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Hematopoietic Cell Transplantation for Tolerance Induction: Animal Models to Clinical Trials¹

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Abstract

The induction of donor-specific immune tolerance is the “holy grail” of transplantation, as it would avoid the toxicities of chronic immunosuppressive therapies while preventing acute and chronic graft rejection. A large number of approaches to tolerance induction have been described in the experimental literature, but only hematopoietic cell transplantation has shown preliminary success for intentional tolerance induction in pilot clinical trials. In this review, I summarize the conditions that allow progress to be made in moving strategies for tolerance induction from the bench to the bedside and discuss the mechanisms by which tolerance may be achieved *via* hematopoietic cell transplantation.

Keywords

Tolerance; Thymus; Chimerism; T-cells

Immune tolerance denotes a state in which the immune system accepts donor organs or tissues but is capable of responding normally to foreign antigens. While recent improvements in immunosuppressive drugs have greatly augmented early organ allograft survival rates, these improvements have had little impact on late graft loss, which is due in large part to chronic rejection. Moreover, a high incidence of malignancies and opportunistic infections as well as drug-specific metabolic and organ-specific toxicities severely limit the tolerability of long-term chronic immunosuppressive therapy. The induction of donor-specific immune tolerance would avoid these complications while also preventing chronic rejection.

A literature search on the topic of transplantation tolerance will yield more than 1700 results, reflecting the many animal studies, mostly in rodents, in which this outcome has been reported. The fact that so many studies, beginning with Medawar’s demonstration that immunologically immature animals can be tolerized by injecting allogeneic cells or tissue (1), have achieved this outcome but almost none have come to clinical fruition, underscores the difficulty in moving this goal from rodents to patients. Currently, the outcomes achieved with chronic immunosuppression, the current standard of care, are, in general, quite good; thus, any major departure from this approach requires that stringent safety and efficacy conditions first be met.

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A major focus of this article will be an analysis of the conditions that have recently been achieved to allow this transition to be made.

CONDITIONS ALLOWING CLINICAL TRIALS OF TOLERANCE STRATEGIES

Any attempt at tolerance induction in humans, which by definition requires departure from the standard of care by removing chronic immunosuppressive therapy, exposes the patient to the risk of rejection. Thus, the expected efficacy of any tolerance approach to be considered in humans must be very high on the basis of animal data before it is evaluated in clinical trials. Fortunately, organ transplant studies in large animals can come reasonably close to mimicking clinical transplantation, so that safety and efficacy evaluations performed may be relevant to humans. A large number of the strategies reported to induce tolerance in rodent models have either failed to achieve tolerance in large animals (2–5) or have not been evaluated. It has become clear over the years that primarily vascularized allografts in rodents are quite tolerogenic, so that a short course of many different kinds of immunosuppression, that prevents initial rejection, is sufficient to allow the inherent tolerogenicity of the graft to prevail. In many cases, regulatory cells have been implicated in this form of tolerance (6,7), and it seems probable that a combination of factors, including relatively low numbers of professional antigen-presenting cells in the graft and possibly exposure of large recipient blood volumes to parenchymal cells of the graft (8,9), help to tip the balance in favor of regulation and tolerance. Unfortunately, these conditions do not appear to be replicated in large animals and humans, in whom rejection of primarily vascularized grafts can be expected in the absence of chronic immunosuppressive therapy. An exception to this generality may occur in the case of renal allografts in pigs, in which a short course of high-dose calcineurin inhibitor has been found sufficient to achieve long-term tolerance across major histocompatibility complex (MHC) barriers (10,11). Of note, this tolerogenicity does not appear to apply to other vascularized organs such as the heart (12,13) unless a vascularized thymus graft is added to it (David H. Sachs, personal communication). The liver has been reported to have tolerogenic properties in rodents and may be less prone to rejection in humans (14). While allogeneic liver grafts were originally reported to be spontaneously accepted in the pig (15), later studies involving pigs with defined MHC antigens indicated that such acceptance requires MHC matching (16) and the original result may be explained by relatedness of the donors and recipients. As will be discussed below, it seems possible that the kidney also has tolerogenic properties in humans. Thus, in order to move from rodent models toward clinical trials of tolerance induction, it is important to first demonstrate efficacy in stringent rodent models such as MHC-mismatched skin grafts, which are among the most immunogenic and least tolerogenic grafts in these species (17). Once this has been achieved, efficacy and safety can be assessed in large animal models.

TOLERANCE INDUCTION BY ALLOGENEIC HEMATOPOIETIC CELL TRANSPLANTATION (HCT)

Allogeneic hematopoietic chimerism was first associated with tolerance when Freemartin cattle (fraternal twins sharing a placental circulation) were shown to be chimeric and tolerant of one another (18). However, achievement of this state in adult recipients with already-established immune systems is challenging, largely due to the immune barrier imposed by recipient T cells (19,20). This barrier can be overcome by recipient conditioning that either eliminates mature host immune cells (21,22), creating an immunological ‘clean slate’, or that permits pre-existing T cells to be rendered tolerant by the donor hematopoietic cells. The latter can be achieved with costimulatory blockade in place of T cell depletion (23). Since newly developing T and B cells are tolerized during their maturation by antigens presented by hematopoietically-derived antigen-presenting cells (APCs), mixed chimerism can educate T and B cells to recognize donor and host antigens as ‘self’, resulting systemic in donor- and host-specific tolerance. Conditioning therapy must be sufficient to overcome both peripheral

and intrathymic T cell-mediated alloreactivity. Under such conditions, both donor- and host-reactive T cells arising de novo in the thymus do so in the presence of donor and recipient APCs, and both donor- and host-reactive T cells are then specifically deleted (24–28). The coexistence of donor and recipient hematopoietic stem cells (HSCs) in the recipient marrow environment leads to life-long mixed chimerism in all hematopoietic lineages and ensures the life-long presence of APCs of each type to induce tolerance among newly-developing lymphocytes. Multilineage mixed hematopoietic chimerism is associated with lifelong central, deletional T cell tolerance, even in senescent animals (24–28), permitting the acceptance of any donor allograft without immunosuppression (22,29,30). B cells (31,32) and NK cells (33) are also tolerized by the induction of mixed chimerism, permitting long-term stability of chimerism once it is achieved.

While the above approach and mechanisms are now well-established in rodents, the development of reliable, non-toxic methods of achieving allogeneic HSC engraftment across MHC barriers in humans is a major challenge. These regimens must be non-myeloablative, so that recipient hematopoiesis can protect the recipient from marrow failure should the HSC graft be rejected. Nevertheless, conditioning must be sufficiently potent to overcome the T cell barrier to HLA-mismatched HSCs and their progeny. While NK cell barriers also reduce HSC engraftment when cell doses are limiting, administration of higher HSC numbers quite readily overcomes NK cell-mediated resistance in rodent models (34). The NK cell barrier to HSC engraftment becomes more significant when T cell suppression is incomplete (35), resulting in reductions in the number of donor stem cells that survive.

Engraftment of HSCs is promoted by myelosuppressive treatments such as a low dose of total body irradiation (TBI) or busulfan. The mechanism by which myelosuppressive host treatment promotes HSC engraftment is not fully understood, and could include both the creation of physical niches and the upregulation of cytokines and other molecules that promote hematopoiesis and HSC expansion (36). However, the need for myelosuppressive therapy can be avoided when very high doses of HSCs are administered (37–39). Nevertheless, some degree of myelosuppression is required for the achievement of mixed chimerism with HSC doses that are currently obtainable from human donors.

To be applicable for the induction of organ allograft tolerance, the state of mixed chimerism must be achieved without the complication of graft-vs-host disease (GVHD), which is currently the major complication of clinical HCT that precludes its routine application across HLA barriers, even in the presence of malignant disease with no other curative option. GVHD is still a significant complication, affecting 40–50% of HCT recipients, even in the HLA-identical setting, and even in recipients of reduced-intensity conditioning regimens who receive pharmacologic GVHD prophylaxis (40,41). This complication, if kept to a manageable level, is acceptable in patients with hematologic malignancies because it is associated with a therapeutic benefit, namely a graft-vs-leukemia (GVL) effect (42). However, this complication is completely unacceptable in patients without malignant disease who need an organ transplant. Unlike HCT for the treatment of hematologic malignancies, solid organ transplantation is routinely performed across extensive HLA barriers. Thus, if HCT is to be used as an approach to organ allograft tolerance induction, the enormous challenge of avoiding GVHD while crossing HLA barriers must be met.

A number of successful HCT protocols developed in animal models involve myelosuppressive and/or immunosuppressive, but not myeloablative, conditioning regimens. In many of the rodent models, GVHD does not develop from the initial HCT because the few donor T cells present in the marrow are exposed to same in vivo treatment that depletes or tolerizes recipient T cells to the donor. Unlike human bone marrow or PBSC, rodent bone marrow does not contain much blood and thus has very little contamination with the type of T cells that most readily

cause GVHD (43). *In vivo* depletion of host CD4⁺ and CD8⁺ T-cells along with TBI (at least 6 Gy) permitted engraftment of allogeneic marrow and induction of skin graft tolerance across complete MHC barriers in mice (44). Adding local thymic irradiation (7 Gy) to the regimen permitted engraftment of fully MHC-mismatched allogeneic marrow in mice receiving only 3 Gy TBI (22). Thymic irradiation is needed because thymocytes, unlike T-cells in the peripheral lymphoid compartment, are not eliminated by the above mAbs. Permanent mixed chimerism and donor- and host-specific tolerance are reliably induced across complete MHC barriers using this regimen (reviewed in (45)). Intrathymic clonal deletion is the major mechanism inducing and maintaining long-term donor-specific tolerance, and is associated with the presence of donor class II^{high} cells in the recipient thymus throughout life, beginning within a few days of BMT (24). Tolerance can be broken by depleting donor cells with donor class I MHC-specific mAbs after stable chimerism has been established and this loss of tolerance correlates with the de novo appearance in the periphery of T-cells bearing donor-reactive TCR (27). However, if the host thymus is removed before depletion of donor cells, or if donor-depleted spleen cells from chimeras are transferred to syngeneic athymic mice, donor-specific tolerance is maintained, and cells with donor-reactive TCR do not appear in the peripheral repertoire (27). These results demonstrate clearly that intrathymic chimerism is essential and sufficient for the maintenance of tolerance in long-term mixed allogeneic chimeras, whereas peripheral chimerism plays no significant role. Since persistent antigen is required to maintain anergy, tolerance cannot be explained by a peripheral anergy mechanism. Moreover, the ease with which new thymic emigrants break tolerance after donor cell depletion by mAb or with which tolerance is broken by infusion of non-tolerant recipient lymphocytes (27) indicates that suppressive cell populations do not play a significant role in maintaining long-term tolerance. Thus, ablation of pre-existing peripheral and intrathymic mature T cells is followed by lifelong central, deletional tolerance in this mouse model.

Conditioning regimens that do not fully deplete T cells have subsequently been developed in murine models. Both thymic irradiation and T cell depleting mAbs in the conditioning regimen discussed above can be replaced by costimulatory blockade (23). Myelosuppressive therapy can also be removed by giving a high dose of donor marrow (46,47). The success of costimulatory blockade in this context is encouraging (23,46–48), as it is difficult to achieve exhaustive T cell depletion with antibodies in large animals and humans. Moreover, if complete T cell depletion could be achieved in humans, T cell recovery from the thymus might be dangerously slow, especially in older patients with minimal thymic function (reviewed in (49)).

Similar to other protocols achieving sustained mixed chimerism, long-term tolerance is maintained by intrathymic deletion in murine mixed chimeras prepared with costimulatory blockade (23,46,48). However, since alloreactive T cells present in the peripheral repertoire at the time of BMT are not depleted prior to transplant, these peripheral T cells must be rendered tolerant. Initial tolerance involves anergy followed by specific deletion of pre-existing peripheral donor-reactive CD4 (50) and CD8 (51,52) T cells. In recipients of BMT with anti-CD154 and 3 Gy TBI, specific donor-reactive CD8 deletion occurs within 1–2 weeks. This rapid CD8 tolerance requires CD4 cells that do not have the characteristics associated with “natural” Tregs (51). Also arguing against a role for “adaptive” or “induced” Tregs, CD4 cells are not required for maintenance of tolerance after this initial 2-week period (51). Thus, while CD4 cells are clearly required for CD8 tolerance in this model, the evidence does not implicate a specific subset of CD4 cells that is differentiated to mediate suppression. The expression of MHC class II on recipient APCs, as well as recipient dendritic cells and B cells, all play an important role in tolerizing pre-existing CD8 cells, but not CD4 cells, in this model (53). Another difference between the mechanism of tolerance of CD4 and CD8 cells is the requirement for PD-1/PD-L1 interactions to tolerize CD8 cells but not CD4 cells (54). Deletion of peripheral donor-reactive CD4 cells occurs more slowly than that of CD8 cells, over 4–5

weeks in chimeras induced with anti-CD154 mAb (50,55). Regulatory cells do not appear to play a major role in inducing or maintaining the tolerance induced by anti-CD154 with BMT (50). Since HSC engraftment ensures complete central deletional tolerance in these long-term chimeras (23,46,48,56), and specific peripheral deletion is quite complete, there may be insufficient donor-reactive T cells present to promote the expansion and maintenance of specific regulatory cells. However, several different allogeneic BMT models using costimulatory blockade in the conditioning may be associated with less complete deletion of pre-existing donor-reactive T cells and seem to involve regulatory mechanisms (57,58).

Durable mixed chimerism has been achieved in several large animal models. In dogs, non-myeloablative regimens have reliably achieved durable chimerism only in the MHC-matched setting (59,60), and this has been associated with renal allograft tolerance (61). In pigs, however, durable mixed chimerism has been achieved across extensive MHC barriers with minimal regimens that include partial in vivo T cell depletion, a very low dose of TBI or thymic irradiation and a short course of post-transplant cyclosporine. This success has relied on the administration of very large doses of donor PBSC. Mixed chimerism is associated with renal and cardiac allograft tolerance in this model (62–64). Since neither donor nor host T cells are fully depleted in recipients of this conditioning regimen, the mechanisms of tolerance that develop must control the residual donor- and host-reactive T cells, and regulatory mechanisms have been implicated. A non-human primate model for mixed chimerism and renal allograft tolerance induction across MHC barriers includes conditioning of cynomolgous monkeys with 3 Gy TBI and 7 Gy thymic irradiation. Since effective T-cell-depleting mAbs are not available for use in primates, polyclonal ATG and a short (28 day) course of cyclosporine are used in its place. A high percentage of splenectomized monkeys receiving class I- and II-mismatched marrow with this protocol develop transient mixed chimerism and donor kidney allograft acceptance (65). The splenectomy is required to avoid anti-donor antibody responses and can be replaced by the use of anti-CD154 mAb (66). The chimerism is only transient in these animals and the mechanisms of tolerance are therefore likely to be more complex than the simple intrathymic deletion process discussed above.

Durable mixed chimerism appears to avoid delayed or chronic rejection in addition to acute rejection of allografts. However, despite several reports of freedom from chronic rejection in cardiac allografts in mixed chimeras (30,32), a special form of isolated graft vasculopathy that does not appear to require T cell alloreactivity has been detected in donor heart grafts in murine mixed allogeneic chimeras (62,67,68). Notably, durable mixed chimeras did not show chronic rejection of kidney or cardiac allografts in large animal porcine models (63,69,70), making it likely that patients with durable chimerism would also be free of this complication.

Mixed chimeras contain, in addition to donor cells, a life-long population of antigen-presenting cells that assures effective antigen presentation to T cells that are positively selected on the recipient thymic epithelium (71,72). However, the exquisite host restriction of virus-specific CTLs in mixed chimeras (72,73) can allow the persistence of a pathogenic viral reservoir in donor cells if the donor and recipient are fully MHC-mismatched. This problem can be avoided by partial MHC sharing between the donor and recipient (73).

ROLE FOR MIXED CHIMERISM IN THE TREATMENT OF HEMATOLOGIC MALIGNANCIES

In recent years, a variety of clinical HCT protocols have been developed for the treatment of hematologic malignancies that utilize reduced intensity conditioning. While mixed chimerism may occur initially in recipients of these protocols, the goal is to achieve full donor chimerism, which is often associated with the complication of GVHD. As is discussed above, a mild to moderate level of GVHD is considered acceptable in this setting, as GVHD is associated with

enhanced anti-tumor effects (74). However, the level of GVHD observed when extensive HLA barriers are transgressed has essentially precluded the routine use of extensively HLA-mismatched HCT.

We have attempted to overcome the limitations to HLA-mismatched HCT for the treatment of hematologic malignancies in a series of clinical protocols that use non-myeloablative conditioning that includes T cell depletion of the recipient and the donor HSC product and aim to achieve initial mixed chimerism without any graft-vs-host response from the HSC transplant. These trials resulted from animal studies showing that conditioning-induced tissue inflammation plays an important role in promoting GVHD. In fact, MHC-directed GVH alloreactivity can be confined to the lymphohematopoietic system when non-tolerant donor T cells are given to mixed chimeras after inflammation induced by the conditioning regimen has subsided. As is discussed above, established mixed chimeras are immunologically tolerant of their original marrow donor. Therefore, GVH reactions resulting from administration of non-tolerant T cells in delayed donor leukocyte infusions (DLI) are not opposed by any host-vs-graft response in mixed chimeras. The unopposed GVH response results in conversion of mixed hematopoietic chimerism to full donor chimerism and strong graft-vs-leukemia/lymphoma (GVL) effects (75,76). However, this powerful GVH alloresponse against lymphohematopoietic cells is not associated with clinical or histological GVHD, even though similar numbers of donor T cells cause rapidly lethal GVHD in freshly conditioned recipients (75,77). Despite clear evidence that they are activated in established mixed chimeras, DLI-derived GVH-reactive T cells do not migrate to the GVHD target tissues, which are mainly epithelial tissues such as skin, intestines and liver. This failure to traffic is due to the absence of inflammatory signals in those tissues (78). Such inflammatory signals, including chemokines and probably adhesion molecules, are induced in GVHD target tissues by conditioning treatment and subside over time (79).

The most potent GVL effects are mediated by GVH-reactive T cells recognizing host MHC alloantigens (76,80) and GVH-reactive T cells become activated and proliferate in established mixed chimeras receiving DLI (78,80). Recipient hematopoietically-derived APCs expressing both class I and class II MHC are required to induce this anti-host reactivity and maximal GVL, which occurs only in mixed and not fully allogeneic chimeras. Fully allogeneic chimeras, which lack host APCs, do not induce activation or expansion of T cells in DLI, which thereby fail to mediate strong GVL effects (76,80).

In efforts to apply this approach to separating GVHD and GVL clinically, proof of principle has been obtained that GVH responses can be confined to the lymphohematopoietic system following delayed DLI and thereby fail to induce GVHD in patients who received non-myeloablative HCT with an initially T cell-depleted HSC graft, even across extensive HLA barriers (81). However, unlike the mice, some patients develop GVHD after receiving DLI. In contrast to mice, T cell recovery in patients is generally poor at the time when DLI are given (82), predisposing them to infections. Activation of toll-like receptors (TLRs), as occurs during infection, can promote the trafficking of DLI-derived T cells to the GVHD target tissues in established mixed chimeras (78). Improved T and B cell reconstitution, which would lead to better control of post-transplant infections and prevent TLR-dependent immune activation, would therefore be likely to improve the ability to separate GVHD and GVL with this approach in patients.

MIXED CHIMERISM: MOVING FROM TREATMENT OF HEMATOLOGIC MALIGNANCIES TO ORGAN ALLOGRAFT TOLERANCE INDUCTION

Studies discussed above, namely the achievement of renal allograft tolerance in a primate model via non-myeloablative mixed chimerism induction (65), combined with clinical results

using the above approach for achieving GVL without GVHD that involved initial mixed chimerism induction (83,84), allowed the first successful trial of organ allograft tolerance induction to be carried out in humans. Clinical safety data obtained with the above non-myeloablative HCT protocols provided an opportunity to attempt to induce transplantation tolerance in patients with a hematologic malignancy, multiple myeloma, and consequent renal failure. Six patients received a simultaneous non-myeloablative bone marrow transplant and renal allograft from an HLA-identical sibling followed by a delayed DLI as part of the anti-malignancy therapy. These patients accepted their kidney allografts without any immunosuppression for follow-ups as long as 10 years. Three of the six patients achieved prolonged complete remissions of their myelomas (85), which was especially surprising in two of the patients, in whom chimerism was only transient (85). Sensitization to minor histocompatibility antigens expressed on hematopoietic cells was observed in some patients who lost their chimerism (85), suggesting that the renal allograft tolerance that was nevertheless achieved might include a role for the kidney graft itself. These patients demonstrated unresponsiveness to donor renal tubular epithelial cells (85), consistent with the possibility that tolerance is specific for minor antigens expressed on the kidney graft itself. In patients with transient chimerism, the pure central, deletional tolerance achieved in murine models is unlikely to be the major mechanism of allograft tolerance.

While results of HCT have generally been poor in the setting of solid tumors, there are case reports of allogeneic HCT performed in hepatocellular carcinoma or cholangiocarcinoma patients receiving liver allografts from the same donor. One patient received myeloablative conditioning and mixed autologous plus donor CD34⁺ cells and achieved mixed chimerism that converted to full donor chimerism following DLI, but succumbed to an opportunistic infection (86). Two additional patients received non-myeloablative conditioning and HLA-mismatched CD34 cell transplantation followed by liver transplantation from the same living donor 40–55 days later. Both were successfully weaned from immunosuppression without allograft rejection despite a lack of durable chimerism, but the tumors recurred (87).

The approach described above in myeloma patients has recently been extended in a pilot study in patients without malignant disease, who received HLA-mismatched haploidentical related donor bone marrow transplantation solely for the purpose of inducing tolerance to the kidney from the same donor. Demonstration of safety in a trial using the same conditioning regimen for HLA-mismatched haploidentical related donor BMT in patients with hematologic malignancies, using the approach of non-myeloablative mixed chimerism induction followed by delayed DLI (81), was critical in allowing this evaluation of tolerance induction in the HLA-mismatched setting. In the trial upon which this study was based, four patients with hematologic malignancies received haploidentical bone marrow grafts following conditioning with cyclophosphamide, thymic irradiation and peri-transplant treatment with a humanized anti-CD2 mAb, a more potent T cell-depleting agent than the equine ATG used in the studies described above for HLA-identical transplantation in myeloma patients. Only transient chimerism was observed in the HLA-mismatched recipients of this initial anti-CD2-based regimen, but loss of chimerism was associated with robust recipient hematopoiesis, demonstrating that the regimen was truly non-myeloablative. Although loss of chimerism was not the desired outcome in this study, these studies documented the important safety parameters of absence of GVHD and preservation of robust recipient hematopoietic potential.

In summary, baseline studies at our institution had established: 1) The ability to achieve initial mixed chimerism without GVHD in the setting of HLA-mismatched HCT; 2) that transient chimerism was associated with renal allograft tolerance when combined MHC-mismatched bone marrow and kidney transplantation were performed in non-human primates; and 3) that transient chimerism was associated with renal allograft tolerance in recipients of combined HLA-identical kidney and BMT in multiple myeloma patients. These observations justified a

second combined kidney/BMT clinical tolerance trial, now using haploidentical related donors in recipients without malignant disease. The HLA-mismatched combined kidney/BMT pilot trial using the non-myeloablative protocol shown in Figure 1 was performed in 5 patients without malignant disease. Follow-up now ranges from about 3 to >6 years. Four of 5 patients were successfully weaned from their initial immunosuppressive monotherapy with calcineurin inhibitor, and graft function has been achieved without immunosuppression for about 2 to 5 years. One graft was lost early due to acute humoral rejection, and this resulted in a modification of the protocol to include anti-CD20 mAb in the conditioning regimen for B cell depletion (88). This trial represents the first successful, intentional achievement of tolerance to an organ allograft across HLA barriers.

In vitro analyses of these patients revealed the progressive development of complete donor-specific unresponsiveness in both mixed lymphocyte responses (MLR) and cell-mediated lympholysis (CML) assays in the four tolerant recipients of combined kidney and BMT. Normal third party alloresponses recovered in all four patients (88), suggesting that the state of donor-specific tolerance was systemic. These observations contrast with those in recipients of HLA-identical combined kidney and bone marrow transplants, who sometimes demonstrated sensitization to donor hematopoietic antigens in association with loss of chimerism (85). These results suggest that the mechanisms of tolerance might differ in the HLA-identical versus the mismatched setting. However, a more unifying explanation is that in both groups tolerance is restricted to antigens expressed by the kidney. In the recipients of HLA-mismatched, haploidentical transplants, the strong pre-existing anti-donor response may disappear after transplant because most allogeneic MHC/peptide complexes inducing direct alloresponses are shared by both the kidney and the hematopoietic cells. Very few T cells may recognize MHC alloantigen/peptide complexes expressed only on hematopoietic cells, and tolerance to those expressed on the kidney would thereby lead to loss of the bulk MLR and CML response. Alternatively, the loss of chimerism in these patients occurred within a few weeks of transplant, when T cells were markedly depleted by the conditioning, and it also remains possible that the loss of chimerism does not reflect a T cell-mediated immune response but instead reflects inadequate donor hematopoietic stem cell engraftment. *In vitro* results differed significantly between recipients of combined haploidentical kidney and BMT and those with hematologic malignancies who received the similar haploidentical BMT regimen without a kidney transplant. In contrast to the combined transplant recipients, those receiving BMT alone showed generally weak alloresponses but nevertheless tended to have stronger anti-donor than anti-third party responses following the loss of chimerism (82). In combination, these results are consistent with a role for the kidney in the tolerance achieved in the recipients of kidney and BMT together.

Given the transient nature of the chimerism achieved, long-term tolerance in the recipients of HLA-mismatched combined kidney and BMT is unlikely to be due to a pure central deletion mechanism. Intra-graft levels of the Treg-associated transcription factor FoxP3 relative to Granzyme B mRNA were increased in tolerant patients compared to conventional transplant patients, raising the possibility that regulatory T cells might play a role in tolerance (88). Regulatory cells are enriched among the T cells initially present in recipients of this regimen for BMT alone (82), and ongoing studies should help to assess the possible role of such cells in the combined transplant recipients. Several of the patients on this trial, including two who achieved operational tolerance, developed donor-specific alloantibodies at various time points; in one case that ultimately achieved tolerance the antibodies developed early and were associated with an acute humoral rejection episode that was successfully treated; in the other case, the antibodies developed later and have not been associated with significant pathology on repeat renal biopsies (88). Thus, the significance of these responses, and their pathogenesis in the presence of apparent T cell tolerance, is currently unclear and requires further investigation.

A different HCT protocol based on the use of ATG and total lymphoid irradiation (TLI), also supported by safety data obtained with a related protocol in patients with hematologic malignancies (89), has recently been used to successfully induce renal allograft tolerance in one of three patients receiving combined hematopoietic and kidney transplantation from HLA-identical related donors (90). This regimen therefore may be less effective than the above ATG/cyclophosphamide-based regimen used to condition myeloma patients for combined kidney and bone marrow transplants (85). A similar ATG/TLI-based protocol was unsuccessful in the HLA-mismatched setting (91,92).

FUTURE DIRECTIONS

HCT has considerable promise for the achievement of organ allograft tolerance in humans and this potential has as yet only just begun to be tapped. Further advances in the development of conditioning regimens that achieve engraftment of hematopoietic cells across HLA barriers without ablative host treatment and without the risk of GVHD should further broaden the applicability of this approach. It will be desirable to extend this approach to other organs, including liver and heart, necessitating further large animal experimentation and the development of protocols whose timing is appropriate for cadaveric transplantation. Ultimately, extension to xenogeneic marrow and organ transplantation, which presents additional immunological and physiological hurdles, would overcome the existing organ allograft shortage. An understanding of the mechanisms by which tolerance is induced and maintained in large animals and pilot clinical studies of combined organ and hematopoietic cell transplantation will be important for the further advancement of this approach.

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Abbreviations

APCs	antigen-presenting cells
BMT	bone marrow transplantation
CML	cell-mediated lympholysis
DLI	donor leukocyte infusion
GVH	graft-vs-host
GVHD	graft-vs-host disease
GVL	graft-vs-leukemia
HCT	hematopoietic cell transplantation

HLA	human leukocyte antigen
HSCs	hematopoietic stem cells
mAbs	monoclonal antibodies
MHC	major histocompatibility complex
MLR	mixed lymphocyte response
NK cells	Natural killer cells
PBSC	peripheral blood stem cells
TBI	total body irradiation
TCR	T cell receptor
TLRs	toll-like receptors

Reference List

1. Billingham RE, Brent L, Medawar PB. "Actively acquired tolerance" of foreign cells. *Nature* 1953;172:603–606. [PubMed: 13099277]
2. Kirk AD, Harlan DM, Armstrong NN, et al. CTLA4-Ig and anti-CD40 ligand prevent renal allograft rejection in primates. *Proc Natl Acad Sci USA* 1997;94:8789–8798. [PubMed: 9238056]
3. Kirk AD, Burkly LC, Batty DS, et al. Treatment with humanized monoclonal antibody against CD154 prevents acute renal allograft rejection in nonhuman primates. *Nature Med* 1999;5:686–693. [PubMed: 10371508]
4. Kirk AD, Tadaki DK, Celniker A, et al. Induction therapy with monoclonal antibodies specific for cd80 and cd86 delays the onset of acute renal allograft rejection in non-human primates I. *Transplantation* 2001;72:377–384. [PubMed: 11502964]
5. Elster EA, Xu H, Tadaki DK, et al. Treatment with the humanized CD154-specific monoclonal antibody, hu5C8, prevents acute rejection of primary skin allografts in nonhuman primate. *Transplantation* 2001;72:1473–1478. [PubMed: 11707732]
6. Kang SM, Tang Q, Bluestone JA. CD4+CD25+ regulatory T cells in transplantation: progress, challenges and prospects. *Am J Transplant* 2007;7:1457–1463. [PubMed: 17511675]
7. Wood KJ, Sakaguchi S. Regulatory Lymphocytes: Regulatory T cells in transplantation tolerance. *Nat Rev Immunol* 2003;3:199–210. [PubMed: 12658268]
8. Sun J, Sheil AGR, Wang C, et al. Tolerance to rat liver allografts. IV. Acceptance depends on the quantity of donor tissue and on donor leukocytes. *Transplantation* 1996;62:1725–1730. [PubMed: 8990351]
9. Sun J, McCaughan GW, Gallagher ND, Sheil AGR, Bishop GA. Deletion of spontaneous rat liver allograft acceptance by donor irradiation. *Transplantation* 1995;60:233–236. [PubMed: 7645034]

10. Rosengard BR, Ojikutu CA, Guzzetta PC, et al. Induction of specific tolerance to class I disparate renal allografts in miniature swine with cyclosporine. *Transplantation* 1992;54:490–497. [PubMed: 1412729]
11. Gianello PR, Lorf T, Yamada K, et al. Induction of tolerance to renal allografts across single-haplotype MHC disparities in miniature swine. *Transplantation* 1995;59:884–890. [PubMed: 7701585]
12. Mezrich JD, Yamada K, Lee RS, et al. Induction of tolerance to heart transplants by simultaneous cotransplantation of donor kidneys may depend on a radiation-sensitive renal-cell population. *Transplantation* 2003;76:625–631. [PubMed: 12973099]
13. Mezrich JD, Benjamin LC, Sachs JA, et al. Role of the thymus and kidney graft in the maintenance of tolerance to heart grafts in miniature swine. *Transplantation* 2005;79:1663–1673. [PubMed: 15973167]
14. Lerut J, Sanchez-Fueyo A. An appraisal of tolerance in liver transplantation. *Am J Transplant* 2006;6:1774–1780. [PubMed: 16889539]
15. Calne RY, White HJ, Yoffa DE, et al. Prolonged survival of liver transplants in the pig. *Br Med J* 1967;4:645–648. [PubMed: 4862477]
16. Flye MW, Pennington L, Kirkman R, Weber B, Sindelar W, Sachs DH. Spontaneous acceptance or rejection of orthotopic liver transplants in outbred and partially inbred miniature swine. *Transplantation* 1999;68:599–607. [PubMed: 10507476]
17. Jones ND, Turvey SE, van Maurik A, et al. Differential susceptibility of heart, skin, and Islet allografts to T cell-mediated rejection. *J Immunol* 2001;166:2824–2830. [PubMed: 11160350]
18. Owen RD. Immunogenetic consequences of vascular anastomoses between bovine twins. *Science* 1945;102:400–401. [PubMed: 17755278]
19. Sharabi, Y.; Sachs, DH.; Sykes, M. T cell subsets resisting induction of mixed chimerism across various histocompatibility barriers. In: Gergely, J.; Benczur, M.; Falus, A., et al., editors. *Progress in Immunology VIII; Proceedings of the Eighth International Congress of Immunology*; Budapest. Heidelberg: Springer-Verlag; 1992. p. 801-805.
20. Hayashi H, LeGuern C, Sachs DH, Sykes M. Alloresistance to K locus mismatched bone marrow engraftment is mediated entirely by CD4+ and CD8+ T cells. *Bone Marrow Transplant* 1996;18:285–292. [PubMed: 8864436]
21. Ildstad ST, Sachs DH. Reconstitution with syngeneic plus allogeneic or xenogeneic bone marrow leads to specific acceptance of allografts or xenografts. *Nature* 1984;307(5947):168–170. [PubMed: 6361574]
22. 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]
23. Wekerle T, Sayegh MH, Hill J, et al. Extrathymic T cell deletion and allogeneic stem cell engraftment induced with costimulatory blockade is followed by central T cell tolerance. *J Exp Med* 1998;187:2037–2044. [PubMed: 9625763]
24. Tomita Y, Khan A, Sykes M. Role of intrathymic clonal deletion and peripheral anergy in transplantation tolerance induced by bone marrow transplantation in mice conditioned with a non-myeloablative regimen. *J Immunol* 1994;153:1087–1098. [PubMed: 8027542]
25. 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]
26. 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]
27. Khan A, Tomita Y, Sykes M. Thymic dependence of loss of tolerance in mixed allogeneic bone marrow chimeras after depletion of donor antigen. Peripheral mechanisms do not contribute to maintenance of tolerance. *Transplantation* 1996;62:380–387. [PubMed: 8779687]
28. Manilay JO, Pearson DA, Sergio JJ, Swenson KG, Sykes M. Intrathymic deletion of alloreactive T cells in mixed bone marrow chimeras prepared with a nonmyeloablative conditioning regimen. *Transplantation* 1998;66:96–102. [PubMed: 9679828]

29. Guo Z, Wang J, Dong Y, et al. Long-term survival of intestinal allografts induced by costimulation blockade, busulfan and donor bone marrow infusion. *Am J Transplant* 2003;3:1091–1098. [PubMed: 12919088]
30. Shirasugi N, Adams AB, Durham MM, et al. Prevention of chronic rejection in murine cardiac allografts: a comparison of chimerism- and nonchimerism-inducing costimulation blockade-based tolerance induction regimens. *J Immunol* 2002;169:2677–2684. [PubMed: 12193741]
31. Yang Y-G, deGoma E, Ohdan H, et al. Tolerization of anti-gal α 1–3gal natural antibody- forming B cells by induction of mixed chimerism. *J Exp Med* 1998;187:1335–1342. [PubMed: 9547344]
32. Ohdan H, Yang Y-G, Shimizu A, Swenson KG, Sykes M. Mixed bone marrow chimerism induced without lethal conditioning prevents T cell and anti-Gal α 1,3Gal-mediated graft rejection. *J Clin Invest* 1999;104:281–290. [PubMed: 10430609]
33. Zhao Y, Ohdan H, Manilay JO, Sykes M. NK cell tolerance in mixed allogeneic chimeras. *J Immunol* 2003;170:5398–5405. [PubMed: 12759414]
34. Lee LA, Sachs DH, Sykes M. Effect of NK cell depletion on long-term, multilineage allogeneic bone marrow engraftment. *Transplant Proc* 1992;25:1246–1247. [PubMed: 8442104]
35. Kean LS, Hamby K, Koehn B, et al. NK cells mediate costimulation blockade-resistant rejection of allogeneic stem cells during nonmyeloablative transplantation. *Am J Transplant* 2006;6:292–304. [PubMed: 16426313]
36. Ito H, Takeuchi Y, Shaffer J, Sykes M. Local irradiation enhances congenic donor pluripotent hematopoietic stem cell engraftment similarly in irradiated and non-irradiated sites. *Blood* 2003;103:1949–1954. [PubMed: 14592817]
37. Tomita Y, Sachs DH, Sykes M. Myelosuppressive conditioning is required to achieve engraftment of pluripotent stem cells contained in moderate doses of syngeneic bone marrow. *Blood* 1994;83:939–948. [PubMed: 7906567]
38. Ramshaw HS, Crittenden RB, Dooner M, Peters SO, Rao SS, Quesenberry PJ. High levels of engraftment with a single infusion of bone marrow cells into normal unprepared mice. *Biol Blood Marrow Transplant* 1995;1:74–80. [PubMed: 9118295]
39. Sykes M, Szot GL, Swenson K, Pearson DA, Wekerle T. Separate regulation of hematopoietic and thymic engraftment. *Exp Hematol* 1997;26:457–465. [PubMed: 9620278]
40. Diaconescu R, Flowers CR, Storer B, et al. Morbidity and mortality with nonmyeloablative compared with myeloablative conditioning before hematopoietic cell transplantation from HLA-matched related donors. *Blood* 2004;104:1550–1558. [PubMed: 15150081]
41. Sorror ML, Storer BE, Maloney DG, Sandmaier BM, Martin PJ, Storb R. Outcomes after allogeneic hematopoietic cell transplantation with nonmyeloablative or myeloablative conditioning regimens for treatment of lymphoma and chronic lymphocytic leukemia. *Blood* 2008;111:446–452. [PubMed: 17916744]
42. Weiden PL, Sullivan KM, Flournoy N, Storb R, Thomas ED. Antileukemic effect of chronic graft-versus-host disease. *New Engl J Med* 1981;304:1529–1533. [PubMed: 7015133]
43. Zeng D, Hoffmann P, Lan F, Huie P, Higgins J, Strober S. Unique patterns of surface receptors, cytokine secretion, and immune functions distinguish T cells in the bone marrow from those in the periphery: impact on allogeneic bone marrow transplantation. *Blood* 2002;99:1449–1457. [PubMed: 11830499]
44. Cobbold SP, Qin S, Waldmann H. Reprogramming the immune system for tolerance with monoclonal antibodies. *Sem Immunol* 1990;2:377–387.
45. Sykes M. Mechanisms of tolerance induced via mixed chimerism. *Front Biosci* 2007;12:2922–2934. [PubMed: 17485269]
46. Wekerle T, Kurtz J, Ito H, et al. Allogeneic bone marrow transplantation with costimulatory blockade induces macrochimerism and tolerance without cytoreductive host treatment. *Nature Med* 2000;6:464–469. [PubMed: 10742157]
47. Durham MM, Bingaman AW, Adams AB, et al. Cutting edge: administration of anti-CD40 ligand and donor bone marrow leads to hemopoietic chimerism and donor-specific tolerance without cytoreductive conditioning. *J Immunol* 2000;165:1–4. [PubMed: 10861026]
48. Ito H, Kurtz J, Shaffer J, Sykes M. CD4 T cell-mediated alloresistance to fully MHC-mismatched allogeneic bone marrow engraftment is dependent on CD40-CD40L interactions, and lasting T cell

- tolerance is induced by bone marrow transplantation with initial blockade of this pathway. *J Immunol* 2001;166:2970–2981. [PubMed: 11207246]
49. Haynes BF, Markert ML, Sempowski GD, Patel DD, Hale LP. The role of the thymus in immune reconstitution in aging, bone marrow transplantation, and HIV-1 infection. *Ann Rev Immunol* 2000;18:529–560. [PubMed: 10837068]
 50. Kurtz J, Shaffer J, Anosova N, Benichou G, Sykes M. Mechanisms of early peripheral CD4 T cell tolerance induction by anti-CD154 monoclonal antibody and allogeneic bone marrow transplantation: Evidence for anergy and deletion, but not regulatory cells. *Blood* 2004;103:4336–4343. [PubMed: 14962909]
 51. Fehr T, Takeuchi Y, Kurtz J, Sykes M. Early regulation of CD8 T cell alloreactivity by CD4+CD25- T cells in recipients of anti-CD154 antibody and allogeneic BMT is followed by rapid peripheral deletion of donor-reactive CD8+ T cells, precluding a role for sustained regulation. *Eur J Immunol* 2005;35:2679–2690. [PubMed: 16082727]
 52. Fehr T, Wang S, Haspot F, et al. Rapid Deletional Peripheral CD8 T Cell Tolerance Induced by Allogeneic Bone Marrow: Role of Donor Class II MHC and B Cells. *J Immunol* 2008;181:4371–4380. [PubMed: 18768896]
 53. Fehr T, Haspot F, Molloy J, Chittenden M, Hogan T, Sykes M. Alloreactive CD8 T cell tolerance requires recipient B cells, dendritic cells and MHC class II. *J Immunol* 2008;181:165–173. [PubMed: 18566381]
 54. Haspot F, Fehr T, Gibbons C, et al. Peripheral deletional tolerance of alloreactive CD8 but not CD4 cells is dependent on the PD-1/PD-L1 pathway. *Blood* 2008;112:2149–2155. [PubMed: 18577709]
 55. Kurtz J, Ito H, Wekerle T, Shaffer J, Sykes M. Mechanisms involved in the establishment of tolerance through costimulatory blockade and BMT: Lack of requirement for CD40L-mediated signaling for tolerance or deletion of donor-reactive CD4+ cells. *Am J Transplant* 2001;1:339–349. [PubMed: 12099378]
 56. Wekerle T, Sayegh MH, Chandraker A, Swenson KG, Zhao Y, Sykes M. Role of peripheral clonal deletion in tolerance induction with bone marrow transplantation and costimulatory blockade. *Transplant Proc* 1999;31:680. [PubMed: 10083292]
 57. Bigenzahn S, Blaha P, Koporc Z, et al. The role of non-deletional tolerance mechanisms in a murine model of mixed chimerism with costimulation blockade. *Am J Transplant* 2005;5:1237–1247. [PubMed: 15888027]
 58. Domenig C, Sanchez-Fueyo A, Kurtz J, et al. Roles of deletion and regulation in creating mixed chimerism and allograft tolerance using a nonlymphoablative irradiation-free protocol. *J Immunol* 2005;175:51–60. [PubMed: 15972631]
 59. Fukuda T, Kerbauf FR, Gooley T, Santos EB, Storb R, Sandmaier BM. Dog leukocyte antigen-haploidentical stem cell allografts after anti-CD44 therapy and nonmyeloablative conditioning in a preclinical canine model. *Transplantation* 2006;82:332–339. [PubMed: 16906030]
 60. Jochum C, Beste M, Zellmer E, Graves SS, Storb R. CD154 blockade and donor-specific transfusions in DLA-identical marrow transplantation in dogs conditioned with 1-Gy total body irradiation. *Biol Blood Marrow Transplant* 2007;13:164–171. [PubMed: 17241922]
 61. Kuhr CS, Allen MD, Junghanss C, et al. Tolerance to vascularized kidney grafts in canine mixed hematopoietic chimeras. *Transplantation* 2002;73:1487–1492. [PubMed: 12023629]
 62. Horner BM, Cina RA, Wikel KJ, et al. Predictors of organ allograft tolerance following hematopoietic cell transplantation. *Am J Transplant* 2006;6:2894–2902. [PubMed: 17294522]
 63. Schwarze ML, Menard MT, Fuchimoto Y, et al. Mixed hematopoietic chimerism induces long term tolerance to cardiac allografts in miniature swine. *Ann Thorac Surg* 2000;70:131–138. [PubMed: 10921697]
 64. Fuchimoto Y, Huang CA, Yamada K, et al. Mixed chimerism and tolerance without whole body irradiation in a large animal model. *J Clin Invest* 2000;105:1779–1789. [PubMed: 10862793]
 65. Kawai T, Cosimi AB, Colvin RB, et al. Mixed allogeneic chimerism and renal allograft tolerance in cynomolgous monkeys. *Transplantation* 1995;59:256–262. [PubMed: 7839449]
 66. Kawai T, Sogawa H, Boskovic S, et al. CD154 blockade for induction of mixed chimerism and prolonged renal allograft survival in nonhuman primates. *Am J Transplant* 2004;4:1391–1398. [PubMed: 15307826]

67. Russell PS, Chase CM, Sykes M, Ito H, Shaffer J, Colvin RB. Tolerance, mixed chimerism and chronic transplant arteriopathy. *J Immunol* 2001;167:5731–5740. [PubMed: 11698446]
68. Uehara S, Chase CM, Kitchens WH, et al. NK cells can trigger allograft vasculopathy: the role of hybrid resistance in solid organ allografts. *J Immunol* 2005;175:3424–3430. [PubMed: 16116237]
69. Fuchimoto Y, Yamada K, Shimizu A, et al. Relationship between chimerism and tolerance in a kidney transplantation model. *J Immunol* 1999;162:5704–5711. [PubMed: 10229802]
70. Lanier LL. Turning on natural killer cells. *J Exp Med* 2000;191:1259–1262. [PubMed: 10770793]
71. Ildstad ST, Wren SM, Bluestone JA, Barbieri SA, Sachs DH. Characterization of mixed allogeneic chimeras. Immunocompetence, in vitro reactivity, and genetic specificity of tolerance. *J Exp Med* 1985;162:231–244. [PubMed: 3159825]
72. Ruedi E, Sykes M, Ildstad ST, et al. Antiviral T cell competence and restriction specificity of mixed allogeneic (P1+P2→P1) irradiation chimeras. *Cell Immunol* 1989;121:185–195.
73. Koehn BH, Williams MA, Borom K, et al. Fully MHC-disparate mixed hemopoietic chimeras show specific defects in the control of chronic viral infections. *J Immunol* 2007;179:2616–2626. [PubMed: 17675525]
74. Weiden PL, Flournoy N, Thomas ED, et al. Antileukemic effect of graft-versus-host disease in human recipients of allogeneic marrow grafts. *New Engl J Med* 1979;300:1068–1073. [PubMed: 34792]
75. 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]
76. 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]
77. Pelot MR, Pearson DA, Swenson K, 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]
78. Chakraverty R, Cote D, Buchli J, et al. An inflammatory checkpoint regulates recruitment of graft-versus-host-reactive T cells to peripheral tissues. *J Exp Med* 2006;203:2021–2031. [PubMed: 16880259]
79. Mapara MY, Leng C, Kim YM, et al. Expression of chemokines in GVHD target organs is influenced by conditioning and genetic factors and amplified by GVHR. *Biol Blood Marrow Transplant* 2006;12:623–634. [PubMed: 16737935]
80. Chakraverty R, Eom HS, Sachs J, 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]
81. Spitzer TR, McAfee S, Dey BR, 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]
82. Shaffer J, Villard J, Means TK, 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. *Exp Hematol* 2007;35:1140–1152. [PubMed: 17588483]
83. Sykes M, Preffer F, McAfee S, 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]
84. Spitzer TR, McAfee S, Sackstein R, 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]
85. Fudaba Y, Spitzer TR, Shaffer J, et al. Myeloma Responses and Tolerance Following Combined Kidney and Nonmyeloablative Marrow Transplantation: In Vivo and In Vitro Analyses. *Am J Transplant* 2006;6:2121–2133. [PubMed: 16796719]

86. Ringden O, Soderahl G, Mattsson J, et al. Transplantation of autologous and allogeneic bone marrow with liver from a cadaveric donor for primary liver cancer. *Transplantation* 2000;69:2043–2048. [PubMed: 10852594]
87. Donckier V, Troisi R, Tougouz M, et al. Donor stem cell infusion after non-myeloablative conditioning for tolerance induction to HLA mismatched adult living-donor liver graft. *Transpl Immunol* 2004;13:139–146. [PubMed: 15380544]
88. Kawai T, Cosimi AB, Spitzer TR, et al. HLA-mismatched renal transplantation without maintenance immunosuppression. *New Engl J Med* 2008;358:353–361. [PubMed: 18216355]
89. Lowsky R, Takahashi T, Liu YP, et al. Protective conditioning for acute graft-versus-host disease. *N Engl J Med* 2005;353:1321–1331. [PubMed: 16192477]
90. Scandling JD, Busque S, Dejbakhsh-Jones S, et al. Tolerance and chimerism after renal and hematopoietic-cell transplantation. *N Engl J Med* 2008;358:362–368. [PubMed: 18216356]
91. Millan MT, Shizuru JA, Hoffmann P, et al. Mixed chimerism and immunosuppressive drug withdrawal after HLA- mismatched kidney and hematopoietic progenitor transplantation. *Transplantation* 2002;73:1386–1391. [PubMed: 12023614]
92. Strober S, Lowsky RJ, Shizuru JA, Scandling JD, Millan MT. Approaches to transplantation tolerance in humans. *Transplantation* 2004;77:932–936. [PubMed: 15077041]

Figure 1.
Protocol for combined HLA-mismatched (haploidentical donor) kidney and bone marrow transplantation used for Immune Tolerance Network trial NKDO3. * components of the regimen that were added after the third transplant (i.e. given only to Patients 4 and 5).