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Transplantation Tolerance in Non-Human Primates and Humans

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Abstract

This review focuses on our recent studies involving non-myeloablative bone marrow transplantation as an approach to inducing organ allograft tolerance across MHC barriers in non-human primates and in patients. The clinical studies are focused on mechanisms of tolerance involved in a protocol carried out at Massachusetts General Hospital in HLA-mismatched haploidentical combinations for the induction of renal allograft tolerance. These studies, in which chimerism was only transient and GVHD did not occur, suggest an early role for donor-specific regulatory T cells in tolerance induction, followed by partial and gradual deletion of donor-reactive T cells. A high throughput sequencing approach to identifying and tracking a significant portion of the alloreactive T cell receptor repertoire has demonstrated biological significance in transplant patients and pointed to clonal deletion as a long-term tolerance mechanism in these recipients. More recently, we adapted this sequencing method to optimally identify the donor-specific regulatory T cell (Treg) repertoire. Interrogation of the early post-transplant repertoire demonstrated expansion of donor-specific Tregs in association with tolerance. The kidney itself plays a key role in tolerance induction in the above patients, who had only transient chimerism. Non-human primate studies indicate that other organs, including the heart, the lungs and the liver, are less readily tolerated following a period of transient mixed chimerism. Our efforts to extend the reach of mixed chimerism for tolerance induction beyond the kidney are therefore focused on the addition of recipient Tregs to the protocol. This approach has the potential to enhance chimerism while further reducing the risk of GVHD.

Keywords

Mixed chimerism; allograft rejection; donor-specific tolerance; clonal deletion; regulatory T cells; kidney; liver

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Introduction

Studies of combined kidney/bone marrow transplantation (CKBMT) across HLA barriers in patients without malignant disease at MGH[1, 2] built on translational studies in murine models, non-human primates and patients with hematological malignancies. Studies in the murine model of mixed chimerism induction aimed to achieve allograft tolerance[3] and required treatment with depleting anti-CD4 and anti-CD8 mAbs, low-dose (3 Gy) TBI and thymic irradiation, which was needed to eliminate intrathymic alloreactivity that otherwise rejected donor progenitors as they entered the thymus, precluding a donor contribution to central deletional tolerance of newly-developing thymocytes[4–6]. A modification of this model for the treatment of hematologic malignancies in mice involved mixed chimerism induction as a platform for delayed donor lymphocyte infusion (DLI), which mediate potent graft-vs-leukemia (GVL) effects without causing graft-vs-host disease (GVHD) in the absence of inflammatory stimuli induced by conditioning[7–12]. We had found that such inflammation provided a critical checkpoint for trafficking of GVH-reactive T cells to the recipient's epithelial GVHD target tissues, where they cause disease[11, 12]. This approach was extended into clinical studies in patients with refractory hematologic malignancies in whom all other treatment modalities failed and was associated with remarkable tumor responses and even cures in patients with bulky, refractory lymphomas and myelomas[13–19]. These potent anti-tumor effects most likely reflected the ability of recipient-derived professional antigen-presenting cells (APCs) to trigger GVH alloreactivity, which is associated with improved GVL effects compared to those elicited in full chimeras lacking recipient APCs[8, 17, 20–2223, 24]. Thus, the strategy of using mixed chimerism as a platform to achieve potent GVL effects without GVHD was successfully translated into the clinic, both in the HLA-identical and HLA-mismatched settings. These studies provided seminal demonstrations that: 1) durable mixed chimerism could be achieved with non-myeloablative conditioning in humans, even with HLA-mismatched donors[13–19], albeit not as reliably as desired; 2) durable or transient mixed chimerism achieved under these conditions could occur without GVHD[13–19], though not as reliably as desired; 3) transient chimerism achieved across HLA barriers with one of these regimens was reliably NOT associated with GVHD, providing a key safety feature that permitted exploration of this approach for CKBMT in patients without malignant disease; and 4) delayed DLI could convert mixed to full chimerism, even across HLA barriers, without inducing GVHD[13–19], though not as reliably as desired.

Early efforts were made to translate the non-myeloablative tolerance protocol used for allograft tolerance in mice to a large animal, non-human primate model[25]. In our view, intermediate large animal models, which model the hurdles to tolerance induction in patients quite faithfully, are ethically necessary before embarking on clinical trials of early immunosuppression withdrawal, which denies the patient standard-of-care anti-rejection treatment. Importantly, these early studies showed that a high proportion (about 60–70%) of animals receiving non-myeloablative CKBMT achieved long-term tolerance to their kidney grafts, despite the transient nature of their chimerism[25, 26]. Meanwhile, the non-myeloablative mixed chimerism/DLI studies in patients with hematologic malignancies had been extended to a group of patients with renal failure due to multiple myeloma and an

available HLA-identical sibling donor. These first human CKBMT studies revealed that both transient and durable mixed chimerism could be associated with tolerance to donor kidneys[27–29]. Remarkably, those patients with transient chimerism, like others in the lymphoma trials discussed above, often enjoyed remarkable tumor responses[18, 27, 28], suggesting that the initial engraftment followed by rejection of donor marrow could elicit anti-tumor immunity, a hypothesis that we tested and confirmed in the murine model[30–33].

Thus, clinical studies in patients with hematological malignancies provided key safety and efficacy data that permitted a trial of HLA-mismatched related donor CKBMT in patients with renal failure and no malignant disease[1, 2, 34]. These were the first trials to successfully achieve allograft tolerance in a high proportion of patients with HLA-mismatched donors: of 10 patients, 7 were successfully removed from immunosuppression for periods of more than 5 to now more than 15 years without rejection. Three patients in the first iteration of the protocol returned to immunosuppressive therapy at 5 to 8 years for low-grade chronic rejection in two cases and recurrent membranoproliferative glomerulonephritis in a third. The three patients who were not successfully removed from immunosuppression included one early kidney graft loss due to preformed donor-specific antibodies (such patients were subsequently excluded from the trial and rituximab was added to the conditioning protocol), an early thrombomicroangiopathy and one acute cellular rejection shortly after immunosuppression withdrawal[1, 2, 34].

Mechanisms of tolerance in CKBMT recipients

In recipients of HLA-mismatched CKBMT in the absence of malignant disease, donor-specific unresponsiveness or hyporesponsiveness was always observed in bulk mixed lymphocyte reaction (MLR) and cell-mediated lympholysis (CML) assays after the transplant and following immunosuppression withdrawal[1, 35, 36]. Limiting dilution assays (LDA) can be informative not only in quantifying an alloresponse but also in pointing to suppressive mechanisms via a “sawtooth” pattern, in which responses paradoxically increase as responder cell numbers are diluted, reflecting the presence of a suppressive cell population present at lower concentration than the responding cells[37, 38]. This pattern was not observed in long-term HLA-mismatched CKBMT recipients[36], arguing against a suppressive mechanism in the maintenance of donor-specific unresponsiveness and kidney allograft tolerance. Consistently, while some anti-donor responsiveness could be revealed by Treg depletion in the first year in some HLA-mismatched CKBMT recipients, long-term (>1 year) MLR and CML responses were not enhanced by depletion of Tregs[35]. These results implicated other mechanisms in the long-term tolerance seen in these patients. The persistent donor-specific unresponsiveness in CKBMT recipients contrasts sharply with the reappearance of anti-donor reactivity *in vitro* after loss of chimerism in recipients of hematopoietic cell transplants alone with similar regimens[39], pointing to a role for the kidney allograft itself in maintaining tolerance in HLA-mismatched CKBMT recipients.

As mentioned above, 1 of 10 patients in our haploidentical CKBMT trial had acute cellular rejection after immunosuppression was withdrawn and eventually lost his allograft. Surprisingly, this patient showed robust donor-specific unresponsiveness in MLR and CML

assays both before and after the rejection[36], indicating that these functional assays cannot be relied upon to specify a tolerant state.

Recipients of non-myeloablative, anti-CD2 (siplizumab)-based, HLA-mismatched hematopoietic cell transplant recipients without a kidney demonstrated enrichment for FOXP3+CD25+CTLA4+ cells among the CD4+ T cells recovering early post-transplant[39]. Similar results were observed in CKBMT recipients with the siplizumab-based regimen, where the enriched CD25+ cells were shown to be CD127- and to correlate with Treg-specific demethylated region (TSDR) demethylation levels, indicating that they are Tregs[40]. Tregs comprised up to 80% of CD4 T cells in the first week post-transplant and gradually declined to baseline percentages over the first year. Absolute concentrations of circulating Tregs recovered to near pre-transplant baseline levels within weeks, in contrast to non-Treg CD4 counts, which took >1 year to recover to baseline levels[40]. In one patient but not the others, TCR sequencing of post-transplant CD4+CD25highCD127- T cells suggested that significant conversion to Tregs of pre-transplant CD4 non-Tregs had occurred[40]. Phenotypic analyses following CKBMT revealed high levels of Treg proliferation early post-transplant, suggesting expansion in response to peripheral lymphopenia induced by conditioning and/or donor antigen. Whether the observed early presence of “resting”-type Tregs[41] expressing CD31 early post-transplant reflects an early wave of emigration from the thymus or simply reflects selective preservation of this Treg subset following conditioning is uncertain, as most Tregs subsequently showed an activated phenotype.

We were able to assess the role of Tregs in the donor-specific hypo- or unresponsiveness in some patients. As mentioned above, persistent anti-donor reactivity was revealed within the first year in some patient samples by depleting Tregs or non-T cells[35]. However, after this timepoint, depletion of Tregs did not enhance anti-donor reactivity in any patients. This result suggested that either deletion or anergy of donor-reactive T cells had occurred over time. Notably, some protocol renal allograft biopsies in these patients contained non-destructive lymphoid clusters that were greatly enriched for FoxP3 expression[1], suggesting that Tregs may play an important local role within the graft in the pathway to tolerance.

Assessing the role of clonal deletion in tolerant patients: a new approach to analyzing the human alloresponse

Following the development of commercially-available platforms for high-throughput sequencing of human TCRβ hypervariable (CDR3) regions, we hypothesized that a large component of the alloreactive T cell repertoire might be identified by sequencing sorted recipient cells that divided in response to donor antigens in an MLR performed prior to transplant, and that these clones could then be tracked post-transplant as a way of discerning the role of deletion in long-term tolerance in CKBMT recipients. Host-vs-Graft (HvG) alloreactive clones were identified as those that expanded in a pre-transplant CFSE-MLR compared to their frequency in the unstimulated CD4 or CD8 T cell pool at the same time. By sampling post-transplant blood and using a uniform frequency threshold for detection of clones to normalize for variability in sample size over time, we were able to compare the frequency of circulating donor-reactive T cells prior to and at various times following transplantation. All tolerant patients showed a significant decline in the number of

circulating donor-reactive CD4 and CD8 clones following the transplant. In contrast, the patient who failed to achieve tolerance despite receiving the same CKBMT regimen did not show any reduction in the number of circulating donor-reactive T cell clones[36], despite showing donor-specific unresponsiveness *in vitro.* These results suggested that the TCR tracking approach was more specific in identifying tolerance than the functional assays[36]. Furthermore, two conventional kidney transplant recipients studied in the same way showed significant increases in the number of circulating donor-reactive CD4 T cell clones following their transplant[36].

The kinetics of loss of circulating donor-specific T cells were somewhat variable among tolerant patients and in some cases suggested an initial expansion of donor-reactive clones before their eventual deletion. Since the regimen used for CKBMT involved extensive peripheral T cell depletion with siplizumab and cyclophosphamide, it was not surprising that comparison of pre- and post-transplant repertoires of these patients revealed considerable turnover of T cells[36]. Conventional transplant recipients, one of whom had received ATG to treat rejection following the transplant, also showed considerable repertoire turnover over the period of one year and nevertheless showed expansion of pre-existing donor-reactive TCRs. Thus, the loss of donor-reactive clones in tolerant patients might be part of a global T cell depletion process. However, the rate of disappearance of donor-reactive CD8 (but not CD4) clones in some cases exceeded that of non-donor-reactive clones. In some instances, donor-reactive CD4 clones initially showed selective expansion compared to all pre-transplant clones[36]. Given the high level of repertoire turnover in these patients, these results suggest that donor antigen drives the selective expansion of donor-reactive T cells under the lymphopenic conditions produced by conditioning. The loss of donor-reactive clones in tolerant patients may therefore be the consequence of both global T cell depletion and the tolerance-inducing conditions that cause their attrition over time. Of note, protocol biopsies did not reveal enrichment of donor-reactive TCR sequences in the grafts, which were free of rejection and did not contain significant infiltrates.

The studies described above strongly suggested that biologically relevant clones are identified as donor-reactive with our TCR sequencing-based approach. Studies in intestinal transplant recipients have further validated the biological relevance of this method, as TCRs identified as donor-reactive were shown to dominate among recipient-mappable TCRs in the allograft itself during rejection episodes[42]. Furthermore, GVH-reactive TCR sequences, also identified with pre-transplant lymphocytes, were found to expand in the graft mucosa in association with replacement of donor APCs with those of the recipient[42]. The use of this technique has provided novel insight into events within the graft itself and the interplay between HvG and GVH alloreactivity in intestinal transplant recipients[42]. The approach deserves exploration in both additional solid organ transplants and in hematopoietic cell transplantation, where it could enhance the tissue diagnosis and provide insight into the pathogenesis of GVHD.

In view of the above studies in human transplant recipients validating the biological significance of TCR sequences identified as alloreactive by our method, we have pursued this approach to obtain a deeper understanding of the human alloresponse[43]. While there have been many estimates of the number and proportion of the T cell repertoire recognizing

a given set of alloantigens, our method provided an opportunity to actually quantify this response. However, we observed that a large proportion of alloreactive TCRs are present in the circulation at very low frequency, suggesting that any given sample is likely to be of insufficient size to allow detection of most of these clones. We therefore developed an extrapolation methodology that took into account the "unseen species" and were able to estimate the cumulative frequency of alloreactive T cells against any extensively HLA-mismatched donor to be in approximately the 0.5%−5% range for both CD4 and CD8 T cells[43]. Moreover, the alloreactive repertoire was highly specific for each unique allogeneic donor and its diversity correlated directly with the degree of HLA mismatching between the responder and the stimulator. The low frequency of most individual alloreactive clones in the circulation suggested that they included mainly naïve T cells. Indeed, very few sequences among a panel of "public" virus-specific TCRs were detected among the alloreactive sequences detected in our studies[43]. Collectively, these results argue against the notion that the alloresponse reflects predominantly a cross-reaction with heterologous pathogen-reactive T cells and instead suggest that almost any TCR is likely to show alloreactivity against some allogeneic donor if a sufficiently large panel could be tested.

More recently, we have adapted the TCR sequencing approach to the detection and fate tracking of donor-specific Tregs, an effort that was needed in order to determine whether the Treg expansions seen early post-transplant in CKBMT recipients were indeed donor-specific. This was a challenging question, as the low abundance of Tregs in peripheral blood made detection of donor-specific Tregs in a given sample likely to be inefficient. Indeed, when we interrogated CFSE-low CD4 cells proliferating in MLRs for the presence of sequences that mapped to the sorted Treg fraction of unstimulated T cells in the same sample, we found that only a very small fraction of proliferating cells were in fact Tregs[44]. However, by expanding Tregs with activated donor B cells, we were able to detect a much broader repertoire of Tregs that had high specificity for that donor and potent suppression of anti-donor reactivity[44]. By subjecting CKBMT recipients' pre-transplant Tregs to this method to identify a significant proportion of the donor-specific Treg repertoire, then interrogating post-transplant specimens for these sequences, we were able to demonstrate that donor-specific Tregs were indeed expanded in the circulation of tolerant patients, but not in that of the patient who failed tolerance induction, at 6 months post-transplant[44]. These results strongly implicate donor-specific Tregs in the tolerance achieved in CKBMT recipients.

Extending allograft tolerance beyond the kidney: Non-human primate studies

As noted above, the kidney plays an active role in promoting tolerance in CKBMT recipients with transient chimerism. The use of a regimen promoting transient chimerism across HLA barriers in these studies was supported by monkey studies demonstrating the efficacy of tolerance induction with this approach[25]. However, studies of lung, heart and islet transplantation in this monkey model have shown that these organs and tissues are less able to promote tolerance in combination with BMT that leads to transient chimerism[45–49]. For this purpose, we believe that induction of durable mixed chimerism will be essential. The approach we have taken to achieve this goal involves infusion of polyclonal recipient Tregs that are expanded and cryopreserved prior to transplantation and infused shortly after the

BMT. Polyclonal recipient Tregs had shown efficacy in promoting durable chimerism in a murine model[50] and this approach held the promise of enhancing the durability of chimerism without increasing the risk of GVHD, in contrast to other approaches to improving HLA-mismatched hematopoietic cell engraftment. Our initial studies with this approach demonstrated its potential in the monkey model, achieving more prolonged chimerism, including donor T cells, and, remarkably, allowing acceptance without immunosuppression of a donor kidney grafted 4 months after the bone marrow transplant[51]. This achievement demonstrated robust tolerance and contrasted with concurrent and historical controls not receiving Tregs, which uniformly reject donor kidneys grafted so long after BMT[51, 52]. However, success with this regimen was limited by CMV reactivation, which occurred uniformly during the initial period when the animals were treated with cyclosporine and which (along with its treatment) had a deleterious effect on donor marrow engraftment[51]. Switching the single agent immunosuppression given for this short initial period (2 months) from cyclosporine to rapamycin has helped to alleviate this problem (P. Alonso-Guallart et al, manuscript in preparation). While many challenges remain, some of which reflect particular challenges in the use of expanded cynomolgus monkey Tregs, studies in progress have confirmed the tolerance-promoting effect of Tregs in this setting and we are optimistic about the clinical potential of this approach.

Because rodent studies have indicated that the liver is a particularly tolerogenic organ[53–56], we hypothesized that the transient chimerism regimen that achieved tolerance in renal allograft recipients (without Treg infusion) would also be successful in promoting liver allograft tolerance. Before testing this in patients, however, we believed it was critical to test this approach directly in the monkey model and therefore took up the challenge of establishing a cynomolgus monkey liver transplant model[57]. Much to our surprise, despite the achievement of robust, transient multilineage chimerism, donor liver grafts were uniformly rejected (S. Chaudhry et al, manuscript submitted). However, when we modified the regimen to include robust *in vivo* depletion of donor and recipient T cells while excluding donor marrow infusion, we achieved transient lymphoid chimerism (from passenger cells in the liver) and long-term liver allograft survival with only a short (2 months) course of immunosuppression (E. Berglund et al, manuscript in preparation). Studies are in progress to refine this protocol and better understand the immune interactions at play while we are considering a clinical protocol based on this approach. These observations underscore the importance and utility of non-human primate studies before embarking on clinical trials of tolerance induction.

Conclusions

Ultimately, we aim to achieve durable mixed chimerism without GVHD to induce long-term deletional tolerance to all types of organs and to islet allografts. In view of the proof of principle that this can be achieved in a handful of patients with haploidentical donors[13, 19], this is a realistic goal. We are optimistic about the potential of expanded recipient Tregs to promote durable chimerism and look forward to further pre-clinical and clinical refinements of this approach.

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Abbreviations

APC	antigen-presenting cell
BMT	bone marrow transplantation
CKBMT	combined kidney and bone marrow transplantation
CML	cell-mediated lympholysis
DLI	donor leukocyte infusions
GVHD	graft-vs-host disease
GVL	graft-vs-leukemia/lymphoma
LDA	limiting dilution analysis
LGVHR	lymphohematopoietic graft-vs-host response
MiHA	minor histocompatibility antigen
MLR	mixed lymphocyte reaction
TBI	total body irradiation
TCR	T cell receptor
Treg	regulatory T cell

References

1. Kawai T, Cosimi AB, Spitzer TR, Tolkoff-Rubin N, Suthanthiran M, Saidman SL et al. HLA-mismatched renal transplantation without maintenance immunosuppression. New England Journal of Medicine 2008; 358(4): 353–361. [PubMed: 18216355]
2. Kawai T, Sachs DH, Sykes M, Cosimi AB, Immune Tolerance N. HLA-mismatched renal transplantation without maintenance immunosuppression. The New England journal of medicine 2013; 368(19): 1850–1852. doi: 10.1056/NEJMc1213779 [PubMed: 23656665]
3. Sharabi Y, Sachs DH. Mixed chimerism and permanent specific transplantation tolerance induced by a non-lethal preparative regimen. J.Exp.Med 1989; 169: 493–502. [PubMed: 2562984]
4. Tomita Y, Khan A, Sykes M. Mechanism by which additional monoclonal antibody injections overcome the requirement for thymic irradiation to achieve mixed chimerism in mice receiving bone marrow transplantation after conditioning with anti-T cell mAbs and 3 Gy whole body irradiation. Transplantation 1996; 61: 477–485. [PubMed: 8610364]
5. Tomita Y, Sachs DH, Khan A, Sykes M. Additional mAb injections can replace thymic irradiation to allow induction of mixed chimerism and tolerance in mice receiving bone marrow transplantation

after conditioning with anti-T cell mAbs and 3 Gy whole body irradiation. Transplantation 1996; 61: 469–477. [PubMed: 8610363]

6. Nikolic B, Khan A, Sykes M. Induction of tolerance by mixed chimerism with nonmyeloblative host conditioning: the importance of overcoming intrathymic alloresistance. Biol.Blood Marrow Transplant 2001; 7(3): 144–153. [PubMed: 11302548]
7. Sykes M, Sheard MA, Sachs DH. Graft-versus-host-related immunosuppression is induced in mixed chimeras by alloresponses against either host or donor lymphohematopoietic cells. J.Exp.Med 1988; 168: 2391–2396. [PubMed: 3264329]
8. Mapara MY, Kim Y-M, Wang S-P, Bronson R, Sachs DH, Sykes M. Donor lymphocyte infusions mediate superior graft-versus-leukemia effects in mixed compared to fully allogeneic chimeras: a critical role for host antigen-presenting cells. Blood 2002; 100: 1903–1909. [PubMed: 12176915]
9. Mapara MY, Kim Y-M, Marx J, Sykes M. DLI-mediated GVL effects in mixed chimeras established with a non-myeloablative conditioning regimen: extinction of GVL effects coincides with loss of alloreactive cells following conversion to full donor chimerism. Transplantation 2003; 76: 297–305. [PubMed: 12883182]
10. Pelot MR, Pearson DA, Swenson K, Zhao G, Sachs J, Yang Y-G et al. Lymphohematopoietic graft-vs-host reactions can be induced without graft-vs-host disease in murine mixed chimeras established with a cyclophosphamide-based non-myeloablative conditioning regimen. Biol.Blood Marrow Transplant 1999; 5: 133–143. [PubMed: 10392959]
11. Mapara MY, Leng C, Kim YM, Bronson R, Lokshin A, Luster A et al. Expression of chemokines in GVHD target organs is influenced by conditioning and genetic factors and amplified by GVHR. Biology of blood and marrow transplantation : journal of the American Society for Blood and Marrow Transplantation 2006; 12(6): 623–634.
12. Chakraverty R, Cote D, Buchli J, Cotter P, Hsu R, Zhao G et al. An inflammatory checkpoint regulates recruitment of graft-versus-host-reactive T cells to peripheral tissues. The Journal of experimental medicine 2006; 203(8): 2021–2031. [PubMed: 16880259]
13. Sykes M, Preffer F, McAffee S, Saidman SL, Colby C, Sackstein R et al. Mixed lymphohematopoietic chimerism and graft-vs-lymphoma effects are achievable in adult humans following non-myeloablative therapy and HLA-mismatched donor bone marrow transplantation. Lancet 1999; 353: 1755–1759. [PubMed: 10347989]
14. Toh HC, Spitzer TR, Preffer F, Alexander SI, MCafee S, Dombkowski D et al. Fluctuating lymphocyte chimerism, tolerance and anti-tumor response in a patient with refractory lymphoma receiving nonmyeloablative conditioning an a haploidentical related allogeneic bone marrow transplant. Cytokines, Cellular and Molecular Therapies 2002; 7: 43–47.
15. Spitzer TR, MCafee S, Sackstein R, Colby C, Toh HC, Multani P et al. The intentional induction of mixed chimerism and achievement of anti-tumor responses following non-myeloablative conditioning therapy and HLA-matched and mismatched donor bone marrow transplantation for refractory hematologic malignancies. Biol.Blood Marrow Transplant 2000; 6: 309–320. [PubMed: 10905768]
16. Dey BR, MCafee S, Sackstein R, Colby C, Saidman S, Weymouth D et al. Successful allogeneic stem cell transplantation with nonmyeloablative conditioning in patients with relapsed hematologic malignancy following autologous stem cell transplantation. Biol.Blood Marrow Transplant 2001; 7: 604–612. [PubMed: 11760148]
17. Dey BR, MCafee S, Colby C, Sackstein R, Saidman S, Tarbell N et al. Impact of prophlactic donor leukocyte infusions on mixed chimerism, graft-vs-host disease and anti-tumor response in patients with advanced hematologic malignancies treated with nonmyeloablative conditioning and allogeneic bone marrow transplantation. Biol.Blood Marrow Transplant 2003; 9: 320–329. [PubMed: 12766882]
18. Dey BR, MCafee S, Colby C, Cieply K, Caron M, Saidman S et al. Anti-tumor response despite loss of donor chimerism in patients treated with nonmyeloablative conditioning and allogeneic stem cell transplantation. British journal of haematology 2005; 128: 351–359. [PubMed: 15667537]
19. Spitzer TR, MCafee S, Dey BR, Colby C, Hope J, Grossberg H et al. Non-myeloablative haploidentical stem cell transplantation using anti-CD2 monoclonal antibody (MEDI-507)-based

conditioning for refractory hematologic malignancies. Transplantation 2003; 75: 1748–1751. [PubMed: 12777868]

20. Chakraverty R, Eom HS, Sachs J, Buchli J, Cotter P, Hsu R et al. Host MHC Class II+ antigen-presenting cells and CD4 cells are required for CD8-mediated graft-versus-leukemia responses following delayed donor leukocyte infusions. Blood 2006; 108: 2106–2113. [PubMed: 16757687]
21. Chakraverty R, Flutter B, Fallah-Arani F, Eom HS, Means T, Andreola G et al. The host environment regulates the function of CD8+ graft-versus-host-reactive effector cells. Journal of immunology 2008; 181(10): 6820–6828.
22. Chakraverty R, Sykes M. The role of antigen-presenting cells in triggering GVHD and GVL. Blood 2007; 110(1): 9–17. [PubMed: 17327406]
23. Childs R, Clave E, Contenin N, Jayasekera D, Hensel N, Leitman S et al. Engraftment kinetics after nonmyeloablative allogeneic peripheral blood stem cell transplantation: Full donor T-cell chimerism precedes alloimmune responses. Blood 1999; 94: 3234–3241. [PubMed: 10556212]
24. Dey BR, MCafee S, Sackstein R, Colby C, Saidman S, Weymouth D et al. Successful allogeneic stem cell transplantation with nonmyeloablative conditioning in patients with relapsed hematologic malignancy following autologous stem cell transplantation. Biology of blood and marrow transplantation : journal of the American Society for Blood and Marrow Transplantation 2001; 7(11): 604–612.
25. Kawai T, Cosimi AB, COLVIN RB, Powelson J, Eason J, Kozlowski T et al. Mixed allogeneic chimerism and renal allograft tolerance in cynomologous monkeys. Transplantation 1995; 59: 256–262. [PubMed: 7839449]
26. Kawai T, Sogawa H, Boskovic S, Abrahamian G, Smith RN, Wee SL et al. CD154 blockade for induction of mixed chimerism and prolonged renal allograft survival in nonhuman primates. American journal of transplantation : official journal of the American Society of Transplantation and the American Society of Transplant Surgeons 2004; 4(9): 1391–1398.
27. Buhler LH, Spitzer TR, Sykes M, Sachs DH, Delmonico FL, Rubin-Tolkoff N et al. Induction of kidney allograft tolerance after transient lymphohematopoietic chimerism in patients with multiple myeloma and end-stage renal disease. Transplantation 2002; 74: 1405–1409. [PubMed: 12451240]
28. Fudaba Y, Spitzer TR, Shaffer J, Kawai T, Fehr T, Delmonico F et al. Myeloma Responses and Tolerance Following Combined Kidney and Nonmyeloablative Marrow Transplantation: In Vivo and In Vitro Analyses. Am.J.Transplant 2006; 6(9): 2121–2133. [PubMed: 16796719]
29. Spitzer TR, Sykes M, Tolkoff-Rubin N, Kawai T, McAfee SL, Dey BR et al. Long-term follow-up of recipients of combined human leukocyte antigen-matched bone marrow and kidney transplantation for multiple myeloma with end-stage renal disease. Transplantation 2011; 91(6): 672–676. [PubMed: 21217460]
30. Rubio MT, Kim YM, Sachs T, Mapara M, Zhao G, Sykes M. Anti-tumor effect of donor marrow graft rejection induced by recipient leukocyte infusions in mixed chimeras prepared with nonmyeloablative conditioning: critical role for recipient-derived IFN-{gamma}. Blood 2003; 102: 2300–2307. [PubMed: 12791660]
31. Rubio MT, Saito TI, Kattelman K, Zhao G, Buchli J, Sykes M. Mechanisms of the anti-tumor responses and host-versus graft reactions induced by recipient leukocyte infusions in mixed chimeras prepared with nonmyeloablative conditioning: a critical role for recipient CD4+ T cells and recipient leukocyte infusion-derived IFN-gamma-producing CD8+ T cells. Journal of immunology 2005; 175: 665–676.
32. Rubio MT, Zhao G, Buchli J, Chittenden M, Sykes M. Role of indirect allo- and autoreactivity in anti-tumor responses induced by recipient leukocyte infusions (RLI) in mixed chimeras prepared with nonmyeloablative conditioning. Clin.Immunol 2006; 120(1): 33–44. [PubMed: 16675304]
33. Saito TI, Rubio MT, Sykes M. Clinical relevance of recipient leukocyte infusion as antitumor therapy following nonmyeloablative allogeneic hematopoietic cell transplantation. Experimental hematology 2006; 34(9): 1271–1277. [PubMed: 16939820]
34. Kawai T, Sachs DH, Sprangers B, Spitzer TR, Saidman SL, Zorn E et al. Long-term results in recipients of combined HLA-mismatched kidney and bone marrow transplantation without maintenance immunosuppression. American journal of transplantation : official journal of the American Society of Transplantation and the American Society of Transplant Surgeons 2014; 14(7): 1599–1611. doi: 10.1111/ajt.12731

35. Andreola G, Chittenden M, Shaffer J, Cosimi AB, Kawai T, Cotter P et al. Mechanisms of Donor-Specific Tolerance in Recipients of Haploidentical Combined Bone Marrow/Kidney Transplantation. American journal of transplantation : official journal of the American Society of Transplantation and the American Society of Transplant Surgeons 2011; 11(6): 1236–1247.

36. Morris HDW S; Robins H; Sprangers B; Locascio SA; Shonts B; Kawai T; Wong W; Yang S;ZUber J; Shen Y; Sykes M Tracking Donor-Reactive T cells: Evidence for Clonal Deletion in Tolerant Kidney Transplant Patients Science translational medicine 2015; 7(272).

37. Bonnefoix T, Bonnefoix P, Mi JQ, Lawrence JJ, Sotto JJ, Leroux D. Detection of suppressor T lymphocytes and estimation of their frequency in limiting dilution assays by generalized linear regression modeling. Journal of immunology 2003; 170(6): 2884–2894.

38. Bonnefoix T, Bonnefoix P, Perron P, Mi JQ, Ng WF, Lechler R et al. Quantitating effector and regulatory T lymphocytes in immune responses by limiting dilution analysis modeling. Journal of immunology 2005; 174(6): 3421–3431.

39. Shaffer J, Villard J, Means TK, Alexander S, Dombkowski D, Dey BR et al. Regulatory T-cell recovery in recipients of haploidentical nonmyeloablative hematopoietic cell transplantation with a humanized anti-CD2 mAb, MEDI-507, with or without fludarabine. Experimental hematology 2007; 35: 1140–1152. [PubMed: 17588483]

40. Sprangers B, DeWolf S, Savage TM, Morokata T, Obradovic A, LoCascio SA et al. Origin of Enriched Regulatory T Cells in Patients Receiving Combined Kidney-Bone Marrow Transplantation to Induce Transplantation Tolerance. American journal of transplantation : official journal of the American Society of Transplantation and the American Society of Transplant Surgeons 2017; 17(8): 2020–2032. doi: 10.1111/ajt.14251

41. Miyara M, Yoshioka Y, Kitoh A, Shima T, Wing K, Niwa A et al. Functional delineation and differentiation dynamics of human CD4+ T cells expressing the FoxP3 transcription factor. Immunity 2009; 30(6): 899–911. [PubMed: 19464196]

42. Zuber JS B;Lau SP;Obradovic A; Fu J; Yang S;,Lambert M; Coley S; Weiner J; Thome JDeWolf S; Farber DL; Shen Y;Caillat-Zucman S; Bhagat G; Griesemer A;Martinez M; Kato T; Sykes M Bidirectional intragraft alloreactivity drives the repopulation of human intestinal allografts and correlates with clinical outcome. Science Immunology 2016; 1(eaah3732): 1–10. e-pub ahead of print 7 October 2016;

43. DeWolf S, Grinshpun B, Savage T, Lau SP, Obradovic A, Shonts B et al. Quantifying size and diversity of the human T cell alloresponse. JCI Insight 2018; 3(15). e-pub ahead of print 2018/08/10; doi: 10.1172/jci.insight.121256

44. Savage TMS BA; Obradovic A; Dewolf S; Lau S; Zuber J; Simpson MT; Berglund E; Fu J; Yang S; Robins H; Ho SH; Tang Q; Turka LA; Shen Y; Sykes M Early expansion of donor-specific Tregs in tolerant kidney transplant recipients JCI Insight 2018; In press.

45. Aoyama A, Ng CY, Millington TM, Boskovic S, Murakami T, Wain JC et al. Comparison of lung and kidney allografts in induction of tolerance by a mixed-chimerism approach in cynomolgus monkeys. Transplant.Proc 2009; 41(1): 429–430. [PubMed: 19249572]

46. Kawai T, Cosimi AB, Wee SL, Houser S, Andrews D, Sogawa H et al. Effect of mixed hematopoietic chimerism on cardiac allograft survival in cynomolgus monkeys. Transplantation 2002; 73(11): 1757–1764. [PubMed: 12084998]

47. Kawai T, Sachs DH, Cosimi AB. Tolerance to vascularized organ allografts in large animal models. Curr.Opin.Immunol 1999; 11: 516–526. [PubMed: 10508708]

48. Oura T, Ko DS, Boskovic S, O'Neil JJ, Chipashvili V, Koulmanda M et al. Kidney Versus Islet Allograft Survival After Induction of Mixed Chimerism With Combined Donor Bone Marrow Transplantation. Cell Transplant 2016; 25(7): 1331–1341. doi: 10.3727/096368915X688966 [PubMed: 26337731]

49. Tonsho M, Lee S, Aoyama A, Boskovic S, Nadazdin O, Capetta K et al. Tolerance of Lung Allografts Achieved in Nonhuman Primates via Mixed Hematopoietic Chimerism. American journal of transplantation : official journal of the American Society of Transplantation and the American Society of Transplant Surgeons 2015; 15(8): 2231–2239. doi: 10.1111/ajt.13274

50. Pilat N, Baranyi U, Klaus C, Jaeckel E, Mpofu N, Wrba F et al. Treg-Therapy Allows Mixed Chimerism and Transplantation Tolerance Without Cytoreductive Conditioning. American journal

of transplantation : official journal of the American Society of Transplantation and the American Society of Transplant Surgeons 2010; 10(4): 751–762.

51. Duran-Struuck R, Sondermeijer HP, Buhler L, Alonso-Guallart P, Zitsman J, Kato Y et al. Effect of Ex Vivo-Expanded Recipient Regulatory T Cells on Hematopoietic Chimerism and Kidney Allograft Tolerance Across MHC Barriers in Cynomolgus Macaques. Transplantation 2017; 101(2): 274–283. doi: 10.1097/TP.0000000000001559 [PubMed: 27846155]
52. Kawai T, Poncelet A, Sachs DH, Mauiyyedi S, Boskovic S, Wee SL et al. Long-term outcome and alloantibody production in a non-myeloablative regimen for induction of renal allograft tolerance. Transplantation 1999; 68: 1767–1775. [PubMed: 10609955]
53. Li W, Kuhr CS, Zheng XX, Carper K, Thomson AW, Reyes JD et al. New insights into mechanisms of spontaneous liver transplant tolerance: the role of Foxp3-expressing CD25+CD4+ regulatory T cells. American journal of transplantation : official journal of the American Society of Transplantation and the American Society of Transplant Surgeons 2008; 8(8): 1639–1651.
54. Li W, Lu L, Wang Z, Wang L, Fung JJ, Thomson AW et al. Costimulation blockade promotes the apoptotic death of graft- infiltrating T cells and prolongs survival of hepatic allografts from FLT3L-treated donors. Transplantation 2001; 72(8): 1423–1432. [PubMed: 11685115]
55. Starzl TE, Murase N, Thomson A, Demetris AJ. Liver transplants contribute to their own success. Nature Med. 1996; 2: 163–165. [PubMed: 8574958]
56. Dangi A, Sumpter TL, Kimura S, Stolz DB, Murase N, Raimondi G et al. Selective expansion of allogeneic regulatory T cells by hepatic stellate cells: role of endotoxin and implications for allograft tolerance. Journal of immunology 2012; 188(8): 3667–3677. doi: jimmunol.1102460[pii];10.4049/jimmunol.1102460 [doi]
57. Kato Y, Griesemer AD, Wu A, Sondermeijer HP, Weiner JI, Duran-Struuck R et al. Novel H-shunt Venovenous Bypass for Liver Transplantation in Cynomolgus Macaques. Comp Med 2017; 67(5): 436–441. e-pub ahead of print 2017/09/25; [PubMed: 28935006]