 subjects with one-year of follow-up have achieved durable chimerism and are off immunosuppression. 5 subjects did not develop stable engraftment and are on immunosuppression, and 2 subjects had allograft loss. No subjects developed GVHD. A separate trial of combined kidney and HSC transplantation in HLA identical patients uses alemtuzumab based conditioning and serial infusions of donor HSC. Of 20 patients, 10 have been withdrawn from immunosuppression.

A completed ITN-sponsored MGH mixed chimerism trial used thymic irradiation combined with antibody-based T cell depletion (the anti-CD2 mAb MEDI-507), rituximab, and cyclophosphamide, with unmodified donor bone marrow infused peri-transplant (3). Operational tolerance was induced in 7 of 10 subjects (the other three lost their grafts), 4 of

whom remain off immunosuppression long-term at 11.5, 5.2, 5.0 and 4.6 years.. Most patients developed “engraftment syndrome”, characterized by severe but transient renal dysfunction in the first week post-transplant. A modified version of the protocol with TBI replacing cyclophosphamide has begun in an effort to prevent engraftment syndrome.

Samsung and Hokkaido Universities (Korea) shared their experience with combined bone marrow and kidney transplantation in 5 subjects. A modified version of the MGH protocol has been used. Engraftment syndrome has been observed in the initial two patients. Although one of them was off immunosuppression for over one year, the patient very recently developed acute rejection. Lastly, the Johns Hopkins group has begun an ITN-sponsored trial in live donor HLA one haplotype-matched patients with a stem-cell transplant protocol that uses high-dose post-transplant cyclophosphamide adapted from their successful experience with sickle-cell disease. An initial subject has been transplanted and has early full donor chimerism.

A panel discussion explored several key points related to tolerance trials: (1) Patient selection - issues explored included exclusion/inclusion criteria, the impact of ethnicity, cognizance and capacity of potential subjects, disease co-morbidities, underlying cause of ESRD, and presensitization. The group all agreed that certain forms of ESRD with a very high risk of recurrence, such as FSGS, as well as pre-existing DSA, should represent an exclusion to enrollment. (2) Study design – there was a spirited discussion about the need for control groups (possibly with randomization) treated with standard immunosuppressive protocols to address the question of whether trying to achieve tolerance leads to superior patient outcomes over the long-term compared with standard of care or drug minimization. Part of this discussion focused as well on whether or not investigators at different institutions would participate in a multi-center trial with a single protocol if the one proposed differed from what they had employed previously. 3) Underlying mechanisms responsible for success or failure in achieving tolerance- most discussants agreed that stable donor chimerism was sufficient to establish donor specific tolerance. Considerable time was spent debating how transient chimerism might establish a form of regulatory tolerance, and how that state might be identified and measured.

Safety issues in drug withdrawal

There was extensive discussion around the issue of making drug withdrawal as safe as possible. One over-arching conclusion of this discussion was the need to be more systematic and standardized in collecting and disseminating clinical and immune monitoring data.

For example, many patients are tolerant already, having been enrolled in a variety of tolerance-induction protocols at different centers. It was agreed that valuable lessons can be learned these experiences, and that wider dissemination and availability of information would help inform future study design.

In keeping with this spirit, a great deal of attention at the meeting was devoted to examining what can already be learned from patients enrolled in the ongoing chimerism clinical trials at MGH, Stanford, Johns Hopkins, and Northwestern (with the first two centers employing mixed chimerism strategies, and the latter two utilizing full-chimerism approaches). Serial

monitoring and quantitation of donor chimerism, usually part of a flow cytometry based immune phenotyping of the peripheral blood, was reported by all 4 centers and felt to be important, although, the loss of chimerism in the peripheral blood did not always predict or lead to graft loss. It was felt that more emphasis on the development of strategies to quantitate the level of chimerism in the graft and bone marrow would be valuable. All centers were performing functional assays, including mixed leukocyte cultures and cell mediated cytotoxicity, to investigate the evolution of donor specific unresponsiveness, although there was agreement that these assays were of very limited predictive value. Tracking donor reactive clones through CDR3 sequencing is being investigated by MGH and Northwestern as a strategy for identifying donor reactive T cells, and the preliminary data suggesting that this tool can be applied to transplantation was felt to be extremely promising.

It was noted that each center currently has its own strategy for sample collection and storage. Implementation of SOP driven sample collection, preparation and storage was agreed unanimously (see recommendations below). Similarly while extensive immune monitoring is being done by each of the clinical centers, it can be difficult to compare results between centers due to a lack of harmonization of assays and assay conditions. It was agreed that creating a baseline set of standard assays and biomarkers that all centers adhered to would be extremely valuable in enabling cross-trial analyses, a particularly important goal given the small patient numbers in any individual trial.

Making the protocols accessible to other patient groups

Non-renal protocols

All organs are not equal when it comes to tolerance induction. It is well known that some organs are tolerance-prone (liver and kidney) while others are tolerance-resistant (heart and lung). The reasons for these organ-specific differences remain unclear, but organ-specific differences in tolerogenicity need to be taken into account while developing tolerance strategies for extra-renal organs and tissues.

Liver

The liver is generally considered the most tolerogenic of transplanted organs and withdrawal of all immunosuppression may be achieved up to 20% of appropriately selected liver allograft recipients without risk of imminent graft loss. In this workshop, the first successful induction of adult liver allograft tolerance using regulatory T cells was reported by a group in Hokkaido University, Japan. Based on results from nonhuman primate studies (4), regulatory T cells were induced and expanded *in vitro* by culturing recipient cells (obtained from splenectomy) irradiated donor PBMCs under costimulatory blockade (using anti-CD80 plus anti-CD86 mAbs). After the recipients were conditioned with cyclophosphamide, the unfractionated cultured cells were infused back into the recipient. It was reported that seven out of ten recipients were currently off immunosuppression for a duration of 2–18 months with stable liver function.

Islets

The specific considerations required for tolerance induction of islet allografts were discussed. The need to protect a very limited mass of β cells from humoral responses, alloantibodies and autoantibodies was shown to be particularly critical for induction of islet allograft tolerance. In a non-human primate system, a predominance of transitional B cells after rituximab and thymoglobulin treatments was associated with long-term islet allograft survival. B cell tolerance may be critically important in inducing islet allograft tolerance. This may beachieved by using anti-BLyS treatment to control B cell maturation.

Heart and lung

Heart and lung are two of the most tolerance-resistant organs. However, studies in swine and non-human primate models at MGH revealed that heart allograft tolerance can be induced by co-transplantation of the renal allograft. Preliminary results suggest that tolerance-prone kidney allografts are able to confer unresponsiveness upon tolerance-resistant heart allografts by expanding Tregs or enhancing their function. Understanding how kidney-specific elements amplify regulatory pathways could result in novel strategies to induce heart allograft tolerance in humans. Most recently, successful induction of tolerance was also achieved in MHC mismatched lung-allograft recipients through a mixed chimerism approach with anti-IL-6R mAb. This suggests that inhibition of anti-inflammatory responses may be critical in achieving lung allograft tolerance.

Application of tolerance induction for children

Applying tolerance induction protocols to children was a significant topic of discussion. As is generally recognized, pediatric patients are the population that has the most to benefit from transplantation in general, and tolerance induction in particular. Although a fully functional thymus, present in all children, seems to be especially helpful in promoting tolerance in experimental systems, there are a number of concerns in applying certain components of current tolerance protocols to children. These include the use of cyclophosphamide and/or radiation which could affect neurologic development and fertility. However, after weighing the pros and the cons, all participants agreed that it would be ethically proper to apply a tolerance protocol proven to be successful in adults to children at the upper end (≥12 years old) of the pediatric age group.

Funding the studies

There was unanimous agreement that difficulties in securing funds for clinical trials was a major impediment to progress. The largest share of US federal funding for this area comes from the National Institute of Allergy and Infectious Diseases (NIAID), and Nancy Bridges, Chief of the Transplantation Branch of the Division of Allergy, Immunity and Transplantation NIAID provided an overview of how that agency supported transplant research. Funding includes areas of basic discovery, translational research and clinical trials (solicited and investigator initiated). Preclinical studies are supported including rodent and non-human primate research. Support for clinical trials includes associated mechanistic studies in adults and children. Dr. Bridges pointed out that the ideal concept of tolerance may not be achievable except in a small subset of patients, that there are safety implications

in tolerance trials, and that striving for absolute, rather than incremental, goals can sometimes impede progress.

Larry Turka represented the Immune Tolerance Network (ITN), an international clinical research consortium funded primarily by NIAID. He explained that the ITN had made an internal decision to focus efforts on HLA mismatched transplants. Areas of interest include kidney, islet and liver transplantation with emphasis on pilot clinical trials that are investigator initiated. ITN has supported the development of immune monitoring assays that can be applied to the clinical trials that are sponsored by the ITN, and is opening to partnerships in which they perform mechanistic assays in trials sponsored by other consortia or agencies.

Tim Schroeder, of CTI Clinical Trial and Consulting, addressed issues of infrastructure and cost for tolerance trials, focusing on some of the challenging aspects of these studies including the limited numbers of patients available, the complexity of protocols, the need for multi-disciplinary approaches and regulatory oversight by IRBs, DSMBs, and the FDA. Most multi-center kidney transplantation trials require an infrastructure developed by contract research organizations, and personnel with regulatory agency experience. The budgets for pivotal trials of drug safety and efficacy are in the range of $50–100,000 per patient, and costs for tolerance studies may be considerably higher (up to $300,000 per patient) due to the need for close patient monitoring, the use of additional non-standard procedures and tests, and longer term follow up. The marked variation among centers in charges for the same procedures/tests is yet another challenge to overcome when developing trial budgets.

Amy Rosenberg of the US Food and Drug Administration discussed how tolerance induction trials in organ transplantation can apply for Expedited Review/Approval in four areas. These included Breakthrough Therapies in which there is substantial improvement over available treatments, Accelerated Approval in which surrogate markers are used to document substantial improvement, Fast Track approval in which therapies address an unmet medical need, and Priority Review in which there is significant improvement in safety or efficacy over available therapies. FDA approvals would allow medical centers to bill insurers for the therapies. The approvals are usually applied for by commercial entities.

It was clear that the costs of the studies are a barrier to progress, and considerable discussion revolved around the conditions that would allow investigators to charge insurance companies and Medicare for the costs of the tolerance induction procedures. There was great enthusiasm to establish a task force to reach out to third party payers to fund costs of tolerance protocols.

Conclusions and Recommendations

This meeting was conceived to address the issue of how to make tolerance induction protocols a standard of care for appropriate transplant recipients. None of the attendees expected a solution to emerge during meeting, but rather all hoped to identify issues and barriers, and to begin to craft a path forward, and indeed by this metric the workshop was a success. There was a frank and open exchange of ideas on a wide range of topics, and the

workshop participants reached five consensus recommendations listed below. Efforts to implement these are already underway and the next gathering of this TTS supported workshop is scheduled for the fall of 2015.

Recommendation 1

Establish a Reporting Standards Committee to develop the standards by which investigators report the results of tolerance trials. The following metrics might be included:

1. Annual self-reported health state
2. Strict reporting standard on histologic findings, with external independent review
3. Functional organ status over time, e.g., estimated creatinine clearance
4. Annual days in hospital and severe adverse events
5. Standards for control groups: this is important since drug minimization protocols may have similar outcomes
6. Precise definitions of the cellular and pharmaceutical intervention

Recommendation 2

Establish standardized protocols for sample collection and storage at agreed time points for all centers undertaking tolerance trials to ensure that comparable samples (in terms of when and how obtained, and types of samples collected) from patients enrolled in different protocols are available for future analysis and comparison.

Recommendation 3

Establish a standard panel of biomarkers and assays agreed upon by the tolerance research community. This will be invaluable for the next phase of clinical investigation to facilitate optimization of current protocols and the design and the implementation of multi-center randomized clinical trials.

Recommendation 4

Children 12 years of age or older should be eligible for enrollment in tolerance-inducing protocols proven to be safe and successful in adults.

Recommendation 5

Establish a task force to engage third party payers in discussions of how to fund costs of tolerance protocols.

Acknowledgments

We thank all of the workshop participants (**please see** Appendix) for their attendance and participation and especially acknowledge Geneviève Leclerc for outstanding administrative assistance and to Francis Delmonico, as President of The Transplantation Society, in the formulation of the plan and agenda of the workshop in close collaboration with the Executive Leadership. We are grateful to The Transplantation Society for organizing the meeting, and to Novartis, Astellas, Genentech, Alexion and Bristol-Myers Squibb for additional support.

Appendix

Meetings attendees

M. Abecassis (Northwestern University School of Medicine), S. Alexander (Children's Hospital at Westmead), J. Allan (Massachusetts General Hospital), S. Bartlett (University of Maryland), G. Benichou (Massachusetts General Hospital), N. Bridges (National Institutes of Health), W. Burlingham (University of Wisconsin), S. Busque (Stanford School of Medicine), A. Chong (University of Chicago), A.B. Cosimi (Massachusetts General Hospital), F. Delmonico (The Transplantation Society), E. Fuchs (The Johns Hopkins University), E. Geissler (University of Regensburg Hospital), G. Hill (Queensland Institute of Medical Research), D. Kaufman (University of Wisconsin), T. Kawai (Massachusetts General Hospital), A. Kirk (Emory University), N. Krieger (Novartis), J. Leventhal (Northwestern University School of Medicine), J. Madsen (Massachusetts General Hospital), J. Markmann (Massachusetts General Hospital), J. Miller (Northwestern University School of Medicine), A. Monaco (Beth Israel Deaconess Medical Center), R. Montgomery (The Johns Hopkins University), A. Naji (University of Pennsylvania), K Newell (Emory University), J.B. Park (Samsung Medical Center), A. Rosenberg (US Food and Drug Administration), G. Ryoichi (Hokkaido University), D. Sachs (Massachusetts General Hospital), B. Sawitzki (Charité Universitätsmedizin Berlin), J. Scandling (Stanford University School of Medicine), T. Schroeder (CTI Clinical Trial and Consulting), J.A. Shizuru (Stanford University School of Medicine), T. Spitzer (Massachusetts General Hospital), S. Strober (Stanford University School of Medicine), M. Suthanthiran (Cornell University), L.J. Swinnen (The Johns Hopkins University), M. Sykes (Columbia University Medical Center), N. Tchao (Immune Tolerance Network), S. Todo (Research Institute of St. Mary's Hospital), S. Tullius (Brigham and Women's Hospital), L. Turka (Massachusetts General Hospital), K. Wood (Oxford University), K. Yamashita (Hokkaido University)

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