Clinical and preclinical tolerance protocols for vascularized composite allograft transplantation

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The field of vascularized composite allografts (VCAs) has undergone significant advancement in recent decades, and VCAs are increasingly common and accepted in the clinical setting, bringing hope of functional recovery to patients with debilitating injuries. A major obstacle facing the widespread application of VCAs is the side effect profile associated with the current immunosuppressive regimen, which can cause a wide array of complications such as infection, malignancy, and even death. Significant concerns remain regarding whether the treatment outweighs the risk. The potential solution to this dilemma would be achieving VCA tolerance, which would allow recipients to receive allografts without significant immunosuppression and its sequelae. Promising tolerance protocols are being studied in kidney transplantation; four major trials have attempted to withdraw immunosuppressive treatment with various successes. The common theme in all four trials is the use of radiation treatment and donor cell transplantation. The knowledge gained from these trials can provide valuable insight into the development of a VCA tolerance protocol. Despite similarities, VCAs present additional barriers compared to kidney allografts regarding tolerance induction. VCA donors are likely to be deceased, which limits the time for significant pre-conditioning. VCA donors are also more likely to be human leukocyte antigen–mismatched, which means that tolerance must be induced across major immunological barriers. This review also explores adjunct therapies studied in large animal models that could be the missing element in establishing a safe and stable tolerance induction method.

Keywords Vascularized composite allografts / Transplantation / Immunosuppression / Face transplantation / Hand transplantation

INTRODUCTION

Vascularized composite allograft (VCA) transplantation can restore significant structural and functional deficits while providing a socially acceptable appearance for patients who have suffered from severe tissue loss and diminished quality of life. Despite the complexity of both technical and immunological aspects of VCA transplantation, advancements in the field have resulted in over 100 cases of hand transplantations and over 40 cases of facial transplantations [1,2]. Other reported VCA transplantations include those of the flexor tendon apparatus, knee, larynx, uterus, and penis [3]. The field has also come a long way...
in the eyes of research administrators. A survey study targeting institutional review boards revealed that most members believed that VCA transplantation would require institutional review board approval only if done in conjunction with a research study [4]. Despite these achievements, many obstacles remain before the widespread application of VCAs. The most daunting roadblock is the need for lifelong immunosuppression (IS) and the associated sequelae.

The complications of IS for solid organ transplantation (SOT) are well studied. These complications include rejection, an increased risk of chronic renal disease, diabetes, infection, osteonecrosis, and malignancy [5-7]. In functional kidney transplantation patients, the transplantation-related death rate can be as high as 25% after 10 years of treatment [8]. In addition to the technical complexity of VCA transplantsations due to the immunogenicity of the skin, VCA patients may require even higher doses of IS therapy than those used in patients undergoing SOT [9]. Therefore, some controversy surrounds VCA transplantation, making otherwise healthy patients vulnerable to similar complications with SOT patients.

Approximately 85% of VCA recipients can experience one or more acute rejection episodes during their first year of transplantation [10]. These episodes are taxing on patients and require high-dose steroid treatment or increased doses of other IS medications [11,12]. Even after acute rejection episodes, chronic rejection (CR) may still develop in many patients [13-15]. Although CR can potentially be kept at bay with consistent IS, a decreased dose may be inevitable due to the development of intolerable or life-threatening side effects, as evident in the literature [14].

The financial burden of IS is also a significant barrier, with treatment potentially costing $14,000 annually for kidney transplant patients [16]. The cost poses an ethical dilemma for patients, clinicians, and society. Who should be allowed to have VCA? Who needs it the most? Is the need justified concerning the potential risk and side effects and the cost to the healthcare system? These concerns highlight the crucial need for a successful tolerance protocol that would allow acceptance of all components of a VCA without the need for high-dose chronic IS.

Allograft tolerance would allow for prolonged survival of the graft without acute or CR episodes and without the need for lifelong IS medications. With a reduction of IS drugs, there would be a substantial decrease in the overall complication rate. Four clinical trials performed at Massachusetts General Hospital, Stanford, Northwestern/Duke, and Samsung Medical Center aimed to achieve kidney allograft tolerance in human leukocyte antigen (HLA)–mismatched transplantations. Complete IS withdrawal has been attempted in these trials [17]. However, tolerance is a fragile state, and stability has yet to be achieved. In efforts to attain donor-specific tolerance, VCA preclinical research focuses on tolerance-inducing treatments, including co-stimulatory blockade, hematopoietic cell transplantation (HCT), cell-based therapy, and other novel approaches [18].

This review examines the current clinical and preclinical studies aimed at developing an effective tolerance strategy. We review the various groups with the most promising clinical tolerance induction protocols in kidney transplantation. We also analyze translational studies on large animals that hold promise for human trials in the future.

METHODS

This narrative review was mapped out by the senior author (DWM) to include relevant areas for the review. Following this outline, JHY and ACJ conducted searches in the PubMed, MEDLINE, and Embase databases. The search strategies included but were not limited to the following keywords: VCA tolerance, VCA rejection, and kidney allograft tolerance. Only studies published in English were considered. No limitations were placed on the time of publication. From the search results, titles and abstracts were reviewed by the authors to determine whether an in-depth review was needed. Disagreements on study inclusion were brought to and settled by the senior author.

CURRENT VCA REGIMENS AND LIMITATIONS

In the first facial transplantation conducted by the Devauchelle group in France, the IS regimen utilized rabbit anti-thymocyte globulin induction (rATG), followed by tacrolimus and mycophenolate mofetil (MMF), with prednisone tapering to a lower dosage. For general infection prevention, amoxicillin-clavulanate was administered. For cytomegalovirus prophylaxis, patients received intravenous ganciclovir followed by valganciclovir. Trimethoprim-sulphamethoxazole was used for Pneumocystis jiroveci pneumonia prevention. Donor nucleated hematopoietic cells were also infused on post-transplant days 4 and 11 [19].

Clinical VCA IS protocols have remained largely similar to established SOT IS protocols, although many groups have introduced modifications intended to reduce side effects and toxicity. Different induction agents have been used, such as humanized IL2 receptor antibody [20,21], alemtuzumab [22], and rituximab [23]. Diaz-Siso et al. [24] worked on steroid-free dual maintenance treatment through gradual weaning of steroids and continuation of tacrolimus and MMF. The Pittsburgh group has used alemtuzumab and methylprednisolone induction followed...
by donor bone marrow infusion and maintenance on tacrolimus monotherapy in upper extremity transplantation [25]. Graham-
mer et al. [26] introduced belatacept, a T cell inhibitor, as a
maintenance agent in hand transplantation to reduce the tacrolim-
us target level. A report of graft loss due to vasculopathy in a
hand transplantation patient on a steroid-sparing protocol has
raised caution regarding withdrawing steroids from treatment
[27]. Given the small number of VCA recipients undergoing
treatment, it is difficult to complete an adequately powered
study to compare different IS regimens at this time.

Acute rejection treatment
Rejections frequently occur in VCA recipients, Hautz et al. [28]
recorded a total of 43 rejection episodes in five hand and fore-
arm transplantation patients. Out of the 43 episodes, the major-
ity were T-cell mediated rejections, along with 12 antibody-me-
diated rejections, one B-cell mediated rejection, and one CR.
Acute rejection is well defined in VCA, and assessment via the
Banff criteria has been developed and applied to the skin [29].
When acute rejection occurs, a steroid bolus is often the first-
line medication used for treatment. Guo et al. [20] reported
three acute rejection incidents at 3, 7, and 17 months after facial
transplantation. Treatment included increasing tacrolimus dos-
age from 15 ng/mL to 25 ng/mL and methylprednisolone pulse
therapy (1 g, 0.5 g, 0.5 g, 0.25 g, 0.125 g) for the first 5 days, fol-
lowed by prednisolone starting at 80 mg daily tapering eventu-
ally to 15 mg daily as a long-term maintenance dose. Pomahac
et al. [30] reported two cases of acute rejection, both successful-
ly treated with pulse doses of methylprednisolone. Basiliximab
use has also been reported in acute rejection, along with alemtu-
zumab reported effective in a steroid-resistant episode [31].
Barret et al. [32] treated acute rejection with 1.5 g/kg of rATG
and replacing MMF with sirolimus. It is worth noting that hy-
peracute rejection, although established in SOT, rarely occurs in
VCA setting and is therefore not well studied [29,33].

Chronic rejection
A significant challenge in discussing CR in VCA transplantation
is the lack of an official definition due to the limited number of
cases and short follow-up time. Another challenge is that the
manifestation of CR in VCA may differ from what is known in
SOT, potentially requiring the establishment of new sets of cri-
eteria [13]. In the first case of human face transplant, the patient
developed CR of the graft with histologic evidence of intimal
thickening, thrombosis of pedicle vessels, and C4d deposits on
the endothelium of dermal vessels. The antibody-mediated re-
jection eventually led to surgical removal of the lower lip, labial
commissures, and right cheek [34]. Petruzzo et al. [14] reported
a case of CR first noticed in the skin, with skin sclerosis at
around year 2 after scheduled IS reduction due to Epstein-Barr
virus-related tumor complications. Overall, the pathogenic
mechanism of CR in VCA requires further research.

Mortality
Death is a feared complication of any medical procedure, partic-
ularly concerning when it occurs due to non-life-threatening
treatment such as VCA transplantation. Cases of VCA-associat-
ed mortality have been reported worldwide. The first reported
mortality occurred in facial VCA transplantation patients, likely
due to medical non-adherence [35]. Multiple other deaths in
VCA recipients have been reported since then [29]. It is difficult
to estimate the mortality rate associated with VCA due to case-
by-case reporting. It would be highly beneficial for the under-
standing of VCA morbidity and mortality if a centralized data-
base and reporting procedure can be adopted.

VCA monitoring
Multiple studies have assessed biomarkers of VCA rejection.
Lian et al. [36] found a significant fluctuation in FoxP3:CD8 ra-
tio that decreased with rejection episodes and reverted to pre-
rejection levels following therapy. Kollar et al. [37] found in a
pilot study that significantly elevated serum matrix metallopro-
teinase three levels, as determined by enzyme-linked immuno-
sorbent assay, were correlated with severe rejection in facial
transplantation patients. Despite these findings, a recent review
by Honeyman et al. [38] outlined the need for a biomarker that
can identify the very early stages of rejection. An assessment
of VCA transplantation rejection via skin or sentinel flap is one
clinical strategy for tolerance monitoring. An earlier approach in
this area involves utilizing skin grafts in hand transplantation.
However, the skin graft was observed to undergo different im-
une responses than VCs, and this practice was discontinued
[39]. A potentially more effective strategy is to used vascular-
ized sentinel flaps at a discreet location such as the inframary
fold, with one report of excellent concordance rate be-
tween the sentinel flap and the facial allograft [40].

CLINICAL PROTOCOLS FOR KIDNEY ALLOGRAFT TOLERANCE

Massachusetts General Hospital
The Massachusetts General Hospital group conducted studies
in 10 HLA haplotype-mismatched kidney transplant recipients.
The conditioning regimen initially consisted of pre-transplant
cyclophosphamide, humanized anti-CD2 monoclonal antibody,
and local thymic irradiation, followed by a post-transplantation
regimen of intravenous donor bone marrow cells (BMCs) infusion with calcineurin inhibitors (CNI) tapering starting at 6 months and complete discontinuation at 9–14 months [41]. Due to the strong humoral response, some patients also received peritransplant rituximab injection. All 10 patients achieved transient mixed chimerism for up to 3 weeks without signs of graft-versus-host disease (GvHD) [42]. Seven out of 10 patients had IS-free renal allograft survival for more than 5 years. Four of these patients had remained IS-free for more than 10 years, while three recipients lost graft function at around 10 years [17,43]. Due to acute kidney injury observed in nine out of 10 recipients, an additional pilot trial was conducted in two patients, replacing cyclophosphamide with low-dose total body irradiation (TBI). One patient had IS-free normal kidney function for 3.75 years, while the other patient failed to develop chimerism. Another pilot trial was conducted using a similar regimen except for rATG and belatacept replacing anti-CD2 mAB and low-dose TBI replacing cyclophosphamide. Both patients failed to develop sufficient chimerism and IS was never withdrawn (Tables 1, 2) [17].

**Stanford**

The Stanford group aimed to establish tolerance through persistent mixed chimerism, which has reduced GvHD and immunodeficiency risk compared to full donor chimerism. IS withdrawal was successful in many HLA-matched patients who developed chimerism. No IS withdrawals were successful in the HLA-mismatched patients. The protocol consists of combined kidney and HCT from the same donor. Fifty-six patients were enrolled, with 29 patients fully matched and 27 patients haplotype-matched. Both groups received similar conditioning protocols, except for the haplotype-matched groups receiving a higher number of T cells. For donors, progenitor cells were collected by apheresis after injection of granulocyte colony-stimulating factors. The recipients received 10 doses of total lymphoid irradiation and five rATG doses over 11 days after transplantation. After completing total lymphoid irradiation, the CD3+ hematopoietic progenitor cells and a defined number of T cells collected from donors were injected into the recipients. Patients were also maintained on MMF and CNI. In patients developing persistent mixed chimerism, MMF was withdrawn after 1 month, and CNI was withdrawn around 1 year. It is worth noting that none of the 56 patients developed severe or chronic infection or GvHD. In the fully matched patients, 23 out of 29 patients have been completely withdrawn from maintenance IS regimen without signs of rejection for up to 14 years. In the HLA haplotype-matched group, the same withdrawal was attempted and resulted in the loss of chimerism; subsequently, no patient in this group was withdrawn entirely from IS regimen. There has been one death associated with stroke and two deaths associated with pulmonary embolism and coronary artery disease in patients with normal graft function. Additionally, two graft losses were seen due to kidney disease relapse [44-46].

**Northwestern/Duke**

The Northwestern/Duke group aimed at inducing durable donor chimerism for tolerance. The study utilized combined kidney and hematopoietic cell transplants in HLA-mismatched liv-

| Table 1. Clinical kidney allograft tolerance protocol conditioning regimen |
|-----------------------------|----------------|----------------|----------------|
| Institution                 | No. of HLA-mismatched patients | Donor cell composition | Recipient radiation treatment | Other recipient treatments used |
| Massachusetts General Hospital | 10 | Whole bone marrow | Local thymic irradiation | Cyclophosphamide, anti-CD2 monoclonal antibody, rituximab, tacrolimus, cyclosporine |
| Stanford                    | 27 | Mobilized CD34 cells+T cells | Total lymphoid irradiation | Anti-thymocyte globulin, tacrolimus, cyclosporine, mycophenolate |
| Northwestern/Duke            | 37 | Mobilized CD34 cells+T cells+facilitator cells | Total body irradiation | Cyclophosphamide, fludarabine, tacrolimus, mycophenolate |
| Samsung Medical Center       | 8  | Whole bone marrow | Local thymic irradiation | Cyclophosphamide, rituximab, fludarabine, anti-thymocyte globulin, tacrolimus, sirolimus |

HLA, human leukocyte antigen.

| Table 2. Clinical kidney allograft tolerance protocol results and complications |
|-----------------------------|----------------|----------------|----------------|
| Institution                 | No. of HLA-mismatched patients | No. of patients with attempted withdrawal of IS | No. of patients off IS drugs | Complications |
| Massachusetts General Hospital | 10 | 8  | 4  | 9 Engraftment syndrome, 6 graft loss |
| Stanford                    | 27 | 6  | 0  | 3 Engraftment syndrome, 3 graft loss, 1 death |
| Northwestern/Duke            | 37 | 26 | 26 | 2 GvHD, 2 graft loss, 3 death |
| Samsung Medical Center       | 8  | 6  | 4  | 7 BK nephropathy, 2 engraftment syndrome, 2 graft loss |

HLA, human leukocyte antigen; IS, immunosuppression; GvHD, graft-versus-host disease.
Chimerism can allow prolonged graft survival in HLA-mismatched animals. However, the risk of GvHD remains high. Treatment with mesenchymal stem cells alone can prolong VCA survival. Adipose-derived stem cells have an immunomodulatory effect and can increase graft survival when added to the conditioning regimen. VCA tolerance studies Many small animals and large animal studies regarding VCA tolerance have been reported. One study identified that the addition of costimulatory blockade can increase graft survival in mice [51]. Another study utilizing costimulatory blockade in mice identified regulatory T cells as a crucial factor for tolerance induction [52]. A recent VCA tolerance study was reported in a nonhuman primate (NHP) model that attempted a delayed bone marrow transplantation after hand or face VCA transplantation [53]. To date, there exists a significant knowledge gap in how best to avoid immune rejection of human VCAs. It will likely require more scientific advances through research in small and large animal models before tolerance induction should be attempted in human VCA recipients.

**LARGE ANIMAL PROTOCOLS**

**Costimulatory blockade**

Costimulatory blockade entails interfering with T cell signaling to prevent activation and clonal expansion of T-cells as well as secretion of inflammatory cytokines. T cell-mediated rejection can be inhibited by blocking these signals, leading to prolonged survival of graft tissue. The use of costimulatory blockade in VCA has also been explored by Freitas et al. in a NHP forearm VCA model [54]. They investigated the use of CTLA4-Ig and LFA3-Ig (which blocks the LFA3-CD2 adhesion pathway) in combination or solely with rapamycin compared to tacrolimus alone. The combination of CTLA4-Ig and LFA3-Ig resulted in graft survival but also fatal cytomegalovirus infections in all three animals, and the use of rapamycin led to increased wound complications. However, subjects treated with CTLA4-Ig had prolonged allograft survival and decreased donor-specific antibody levels compared to CNI alone [54]. As with solid organ regimens, it seems that costimulatory blockade will remain an additional medication in the IS regimen in VCA treatment (Table 3).

**Infusion of donor bone marrow**

Barth et al. [55] speculated that a vascularized bone marrow (VBM) component of a VCA would allow for donor cell engraftment to increase the likelihood of tolerance to the transplanted graft. They compared NHP heterotopic fascial allografts

| Table 3. Summary of large animal protocols |
|------------------------------------------|
| **Treatment category** | **Animal model** | **Summary of findings** |
|------------------------|-----------------|------------------------|
| Costimulatory blockade | NHP             | The addition of costimulatory blockade increased VCA survival and decreased donor-specific antibody levels compared to standard treatment. |
| Donor bone marrow infusion | NHP             | Vascularized bone marrow infusion increased graft survival while on IS. However, withdrawal of IS led to graft rejection. |
| Chimerism              | Swine, canine   | Chimerism can allow prolonged graft survival in HLA-mismatched animals. However, the risk of GvHD remains high. Same-day conditioning for chimerism induction may be possible in VCA transplantation. |
| Cell-based therapy     | Swine           | Treatment with mesenchymal stem cells alone can prolong VCA survival. Adipose-derived stem cells have an immunomodulatory effect and can increase graft survival when added to the conditioning regimen. |

NHP, nonhuman primate; VCA, vascularized composite allograft; IS, immunosuppression; HLA, human leukocyte antigen; GvHD, graft-versus-host disease.
with a VBM component without subsequent tacrolimus and MMF IS. The animals that received the facial allograft with VBM had prolonged survival of the graft while on IS, but rejected their grafts when IS was discontinued. However, alloreactive antibodies were not found in the VBM group, revealing a protective effect of VBM.

In a later study by the same group, Brazio et al. [56] completed a non-pretreated bone marrow infusion at the time of VCA in three experimental animals. They found no protective effects of the bone marrow infusion, and the allografts were rejected as early as postoperative day 28. There were no signs of chimerism in these animals, and alloantibodies were also detected, although to a lesser amount than the group with no donor cell treatments. At this time, 2011 and on, the concept of donor bone marrow presence to promote tolerance to a VCA was continually investigated and refined to the pivotal large animal studies that achieved tolerance across major histocompatibility complex (MHC) barriers.

**Stable and transient chimerism**

Stable and transient chimerism can be achieved after HCT. HCT is the intravenous infusion of hematopoietic cells containing stem and progenitor cells into the recipient. Once infused, transplanted donor hematopoietic stem cells travel to the recipient’s bone marrow compartment, take up residence, differentiate, and proliferate into all blood lineages. When lineages of donor and recipient cells co-exist, chimerism has been achieved.

Several large animal studies in swine and canines have demonstrated that HCT leading to chimerism is a key factor in VCA tolerance [18]. The first study showing tolerance across MHC barriers using HCT was that by Hettiaratchy et al. [57] in a pig VCA model. The animals were pretreated with a porcine CD3-immunotoxin T-cell depleting agent, split into two groups, and transplanted with cytokine-mobilized peripheral blood mononuclear cells (CM-PBMCs) or BMCs, and treated with a 30-day course of cyclosporine A (CsA). Both groups achieved chimerism, but the CM-PBMC group maintained stable chimerism while the BMC group experienced transient chimerism. The donor musculoskeletal components of three CM-PBMC VCA recipients and two BMC VCA recipients survived long-term, including one fully mismatched animal. Unfortunately, the animals that achieved stable chimerism ultimately developed cutaneous GvHD.

All animals in the study by Hettiaratchy et al. rejected the skin component of the graft, underscoring donor skin immunogenicity as a major obstacle for VCA tolerance [18,57]. Horner et al. [58] investigated the outcomes of a vascularized fasciocutaneous transplant in swine with established stable chimerism, using a conditioning protocol consisting of CD3-immunotoxin, 100 cGy TBI before HCT, and a 45-day course of CsA post-HCT that successfully resulted in long-term acceptance of vascularized organ allografts. They achieved complete VCA tolerance for over 300 days in a stably chimeric HCT recipient who had not received IS for 7 weeks, the first published skin tolerance achievement. Leonard et al. [59] further extended this protocol to assess the ability to achieve complete tolerance when the VCA is transplanted at the same time as the HCT. Stable chimerism was achieved, as was tolerance across MHC barriers. However, two animals suffered cutaneous GvHD. The incidence of GvHD was associated with higher levels of donor chimerism.

In a subsequent canine study by Chang et al. [60], BMC- or granulocyte-colony stimulating factor (G-CSF)-mobilized stem cells in peripheral blood were transplanted to recipients preconditioned with 4.5 Gy TBI at a rate of 7 cGy/min. Subjects received VCA and HCT simultaneously, followed by MMF for 28 days and CsA for 35 days. One animal that received BMCs achieved stable chimerism and long-term tolerance of their allograft (52 weeks), while all animals who received G-CSF-mobilized stem cells achieved stable chimerism and long-term tolerance (up to 94 weeks).

The animals in the studies by Hettiaratchy et al. (swine) [57] and Chang et al. (canines) [60] experienced transient chimerism. All animals that received BMC in the study of Hettiaratchy et al. [57] experienced transient chimerism, including one fully mismatched animal with graft survival for 132 days until the graft was purposely removed. The canine that remained tolerant to its graft for 94 weeks in the Chang et al. study lost granulocyte chimerism at 10 weeks and mononuclear cells at 15 weeks [60]. Animals with transient chimerism did not develop GvHD, while animals with stable chimerism did. Swine and canine HCT carry a similar risk of GvHD, and higher donor cells’ levels increase the risk [57,60]. As such, transient chimerism may be a pivotal component to tolerance without the risk of GvHD if it could be harnessed safely and consistently.

**Cell-based therapy**

Alternative cell infusions have been investigated to avoid the risk of GvHD. Mesenchymal stem cells (MSCs) are multipotent nonhematopoietic progenitor cells residing in the bone marrow. These cells lack immunogenic surface receptors for alloreactive T cells to respond to [61]. Kuo et al. [62] first investigated MSC therapy on outbred swine. They found that with TBI, bone marrow transplant, CsA, and MSC treatment, composite grafts survived for over 200 days in three animals. There were no histological signs of rejection of the skin and muscle at 35 weeks,
shown that it may be possible to induce durable chimerism with chimerism dominance. The Northwestern/Duke group has induced different levels of donor chimerism in the recipients. The major protocols all based their strategy on adequate risk assessment with successful tolerance induction. The least intrusive chimerism that would allow for long-term graft survival without IS treatment should be favored. All protocols consistently employ radiation treatment as part of the conditioning regimen along with donor HCT. These treatments are crucial for the induction of tolerance. It is also clear that other adjunct treatments would be necessary to tilt the balance towards a safer and stable tolerance state. It is worth noting that the kidney transplantation tolerance trials were performed with living donors, while VCA donors are likely deceased. This presents an additional barrier to the development of VCA tolerance protocol because there would be minimal time for pre-conditioning. VCA transplantation is also more likely to be HLA-mismatched than kidney transplantation, requiring tolerance induction over more significant immunological barriers. Large animal models serve as the bridge for new therapies to demonstrate effectiveness before being adopted into human clinical trials [69]. Studies in swine, canine, and NHP continue to provide insight into clinical tolerance protocols. A study done in haploidentical canine models demonstrated the possibility of same-day conditioning for VCA transplantation [60]. The elimination of significant pre-conditioning would open up the potential donor pool for VCA and significantly increase its feasibility and application, as living donor for VCA is unlikely. The study involving belatacept as part of the conditioning regimen for renal allograft transplant in NHP has successfully reported long-term graft survival without IS [70]. VCA transplantation utilizing belatacept has also been shown to increase rejection-free graft survival in NHP [54]. Another promising treatment in large animal studies is T cell depletion through CD3 immunotoxin, which, unlike rATG or alemtuzumab, is capable of depleting T cells within the lymphoid tissues as well as the blood [71,72]. The human equivalent of the porcine CD3-immunotoxin is currently in clinical trials and is being marketed by Angimmune LLC as Resimmune [73]. Recipient T cell depletion using CD3 immunotoxin for VCA transplantation in miniature swine has been shown to allow long-term IS-free graft survival [57,59]. Sphingosine 1-phosphate receptor modulators are another class of medication with therapeutic potential in transplantation patients. One of these is FTY720 (fingolimod), which was reported to reduce cardiac fibrosis in rat studies [71]. However, two phase-3 clinical trials using FTY720 in renal transplant-
tation were performed without meeting the endpoint, and further research was discontinued [74,75]. ASP0028, an S1P1/ S1P5 selective agonist, has shown efficacy and safety in an NHP renal transplantation model and could have future therapeutic potential [76].

CONCLUSIONS

The future of the clinical application of VCAs is dependent on shifting the risk-benefit ratio. Reducing or eliminating acute and CR would increase VCA feasibility and applicability by removing the double-edged sword of IS treatment. Animal model studies are crucial for further understanding of the immunological barrier faced with HLA-mismatched VCA transplantation. A successful tolerance induction protocol should contain low toxicity to the recipient, require minimal or same-day preconditioning, and utilize short-term IS medications.

NOTES

Conflict of interest
No potential conflict of interest relevant to this article was reported.

Author contribution
Conceptualization: S Colakoglu, CA Huang, DW Mathes. Data curation: JH Yang, AC Johnson. Methodology: CA Huang, DW Mathes. Project administration: S Colakoglu, DW Mathes. Writing - original draft: JH Yang, AC Johnson. Writing - review & editing: JH Yang, S Colakoglu, CA Huang, DW Mathes.

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