

AWARD NUMBER: W81XWH-15-1-0281

TITLE: Local Tacrolimus (FK506) Delivery for Prevention of Acute Rejection in the Non-Human Primate Delayed Mixed Chimerism Vascularized Composite Allograft Tolerance Induction Protocol

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Fort Detrick, Maryland 21702-5012

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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						Rutgers, The State University of New Jersey 145 Bevier Road, Piscataway, NJ 08854-8087		
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14. ABSTRACT The focus for Year 3 was to study the controlled release of tacrolimus (FK506) from a polymeric local delivery system (PLDS) both <i>in vitro</i> and <i>in vivo</i> and the stability of the devices in storage. In the <i>in vivo</i> studies, the PLDS released a large amount of tacrolimus, a first in the field which often suffers from low levels of release. Laminated PLDS devices were developed to reduce the initial burst of drug <i>in vivo</i> while improving the controlled release profile. A subcutaneous rat model was initiated to efficiently characterize the <i>in vivo</i> release profile of the reformulated devices, which now show burst-free controlled release over 7 days <i>in vivo</i> . The release profile observed <i>in vivo</i> correlates to prior <i>in vitro</i> results which is beneficial for screening devices in the future. Long-term storage of the devices leads to a reduction in the release rate of the drug through loss of the molecular dispersion within the film. This helps control the early release rate and provide a more consistent release over time.								
15. SUBJECT TERMS Drug delivery, immunosuppression, tacrolimus, FK506, vascularized composite allografts, immune rejection, preclinical, transplant, nonhuman primate model, degradable polymer, tyrosine-derived polycarbonate								
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1. INTRODUCTION: The purpose of this research is to develop an intraoperative, implantable, biomaterials-based, controlled release system for the local delivery of tacrolimus (a potent immunosuppressive drug) to prevent acute rejection episodes of vascularized composite allografts (VCAs) in non-human primates until delayed mixed chimerism can be established and subsequent withdrawal of immunosuppression can be safely performed. VCA provides the opportunity to restore complex anatomical and functional units, such as the hands or face, following devastating injury. These procedures have the potential to revolutionize the treatment of wounded warriors with extremity amputations and severe craniofacial injuries. However, minimization of immunosuppression and/or induction of tolerance represent the major impediment to widespread application of VCA procedures. In this work, a polymer-based device loaded with tacrolimus will be tested and analyzed using *in vitro* and *in vivo* techniques. *In vivo* models include a non-human primate model, which will be used for studies of VCA immunosuppression and development of tolerance induction protocols. These studies, if successfully completed, will allow us to initiate enabling studies required for a regulatory application.

2. KEYWORDS: Drug delivery, immunosuppression, tacrolimus, FK506, vascularized composite allografts, immune rejection, preclinical, transplant, nonhuman primate model, degradable polymer, tyrosine-derived polycarbonate

3. ACCOMPLISHMENTS:

Our overall objective is to demonstrate the *in vivo* safety and efficacy of the local site-specific administration of tacrolimus via an implantable polymeric local delivery system (PLDS) that will enable the reduction of systemic immunosuppression levels and avoid immunosuppression-related morbidity and rejection episodes that result in either graft loss or sensitization to donor bone marrow. The hypothesis is that the application of a PLDS for the controlled and sustained release of tacrolimus directly at the host-donor skin interface of the vascularized composite allotransplant (VCA) will prevent acute rejection episodes, reduce the need for systemic immunosuppression, and serve as a bridge to the induction of immunologic tolerance by delayed mixed chimerism. The project's specific aims are (1) to develop and characterize PLDS for tacrolimus and profile the *in vivo* release kinetics and local tissue distribution of tacrolimus in a small animal model; (2) optimize the adjunctive tacrolimus implant dose necessary for the reduction of systemic immunosuppression for upper extremity allotransplantation in a nonhuman primate (NHP) model; and (3) provide adjunctive local immunosuppression with the tacrolimus implant as a bridge to tolerance induction by delayed mixed chimerism for upper extremity allotransplantation in an NHP model. The study design builds upon a substantial amount of preliminary data on the development of a controlled release system for the local delivery of calcineurin inhibitors (CNIs), and its completion will enable the initiation of investigational new drug-enabling studies with the belief that this technology will smoothly segue into clinical trials and be translated into a revolutionary immunosuppressive therapy directly beneficial to the success of currently ongoing clinical VCA tolerance protocol for hand transplantation.

What were the major goals of the project?

Specific Aim 1: To develop and characterize Polymeric Local Delivery Systems for tacrolimus (FK506) and profile the in vivo pharmacokinetics of tacrolimus in a small animal model.

Specific Aim 2: To optimize the tacrolimus implant + systemic immunosuppression dose regimen necessary for rejection-free, infection-free facial allotransplantation in a non-human primate (NHP) model.

Specific Aim 3: To provide adjunctive local immunosuppression with the tacrolimus implant as a bridge to tolerance induction by delayed mixed chimerism for facial allotransplantation in a non-human primate model.

Table 1. Statement of Work for MGH

Task	Timeline (months)	Comments
Task 1. To develop and characterize Polymeric Local Delivery Systems for Tacrolimus (FK506) and profile the in vivo pharmacokinetics of tacrolimus in a small animal model	1-36	
1.1. Formulate polymeric local delivery systems (PLDS) for tacrolimus and characterize in vitro.	1-36	
1.1.1. Polymer synthesis and characterizations	1	100% complete
1.1.2. Investigate thermal stability of tacrolimus in the presence of polymer	8-15	100% complete
1.1.3. Fabricate tacrolimus-loaded polymeric local delivery system (tacrolimus-PLDS) devices	2-27	100% complete
1.1.4. Investigate the irradiation stability of tacrolimus-PLDS	18-36	100% complete
1.1.5. Perform in vitro polymer degradation and release study of tacrolimus	12-36	100% complete
1.2. Demonstrate in vivo safety and profile the in vivo release and local tissue distribution of tacrolimus from PLDS in a small animal model.	1-36	
1.2.1. Obtain IACUC and ACURO approval of animal subcutaneous implantation protocol	1-3	100% complete
1.2.2. Cohort 1 - Demonstrate in vivo safety of tacrolimus (15wt%) loaded PLDS for 2 weeks and 3 weeks in a rat subcutaneous model. (Fabrication of devices + 3 weeks animals in life + analyses).	5-16	100% complete
1.2.3. Cohort 2 - Optimize methods for tacrolimus quantification by testing tissue samples of NHP that have been treated with systemic tacrolimus.	16-21	100% complete
1.2.4. Cohort 3 - Optimize and finalize test methods for tacrolimus quantification by testing tissue samples of NHP that have been treated with drug-loaded PLDS.	19-36	100% complete
Task 2. To optimize the tacrolimus implant + systemic immunosuppression dose regimen necessary for rejection-free, infection-free partial facial allotransplantation in a nonhuman primate (NHP) model.	12-27	
2.1. Obtain IACUC and ACURO approvals.	1-6	100% complete
2.2. Fabricate and characterize FK506-PLDS for implantation in	12-27	100% complete

vivo		
2.3. Partial heterotopic face transplants on SIS protocol (n=6).	12-24	100%
2.4. Investigate VCA survival, frequency of rejection and complications, document rejection process clinically and histologically.	12-54	100%
2.5. Summarize optimal immunosuppressive requirements for VCA survival in NHPs. Analyse and summarize data on VCA rejection. Year 1 report.	12-24	100%
Task 3. To provide adjunctive local immunosuppression with the tacrolimus implant as a bridge to tolerance induction by delayed mixed chimerism for partial facial allotransplantation in a non-human primate model.	24-54	
3.1. Delayed tolerance induction protocol, wean immunosuppression.	10-54	75%
3.2. Partial heterotopic face transplants on 2 months SIS (n=4).	24-36	50%
3.3. Investigate chimerism, in vitro immune status, VCA survival outcomes following weaning of immunosuppression.	24-36	75%
3.4. Fabricate and characterize FK506-PLDS for implantation in vivo	36	100%
3.5. Summarize preliminary data/progress on DTIP transplants for inclusion in annual report		75%

3.2 What was accomplished under these goals?

Year 4 MGH

In group 3, we transplanted (heterotopic partial face transplant) 2 recipients NHP; M8318 and M8418. This report describes the follow-up postoperatively. M8318 was transplanted on 2/19/19 (donor M8218). The surgery was uneventful. Tacrolimus was injected IM daily at the dose of 0.1mg/kg from POD 0 to POD 3. The rest of the immunosuppressive regimen was started as well (ATGAM, MMF, methylprednisolone), and Heparin was administered the first week. The tacrolimus level in the peripheral blood was at 79.1ng/mL on POD 3 when the injections were stopped. On POD 5, an erythema started on the VCA associated with a leukocytosis (11.2G/L), that was treated by administration of Ceftriaxone 50mg/kg IM in the hypothesis of a cutaneous infection (after discussion with the veterinarian staff). Under this antibiotic course, the leukocytosis decreased, and the erythema decreased homogenously but remained at the distal part of the flap. The bacteriological samples came back positive for Enterobacter Cloacae and MRSA. Decision was made to treat by Vancomycin IV infusion every other day until CBC became normal. After 3 IV infusion (20mg/kg), the flap was looking totally healthy, and the WBC went back to a normal range (6.8G/L). The skin biopsy taken during the erythema episode showed no sign of acute rejection according to the Banff scale. On POD 10, Tacrolimus injection were started again at 0.1mg/kg because of tacrolimus blood levels continuously decreasing from 79.1ng/mL (POD 3) to 26.6ng/mL (POD 10).

Of note, he continuously lost weight during the post-operative period despite high protein diet (4.5kg to 4.14kg), then stabilized on POD 29 and went back to 4.46kg on POD 50, helped by oral dronabinol 2.5mg daily. He received total body irradiation (TBI 1.5Gy) on POD 57 and 58, and thymic irradiation on POD 62. Bone marrow transplant on POD 63 was uneventful and 1.023×10^9 viable cells were infused in the OR under monitoring. After that, the weight dropped

again to less than 4 kg. Prophylactic antibiotics (Baytril) were started because of leucopenia. On POD 72, he started gaining weight again. On POD 77, he was sedated for IV infusion of anti-IL6R. After sedation he showed poor recovery, his CBC show 37.4G/L leucocytes and the decision was taken with the vets to euthanize him in front of this suspicion of post-transplant lymphoproliferative disorder (PTLD). The histology confirmed the diagnosis of PTLD and the FACS analysis revealed that it was from recipient origin (Figure 2).

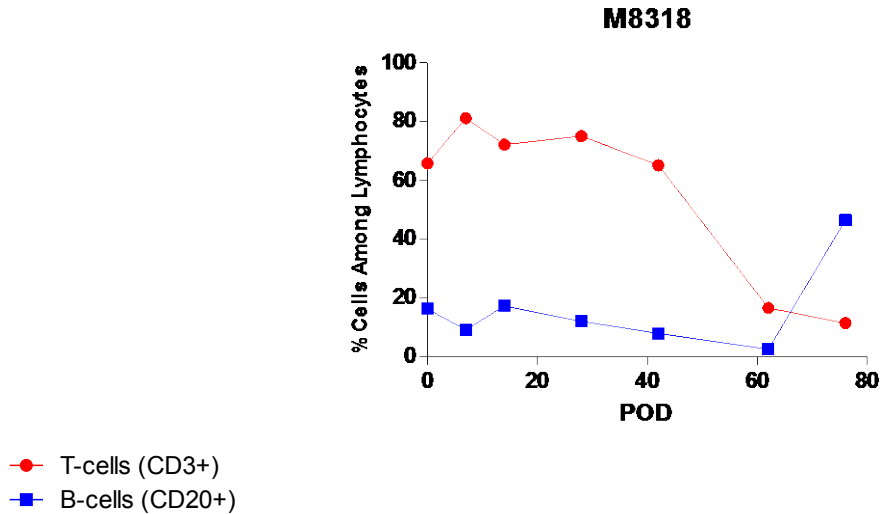


Figure 1: Whole blood analysis of M8318. Graphs represent % of T and B-cells in the lymphocyte gate at each time point. M8318 B-cell increase between POD60-EOS likely PTLD.

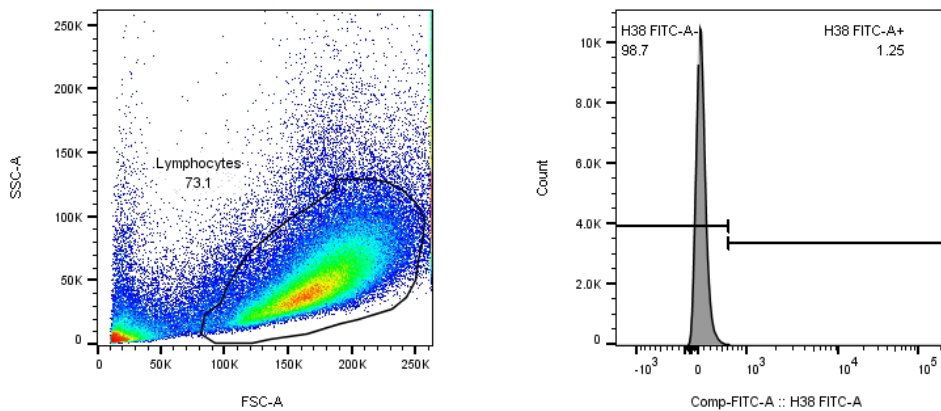


Figure 2: only H38- cells were detected on the bloodstream demonstrating the recipient origin of the PTLD

M8418 was transplanted on the 2/20/19 (donor M8518). The surgery was uneventful. Tacrolimus was injected IM daily at the dose of 0.1mg/kg from POD 0 to POD 3. The rest of

the immunosuppressive regimen was started as well (ATGAM, MMF, methylprednisolone), and Heparin was administered the first week. The tacrolimus level in the peripheral blood was at 112.4ng/mL on POD 3 when the injections were stopped. On POD 4, an erythema was observed on the periphery of the VCA, and then on POD7 the whole flap showed erythema with leukocytosis (19.3G/L) that was treated by Baytril 5mg/kg IM in the hypothesis of a cutaneous infection. On POD 8, a wound dehiscence happened on the distal part of the flap, that was trimmed and sutured with approval from the veterinarians' team. On POD 10 the wound opened again in the same place, we decided to let it heal secondarily. The bacteriological samples came back positive for Staphylococcus sp, decision was made to switch to Trimethoprim 138mg. The erythema disappeared and the wound healed without issue. On POD 14 WBC were normalized. On POD 24, a mild scrotal edema started. Our hypothesis was a side effect of the high tacrolimus level released by the discs. Decision was made to monitor and treat on demand with Furosemide IM. On POD 29, he started looking slower and his WBC increased again to 14G/L, Baytril IM was started again. We discovered a diabetes with glycemia 389mg/dL but no ketones in urines. We started insulin and diabetes diet. After 10 days of antibiotics with still leukocytosis, Ceftriaxone was added. On POD 50, Dronabinol was added to stimulate appetite, and insulin was stopped. During that period of time he significantly lost weight. From POD 50 to POD 60, he started gaining weight. He received total body irradiation (TBI 1.5Gy) on POD 56 and 57, and thymic irradiation on POD 61. Bone marrow transplant on POD 62 was uneventful and 3.21×10^9 viable cells were infused in the OR under monitoring. On POD 65, tacrolimus IM injection were started again because tacrolimus level was dropping under 20ng/mL. After that, the weight dropped again to less than 4 kg. Prophylactic antibiotics (Baytril) were started because of leucopenia on POD 69. On POD 76, he received a whole blood transfusion to treat his anemia (HBG 6.4g/dL). On POD 72, he started gaining weight again. On POD 83, his weight dropped.

On POD 86, sedation planned with the veterinarians for clinical examination and weight assessment. He was still losing weight with a total weight loss of 31% since surgery and NHP looking uncomfortable. Decision was taken to euthanize the monkey. Necropsy showed very unhealthy lungs. The VCA itself contained some lumps. Some centimetric lymph node found in inguinal both sides, mesenteric and para aortic. The blood chimerism analysis showed for a high level of chimerism from POD 60 (Figure 3).

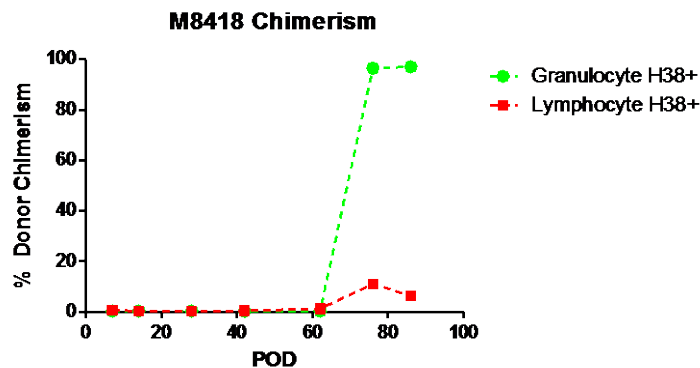


Figure 3: %H38+ donor cells in the granulocyte and lymphocyte gates of M8418 throughout the study. BMT was performed on POD62

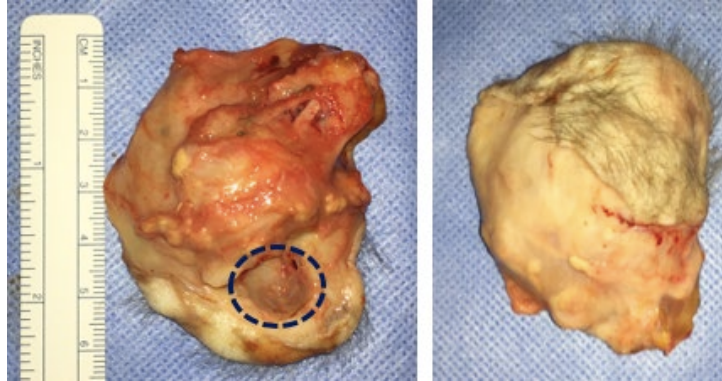


Figure 4: M8418 developed a posttransplant lymphoma disease (PTLD) inside the VCA.
 (left) Posterior view of the VCA (PTLD surrounded by black dashes)
 (right) Anterior view of the VCA



Figure 5: Intestinal location of PTLD (PTLD surrounded by black dashes)

3.3 Specific Objectives

Significant results/ key outcomes

- a) PLDS devices were manufactured, sterilized and stored for future use
 - a. Stability testing was carried out on stored samples finding that release decreased with storage.
 - b. Reduction in release was related to crystallization of the drug within the films and loss of a molecular dispersion.
 - c. A variety of methods were inacted to prevent this from occurring. The most promising being the incorporation of drug-loaded nanospheres into water soluble films.

- b) *In vitro* modelling of the release profile.

- a. The HPLC protocol developed in Year 1 for tacrolimus was used to great effect to quickly and efficiently determine the release profile of the original and modified devices *in vitro* up to 140 days.
 - b. The *in vitro* modelling allowed for the characterization of aged PLDS devices which showed reduced release and prolonged the period of controlled release.
- c) *In vivo* modelling of the release profile.
- a. A subcutaneous rat model was initiated under existing MR141092.01 (IACUC #94-048) to determine the *in vivo* release profile of the devices.
 - b. *In vivo* release was found to correlate to the *in vitro* release profile with a high early release transitioning to a lower late-stage release.

What opportunities for training and professional development has the project provided?
Nothing to report.

How were the results disseminated to communities of interest?

Results were presented as a full-length research talk at the Society for Biomaterials Annual Meeting in Atlanta, Georgia on April 11-14, 2018

Molde, J., Steele, J., Ortiz, O., Iovine, C., Dube, K., Merolli, A. Yang Ng, Z., Certulo Jr., CL., & Kohn, J. Development of Tacrolimus-Loaded Polymeric Delivery System for Localized Immunosuppression. Talk presented at the Society for Biomaterials Annual Meeting; 2018 April 11-14; Atlanta, GA.

Results were presented as a research talk at the 14TH Congress of the international society of vascularized composite allotransplantation ISVCA, October 1st, 2019 at the Main Conference India Exposition Mart, Greater Noida, New Delhi.

Taveau C, Lellouch AG, Andrews AR, Molde J, Ng ZY, Tratnig P, Jonczyk MM, Randolph MA, Kohn J, Cetrulo CL Jr Local FK506 Implant Technology in VCA – Successful Bridge to Delayed Mixed Chimerism 14TH Congress of the international society of vascularized composite allotransplantation ISVCA, October 1st, 2019 at the Main Conference India Exposition Mart, Greater Noida, New Delhi.

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

We planned to perform a third and last heterotopic partial face transplantation in group 3 to complete data on FK release profile *in vivo* and on the delayed tolerance induction.

We have ordered 2 non-human primates (NHPs) and plan to perform the VCA transplantation (group 3, disc + induction of tolerance protocol) on December 9th, 2019.

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.

Storage concerns have been addressed and the project is proceeding.

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.

Molde, J., Steele, J., Ortiz, O., Iovine, C., Dube, K., Merolli, A. Yang Ng, Z., Certulo Jr., CL., & Kohn, J. Development of Tacrolimus-Loaded Polymeric Delivery System for Localized Immunosuppression. Talk presented at the Society for Biomaterials Annual Meeting; 2018 April 11-14; Atlanta, GA.

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.

Massachusetts General Hospital

Name	Project Role	Person month worked	Contribution to the project
Curtis Cetrulo	PI	0.36	Overall design and direction of proposed studies, interpretation of results
Alex Lellouch	Research Fellow	1.4	Assist in surgical procedures, analyses of immune responses, interpretation of results
Alec Andrews	Research Technologist	4	Assistance in pre/post operative animal care and overall study coordination

Rutgers University

Name	Project Role	Person month worked	Contribution to the project
Joachim Kohn	PI	0.60	Administrative and technical oversight of Rutgers team
Sangya Varma	Program Manager	0.30	Grant management
Sanjeeva Murthy	Scientist	0.12	Worked on Polymer design, synthesis & experimental design

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
Current Support Changes for the PI, Co-I or Other Senior/Key Personnel Changes in Current Support	
Joachim Kohn	Change: Extended DoD grant W81XWH-15-C-0043 “IND Filing for Intravenous cP12 and Pre-IND Studies of Intravenous and Topical cNP5 to Limit Burn Injury Progression” Role: PI Effort: 1.5%

	Date: 09/24/15-03/31/20 No impact
Joachim Kohn	Change: Extended DoD grant W81XWH-15-DMRDP-CRM RP “Tissue Engineering Strategies to Maintain Distal Target Efficacy and Promote Full Functional Recovery Following Major Peripheral Nerve Injury” Role: PI Effort: 1.5% Date: 09/30/16-09/29/20 No impact
Joachim Kohn	Change: Extended NSF/DMR grant 1608072 “New Polymeric Biomaterials Inks for 3D Printing” Role: PI Effort: N/A Date: 08/15/16-07/31/20 No impact
Joachim Kohn	Change: Received new NIH award “Rutgers Optimizes Innovation (ROI)” Role: MPI Effort: 7.5% Date: TBD No impact
Joachim Kohn	Change: Received new Rutgers Techadvance award “Discovery and Validation of Polymers meeting Targeted Product Profile Specifications” Role: PI Effort: N/A Date: 12/16/18-12/05/19 No impact
Joachim Kohn	Change: Completed NIH award P41EB001046 “RESBIO III” Role: PI Effort: N/A Date: 06/01/13-05/31/19 No impact
Joachim Kohn	Change: Completed DoD award W81XWH-08-2-0034 “Armed Forces Institute for Regenerative Medicine (AFIRM)” Role: PI Effort: N/A Date: 03/10/08-09/30/18 No impact
Joachim Kohn	Change: Completed Rutgers Techadvance award “Targeted Delivery of Vitamin E acetate to skin using Rutgers TyroSpheres” Role: PI Effort: N/A Date: 08/17/18-08/16/19 No impact

What other organizations were involved as partners?

Nothing to report.

8. SPECIAL REPORTING REQUIREMENTS

Nothing to report.

QUAD CHARTS: Updated and submitted as attachment.

Local Tacrolimus (FK506) Delivery for Prevention of Acute Rejection in the Non-Human Primate Delayed Mixed Chimerism Vascularized Composite Allograft Tolerance Induction Protocol

Log Number: **MR141092**

Award Number: **W81XWH-15-1-0281**



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

(Prime) Org: Massachusetts General Hospital

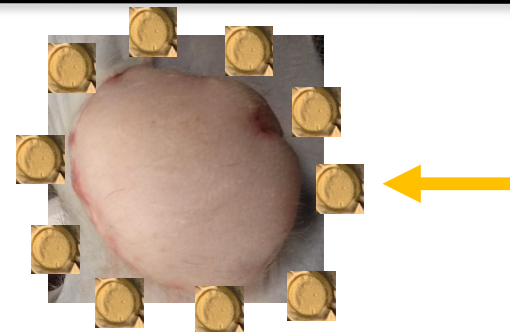
Award Amount: \$1,042,283

Study Aim(s)

- To develop and characterize Polymeric Local Delivery Systems for tacrolimus and profile the in vivo release kinetics and local tissue distribution of tacrolimus in a small animal model (Rutgers)
- To optimize the tacrolimus implant + systemic immunosuppression dose regimen necessary for rejection-free, infection-free heterotopic partial face allotransplantation in a non human primate (NHP) model
- To provide adjunctive local immunosuppression with the tacrolimus implant as a bridge to tolerance induction by delayed mixed chimerism for heterotopic partial face allotransplantation in a NHP model

Approach

By developing an intraoperative, implantable, biomaterials-based, controlled release system for the local administration of tacrolimus into the VCA, the following advantages can be achieved: (1) ensure recipient compliance (2) potential reduction of systemic dose and resultant decrease in risks of systemic side effects and infections (3) localized delivery of the therapeutic at the point of immune cell interaction between donor face and recipient skin immune system. This approach builds upon the preliminary work on device development at Rutgers/NJCB. This technology represents a critical adjunct to the safety profile and efficacy of the delayed tolerance protocol for VCA that is under development at our center at MGH.



Local, skin-specific immunosuppression with subcutaneously placed sustained-release tacrolimus-eluting discs (orange arrow points to schematic representation of subcutaneously placed discs along host-donor suture line).

Accomplishment: Fabrication of FK506-PLDS and in vivo implantation on NHPs

Timeline and Cost

Activities	CY	15	16	17	18-20
To develop and characterize Polymeric Local Delivery Systems for Tacrolimus (FK506) and profile the in vivo pharmacokinetics of tacrolimus in a small animal model		■	■		
To optimize the tacrolimus implant + systemic immunosuppression dose regimen necessary for rejection-free, infection-free VCA in NHPs			■	■	
To provide adjunctive local immunosuppression with the tacrolimus implant as a bridge to delayed tolerance induction of VCA in NHPs					■
Summarize optimal immunosuppressive requirements for VCA survival in NHPs. Analyse and summarize data on VCA rejection.					■
Estimated Budget (\$)		\$3	\$135	\$271	\$333

Goals/Milestones

CY15 Goal – System demonstration

- ✓ Demonstrate in vivo safety and profile the in vivo release and local tissue distribution of tacrolimus from PLDS in a small animal model

CY16 Goals – System validation, Production Readiness

- ✓ Demonstrate in vivo safety and profile the in vivo release and local tissue distribution of tacrolimus from PLDS for 2 months.
- ✓ Fabricate and characterize FK506-PLDS for implantation in vivo

CY17 Goal – Product Testing

- ✓ Investigate VCA survival, frequency of rejection and complications, document rejection process clinically and histologically

CY18-19 Goal – Suitability testing

- ✓ Test new tacrolimus disc for group 3 (tolerance induction protocol)
- ✓ Investigate chimerism, in vitro immune status, VCA survival

Comments/Challenges/Issues/Concerns

- ✓ Monitoring of the tacrolimus level

Budget Expenditure to Date

Projected Expenditure: \$959,595

Actual Expenditure: \$959,595