AWARD NUMBER: W81XWH-22-1-0959

TITLE: Stable Mixed Chimerism and Tolerance of Vascularized Composite Allografts via Bcl-2 Inhibition

PRINCIPAL INVESTIGATOR: Dr. Alexandre Lellouch, MD

CONTRACTING ORGANIZATION: Massachusetts General Hospital, Boston, MA

REPORT DATE: October 2023

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Development 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 2023	Annual	30Sep2022-29Sep2023

4. TITLE AND SUBTITLE

Stable Mixed Chimerism and Tolerance of Vascularized Composite Allografts via Bcl-2 Inhibition

5a. CONTRACT NUMBER
W81XWH-22-1-0959

5b. GRANT NUMBER
RT210047P2

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Dr. Alexandre Lellouch, MD

E-Mail: ALELLOUCH@mgh.harvard.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Massachusetts General Hospital
55 Fruit Street
Boston, MA 02114-2621

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Development 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

The project aims to develop *a clinically applicable nontoxic conditioning regimen with selective Bcl-2 inhibition for induction of mixed chimerism and VCA tolerance.*

In the first year, the focus was on preparing the field (getting the protocols approuved, screening acquiring non-human primates, drugs and reagents) and starting the surgeries .

15. SUBJECT TERMS
Tolerance induction, VCA transplantation, face transplantation, mixed chimerism

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

TABLE OF CONTENTS

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

Vascularized composite allotransplantation (VCA), has revolutionized reconstructive surgery. However, patients require life-long systemic immunosuppression to permit the tissues to survive. But still more than 90% of patients experience acute rejection episodes within the first year that are at risk of developing chronic rejection. Tolerance protocols have been investigated to avoid the need for long-term immunosuppressive drugs. Induction of hematopoietic chimerism by donor bone marrow transplantation is currently the only method reproducibly achieving tolerance in clinical transplantation. Our goal is to develop a nontoxic regimen to induce immune tolerance whereby the patient will not mount an immune reaction to the allograft by generating mixed chimerism.

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

VCA, tolerance, face, mixed chimerism, transplantation, Tregs, BCL2 inhibition

3. **ACCOMPLISHMENTS:** *The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official 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.

1. *Milestone # 1 IACUC, ACURO approval obtained*. **100% complete**
2. *Major task 1:*
   Subtask 1: *Perform transplants in Aim 1.* **30% Complete**
   Subtask 2: *Perform analysis of Aim 1 result:* **30% Complete**
3. *Major Task 2*
   Subtask 1: *Perform in vitro assay in Aim 2:* **Blood samples frozen for future analysis**
   Subtasck 2: *Perform analysis of Aim 2 results.* **Nothing to report**

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) Animal acquisition:

After getting our protocol approved for both delayed and simultaneous protocols, we started screening from the vendor non-human primate candidates for potential donors and recipients. A total of 16 animals were screened from the vendor (Alpha Genesis). At the same time, new personnel accreditation and training, as well as drug acquisition, was achieved.

Figure 1. 16 animals screened for potential donors and recipients.

	Donors					Recipients					
ID Number	Age	Overall appearance	Weight KG	H38	ABO	ID Number	Age	Overall appearance	Weight KG	H38	ABO
MB1342	11,9	3/13/22 excellent	7,25	+	AB	BC2010	7,4	8/18/22 excellent	7,82	-	A
MB1379	10,9	3/9/23 Good	7,69	+	A	FR2490	9,3	3/9/23 excellent	7,50	-	AB
MB1937	7,8	Physical exam not available	7,18	+	AB	**FR2499**	9,1	3/9/23 Good	8,16	-	A
MB2190	9,10	10/12/22 Excellent	8,42	+	AB	FR2718	8,9	10/12/22 Excellent	7,54	-	A
NR872	10,0	10/12/22 Excellent	7,31	+	AB	**UG2893**	10,3	3/9/23 Good	8,52	-	AB
NR970	9,10	10/12/22 Excellent	7,96	+	B	NV1169	9,6	3/13/22 excellent	7,33	-	AB
NV1303	7,5	08/18/22 Excellent	7,93	+	B	MB2793	7,11	3/ç/22 good	7,80	-	AB
NV1491	9,7	10/12/22 Excellent	8,21	+	AB						
UG3195	9,10	10/12/22 Excellent	7,37	+	B						

This screening included (Figure 1):

- Checking veterinary animal records
- H38 status to define donors (H38+) and potential recipients (H38-).
- ABO groups for blood compatibility between donors and recipients.
- MHC screening to define pairs of monkeys based on their compatibility (either full MHC mismatch or MHC haplo-matched)

Figure 2. Animals selected based on their H38, ABO, and MHC status. By convention, and to allow chimerism data interpretation, all donors were selected H38+ and recipients H38-. Recipients were choosed to be universal blood recipients (ABO group AB) so they could be matched with all potential donors to create pairs with either full MHC mismatch or haplomatch.

	Donors				Recipients				
Animal n°	H38	ABO	MHC HAPLO1	MHC HAPLO2	Animal n°	H38	ABO	MHC HAPLO1	MHC HAPLO2
6923 (MB2190) 9.85kg	+	AB	**M1**	**M4**	**7523 (NV1169) 7.65 kg**	-	AB	**M6**	**recM6M3**
7023 (NV1303) 8.15 kg	+	B	**M2**	**M5**	**7423 (UG2893) 7.8kg**	-	AB	**M1**	**recM3M2**
7123 (NV1491) 8.95 kg	+	AB	**M3**	**recM5M4**	**7323 (FR2490) 7.95**	-	AB	**M3**	**M6**
7223 (UG3195) 7.85kg	+	B	**M3**	**recM4M6**	**7623 (MB2793) 7.9kg**	-	AB	**M1**	**M2**

2) Blood draws for Peripheral Bone Marrow Cell (PBMC) isolation and banking:

All donors and recipients scheduled for surgery underwent multiple blood draws to bank peripheral bone marrow cells (PBMC) and serum. This is crucial for later in vitro assays and fulfilling Major Task 2. Each donor and recipient underwent a minimum of 2 blood draws. The blood volumes collected adhered to the protocol limits of 1% of body weight every two weeks or 0.5% of body weight per week, with a mandatory 15-day interval free from any blood draws preceding the surgery. Additionally, blood samples were also banked on the day of surgery for both the donors and recipients.

3) Surgeries performed

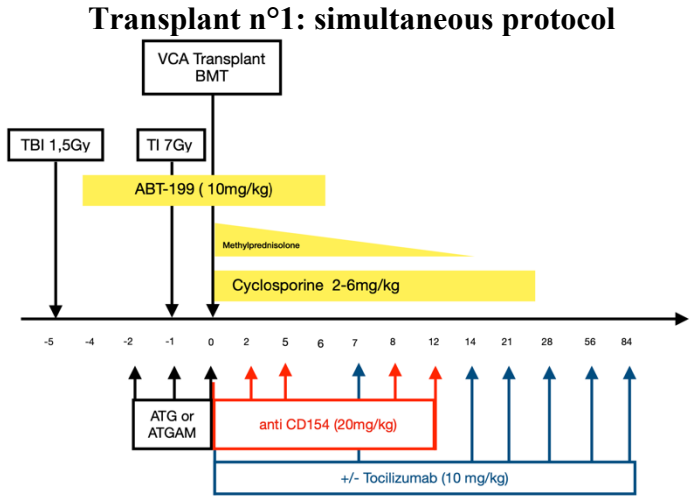


Figure 3. Simultaneous protocol diagram. The heterotopic face transplantation and the bone marrow infusion were performed simultaneously in this protocol. Recipient conditioning started 5 days prior to the face transplantation. TBI: total body irradiation. TI: thymic irradiation. VCA: Vascularized composite allotransplant. BMT: Bone marrow transplant. Medications used included: ABT199 (Venetoclax), Methylprednisolone, Cyclosporine, ATG (Thimoglobuline), AntiCD-154 (costimulat!ion blocade), Tocilizumab (antiIL-6)

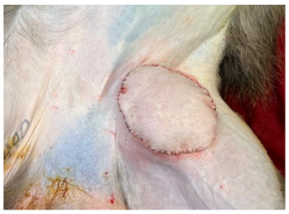
Recipient conditioning. The recipient received total body irradiation 5 days prior to the surgery at a dose of 1.5 Gy, and thymic irradiation one day before the surgery at a dose of 7 Gy. He also received Thymoglobulin and Venetoclax, as noted in the protocol. The recipient tolerated the conditioning well, as shown by the CBC results, weight, and clinical assessment of the animal.

CBC:
08/22/23: HTC: 42.2% /WBC: 8.5 x 10^3 /µl / PLT: 216 x 10^3 /µl
09/06/23: HTC: 38% / WBC: 8.8 x 10^3 /µl / PLT: 113 x 10^3 /µl
09/26/23: HTC 24.4%/ WBC: 14.6 113 x 10^3 /µl / PLT: 261 x 10^3 /µl

Animal weight :
09/22/2023: 7.9kg
09/25/23: 7.8 kg

Procedure The procedure was performed on September 27th, 2023. The procedure included a heterotopic face transplant followed by the infusion of the bone marrow cells isolated from the donor vertebrae. The VCA looked great during the immediate post-operative course, but a day later, a veinous thrombosis was discovered. This caused the end of the study for this subject. Chimerism is typically observable around two weeks after the bone marrow transplantation, so this was too early to assess the establishment of a potential chimerism just one day after the conditioning and bone marrow transplant. The autopsy revealed that there had been a veinous thrombosis starting in the femoral vein of the recipient.

D0



POPD1

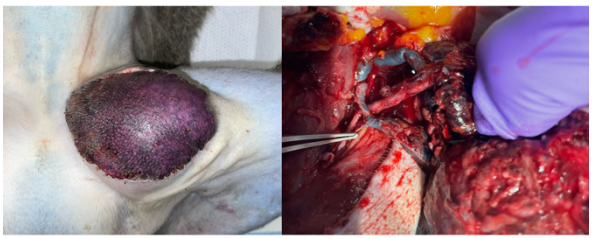


Figure 4. VCA pictures were taken on the day of the transplant. And at postoperative day 1, where a venous thrombosis of the female was seen.

Transplant n°2: delayed protocol

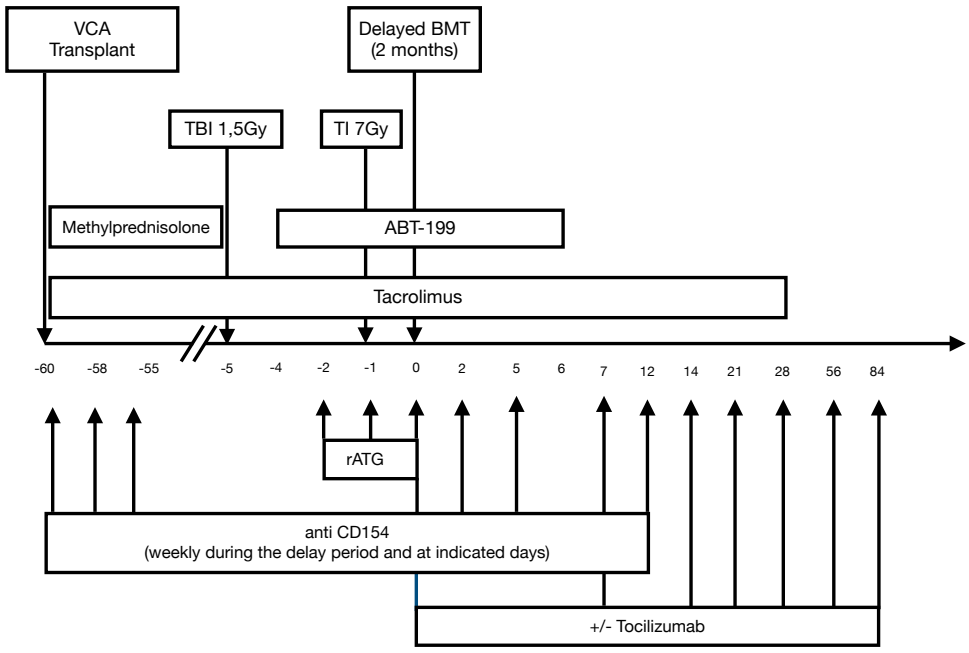


Figure 5. Delayed protocol diagram. The heterotopic face transplantation to be performed two months before the bone marrow infusion. Recipient conditioning is started 2 months after the face transplantation. TBI: total body irradiation. TI: thymic irradiation. VCA: Vascularized composite allotransplant. BMT: Bone marrow transplant. Medications used included: ABT199 (Venetoclax), Methylprednisolone, Cyclosporine, ATG (Thimoglobuline), AntiCD-154 (costimulat!ion blocade), Tocilizumab (antiIL-6)

Translant surgery and clinical course:

The heterotopic face transplant was performed on October 3rd, 2023. The immediate postoperative period was uneventful. A wound dehiscence was noticed on POD3, and after discussion with the veterinary staff, the decision was made to suture it, as it had been open for less than an hour, and the monkey had been under very close clinical assessment. This helped prevent further extension of the disunion, and complete closure was achieved without any acute rejection episode. This wound dehiscence led to the recommendation by the veterinary staff to continue antibiotics for an additional week after completing the initial week. Weight loss was also observed, as expected, and improvement began at POD 26 after appetite stimulants and close veterinary care were administered to the recipient. Chimerism was not assessed, as in this delayed protocol, the bone marrow infusion is scheduled 2 months after the surgery.

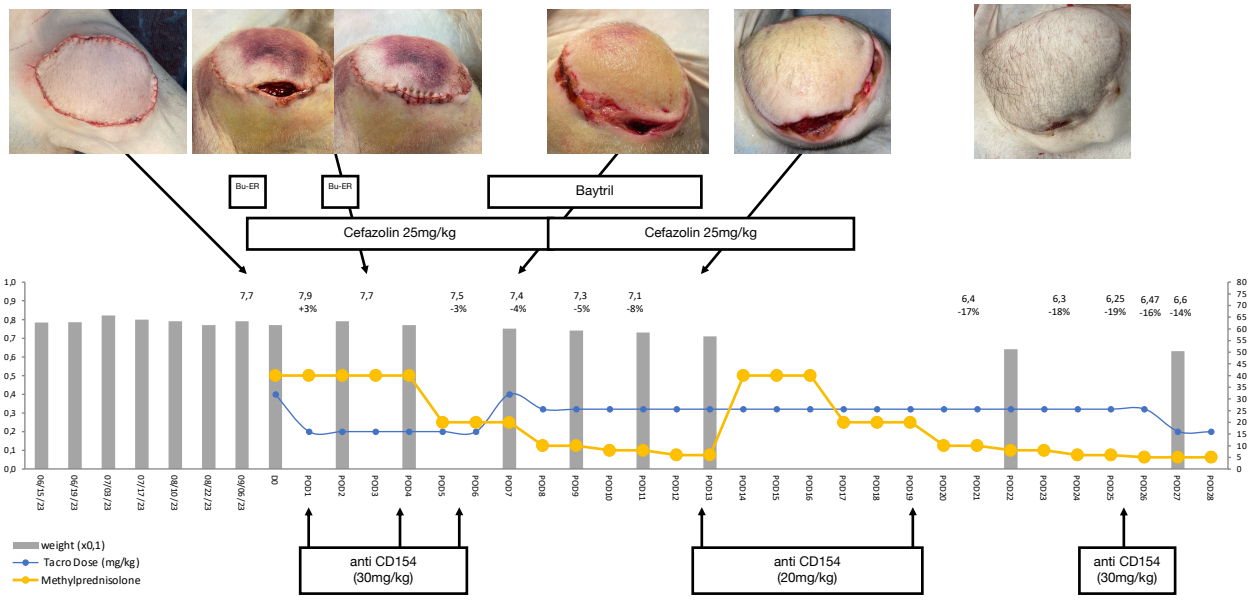


Figure 5. Clinical course and medications administered to the recipient.

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.

Two post-doctoral research fellows, one PhD candidate and one medical student were trained. Training topics included surgical techniques of heterotopic partial face transplant in the NHP model (attending plastic surgeon Dr Lellouch and Vice Chair of MGH IACUC Mark Randolph), bone marrow harvesting and processing, animal care and drug administration, performing animal sedation for IV drugs blood draw and procedures, performing Irradiation, as well as scientific writing, experimental design, and various data analysis techniques.

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.

Experiments are still ongoing; nothing to report to date. Fellows participated in several lab meetings and were able to expose the project to their collegues

What do you plan to do during the next reporting period to accomplish the goals?
If this is the final report, state "Nothing to Report."

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

More surgeries are going to be performed. This will allow to fully test both simultaneous and delayed. Also, chimerism detection and tolerance monitoring will be performed. In vitro assays (Major Task2) will be performed.

4. **IMPACT:** D*escribe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:*

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.

As an interdisciplinary project, the results are expected to have impact on the fields of plastic surgery, transplantation and immunology.

If there is nothing significant to report during this reporting period, state "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 PD/PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official 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.

Nothing to report.

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.

Due to a sharp increase in animal price from what had been initially accounted for, less animals were purchased during this reporting period. Animal price had increased from 6000$ to 35000$ fater the pandemic period.

objectives at less cost than anticipated.

Nothing additional 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

Not applicable.

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 yet.

  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 yet.

- **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. Describe the technologies or techniques were 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. 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;*
  - *physical 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".

Example:

Name: *Mary Smith*
Project Role: *Graduate Student*
Researcher Identifier (e.g. ORCID ID): *1234567*
Nearest person month worked: *5*

Contribution to Project: *Ms. Smith has performed work in the area of combined error-control and constrained coding.*
Funding Support: *The Ford Foundation (Complete only if the funding support is provided from other than this award.)*

Name: *Tatsuo Kawai*
Project Role: *PI*
Researcher Identifier (e.g. ORCID ID):
Nearest person month worked: *1.0*
Contribution to Project: *Dr. Kawai has been working on the design of the study, administrative management of the project, grant submission and scientific advising, performing the surgeries, medication protocol and animal care.*

Name: *Alexandre G. Lellouch*
Project Role: *Co-PI*
Researcher Identifier (e.g. ORCID ID):
Nearest person month worked: *1.0*
Contribution to Project: *Dr. Lellouch has been working on the design of the study, administrative management of the project, grant submission and scientific advising, performing the surgeries, medication protocol and animal care.*

Name: *Curtis J. Cetrulo*
Project Role: *Co-PI*
Researcher Identifier (e.g. ORCID ID):
Nearest person month worked: *1.0*
Contribution to Project: *Dr. Cetrulo has been working on the design of the study, administrative management of the project, grant submission and scientific advising, performing the surgeries, medication protocol and animal care.*

Name: *Haizam Oubari*
Project Role: *Research Fellow*
Researcher Identifier (e.g. ORCID ID): *0000-0001-8927-3707*
Nearest person month worked: *5*
Contribution to Project: *Mr. Oubari has performed work on the IACUC protocol submission and amendments, testing and purchasing the animals and grugs, on administrative management of the project, performing the surgeries and animal, medication protocol andanimal care.*

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.

Nothing to Report

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 Principal Investigator (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.*

See attached quad chart.

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.*

No additional document to report.