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Summary of the Third International Workshop on Clinical Tolerance

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Abstract

The Third International Workshop on Clinical Tolerance was held in Stanford, California, September 8–9, 2017. This is a summary of Workshop presentations of clinical trials designed to withdraw or minimize immunosuppressive (IS) drugs in kidney and liver transplant patients without subsequent evidence of rejection. All clinical protocols had in common the use of donor or recipient cell therapy combined with organ transplantation. Tolerance to HLA matched and mismatched living donor kidney transplants with complete withdrawal of IS drugs without subsequent rejection for up to 14 years of observation was achieved in more than 50 patients enrolled in trials in 4 Medical Centers after the establishment of transient or persistent chimerism. Complete IS drug withdrawal without chimerism was reported in a prospective trial of liver transplantation combined with injection of regulatory T cells. IS drug minimization without rejection was reported in recipients of living donor kidney transplants enrolled in the One Study consortium after injection of recipient regulatory T cells, or injection of donor regulatory monocytes or dendritic cells. In conclusion, considerable progress has been made in achieving IS drug withdrawal after cell therapy in recipients of organ transplants.

Introduction

The goal of The Third Workshop was to update the progress of studies of the safe withdrawal of immunosuppressive drugs in kidney and liver transplant patients by investigators who are actively engaged in or planning interventionist clinical trials.

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Summary of clinical tolerance studies of living donor kidney transplantation at Stanford, Northwestern/Duke, and Samsung Medical Centers, and the Massachusetts General Hospital

The four medical centers presented updates of their tolerance induction regimens and outcomes of recipients. A summary of recipients without hematologic malignancies is shown in Table 1. The approach for renal allograft tolerance developed by the Stanford group (Stanford, California), presented by Dr. John Scandling, is based on decades long basic and clinical studies using a total lymphoid irradiation (TLI) conditioning regimen [1–5]. The conditioning protocol in combined hematopoietic cell and kidney transplantation consists of TLI (80–120 cGy/day 10 daily doses starting on post-operative day one after kidney transplantation) and rabbit anti-thymocyte globulin (rATG); (1.5 mg/kg/day, 5 daily doses starting on day 0) (Table 1). Following the last dose of TLI, $CD34^+$ enriched donor "mobilized" peripheral blood mononuclear cells were infused into the with a defined dose of donor T cells. The kidney transplant recipients were maintained with calcineurin inhibitor (CNI), mycophenolate mofetil (MMF) for 6 to 14 months before discontinuation in 29 HLA matched subjects. Chimerism was induced in 28 subjects studied, and 24 patients met the immunosuppression (IS) withdrawal criteria, which requires persistent chimerism for at least 6 months, absence of rejection on protocol biopsy and no evidence of GVHD (Table 1). Among these 24 recipients, 10 with stable chimerism and 14 with transient chimerism were off IS for 2–113 months (median 43 months). There were 2 graft losses due to kidney disease relapse. There was 1 death associated with stroke after graft loss due to lupus at 10 years posttransplant. There were 2 deaths associated with pulmonary embolism, and coronary artery disease in patients with normal graft function (Table 2).

The most recent cohort included 19 recipients of HLA-haplotype-matched kidneys. An escalating dose of infused $CD34^+$ and $CD3^+$ T cells (3, 10, 20, 50 and 100 × 10^6/Kg, compared to 1 × 10^6/kg in the prior two cohorts) was used in the effort to promote mixed chimerism induction. Persistent chimerism for at least 12 months was achieved in 9 of 18 patients followed for at least 1 year. In these 9 patients, MMF was discontinued at 9 to 12 months after which the patients remained on tacrolimus monotherapy. During the second-year discontinuation of tacrolimus was performed in 6 patients with low levels (<30%) of chimerism resulting in loss of chimerism in all 6 with rejection episodes in 2 (Table 1). All 6 patients were returned to tacrolimus therapy with or without MMF, and 3 chimeric patients were continued on tacrolimus monotherapy. At present, none of the mismatched patients have been successfully withdrawn from IS drugs. Ongoing studies will determine whether high levels of chimerism (>50%) in mismatched patients allow for discontinuation of tacrolimus, and persistent chimerism and tolerance.

The approach for tolerance by the Northwestern/Duke group (Chicago, Illinois, Durham, NC), presented by Dr. Joseph Leventhal, was based on the establishment of persistent donor chimerism. Their nonmyeloablative conditioning included fludarabine (30mg/m2, days −5, −4, and −3), cyclophosphamide (50mg/kg/dose, days −3 and +3), 200cGy of total body irradiation (TBI) (day −1), followed by the living donor kidney transplant (Table 1) [6]. A "mobilized" peripheral blood mononuclear cell product was processed to contain defined

quantities of T cells, CD34+ cells and facilitator (FCRx) cells, and cryopreserved until administration the day after the kidney transplant. Recipients are initially maintained on tacrolimus and mycophenolate-based IS. Their immunosuppression is weaned over the 12 months and fully withdrawn at one year [6]. In their program, 42 subjects have been enrolled and 37 have been transplanted as part of the Phase 2 trial (Table 1). Among the first 31 subjects who have reached > 12 months, durable donor chimerism was established in 23 subjects and immunosuppression was successfully discontinued in 22 subjects with immunosuppression free survival ranging from 8 to 81 months (Table 1). Full donor chimerism (> 98% whole blood and T cell lineage) was observed in 19 of these 22 subjects. Five subjects developed transient chimerism and were maintained on low-dose immunosuppression with normal renal function. Two subjects lost their renal allografts within the first-year post-transplant related to the development of opportunistic infections (Table 2). GVHD was observed in two subjects, both of which occurred in highly HLA mismatched living unrelated kidney from multiparous female donors (Table 2). One patient is currently stable with grade 1–2 ocular/musculoskeletal chronic GVHD but the other died due to Grade 3 GI GVHD plus CMV Colitis at 11 months after transplantation. The Northwestern group also provided an update on a separate trial of tolerance induction in HLA-identical living donor kidney transplant recipients [7] Recipients in this nonchimeric operational tolerance protocol were given alemtuzumab, tacrolimus/MPA with early sirolimus conversion and were multiply infused with donor hematopoietic CD34(+) stem cells. Immunosuppression was withdrawn by 24 months. Twelve months later, operational tolerance was confirmed by rejection-free transplant biopsies. Six of 15 evaluable patients achieved operational tolerance with this approach[7]. Patients with evidence of rejection were not withdrawn from or were returned to IS drugs. Tolerant subjects demonstrated time-dependent increases of circulating CD4(+) CD25(+) CD127(−) FOXP3(+) Tregs versus losses of Tregs in nontolerant subjects. In addition, serial gene expression profiles were observed in the whole blood and protocol biopsy samples that were highly associated with operational tolerance [7].

Massachusetts General hospital (Boston, Massachusetts) clinical trials for induction of renal allograft tolerance conducted on two different patient populations was summarized by Dr. Tatsuo Kawai; 1) Patients with end-stage renal disease (ESRD) without malignancy and 2) ESRD patients with hematologic malignancies [8–12]. The initial conditioning regimen for 10 HLA-haplotype mismatched kidney transplantation patients without malignancy consisted of cyclophosphamide, humanized anti-CD2 mAb (MEDI-507) and local thymic irradiation (700 cGy). On Day 0, kidney transplantation was performed, followed by i.v. infusion of whole donor bone marrow cells. CyA was administered postoperatively and then slowly tapered after 6 months and completely discontinued by 9–14 months (Table 1) [8]. This regimen was subsequently modified by adding two pretransplant or four peritransplant rituximab injections. Transient mixed chimerism for up to 3 weeks was induced in all recipients, without any evidence of GVHD.

Immunosuppression was successfully discontinued in 7 of the 10 recipients. Four have remained off immunosuppression with normal kidney function, after more than 7 to 14 years. In the other three, immunosuppression was resumed after 5, 7 and 8 years (i.e. with immunosuppression-free graft survivals of 4, 6 and 7 years), due either to recurrence of the

original kidney disease or development of chronic rejection.[8] Three patients had graft loss due to preformed antibody, CNI related TMA and acute rejection (Table 2). Associated with the early recovery of host T cells and loss of chimerism, transient acute kidney injury (AKI), as a major clinical symptom of the engraftment syndrome, was observed in 9 of 10 study subjects[9] (Table 2). To overcome AKI, a pilot study, in which cyclophosphamide was replaced with low-dose TBI, was recently conducted in three recipients. These three recipients did well without AKI with IS being discontinued for more than 2 years in one and being tapered in the last patient (manuscript in preparation).

Ten patients with multiple myeloma and ESRD have undergone HLA-matched kidney and bone marrow transplantation with follow-up time of up to 19 years [10–12]. Five of 10 patients are alive, two with no evidence of myeloma for 7 to 19 years posttransplant. Three patients have normal or near-normal renal function without needing systemic IS. Two patients with normal renal function off IS were returned to immunosuppressive therapy because of chronic GVHD [10–12]. This approach has been extended to 5 HLA haplotype mismatched transplantation with IS being off in two patients.

The study at the Samsung Medical Center (Seoul, Korea) was summarized by Dr. Jae Berm Park. This was performed on 7 combined kidney and bone marrow transplantations from HLA mismatched living donors to induce allograft tolerance via the mixed chimerism approach between December 2011 and May 2014. Median follow-up was 46 months (range, 42~70) after transplant. The conditioning regimen used in the majority of subjects consisted of cyclophosphamide (22.5mg/kg, −5 and −4 days before transplantation), fludarabine (10mg/m^2, −6, −5, −4, −3 days), rituximab (375mg/m^2, −7 and −2 days before transplantation), and anti-thymocyte globulin (1.5mg/mg/day on days −1, 0 and +1). Maintenance IS was with tacrolimus and steroids, which was slowly tapered over 6~12 months. All the recipients developed chimerism at least for 3 weeks after transplantation. Immunosuppression was tapered off in four out of the seven patients. Among these four, two patients had rejection episodes at 2 and 16 months after IS withdrawal, which required the resumption of their IS. The other two patients maintained stable graft function for 25 and 35 months after IS withdrawal. Among the other three patients on IS, IS withdrawal has been in progress in one patient but IS was never tapered in two patients because of acute rejection in early post-transplantation period and severe BK virus associated nephropathy (BKVAN). The latter 5 patients had significant BK viremia (> 4 log in blood PCR) during their post-transplant period with one graft loss (Table 2).

Dependence of Tolerance on Chimerism

The Stanford and Northwestern groups concluded that persistence of chimerism was necessary to achieve tolerance in their HLA mismatched kidney transplant recipients. IS drugs were not successfully withdrawn from mismatched recipients who failed to develop chimerism or who lost chimerism. The Stanford group is continuing to escalate the dose of donor T cells given to HLA mismatched recipients to increase the levels and duration of mixed chimerism. The MGH group is also attempting to increase the levels and duration of chimerism by including TBI in their conditioning regimen.

Tolerance in liver transplantation

The clinical trial performed at Hokkaido Univ. (Sapporo, Japan), and summarized by Dr. Kawai, is a prospective interventionist cell therapy trial that attempted to induce liver transplant tolerance [13]. The conditioning regimen is based on previously reported non-human primate studies for renal allograft tolerance, which included splenectomy at the time of liver transplant with conventional triple IS. Cyclophosphamide (40mg/kg) was administered on day 5, followed by adoptive transfer of an ex vivo-generated regulatory T-cell-enriched cell product on day 13. All 10 patients enrolled into the study are currently doing well and 7 of them have completed successful weaning and cessation of their IS. IS free survival is ranging for 16–33 months. The other 3 patients with autoimmune disease failed to achieve complete cessation of their IS drugs.

Discussion of immunosuppressive drug minimization and of discontinuation in trials of HLA matched and mismatched patients

The comparisons of risks and benefits of immunosuppressive drug minimization versus discontinuation in kidney and liver transplantation trials was discussed extensively by Drs. Edward Geissler, David Sachs, and Kenneth Newell. There was a consensus that graft injury and loss in these trials should be no greater than in standard of care patients, and prevention can be improved by use of biopsies just before drug minimization, and the use of biomarkers of tolerance [14–24].

One of the critical questions for clinical trials of tolerance in living donor kidney transplantation is whether to include HLA matched related, haplo-identical related or HLA unrelated mismatched recipients. The case was made very clearly that each of these donor-recipient combinations can yield very valuable information and the choice may be dictated by the tolerance induction protocol applied [5,7,25,26].

Kidney transplantation studies with regulatory immune cell therapy

Dr. Edward Geissler, the coordinator for the outcome office of the European Union funded ‘ONE Study’ (www.onestudy.org), a novel multi-center international living donor kidney transplant trial was presented. The goal of the trial is to investigate the safety and feasibility of infusions of polyclonal regulatory T cells (Oxford University/King’s College London, Charite, Berlin), donor alloantigen reactive regulatory T cells (UCSF, MGH), regulatory donor derived macrophages (University of Regensburg), and dendritic cells (Nantes University) into transplant patients [27]. The design of the ONE Study protocol allows for intra as well as inter-center comparisons, thus, enabling safety and feasibility studies using different forms of immune regulatory cell therapies to be performed in small numbers of patients to determine which cell therapies should be taken forward for efficacy studies.

All centers participating in the ONE Study have completed patient enrollment and follow up in the reference group where all patients were treated with the same immunosuppressive regimen – Basiliximab induction, tracrolimus, MMF, and steroids. A comprehensive program of validated and quality controlled immune monitoring assays were performed on serial pre and post-transplant patient samples either locally at each participating center or

centrally in Berlin, including a real time whole blood flow cytometry phenotypic analysis of peripheral blood leukocytes [28].

The strategy and hurdles for manufacturing of donor alloantigen reactive regulatory T cells were outlined by Dr. Qizhi Tang from University of California San Francisco (UCSF) [26–28]. Technical issues were overcome using EBV transformed donor B cells as the stimulator cells for the generation of the donor reactive regulatory T cells in the UCSF GMP protocol.

Data from the UK ONE study component (University of Oxford and King's College London) was summarized by Dr. Kathryn Wood. Twelve living donor renal transplant recipients have been treated with ex vivo expanded polyclonal regulatory T cells 5 days after transplantation in a dose escalation protocol; n=3 patients at each dose 1×10^6 per Kg; 3×10^6 per Kg, 6×10^6 per Kg and 10×10^6 per Kg. No adverse events were noted at either the time of cell infusion or during the clinical follow up period to date. No cases of biopsy proven rejection have occurred to date.

Safety issues in HLA matched and mismatched kidney and hematopoietic cell transplant trials

Dr. Ephraim Fuchs presented an overview of the risks and benefits of HLA haploidentical bone marrow transplantation (BMT) for patients with hematologic malignancies at the Johns Hopkins Medical Center with a focus on adverse events.

The importance of eliminating alloreactive T cells to reduce the risk of GVHD was emphasized, and details regarding the evolution of the Hopkins cyclophosphamide based conditioning regimen for this purpose was presented. He suggested that ATG be added to conditioning regimens for BMT for nonmalignant diseases (such as tolerance induction) to reduce the risk of GVHD further. That said, he indicated that we are currently unable to eliminate the risk of GVHD or conversely of graft failure entirely.

He emphasized the inherent instability of mixed chimerism in HLA mismatched BMT which may have implications for protocols choosing mixed chimerism as a destination for engraftment. He also pointed to the importance of viewing combined BMT and solid organ transplantation patients with complete chimerism as bone marrow transplant recipients first and foremost as relates to adverse event risk and management.

Dr. Joseph Leventhal (Northwestern) gave an overview of adverse events experienced in the Phase 2 Facilitating Cell (FCRx) trial conducted at Northwestern/Duke/Louisville (NCT00497926) [6,25].

All subjects experienced a nadir period of cytopenia, with expected severe leukopenia and thrombocytopenia that extended no more than 2 weeks and could be managed in the outpatient clinics. Two subjects failed to develop donor chimerism; these were highly HLA mismatched (0/6) living unrelated donor/recipient pairs, with one subject being highly sensitized (PRA > 50%). Transient chimerism was seen in six of the Northwestern subjects, and was associated with suboptimal cell dosing, intercurrent infection, or recipient HLA sensitization. Subjects with transient chimerism developed autologous reconstitution. There

have been two cases of biopsy proven acute GVHD (Day 95 and Day 134 post-transplant). Both occurred in highly HLA mismatched unrelated transplants from multiparous female donors (4/6 and 5/6 mismatched respectively). The first subject was promptly diagnosed and successfully treated with corticosteroids. The second subject had a delay in diagnosis and initiation of treatment which contributed to the development of treatment resistant GI gastrointestinal GVHD and cytomegalovirus; he expired at 11 months posttransplant.

The overall spectrum of infectious complications has been comparable to that seen in standard of care kidney and BMT transplant recipients. There have been two renal allograft losses related to infectious complications within the first year post transplant. Two deaths have occurred – the aforementioned case of treatment GVHD and a tolerant subject with a heavy smoking history who developed late stage lung cancer 5 years post transplant.

Dr. Stephan Busque (Stanford) gave an overview of the 48 subjects who have been treated with the TLI based tolerance induction regimen at Stanford over 12 years as relates to adverse events [5]. They did not observe a nadir period with severe neutropenia and thrombocytopenia with their treatment regimen. There were no cases of GVHD. Infectious complications were described and appeared consistent with standard of care transplant recipients. No graft losses due to rejection or chronic rejection was seen. The establishment of mixed chimerism did not appear to protect against kidney disease recurrence in their experience.

Dr. Tatsuo Kawai gave an overview of the MGH kidney tolerance experience. He detailed the phenomenon of “engraftment syndrome” that occurred in the majority of patients. Cyclophosphamide has been replaced by TBI in their clinical protocols and the change appears to have eliminated the “engraftment syndrome”.

Update on immune monitoring of tolerance trials

Dr. Megan Sykes (Columbia University) gave an update on immune monitoring of subjects from the MGH experience [26]. Enrichment for CD4+CD25+Foxp3+ Tregs in the blood of transiently chimeric kidney and BMT subjects who became tolerant was seen. High throughput screening of the TCR repertoire pre and post transplant provided evidence for Treg expansion in tolerant subjects. Tracking alloreactive cells with this approach provided evidence for clonal deletion at later time points. She suggested that tolerance which develops in their combined kidney and BMT patients may evolve through a period of robust Treg dependent immunoregulation, followed by clonal deletion.

Dr. Samuel Strober (Stanford) focused upon the phenomenon of mixed chimerism and in relationship to tolerance [5]. In their HLA-identical recipients, a critical period of multilineage mixed chimerism allows for the development of stable operational tolerance. Most of these subjects lost this mixed chimerism following the withdrawal of immunosuppression, yet operational tolerance persisted. In contrast mismatched (haploidentical) subjects with immunosuppression dependent mixed chimerism for at least 1 year did not become tolerant after loss of chimerism. Modifications to their protocol to allow for the establishment of immunosuppression independent mixed chimerism in mismatched subjects is being

performed by increasing the levels of donor cell chimerism to above 50% before immunosuppressive drug withdrawal.

Dr. Robert Colvin (MGH) focused upon the histopathologic features of tolerant renal allografts. He discussed Treg Organized Lymphoid Structures (TOLS) and their development in tolerant renal allografts in preclinical nonhuman primate models, and emphasized the importance of renal biopsy confirmation of tolerance, which should include the systematic evaluation of protocol biopsies from clinical and preclinical trials using immunophenotypic and molecular analyses.

Dr. James Mathew (Northwestern) gave an overview of cellular and molecular assays used for the study of subjects in ongoing tolerance trials at Northwestern [7]. A tolerance induction trial using serial infusions of donor hematopoietic cells in HLA-identical kidney transplants has identified sustained increased numbers of circulating Tregs in the blood as an indicator of operational tolerance. Dr. Mathew presented results from a Phase 1 trial of polyclonally expanded Tregs in living donor kidney transplant recipients conducted at Northwestern. Subjects were shown to have normal protocol biopsies a month after Treg infusion and sustained circulating increased numbers of Tregs.

Future Directions

A discussion of future directions for clinical tolerance research and cell therapy was led by Dr. Carlos Esquivel (Stanford). There was agreement to attempt to design future clinical tolerance induction protocols to combine the most desirable features of the single center protocols. Three of the single centers discussed near term strategies for improvement of safety and efficacy. The Northwestern group plans to exclude donor-recipient pairs where the potential donor is a mismatched unrelated multiparous female with an intended male recipient in order to reduce the incidence of GVHD. The MGH group altered their conditioning regimen by replacing cyclophosphamide with low dose TBI to eliminate the “engraftment syndrome”, and enhance persistence of mixed chimerism. The Stanford group will continue to escalate the dose of donor T cells infused into recipients to increase the levels and duration of mixed chimerism to achieve tolerance.

There was also general interest in extending the tolerance clinical trials to recipients of deceased donor organ transplants including heart and lung transplants. This requires the use of a completely posttransplant conditioning regimen or a delay of the conditioning regimen after bridging with standard of care IS drugs. The Stanford group is currently evaluating the use of deceased donor vertebrate body marrow for this approach.

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Abbreviations

AKI	acute kidney injury
BKVAN	BK virus associated nephropathy
BMT	bone marrow transplantation
CNI	calcineurin inhibitor
ESRD	end-stage renal disease
IS	immunosuppressive
MMF	mycophenolate mofetil
rATG	rabbit anti-thymocyte globulin
TLI	total lymphoid irradiation
TOLS	Treg Organized Lymphoid Structures

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Table 1.

Tolerance Induction Regimens

Patients and Outcomes	Medical Center			
	Northwestern University*	Stanford University	Massachussetts General Hospital	Samsung Medical Center
HLA matched or mismatched	mismatched	matched/mismatched	mismatched	mismatched
Recipient conditioning regimen	TBI+CY+Flu	TLI+ATG/TLI+ATG	LTI+CY+anti-CD2mAb	CY+ATG
Donor cell composition	mobilized blood CD34 cells + T cells + facilitator cells	mobilized blood CD34 cells + T cells	whole bone marrow	whole bone marrow
No. patients enrolled and given kidney transplants	37	29/19	10	7
No. patients with withdrawal of IS drugs attempted	22	24/6**	7	4
No. patients continuously off IS drugs	22	21/0	4	2
Duration continuously off IS drugs	8–81 mos		84–168 mos	25–35 mos
No. patients off IS drugs with return to IS drugs due to rejection or relapse	0	3/2	3	2
Duration off IS drugs before return to IS drugs	-	12–63 mos/3–5 mos	48–84 mos	2–16 mos

TBI-200 cGy total body irradiation; TLI-80–120 cGyx10 doses total lymphoid irradiation; CY-cyclophosphamide; ATG-anti-thymocyte globulin; Flu-fluorabine; CD34 cells; G-CSF; mobilized blood with enriched hematopoietic progenitor T cells from immobilized blood, mAb-monoclonal antibody, LTI-700cGy local thymic irradiation.

*One of 37 recipients was enrolled and transplanted at Duke University

**4 patients returned to therapeutic levels of Tac after loss of chimerism

Table 2.

Adverse Events in Tolerance Induction Regimens

Medical Center				
Patients and Adverse Events	**Northwestern University***	**Stanford University**	**Massachussetts General Hospital**	**Samsung Medical Center**
HLA matched or mismatched	mismatched	matched/mismatched	mismatched	mismatched
No. patients given transplants	37	29/19	10	7
No. with BK viremia/nephropathy	ND**	0/0	0	7
No. with GVHD	2	0/0	0	0
No. with engraftment syndrome	0	0/2	9	2
No. with graft loss (rejection/disease relapse/infection)	2***	2/0	3	1***
No. of deaths	1	3/0	0	0

* One of 37 recipients was enrolle and transplanted at Duke University

** ND-not discussed

*** graft loss due to infection