Proceedings: Immune Tolerance and Stem Cell Transplantation: A CIRM Mini-Symposium and Workshop Report

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ABSTRACT

The mission of the California Institute for Regenerative Medicine (CIRM) is to accelerate stem cell treatments to patients with unmet medical needs. Immune rejection is one hurdle that stem cell therapies must overcome to achieve a durable and effective therapeutic benefit. In July 2014, CIRM convened a group of clinical investigators developing stem cell therapeutics, immunologists, and transplantation biologists to consider strategies to address this challenge. Workshop participants discussed current approaches for counteracting immune rejection in the context of organ transplant and cellular therapy and defined the risks, challenges, and opportunities for adapting them to the development of stem cell-based therapeutics. This effort led to the development of a Roadmap to Tolerance for allogeneic stem cell therapy, with four fundamental steps: (a) the need to identify “tolerance-permissive” immune-suppressive regimens to enable the eventual transition from current, drug-based approaches to a newer generation of technologies for inducing tolerance; (b) testing new biologics and small molecules for inducing tolerance in stem cell-based preclinical and clinical studies; (c) stimulation of efforts to develop novel therapeutic approaches to induce central and peripheral tolerance, including manipulation of the thymus, transplantation of purified stem cells, and cell therapy with T-regulatory cells; and (d) development of robust and sensitive immune monitoring technologies for identifying biomarkers of tolerance and rejection after allogeneic stem cell treatments in the clinical setting.

INTRODUCTION

For development of effective allogeneic or modified autologous cell therapy products that require engraftment to achieve a biologic effect, a major roadblock is immune acceptance of the cellular graft. Therefore, achievement of immune tolerance to cellular grafts will both accelerate the delivery of therapies to patients and improve the likelihood of success. The California Institute for Regenerative Medicine (CIRM) has funded and is continuing to fund research on developing approaches to immune tolerance through a variety of mechanisms, both through dedicated projects and as subcomponents of programs in our translational pipeline. However, a question is raised as to whether CIRM can more efficiently and effectively have an impact on this roadblock.

To address this question, CIRM convened a meeting of clinical investigators developing cellular therapeutics and individuals working on approaches to transplantation tolerance. The participants discussed immunosuppressive regimens that are currently used to achieve graft acceptance in the context of chronic immunosuppressive therapy and experimental tolerance induction protocols to achieve graft acceptance without the need for chronic immunosuppressive therapy. Although much of the discussion drew from the more established organ transplantation and hematopoietic transplantation fields, this symposium emphasized the current approaches to stem cell graft acceptance, both in general and more specifically, to projects in CIRM’s development portfolio.

STATE OF THE FIELD

When tissue or cells are transplanted into a genetically distinct host, the recipient (host) immune system recognizes alloantigens in the donor graft as foreign and mounts an immune response that results in graft rejection. To prevent rejection of solid organs or cellular grafts, patients are typically placed on long-term immunosuppressive drug regimens, which are associated with adverse effects on health and quality of life. As an alternative, the transplantation biology field has long sought approaches to
establishing clinical tolerance, or a specific lack of immune responsiveness to a given tissue or cell type. Such tolerance could be achieved through mitigation of T-cell reactivity, a process that occurs naturally in the mammalian immune system through two mechanisms: central tolerance and peripheral tolerance. Central tolerance involves selective elimination of autoreactive T cells in the thymus (called thymic deletion or thymic education). Peripheral tolerance is effected through numerous different processes, including functional inactivity of T cells (anergy), suppression of T cells by other cells (regulation), and peripheral deletion of alloreactive T cells by apoptosis [1]. Tolerance induction protocols attempt to harness these naturally occurring mechanisms to control immune reactivity. One of the classic experimental approaches to tolerance induction is to replace the host immune compartment with donor immune cells to achieve full chimerism, and thus the immune system would recognize donor cells as “self.” In other approaches, mixed chimerism is established (presence of both donor and host immune cells), which results in the directed education of recipient T cells to recognize donor antigens as self (i.e., by thymic deletion of donor alloreactive T cells). There has also been progress on tolerance induction protocols that rely on peripheral tolerance, using pharmacologic regimens or by transplanting regulatory T cells.

Despite progress in the field, tolerance induction protocols are not yet in wide clinical application for organ transplantation. Outlined below are a number of current approaches to establishing tolerance that are under development and that were discussed at the CIRM mini-symposium. Some of them are closer to clinical testing than others, but all represent promising advances in the field of transplantation tolerance. More information on these and other approaches can be found in several recent reviews [2, 3].

**CURRENT APPROACHES FOR INDUCING TOLERANCE TO CELL THERAPIES**

**Hematopoietic Chimerism**

One major strategy that has become mainstream practice in the regenerative medicine field relies on the phenomenon of hematopoietic chimerism, which can be achieved via successful bone marrow transplantation. In this procedure, blood stem cells from a donor are transplanted into a recipient host whose own blood forming system has been partially depleted or totally ablated. This procedure enables the eventual replacement of the host’s immune system with one of donor origin, resulting in full hematopoietic chimerism and the establishment of tolerance to both host and donor tissues. Unfortunately, the widespread use of this technique is limited by the transplantation procedure itself, which carries a very high risk of immunological complications during the initial period of ablation and recovery and is therefore not feasible as a generalizable approach for achieving tolerance to stem cell-derived tissues. Moreover, this approach requires donor hematopoietic cells that are fully matched with the intended stem cell therapy product, which, although achievable, imposes additional hurdles. Despite these ongoing challenges, there has been progress in developing tolerance induction regimens for organ transplantation that use nonmyeloablative regimens to achieve mixed chimerism. For example, Scandling et al. [4] reported on kidney transplant recipients who achieved mixed chimerism, donor-specific unresponsiveness, and the ability to withdraw immunosuppressive medications. These patients were infused with donor hematopoietic progenitor and T-cell populations (CD34+, CD3+) after total lymphoid irradiation and antithymocyte globulin treatment prior to receiving their kidney transplants [4]. The underlying mechanism of action is purported to involve peripheral mechanisms, including regulatory T cells and natural killer T cells, which are less susceptible to lymphoid irradiation than other lymphocytes.

**Depletion or Modulation of Effector Cell Response**

Another tolerance induction strategy relies on depleting key cell populations, such as reactive lymphoid cells, or modifying the activity of reactive (effector) cell populations through pharmacologic, antibody-based, or cell-based strategies. For example, the monoclonal antibody alemtuzumab binds to mature lymphocytes and targets them for destruction [5], the fusion protein abatacept (Orencia; Bristol-Myers Squibb, New York, NY, http://www.bms.com) blocks costimulation of T cells and thus inhibits T-cell activation [6], and the use of pharmacological agents influences the phenotype and behavior of tolerogenic/immunogenic cell populations such as dendritic cells [7]. Costimulation blockade strategies with other combinations of proteins or antibodies are being used to block T-cell activation or induce T-cell anergy [8]. Finally, the adoptive transfer of specific cell populations to modify the immune response represents another intriguing approach for promoting tolerance, with many hypothetical advantages over single- or single-biologic regimens, such as an ability to sense and integrate cellular signals and an ability to execute complex responses, including the downregulation of detrimental molecular pathways (Table 1). Regulatory T cells (Tregs), which can include central and peripheral subsets, represent a particularly promising strategy for adoptive immunotherapy, as they are amenable to genetic modification and can be manufactured in large numbers. They are especially attractive for their pleiotropic functions, including suppressive cytokine production, effector cell killing, and metabolic disruption. Importantly, Tregs are able to modify the activity of cells around them (bystander effect) and “educate” neighboring cells to become tolerant. One of the challenges with this approach is the potential incompatibility with use of some of the standard immunosuppressive agents such as calcineurin inhibitors, which prevent the proliferation of these Tregs.

**Table 1. Antibody- and cell-based immune tolerance induction strategies discussed at the CIRM Immune Tolerance Mini-Symposium**

| Therapeutic | Mechanism(s) of action | Reference |
|-------------|------------------------|-----------|
| Antibody-based | | |
| Alemtuzumab | Mature lymphocyte depletion | [5] |
| Abatacept | Costimulation blockade | [6] |
| Cell-based | | |
| Regulatory T cells | Downregulation of detrimental molecular pathways | [9] |
| Tolerogenic dendritic cells | | |
| Myeloid-derived suppressor cells | | |
| Mesenchymal stromal cells | | |
| Natural killer T cells | | [14] |

Several completed and ongoing clinical trials have presented opportunities to evaluate the potential of tolerance-induction
approaches for facilitating cell transplantation or for suppressing self-reactive effector cells in autoimmune disorders. Dr. Maria-Grazia Roncarolo discussed recent results from the ALT-TEN trial testing a human Treg subpopulation, named Tr1, for its ability to promote immune recovery without severe graft-versus-host disease (GVHD) after haploidentical hematopoietic stem cell (HSC) transplant in patients with hematologic malignancies. Several transplanted patients showed long-term disease remission and the presence of donor-derived T cells, antigen-specific anergy, and other signs of Treg activity, demonstrating the safety and feasibility of this approach and providing preliminary evidence of a tolerance signature [15]. Treg-based trials, using FOXP3+ Tregs, have been proven to be safe and to reduce GVHD in patients receiving allogeneic major histocompatibility complex-mismatched HSCs [16]. Dr. Jeffrey Bluestone (University of California, San Francisco [UCSF]) discussed other new tests of Tregs for modulating autoimmune diseases, such as type 1 diabetes and Crohn’s disease, with some early suggestions from his own studies that the transplanted Tregs are safe and, in some cases, can persist for at least 6 months. In addition to these early trials, alloreactive ex vivo expanded Tregs are currently being developed for kidney and liver organ transplantation and other disorders (the ONE Study).

The Immune Tolerance Network (ITN) has tested a variety of approaches in clinical trials with the goals of defining and elucidating mechanisms of operational tolerance. Dr. Gerald Nepom provided four illustrative examples of such studies in the context of pancreatic transplantation for type 1 diabetes, autologous HSC transplantation for refractory multiple sclerosis, pediatric liver transplantation, and the use of alefacept and/or thymoglobulin for modifying the anti-islet autoimmune response in patients with type 1 diabetes. By monitoring the effects of immune suppression withdrawal or various tolerance approaches on Tregs and other immune cell populations in these trials, it has become clear that although tolerance is achievable in theory, current methods for immunomodulation are transient and work only to a degree. Immune regulation can be helpful but is underappreciated, and in fact many drugs currently used for immunosuppression likely inhibit such regulation. Dr. Nepom suggested that a general path forward could comprise a strategy to (a) ablate, exhaust, or energize the effector response; (b) deviate the induced response; and (c) boost or enhance the regulatory response. Clinically, this translates to inducing remission while avoiding interference with regulation, pushing the system toward a regulated, tolerogenic environment while in remission and the inflammatory milieu is quiescent. Potential strategies to achieve this approach could include antibodies to inflammatory cytokines with costimulation blockade or followed by an antigen-containing nanoparticle, or lymphocyte depletion followed by an antigen to hemostatic cytokines [17].

**Novel Stem Cell-Based Approaches to Induce Tolerance to Stem Cells or Their Derivatives**

The major organ involved in inducing central immune tolerance is the thymus, the generative organ for T-cell development. Within the thymus, a key population of cells called thymic epithelial cells (TECs) provide developmental support and guide T-cell development by imposing both positive and negative selection steps, thereby eliminating autoreactive T-cell subsets while promoting survival of regulatory T cells that can actively suppress immune responses in an antigen specific manner. Given the key role of TECs in establishing self-tolerance, differentiation of a functional thymus from stem cells has the theoretical potential to prevent rejection of stem cell-derived tissue. Thus, reprogramming the immune system through induction of graft specific immune tolerance represents an appealing alternative to aggressive immunosuppression. A recent development in this area was presented by Dr. Mark Anderson (UCSF), whose laboratory has successfully developed a novel method to generate thymic epithelial progenitor cells (TEMps) from human pluripotent stem cells (hPSCs) [18]. hPSC-derived TEMps are functional upon transplantation in athymic mice and acquire characteristics of mature TECs that allow them to support multistage T-cell development. In addition, hPSC-derived TEM grafts placed under the renal capsule support the generation of functional T cells capable of mounting allogeneic immune responses and promoting the formation of Tregs. In a related area, Dr. Gay Crooks (University of California, Los Angeles) discussed her own research on the development of implantable thymic microenvironments composed of two human thymic stromal populations critical for thymopoiesis: TECs and thymic mesenchyme (TM) [19]. Organoids formed from ex vivo expanded human TECs and TMs can support thymopoiesis from human CD34+ cord blood cells after implantation into immune-deficient mice and produce functional T cells with a diverse T-cell receptor repertoire. Understanding the role of non-epithelial components of the thymic microenvironment in supporting the survival and function of TECs and developing the technology to incorporate them into a surgically implantable three-dimensional thymic organoid will be essential if pluripotent stem cell-derived TECs are to be used clinically.

Dr. Yang Xu’s laboratory at the University of California, San Diego, is exploring ways to suppress the human immune response to allogeneic human embryonic stem cell (hESC)-derived cells by exploiting inhibitory signaling pathways that restrain T-lymphocyte activity. They have genetically modified hESCs by “knocking-in” genes for ligands for two potent inhibitory receptors expressed by T lymphocytes (CTLA4 and PD-1). A humanized mouse, with a functional human immune system that could mount allogeneic immune rejection of hESC-derived cells, was used to demonstrate the tolerance of the CTLA4 Ig/PD-L1 engineered cells, in contrast to unmodified cells that were rapidly rejected [20]. One potential risk of this approach is that it would allow an hESC-derived graft that developed tumorigenic potential to escape immune surveillance. To circumvent this possibility, a suicide gene approach is being investigated.

Targeting and removing defective stem cells from their niche is an essential component of successful stem cell therapy for allogeneic hematopoietic cell transplantation, which is widely applied for the treatment of hematological malignancies and genetic diseases. At present, no specific reagent exists that targets endogenous HSCs, and thus the standard of care is to receive chemotherapy and/or radiation to create space for the donor HSC engraftment. Dr. Judith Shizuru (Stanford University) discussed an antibody approach to achieve a chemotherapy/radiation-free conditioning regimen under development for allogeneic stem cell transplantation. CD117 is a cytokine receptor expressed on HSCs whose ligand, stem cell factor, promotes survival, self-renewal, differentiation, and proliferation of HSCs. Anti-human CD117 inhibits human HSC proliferation in vitro and results in human HSC depletion in humanized NOD-scid IL2R<sup>null</sup> (NSG) mice [21]. Dr. Tippi MacKenzie (UCSF) discussed her own studies in which ACK2, an antibody against the murine CD117, depletes fetal
host HSCs and increases space within the hematopoietic niche for donor cell engraftment. Her results suggest that in utero depletion of fetal hematopoietic stem cells by anti-CD117 antibody improves engraftment after neonatal transplantation in mice by effectively depleting HSCs within bone marrow with minimal toxicity [22].

**CURRENT APPROACHES IN MONITORING OF HUMAN IMMUNE RESPONSE**

An essential tool for achieving tolerance is the ability to monitor rejection and tolerance in a rapid, reliable, and relatively noninvasive way, which can be particularly challenging when studying rejection of a small number of transplanted (noncirculating) cells. Therefore, more sensitive assays are needed for assessing immune responses to transplanted tissues. To this end, Dr. Mark Davis (Stanford University) discussed recent results from his laboratory on the development and use of a number of sensitive assays to measure immune responses in human subjects. In one example, the analysis of T-cell repertoires revealed the presence of T cells reactive against viruses and pathogens in individuals who have never been exposed to these pathogens, such as a high frequency of memory T cells for HIV in healthy blood bank donors who were HIV-seronegative, illustrating the phenomenon previously referred to as “heterologous immunity” [23]. A further conclusion from these studies is that peripheral tolerance works, and thus tolerance strategies to induce peripheral tolerance to allogeneic cells and tissues may be possible.

Dr. Davis also discussed recent innovations in cell labeling and flow cytometry, which have led to the development of powerful new approaches for high-throughput analysis and monitoring of individual cell phenotypes within a population. The technique of mass cytometry, or CyTOF, has proven extremely useful for identifying unique gene expression signatures among various types of immune cells [24]. In this technique, multiple proteins within a cell are labeled using panels of heavy metal-tagged antibodies, and thus each cell within a population will reflect a distinct mass that is determined by the combination and quantity of bound antibodies. Cells are sorted by flow cytometry, and their labels are quantified and deconvoluted via time-of-flight mass spectrometry and bioinformatics approaches. It is now possible to label cells with up to 40 different mass-tagged antibodies, enabling up to 60 billion different marker combinations to be distinguished from one another. As CyTOF expands into more mainstream use, the ability to identify unique gene expression signatures at the individual cell level holds promise for revealing novel mechanistic insights about the function and behavior of the immune system and for enabling development of improved procedures for tracking and monitoring cell fate upon transplantation.

**CLINICAL EXPERIENCE AND CONSIDERATIONS FOR SUPPRESSING AND MONITORING IMMUNE RESPONSE**

In early-stage stem cell therapy trials, where the focus is on safety and early efficacy, the issue of immune rejection has been an important component of clinical trial design, with immunosuppressive regimens generally being informed by clinical experience with organ and bone marrow transplantation. Nonpharmacologic approaches, such as ViaCyte’s encapsulated cell product to be described below, have also been considered as a means of avoiding immune rejection, which is paramount in cases where biologic activity and therapeutic efficacy are dependent on long-term graft survival and function. It is not yet clear that current immunosuppressive regimens will be sufficient for the various stem cell products in development. Therefore, effective readouts to monitor the host immune response, as well as methods to monitor stem cell survival and function, are critical in these stem cell trials.

Dr. Jane Lebkowski reported on the interval experience with the first-in-human clinical trial of an hESC-based product in the setting of thoracic spinal cord injury. First developed at Geron, Inc., and currently being developed by Asterias Biotherapeutics, Inc., this cell product, now referred to as AST-OPC1, is a mixed population of hESC-derived oligodendrocyte progenitors that can remyelinate axons, produce neurotrophic factors, and promote neovascularization. The immunosuppression regimen chosen for this trial was largely based on published findings and in vitro characterization of the AST-OPC1 product. These cells are reportedly human leukocyte antigen (HLA) class I-low and HLA class II-low, do not induce natural killer cell-mediated killing upon exposure to sera from normal healthy volunteers, and exhibit low T-cell allostimulatory activity in mixed lymphocyte reaction (MLR) cultures and enzyme-linked immunospot (ELISPOT) assays.

In the Geron clinical protocol, immune parameters were not used as exclusion criteria, and there was no requirement for any degree of HLA matching between the cell product and the recipient. In fact, in the closest donor-recipient match, only 5 out of the 10 antigens were matched, and there was a complete HLA mismatch in 2 subjects. Implantation was performed in 5 subjects during the acute postinjury phase; these subjects received a calcineurin inhibitor, targeting low trough drug levels, which was tapered off at a defined point post-transplantation. There were no serious adverse events reported in the study, nor was there evidence of an inflammatory response based on imaging studies. Similarly, there was no evidence of serum antibody binding to oligodendrocyte progenitor cells as assessed by fluorescein-activated cell sorting analysis, nor was there an indication in the cerebrospinal fluid of antibody mediated immune response to the AST-OPC1 product or of cell-mediated immune response by ELISPOT at 1 year. Although some findings were consistent with prevention of lesion cavity formation, there was no direct evidence of AST-OPC1 survival, and thus it is unclear whether this absence is a consequence of immune rejection.

Dr. Clive Svendsen reported on his efforts to develop a stem cell therapy for amyotrophic lateral sclerosis (ALS) using fetal-derived neural stem cells (astrocytes) that are genetically modified to secrete glial cell-derived neurotrophic factor (GDNF). The clinical trial design entails unilateral injection to the spinal cord (using the contralateral side as control), assuming that disease progression will be the same on both sides. Cerebrospinal fluid collection and magnetic resonance imaging will be used to evaluate efficacy. In addition, they plan to monitor production GDNF as a measure of both efficacy and as evidence of cell survival. For this trial, Dr. Svendsen proposes an intensive immunosuppressive regimen consisting of tacrolimus, mycophenolate mofetil, and basiliximab to be tapered over time, with monitoring of humoral and cellular immune responses. The goal of this approach is to protect transplanted cells from immune-mediated destruction so that mid- to long-term safety and preliminary efficacy of cells can be realistically evaluated. Although it was possible to extend cell survival in preclinical studies using similar approaches, Dr. Bluestone and the others cautioned against the use of basiliximab (targeting CD25), as it may result in the loss of tolerance facilitating Tregs.
Dr. Svendsen referred to a separate experience with Neuralstem’s unmodified human neural stem cell product for ALS, noting that one patient exhibited clinical improvement that is now attributed to amelioration of an underlying autoimmune issue by the immunosuppressive medications given for the trial. Based on this observation, there is currently a clinical study at Emory University evaluating the effect of immunosuppressive medications (independent of any cell transplantation product) in ALS.

Dr. Stephen Huhn from StemCells, Inc., reviewed his company’s clinical experience with human central nervous system stem cell (HuCNS-SC) transplantation in a variety of target indications including the neurodegenerative lysosomal storage disease neuronal ceroid lipofuscinosis (NCL), the rare genetic demyelination disorder Pelizaeus-Merzbacher disease (PMD), the blinding eye disease age-related macular degeneration (AMD), and spinal cord injury (SCI). HuCNS-SC cells are derived from donated fetal brain tissue, are restricted to the central nervous system (CNS) lineage and have been shown in preclinical studies to express a brief course of perioperative corticosteroids and short-term maintenance doses of tacrolimus and mycophenolate. He noted that there have been reports in postmortem studies of long-term allograft survival in CNS up to 16 years after transplantation. Nonetheless, there also have been case reports of possible alloimmunization to donor antigens following fetal neural grafts transplanted into subjects with Huntington’s disease [25].

Although there may be temporary disruption of the blood-brain barrier during surgical delivery of cells into the brain, spinal cord, or retina, CNS is widely considered to be an immune-privileged site. This immune-privilege is not absolute, however, and thus the clinical protocols of StemCells, Inc., include immunosuppressive regimens that provide coverage until the blood-brain barrier can be expected to reestablish. The general regimen consists of a brief course of perioperative corticosteroids and short-term maintenance doses of tacrolimus and mycophenolate mofetil. Tacrolimus was discontinued after 1 year in the NCL trial, 9 months for the PMD trial, 8 months in the SCI trial, and 3 months in the AMD trial. Mycophenolate mofetil was discontinued after 1 month in all studies.

Immune monitoring to assess allorecognition, particularly after immunosuppressive medication withdrawal and/or any association with signs of clinical efficacy, is still under development. Based on gross clinical parameters and lack of inflammatory signs on imaging, StemCells, Inc., reports that no evidence has been seen of graft rejection after immunosuppression withdrawal.

Dr. Kevin D’Amour from ViaCyte presented his company’s strategy for avoiding the host immune response. ViaCyte’s VC-01 product candidate consists of hESC-derived pancreatic endoderm cells, called PEC-01, which are encapsulated within ViaCyte’s proprietary Encaptra device and implanted subcutaneously where they mature and differentiate in vivo into glucose-responsive insulin-producing \( \beta \) cells and other endocrine cells. To avoid the need for immunosuppressive medications, the device is designed to exclude host cell contact with the implanted PEC-01 cells. In preclinical studies, the Encaptra device protected allogeneic newborn mouse pancreas from immune destruction even in the setting of immune sensitization, and it protected pancreatic cells from autoimmune response in nonobese diabetic mice. Although the device can exclude cells, it does not stop the flow of antibodies. In the planned clinical trial, therefore, host immune response will be monitored longitudinally by tracking panel reactive antibodies, reactivity to islet autoantigens, and evaluating T cell-mediated response by MLR and a tetramer assay. The plan is to correlate clinical efficacy and safety data with these immunologic monitoring studies.

**CONCLUSION**

In the context of stem cell transplantation, where engraftment of biologically active cells is desired, control of immune rejection is essential and, ultimately, immune tolerance is the goal. Toward achieving this goal, workshop participants identified the following universal challenges. (a) As yet, there is no clear approach to determine which of the immunosuppressive regimens that have emanated from the organ transplant and hematopoietic transplant experience are appropriate for a given stem cell therapeutic product. (b) It is not feasible to expect companies that are developing stem cell products to conduct novel experiments (i.e., with tolerance induction approaches) at the same time that they are trying to test their product candidate. (c) In vivo preclinical models are often not predictive of the clinical scenario. (d) Sensitive cell tracking technology for clinical applications is not yet in place, limiting the ability to correlate cell survival and engraftment with clinical efficacy measures, and preventing reliable understanding of innate and adaptive immune responses to cellular grafts.

These challenges notwithstanding, this meeting recognized four key steps toward the goal of prevention of graft rejection and induction of tolerance to stem cell-based tissues, comprising a Roadmap to Tolerance. First, it will be important to identify tolerance-permissive immune-suppressive regimens that can be used in the short term to overcome immediate rejection, and these regimens must not otherwise impede or interfere with cellular and molecular mechanisms necessary for operational tolerance. In this way, these regimens would serve as a “bridge to tolerance” until either operational tolerance or a tolerance induction protocol allows full removal of the immunosuppressive drugs. Second, new biologics and small molecules for inducing tolerance, such as those evaluated in clinical trials sponsored by the ITN, should be incorporated within stem cell-based preclinical and clinical studies. Stem cell transplantation may provide unique opportunities to investigate relationships between cell type, transplantation site, mechanism of action, and other factors affecting therapeutic potential. Third, novel therapeutic approaches for inducing central and peripheral tolerance should continue to be pursued, as they may be particularly relevant to cell therapy approaches. These approaches include manipulation of thymus, transplantation of purified stem cells, and cell therapy with T regulatory cells, all of which are highly promising but very preliminary in terms of their clinical translation. Finally, investigators are urged to continue developing and implementing robust immune monitoring technologies for identifying early biomarkers of rejection and acceptance, and for evaluating the effectiveness of immunosuppressive regimens and their correlation to therapeutic efficacy.

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AUTHOR CONTRIBUTIONS

S.T.: conception and design, manuscript writing, final approval of manuscript; M.T.M., R.L.J., and K.A.S.: conception and design, manuscript writing.

DISCLOSURE OF POTENTIAL CONFLICTS OF INTERESTS

The authors indicated no potential conflicts of interest.