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Author manuscript

*Am J Transplant.* Author manuscript; available in PMC 2016 August 01.

Published in final edited form as:

*Am J Transplant.* 2015 August ; 15(8): 2240–2249. doi:10.1111/ajt.13379.

## Studies Introducing Costimulation Blockade for Vascularized Composite Allografts in Non-Human Primates

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### Abstract

Vascularized composite allografts (VCAs) are technically feasible. Similar to other organ transplants, VCAs are hampered by the toxicity and incomplete efficacy associated with conventional immunosuppression. Complications attributable to calcineurin inhibitors remain prevalent in the clinical cases reported to date, and these loom particularly large given the non-lifesaving nature of VCAs. Additionally, acute rejection remains almost ubiquitous, albeit controllable with current agents. Costimulation blockade offers the potential to provide prophylaxis from rejection without the adverse consequences of calcineurin-based regimens. In this study, we used a non-human-primate model of VCA in conjunction with immunosuppressive regimens containing combinations of B7-specific costimulation blockade with and without adhesion blockade with LFA3-Ig to determine what adjunctive role these agents could play in VCA transplantation when combined with more conventional agents. Compared to tacrolimus, the addition of belatacept improved rejection free allograft survival. The combination with LFA3-Ig reduced CD2<sup>hi</sup> memory T cells, however did not provide additional protection against allograft rejection and hindered protective immunity. Histology paralleled clinical histopathology and Banff grading. These data provide the basis for the study of costimulation blockade in VCA in a relevant preclinical model.

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### Disclosure

The authors of this manuscript have no conflicts of interest to disclose as described by the *American Journal of Transplantation*.

## Introduction

To date over 150 patients have received VCAs (1). Similar to other transplants, recipients require long-term immunosuppression incurring lifelong risks of infection and metabolic disturbances. Given that VCAs are used to enhance quality of life, these risks impose a major concern for the appropriate use of VCAs. Typical complications reported for VCAs are attributable to calcineurin inhibitors (CNIs) and steroids (2, 3). Additionally, rejection rate in VCA is 85-90% within the first year (4, 5). Thus, the optimal regimen for VCA transplantation remains to be defined.

Costimulation blockade (CoB) has emerged as a promising strategy to prevent allograft rejection and avoid the deleterious side effects of CNIs. Belatacept (LEA29Y) is a second-generation CTLA-4Ig molecule blocking the CD28:CD80/CD86 pathway, with increased affinity for the B7 molecules and more potent immunosuppression.(6) Calcineurin and steroid free CD28 blockade-based therapies can prolong allogeneic islet and kidney survival in non-human primates (NHPs).(7, 8) Studies using CoB in NHPs have typically used the mTOR inhibitor sirolimus rather than CNIs, based on mechanistic synergies shown in lower species.(9)

Clinical experience using belatacept instead of CNIs has had higher rates of acute rejection attributable to memory T cells as a major component in CoB-resistant rejection.(10) The most alloreactive subset of effector memory T cells in humans is characterized as CD8<sup>+</sup>CD28<sup>hi</sup>CD28<sup>-</sup> cells,(10) and application of the CD2-specific LFA-3Ig (alefacept) has been demonstrated to improve renal allograft survival in NHPs by depleting this population. (10, 11) This is thought to be because T effector memory cells express higher concentrations of CD2 than naïve T cells, with LFA3-CD2 signaling as an essential component of activation. Alefacept interrupts this interaction on the T cell reducing activation and exerting a depletion effect on the memory T cell compartment via its Fc region.(12) For skin-containing VCAs, this effect may be particularly beneficial given its proven efficacy in eliminating autoimmune dermatopathology. (12) Additionally, this agent can act in conjunction with CD28 CoB agents by abrogating the memory T cell compartment to prevent graft rejection (11).

## Materials and Methods

### Animals

All procedures conformed to principles within the *Guide for the Care and Use of Laboratory Animals*, were approved by the Institutional Animal Care and Use Committee, and were conducted in accordance with Yerkes National Primate Research Center guidelines. Rhesus macaques (*Macaca mulatta*) were obtained from either the Yerkes National Research Center field station (Lawrenceville, GA) or AlphaGenesis, Inc (Yemassee, SC), quarantined, and housed under standard conditions at the Yerkes National Primate Research Center (Atlanta, GA). Animals were tuberculin non-reactive and seronegative for SIV, STLV, SRV, and Herpes B virus.

## Procedure

Pairs were selected based on ABO compatibility and MHC Class I non-identity. A radial forearm graft was performed as previously described excluding the bone.(13)

## Groups

Animals in all treatment groups received methylprednisolone 15 mg/kg  $\times$  3 days with a taper over one month. Experimental groups had the following agents added: Group I tacrolimus (T); Group II CTLA-4Ig, alefacept, and sirolimus; Group III CTLA-4Ig, alefacept, and tacrolimus (CAT) with conversion to sirolimus; Group IV belatacept (replacing CTLA4-Ig), in addition to alefacept, and tacrolimus (BAT) with conversion to sirolimus; and Group V belatacept and tacrolimus (BT) with conversion to sirolimus (Figure 1). Intramuscular tacrolimus dosing was adjusted for target trough levels of 8-12 ng/mL and intramuscular sirolimus dosing was targeted for a range of 8-12 ng/mL with weekly monitoring of drug levels(13,24).

## Monitoring

Allografts were inspected for wound healing, and changes in epidermal and hair growth. Skin biopsies were performed with visual changes and monthly. Viral reemergence was observed in the initial transplants, which prompted cytomegalovirus (CMV) prophylaxis with oral valganciclovir and weekly monitoring of CMV levels by polymerase chain reactions (PCR) testing (Table 1). Animals received intramuscular ganciclovir at 6mg/kg when levels exceeded 10,000 copies/mL of whole blood.

## Polychromatic flow cytometry (PFC)

PFC was used to monitor the immunologic profile of the peripheral blood. Surface staining for CD2, CD3, CD4, CD8, CD20, CD28, and CD95 was carried out weekly with commercially available anti-human or anti-rhesus antibodies (BD Biosciences, San Jose, CA, USA). Cytometry was performed on a LSR II flow cytometer (BD Biosciences) and analyzed using FlowJo software version 9 (Tree Star, Ashland, OR, USA).

## Donor-specific antibody (DSA) detection

DSA was measured at baseline and weekly for one month after transplant, then monthly. Donor lymphocytes were blocked with goat IgG (Jackson Immuno Research Laboratories, West Grove, PA, USA). Cells were then incubated with recipient serum followed by FITC-labeled goat anti-monkey IgG (KPL, Gaithersburg, MD, USA) and analyzed using flow cytometry. Positive control samples were obtained from historic NHP serum sensitized from allogeneic skin grafts. A positive DSA assay was determined to be alloantibody mean fluorescent intensity (MFI) twice the level of baseline control.

## Statistical analysis

To eliminate lag-time bias due to variations from visual changes to biopsy, the time point of initial visual changes prior to a biopsy with confirmed rejection was used as the time point for analysis. Kaplan-Meier death censored rejection-free survival analysis was performed using a log-rank (Mantel-Cox) test using GraphPad Prism<sup>®</sup> version 6 (San Diego, CA).

## Results

We have shown that untreated VCAs reject promptly and that CNIs can marginally extend rejection free allograft survival (Table 2B) (13). As has been seen clinically, most VCAs are performed on CNI-based regimens and nevertheless have early rejection requiring rescue therapy. In order to revalidate these findings with contemporaneous controls, we performed three VCAs in NHPs using a tacrolimus-based regimen as described in the methods.

Three animals received VCAs using the basic methylprednisolone and tacrolimus regimen. All animals rejected within the first 15 days after transplant. One animal developed DSAs. All animals received CMV prophylaxis and serum viral loads did not approach treatment threshold.

To mirror prior studies using CoB in kidney transplantation in lieu of tacrolimus, we performed two transplants using CTLA4-Ig, alefacept, and sirolimus. (10, 11) Both allografts failed to heal at the skin edges and the deeper tissues lasting 14 days, and wound failure was seen in native and allograft skin (Figure 2) indicating that this was a non-allospecific wound failure. Histology demonstrated infiltrates consisting predominately of monocytes with fewer lymphocytes, supporting a generalized inflammatory response and inconsistent with rejection. Thus, while this regimen might be efficacious in preventing rejection, the use of sirolimus, with its known impediments related to wound healing, were impractical for VCAs. Consequently, we modified succeeding regimens to start with tacrolimus and convert to sirolimus.

We performed six transplants using CTLA4-Ig, alefacept, and tacrolimus with sirolimus conversion for long-term survivors. Two transplants CAT1 and CAT2, remained rejection free until day 62 and 63 respectively. Histology for both biopsies revealed Banff 1. Serum CMV levels were not obtained in these animals.

Two transplants, CAT3 and CAT4, were sacrificed on days 41 and 65 for weight loss due to viral illness. Peak CMV viral loads were  $2 \times 10^7$  and  $1.3 \times 10^4$  copies/mL respectively (Table 1). Both allografts were free of macroscopic signs of rejection and demonstrated Banff 0.

One transplant (CAT5) remained without gross changes for over two months, with a protocol biopsy on POD 77 demonstrating Banff 0. A petechial rash arose on POD 84. This progressed to a more severe lesion including erythema, induration, petichiae, scaling/flaking, epidermal necrosis, and hair loss. Biopsy at this time on POD 92 revealed a Banff III.

One transplant (CAT6) began developing a diffuse scaly rash on POD 42. The gross appearance progressed to include edema and mild epidermal sloughing, biopsy performed on POD 49 demonstrated Banff III.

Out of this study group, only four animals were monitored for CMV infection, with all 4 developing detectable CMV viremia. Two animals were ultimately sacrificed for weight loss consistent with systemic illness. Other infectious complications included a gram-positive bacteremia in one animal, and *Entamoeba histolytica* diarrhea in one animal.

As these studies were taking place, the FDA approved belatacept for kidney transplantation. Thus, we chose to change from CTLA4-Ig to belatacept, to make our studies more clinically translatable and to mirror subsequent experience in NHP renal transplantation (8). Four animals received belatacept, alefacept and all succumbed to problems associated with control of protective immunity. The first transplant (BAT1) was sacrificed on POD 56 due to weight loss. This animal did not receive CMV prophylaxis and had a peak of  $2.3 \times 10^7$  viral copies/mL at the time of sacrifice. The allograft was macroscopically similar to baseline and demonstrated Banff 0 skin histology (Figure 3).

The second transplant (BAT2) experienced weight loss and viral illness with immunosuppression adjusted with reduction by POD 78. CMV viral copies/mL peaked to  $3 \times 10^6$ . No visual skin changes were observed until after POD 84. Due to progression of weight loss, the animal was sacrificed on POD 90, with histology demonstrating Banff III. The third transplant (BAT3) was sacrificed on day 73 due to significant weight loss, with an allograft devoid of skin changes and Banff 0.

The fourth transplant (BAT4) had a rash on POD 21 with a Banff II rejection (Figure 4). Adjustment of tacrolimus dosing to target the upper therapeutic limit resulted in improvement of the rash similar to BT1. The graft did not demonstrate any visual changes until day 105. At this time the animal was sacrificed due to significant weight loss and a CMV infection with a maximum of  $1.4 \times 10^4$  viral copies/mL. Macroscopically the skin demonstrated small areas of light pink rash with few scattered petichiae and preserved hair growth. Histology revealed Banff II rejection with prominent perivascular inflammation with notable endarteritis and fibrinoid change.

To reduce problems with protective immunity we excluded alefacept from four animals that received VCAs with methylprednisolone, tacrolimus and belatacept, with sirolimus conversion for long-term survivors. Graft survival was significantly prolonged compared to Group I (mean rejection free survival = 78.8 days,  $p = 0.01$ ). These grafts proceeded as follows. The first transplant (BT1) presented a patchy erythematous rash on POD 28, biopsy demonstrated Banff I. Tacrolimus trough level was 3.6 mg/dL, subsequently the dose was adjusted to target levels and the rash disappeared. Significant weight loss led to the sacrifice of the animal on POD 44 with an excisional biopsy demonstrating Banff 0.

The second transplant (BT2) had immunosuppression withdrawn and graft excision on POD 77 due to weight loss from viral infection. No visual changes were observed, histology demonstrated Banff I. (Figure 5).

The third transplant (BT3) had a macroscopically normal appearing allograft up to 4 months post-transplant. At the time of graft excision on day 133, the graft was swollen with erythematous, indurated skin and significant hair loss. Histology demonstrated significant lymphocytic infiltration with epithelial dyskeratosis - a Banff III rejection.

The fourth transplant (BT4) had sparse light pink macules on allograft skin on day 28, which was accompanied by a similar rash at the ipsilateral axilla. Skin biopsy on POD 35 was Banff 0. The rash resolved without treatment. A protocol biopsy on POD 100 was Banff 0, with no gross abnormalities of the skin present. On POD 112 the graft developed areas of

erythema and induration with epidermal scabbing. The graft was excised on POD 140, histology demonstrated Banff III.

A summary is depicted in Table 2.

### **The addition of costimulation blockade delays rejection in VCA**

Overall comparison of the four treatment groups revealed that the addition of CoB improved rejection free survival compared to tacrolimus with a steroid taper alone (T vs BT  $p=0.01$ , vs BAT  $p=0.01$ , vs CAT  $p<0.01$ ). Alefacept combined with belatacept did not benefit costimulation blockade when compared to belatacept alone (BT vs BAT  $p=0.60$ , BT vs CAT  $p=0.34$ ). When adding the historic control data the  $p$ -value for the log rank comparing all animals treated with CNI's based therapy to all animals treated with CoB was .0143 (Figure 6).

### **Alefacept diminishes CD2<sup>hi</sup> CD8<sup>+</sup> T cells long term but only transiently reduces CD2<sup>+</sup> memory effector T cells when added to belatacept**

Belatacept therapy without alefacept demonstrated a bimodal distribution of CD2<sup>lo</sup> and CD2<sup>hi</sup> CD8<sup>+</sup> T cells (Figure 7). The administration of alefacept resulted in the loss of a distinct CD2<sup>hi</sup> CD8<sup>+</sup> T cell population. Despite this observation, longitudinal monitoring of CD8<sup>+</sup>CD28<sup>-</sup>CD95<sup>+</sup>CD2<sup>+</sup> T<sub>em</sub> cells demonstrated a transient decrease which was most profound on POD 3, with prompt reconstitution to baseline levels despite continued treatment. This suggests that although CD2<sup>+</sup>CD8<sup>+</sup> T cells were effectively targeted by alefacept, the CD8<sup>+</sup> T<sub>em</sub> population may not have been greatly reduced in the long term.

### **Costimulation blockade regimens prevented DSA formation**

No alloantibody formation was observed in CoB groups. One of three animals treated with tacrolimus and steroids and six out of seven historic controls treated with CNIs based regimens developed DSA (Figure 8). T2's graft was excised on POD 7 with Banff I and was not found to have positive alloantibody. This may be due to early cellular rejection prior to DSA formation by the experimental endpoint. T3 did not develop DSA by graft excision on POD 14.

## **Discussion**

VCA has the potential to provide life-improving function to people suffering from tissue loss unable to be corrected through traditional reconstructive techniques. Severe complications and mortality have been reported after VCA (2, 14, 15). These complications loom particularly large given the non-life saving nature of VCA. In this study, we used our established model of VCA (13). These studies represent a judicious, extensive initial testing of CoB-based regimens in VCA, which draw from other successful experiences using CoB in NHPs to test both B7 blockade and CD2 blockade (8, 11, 16). Rejection was diagnosed using the 2007 VCA-Banff working classification,(17) which was comparative to clinical VCA rejection. Based on our observations and similar to clinical cases, CNI's are ineffective in preventing rejection. Although rejection may have been reversed, treatment of rejection was outside the scope of this study.



Our first study regimen included sirolimus starting on the day of transplant. Both grafts experienced wound healing complications ultimately leading to engraftment failure. These healing related complications are well-documented in other surgical settings but have not been specifically tested VCAs. Thus, these studies provide objective documentation that de novo sirolimus might be a particular risk for VCAs (18-22). As opposed to other solid organ transplants, in a skin-containing VCA, the skin is a component of the transplant and the wound healing complications prevent engraftment. Thus, our results with sirolimus in VCA may be clinically relevant and demonstrate that sirolimus is not an appropriate agent to be used immediately after a skin containing-VCA. The conversion of tacrolimus to sirolimus avoided these healing problems in our studies, thus providing a clinically relevant alternative to consider.

The combination of CTLA-4Ig and alefacept provided additional protection to the allograft and delayed rejection significantly compared to tacrolimus and steroids alone. However, the combination of these agents led to a failure to thrive in several animals ultimately requiring euthanasia. The necropsy reports described no gross abnormality. Significantly elevated CMV in euthanized animals suggests that the compromise of protective immunity led to death due to a viral illness. This diminution of protective immunity from alefacept has been previously reported in the NHP islet and kidney transplantation experience(8, 16). Modification of this regimen to use belatacept with alefacept and tacrolimus instead of CTLA-4Ig resulted in similar outcomes. With animals that succumbed to viral illness having histology devoid of rejection in both these study groups, it is likely that the combination of alefacept and CD28 blockade was too effective at preventing rejection with a significant cost to protective immunity.

Withdrawal of alefacept and treatment with belatacept and tacrolimus alone resulted in similar protection against allograft rejection. However, the impact in protective immunity did not differ when compared to either CoB group using alefacept. Thus, in our studies, alefacept did not provide additional benefit to B7 CoB in VCA despite the risks. Additionally, alefacept was recently withdrawn from the market. Study groups using CoB did not develop detectable DSA, which is consistent with prior reports (6, 23). Our rationale was to continue with a group consisting of belatacept and steroids. Our preliminary data from one NHP VCA recipient resulted in a rejection free survival of 28 days and a Banff III (data not shown). Further results on this group are forthcoming.

## Summary

CoB are capable of improving rejection free survival of VCAs when compared to a standard CNI-based therapy. Alefacept, although effective at reducing the memory T cell compartment, did not improve outcomes and ultimately hindered protective immunity. Sirolimus immediately post-transplant of VCAs led to immediate post-operative wound healing complications. DSA development was not observed in CoB groups, which correlates to current experience with CoB in other solid organ transplants. These data provide initial results of CoB-based regimens in NHPs in VCA. Further refinements of how best to use belatacept in VCA require more experimentation.

## Acknowledgments

The authors wish to acknowledge Jennifer Cheeseman, Sebastian Perez, Stephanie Monday, Benjamin Kramer, Andrew Page, and Adriana Gibby of the Emory Transplant Center for their technical assistance.

This study was supported by a grant from the Department of Defense administered through the Navy Bureau of Medicine and Surgery's Medical Development Program, by the American Society of Transplant Surgeons, by the Melina Nakos Foundation, and by resources at the Atlanta VA Medical Center.

## Abbreviations

<b>VCA</b>	vascularized composite allograft
<b>NHP</b>	non-human primate
<b>CMV</b>	cytomegalovirus
<b>PCR</b>	polymerase chain reaction
<b>CoB</b>	costimulation blockade
<b>PFC</b>	polychromatic flow cytometry
<b>DSA</b>	donor-specific antibody

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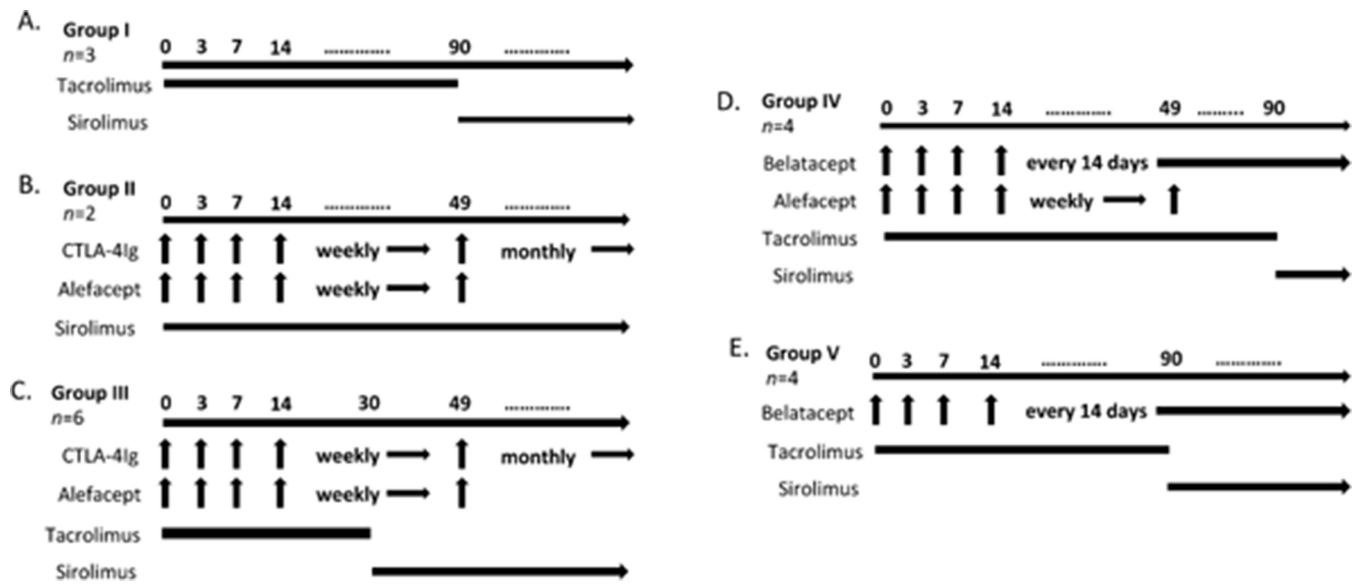
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**Figure 1.**

Immunosuppression treatment groups.

A. Group I – Tacrolimus (T, n=3). Tacrolimus given twice daily intramuscularly.

B. Group II – CTLA-4 Ig, alefacept, sirolimus (CAS, n=2). CTLA-4 Ig 20 mg/kg and alefacept 1 mg/kg intravenously on days -1, 3, 7, and weekly thereafter. Alefacept was last administered on day 49 and CTLA-4Ig converted to monthly dosing at that time.

Intramuscular sirolimus given daily.

C. Group III – CTLA-4 Ig, alefacept, tacrolimus conversion to sirolimus (CAT, n=6).

CTLA-4 Ig 20 mg/kg and alefacept 1 mg/kg intravenously on days -1, 3, 7, and weekly thereafter. Alefacept was last administered on day 49 and CTLA-4Ig converted to monthly dosing at that time. Intramuscular tacrolimus given twice daily for 30 days, followed by daily sirolimus.

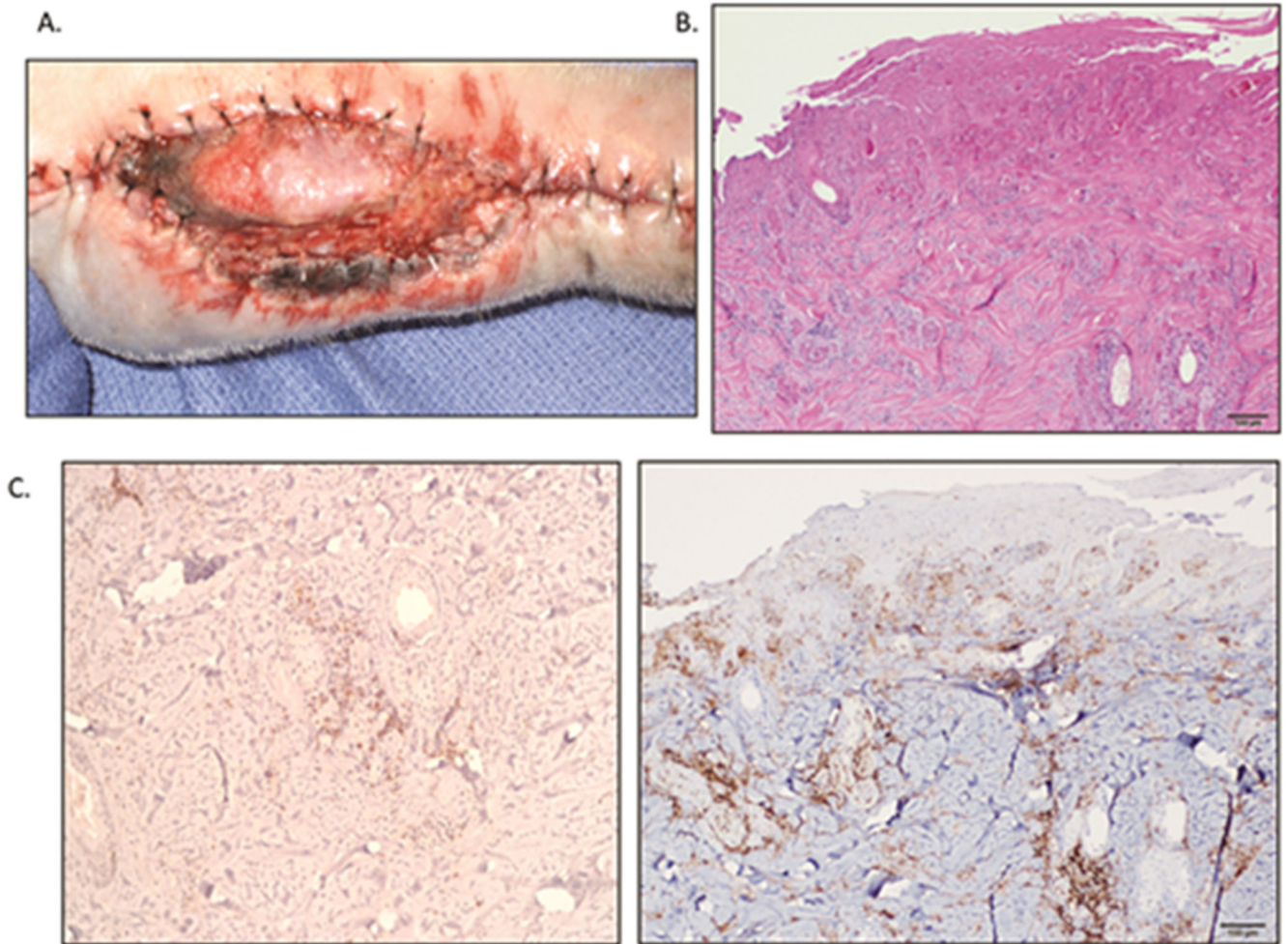
D. Group IV – Belatacept, alefacept, tacrolimus conversion to sirolimus (BAT, n=4).

Belatacept 20 mg/kg intravenously on days 0, 3, 7, 14, and every other week thereafter.

alefacept 1 mg/kg was administered intravenously on days -1, 3, 7, and continued weekly until last dose on day 49. Intramuscular tacrolimus given twice daily for 90 days, followed by daily sirolimus.

E. Group V – Belatacept, tacrolimus conversion to sirolimus (BT, n=4). Belatacept 20 mg/kg

intravenously on days 0, 3, 7, 14, and every other week thereafter. Intramuscular tacrolimus given twice daily for 90 days, followed by daily sirolimus.



**Figure 2.**

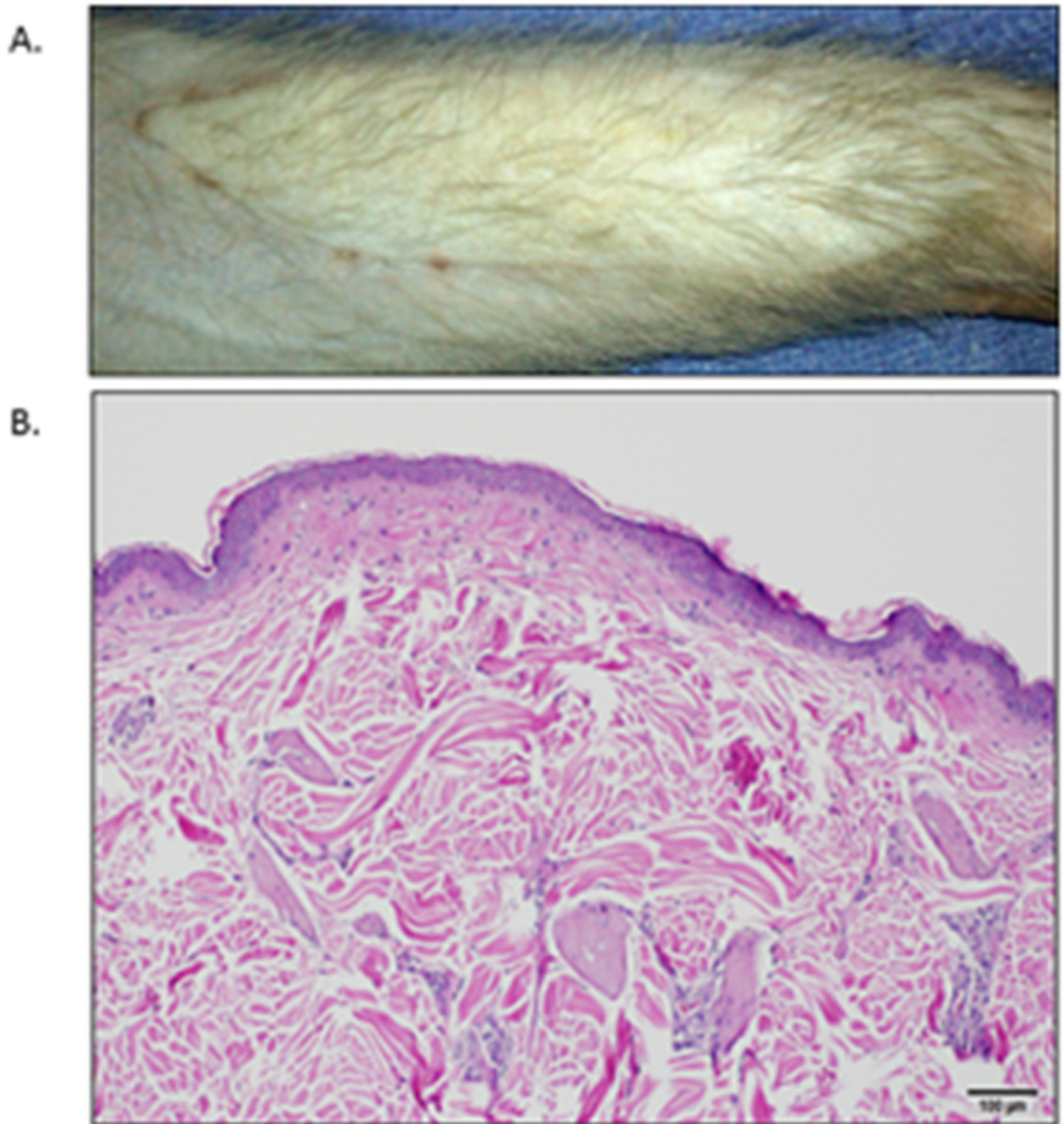
Wound healing complications of a VCA treated with sirolimus.

A. Macroscopic appearance of CAS1 on POD 14. Note the poorly healed skin edges and edema not only on the graft-native border, but also between the native skin edges as well. There is poor granulation of the exposed tissues in the open wound.

B. Biopsies taken from (A) demonstrate significant infiltrates on skin H&E stain (5× magnification).

C. Further immunohistochemistry analysis of the biopsy revealed a far greater macrophage (left image) than T-cell (right image) response, suggesting a generalized inflammatory response as opposed to rejection.



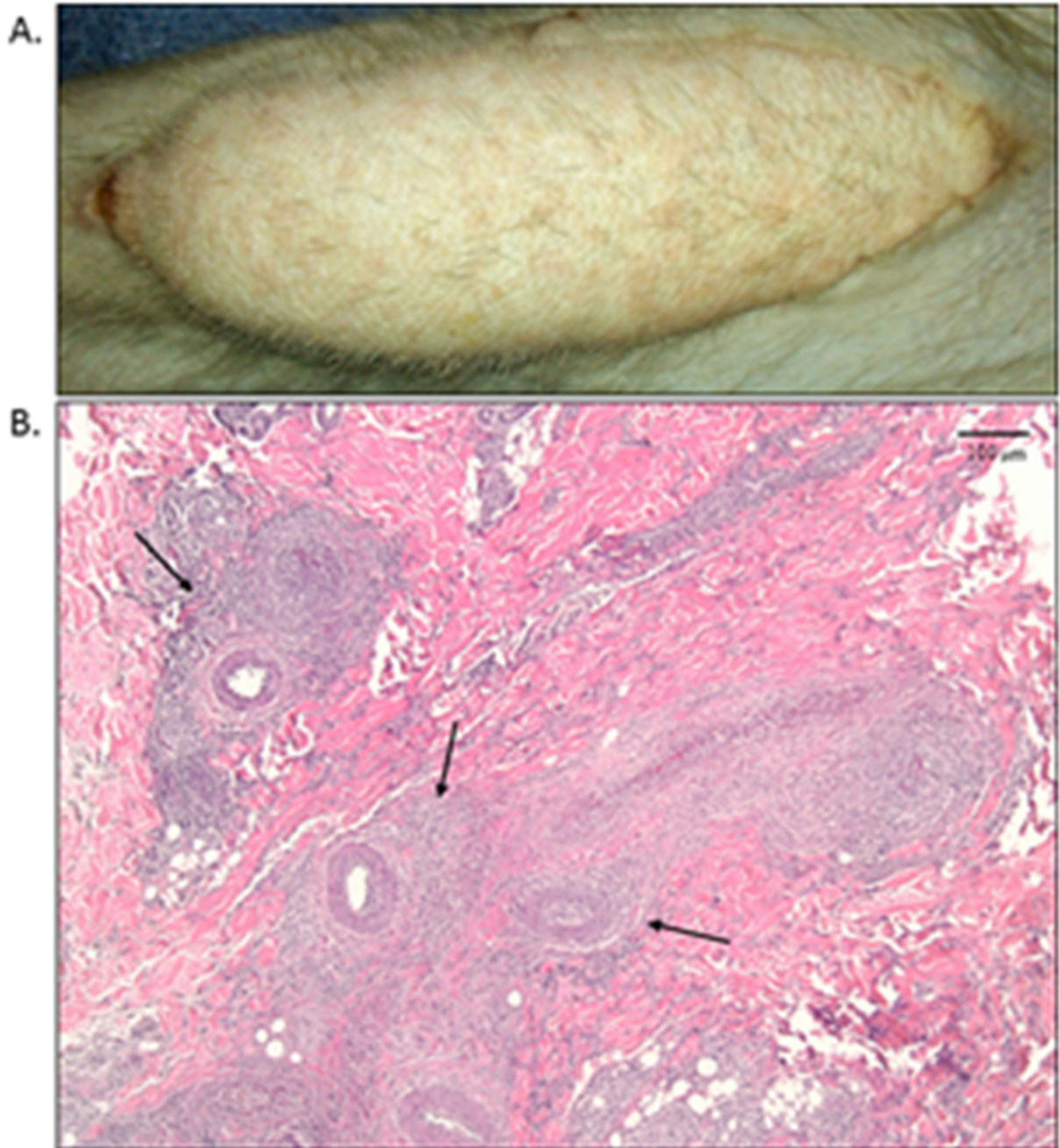


**Figure 3.**

Engraftment without rejection.

A. Macroscopic appearance of BAT1 on POD 56 at the time of sacrifice. This allograft has well healed skin edges with no hair loss and no gross abnormalities or rash present.

B. Skin biopsy from (A) with H&E staining shown (5× magnification). No leukocyte infiltrate and normal appearance of epithelial and dermal tissues demonstrate Banff 0.



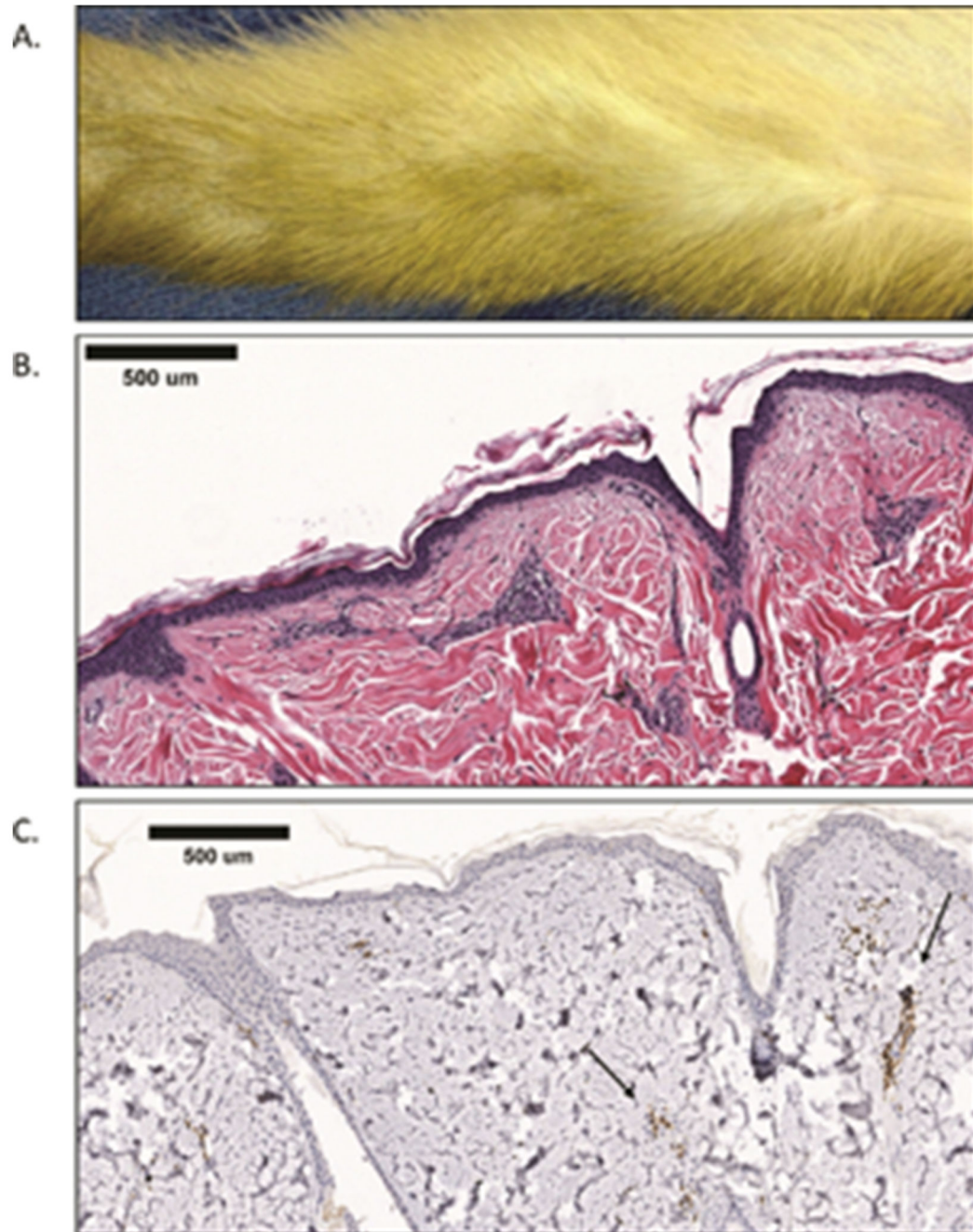
**Figure 4.**

Rejection of a VCA treated with dual costimulation blockade.

A. Macroscopic appearance of BAT4 on POD 21. A petechial rash is newly present, however no significant hair loss is noted from baseline.

B. Skin biopsy from image (A) with H&E staining shown (5× magnification). Significant perivascular infiltration is noted (arrows) with no epithelial involvement, demonstrating a Banff II.





**Figure 5.**

VCA treated with belatacept and tacrolimus.

A. Macroscopic appearance of BT2 on POD77 at time of sacrifice. No significant changes were noted on gross inspection, minimal hair loss, well healed skin edges, and no rash was observed.

B. Protocol skin biopsy was taken from (A) with H&E staining shown (10× magnification). Mild perivascular infiltration is present with no significant epithelial abnormalities noted demonstrating a Banff I.



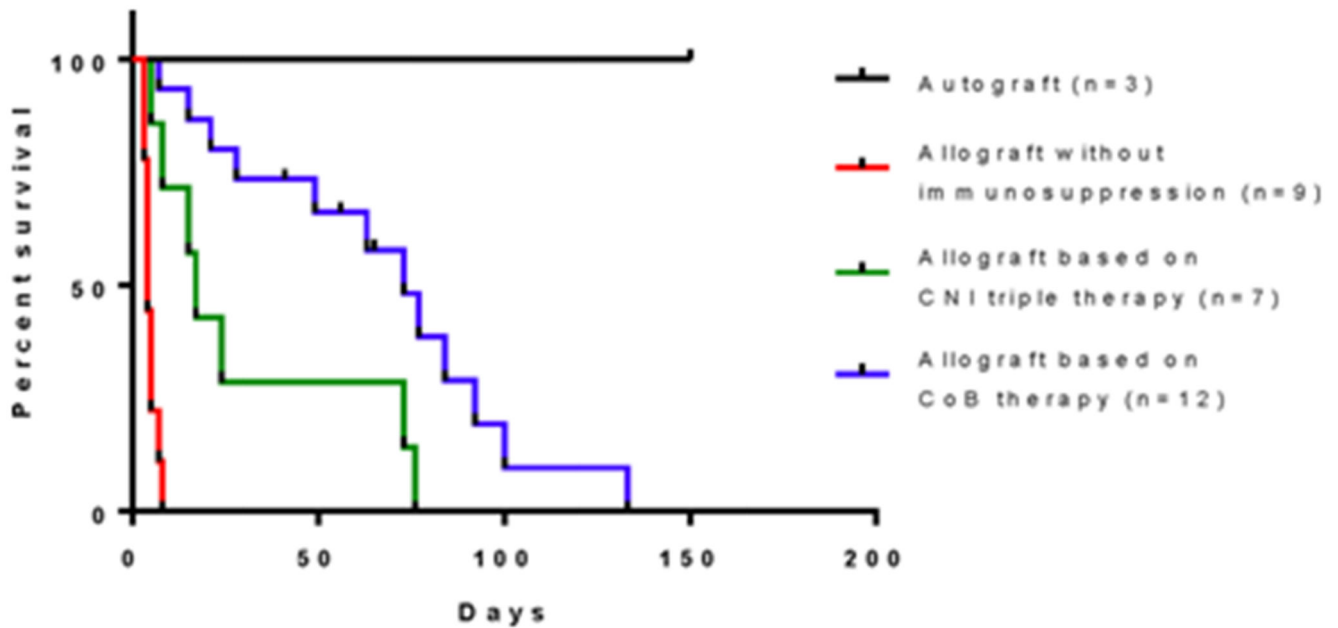
C. Immunohistochemistry from biopsy (B) using a CD3 stain confirms lymphocyte infiltration as demonstrated by arrows.

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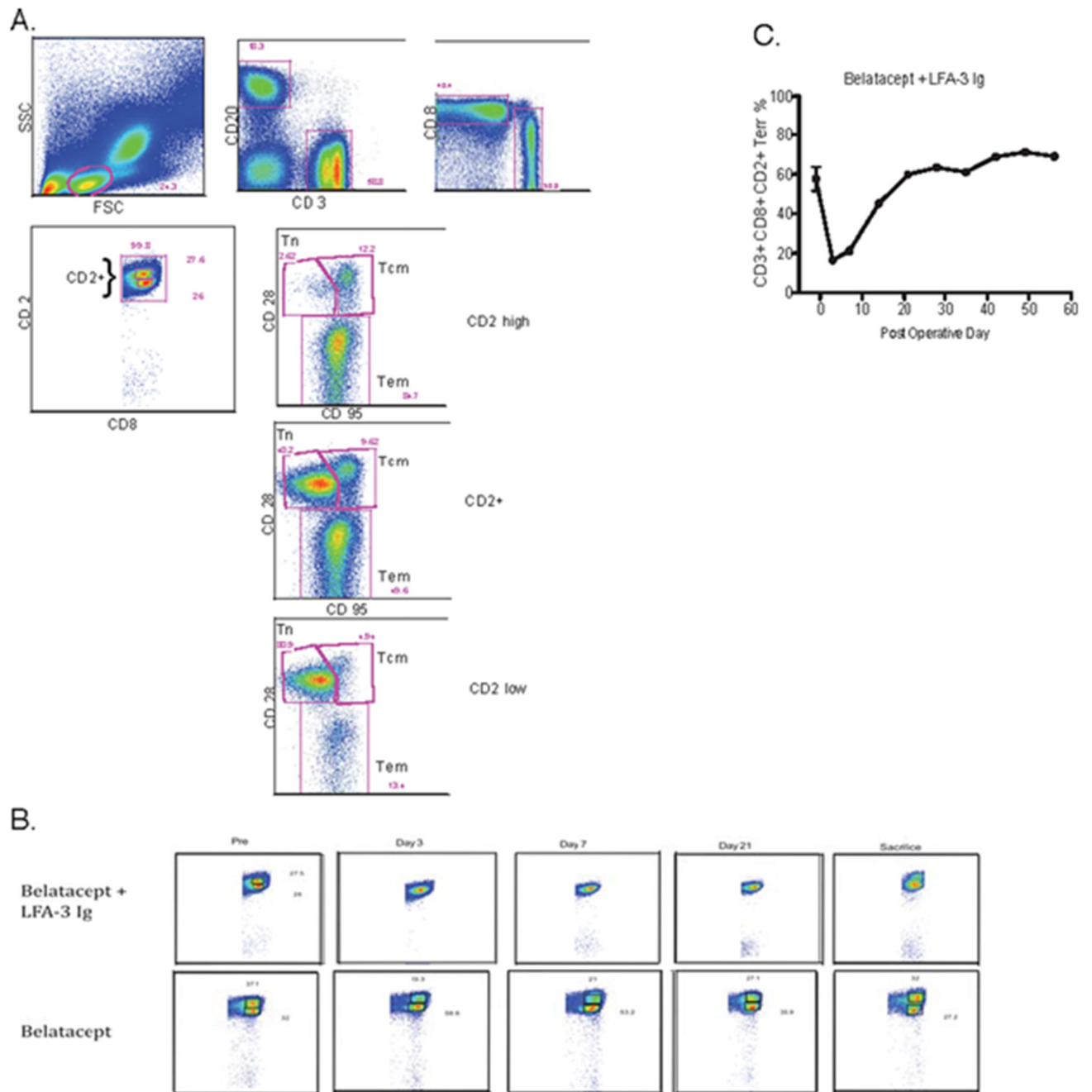
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**Figure 6.**

Overall rejection-free survival of vascular composite allografts. Rejection in this analysis was determined by time of initial visual changes leading to a biopsy proven rejection to minimize variability from time of visual changes to biopsy. Addition of CoB to tacrolimus resulted in improved rejection-free survival when compared to historic controls ( $p=0.0143$  vs combined CoB).



**Figure 7.** Alefacept in combination with belatacept abrogates CD2<sup>hi</sup>CD8<sup>+</sup> T cells without long term effect on the CD2<sup>+</sup> effector memory T-cell compartment. Polychromatic flow cytometry was used to evaluate the effect of alefacept on peripheral blood CD2<sup>+</sup>CD8<sup>+</sup> T-cells, representative findings depicted here.  
 A. PFC gating algorithm to identify the memory T-cell compartment.  
 B. Distinct CD2<sup>hi</sup> and CD2<sup>low</sup> populations within CD8<sup>+</sup> T-cells can be identified throughout the entire course in animals receiving belatacept without alefacept. Contrary to this,

alefacept administration results in decrease of CD2<sup>hi</sup>CD8<sup>+</sup> that is sustained throughout treatment

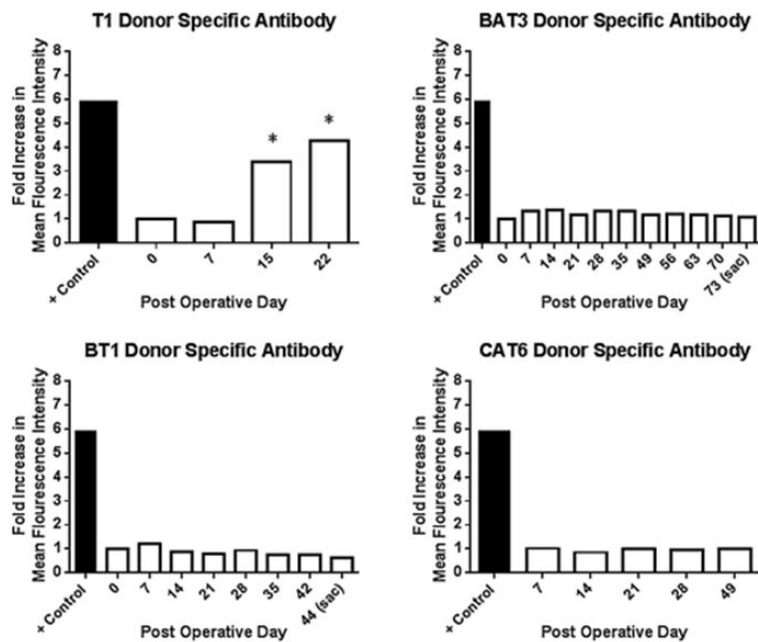
C. Effector memory T-cells (CD8<sup>+</sup>CD28<sup>-</sup>CD95<sup>+</sup>CD2<sup>+</sup>) nadir 3 days after administration of alefacept and subsequently rebounds to pre-treatment levels thereafter.

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**Figure 8.** Costimulation blockade prevents formation of donor specific antibodies. Representative data shown from each study group. No alloantibody was observed in any costimulation blockade based regimen, however it was observed in T1 by POD 15 when the graft was excised for rejection. Alloantibody persisted one week after excision as noted on POD 22.

**Cytomegalovirus monitoring**

**Table 1**

Cytomegalovirus (CMV) levels (copies/mL) were monitored in animals on a weekly basis, the table depicts the maximum level detected during the time course of the allograft. Not all animals received valganciclovir prophylaxis; this was instituted after deficits were noted in protective immunity affecting experimental endpoints. CAT1 and CAT2 did not have CMV levels consistently monitored and therefore are not shown.

Group	Animal ID	Highest CMV level (copies/mL)	On prophylaxis (Y/N)
Group I	T1	500	Y
	T2	1500	Y
	T3	1000	Y
Group III	CAT3	20,176,950	N
	CAT4	13,050	N
	CAT5	230,225	N
	CAT6	350	N
	BAT1	23,000,000	N
	BAT2	3,070,050	N
Group IV	BAT3	850	Y
	BAT4	14,150	Y
	BT1	2800	Y
	BT2	6000	Y
Group V	BT3	1500	Y
	BT4	200	Y

Summary of outcomes. (A) Study groups—all immunosuppression treated groups included a steroid taper over a course of 1 month. Animals that were sacrificed due to viral illness and failure to thrive did not demonstrate gross abnormalities on pathologic evaluation. (B) Historical controls previously published (13)

**Table 2**

(A) Group and regimen*	Animal ID	Initial visual changes prior to biopsy (POD)	Biopsy (POD)	Banff grade	Study endpoint	DSA (Y/N)
Allograft control	Allo1	2			rejection	N/A
No immunosuppression	Allo2	5			rejection	N/A
Autograft control	Auto1	negative	N/A	-	survival	N/A
No immunosuppression	Auto2	negative	N/A	-	survival	N/A
	Auto3	negative	N/A	-	survival	N/A
Group I	T1	14	15	1	rejection	Y
Tacrolimus	T2	7	7	1	rejection	N
	T3	13	14	2	rejection	N
Group II	CAS1	14	14	N/A	graft failure	N
CTLA-4Ig, alefacept, sirolimus	CAS2	5	9	N/A	graft failure	N
Group III	CAT1	56	62	1	rejection	N
CTLA-4Ig, alefacept, tacrolimus with conversion to sirolimus	CAT2	63	63	1	rejection	N
	CAT3	none	41	0	failure to thrive (cuthanasia)	N
	CAT4	none	65	0	failure to thrive (cuthanasia)	N
	CAT5	84	92	3	rejection	N
	CAT6	42	49	3	rejection	N
Group IV	BAT1	none	56	0	failure to thrive (cuthanasia)	N
Belatacept, alefacept, tacrolimus with sirolimus conversion	BAT2	85	90	3	rejection	N
	BAT3	none	73	0	failure to thrive (cuthanasia)	N
	BAT4	21	21	2	rejection	N
Group V	BAT1	28	28	2	rejection	N
Belatacept, tacrolimus with sirolimus conversion	BAT2	none	77	1	failure to thrive (cuthanasia)	N



(A) Group and regimen*	Animal ID	Initial visual changes prior to biopsy (POD)	Biopsy (POD)	Banff grade	Study endpoint	DSA (Y/N)
	BAT3	98	133	3	rejection	N
	BAT4	112	140	3	rejection	N

(B) Group and regimen*	Animal ID	Day of rejection	Donor specific antibody (Y/N)
I. DSA, donor-specific antibody; MMF, mycophenolate mofetil; POD, postoperative day.			
Allograft controls without immunosuppression			
	Hist1	3	Y
	Hist2	4	N
	Hist3	4	N
	Hist4	3	N/A
	Hist5	7	Y
	Hist6	4	Y
	Hist7	8	Y
	Hist8	15	Y
	Hist9	5	N
	Hist10	17	Y
	Hist11	24	Y
	Hist12	8	Y
	Hist13	73	Y
	Hist14	76	Y

Triple therapy (tacrolimus, MMF, and steroid taper)			
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