# Tolerance induction via mixed chimerism in vascularized composite allotransplantation: is it time for clinical application?

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**Purpose of review**
The present review summarizes current data on the induction of immunologic tolerance through mixed hematopoietic chimerism relevant to applying this approach to vascularized composite allotransplantation.

**Recent findings**
Clinical allograft tolerance has been achieved recently for kidney transplants, using nonmyeloablative conditioning regimens and bone marrow transplantation from living donors. The mixed chimerism attained in these studies was either transient or durable, and both permitted tolerance of the renal allografts to be achieved across MHC-matched and MHC-mismatched barriers. In order to extend these protocols to deceased donor transplants across full MHC-mismatched combinations, as will be required for vascularized composite allografts (VCA), a delayed tolerance protocol has recently been developed, in which the donor bone marrow is given 4 months posttransplant. Recent primate studies of kidney transplants using this protocol have been successful and have demonstrated that strategies to abrogate memory T cells may be helpful.

**Summary**
Induction of tolerance in renal allograft transplantation has been achieved clinically, via mixed chimerism protocols. Modifications of these protocols for transplants, which require use of deceased donors across full MHC mismatches, have shown promise in preclinical models. It is therefore appropriate to consider evaluation of these protocols in clinical trials for kidney transplants, and if successful, for VCA.

**Keywords**
bone marrow transplantation, mixed chimerism, tolerance, vascularized composite allograft

## INTRODUCTION

Vascularized composite allotransplantation (VCA) is now an established treatment modality in the management of disfiguring injury, tissue loss, and amputation. Transplantation of VCA, such as the hand, face, or abdominal wall, offers patients remarkable restoration of form and function. The latest report of the International Registry on Hand and Composite Tissue Transplantation undergoing VCA indicates that these procedures offer patients a significant improvement over conventional reconstruction and/or prostheses with respect to functional outcomes, patient satisfaction, and quality of life [1,2]. However, these significant benefits must be weighed against the risks of long-term immunosuppression required to prevent rejection of VCA, which include infection, malignancy, diabetes, and renal insufficiency. The induction of immune tolerance of VCA would significantly improve this risk–benefit ratio and would also potentially broaden the indications for vascularized composite transplantation to include, for example, reconstruction following resection of malignant disease or the treatment of congenital anomalies, and permitting novel tissues

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## KEY POINTS

- Mixed hematopoietic chimerism – a state in which host-derived and donor-derived hematopoietic cells coexist within an individual – has been demonstrated to achieve robust tolerance of solid organs.
- Clinical tolerance of kidneys has been achieved through mixed chimerism across MHC-matched and haplomatched barriers.
- Full chimerism, obtained with facilitating cells, has permitted acceptance of renal allografts, in some instances across full MHC-matched barriers in a clinical study.
- Tolerance across full mismatch barriers would need to be achieved for the deceased donor transplant scenario required for VCA transplantation, likely requiring more severe preconditioning protocols then those which have been successful for living donor renal transplants to date.
- A delayed tolerance induction protocol that minimizes the morbidity of the combined VCA transplant and the donor bone marrow transplant (DBMT) by delaying the DBMT has been tested in large animal models and is under evaluation in clinical trials for both solid organs and VCA.
- These advances in clinical organ transplant tolerance and in animal models for VCA tolerance suggest that the time will soon be appropriate for VCA clinical tolerance trials.

to be transplanted such as lower extremities, periorbital structures, genitourinary tissue, or smaller complex anatomic units such as digits, ears, lips, or a nose [3▪▪,4]. The purpose of this review is to summarize current progress in VCA tolerance strategies and to outline developments in the induction of organ transplant tolerance through hematopoietic chimerism, on which further advances in VCA tolerance will undoubtedly depend.

## MIXED CHIMERISM

Mixed hematopoietic chimerism, a state in which host-derived and donor-derived hematopoietic cells coexist within an individual after transplantation of donor hematopoietic stem cells from bone marrow (Fig. 1), has led to clinical tolerance for kidneys across MHC matched and mismatched barriers (summarized in the article of Kawai *et al.* [3▪▪]). To achieve mixed chimerism, nonmyeloablative conditioning regimens have been utilized. Mixed chimerism is distinguished from the full (i.e., 100%) chimerism observed after myeloablative conditioning. Mixed chimerism was developed to mitigate

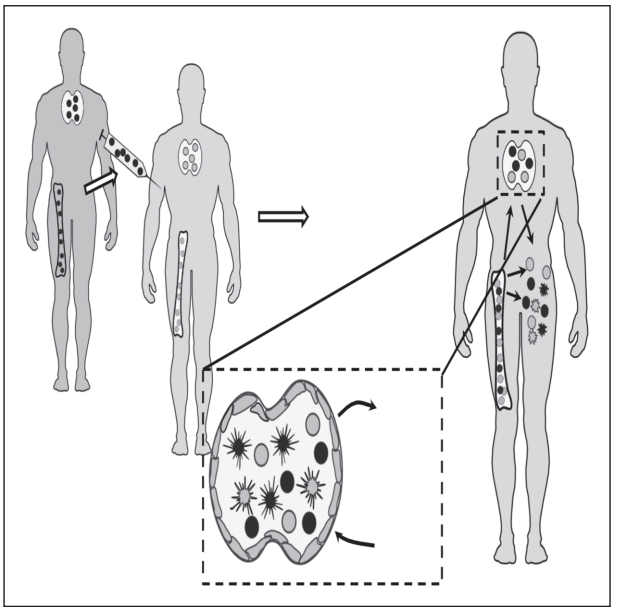

**FIGURE 1.** Mixed chimerism donor hematopoietic stem cells (black) are transplanted to the vascularized composite allografts (VCA) recipient (light grey) and migrate to the thymus where both donor and recipient alloreactive cells undergo deletion thereby permitting organ or VCA allograft acceptance.

against graft-versus-host disease and immunoincompetence, frequently seen with full chimerism [5].

## THE USE OF BONE MARROW STEM CELL TRANSPLANTATION IN CLINICAL VASCULARIZED COMPOSITE ALLOTRANSPLANTATION

In clinical VCA, bone marrow infusion has been utilized by Dubernard's group in conjunction with face allotransplantation, and by the Pittsburgh/Johns Hopkins group for hand allotransplantation. Dubernard reported no macrochimerism or microchimerism, and it is not clear that a reduction in immunosuppression requirement resulted [6].

Bone marrow infusion used in conjunction with hand transplantation represents an element of the Pittsburgh Protocol, described by Lee *et al.* [7]. In their series, bone marrow infusion 7–21 days post VCA was used as a potential means of preventing graft loss to acute rejection. Skin rejection crises that were observed responded well to topical immunosuppression. The efficacy and safety of their protocol is not yet clear, and will require long-term follow-up. No hematopoietic macrochimerism or microchimerism was detected in either of the two patients reported [7]. Nonetheless, the authors suggested

that early immunomodulation occurred on the basis of the low levels of maintenance immunosuppression required in these patients. Direct evidence for immunomodulation by a mixed chimerism approach has been reported in a swine model [8▪▪]. Despite these data, a protective effect of infused bone marrow effect against VCA rejection was not observed in a recent primate study [9▪▪].

## CLINICAL RENAL ALLOTRANSPLANTATION TOLERANCE PROTOCOLS

Clinical renal allograft tolerance has been successfully achieved by three centers – MGH, Northwestern, and Stanford – all of which have investigated approaches to induce tolerance using HSCT across HLA-matched or HLA-mismatched kidney transplantation. At MGH, the protocols utilized cyclophosphamide, local thymic irradiation (7 Gy), anti-CD2 mAb, and concomitant kidney and bone marrow transplantation [10▪▪]. Patients were maintained on a calcineurin inhibitor for 8- to 14-month posttransplant. All 10 recipients in this protocol developed transient mixed chimerism for 7 to 21 days, and seven were weaned completely from immunosuppression by14 months posttransplantation. Four of the seven successful transplant patients have remained immunosuppression free for 5–12 years, whereas the other three resumed immunosuppression at 5, 7, and 8 years after transplantation due either to recurrence of the original disease or to chronic rejection [10▪▪].

In this study, the development of unanticipated de-novo donor-specific antibodies (DSA) was observed, possibly related to the extent of B-cell depletion that resulted from the conditioning regimen. Allograft vasculopathy was not observed, however. The MGH investigators hypothesized that depletion of B cells leads to enhanced B-cell activating factor (BAFF) production, which leads to residual or recovering B-cell activation after transplantation. Addition of rituximab to the regimen prevented de-novo DSA production, provided that patients received four doses (DSA formation was not prevented in those patients who received none or two doses) [10▪▪]. This de-novo production of DSA has not been observed in swine or nonhuman primate models, or has allograft vasculopathy in tolerant animals [11▪▪,12]. Further investigation is needed to decipher the relationship between B-cell depletion, BAFF, and de-novo DSA formation and the effect on tolerance.

Northwestern University has reported success with induction of tolerance in HLA-mismatched kidney transplant recipients through replacement of recipient hematopoietic cells with donor cells (i.e. full chimerism). Their protocol included total body irradiation (200 Gy), fludarabine (30 mg/kg day 3), and cyclophosphamide (50 mg/kg on day 3). The kidney transplantation took place on day 0 with administration of mycophenolate mofetil and tacrolimus. Donor HSCs combined with 'facilitating cells' produced by a patented enrichment procedure were given on day 1, and cyclophosphamide (50 mg/ kg) was administered on day 3. This protocol was designed as a modification of a protocol developed at Johns Hopkins [13] for HLA-mismatched bone marrow transplantation for treatment of hematologic malignancies with reduced risk of GVHD. In this protocol, antihost T cells are suppressed by introducing cyclophosphamide on days 3 and 4 after HSCT. Facilitating cells was added to the Johns Hopkins protocol on the basis of their reported effectiveness in mouse models [14]. Fifteen patients were treated under this protocol, of which nine developed full donor chimerism and in six patients immunosuppression could be discontinued completely. Longer-term follow-up will be needed to determine the risk–benefit ratio of this approach [15].

At Stanford, a total lymphocyte irradiation (TLI)-based regimen has been assessed, utilizing TLI (80–120 cGy, 10 doses total on days 0–9), rabbit antithymocyte globulin (1.5 mg/kg, 5 days total on days 0–4), followed by HLA-matched peripheral blood $CD34^+$ stem cell, and $CD3^+$ cell infusion on day 11. Both mycophenolate mofetil and CyA were started on day 0 and lowered over the next 6 months. This regimen achieved success in HLA-matched kidney transplantation in which 19 of 22 patients receiving the protocol developed persistent mixed chimerism, and 16 were weaned completely off immunosuppression. However, this protocol was not successful in HLA-mismatched kidney transplant patients and an attempt to induce stable mixed chimerism and renal allograft tolerance failed. The newest Stanford experimental regimen includes increased $CD34^+$ cell and $CD3^+$ cell doses is currently being tested [16–20].

## ADVANTAGES OF CLINICAL RENAL TRANSPLANT TOLERANCE

The results of the MGH tolerance protocol were compared with 21 matched living donor kidney transplant recipients with conventional immunosuppression. During a 10-year period in patients with conventional immunosuppression, four patients lost their kidney grafts from rejection and many suffered posttransplant morbidities, including hypertension requiring medical management (85%), hyperlipidemia (65%), new-onset insulin-

dependent diabetes (35%), and serious infectious complications (25%). Comparing this group to the original cohort of patients in whom tolerance was induced, less than half of the recipients are currently on antihypertensive medications and none has developed serious events or malignancies; a good indication can be made that induction of tolerance is useful for sustaining health and graft survival [10▪▪].

## EXTENSION OF TOLERANCE PROTOCOLS TO FULLMISMATCHES AND DECEASED DONOR TRANSPLANTS: IS STABLE MIXED CHIMERISM REQUIRED?

As mentioned above, extension of these protocols to full MHC-mismatched, deceased-donor combinations will be required for VCA and for many organ allografts other than kidneys. Therefore, an approach to such combinations is currently a major goal in our center and others.

Observations from in-utero models suggested that there may be a need for stable mixed chimerism for nonrenal transplants [21▪▪,22]. Consistent with this prediction, and in contrast to preclinical and clinical renal allotransplantation data, in which transient chimerism was sufficient to induce tolerance, stable mixed chimerism was required to achieve tolerance of all components of vascularized composite allografts across a single haplotype mismatch in miniature swine [11▪▪]. Stable mixed chimerism and VCA tolerance were achieved across MHC barriers when recipients were previously rendered chimeric, or when treated with simultaneous VCA and chimerism induction [11▪▪].

The increased conditioning and resulting toxicity required for achievement of mixed chimerism across a *full* mismatch barrier in both swine and primates has led to the development of a delayed tolerance approach in which the allograft is transplanted and donor bone marrow stored at the time of transplant. Primate studies using this protocol for renal allotransplantation have suggested a window for chimerism induction of 4 months posttransplant, when the perioperative inflammatory state has subsided. At this time point, the mixed chimerism-conditioning regimen is initiated and the donor bone marrow given [12]. This approach has been successful in inducing tolerance for MHC-mismatched renal [23] and lung [24▪▪] allografts.

In the lung study, three of four nonhuman primates became tolerant of their allografts, with two of three tolerant animals exhibiting stable mixed chimerism and the third tolerant animal demonstrating transient chimerism but remaining tolerant of the lung allograft. The two tolerant animals that achieved stable mixed chimerism were transplanted across an MHC-haploidentical mismatch, whereas the animal that developed transient chimerism was transplanted across a higher degree of MHC matching (two out of four matches at class I, and two out of four matches at class II). A fully MHC-mismatched animal did not exhibit chimerism or tolerance in this study. Nonetheless, this study is particularly exciting as it represents the first demonstration of stable mixed chimerism in a primate chimerism protocol [24▪▪].

## DELAYED TOLERANCE AND MEMORY

Other recent primate studies have demonstrated that strategies to abrogate allospecific memory T cell (Tmem) responses may be required for clinical tolerance induction using delayed tolerance protocols. In these studies, levels of allospecific Tmem varied significantly between donor–recipient pairs. Interestingly, MHC matching was not always associated with a low memory alloreactivity, with some donor–recipient pairs exhibiting high Tmem alloresponsiveness even with favorable MHC matching and vice versa. Therefore, strategies for selecting donor–recipient pairs with low Tmem alloreactivity may be an important element for the successful induction of tolerance using the delayed tolerance approach [25–27].

## MIXED HEMATOPOIETIC CHIMERISM AND THE SKIN IMMUNE SYSTEM

The observation of 'split tolerance', in which the host is tolerant of some components of the VCA – that is muscle, bone – but not others (usually skin) has been observed in preclinical VCA experimental models [28–31]. We and others have noted such outcomes, even when stable mixed chimerism was achieved and donor-specific unresponsiveness in in-vitro assays of peripheral blood-derived leukocytes was evident, suggesting that independent skin-specific mechanisms that escape tolerization through mixed hematopoietic chimerism may be responsible [29,32,33]. Indeed, an appreciation for the complexity of the skin immune system is exemplified by the recent quantification of the T cells in normal, resting human skin, a concentration twice that found in the circulating blood volume [32,33]. T effector and T regulatory populations that permanently reside in the skin have been described and their immune functions characterized in more detail in recent work by Clark and others [34–36]. For example, Langerhans' cells have been shown to modulate local homeostasis by inducing proliferation of skin-resident T regulatory cells under

normal conditions or stimulating skin-resident T effector memory cells when exposed to pathogens [37,38]. These new insights underscore the importance of further study of skin-resident immune populations to elucidate mechanisms for tolerance of all components of a VCA, including skin, when mixed chimerism is achieved.

## CONCLUSION

Induction of tolerance in clinical renal transplantation has been achieved via hematopoietic chimerism approaches. Modification of these successful protocols will be necessary for transplants of VCA that will generally require a deceased donor and a full MHC mismatch. A delayed tolerance approach has shown promise in preclinical models and is ready for evaluation in clinical trials for both renal transplantation and VCA. Further strategies aimed at well-tolerated induction of stable mixed chimerism, including allospecific Tmem suppression and favorable donor–recipient Tmem repertoires are under investigation. In addition, new insights into the unique immunobiology of the skin immune system will be important in future protocols for VCA tolerance.

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### Conflicts of interest

*There are no conflicts of interest.*

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