Special Feature

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Meeting Report: The Sixth International Sam Strober Workshop on Clinical Immune Tolerance

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INTRODUCTION

The Clinical Tolerance Workshop series was established by the late Dr Sam Strober and colleagues with the goal of bringing together investigators working in the field of transplantation tolerance. For the first time, the meeting was held outside the United States. The Sixth International Workshop was run from the March 25–26, 2024 at Exeter College, University of Oxford. International world-leading scientists and clinicians gave updates on their ongoing research into tolerance induction through cell therapies and chimerism, with the goal of immunosuppression (IS) withdrawal. Invited speakers from the field of autoimmunity gave insights into the translational intersections between transplant tolerance and autoimmune regulation. Given the nature and length of these trials, regular dissemination is crucial for current investigators designing trials in

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the field. This report provides a comprehensive summary of these ongoing efforts.

CHIMERISM TRIALS

Table 1 provides a summary of ongoing chimerism trials. Complete withdrawal from pharmaceutical IS and achieving long-term tolerance are the ultimate aims of combining hematopoietic chimerism with solid organ transplantation. Different patient-conditioning protocols give rise to hematopoietic chimerism that can be transient or persistent, mixed where recipient and donor cells coexist, or full when the hematopoietic cells are completely donor derived.

The Massachusetts General Hospital protocols require initial conditioning with cyclophosphamide, thymic irradiation, the anti-CD2 monoclonal antibody siplizumab, with rituximab for combined kidney and bone marrow transplant (CKBMT), followed by progressively tapered standard IS. This results in a transient mixed chimerism that lasts on average a few weeks. Siplizumab is an antibody that depletes T cells through antibody-dependent cellular cytotoxicity, while relatively sparing regulatory T-cell (Treg) populations important for immune regulation.[1,2] This results in a transient mixed chimerism. Leonardo Riella described the current revision of their conditioning protocols to reduce the rate of chimerism transition syndrome (CTS). This immunologic event, characterized by severe acute kidney injury, fevers, loss of chimerism, and reconstitution of the recipient's immune system, typically occurs 2 wk after transplantation.[3] The 2022 revision adds fludarabine, reduces the dosage of cyclophosphamide and removes posttransplant doses of rituximab (NCT04540380). This protocol continues to establish mixed chimerism with only 1 of 8 patients developing CTS, without any graft loss. The interval between cyclophosphamide treatment and hemodialysis also impacted white blood cell depletion and the degree and duration of transient chimerism (unpublished).

Joshua Weiner (Columbia University) introduced the PANORAMA trial (A Study of TCD601 in the Induction of Tolerance in Renal Transplantation) (NCT04803006), which similarly aims to reduce the incidence of CTS through modifications to previous CKBMT protocols. The updated regimen deepens T-cell depletion and broadens the period of depletion by adding mycophenolate mofetil and moving siplizumab dosing earlier in the pre-transplant period, with the goal of flattening the curve of recipient immune reconstitution. Results have thus far been positive. The first 3 patients have been fully weaned off IS with good graft function and no evidence of rejection, with all showing early chimerism and early enrichment in circulating Treg. One patient had a decline in donor responsive T-cell clones, with data on the remaining patients awaited. A fourth patient is weaning, and 2 more pairs are being planned. There have been no significant episodes of CTS, with 2 patients experiencing mild episodes marked by borderline elevated creatinine and elevated serum IL-6 without any other signs or symptoms. All episodes resolved without long-term sequelae after a single dose of tocilizumab. The mechanisms underlying the tolerance facilitated by transient chimerism after CKBMT are not fully understood. High throughput T-cell receptor (TCR) sequencing has allowed the identification of donor-reactive T cells pre-transplantation, which can then be tracked in the posttransplant period.[4] Gradual deletion of preexisting donor-reactive T cells following an early expansion of donor-specific Treg, also identified by a TCR tracking mechanism, was shown to be mechanisms involved in the tolerance of CKBMT recipients.[5] Megan Sykes (Columbia University) discussed mechanistic studies looking to identify donor reactive T-cell effector and Treg clones and track these cells in the circulation and graft.

At Stanford, the CKBMT approach instead results in persistent mixed chimerism through conditioning with antithymocyte globulin (ATG) and total lymphoid irradiation (TLI) at kidney transplantation, followed by tapering standard IS and a later bone marrow transplant (BMT). Stephan Busque summarized the outcomes of 74 matched and mismatched patients that have been treated with a TLI- and ATG-based conditioning protocol for CKBMT since 2005.[6] The 5-y survival rate was 97% and 94.7% for patients and grafts, respectively. The matched protocol resulted in >80%

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TABLE 1.

Complete IS withdrawal (tolerance)

Institution/trial	Protocol	BMC PBSC Treg	HLA mismatch	Chimerism	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 *dosed	0	26 (72%) *unknown how long			12	GvHD (2)
Talaris Phase III FCR001 FREEDOM-1 trial	CY 50 mg/kg pre- and post-Tx, Fludara 30 mg/m^2 × 3, TBI 200 cGy	PBSC Facilitating cells, αβTCR + T cells	Haplo-matched or mismatched	Full or mixed persistent	9 dosed	0	6	0	0	3	Severe GvHD (1) Infection/death (1)
Northwestern	Alemtuzumab induction (30 mg IV d 0 and 4) mTOR conversion	BMC/PBSC CD34 selected	Matched	Transient	15	0			6	13.5	Disease recurrence (3) FSGS/IgA nephropathy
Stanford 1	TLI (1200 cGy) + rATG	PBSC CD34$^+$ T cell 10^6/kg	Matched	Mixed persistent or transient	29	0	0	4	13	16	Zoster (8)
Stanford 2	TLI (1200 cGy) + rATG	PBSC CD34$^+$ cells + T cells 3–150 (×10^6/kg)	Haplo-matched	Mixed persistent	27	0	0	0	0	—	Zoster (5) Transient AKI (2) Haemolytic anemia (1)
Stanford 3	TLI (1080 cGy) + TBI (40–80 cGy) + rATG	PBSC CD34$^+$ cells + T cells 50 (×10^6/kg)	Haplo-matched	Mixed persistent	6	3	1	0	0	3	Acute GvHD (1) Skin, grade 2 (resolved)
Zurich, Tel Aviv ad UCLA *Stanford protocol	TLI (1200 cGy) + rATG	PBSC CD34$^+$ T cell 10^6/kg	Matched	Mixed persistent or transient	17	—		16		—	
UCLA 1	Delayed tolerance. TL1 + rATG	PBSC CD34$^+$ T cell		Mixed persistent or transient	3	1		2		—	
Stanford Paediatric	TBI (200 cGy) + rATG + Rituximab + Fludarabine + Cyclophosphamide	Ab T cell and CD19 B depleted PBSC + T cells	Haplo-matched	Full or mixed persistent	6	—		5		—	GvHD *Not stated for every patient
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	6	2	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)

Continued next page

TABLE 1. (Continued)

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
MGH 1	CY 60 mg/kg × 2, rituximab, TI, anti-CD2, CNI	BMC	Haplo-matched or mismatched	Mixed transient	10	0	0	7	7	17	Transient AKI (9)
MGH 2	CY 22.5 mg/kg × 2, rituximab, Fludara 10 mg/m² × 3, TI, anti-CD2, CNI	BMC	Haplo-matched or mismatched	Mixed transient	8	3	0	0	0	0	Transient AKI (1)
Columbia PANORAMA trial	CY 60 mg/kg × 2, rituximab x4, Fludara 10 mg/m² × 3, TI, anti-CD2, CNI, MMF	BMC	Haplo-matched	Mixed transient	4	1	3	0	0	0.42	Transient AKI (2)
Samsung Medical Center	CY 22.5 mg/kg × 2, Fludara10 mg/m² × 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, fuludarabine; Full (>98%) whole blood/T-cell chimerism; GvHD, graft-versus-host disease; IgAN, IgA nephropathy; IS, immunosuppression; 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; UCLA, University of California, Los Angeles; Zoster, high initial incidence of single dermatome zoster reduced with prolonged acyclovir prophylaxis.

of patients withdrawn from their IS and 65% remaining off IS for 5–16 y. The TLI-ATG protocol applied to haploidentical patients resulted in only 40% of patients with sustained chimerism of more than a year and reduction of IS to monotherapy of tacrolimus. The recent addition of a single low dose of total body irradiation (TBI) resulted in high sustained chimerism in 5 of 6 patients with 2 patients off IS for 3 y and 5 mo, respectively, and 3 patients in taper. The Stanford human leukocyte antigen (HLA) matched protocol has now been used in Zurich, Tel Aviv, and at the University of California Los Angeles with similar results.

This protocol has been adopted by Medeor Therapeutics, presented by Daniel Brennan, in their Phase III multicenter randomized trial (NCT03363945) on the safety and efficacy of the cell product, MDR-101 (donor bone marrow enriched for $CD34^+$ cells and adjusted for T-cell content), in the induction of functional tolerance in HLA-matched CKBMT. As of March 2024, 19 out of 20 infused patients developed chimerism and 15 patients had been weaned from IS for at least 2 y. Acute rejection was observed in 2 patients, but no cases of de novo donor-specific antibodies (dnDSA), graft-versus-host disease (GvHD), or graft loss were observed. Jeffrey Veale from University of California, Los Angeles described their experience in successfully weaning 6 HLA-matched patients from IS after simultaneous CKBMT, but also in adapting this protocol to establish the first cases of delayed tolerance (NCT05525507) in 3 patients who received a BMT 1–5 y postkidney transplant (KTx).

Alice Bertaina (Stanford University) applied a new protocol combining ab T-cell depletion of peripheral blood stem cells (PBSC) collected from haploidentical donors, a reduced intensity conditioning regimen, and no GvHD pharmacological prophylaxis for CKBMT for pediatric patients with Schimke immuno-osseous dysplasia (SIOD). The 3 patients achieved full donor chimerism and a functional tolerance that has enabled IS-free survival for at least 42 mo after KTx.[7] This success has instigated a Phase Ib/IIa trial (NCT05508009) in pediatric patients with SIOD, cystinosis, focal segmental glomerulosclerosis, systemic lupus erythematosus nephritis and chronic kidney disease Stage 4 of CKBMT to tackle the inadequacy of lifelong IS to enable allograft survival in children.

Ephraim Fuchs presented work from ImmunoFree, Inc., who took over sponsorship of the terminated FREEDOM-1 study (NCT03995901) from Talaris Therapeutics in 2023. The Phase III trial was halted early due to perceived excessive rates of GvHD among the first 9 patients. A root cause analysis identified only one clear case of severe GvHD among the 9 patients and suggested that the degree of HLA mismatch between donor and recipient and the doses of $CD34^+$ and $TCR\alpha\beta^+$ cells in the donor hematopoietic stem cell graft were associated with major outcomes including GvHD and achievement of tolerance. ImmunoFree, Inc. is developing a Phase II single-arm, multicenter study (the "RELIEF" trial) of a re-designed engineered cell product, IF001, to establish sustained hematopoietic chimerism and delayed tolerance in prior recipients of kidneys from partially HLA-matched (≥4/8 HLA) living donors.

Northwestern University has conducted a clinical trial designed to establish persistent donor chimerism in mismatched donor/recipient pairs using a manipulated donor stem cell graft and nonmyeloablative conditioning with a regimen of fludarabine, cyclophosphamide and TBI before

CKBMT, posttransplant cyclophosphamide, and tapered IS. Joseph Leventhal presented that in hematopoietic chimerism-based regimens for IS withdrawal, analysis of HLA molecular mismatches revealed that patients who were able to wean off all IS had lower HLA-DRB1 3-dimensional electrostatic mismatch scores in the host versus graft direction.[8] Long-term (>15 y) follow-up has demonstrated improved renal function and fewer metabolic and infectious complications in tolerant subjects as compared with well-matched controls. Single-cell RNA-seq analysis demonstrated that immune cell populations were highly heterogenous among patients participating in the trial, while proteoforms (different molecular forms of a protein arising from a single gene) differed between donor and recipient peripheral blood mononuclear cells (PBMC) in tolerant patients. A separate Phase 1 clinical trial to investigate the use of Treg and hematopoietic mixed chimerism to promote donor engraftment and kidney organ tolerance in collaboration with Stanford University is ongoing. A new Phase 1 trial to explore the safety and efficacy of extracorporeal photopheresis (ECP) treated cell infusion and posttransplant ECP for the prevention of rejection in living donor kidney transplantation (LDKT) was presented.

REGULATORY T-CELL TRANSFER THERAPIES

Table 2 provides a summary of ongoing cell therapy trials. Qizhi Tang (University California San Francisco) reported outcomes from the Treg Adoptive Therapy in Subclinical Inflammation in Kidney Transplantation (TASK) study, a multicenter randomized controlled trial investigating the safety and efficacy of autologous, polyclonal regulatory T cells (apTreg) to reduce subclinical inflammation detected during surveillance biopsies (NCT02711826). Sixteen subjects were randomized and 7 out of 8 subjects in the cell therapy group received Treg infusions. No safety concerns were detected. There were no differences in the primary efficacy endpoint, defined as the percentage change in inflammation in the kidney cortex on repeat biopsy at 6 mo with both treatment arms showing a similar decrease from the enrollment baseline. Of note, the cell manufacturing process was modified by pre-enriching for CD25 cells using magnetic-activated cell sorting to fluorescence-activated cell sorting purification for the $CD4^{+}CD127^{lo/-}$ Treg, significantly increasing the yield of apTreg.

Koichiro Uchida (Juntendo University) presented long-term follow-up data from a previous pilot study aimed to induce tolerance using induced T cells with suppressing functions, which is a costimulatory blockade induced, donor antigen reactive autologous CD4 and CD8 Treg cell-based product, in living donor liver transplantation (LDLT).[9] Three of 10 patients developed mild acute cellular rejection during IS weaning and were maintained on conventional low-dose IS, while 7 patients remained IS-free for 5.4–10.4 y. An ongoing Phase I/II multicenter study using the same protocol has now completed recruitment and IS weaning is ongoing for the majority of the 10 recruited patients (NCT04950842). Preliminary analyses of liver biopsy samples demonstrated the formation of lymphoid-rich cellular aggregates which are positive for Foxp3 and highlighted the difficulty in grading these biopsies using current Banff criteria.

Thomas Wekerle (Medical University of Vienna) reported interim outcomes of the Vienna Trex001 study (NCT03867617), a single-center phase I/IIa trial with a non-randomized open-label control group.[10] In the trial, unseparated donor bone marrow cells and expanded polyclonal recipient Treg were transferred at day 0–3 post-LDKT without any myelosuppressive recipient conditioning (no irradiation, no cytotoxic drugs). The addition of Treg transfer to a BMT is intended to promote chimerism and allograft tolerance. Six subjects in the cell therapy arm received both cell products without any infusion-related adverse events. One additional subject was withdrawn before Treg administration as the Treg product was out of specification. Total leukocyte and lineage-specific chimerism will be evaluated by droplet digital polymerase chain reaction and flow cytometry. Episodes of early acute T-cell-mediated rejection were observed in 3 patients. Of note, 5 out of 7 subjects in the cell therapy arm who received ATG as monotherapy 2 wk before the kidney transplant developed serum sickness.[11] All patients are doing well at the latest follow-up.

Fadi Issa (University of Oxford) provided an update on the United Kingdom arm of the ONE study (A Unified Approach to Evaluating Cellular Immunotherapy in Solid Organ Transplantation) and the ongoing TWO study (Transplantation Without Overimmunosuppression), which utilizes ex vivo expanded apTreg in LDKT.[12-14] At 7-y follow-up, all 12 patients in the cell therapy group of the ONE study have maintained graft survival. In the TWO study, the initial protocol utilized alemtuzumab induction followed by cell product administration at 6 mo after renal transplantation. However, this protocol was modified to transfer of the cell therapy product at day 5 posttransplantation without induction IS and administration of basiliximab induction in the control group, due to concerns about lymphodepletion in low immunologic risk KTx recipients during the COVID-19 pandemic. No safety concerns or cell therapy-related adverse events such as rejection have been detected to date.[15]

James Mathew (Northwestern University) described modifications in their apTreg cell product manufacturing since the TRACT study (NCT02145325). These included replacing CD3/CD28 microbeads with a CD3/CD28 nanomatrix to avoid cell loss due to bead removal during harvesting and replacing sirolimus with everolimus, which improved cell yield and maintained a stable Treg phenotype.[16,17] There are currently plans for a Phase II study utilizing these cell products in KTx. An optimized protocol for expansion of donor-specific Treg by sequential stimulation of Treg with expanded donor B cells on day 0 and 7, then polyclonal stimulation on day 14, was also described and a Phase 1 study using these cell products is currently being planned.[18]

Giovanna Lombardi (Kings College, London) gave an overview of the work being undertaken in collaboration with her team. The GAMECHANgER-1 study (EudraCT 2021–001,664-23) will recruit 21 pre-sensitized patients due to receive a KTx for polyclonal Treg infusion, with the primary endpoint being the of in vitro inhibition of anti-HLA T and B-cell responses.[19] In the planned ATT-Heart study, 9 children requiring heart transplantation will be recruited to receive autologous thymic Treg cells, with the aim of preventing cardiac allograft vasculopathy.[20] In this phase 1 trial, there is no plan to taper current standard of care IS doses, as the main aim is to

TABLE 2.

Ongoing cell therapy clinical trials for transplantation

IS minimization/ withdrawal

Study	Setting	Cell product and protocol	Recruitment target/ recruited/ completed study/ ongoing follow-up	IS taper in progress	IS taper completed	Other results	Adverse events in CTG
Juntendo University	Multicenter single-arm study HLA-mismatched LDLT	D5: cyclophosphamide D13: co-stimulatory blockade induced, donor antigen reactive autologous CD4 & CD8 Treg based product x1 dose 0–1 mo: corticosteroids/ MMF withdrawal 6–18 mo: CNI weaning	10/ 10/ 1/ 9	9	1	Nil	Elevated liver enzymes (n = 3)
Medical University of Vienna (Trex001)	Single-center intervention arm with nonrandomized control group HLA-mismatched LDKT	D-14: ATG D0 to 3: apTreg with donor BM cells D0 to 21: Tocilizumab x4 doses 6–9 mo: Rapamycin withdrawal 9–12 mo: corticosteorids withdrawal From 12 mo: belatacept monotherapy with interval spacing (up to 8 wk) vs SOC with ATG induction D0, no IS reduction	12/ 12/ 9/ 2	3	2 (belatacept mono-therapy)	3 rejections	None
University of Oxford (TWO study)	Single-center RCT LDKT	No induction D5: apTreg x1 dose 0–14 wk: CS withdrawal 0–48 wk: MMF withdrawal From week 48: tacrolimus monotherapy vs SOC with basiliximab induction	68/ 44/ —/ —	—	—	Nil	None
University of Pittsburgh	Single-center; single-arm study LDLT	Week -1: donor-derived DCreg x1 dose No basiliximab induction D0-7: Corticosteroid withdrawal 6–12 mo: MMF withdrawal 12–18 mo: Tacrolimus withdrawal	16/ 15/ 12/ 1	0	4 (of 8 patients eligible for weaning)	3 of these 4 patients off all IS for > 2. 5 y; 1patient resumed IS after BPAR at 8. 5 mo off all IS	None
University of Pittsburgh	Single-center; single-arm study Stable LDLT patients, 1–3 y post-Tx	Donor-derived DCreg; From D7 postinfusion: begin immunosuppression withdrawal	16/ 15/ 3/ 12	0	5	4 of these 5 patients off all IS for 1.5–3 y; 1 patient resumed IS after 1 y off all IS	None

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TABLE 2. (Continued)

IS minimization/ withdrawal							
Sangamo Therapeutics (STEADFAST)	Multicenter LDKT	Autologous chimeric antigen receptor Treg (TX200)	14/11/2/9	—	—	Prelim observation of TOL like structures in kidney biopsy at dose levels 3 and 4	None considered related to TX200
Quell Tx (LIBERATE)	Multicenter 1-5 y postliver Tx	Autologous chimeric antigen receptor Treg (QUEL-001)	18/—/—/—	—	—	Confirmed CAR Treg in liver biopsy at 28 d	No drug related AEs noted
GOSH/ Kings(ATT-Heart)	Single-center pediatric heart transplant	Thymic derived Treg	9/0/0/0 Not yet started, awaiting MHRA approval	No IS taper planned	NA	NA	NA
Kings (GAMECHAN gER-1)	Multicenter sensitized patients post HLA-Ab incompatible kidney Tx	Autologous polyclonal Treg	21/2/0/0	No IS taper planned	NA	None to date	No drug related AEs noted
Nantes University Hospital (EIGHT TREG Study)	Single-center LDKT	No induction D-1 : Autologous CD8+/CD45RC low/- Treg Standard maintenance – corticosteroid, tacrolimus and MPA	9/0/0/0 Recruitment not started yet	NA	NA	NA	NA
Northwestern University (ELIMINATE)	Multicenter Liver Tx	Standard immunosuppression + everolimus, aim to wean one arm to everolimus monotherapy	340 ?started				NA
MGH (PANORAMA)	Single-center CKBMT HLA mismatch	Standard MGH protocol for CKBMT, except earlier siplizumab	?/4/?/?	3	1	Transient chimerism in all Treg enrichment at 6 mo	NA
Treatment of subclinical rejection							
Study	Setting	Cell product and protocol					
University California San Francisco (TASK)	Multicenter RCT Stable graft function with subclinical inflammation on kidney allograft 6-mo surveillance biopsies	D41 postbiopsy: apTreg x1 dose ±conversion to everolimus vs SOC (no additional treatment)	16/ 16/ 12/ 3	NA	NA	No difference in primary endpoint (% change in inflammation at D226 repeat biopsy)	None
Charité – Universitätsmedizin Berlin (ProTreg21)	Single-center	Treatment group: 2 doses ATG, Treg02 cell product infusion (day 18 ± 2) postdeceased donor kidney transplantation immunosuppressive triple therapy gradually tapered Control group: 2 doses of ATG, Standard immunosuppressive triple therapy	18/ 0/ recruitment started/ 0	0	0	0	0
Charité – Universitätsmedizin Berlin (TregTacRes)	Single-center	TregTacRes in living donor kidney transplant recipients and gradual tapering of standard triple immunosuppressive therapy to low-dose tacrolimus monotherapy	24/ 0/ 0/ 0 Recruitment not started yet	0	0	0	0

Ag, antigen; apTreg, autologous polyclonal regulatory T cells; ATG, anti-thymocyte globulin; BPAR, biopsy-proven acute rejection; CKBMT, combined kidney and bone marrow transplant; CTG, cell therapy group; IS, immunosuppression; LDKT, living donor kidney transplant; LDLT, living donor liver transplant; NA, not applicable; RCT, randomized controlled trial; SOC, standard of care.

establish feasibility of generating adequate doses of the Treg product for each child from their thymus. In addition, they plan to determine safety of the expanded Treg product at escalating doses when given to children at 3 mo posttransplant.

NEXT-GENERATION REGULATORY T-CELL THERAPIES

There is increasing interest in the generation of donor antigen-specific chimeric antigen receptor (CAR) Treg to enhance the specificity of immune regulation towards the donor organ.[18] Katharina Schreeb (Sangamo Therapeutics) provided an update on the STEADFAST trial (NCT04817774), a Phase I/II trial of HLA-A2-specific CAR Treg (TX200; autologous product) in KTx. At the time of the datacut, there were 11 patients recruited, 8 to treatment, and 3 controls. Of these, 6 had been treated at the time of the cutoff. Following the dosing of the first 3 patients, the initial 3 + 3 protocol was switched to a Bayesian Design, allowing for dosing at levels 18x higher than the starting dose level. No treatment-related adverse events have been noted as of December 2023, including no evidence of increases in cytokines or cytokine release syndrome. Most nonserious adverse events were grade 1–2. There are preliminary observations of Treg-rich organized lymphoid structures in the biopsies of patients (n = 2) treated with higher doses of TX200.

Luke Devey and Marc Martinez-Llordella from Quell Therapeutics reported on the LIBERATE trial (NCT05234190), a Phase I/II trial of HLA-A2-specific CAR Treg in liver transplantation. HLA-A2$^-$ patients who have received an HLA-A2$^+$ liver transplant within the past 1–5 y are being recruited. Autologous Treg are isolated, expanded, and then transduced with the CAR. Initial analysis has found the CAR Treg in the peripheral blood and the liver donor biopsy at 28 d posttreatment. There were no drug-related adverse events and recruitment is ongoing to meet the 18-patient target.

While initial Treg therapies have good safety profiles and hints for efficacy in clinical trials, there are still challenges in establishing their efficacy in all patients, such as Treg sensitivity to concurrent IS and their decreased effectiveness in patients with preformed effector T cells.[21,22] Petra Reinke's (Charité, Berlin) group has developed a tacrolimus-resistant Treg, using CRISPR-Cas9 technology to knockout FKBP-12, which is currently entering a First-in-Class clinical trial. Further development work towards a new product has demonstrated that better depletion of effector memory Treg from the starting material can lead to a more stable Treg phenotype. Another topic is to redirect the specificity of the Treg by modifying the CD3 epsilon gene to induce physiological rather than tonic signaling. In addition, a novel strategy has been developed to overcome the issue of alloimmunogenicity for off-the-shelf Treg products.

Carole Guillonneau (Nantes University) gave an update on her team's work to translate a CD8$^+$ Treg therapy in the clinic. Her team has previously demonstrated effectiveness of CD8$^+$ Treg in a humanized mouse model of skin graft rejection and GvHD.[23] They have now shared evidence of successful manufacture of functional and high-purity CD8$^+$ Treg Good Manufacturing Practices product and confirmed the feasibility of producing CD8$^+$ Treg from patients with kidney failure. They are now planning a Phase I/II clinical trial to evaluate the safety and efficacy of this product in kidney transplant patients.

DENDRITIC CELL THERAPIES

Angus Thomson (University of Pittsburgh) summarized the outcomes from 2 clinical trials using donor-derived regulatory dendritic cells (ddDCregs) to induce tolerance, either 1 wk before LDLT (NCT03164265) or 1–3 y after transplant in stable LDLT recipients (NCT04208919). No cell product-related adverse events were reported. In patients given ddDCregs before transplant, 4 of 13 patients were weaned off all IS; 3 of these patients remain off all IS for > 2.5 y, with 1 patient developing rejection after 8.5 mo off all IS. Patients given ddDCregs suppressed peripheral Th1 cytokine responses and donor alloreactive effector T cells, as well as reducing the frequency of CD8$^+$ T and NK cells within the liver allografts compared with standard of care LDLT patients.[24] After administration of ddDCregs 1–3 y posttransplant, 5 of 14 patients were weaned off all IS. 4 of these patients remain stable, off all IS for between 1.5 and 3 y. 1 patient resumed standard of care IS after 1 y off all IS.

Aurélie Moreau (University of Nantes) presented their group's experience implementing autologous tolerogenic dendritic cells (ATDC) from preclinical studies to human clinical trials in the ONE study.[25] In this trial of kidney transplantation, no toxicity associated with cell therapy was observed. In the ATDC group, mycophenolate was successfully reduced or stopped in 5 of 8 patients, allowing tacrolimus monotherapy for 2 of them. The immune monitoring highlighted a reduced expression of CD8$^+$ T-cell activation markers and a transient increase of Foxp3 expression in the blood of ATDC-treated patients.[25]

IMMUNOSUPPRESSION WEANING/BIOMARKERS AND MONITORING

Several studies have previously investigated IS weaning in liver transplantation.[26-29] Josh Levitsky (Northwestern University) gave an overview of the field, summarizing that most are single-center, deceased donor liver transplants with a baseline regimen of calcineurin inhibitor IS, and with varied overall success rates.[30] Biomarkers of immune activation versus quiescence are needed to guide which patients can be weaned, by how much, and at which timepoint. There have been a few recent studies in this area, including CTOT-14 which used a 38 gene mRNA signature to identify acute rejection versus stable transplant,[31] as well as studies of donor-derived cell-free DNA to identify early graft injury.[32] Professor Levitsky introduced the new multicentre ELIMINATE study which aims to conduct liver transplant IS minimization with everolimus in 340 patients. Participants will receive a calcineurin inhibitor and everolimus, with a cohort aiming to wean onto everolimus monotherapy.

Studies have shown that there are differences in histology and transcriptional profiles in liver biopsies between tolerant and non-tolerant patients.[33] Jake Demetris (University of Pittsburgh) highlighted the identification of

T-cell/antigen-presenting cell pairs as a predictor for rejection following IS withdrawal in liver transplant patients. This histological observation has implications for the fundamental understanding of alloreactivity. In recent years, the development of next-generation pathology techniques, including spatial transcriptomics, has offered new ways to identify and dissect these potential biomarkers for rejection or tolerance.[34]

Alberto Sanchez-Fueyo (Kings College London) reported on their work to characterize intragraft Treg in healthy livers, chronic liver disease, and hepatocellular carcinoma, comparing these to peripheral blood cells. Initial results found significant differences in the numbers of Treg between these groups. scRNAseq is now being used to characterize these Treg further, noting evidence for compromised Treg in the liver niche in disease, with specific differences in mTOR, TCR signaling, and IL2/STAT5 pathways.

Further work highlighting the importance of analyzing intragraft immune cell populations was presented by Alessandro Alessandrini (Massachusetts General Hospital). It has been recognized that Treg-rich organized lymphoid structures (TOLS) may be found in accepted kidney allografts.[35] Their group has used temporal scRNAseq on accepted murine kidney allografts to identify the composition of TOLS with increased populations of B cells and $CD8^+$ T cells.[36] They noted that while $CD8^+$ T cells were the main component intragraft initially, this decreased over time while they increased regulatory markers. They introduced the concept of defensive tolerance, which they defined as the ability to render infiltrating immune cells anergic or to reprogram them to a regulatory phenotype of cell capable of promoting tolerance.[37]

Treg are thought to have several mechanisms for inducing tolerance, 1 of which is infectious tolerance.[38] Megan Levings (University of British Columbia) reported on a mouse model of islet transplantation,[39] which was chosen so that both autoimmunity and alloimmunity could be studied. In this model, $HLA\text{-}A2^+$ NOD islet cells were transferred to the anterior chamber of the eye in $HLA\text{-}A2^-$ NSG mice. BDC2.5 $CD4^+$ T cells were then transferred either alone or with polyclonal or HLA-A2-specific CAR Treg. The group that received HLA-A2-specific CAR Treg survived for the longest without developing hyperglycemia. Non-diabetic mice had suppressed expansion of BDC2.5 $CD4^+$ T cells. Diabetic control was maintained, even after resection of the eye with the $HLA\text{-}A2^+$ islet transplant. The HLA-A2-specific CAR Treg appeared to convert BCD2.5 T cells to Treg, therefore protecting the endogenous $A2^-$ islets.

Gilbert Fruhwirth (King's College London) described the potential utility of imaging cell-based immunotherapies with techniques including ex vivo cell labeling and reporter gene-afforded cell labeling. While ex vivo cell labeling can accurately quantify the biodistribution of a cell therapy product in the short term, it presents significant challenges when long-term tracking or monitoring is required. In contrast, reporter gene-afforded cell labeling is clinically compatible when used with the correct reporter/imaging agent combination. However, it relies on genetic engineering and therefore, is only considered a viable option for cell products that already require cell engineering for efficacy.[40] Importantly, their laboratory has demonstrated in preclinical studies reporter gene-afforded Treg tracking over a month using a human radionuclide reporter and a matching radiotracer pair that is already clinically translated.[41] Furthermore, they have also demonstrated that CAR-Treg can also be engineered with this toolbox.[42] They now plan a Phase I-clinical trial aimed at demonstrating safety of reporter gene-afforded CAR Treg tracking in humans.

AUTOIMMUNITY

The principles in treating autoimmunity run in parallel to that of alloimmunity as both aim to mitigate deleterious immune responses towards vital tissues identified as "non-self." The remission, control, and assessment of autoimmune responses may provide translatable insights for the field of transplantation.

Piotr Trzonkowski (Medical University of Gdańsk) gave an update on the application of apTreg and next-generation Treg to treat pediatric patients with type 1 diabetes (T1D). Treg-treated patients in the TregVac1.0 trial (ISRCTN06128462) successfully produced more insulin 2- and 5-y postinfusion.[43] To better prevent beta cell loss, patients were selected to have higher insulin levels at the trial start for the TregVac2.0 trial (EduraCT 014-004319-35). Cotreatment of these patients with rituximab and apTreg better-preserved insulin secretion and produced higher remission rates than apTreg alone.[44] Reduced required insulin doses were associated with increased levels of $PD\text{-}1^+$ T cells after therapy, suggesting these cells may be a biomarker of therapeutic outcomes.[45] The preTREG trial (Eudra 2023-505226-33-00) now aims to treat T1D patients at a much earlier stage with polyclonal Treg to have the highest impact on beta cell conservation. The next innovations are antigen-reactive Treg, such as the PolTREG PTG-020 product and TCR-Treg through the ARTiDe program.

Elmar Jaeckel (University of Toronto) described the development of A2-specific CAR Treg engineered to have membrane-bound IL-2. This modification to antigen-specific T cells promoted Treg survival and their resistance to calcineurin inhibitors.

John Isaacs (Newcastle University) explored the concept of reversible autoimmunity in the context of rheumatoid arthritis patients who achieve drug-free remission after acute treatment.[46] These patients were characterized by higher frequencies of Treg in baseline blood samples when compared with patients who develop arthritic flares. The onset of arthritic flares was characterized by increased frequencies of $CD45RO^+PD1^{hi}$ T cells and $CD27^+CD86^+CD21^-$ B cells.[47]

Tim Tree (Kings College London) and his team have developed an activation-induced marker-10x single-cell sequencing workflow for the multiomics assessment of antigen-specific T cells. The value of this approach was demonstrated in the preliminary analysis of samples from the phase II IMPACT trial (NCT04524949) in type 1 diabetes. Islet-specific T cells were transcriptionally distinct from vaccine-specific T cells, and the therapeutic intervention modified the frequency and phenotype of islet-specific T cells.

CONCLUSIONS AND FUTURE DIRECTIONS

The Sam Strober Clinical Immune Tolerance Workshop remains a key meeting in the field. This year, it highlighted the breadth of ongoing clinical trials in both chimerism and cellular therapy. Shared messages underscored the need for refining patient selection methods to identify those most likely to benefit from specific therapies.

It is becoming increasingly clear that deeper analyses of transplant biopsies are essential for gaining insights into therapy effectiveness. In particular, the identification of lymphoid-rich aggregates across different cell therapies in the biopsies of patients who received cell therapies, has drawn attention to the dynamic nature of intratissue immune activity, which is not always an indicator of rejection. How these cellular aggregates contribute to stable clinical outcomes or even tolerance remains unclear. However, they are likely to provide mechanistic insights and could serve as early proxy measures of clinical outcomes, given the challenges of detecting improvements without lengthy follow-up period, especially in small and underpowered patient cohorts.

Technological advances in tissue analysis now allow for the precise mapping of immune populations in situ, offering opportunities to dissect their functional states and interactions. Comparing biopsies across trials and institutions may help identify shared immunologic mechanisms. Additionally, advances in the identification of alloreactive T- and B-cell clones within biopsies will likely add further mechanistic detail, potentially providing early and sensitive measures of treatment effects.

Reflecting on the ONE study, Edward Geissler (University Hospital Regensburg) called for greater international collaboration in the setting up of consensus trials to establish global standards for future cell therapy and tolerance clinical trials, which will be important in defining comparable protocols, outcomes, and acceptable risks for different solid organ transplants as therapies continue to evolve. We anticipate revisiting these critical advances in the next workshop, scheduled for 2026 in Boston, where ongoing trials may shed light on the long-term clinical relevance of these mechanistic insights.

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REFERENCES

1. Podestà MA, Binder C, Sellberg F, et al. Siplizumab selectively depletes effector memory T cells and promotes a relative expansion of alloreactive regulatory T cells in vitro. *Am J Transplant*. 2020;20:88–100.
2. Sprangers B, DeWolf S, Savage TM, et al. Origin of enriched regulatory T cells in patients receiving combined kidney-bone marrow transplantation to induce transplantation tolerance. *Am J Transplant*. 2017;17:2020–2032.
3. Kawai T, Sachs DH, Sprangers B, et al. Long-term results in recipients of combined HLA-mismatched kidney and bone marrow transplantation without maintenance immunosuppression. *Am J Transplant*. 2014;14:1599–1611.
4. Morris H, DeWolf S, Robins H, et al. Tracking donor-reactive T cells: evidence for clonal deletion in tolerant kidney transplant patients. *Sci Transl Med*. 2015;7:272ra10.
5. Savage TM, Shonts BA, Obradovic A, et al. Early expansion of donor-specific Tregs in tolerant kidney transplant recipients. *JCI Insight*. 2018;3:e124086.
6. Busque S, Scandling JD, Lowsky R, et al. Mixed chimerism and acceptance of kidney transplants after immunosuppressive drug withdrawal. *Sci Transl Med*. 2020;12:eaax8863.
7. Bertaina A, Grimm PC, Weinberg K, et al. Sequential stem cell–kidney transplantation in Schimke immuno-osseous dysplasia. *N Engl J Med*. 2022;386:2295–2302.
8. Senev A, Tambur AR, Kosmoliaptsis V, et al. HLA molecular mismatches and induced donor-specific tolerance in combined living donor kidney and hematopoietic stem cell transplantation. *Front Immunol*. 2024;15:1–12.
9. Todo S, Yamashita K, Goto R, et al. A pilot study of operational tolerance with a regulatory T-cell-based cell therapy in living donor liver transplantation. *Hepatology*. 2016;64:632–643.
10. Oberbauer R, Edinger M, Berlakovich G, et al. A prospective controlled trial to evaluate safety and efficacy of in vitro expanded recipient regulatory T cell therapy and tocilizumab together with donor bone marrow infusion in HLA-mismatched living donor kidney transplant recipients (Trex001). *Front Med (Lausanne)*. 2021;7:1–11.
11. Muckenhuber M, Mucha J, Mengrelis K, et al. Optimum timing of antithymocyte globulin in relation to adoptive regulatory T cell therapy. *Am J Transplant*. 2023;23:84–92.
12. Harden PN, Game DS, Sawitzki B, et al. Feasibility, long-term safety, and immune monitoring of regulatory T cell therapy in living donor kidney transplant recipients. *Am J Transplant*. 2021;21:1603–1611.
13. Brook MO, Hester J, Petchey W, et al. Transplantation Without Overimmunosuppression (TWO) study protocol: a phase 2b randomised controlled single-centre trial of regulatory T cell therapy to facilitate immunosuppression reduction in living donor kidney transplant recipients. *BMJ Open*. 2022;12:e061864–e061868.
14. Sawitzki B, Harden PN, Reinke P, et al. Regulatory cell therapy in kidney transplantation (The ONE Study): a harmonised design and analysis of seven non-randomised, single-arm, phase 1/2A trials. *Lancet*. 2020;395:1627–1639.
15. Brook MO, Hennessy C, Hester J, et al. Late treatment with autologous expanded regulatory T-cell therapy after alemtuzumab induction is safe and facilitates immunosuppression minimization in living donor renal transplantation. *Transplantation*. 2024;108:2278–2286.
16. Levitsky J, Miller J, Huang X, et al. Immunoregulatory effects of everolimus on in vitro alloimmune responses. *PLoS One*. 2016;11:e0156535–e0156514.
17. Mathew JM, H-Voss J, LeFever A, et al. A phase i clinical trial with ex vivo expanded recipient regulatory t cells in living donor kidney transplants. *Sci Rep*. 2018;8:1–12.
18. Mathew JM, Voss JH, McEwen ST, et al. Generation and characterization of alloantigen-specific regulatory T cells for clinical transplant tolerance. *Sci Rep*. 2018;8:1–14.
19. Dudreuilh C, Jarvis P, Beadle N, et al. Can regulatory T cells improve outcomes of sensitised patients after HLA-Ab incompatible renal transplantation: study protocol for the Phase IIa GAMECHANgER-1 trial. *BMC Nephrol*. 2023;24:1–13.
20. Romano M, Sen M, Scottà C, et al. Isolation and expansion of thymus-derived regulatory T cells for use in pediatric heart transplant patients. *Eur J Immunol*. 2021;51:2086–2092.
21. Roemhild A, Otto NM, Moll G, et al. Regulatory T cells for minimising immune suppression in kidney transplantation: Phase I/IIa clinical trial. *The BMJ*. 2020;371:m3734.
22. Landwehr-Kenzel S, Müller-Jensen L, Kuehl JS, et al. Adoptive transfer of ex vivo expanded regulatory T cells improves immune cell engraftment and therapy-refractory chronic GvHD. *Mol Ther*. 2022;30:2298–2314.
23. Bézie S, Meistermann D, Boucault L, et al. Ex vivo expanded human non-cytotoxic CD8+CD45RClow/− Tregs efficiently delay skin graft rejection and GVHD in humanized mice. *Front Immunol*. 2018;8:2014.
24. Tran LM, Macedo C, Zahorchak AF, et al. Donor-derived regulatory dendritic cell infusion modulates effector CD8+ T cell and NK cell responses after liver transplantation. *Sci Transl Med*. 2023;15:1–16.
25. Moreau A, Kervella D, Bouchet-Delbos L, et al; DIVAT Consortium. A phase I/IIa study of autologous tolerogenic dendritic cells immunotherapy in kidney transplant recipients. *Kidney Int*. 2023;103:627–637.
26. Levitsky J, Burrell BE, Kanaparthi S, et al. Immunosuppression withdrawal in liver transplant recipients on sirolimus. *Hepatology*. 2020;72:569–583.

27. Feng S, Demetris AJ, Spain KM, et al. Five year histological and serological follow-up of operationally tolerant pediatric liver transplant recipients enrolled in WISP-R conclusion-operationally tolerant pediatric liver transplant recipients maintain generally stable allograft histology in sp. *Hepatology*. 2017;65:647–660.
28. Shaked A, DesMarais MR, Kopetskie H, et al. Outcomes of immunosuppression minimization and withdrawal early after liver transplantation. *Am J Transplant*. 2019;19:1397–1409.
29. Benítez C, Londoño MC, Miquel R, et al. Prospective multicenter clinical trial of immunosuppressive drug withdrawal in stable adult liver transplant recipients. *Hepatology*. 2013;58:1824–1835.
30. Levitsky J, Feng S. Tolerance in clinical liver transplantation. *Hum Immunol*. 2018;79:283–287.
31. Levitsky J, Asrani SK, Schiano T, et al; Clinical Trials in Organ Transplantation - 14 Consortium. Discovery and validation of a novel blood-based molecular biomarker of rejection following liver transplantation. *Am J Transplant*. 2020;20:2173–2183.
32. Levitsky J, Kandpal M, Guo K, et al. Donor-derived cell-free DNA levels predict graft injury in liver transplant recipients. *Am J Transplant*. 2022;22:532–540.
33. Bohne F, Martínez-Llordella M, Lozano JJ, et al. Intra-graft expression of genes involved in iron homeostasis predicts the development of operational tolerance in human liver transplantation. *J Clin Invest*. 2012;122:368–382.
34. Wood-Trageser MA, Lesniak D, Gambella A, et al. Next-generation pathology detection of T cell-antigen-presenting cell immune synapses in human liver allografts. *Hepatology*. 2023;77:355–366.
35. Rosales IA, Yang C, Farkash EA, et al. Novel intragraft regulatory lymphoid structures in kidney allograft tolerance. *Am J Transplant*. 2022;22:705–716.
36. Guinn MT, Szuter ES, Yokose T, et al. Intragraft B cell differentiation during the development of tolerance to kidney allografts is associated with a regulatory B cell signature revealed by single cell transcriptomics. *Am J Transplant*. 2023;23:1319–1330.
37. Yokose T, Colvin RB, Alessandrini A. Dysfunction of infiltrating cytotoxic CD8 + T cells within the graft promotes murine kidney allotransplant tolerance. *J Clin Investig*. 2024;134:e179709.
38. Waldmann H, Adams E, Fairchild PJ, et al. Infectious tolerance and the long-term acceptance of transplanted tissue. *Immunol Rev*. 2006;212:301–313.
39. Mojibian M, Harder B, Hurlburt A, et al. Implanted islets in the anterior chamber of the eye are prone to autoimmune attack in a mouse model of diabetes. *Diabetologia*. 2013;56:2213–2221.
40. Ashmore-Harris C, Iafrate M, Saleem A, et al. Non-invasive reporter gene imaging of cell therapies, including T cells and stem cells. *Mol Ther*. 2020;28:1392–1416.
41. Jacob J, Nadkarni S, Volpe A, et al. Spatiotemporal in vivo tracking of polyclonal human regulatory T cells (Tregs) reveals a role for innate immune cells in Treg transplant recruitment. *Mol Ther Methods Clin Dev*. 2021;20:324–336.
42. Mohseni YR, Saleem A, Tung SL, et al. Chimeric antigen receptor-modified human regulatory T cells that constitutively express IL-10 maintain their phenotype and are potently suppressive. *Eur J Immunol*. 2021;51:2522–2530.
43. Marek-Trzonkowska N, Myśliwiec M, Iwaszkiewicz-Grześ D, et al. Factors affecting long-term efficacy of T regulatory cell-based therapy in type 1 diabetes. *J Transl Med*. 2016;14:1–11.
44. Zieliński M, Żalińska M, Iwaszkiewicz-Grześ D, et al. Combined therapy with CD4+CD25highCD127– T regulatory cells and anti-CD20 antibody in recent-onset type 1 diabetes is superior to monotherapy: Randomized phase I/II trial. *Diabetes Obes Metab*. 2022;24:1534–1543.
45. Zieliński M, Sakowska J, Iwaszkiewicz-Grześ D, et al. PD-1 Receptor (+) T cells are associated with the efficacy of the combined treatment with regulatory t cells and rituximab in type 1 diabetes children via regulatory t cells suppressive activity amelioration. *Int Immunopharmacol*. 2024;132:111919.
46. Baker KF, Skelton AJ, Lendrem DW, et al. Predicting drug-free remission in rheumatoid arthritis: A prospective interventional cohort study. *J Autoimmun*. 2019;105:102298.
47. Baker KF, McDonald D, Hulme G, et al. Single-cell insights into immune dysregulation in rheumatoid arthritis flare versus drug-free remission. *Nat Commun*. 2024;15:1–16.