AWARD NUMBER: W81XWH-21-1-0734

TITLE: Using Bioinspired Next-Generation Cryoprotectants to Advance Ex Vivo Preservation of Vascularized Composite Allografts at High Subzero Temperature

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CONTRACTING ORGANIZATION: Johns Hopkins University School of Medicine
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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	30SEPT2022 - 29SEPT2023

4. TITLE AND SUBTITLE

Using Bioinspired Next-Generation Cryoprotectants to Advance Ex Vivo Preservation of Vascularized Composite Allografts at High Subzero Temperature

5a. CONTRACT NUMBER
W81XWH-21-1-0734

5b. GRANT NUMBER
RT200031P1

5c. PROGRAM ELEMENT NUMBER

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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
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Baltimore, MD 21205-1832

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

Advancements in tissue transplantation are tightly constrained by the limited preservation time of vascular composite allografts. Innovative biopreservation solutions combined with subzero storage protocols can allow and demonstrate the short-term suspended animation of extremity grafts. Our objective with this grant was to apply X-Therma's scaled-up product (XT-ViVoTM) intended for sub-zero organ preservation and explore its advanced preservation capabilities to extremities such as whole rat forelimbs and swine hind limbs for up to 72h periods of ischemia resulting in full restoration of form and function post-transplant.

Based on this reporting period, we have continually instituted and maintained scale-up practices at X-Therma within our new cleanroom and facility, successfully devised to produce thea method to scale up production of the biomimetic peptoid at a 100g scale,, formulate it within the XT-ViVo® preservation solution at a multi-liter scale adequate for all in vivo studies. The final XT-ViVo® solutions meet stringent quality requirements and pass robust release testing criteria. With our XT-ViVo® formulation, our partners at JHU have successfully preserved the limbs of a total of 35 animals. Analysis included typical parameters including (1) macroscopic observation, (2) external skin regeneration, (3) nerve regeneration, (4) overall grip strength and (5) overall inflammation.

15. SUBJECT TERMS
Cryoprotectants, High Subzero, Limb Transplant, Extended Preservation, Biomimetics, Vascular composite allografts

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

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

Vascular composite allotransplantation (VCA) can fully restore missing limbs with functionally and anatomically equivalent tissue. However, the current gold standard in tissue/organ preservation (static cold storage at 4°C) is insufficient and limits critical ischemia time (CIT) to a narrow window, that can result in graft degradation, delayed function, and even complete loss of primary non-function. We propose that the transport and storage of tissues at high subzero temperatures in a non-frozen state will fundamentally improve and transform organ transplantation by providing greatly extended CIT to increase graft utility. Within the scope of this contract, we will evaluate the use of XT-ViVo®, a preservation solution using anti-freeze protein mimics (peptoids), previously used in heart, kidney and penis transplants, to preserve extremity grafts (rat and swine hindlimbs) at subzero (<0°C) conditions for up to 72h periods of ischemia resulting in full restoration of form and function post-transplant. Successful execution will lay the foundation to further develop XT-ViVo® for clinical use, significantly reducing geographic restrictions, increasing transplant availability and success rates, of particular benefit for personnel with combat-related injuries.

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

Biopreservation, Organ Transplant, Limb transplant, Vascular composite allografts, high subzero, XT-ViVo, Biomimetics, Peptoids, Ice-free, Critical Ischemia time

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.*

Overall objective: Apply our advanced preservation capabilities to transplant extremities such as whole rat forelimbs (small animal) and swine hind limbs (large animals) after ischemia for up to 72 hours, minimizing ischemic damage and resulting in the full restoration of form and function post-transplant. The award is broken up into three major aims, with each aim being divided into its own subtasks.

Aim 1: Peptoid synthesis and optimization of XT-ViVo production. (Target: Months 0-36)

- **Task 1 and 2:** Peptoid synthesis and XT-ViVo preparation and development (Target: Months 1-36, Level of completion: 70%)
- **Task 3:** Ex vivo testing in rat forelimb tissue (Target dates: Month 6, Level of completion: 100%)

Milestone 1: Working protocol for ex-vivo tissue preservation is complete. Promising perfusion methods can be transferred to the Partnering PI for in vivo transplant investigation. (Target: Month 6, Level of completion: 90%)

Aim 2: Whole limb preservation and transplant (Target: Months 4-18)

- **Task 1:** Preserve rat forelimbs and perform transplantation. (Target: Months 4-18, Level of completion: 100%)
- **Task 2:** Animal monitoring and data collection. (Target: Months 4-18, Level of completion: 85%)

Milestone 2: Successful subzero preservation of whole rat forelimbs for up to 72 hours of ischemia followed by transplantation will demonstrate a new standard in preservation length of highly functioning tissue. (Target date: Month 18, Level of completion: 85%)

Aim 3: Translation to pre-clinical large animal and human-sized constructs (Target: Months 19-36)

- Task 1: Preservation and transplantation of swine hind limb tissue (Target: Months 12-34, Level of completion: 0%. (The IACUC for the swine transplant model has been approved, and ACURO is pending approval).
- Task 2: Animal monitoring and data collection. (Target: Months 19-34, Level of completion: 0%)

Milestone 3: Project completion. Successful preservation of swine hind limb tissue using XT-ViVo will provide the

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.

Aim 1, Tasks 1 and 2: As required in Task 1 and 2, we continue to producesuccessfully scaled-up functional peptoids no longer at a100-200 mg scale sufficient for R&D, but nowat a scale of 200g, moving from traditional vessel synthesis to preparing peptoid in large-scale 20 L reactors and using advanced-level equipment (e.g. high throughput HPLC) to assess quality metrics (**Fig 1**). The peptoid has also been formulated within the XT-ViVo® solution, in a manner that passes our internal quality release tests. This has allowed us to manufacture volumes at multi-liter scales, which continue to help us meet meet our 100L-scale future manufacture needs and ensure stable supply of all raw materials, supply chain, facilities preparation, etc. is completed for project. In addition to the successful scale-up using the solid-support phase synthesis above, X-Therma has also developed an alternate solution phase-based approach. The solution-based approach allows production at an existing scale, without the need for solid support or resin requiring laborious downstream purification processes. The approach also allows the desired peptoid sequence and the intermediates to be manufactured in a stable form and purified as needed, streamlining the manufacturing process and increasing the purity of the final product. We expect that the new method will continue to meet the market demand as well as the peptoid yield, purity and reproducibility needed to achieve effective preservation.

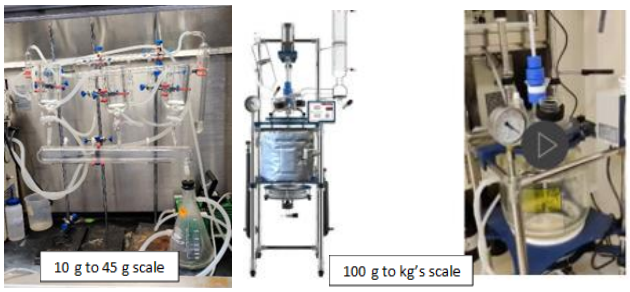
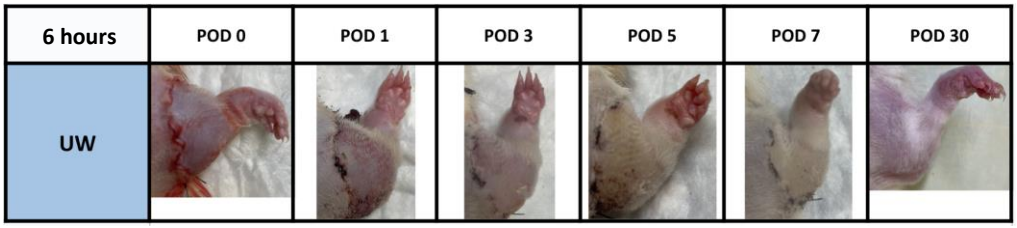


Fig 1. Small (left) and large (right) scale peptoid synthesis capabilities at X-Therma Inc.

Aim 1, Task 3: This milestone is completed and has been reported in previous reports.
Aim 2, Task 1: Subtask 1 is now successfully completed. Based on the ACURO approval, all control and experimental groups have successfully undergone transplantation and reached their endpoints, yielding a total of 35 successful forelimb transplants. As mentioned above in Aim 2, Task 1, the team at JHU performed syngeneic transplantation in the control groups (UW solution, stored at 4°C) and experimental groups (XT-ViVo®, stored at -5°C). To date, all animals from both control and experimental groups (n=5 per group and storage condition) have undergone successful forelimb transplantation, have reached endpoint, and have been sacrificed for analysis highlighted in Task 2.

Aim 2, Task 2: Comprehensive histological and functional assessments were performed on the tissues harvested from each of the animals. This included (1) post-operative macroscopic images of the transplanted forelimbs to assess overall healing and skin quality, (2) Luminex assay to assess for markers of angiogenesis, wound healing, and inflammation in the serum (IL-1b, IL-6, IL-10, MCP-1, VEGF, TNFα), (3) Masson's-Trichrome for percentage of collagen and fibrosis, (4) Muscle atrophy anti-laminin-y and nerve staining, and finally (5) functionality based on stimulated grip strength testing of animals post-healing. The histological analysis is underway, and all other data is presented below.

A)



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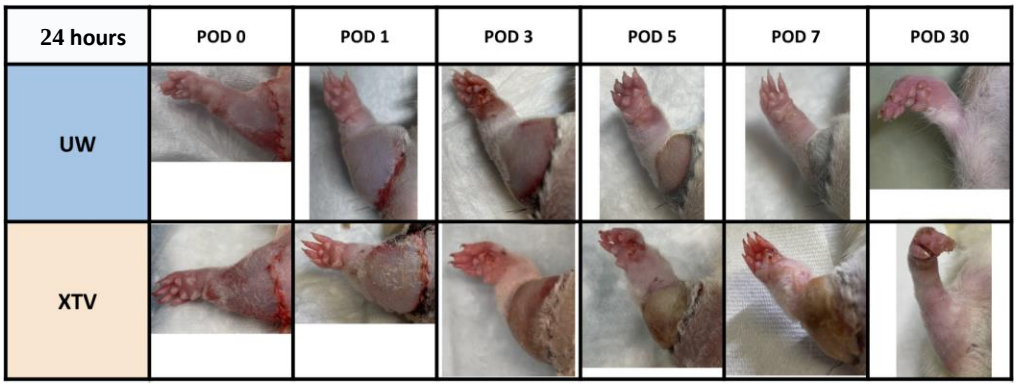

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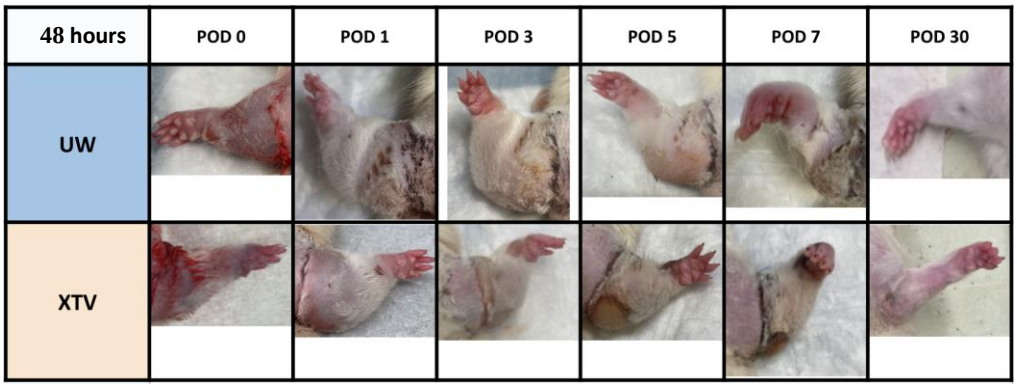

D)

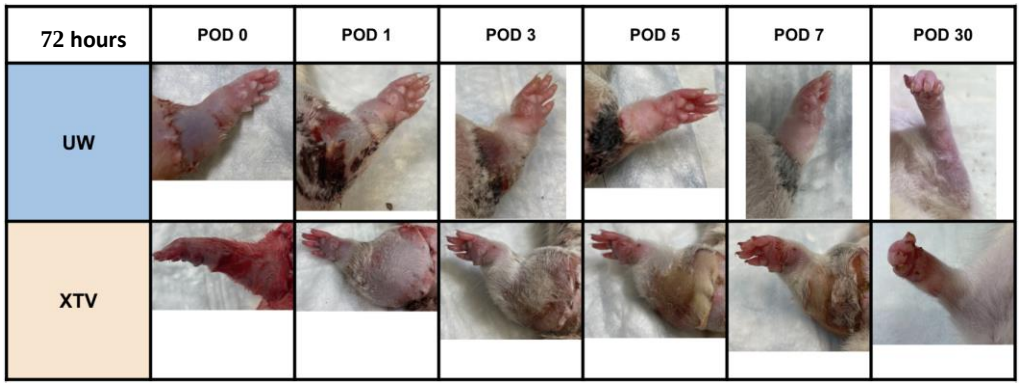

Figure 2. Representative images of POD 0, 1, 3, 5, 7, and 30 (endpoint) for all groups. A) UW-6 h; B) top row: UW-24 h, bottom row: XTV-24h C) top row: UW-48h, bottom row: XTV-48h; C) top row: UW-72h, bottom row: XTV-72h.

Macroscopic evaluation of all paws suggested storage up to 72 hours in both solutions continue to look well perfused without any signs of skin necrosis (Figure 2). Interestingly, all XTV limbs reperfused more quickly on average than their UW counterparts: 18.3 vs 20.7 seconds at 24 hours of CIT, 7.7 vs 20 seconds at 48 hours of CIT, and 15 vs 30 seconds at 72 hours of CIT. Skin necrosis however, while equal in both groups, was positively correlated with storage time. By POD3, skin necrosis was significant in both control (UW) and XT-ViVo®.

Luminex data evalauting the general inflammation and angiogenesis demonstrate similar trends (Figure3). In general, early-stage inflammation is enhanced, but recovers quickly by the endpoint in preservation timeframes <72 h. However, 72 h storage in both XT-ViVo® and UW results in lower levels of inflammation that did not recover completely by the endpoints (POD30). Angiogenesis in all groups were the same.

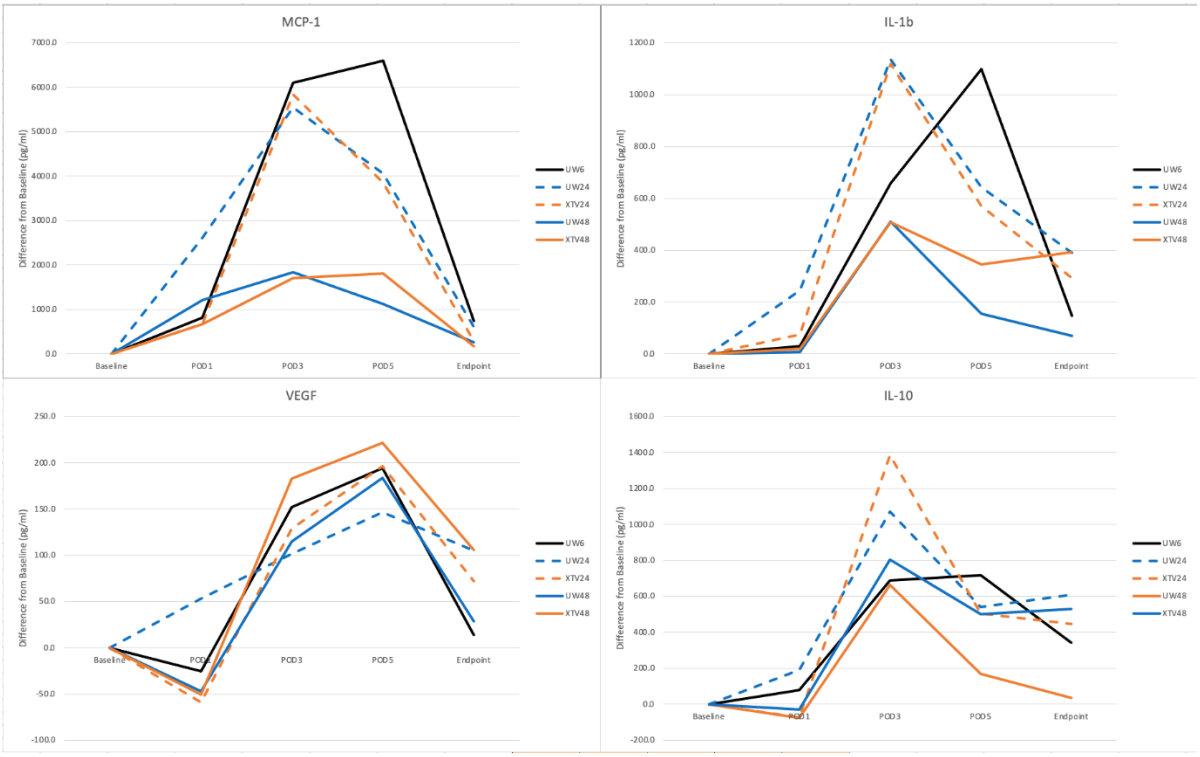

Figure 3. Luminex assay for biomarker assessment for UW6, UW24, UW48, XTV24, and XTV48 groups (n=3 per group) at baseline, POD1, 3, 5, and 30 (endpoint). A) MCP-1 levels B) IL-1b levels C) VEGF levels D) IL-10 levels.

Grip Strength (N)		
	UW	**XTV**
6 hours	0.54 (±0.20)	
24 hours	0.19 (±0.12)	0.18 (±0.13)
48 hours	0.22 (±0.25)	0.08 (±0.13)
72 hours	0.1 (±0.11)	0.08 (±0.16)

Table 1. Grip strength test results from POD30 (endpoint) for all groups.

Functionally, each group demonstrated the ability to form a fist for grip strength testing (Table 1), but there were no significant differences between their strengths at the 4 week time point. Further, a loss in grip strength was observed relative to native (pre-operative) animals. Further statistics will be needed to evaluate the true impact of loss of grip strength.

G-Ratio		
	UW	**XTV**
6 hours	0.46 (±0.15)	
24 hours	0.54 (±0.16)	0.44 (±0.13)
48 hours	0.42 (±0.12)	0.42 (±0.16)
72 hours	0.50 (±0.16)	0.52 (±0.12)

Table 2. G-ratio of toluidine blue-stained median nerves from POD30 (endpoint) for all groups.

Analysis of G-ratio (total fiber diameter) in sections that were stained with toluidine blue (for nerves) suggested histologic evidence of similar level of nerve regeneration in every group (Table 2). Nerve generation post-surgery is a crucial parameter affecting the overall functionality of the transplanted limb. Similar patterns were observed with collagen in sections, with no excessive fibrosis observed (Table 3).

% Collagen		
	UW	**XTV**
6 hours	25.48 (±12.04)	
24 hours	31.83 (±11.11)	23.43 (±13.97)
48 hours	33.48 (±13.57)	26.64 (±8.08)
72 hours	31.97 (±12.24)	25.98 (±5.09)

Table 3. Fibrosis analysis of Masson's Trichrome stained extrinsic flexors from POD30 (endpoint) for all groups.

Aim 3: IACUC has been approved. ACURO approval is pending.

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.

While the X-Therma team has significant experience in molecular techniques, tissue engineering, cryopreservation and pharmacology, the close interactions with the JHU team, extensive literature reviews of the transplant field (pre-clinical and clinical) and histopathologists at the CRO, has resulted in a significant advancement in knowledge for the participants. Our partners' on the JHU side were able to present the work from Milestone 2 at two conferences. NESPS and Hopkins resident research day.

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

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.

Aims 2 and 3: Throughout the subsequent reporting periods, we will provide preservation solutions (XT-ViVo®) to JHU that meet their volume requirements and internally defined quality release criteria.

Aim 2, Task 2: Over the next period, any remaining histological data will be analyzed and run through statistical tests to assess for any significant differences between control and experimental groups on the Johns' Hopkins side.

Aim3, Task 1: We will continue to wait for ACURO approval before beginning any large swine experiments.

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).

The preliminary findings in this grant indicate that preserving forelimbs in XT-ViVo® for up to 72 hours at subzero conditions is feasible and that grafts can maintain perfusion for 30 days. Moreover, nerves can survive, regenerate, and reinnervate muscle targets after these conditions. This builds to foundation to further optimize this technology for prolonged preservation of

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.

Overall, success with preserving VCA constructs with XT-ViVo® even at short CIT timepoints (6h) similar to the current standard, demonstrates the broad applicability of the XT-ViVo® technology originally intended for organs, now in complex VCA products. Developing potential preservation alternatives that do not modify the peri-transplant workflow, would naturally increase availability, and improve access to tissues despite in the absence of comparable alternatives.
Further, it suggests potential translatability of the XT-ViVo® technology for use in subzero preservation a range of biologics irrespective of complexity, size, and function, including cell and gene therapy, tissue engineered constructs and grafting, although this remains to be confirmed through further testing.

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 as of now.

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.

Aim 3, Task 1: We require ACURO approval to begin any large animal swine work that was proposed in Aim 3. Any delay in obtaining ACURO approval could potentially result in a compromised timeline. However, we have experienced surgeons and an efficient team that we expect will be able to perform surgeries quickly and perform downstream analyses in parallel. Any preliminary training for surgeons on the large swine model of VCA transplant can be performed on additional protocols that already exist within the partner institution.

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.

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."*

- **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.*

Muss T, Guo Y, Zhang Y, et al. Using Bioinspired Nex-Generation Cryoprotectants to Advance Ex Vivo Preservation of Vascularized Composite Allografts at High Subzero Temperature. Northeastern Society of Plastic Surgeons Annual Meeting; September 2023; Washington, DC, USA.

Muss T, Guo Y, Zhang Y, et al. Extending Ex Vivo Preservation of Vascularized Composite Allografts with Bioinspired Cryoprotectants At High Subzero Temperature. The 15th Annual JHU/UMMS Plastics and Reconstructive Surgery Research Symposium; June 2023; Baltimore, MD, USA.

- **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".

Name: Mark Kline
Project Role: CTO
Nearest person month worked: 5
Contribution to Project: Dr. Mark Kline was involved in the conception of the project, grant writing, budgeting, allocation of resources, direction of chemistry and all finance-related tasks.

Name: Arthi Shridhar
Project Role: Scientific Program Lead
Nearest person month worked: 5
Contribution to Project: Dr. Arthi Shridhar has worked on the project planning, ensuring timely execution of the project and is also the primary surgeon on the limb extraction. She is also in charge of all correspondence in the form of reporting with the DOD.

Name: Purna Vasireddy
Project Role: Synthetic Chemist
Nearest person month worked: 5
Contribution to Project: Dr. Purna Vasireddy has worked on optimizing the scale up of the peptoid, ensuring a steady supply is manufactured for formulation into XT-ViVo®.

Name: Mussie Gide
Project Role: Synthetic Chemist
Nearest person month worked: 5

Contribution to Project: Mr. Mussie Gide has worked peptoid manufacturing and downstream characterization of the synthesized peptoids, using advanced-level equipment (e.g. high throughput HPLC) to assess quality metrics

Name: Gamid Abatchev
Project Role: Formulation Scientist
Nearest person month worked: 5
Contribution to Project: Gamid Abatchev has worked on designing and manufacturing the formulations at a multi-liter scale using our GMP cleanroom facilities. He has also worked on characterizing final XT-ViVo® formulations using internal quality release tests.

Name: Kartheek Pothana
Project Role: Research Associate
Nearest person month worked: 5
Contribution to Project: Mr. Kartheek Pothana is the secondary surgeon and has been trained on the ex vivo limb extraction and perfusion. He will also be in charge of performing all other histological evaluations to be performed on site at X-Therma Inc.

Name: Gerald Brandacher, MD
Project Role: Principle Investigator
Nearest person month worked: 1 (through 08/31/2023)
Contribution to Project: Oversight of all research activities

Name: Byoung Chol Oh
Project Role: Co-Investigator
Nearest person month worked: 1
Contribution to Project: Experimental Design, data collection and data interpretation

Name: Jaimie Shores
Project Role: Co-Investigator
Nearest person month worked: .12
Contribution to Project: Experimental Design for large animal study, data interpretation

Name: Eleni Drivas
Project Role: Research Fellow
Nearest person month worked: 2
Contribution to Project: Experimental design, performed small animal transplant procedures, data collection, data interpretation

Name: Amanda Loftin
Project Role: Medical Student, Research Fellow
Nearest person month worked: 6
Contribution to Project: Experimental Design, assistance with animal surgeries, data collection, and data interpretation

Name: Yichuan Zhang
Project Role: Medical Student, Research Fellow
Nearest person month worked: 6
Contribution to Project: Experimental Design, assistance with animal surgeries, data collection, and data interpretation

Name: Amy Bodine
Project Role: Research Specialist
Nearest person month worked: 1 (through 05/18/2023)
Contribution to Project: Provided assistance with data collection and data interpretation

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

Gerald Brandacher	Changes: Ended - Human iPSC-derived EGFR+ functional Schwann Cells to Enhance Nerve Regeneration and Improve Functional Outcomes in VCA Role: Co-I

	Effort: 2% Dates:06/30/2020 – 06/29/2023
Gerald Brandacher	Changes: Ended - Ethical Factors Impacting Patients' Decisions to Pursue VCA Role: PI Effort: 1% Dates: 09/30/2019 – 12/31/2022
Gerald Brandacher	Changes: Ended - Cell-targeted glutamine antagonists as a novel therapy for lymphoma Role: Co- I Effort:2.5% Dates: 07/02/2018-06/30/202
Gerald Brandacher	Changes: Ended - Novel brain penetrant metabolic inhibitors to treat MYC-driven medulloblastoma Role: Co-I Effort: 2.5% Dates: 08/01/2018- 05/31/2022
Gerald Brandacher	Changes: Ended - Understanding and Supporting Public Information Needs about VCA Donation Role: PI Effort: 1% Dates:09/30/2018 – 09/29/2022
Gerald Brandacher	Changes: Ended - A Biocompatible Therapeutic Platform for Precise Regulation of Vascularized Composite Allotransplant Rejection via Enhanced Costimulation Blockage Role: Co-I Effort: 2% Dates:09/30/2018 – 03/30/2023
Gerald Brandacher	Changes: Ended - Non-Invasive Immune Monitoring to Improve Outcomes in Composite Tissue Transplantation Role: PI Effort: 2% Dates: 09/18/2013 – 09/17/2022
Gerald Brandacher	Changes: Ended - Post-Transplantation Cyclophosphamide to Promote Immune Tolerance after Reconstructive Transplantation Role: PI Effort: 2% Dates: 09/18/2013-09/17/2022
Gerald Brandacher	Changes: Ended - Non-invasive Immune Monitoring Biomarkers using Plasma microRNAs in VCA Role: Co-I Effort: 1% Dates: 06/15/2019 – 06/14/2023
Gerald Brandacher	Changes: Ended - Isochoric Pressure Based Preservation of Cells, Tissues and Organs Role: PI Effort: 3.75% Dates: 01/01/2022 – 03/31/2023
Gerald Brandacher	Changes: Pending role change from PI to consultant. Effort ends on award 08/31/2023 Quantitative Ambulatory Assessment and Prognosis of the Impact of Severe Upper Limb Injuries on Real World Behavior
Gerald Brandacher	Changes: Pending role change from PI to consultant. Effort ends on award 08/31/2023 Assessing the Comparative and Longitudinal Benefits of Vascularized Composite Allotransplantation of the Hand
Gerald Brandacher	Changes: Pending role change from PI to consultant. Effort ends on award 08/31/2023 A Novel Application of Normothermic Machine Perfusion for Face Recovery to Reduce Intragraft Inflammation and Optimize Organ Viability
Gerald Brandacher	Changes: Pending role change from PI to consultant. Effort ends on award 08/31/2023 High-Dose Post-Transplantation Cyclophosphamide to Induce Delayed Immune Tolerance after Reconstructive Transplantation

Gerald Brandacher	Changes: Pending role change from PI to consultant. Effort ends on award 08/31/2023 Preclinical Development of a Novel Small Molecule T cell Metabolism Inhibitor to Prevent Rejection and enable Calcineurin Inhibitor-free Allograft Survival in VCA
Gerald Brandacher	Changes: Pending role change from PI to consultant. Effort ends on award 08/31/2023 Targeting Ischemia Reperfusion Injury With HO-1 Gene Therapy to Improve VCA Outcomes
Gerald Brandacher	Changes: Pending role change from PI to consultant. Effort ends on award 08/31/2023 Feasibility of Expanding Ischemia Time for Hearts Destined for Transplantatio
Gerald Brandacher	Changes: Effort ends on award 08/31/2023 Improving Acceptability and Outcomes for Upper Extremity Transplantation in Service Members and Veterans
Gerald Brandacher	Changes: Pending role change from PI to consultant. Effort ends on award 08/31/2023 A Novel and Clinically Feasible Co-therapy of Deceased Donor Bone Marrow Combined With Donor-Matched Mesenchymal Stem Cells to Establish Immune Tolerance
Gerald Brandacher	Changes: Pending role change from PI to consultant. Effort ends on award 08/31/2023 Using Bioinspired Next-Generation Cryoprotectants to Advance Ex Vivo Preservation of Vascularized Composite Allografts at High Subzero Temperature
Gerald Brandacher	Changes: Pending role change from PI to consultant. Effort ends on award 08/31/2023 Taming Endothelial Activation and Sterile Inflammation to Rescue VCA's from Preservation Injury
Gerald Brandacher	Changes: Effort ends on award 08/31/2023 Myocardial-Associated B Lymphocytes and Inflammatory Injury
Gerald Brandacher	Changes: Effort ends on Award 08/31/2023 Synergistic Validation of Polyethylene Glycol mediated fusion (PEG Fusion) autograft re-construction in large animal model of Segmental Nerve Injury (SNI)
Byoung Oh	Changes: Ended W81XWH-19-1-0234 Non-Invasive Immune Monitoring Biomarkers using Plasma microRNAs in VCA Role : PI Effort: 5% Dates: 06/15/2019 – 06/14/2023
Byoung Oh	Changes: Ended - Human iPSC-derived EGFR+ functional Schwann Cells to Enhance Nerve Regeneration Role: PI Effort:15% Dates:06/30/2020 – 06/29/2023
Byoung Oh	Changes: Ended - A Novel and Clinically Feasible Co-therapy of Deceased Donor Bone Marrow Comb. Role: Co - I Effort: 5% Dates: 07/01/2020 – 06/30/2023
Byoung Oh	Changes: Received Extended Limb Preservation Employing an Optimization Strategy for Stabilization Role: Co-I Effort: 5% Dates 07/01/2022 – 03/31/2024
Byoung Oh	Changes: Received - W81XWH2210726 A Novel Universal and Portable Normothermic Machine Perfusion Device for Vascularized Composite Allografts Role: Co-I Effort: 10% Dates 09/30/2022 – 09/29/2025
Byoung Oh	Changes: Received Engineering thymic selection to control the development of

	alloreactive T cells and promote VCA acceptance Role: Co-I Effort: 10% Dates 09/30-2022 – 09/29/2025
Byoung Oh	Changes: Received Fertility Protection in Cancer: Recovery of Whole Ovaries enabled by Next... Role: Co-I Effort: 2% Dates 01/01/2023 – 07/31/2023
Byoung Oh	Changes: Received HT94252310593- Immunogenicity in subzero stored VCA Role: PI Effort: 20% Dates 09/01/2023 – 02/28/2025
Byoung Oh	Changes: Received- Use of extracellular vesicles from metabolically engineered Mesenchymal Stem cell Role: Collaborator Effort: 2% Dates 09/01/2023 – 05/31/2025
Byoung Oh	Changes: Received - R44DK133013 Mechanistic approach to optimization of a kidney preservation solution Role: PI Effort: 2% Dates 07/01/2023 – 06/30/2024

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.*

Organization Name: Johns Hopkins University
Location of Organization: Baltimore, Maryland
Contribution: Collaboration and Personnel exchanges (Project ideation, Statement of work preparation, Surgical training)

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: *N/A*

QUAD CHARTS: *N/A*

9. APPENDICES: *N/A*