**AWARD NUMBER:** W81XWH-17-1-0454

**TITLE:** GalT-KO Porcine Nerve Xenograft for Reconstruction of Large Nerve Gaps

**PRINCIPAL INVESTIGATOR:** Curtis L. Cetrulo Jr, MD

**RECIPIENT:** Massachusetts General Hospital
**Boston, MA 02114**

**REPORT DATE:** October 2018

**TYPE OF REPORT:** Annual

**PREPARED FOR:** U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

# REPORT DOCUMENTATION PAGE

*Form Approved*
*OMB No. 0704-0188*



| **1. REPORT DATE** | **2. REPORT TYPE** | **3. DATES COVERED** |
|---|---|---|
| October 2018 | Annual | 15 Sept 2017 – 14 Sept 2018 |

**4. TITLE AND SUBTITLE**
GalT-KO Porcine Nerve Xenograft for Reconstruction of Large Nerve Gaps

**5a. CONTRACT NUMBER**

**5b. GRANT NUMBER**
W81XWH-17-1-0454

**5c. PROGRAM ELEMENT NUMBER**

**6. AUTHOR(S)**
Curtis Cetrulo, Jr, MD

**5d. PROJECT NUMBER**

**5e. TASK NUMBER**

**5f. WORK UNIT NUMBER**

**E-Mail:** ccetrulo@partners.org

**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**

**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)**

**11. SPONSOR/MONITOR'S REPORT NUMBER(S)**

**12. DISTRIBUTION / AVAILABILITY STATEMENT**
Approved for Public Release; Distribution Unlimited

**13. SUPPLEMENTARY NOTES**

## 14. ABSTRACT

Injuries to peripheral nerves can be a devastating component of a traumatic military injury. We have developed a herd of genetically modified swine (GalT-knockout) whose tissues are able to avoid hyperacute rejection, and are immunologically similar to allografts. Nerve allografts and xenografts both require some immunosuppressive therapy, however this requirement is temporary as autologous cells migrate into the grafts over time, replacing donor cells. Conveniently, treatment with the immunosuppressant FK506 has been shown to independently promote nerve regeneration. This study will compare functional recovery after nerve gap reconstruction using xenograft vs. autograft with FK506 immunosuppression in a nonhuman primate model. Pursuing this project will advance a promising new option for nerve reconstruction in a high quality model of functional outcome, and will result in a technology poised for subsequent clinical investigation.

**15. SUBJECT TERMS**
peripheral nerve regeneration, xenotransplantation, immunosuppression, tacrolimus, FK506, nerve allograft, preclinical, transplant, nonhuman primate model

| **16. SECURITY CLASSIFICATION OF:** | | | **17. LIMITATION OF ABSTRACT** | **18. NUMBER OF PAGES** | **19a. NAME OF RESPONSIBLE PERSON** |
|---|---|---|---|---|---|
| **a. REPORT** | **b. ABSTRACT** | **c. THIS PAGE** | | | **19b. TELEPHONE NUMBER** *(include area code)* |
| Unclassified | Unclassified | Unclassified | Unclassified | 12 | USAMRMC |

## TABLE OF CONTENTS

## INTRODUCTION:

In high-energy trauma such as motor vehicle collision or ballistic trauma, there is often lost or devitalized tissue and a resulting nerve gap. There are several graft and conduit products that are effective for reconstruction of small nerve gaps, but ineffective in larger gaps (>3cm). The gold standard for reconstructing large nerve gaps is autograft, or expendable nerves harvested from the patient's own body. They contain viable Schwann Cells, which promote nerve regeneration. Other currently available products are either synthetic or lack cells, and do not support regeneration to the same degree. The use of allograft, grafts harvested from human cadavers, has been investigated with some success, but availability and ethical concerns related to sourcing of human tissues discourage their use.

Because no nerve reconstruction product provides efficacy comparable to autografting for long nerve defects, and the amount of autologous nerve available for use as a graft material is often insufficient, functional outcomes for these injuries are generally poor. Unfortunately, injuries sustained by soldiers at war are skewed toward the high-energy trauma mechanisms that result in large nerve gaps, often with multiple major nerves affected. In these cases autografts are vastly inadequate in number, leaving members of the military disproportionately affected by poor outcomes from peripheral nerve injury and the currently available autograft- alternatives. While development of improved nerve reconstruction options will benefit anyone with a major nerve injury, the severe nerve injuries in members of the military place them in a particular position to benefit.

One possible solution lies in the field of xenotransplantation, using tissues from a nonhuman species. Nerve xenografts have previously been impossible beyond mouse studies because humans and nonhuman primates possess a preformed immune response to a molecule on the cells of other species resulting in hyperacute rejection when xenogeneic tissues are transplanted. We have developed a herd of genetically modified swine (GalT-knockout) whose tissues are able to avoid hyperacute rejection, and are immunologically similar to allografts. Nerve allografts and xenografts both require some immunosuppressive therapy, however this requirement is temporary as autologous cells migrate into the grafts over time, replacing donor cells. Conveniently, treatment with the immunosuppressant FK506 has been shown to independently promote nerve regeneration. Additionally, we have already completed proof-of-concept studies using skin from GalT-knockout swine for wound coverage in nonhuman primates, which performed equivalently to allogeneic skin grafts, and for which we are preparing to begin a clinical trial.

Based on this progress, this study will compare functional recovery after nerve gap reconstruction using xenograft vs. autograft with FK506 immunosuppression in a nonhuman primate model. It will also examine the effect of removing immunosuppression at the 6 month time point on recovery and graft survival. While graft rejection after withdrawal of immunosuppression is not anticipated based on small animal data, it is an important risk to characterize further. Pursuing this project will advance a promising new option for nerve reconstruction in a high quality model of functional outcome, and will result in a technology poised for subsequent clinical investigation. Following a clinical trial for GalT-KO skin grafts from this herd in 2017, along with successful completion of this and other final preclinical investigations in 2019, we plan for human clinical trials for xenogeneic nerve grafts to begin as soon as 2020.

**KEYWORDS:** peripheral nerve regeneration, xenotransplantation, immunosuppression, tacrolimus, FK506, nerve allograft, preclinical, transplant, nonhuman primate model

**ACCOMPLISHMENTS:**

1. The major accomplishment of this period of the grant was the development and implementation of Good Laboratory Practice protocols for this project for preparation to implement the study in a GLP facility (Biomere). This plan will allow the data acquired to be immediately "FDA-ready" and will be implemented into an IND application for this technology to be tested in clinical trials. We will save years on development costs with this approach and we believe it will facilitate getting our nerve grafts into wounded warriors much sooner than a traditional non-GLP approach. This strategy was discussed and approved of by our Program Officers and we are now ready to finish the study under GLP conditions.

**What were the major goals of the project?**

Based on the progress we have made in the field of xenotransplantation, our previous work leading to imminent human clinical trials for GalT-KO skin grafts from this herd, which opens the door for further development of xenotransplantation products, and the persistent need for a high quality alternative to autologous nerve graft for peripheral nerve reconstruction, we plan to begin preclinical investigation of cryopreserved GalT-KO nerve xenografts in a nonhuman primate model as described below.

**Specific Aims and Hypotheses**.

***Specific Aim 1:*** The primary aim of this study is to evaluate of the effectiveness of the GalT-KO xenogeneic nerve grafts for reconstruction of long segmental nerve gaps in non-human primates based on functional outcome. We also plan to collect electrophysiologic data and perform histological analysis on biopsies of the graft material to corroborate the functional outcome data. *Hypotheses*: - GalT-KO porcine nerve xenografts will perform in non-inferior fashion to non-human primate autografts on the basis of functional recovery at 6 months when used for bridging of long (4 cm) radial nerve gaps. - Electrophysiological and histological data will corroborate functional data, indicating that GalT-KO porcine nerve xenografts perform non-inferiorly to autografts in this model

***Specific Aim 2:*** The secondary aim of this study is to evaluate the effect on functional recovery of withdrawing immunosuppression after axonal regeneration through the graft is complete. The hypotheses for investigation of these aims is as follows: *Hypothesis*: Removal of immunosuppression at 6 months post-reconstruction will not result in loss of functional recovery at 9 months post-reconstruction.

**Study Design:** 10 NHP will receive bilateral 4cm radial nerve defects. For each, one will be reconstructed using xenograft and one using autograft as control. All NHP will receive FK506 immunosuppression for 6 months. At 6 months, recovery of radial nerve motor function (wrist extension) will be assessed for both groups. Group 1 will also undergo electrophysiological

(EP) testing and graft harvest for histological assessment. For Group 2, FK506 will be withdrawn and motor recovery will be followed periodically for 3 months. At 9 months, they will undergo EP testing and graft harvest for histological assessment. Prior studies in this model showed acellular allograft, a clinically acceptable nerve graft material, resulted in approximately 75% worse motor recovery. With this in mind, we set the noninferiority limit for the primary outcome to be 35% different from the autograft control group, and the power of the study at 90%.

**SOW:**

| **Specific Aim 1** | **Timeline** | **Site** |
|---|---|---|
| **Major Task 1** | Months | |
| Subtask 1 – Obtain IACUC/ACURO approvals | 1-3 | MGH |
| Subtask 2 – Identify NHP and assign to Group 1 or 2 | 3-9 | MGH |
| Subtask 3 – Begin Surgical Procedures | 4-12 | MGH |
| Subtask 4 – Assess functional recovery monthly | 5-12 | MGH |
| Subtask 5 – For Group 1, perform EP testing and graft harvest for histology 6 months postoperatively | 10-12 | MGH |
| Milestone(s) Achieved: Completion of Year 1 work | 12 | MGH |
| **Specific Aim 2** | | |
| **Major Task 2** | | |
| Subtask 1 – Complete remaining surgical procedures | 12-15 | MGH |
| Subtask 2 – Continue assessment of functional recovery | 12-21 | MGH |
| Subtask 3 – For Group 1, perform EP testing and graft harvest for histology 6 months postoperatively. | 12-21 | MGH |
| Milestone(s) Achieved: Completion of Work for Specific Aim 1 | 21 | MGH |
| Milestone(s) Achieved: Project complete, prepare manuscript write-up | 35-36 | MGH |
| **Specific Aim 2**<br>**Specific Aim 2 begins at 6 months postoperatively** | **Timeline** | **Site** |
| **Major Task 1** | Months | |
| Subtask 1 – Continue Surgical Procedures and assessment as per Specific Aim 1. | 10-12 | MGH |
| Subtask 2 – Identify NHP assigned to Group 2 | 10-12 | MGH |
| Subtask 3 – For NHP in Group 2, withdraw FK506 immunosuppression at 6 months postoperatively | 10-12 | MGH |
| Milestone(s) Achieved: Completion of Year 1 | 12 | MGH |

| | | |
|---|---|---|
| work | | |
| **Specific Aim 2** | | |
| **Major Task 2** | | |
| Subtask 1 – Complete remaining surgical procedures | 12-15 | MGH |
| Subtask 2 – Continue assessment of functional recovery | 12-24 | MGH |
| Subtask 3 – For Group 2, perform EP testing and graft harvest for histology 9 months postoperatively. | 13-24 | MGH |
| Milestone(s) Achieved: Completion of Work for Specific Aim 2 | 24 | MGH |
| Milestone(s) Achieved: Project complete, prepare manuscript write-up | 24 | MGH |

- IACUC: Institutional Animal Care and Use Committee
- ACURO: Animal Care and Use Review Office (of the US Army Medical Research and Materiel Command)
- NHP: Nonhuman Primate
- EP: Electrophysiologic

### 3.2 What was accomplished under these goals?

**Major Activities**

Major activities: development and implementation of Good Laboratory Practice protocol for this project for preparation to implement the study in a GLP facility (Biomere). We are prepared to operate on 5 of the 10 monkeys in mid-Novemebr 2018 at Biomere. Biomere's IACUC approval has been obtained and we are currently awaiting ACURO approval. This strategy was discussed and approved of by our Program Officers and we are now ready to finish the study under GLP conditions.

We have designed a protocol that is ready for FDA IND submission upon completion. We learned from previous experience with GalT-KO skin experiments - after completion of studies at MGH and publication, we were required to repeat many of these studies at a GLP laboratory before the data could count toward an FDA IND application. In order not to repeat this process, we have elected to perform the initial studies of this project under GLP conditions.

**Specific Objectives**

Development and implementation of Good Laboratory Practice- approved protocols

**Significant results/key outcomes**

**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?**
The goals of the next reporting period are to complete the *in vivo* xenograft and allograft nerve experimenst in NHPs at Biomere under GLP conditions.

**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:**

**Actual or anticipated problems or delays and actions or plans to resolve them.**
We obtained timely IACUC/ACURO approval of our original protocols from MGH. However, in order to move the project to Biomere- for GLP standards- we resubmitted to Biomere's IACUC and then to ACURO a second time. We are currently awaiting ACURO approval of Biomere's IACUC-approved protocol. A vendor agreement needed to be arranged between MGH and Biomere, which resulted in some delays, however, we are on track for completion of all surgeries by 1/1/2019. Nerve outcomes will then be reported Spring 2019 as the animals are followed over the ensuing winter months.

**Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents.**
Nothing to report.

**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."

Nothing to report.

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

Nothing to report.

**Other publications, conference papers, and presentations**.

**Local Invited 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**

**Table 2: Personnel efforts and person month worked.**

| Name | Project Role | Person month worked | Contribution to the project |
|---|---|---|---|
| Curtis Cetrulo | PI | 0.12 | Overall design and direction of proposed studies, interpretation of results |
| Josef Kurtz | Investigator | 2.75 | Assessment of transplant recipients, supervision of work performed by research fellow, assists with interpretation of results. |
| Mark Randolph | Investigator | 0.42 | Assessment of transplant recipients, supervision of work performed by research fellow, assists with interpretation of results. |
| Alexandre Lellouch | Research Fellow | 1.1 | Assist in surgical procedures, analyses of immune responses, 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<br>Role: PI<br>Effort: N/A<br>Date: 12/15/16-05/31/18<br>No impact |
| Curtis Cetrulo | Change: Ended Shire HGT, Inc. Sponsored Research Agreement<br>Role: PI<br>Effort: N/A<br>Date: 11/01/16-5/14/18<br>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 VCA Tolerance Protocol"<br>Role: PI<br>Effort: 3%<br>Date: 09/15/15-09/14/19<br>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"<br>Role: PI<br>Effort: 2%<br>Date: 09/15/16-09/14/19<br>No impact |
| Curtis Cetrulo | Change: Ended DoD grant W81XWH-13-2-0062 "Tolerance in Nonhuman Primates by Delayed Mixed Chimerism"<br>Role: PI<br>Effort: 1%<br>Date: 09/15/13-09/14/17<br>No impact |
| Curtis Cetrulo | Change: Ended DoD grant W81XWH-13-2-0060 "Immunomodulation Tolerance Induction after VCA Using Biologic Agents (CTLA4-IG) and Donor BM Cells"<br>Role: PI- subcontract<br>Effort: 1%<br>Date: 09/15/13-09/14/17<br>No impact |
| Curtis Cetrulo | Change: Ended DoD grant W81XWH-12-2-0037-P00003 "A Novel Protocol for Upper Extremity Restoration by Transplantation with Intent for Tolerance Induction"<br>Role: PI- subcontract<br>Effort: 1%<br>Date: 09/30/12-09/29/17<br>No impact |

| | |
|---|---|
| Curtis Cetrulo | Change: Ended DoD grant W81XWH-13-2-0053 "Towards a Preclinical Large Animal Tolerance Protocol for Vascularized Composite Allotransplantation in Swine"<br>Role: PI- subcontract<br>Effort: 1.5%<br>Date: 09/18/13-09/17/17<br>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"<br>Role: PI<br>Effort 10%<br>Date: 01/01/18-12/31/20<br>No impact |
| Curtis Cetrulo | Change: Received DoD/Fred Hutchinson Cancer Research Center grant W911NF-17-1-0360 "Improving Outcome in Ischemia and Ischemia Reperfusion Injury Using Elemental Reducing Agents"<br>Role: PI (Subaward)<br>Effort 1%<br>Date: 09/01/17-11/30/20<br>No impact |

**What other organizations were involved as partners?**
Nothing to report.

## 8. SPECIAL REPORTING REQUIREMENTS

Nothing to report.

**QUAD CHARTS:** See attached

# GalT-KO Porcine Nerve Xenograft for Reconstruction of Large Nerve Gaps

Log No.: OR160211
W81XWH-17-1-0454

**PI:** Curtis L. Cetrulo, Jr., M.D., FACS **Org:** Massachusetts General Hospital **Award Amount:** $500,000

## Study/Product Aim(s)

1) Demonstrate the efficacy of GalT-KO porcine nerve xenograft based on functional outcome compared to historical autograft control
   - Correlate functional outcome data with elecrophysiological (EP) studies and histological analysis of grafts for regeneration/rejection
2) Investigate effect of immunosuppression withdrawal on recovery

## Approach

This study includes 10 NHP. Each receives a 4cm radial nerve defect, reconstructed with xenograft

Each NHP will receive immunosuppression via systemic FK506, which independently improves nerve regeneration

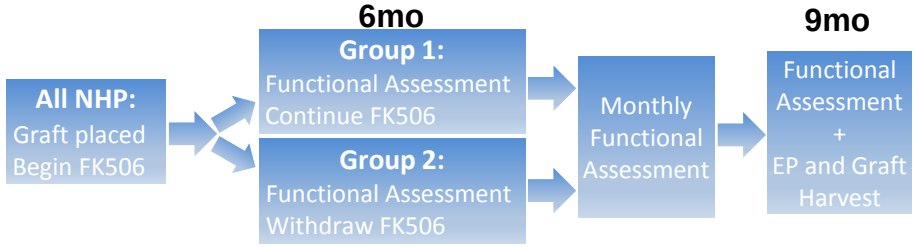
Functional recovery is the primary outcome. This is measured directly, as, while important, it does not always correlate with other endpoints

**Aim 1**: Functional assessment of both groups (n=10) at 6mo

**Aim 2**: In Group 2, withdraw FK506 at 6mo. Monthly functional analysis for 3 further months.

**Both Aims:** EP testing and graft harvest for histology at 9mo.

**Study Design** 10 NHP will have radial nerve injury repaired with xenograft. All will receive FK506 and be treated identically for the first 6 months. At six months, both groups will undergo functional assessment. At that time, *Group 1* will continue with standard FK therapy, while in *Group 2*, FK506 will be withdrawn. All animals will be monitored 3 additional months to assess durability of recovery without immunosuppression. At 9mo, both groups will undergo EP studies, and the grafts will be harvested for histology.




Accomplishment: We recently obtained IACUC/ACURO approval for this study. Previously, we successfully demonstrated proof-of-concept using GalT-KO skin grafts, with which we have completed preclinical testing and are preparing for human trials for the application of severe burn coverage.

## Timeline and Cost

| Activities CY | 17 | 18 | 19 | 20 |
|---|---|---|---|---|
| Nonhuman Primate Purchase | | | | |
| Surgical procedures for all animals | | | | |
| Follow up for Functional outcome | | | | |
| Histology and Electrophysiological Studies | | | | |
| **Estimated Budget ($K)** | **$18** | **$45** | **$437** | |

**Updated:** 10/14/2018

**Goals/Milestones**

**Pre-Study Goal**– Proof of concept

☑ Demonstrate efficacy of other GalT-KO xenograft products (skin)

**CY18 Goals** –

☐ Obtain IACUC/ACURO approval
☐ Begin surgical procedures (nerve grafting) for both groups
☐ Begin post-operative functional monitoring at 1mo

**CY19 Goals** –

☐ Complete surgical procedures for both groups
☐ Complete 6mo functional testing for all animals
☐ Begin EP analysis and harvest of graft material for histological analysis
☐ For Group 2, withdraw FK506 at 6mo

**CY20 Goal** –

☐ Complete functional monitoring, EP and histologic analysis by 2nd Quarter of 2020

**Comments/Challenges/Issues/Concerns**

**Budget Expenditure to Date:**

Projected Expenditure: $59,383
Actual Expenditure: $59,383