

AWARD NUMBER: W81XWH-16-1-0702

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

PRINCIPAL INVESTIGATOR: Curtis L Cetrulo, MD, FACS

RECIPIENT: Massachusetts General Hospital

Boston, MA 02114-2621

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Fort Detrick, Maryland 21702-5012

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4. TITLE AND SUBTITLE Optimization of Delayed Tolerance Induction in Swine: A Clinically-Relevant Protocol for Immunosuppression-Free Vascularized Composite Allotransplantation	5a. CONTRACT NUMBER
	5b. GRANT NUMBER W81XWH-16-1-0702
	5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S) Curtis Cetrulo, MD E-Mail: ccetrulo@partners.org	5d. PROJECT NUMBER
	5e. TASK NUMBER
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7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) AND ADDRESS(ES) The Massachusetts General Hospital 55 Fruit Street Boston, MA 02114-2696	8. PERFORMING ORGANIZATION REPORT NUMBER
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9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012	10. SPONSOR/MONITOR'S ACRONYM(S)
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13. SUPPLEMENTARY NOTES

14. ABSTRACT .
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.

15. SUBJECT TERMS
See below

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT Unclassified	18. NUMBER OF PAGES 15	19a. NAME OF RESPONSIBLE PERSON USAMRMC
a. REPORT Unclassified	b. ABSTRACT Unclassified	c. THIS PAGE Unclassified			19b. TELEPHONE NUMBER (include area code)

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1. **INTRODUCTION:** Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.

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:** Provide a brief list of keywords (limit to 20 words).

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

3. **ACCOMPLISHMENTS:** The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency Grants Officer whenever there are significant changes in the project or its direction.

What were the major goals of the project?

List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.

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.

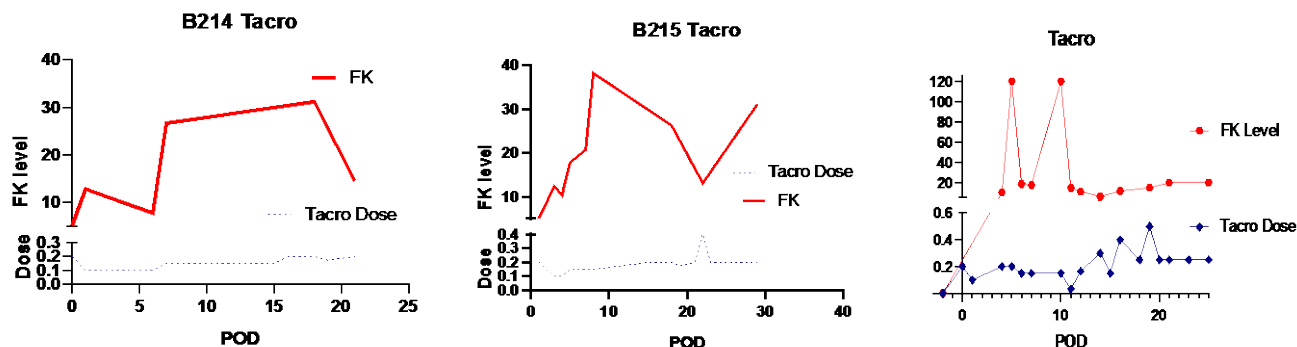
What was accomplished under these goals?

For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.

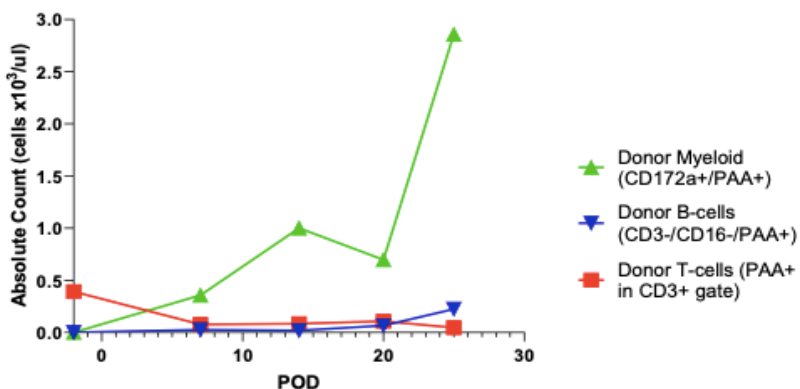
- 1) 3 VCA transplantations were performed in full-mismatch compatibility (Aim 2 group 3) Belatacept® was unavailable on market and we had to switch to abatacept®. Both are co-stimulator blockade (anti CTLA4 Ig)
- 2) Surgery performed in full-mismatch compatibility:
 - **B214:** Full Mismatch (AA→GG) Female to Female 4/25/19
 - BM- Stem Cell transplant count: $1.7 \times 10^9/\text{kg}$
 - **B215:** Full Mismatch (AA→GG) Male to Male 4/26/19
 - BM- Stem Cell transplant: $1.7 \times 10^9/\text{kg}$
 - **B246:** Full Mismatch (CC→AA) Male to Male 8/29/19
 - BM- Stem Cell transplant: $1.7 \times 10^9/\text{kg}$
- 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?

If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. "Training" activities are those in which individuals with advanced professional skills and experience assist others in attaining greater proficiency. Training activities may include, for example, courses or one-on-one work with a mentor. "Professional development" activities result in increased knowledge or skill in one's area of expertise and may include workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.

Nothing to report

How were the results disseminated to communities of interest?

If there is nothing significant to report during this reporting period, state “Nothing to Report.”

Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.

Nothing to report

Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

- 4. We will perform surgeries per our SOW.

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

If there is nothing significant to report during this reporting period, state “Nothing to Report.”

Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).

Nothing to report

What was the impact on other disciplines?

If there is nothing significant to report during this reporting period, state “Nothing to Report.”

Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.

Nothing to report

Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:

- *transfer of results to entities in government or industry;*
- *instances where the research has led to the initiation of a start-up company; or*
- *adoption of new practices.*

Nothing to report

What was the impact on society beyond science and technology?

If there is nothing significant to report during this reporting period, state “Nothing to Report.”

Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:

- *improving public knowledge, attitudes, skills, and abilities;*
- *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*
- *improving social, economic, civic, or environmental conditions.*

Nothing to report

- 5. CHANGES/PROBLEMS:** The Project Director/Principal Investigator (PD/PI) is reminded that the recipient organization is required to obtain prior written approval from the awarding agency Grants Officer whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, “Nothing to Report,” if applicable:

Changes in approach and reasons for change

Describe any changes in approach during the reporting period and reasons for these changes.

Remember that significant changes in objectives and scope require prior approval of the agency.

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

Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

We are actively working to resolve procurement difficulties with regard to the swine and Belatacept®.

Changes that had a significant impact on expenditures

Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.

Nothing to report

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

Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.

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: List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state “Nothing to Report.”

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

Report only the major publication(s) resulting from the work under this award.

Journal publications. *List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal; volume; year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

Nothing to report

Books or other non-periodical, one-time publications. *Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: Author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

Nothing to report

Other publications, conference papers, and presentations. *Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.*

Nothing to report

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

List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.

Nothing to report

- **Technologies or techniques**

Identify technologies or techniques that resulted from the research activities. In addition to a description of the technologies or techniques, describe how they will be shared.

Nothing to report

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

Identify inventions, patent applications with date, and/or licenses that have resulted from the research. State whether an application is provisional or non-provisional and indicate the application number. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.

Nothing to report

- **Other Products**

Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment, and/or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:

- *data or databases;*
- *biospecimen collections;*
- *audio or video products;*
- *software;*
- *models;*
- *educational aids or curricula;*
- *instruments or equipment;*
- *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
- *clinical interventions;*
- *new business creation; and*
- *other.*

Nothing to report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate “no change.”

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.
Alex Lellouch	Research Fellow	1.2	Dr. Lellouch is responsible for assisting Dr. Cetrulo in leading the surgical procedures on transplant donors and recipients, in analyses of immune responses in the recipients and interpretation of results.
Alec Andrews	Research Technologist	3.0	Mr. Andrews is responsible for pre- and post-operative animal care and for assisting Drs. Cetrulo and Lellouch in study coordination.
Natacha Carnel	PhD Researcher	2.0	Ms. Carnel is performing in vitro assays, MLR flow cytometry, preparing antibodies and assisting in study coordination.

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: 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: 09/15/16-09/14/20 No impact
Curtis Cetrulo	Change: Extended DoD grant W81XWH-15-1-0281 “Local Tacrolimus (FK506) Delivery for Prevention of Acute Rejection in the Non-Human Primate Delayed Mixed Chimerism Vascularized Composite Allograft Tolerance Induction Protocol” Role: PI Effort: 3% Date: 09/15/16-03/14/20 No impact
Curtis Cetrulo	Change: Extended DoD grant W81XWH-17-1-0454 “GalT-KO Porcine Nerve Xenograft for Reconstruction of Large Nerve Gaps” Role: PI Effort: 1%

	Date: 09/15/17-03/14/20 No impact
Curtis Cetrulo	Change: Received W81XWH-19-1-0437 “Supercooled Ex-Vivo Porcine VCA Preservation to Extend the Timeline Between Procurement and Transplantation and Enable Tolerance Induction to Eliminate Immunotherapy Needs and Risks” Role: PI Effort: 2% Date: 08/01/19-07/31/22 No impact
Curtis Cetrulo	Change: N/A – Retained W911NF-17-1-0360 P00005 “Improving Outcome in Ischemia and Ischemia Reperfusion Injury Using Elemental Reducing Agents” Role: PI, MGH Subaward Effort: 1% Date: 09/01/17-11/30/20 No impact
Curtis Cetrulo	Change: N/A - Retained 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
Curtis Cetrulo	Change: N/A - Retained Musculoskeletal Transplant Foundation grant “Costimulation Blockade-Based Regimens of Mixed Chimerism to Overcome Split Tolerance in VCA” Role: PI Effort 10% Date: 08/01/17-07/31/20 No impact

What other organizations were involved as partners?

If there is nothing significant to report during this reporting period, state “Nothing to Report.”

Describe partner organizations – academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or domestic) – that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed.

Provide the following information for each partnership:

Organization Name:

Location of Organization: (if foreign location list country)

Partner’s contribution to the project (identify one or more)

- *Financial support;*
- *In-kind support (e.g., partner makes software, computers, equipment, etc., available to project staff);*
- *Facilities (e.g., project staff use the partner’s facilities for project activities);*

- *Collaboration (e.g., partner's staff work with project staff on the project);*
- *Personnel exchanges (e.g., project staff and/or partner's staff use each other's facilities, work at each other's site); and*
- *Other.*

Nothing to Report

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: For collaborative awards, independent reports are required from BOTH the Initiating PI and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to <https://ers.amedd.army.mil> for each unique award.

QUAD CHARTS: If applicable, the Quad Chart (available on <https://www.usamraa.army.mil>) should be updated and submitted with attachments.

9. **APPENDICES:** Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.

None

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

Log Number: RT150065

Award Number: W81XWH-16-1-0702

PI: Curtis L. Cetrulo, Jr., M.D., FACS

Org: Massachusetts General Hospital

Award Amount: \$449,995

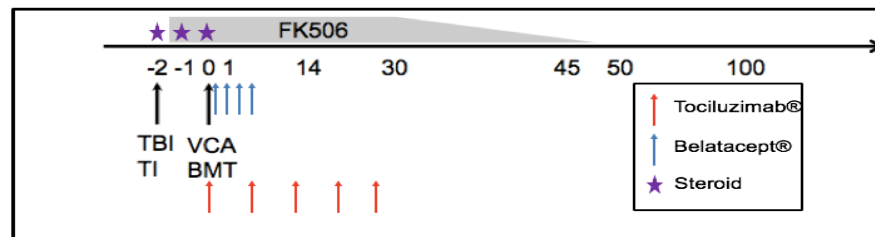


Study/Product Aim(s)

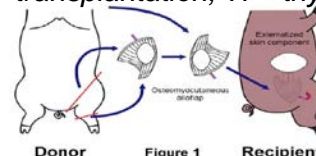
- To achieve proof-of-concept of the day 0 induction protocol in generating durable mixed chimerism for immunosuppression-free VCA tolerance and survival in swine
- To investigate the role of difference MHC class mismatches in VCA

Approach

We will modify our previously successful tolerance induction protocol using CM-PBMC into a clinically-relevant, mixed chimerism approach by bone marrow transplantation across various MHC barriers to mirror the clinical challenges of MHC matching for donor-recipient pairs in VCA.



Tolerance induction protocol in swine. Tolerance induction protocol based on bone marrow transplantation. *FK506* = Tacrolimus, *TCR* = T cell receptor, *VCA* = vascularized composite allotransplantation, *TBI* = total body irradiation, *BMT* = bone marrow transplantation, *TI* = thymus irradiation



No sign of rejection after more than 200 days off immunosuppression

Timeline and Cost

Activities	CY	16	17	18	19	20
Perform VCA in class I mismatch recipients					■	■
Perform VCA in full MHC mismatch					■	■
Investigate chimerism, VCA survival, complications, <i>in vitro</i> immune status					■	■
Complete analysis, prepare manuscript for submission					■	■
Estimated Budget (\$K)		\$9	\$58	\$99	\$206	\$78

Goals/Milestones

CY16 Goals

- ✓ Obtain IACUC and ACURO approval
- ✓ Establish induction protocol for immunosuppression-free VCA tolerance in full mismatch swine

CY17 Goal

- ✓ Validate induction protocol across various MHC mismatches
- ✓ IACUC and ACURO approved

CY18-19 Goal

- ✓ Pairs identified for surgeries
- Identify possible mechanisms behind VCA acceptance or rejection in the context of different MHC mismatches

Comments/Challenges/Issues/Concerns

- Belatacept® obtained. Abatacept stopped due to PTLD

Budget Expenditure to Date

Projected Expenditure: \$358,322

Actual Expenditure: \$358,322