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TITLE: Optimization of Delayed Tolerance Induction in Swine: A Clinically Relevant Protocol for Immunosuppression-Free Vascularized Composite Allotransplantation

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Optimization of Delayed Tolerance Induction in Swine: A Clinically Relevant Protocol for Immunosuppression-Free Vascularized Composite Allotransplantation

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13. SUPPLEMENTARY NOTES

14. ABSTRACT
Tolerance of *kidney* allografts has been achieved in nonhuman primates (NHPs) using a the delayed period protocol, i.e combination of post-transplant non-myeloablative conditioning and donor bone marrow transplantation four months later (DBMT). That results in transient mixed hematopoietic chimerism and then led transplant tolerance. A similar protocol has recently been successfully extended to human recipients of HLA-mismatched living-related renal allografts. Clearly, mixed chimerism represents a powerful and clinically relevant approach to tolerance induction. Unfortunately the reproducibility of such protocol constitutes a bottleneck because of the risk to develop an acute rejection (AR) episode during the delayed period. Any episode of AR sensitize le patient and then decrease the chance of bone marrow engraftment. In this protocol we want to perform the BMT the day of the surgery before any episode of acute rejection.Recently co-stimulatory blockade has gained considerable attention for its possible beneficial and critical effects in tolerance inducing regimens. However, unequivocal evidence demonstrates that CTLA4-Ig or belatacept on its own are not capable of inducing tolerance in either stringent rodent models, or in non-human primate solid organ transplantation. Thus, CTLA4-Ig needs to be combined with other strategies if tolerance is the ultimate goal. Concomitant administration of donor HSCs in the form of DBMT with CTLA4-Ig appears to be the most promising approach to induce tolerance as indicated in various small and large animal solid organ transplant models. This is particularly attractive for VCA due to the fact that many of these types of transplants already include a vascularized BM component. Moreover, recent evidence suggests that augmentation with CTLA4-Ig promotes engraftment following DBMT for the induction of stable mixed chimerism and thus transplantation tolerance, and helps overcome split tolerance of transplanted skin. We hypothesize that the attainment of transplantation tolerance, defined as the absence of destructive immune responses against a transplanted organ or tissue without the requirement for immunosuppression, would not only allow successful withdrawal of immune medications but also potentially negate the development of chronic rejection.

15. SUBJECT TERMS
See below

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
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Unclassified	Unclassified	Unclassified	Unclassified	10	**19b. TELEPHONE NUMBER** *(include area code)*

TABLE OF CONTENTS

1. INTRODUCTION:

Vascularized composite allotransplantation (VCA) has emerged as a viable option for restoring form and function in patients with devastating soft tissue defects. To date, 44 faces and 120 hand/upper extremity transplants have been performed worldwide, with promising short- to intermediate-term functional and immunological outcomes. Nevertheless, the requirement for long-term immunosuppressive therapy to maintain the allograft increases the risk of related side effects such as infections, metabolic complications or even malignancies. Consequently, it is essential to develop a strategy to achieve immune tolerance, to obviate the requirement for long-term maintenance immunosuppression. T cell co-stimulation blockade (CoB) arose as an attractive concept to induce transplant tolerance in the 1990s and has been developed and used successfully in murine heart and islet cell transplant models. The administration of donor bone marrow (BM) cells in combination with CoB appears to be the most promising approach to achieve a state of tolerance through mixed chimerism, as indicated by various small and large animal solid organ transplant models. We propose to develop clinically relevant strategies using CoB (belatacept) and donor bone marrow cells to induce mixed chimerism in an established swine model of VCA.

We hypothesize that the attainment of transplantation tolerance, defined as the absence of destructive immune responses against a transplanted organ or tissue without the requirement for immunosuppression, would not only allow successful withdrawal of immune medications but also potentially negate the development of chronic rejection.

2. KEYWORDS:

Vascularized composite allotransplantation, mixed chimerism, co-stimulatory blockade, bone marrow transplant, immunologic tolerance, fasciocutaneous flap

3. ACCOMPLISHMENTS:

What were the major goals of the project?

Our objectives are: (1) to add co-stimulatory blockade to promote both successful engraftment after donor bone marrow cell infusion to achieve mixed chimerism and thus tolerance of VCA and (2) to apply the day 0 protocol across the range of MHC barriers that may be encountered clinically to demonstrate the robustness of this approach.

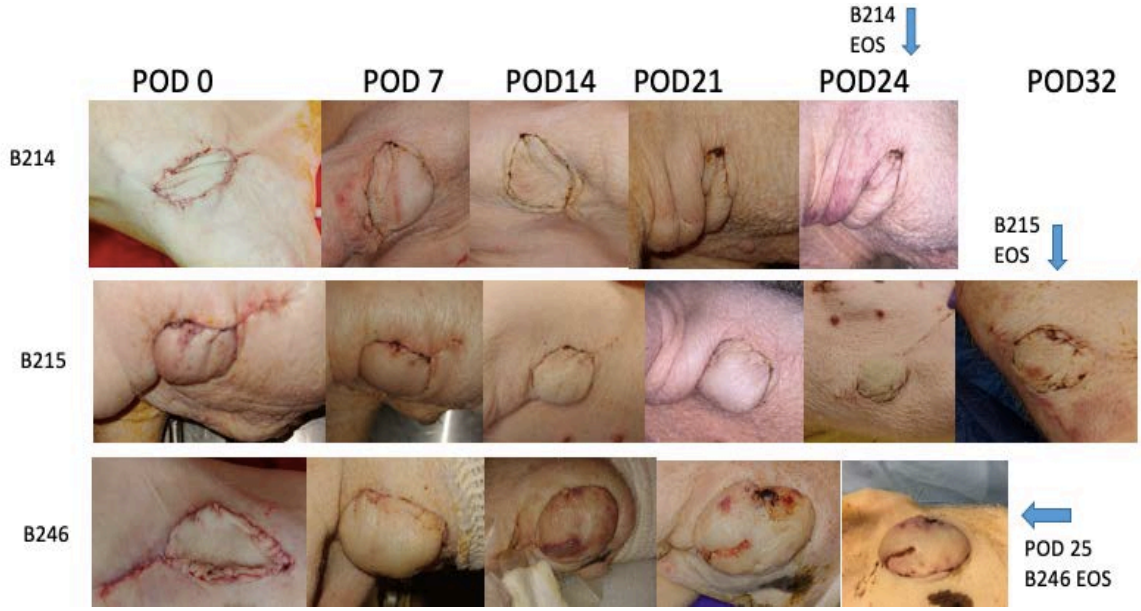
What was accomplished under these goals?

1) 3 VCA transplantations were performed in full-mismatch compatibility (Aim 2 group 3) Belatacept® was unavailable on market and we had to switch to abatacept®. Both are co-stimulator blockade (anti CTLA4 Ig)

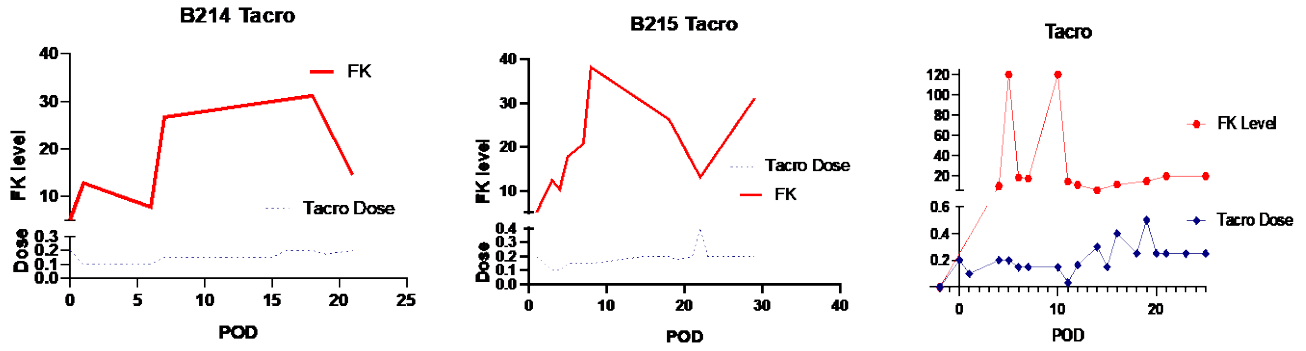
2) Surgery performed in full-mismatch compatibility:

- **B214**: Full Mismatch (AA→GG) Female to Female 4/25/19
  - BM- Stem Cell transplant count: 1.7x10^9/kg
- **B215:** Full Mismatch (AA→GG) Male to Male 4/26/19
  - BM- Stem Cell transplant: 1.7x10^9/kg
- **B246**: Full Mismatch (CC→AA) Male to Male 8/29/19
  - BM- Stem Cell transplant: 1.7x10^9/kg

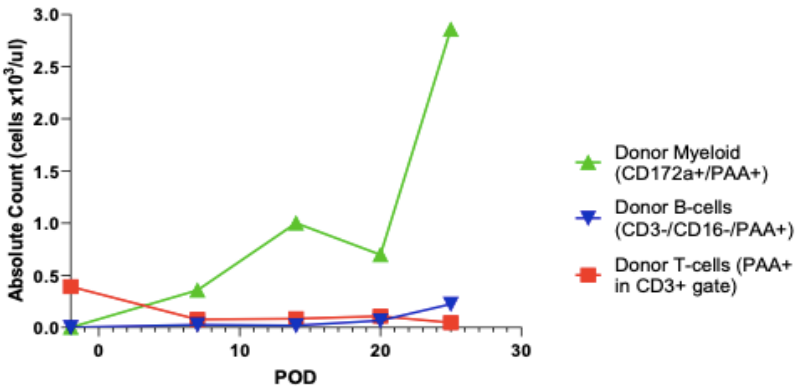
3) The clinical course of the three recipients were comparable. No rejection episodes were detected clinically and on histology. In the other hand, the 3 recipients developed a posttransplant lymphoma disease (PTLD). Previously, in class-I-mismatch, we did not notice the development of this disease. The only difference in this protocol was the use of Abatacept® since we were unable to obtain Belatacept®. Given the similar evolution of these 3 recipients we decided to stop abatacept® administration and switch to Belatacept®. After contacting Bristol Mayer Squibb (BMS) company, we finally a donation of Belatacept® (10g) for our research purposes.



Tacrolimus course of the 3 recipients (tacrolimus range: 20-30ng/ml):



The pig B246 shows the establishment of a macrochimerism (Figure below, representative data), with a rapid and important increase of donor myeloid population (about 80% of cells in 20 days post VCA). The chimerism for T and B cells is more moderate with about 10-15% of donor T cells, mostly composed of CD4+ cells, and less than 2% of donor B cells (mainly resting B cells CD3-/CD16-) in the lymphocyte population.



What opportunities for training and professional development has the project provided?

Nothing to report

How were the results disseminated to communities of interest?

Nothing to report

What do you plan to do during the next reporting period to accomplish the goals?

Respond to reviewer's comments (manuscript ongoing)

4. IMPACT:

What was the impact on the development of the principal discipline(s) of the project?

Nothing to report

What was the impact on other disciplines?

Nothing to report

What was the impact on technology transfer?

Nothing to report

What was the impact on society beyond science and technology?

Nothing to report

5. CHANGES/PROBLEMS:

Changes in approach and reasons for change

Due to the promising results we have produced in our laboratory with the day 0 protocol, we amended the SOW to replace the originally planned delayed tolerance protocol. We managed to induce long-term survival immunosuppression-free with development of mixed chimerism in three swine (class 1 mismatch).

Actual or anticipated problems or delays and actions or plans to resolve them

Nothing to report

Changes that had a significant impact on expenditures

Nothing to report

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Significant changes in use or care of human subjects

Nothing to report

Significant changes in use or care of vertebrate animals.

Nothing to report

Significant changes in use of biohazards and/or select agents

Nothing to report

6. PRODUCTS:

- **Publications, conference papers, and presentations**

  Journal publications.

  Nothing to report

  Books or other non-periodical, one-time publications.

  Nothing to report

  Other publications, conference papers, and presentations.

  Nothing to report

- **Website(s) or other Internet site(s)**

  Nothing to report

- **Technologies or techniques**

Nothing to report

- **Inventions, patent applications, and/or licenses**

Nothing to report

- **Other Products**

Nothing to report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name	Project Role	Person month worked	Contribution to the project
Curtis Cetrulo	PI	0.24	Overall design and direction of proposed studies, interpretation of results.
Josef Kurtz	Co-Investigator	2.75	Assessment of transplant recipients, supervision of work performed by research fellow, assists with interpretation of results.

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Current Support Changes for the PI, Co-I or Other Senior/Key Personnel	Changes in Current Support
Curtis Cetrulo	Change: Ended XenoTherapeutics, Inc. Sponsored Research Agreement Role: PI Effort: N/A Date: 12/15/16-05/31/18 No impact
Curtis Cetrulo	Change: Ended Shire HGT, Inc. Sponsored Research Agreement Role: PI Effort: N/A Date: 11/01/16-5/14/18 No impact
Curtis Cetrulo	Change: Extended DoD grant W81XWH-16-1-0702 “Optimization of Delayed Tolerance Induction in Swine: A Clinically-Relevant Protocol for Immunosuppression-Free Vascularized Composite Allotransplantation”

	Role: PI Effort: 2% Date: 09/15/16-09/14/19 No impact
Curtis Cetrulo	Change: Received Shriners Hospital for Children, Boston grant 85103-BOS-18 "Role of the Thymus in Tolerance of Vascularized Composite Allotransplantation" Role: PI Effort 10% Date: 01/01/18-12/31/20 No impact

What other organizations were involved as partners?

Nothing to Report

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS:

QUAD CHARTS:

9. APPENDICES:

None