Meeting Report: The Fifth International Samuel Strober Workshop on Clinical Immune Tolerance

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INTRODUCTION

Chronic graft rejectionand toxicities of long-term immunosuppressive therapies impede the advancement of organ transplantation. Establishment of a state of tolerance, in which the immune system remains functional and competent but treats the donor as "self," would prevent chronic rejection and obviate the need for long-term immunosuppression (IS). In the past 2 decades, several clinical protocols have achieved organ allograft tolerance. Furthermore, new biological compounds and cell therapies aimed at tolerance induction have begun to be clinical in tolerance trials.

The biennial Workshop brings together leaders in the field of transplant tolerance and its underlying science to exchange progress and ideas through an interactive format. The Fifth International Workshop was held from April 27 to 29, 2022, in Jersey City, New Jersey, and was renamed in honor of one of its earliest proponents and founders, Samuel Strober, who passed away in 2022. The 2-d meeting covered the status of pre-clinical and clinical trials designed to minimize or withdraw immunosuppressive drugs in kidney, liver, intestinal, and lung transplantation, and mechanistic studies to understand tolerance and identify potential predictors and biomarkers. Here we summarize the many exciting advances presented at the Workshop.

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TOLERANCE TRIALS: KIDNEY

During the last 2 decades, hematopoietic chimerism-based regimens aiming at complete IS withdrawal (ISW) have been evaluated (Table 1). These regimens involve induction of either full (persistent) or mixed chimerism (persistent or transient) via bone marrow transplantation (BMT) or peripheral blood stem cell (PBSC) transplantation. Suzanne Ildstad (Talaris) updated the outcomes of a trial for extensively HLA-mismatched kidney transplantation (KTx) plus PBSC and "facilitating cell" transplantation at Northwestern University.[1,2] With persistent high levels of donor chimerism, 26 of 37 recipients were successfully removed from IS, and 6 are currently IS-free for >10 y. A randomized, multicenter, phase III trial is now underway. Joseph Leventhal (Northwestern University) reported operational tolerance in 6 of 15 recipients of HLA-identical KTx with donor CD34 cell infusion following alemtuzumab induction without myelosuppressive conditioning.[3] Stephan Busque (Stanford U) summarized a total lymphoid irradiation/antithymocyte globulin (TLI/ATG)–based protocol in which 24 of 29 HLA-identical KTx recipients discontinued IS for >5 y and 7 for >10 y.[4,5] In an HLA-mismatched cohort, no IS-free allograft survival has yet been achieved. The addition of low-dose total body irradiation (TBI) or regulatory T cell (Treg) infusion is being evaluated. Daniel Brennan (Medeor) reported on a similar TLI/ATG/CD34 cell infusion trial in HLA-identical KTx recipients. Nineteen of 20 recipients successfully discontinued IS up to 2.6 y of follow-up, of which 2 with recurrent disease returned to IS. Tatsuo Kawai Massachusetts General Hospital (MGH) updated the trial of HLA-mismatched combined KTx and BMT (CKBMT).[6-8] Using cyclophosphamide (CY), thymic irradiation, and anti-CD2 monoclonal antibodies with or without rituximab, 7 of 10 achieved IS-free allograft survival for >5 y and 4 for >10 y, with the longest at 17 y. Two recipients of a new conditioning regimen with additional fludarabine developed transient mixed chimerism without complications, and IS tapering is in progress. Dr Kyo Won Lee summarized trials at the Samsung Medical Center (Seoul).[9] With their most recent regimen (CY, rituximab, fludarabine, thymic irradiation, and ATG), ISW was achieved in 11 of 19 recipients with transient mixed chimerism.

To minimize IS in HLA-mismatched KTx recipients, clinical trials with Treg have begun (Table 2). The Northwestern group infused Treg following alemtuzumab induction, and Thomas Wekerle (Medical U of Vienna) combined polyclonal

TABLE 1.

Complete IS withdrawal (tolerance)

Institution/trial	Protocol	BMC PBSC Treg	HLA mismatch	Chime rism	Participants (N)	IS taper in progress	Off IS 1–5 y	Off IS 5–10 y	Off IS >10 y	Longest off IS (y)	Adverse events
Northwestern/Talaris Phase 2 FCR001	CY 50 mg/kg pre- and post-Tx, Fludara 30 mg/m^2 × 3, TBI 200 cGy	PBSC Facilitating cells, αβTCR+T cells	Mismatched	Full or mixed persistent	37	0	7	13	6	12	GVHD (2)
Northwestern	Alemtuzumab induction (30 mg IV d 0 and 4) mTOR conversion	BMC/PBSC CD34 selected	Matched	Transient	20	0			6	13.5	Disease recurrence (2) (FSGS/IgAN)
Stanford 1	TLI (1200 cGy) + rATG	PBSC CD34$^+$ T cell 10^6/kg	Matched	Mixed persistent or transient	29	0	7	10	7	14	Zoster (8)
Stanford 2	TLI (1200 cGy) + rATG	PBSC CD34$^+$ cells + T cells 3–150 (×10^6/kg)	Haplo-matched	Mixed persistent or transient	26	1	0	0	0	–	Zoster (5) Transient AKI (2) Hemolytic anemia (1)
Stanford 3	TLI (1080 cGy) + TBI (40–80 cGy) + rATG	PBSC CD34$^+$ cells + T cells 50 (×10^6/kg)	Haplo-matched or mismatched	Mixed persistent or transient	6	2	1	0	0	–	Acute GVHD (1) Skin, grade 2 (resolved)
Stanford-Northwestern	TLI (1320 cGy) + rATG	PBSC CD34$^+$ cells + T cells 100 (×10^6/kg) Recipient's Treg 2–5 (×10^6/kg)	Haplo-matched	Mixed persistent or transient	3	0	0	0	0	–	Transient AKI (1)
Medeor Trial	TLI + rATG, CS (off D10), MMF (off D39), CNI taper beginning D181–D365 (off D366)	PBSC	Matched	Mixed persistent or transient	20	0	18	0	0	2.77	None Disease recurrence IgAN (2)
MGH 1	CY 60 mg/kg × 2, rituximab, TI, anti-CD2, CNI	BMC	Mismatched	Mixed transient	10	0	0	3	4	17	Transient AKI (9)
MGH 2	CY 22.5 mg/kg × 2, rituximab, Fludara 10 mg/m^2 × 3, TI, anti-CD2, CNI	BMC	Mismatched	Mixed transient	2	2	0	0	0	0	None
Samsung Medical Center	CY 22.5 mg/kg × 2, Fludara10 mg/m^2 × 4, rituximab, rATG, CNI	BMC	Mismatched	Mixed transient	19	6	2	3	0	7	GVHD (1), PTLD (2)

AKI, acute kidney injury; BMC, bone marrow cell; CD, cluster of differentiation; CNI, calcineurin inhibitor; CS, corticosteroid; CY, cyclophosphamide; FSGS, focal segmental glomerulosclerosis; Fuldara, fuuldarabine; GVHD, graft-vs-host disease; IgAN, IgA nephropathy; IS, immunosuppression; Full, full (>98%) whole blood/T-cell chimerism; MGH, Massachusetts General Hospital; MMF, mycophenolate mofetil; mTOR, mammalian target of rapamycin; PBSC, peripheral blood stem cells; PTLD, posttransplant lymphoproliferative disease; rATG, rabbit antithymocyte globulin; TBI, total body irradiation; TCR, T-cell receptor; TI, thymic irradiation; TLI, total lymphoid irradiation; Treg, regulatory T cell; Tx, transplantation Zoster, high initial incidence of single dermatome zoster reduced with prolonged acyclovir prophylaxis.

TABLE 2.
IS minimization

Institution/ trial	Protocol	BMC PBSC Treg	HLA mismatch	Chime-rism	Partici-pants (N)	IS taper in progress	Off IS 1–5 y	Off IS 5–10 y	Off IS >10 y	Longest off IS (y)	Adverse events
Northwestern	Alemtuzumab induction (30 mg IV d 0 and 4) mTOR conversion	Treg	Mismatched	N/A	9	9	N/A	N/A	N/A	N/A	None
Medical University of Vienna (Trex001)	Polyclonal recipient Treg (1.5×10^7/kg) anti–IL-6R, ATG belatacept, sirolimus, CS IS taper after 6 mo	Donor BMC Recipient Treg	Mismatched	Mixed tran-sient	5	3	N/A	N/A	N/A	N/A	
STEADFAST Trial	HLA A2 CAR-Treg	Treg	Mismatched	N/A	1	N/A	N/A	N/A	N/A	N/A	None
TWO Study	No induction, Treg d5, Tac reduction from w 38, MMF withdrawal w48.	Treg	Mismatched	N/A	32	4	N/A	N/A	N/A		None

ATG, antithymocyte globulin; BMC, bone marrow cell; CAR-Treg, chimeric antigen receptor-engineered regulatory T cell; CS, corticosteroids; IL, interleukin; IS, immunosuppression; MMF, mycophenolate mofetil; mTOR, mammalian target of rapamycin; N/A, the goal is to minimize immunosuppression; PBSC, peripheral blood stem cell; Treg, regulatory T cell; w, posttransplant week; TWO, Transplantation Without Overimmunosuppression; STEADFAST, Safety & Tolerability Study of Chimeric Antigen Receptor T-Reg Cell Therapy in Living Donor Renal Transplant Recipients.

recipient Treg with donor BMT.[10] Dr Katharina Schreeb (Safety & Tolerability Study of Chimeric Antigen Receptor T-Reg Cell Therapy in Living Donor Renal Transplant Recipients [Steadfast] Trial, Sangamo Therapeutics) described their trial using HLA-A2–specific chimeric antigen receptor-engineered Treg, and Fadi Issa (Oxford U) described the Transplantation Without Overimmunosuppression Study, in which Treg are infused without induction therapy.[11]

TOLERANCE TRIALS: LIVER

Angus Thomson (U of Pittsburgh) reported on a phase I/II trial of pretransplant infusion of donor-derived regulatory dendritic cells in living donor liver transplantation (LTx) recipients. Fifteen patients received regulatory dendritic cells 1 wk before transplant[12] under triple IS. Eligible participants underwent ISW based on 1-y protocol graft biopsy. Three of 13 enrolled patients have remained off all IS for >1 y so far, with clean 1-y biopsies.

Qizhi Tang (University of California, San Francisco) summarized their decade-long Treg therapy experiences demonstrating safety, persistence, and stability of Treg after infusion. She reported on the recently completed phase I/II trial ("Donor Alloantigen Reactive Tregs (darTregs) for Calcineurin Inhibitor (CNI) Reduction [ARTEMIS]"), designed to assess the ability of donor-specific Treg to enable IS minimization 2 to 6 y following living donor LTx. Safety and persistence of infused Treg were demonstrated, but poor Treg expansion precluded sufficient enrollment to assess efficacy. Generalized Treg activation, senescence, and selective reduction of donor reactivity after LTx may be responsible for the manufacturing challenge. Functional evidence for loss of donor-reactive conventional CD4 and CD8 T cells[13] confirms alloreactive T-cell receptor (TCR) tracking studies demonstrating deletion of donor-reactive clonotypes.[14]

Sandy Feng (UCSF) reported on ISW trials in children with LTx. Significant proportions of eligibility biopsies show subclinical inflammation with interface activity and a transcriptional profile of attenuated T cell–mediated rejection.[15] Among subjects who successfully discontinue IS and meet biochemical criteria for operational tolerance, some biopsies fail histological criteria for tolerance.[16] Two novel predictors of failed ISW have emerged: (1) de novo donor-specific antibodies during IS reduction[16,17] and (2) an increased number of immunological synapses between antigen-presenting cells and lymphocytes[16,18] in the eligibility biopsy.

Josh Levitsky (Northwestern University) reported results of the Evaluation of Donor Specific Immune Senescence and Exhaustion as Biomarkers of Tolerance Post Liver Transplantation (OPTIMAL) trial (ITN056ST; PI: J. Markmann), a multicenter, prospective, open-label, noncontrolled study in which 61 liver recipients underwent gradual ISW without immunomodulation. Patients expected to have a >50% rate of ISW success based on age and time posttransplant in a prior study[19] were recruited. Only 16% achieved full ISW without rejection, comparable with results of the Liver Immunosuppression Free Trial (LIFT) in Europe (13/80 successfully withdrawn; A. Sanchez-Fueyo et al, unpublished personal communication, April 28, 2022). These data, along with the A-WISH study,[20] support the need for immunomodulatory strategies to achieve full ISW. James Markmann (MGH) reported on the generation of donor-specific Treg generated in mixed lymphocyte reaction cultures supplemented with belatacept.[21] Three patients in The ONE Study KTx trial and 3 in the "Liver Transplantation With Tregs at MGH (LITTMUS)" LTx trial received Treg. All 3 recipients of Treg early post-KTx have successfully been weaned to tacrolimus monotherapy by 11 mo and remain rejection-free now for >6 y posttransplant. Notably, each patient demonstrated Treg-rich aggregates on biopsy, suggesting homing of the donor-specific Treg to the graft. In the "LiTTMUS" trial, Treg were given for >6 mo posttransplant, following lymphodepletion by cytoxan. Of 36 consented patients, 19 failed screening criteria, often because of the unavailability of splenocytes from deceased donors. Three patients have received cell infusions, of whom 2 have been weaned but later returned to IS. The third patient was successfully weaned <1 y ago.

Alberto Sanchez-Fueyo (King's College London) provided an update on 3 clinical trials of ISW in stable

LTx recipients. The biomarker-guided "LIFT" ISW trial in patients for >3 y posttransplant has just been completed. "Low-dose IL-2 for Treg Expansion and Tolerance (LITE)" explored the capacity of low-dose interleukin-2 given 2 to 6 y posttransplant to promote Treg expansion in liver recipients on tacrolimus monotherapy. Although low-dose interleukin-2 effectively expanded circulating Treg, it did not promote their trafficking to the transplanted liver but primed the allograft for rejection, which led to the trial's early termination.[22] "Safety and Clinical Activity of QEL-001 in A2-mismatch Liver Transplant Patients (LIBERATE)" is an ongoing phase I/IIa trial sponsored by Quell Therapeutics that explores the safety and biological activity of anti–HLA-A2 chimeric antigen receptor-engineered Treg[23] in patients 1 to 5 y posttransplant.

TOLERANCE TRIALS: OTHER ORGANS

Tomoaki Kato (Columbia University) summarized mechanistic studies suggesting that the infusion of donor $CD34^+$ cells into intestinal transplantation (ITx) recipients at the time of peak lymphohematopoietic graft-versus-host response (LGVHR) might lead to durable mixed chimerism and tolerance in ITx recipients.[24–27] In a pilot study (Ossium Health), 1 patient received donor CD34 cell infusion 11 d following transplantation and demonstrated high levels of multilineage peripheral blood chimerism.

Paul Szabolcs (University of Pittsburgh) reported preliminary studies in patients with primary immunodeficiency diseases and pulmonary failure who received donor BM following bilateral orthotopic lung transplantation. Following a previous successful 2010 index case, 6 patients received BMT subsequent to deceased donor lung transplants. Three of the 6 recipients are alive, and 1 patient with persistent mixed chimerism has been off IS for >3 y, demonstrating successful tolerance induction in a challenging disease setting.

TOLERANCE TRIALS: NONHUMAN PRIMATES

Strategies to induce tolerance in nonhuman primate (NHP) allogeneic liver, kidney, and islet transplantation were discussed. Previous data suggested that transient chimerism induces tolerance in approximately 70% of recipients of CKBMT but not in recipients of BMT with nonrenal organ transplants. Achieving durable chimerism without graft-versus-host disease (GVHD) across major histocompatibility complex (MHC) barriers has been a major challenge.

Adam Griesemer (Columbia University) reported long-term survival off IS among NHPs receiving LTx with or without BMT and peritransplant depletion of memory T cells. Dixon Kaufman (University of Wisconsin) showed that belatacept augmented transient mixed chimerism rates in TLI/ATG-treated monkeys receiving haplotype mismatched KTx, although this approach did not improve tolerance induction. Kazuhiko Yamada (Columbia University) demonstrated >365 d of durable multilineage mixed chimerism without GVHD with kidney allograft tolerance following high-dose PBSC transplantation and delayed KTx across haplotype MHC barriers. This first-in-primate achievement involved minimal TBI with a short course of costimulatory blockade and tacrolimus. Thomas Fehr (University of Zurich) presented his work with Tatsuo Kawai using inhibitors of the antiapoptotic protein B-cell lymphoma 2 to promote chimerism with minimal TBI, achieving renal allograft tolerance in 5 of 6 monkeys. Bernhard Hering (University of Minnesota) reported long-term tolerance of MHC class-I disparate islet allografts in NHPs using a nonchimeric tolerance strategy involving peritransplant infusion of apoptotic donor leukocytes under short-term anti-CD40, rapamycin, etanercept, and tocilizumab treatment.

IMPROVING HEMATOPOIETIC STEM CELL TRANSPLANTATION IN PATIENTS WITH MALIGNANCIES

Thomas Spitzer (MGH) presented his experience with CKBMT in patients with hematologic malignancies and end-stage renal disease in HLA-identical and, with posttransplant CY (PTCy), haploidentical donors. This approach can attack the malignancy through a graft-versus-tumor effect while permitting KTx that would otherwise be precluded because of the underlying malignancy. Immune tolerance has been achieved with transient or durable chimerism.

Ephraim Fuchs (Johns Hopkins University) discussed the use of PTCy to control GVH and host-versus-graft (HVG) responses after partially HLA-mismatched (HLA-haploidentical)–related donor PBSC or BMT. The PTCy platform was successful in the treatment of hematologic malignancies and nonmalignant hematologic disorders. For nonmalignant indications, the combination of pretransplantation rabbit ATG and PTCy achieved durable engraftment with <10% acute or chronic GVHD, suggesting an approach to achieving tolerance to solid organ allografts.

Ran Reshef (Columbia University) focused on strategies to modify lymphocyte migration for GVHD prevention by blocking chemokine receptors such as C-C motif chemokine receptor 5, which seems to reduce visceral GVHD. Whereas short-term C-C motif chemokine receptor 5 blockade did not achieve statistically significant benefit in a prospective randomized trial, further studies suggested that extended administration may be advantageous. Trials of integrin blockade and fingolimod were also discussed.

Everett Meyer (Stanford University) presented updates on the development of Treg-based therapies for the prevention of GVHD. Prophylactic Treg administration improved GVHD- and relapse-free survival compared with contemporaneous controls.

MECHANISTIC STUDIES AND IMMUNE MONITORING

Megan Sykes (Columbia University) summarized a TCR sequencing–based method identifying and tracking alloresponses. Long-term tolerant renal recipients showed the deletion of donor-specific T cells[28] and early expansions of circulating donor-specific regulatory T cells.[29] TCR tracking in both the GVH and HVG directions has provided evidence that LGVHR promotes multilineage chimerism in ITx recipients while attenuating HVG-reactive T cells.[24,26,27] Single-cell RNA sequencing demonstrated the effector function of GVH-reactive T cells migrating to recipient BM, demonstrating LGVHR in situ.

Manikkam Suthanthiran (Cornell University) described how urine provides a glimpse into the status of the entire renal allograft. Urine-based mRNA profiling studies have demonstrated that (1) increased abundance of granzyme-B mRNA and perforin mRNA is associated with rejection; (2) increased urinary cell FOXP3 mRNA correlates with successful resolution of rejection; (3) a urinary cell 3-gene signature of T-cell CD3ε chain, interferon-gamma-inducible protein 10, and 18s rRNA is diagnostic of subclinical and clinical cellular rejection; and (4) increased urinary CD20 mRNA and increased ratios of cytotoxic T-lymphocyte–associated protein 4 to granzyme-B mRNA are associated with kidney allograft tolerance.[30,31]

James Mathew (Northwestern University) summarized studies addressing the hypothesis that sustained and sequential immunoregulation, anergy, exhaustion, and senescence may lead to deletional tolerance.[32] He shared preliminary data suggesting increased donor-reactive T cells in blood and urine as diagnostic/predictive of rejection.

Emmanuel Zorn (Columbia University) discussed what alloantibodies "really" recognize. Donor HLA and minor histocompatibility antigens and self-antigens are all targets of antibodies associated with graft loss.[33,34] Antibodies secreted by graft-infiltrating plasma cells during cardiac allograft vasculopathy recognize chemical modifications on self-macromolecules. These studies underscore the complexity of B-cell immunity to solid organ transplants.

NATIONAL INSTITUTES OF HEALTH UPDATES

Drs Bridges, Chandran, and Shaw (National Institutes of Health) presented updates from the National Institute of Allergy and Infectious Diseases Clinical Trials in Organ Transplantation, the Immune Tolerance Network, and the NHPCSG, respectively. Clinical Trials in Organ Transplantation in Children and Adults awards in 2021 included investigations of drug minimization, tolerogenic strategies, and reduction of IS-related morbidity in adults and children. The Immune Tolerance Network update focused on the design of future adaptive platform trials of LTx tolerance. The Nonhuman Primate Transplantation Tolerance Cooperative Study Group aims to develop safe and effective transplant tolerance protocols in preclinical NHP models. Projects supported through this consortium have led to the development or refinement of approaches used in multiple clinical trials.

CONCLUSIONS AND FUTURE DIRECTIONS

The meeting underscored the rapid progress being made in understanding tolerance and rejection, developing cell therapy and biologic approaches, and bringing them to the clinic. The authors look forward to the next iteration of this workshop, anticipated in 2024, to discuss further advances in cell therapy and other novel modalities to facilitate ISW and durable hematopoietic chimerism across HLA barriers.

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