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14. ABSTRACT

Close to 40% of combat injuries sustained in Operation Iraqi Freedom and Operation Enduring Freedom involve severe extremity and craniofacial trauma. For many devastating injuries where conventional reconstruction is not possible, vascularized composite allotransplantation (VCA) has become a viable alternative, providing new, exciting options for Wounded Warriors that could better restore the appearance, anatomy, and function. However, clinical management of these injuries prior to reconstruction frequently requires multiple blood transfusion or skin allografts resulting in the formation of alloantibodies (anti-HLA IgG Abs, donor specific antibodies or DSA) and a high degree of sensitization. The role of DSA and mechanisms of antibody mediated rejection (AMR) in VCA are still largely unknown. To date, there is only one single experimental study published that has recently attempted to define the role of DSA in a rat model of vascularized osteomyocutaneous flap allotransplantation. As such, this project aims to comprehensively investigate the mechanisms and impact of pre-existing and de-novo DSA and AMR in in VCA. The goal is to develop a clinically translatable desensitization protocol that will subsequently broaden the eligible population for reconstructive transplantation to include those patients who have become sensitized to foreign antibodies

15. SUBJECT TERMS

vascularized composite allotransplantation, sensitization, autologous hematopoietic stem cell transplantation, antibody mediated rejection, donor specific antibodies

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1. INTRODUCTION

For many devastating combat injuries where conventional reconstruction is not possible, Vascularized Composite Allotransplantation (VCA) has become a viable alternative. This approach provides new, exciting options for Wounded Warriors that could restore appearance, anatomy, and function better than other available treatment options. However, clinical management of these injuries prior to transplantation frequently requires multiple blood transfusion or skin grafts resulting in the formation of alloantibodies (anti-HLA IgG Abs) and sensitization. In solid organ transplantation (SOT), such pre-sensitization is the greatest risk factor for allograft rejection and long-term graft failure, and causes patients to be excluded as candidates for transplantation. However, the role of donor-specific antibodies (DSA) and mechanisms of antibody-mediated rejection (AMR) in VCA are largely unknown. Thus, there is an imminent need to develop a better understanding of the mechanisms related to DSA and AMR after VCA as well as to implement novel clinically relevant desensitization protocols that would be applicable to a cadaveric donor setting.

The objective of this project therefore is to comprehensively investigate the mechanisms and impact of pre-existing and de-novo DSA and AMR in VCA and to develop a clinically relevant desensitization protocol that will subsequently broaden the population of sensitized patients eligible for reconstructive transplantation. The investigators will test their central hypothesis that that the impact and mechanisms, of AMR in reconstructive transplantation as well as the cadaveric donor setting will require specifically tailored desensitization strategies and treatment regimens in order to improve access and outcomes for highly sensitized VCA candidates in a pre-clinical large animal model.

2. KEYWORDS

vascularized composite allotransplantation, sensitization, autologous hematopoietic stem cell transplantation, antibody mediated rejection, donor specific antibodies

3. ACCOMPLISHMENTS

During this reporting period a total of 2 VCA (i.e. swine heterotopic hind limb) transplantations were performed using fully SLA mismatched hind limb donor, recipient pairs and a donor MHC matched bone marrow transplant. The recipient animals were sensitized with SLA disparate donor skin grafts to achieve donor-specific presensitization prior to transplantation. A desensitization protocol containing of a 7-day course of Fludarabine combined with 800cGy total body irradiation followed by a donor MHC matched bone marrow transplantation was subsequently applied 46 days post skin transplantation.

However, due to the COVID-19 pandemic and the associated shut down of our research program at Johns Hopkins for several months our team experienced significant delays with regard to meeting the proposed milestones on this project. This required us to seek another No Cost Extension (NCE) for this project which has been approved on November 19, 2020.

A. Major Goals

The major goals of this project for Year 2 as stated in the approved SOW were:

Major Task 1: Identify the role of presensitization, DSA, and mechanisms of AMR in VCA
Major Task 2: Identify the role of de-novo DSA and impact on AMR in VCA

Major Task 3: Implement a novel clinically relevant desensitization protocol using autologous hematopoietic stem cell transplantation (HSCT)

Table 1: Progress against the SOW

Task	**Start Date**	**End Date**	**% Complete**	**Comments**
Major Task 1	October 2017	October 2018	100 %	Major task 1 has been completed as detailed in the previous quarterly reports with three recipients successfully performed in Group 1 and two recipients in Group 2, respectively. Although there has been a total of five recipients proposed in Group 2, the clinical outcome in the first two animals was consistent and as expected with rapid graft loss in the sensitized animals. We therefore propose to not perform any additional control animals in this group as outlined in our submitted NCE and change in scope of work request.
Major Task 2	-	-		Given the significant increase in price per animal we will have to reduce the overall number of animals/transplants proposed in the original SOW. In order to be able to not compromise statistical power in the individual experimental groups we therefore propose to forgo Major task 2 and to focus on completion of the clinically more relevant Major task 3 for the remainder of the project.
Major Task 3	September 2018	October 2021	40 %	Major task 3 will be performed as originally proposed with a total of six transplants performed in Group 5. However, according to our preliminary study on harvesting autologous bone marrow in Group 5 to obtain sufficient bone marrow cell numbers for autologous BM transplantation, we will require three additional SLA-matched bone marrow donor animals at a ratio of 1 donor per 2 transplant recipients.

B. Accomplishment of Goals

Major Task 3: Implement a novel clinically relevant desensitization protocol using autologous hematopoietic stem cell transplantation (HSCT)

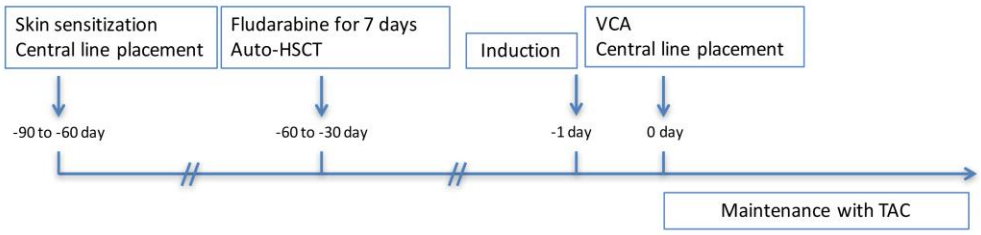

Figure 1: Treatment regimen of the recipient animal

Over the course of the past year, both animal availability issues as well as the COVID-19 pandemic have resulted in significant delays in this project. However, the team was still able to work on Major Task 3, group 5, as outlined in **Figure 1**.

While encouraging data from Major Task 1 sustain our enthusiasm for the continuation of the originally proposed experiments, 2 recipient animals in the treatment arm unfortunately suffered significant radiation related toxicity as part of the protocol. Due to this outcome, attention was directed at a full, post-mortem etiological work-up. Necropsy results demonstrated severe neutropenia, radiation induced bone marrow suppression, followed GI toxicity and tissue damage, secondary bacterial translocation, and radiation-induced lung injury confirmed by veterinary pathologists (**Figure 2**).

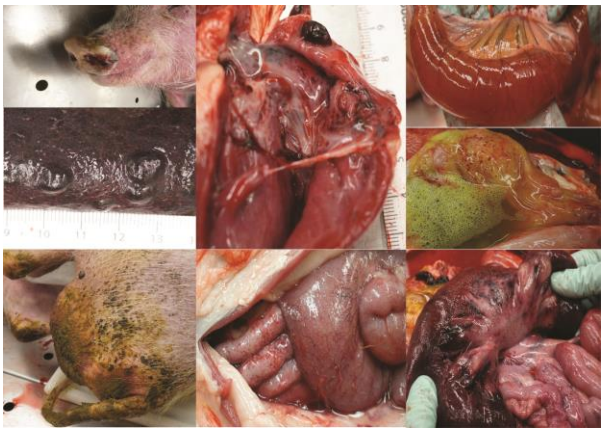

Figure 2: Radiation-induced toxicities and pathological findings in recipient animals

While this was not an anticipated outcome, this work demonstrated a need to carefully re-evaluate our radiation dosing strategy as well as post bone marrow transplant supportive care of recipient animals. At this time, meetings are ongoing with on-site medical physics experts to adjust the irradiation dose and methodology and compare it against the published data. Bone marrow harvest techniques are again being revisited and perfected, and discussions with our veterinary colleagues have been carried out to further optimize post- irradiation supportive care for recipient animals. As such this careful re-evaluation is laying the groundwork for the rest of the group 5 animals required to complete this subtask.

C. Training and Professional Development

Large animal experiments are a critical component of validating proposed protocol prior to a potential application in the clinical VCA setting. Proposed regimen requires fine tuning to reduce radiation toxicity.

D. Result Dissemination

Nothing to report

E. Future plan

The start of the large animal transplant experiments as outlined by the SOW under Task 3 was significantly delayed due to limited animal availability from the breeder at Columbia/MGH. However, over the past couple of months, we have made significant progress in satisfying our demands for the remainder of the project. To carefully address the specific needs of the experiments (SLA type, gender, size, age) as outlined by the SOW specific breeding pairs to allow for reproducibility are required and the investigators have been assured that those will be provided for this project.
In light of irradiation toxicity noted with these animals, our team has taken and is continuing to take every possible precaution to prepare and optimize processes (irradiation, bone marrow harvest and processing) associated with the translational large animal experiments outlined by the SOW under Task 3. During the last year despite all the encountered challenges, progress has been made with the first *in-vivo* pilot trials using a desensitization protocol. Based on these initial results, the remaining time and experiments during the NCE

period will focus on further adapting and optimizing the components of the protocol to achieve VCA graft survival in the setting of sensitization.

4. IMPACT

A. Impact on the Development of the Principal Discipline(s) of the Project

The development of specifically designed animal models as proposed in this study will be a prerequisite to pave the way to the acquisition of the lacking DSA and AMR data indispensable to the further advancement of field of VCA. The insights gained from this project will lead to a better understanding of the molecular and pathological sequelae of DSA and AMR in VCA. This will bring us closer to developing specific, targeted, and clinically applicable treatment modalities for AMR. In particular, the use of autologous HSCT as a novel, rapidly translatable desensitization approach will have a significant impact on our discipline and will allow us to successfully perform VCA in highly sensitized patients who otherwise would be excluded as candidates for transplantation.

B. Impact on Other Disciplines

A better understanding of DSA and AMR in VCA, along with the development of clinically applicable desensitization protocols, will not only contribute greatly to the advancement of the field of reconstructive transplantation but also be applicable to other types of solid organ transplantation to enable desensitization in a cadaveric donor setting.

C. Impact on Technology Transfer

Nothing to Report

D. Impact on Society beyond Science and Technology

Nothing to Report

5. CHANGES/PROBLEMS

Nothing to Report

A. Changes in Approach and Reasons for Change

Nothing to Report

B. Actual or Anticipated Problems or Delays and Actions or Plans to Resolve Them

Due to the COVID-19 pandemic, Johns Hopkins University has put in place a complete research laboratory shutdown on March 18, 2020, causing these plans to be abruptly halted. Although most recently our research program has been reopened, we are still expecting further delays over the next couple of months during a staged reopening phase that only allows our program to operate at reduced capacity. In order to address these challenges and to make up for the experienced unexpected disruption due to the COVID-19 pandemic we requested an additional one-year extension without funds which was approved on November 19, 2020.
Additionally, as previously mentioned, radiation induced toxicity issues are being tackled with the help of Johns Hopkins radiation oncologists and medical physicists, and post radiation care strategies have been discussed at length with our veterinary colleagues.

C. Changes that had a Significant Impact on Expenditures

Nothing to Report

D. Significant Changes in Use or Care of Human Subjects, Vertebrate Animals, Biohazards, and/or Select Agents

Nothing to Report

6. PRODUCTS

Nothing to Report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

A. Participants & Collaborators

Name (First and Last)	Description
Gerald Brandacher, M.D.	Project Role: PI Nearest person month worked: 1 Contribution to Project: As the PI, Dr. Brandacher has overseen performance and execution of in-vivo and in-vitro studies and contributed to the surgery.
Zhaoli Sun, M.D.	Project Role: Co-PI Nearest person month worked: 1 Contribution to Project: As a Co-PI Dr. Sun has designed and supervised all in-vitro and in-vivo experiments as related to and outlined by the SOW.
Byoungchol Oh, D.V.M. Phd	Project Role: Co-I Nearest person month worked: 1 Contribution to Project: As a co-investigator Dr. Oh has performed all in-vitro and in-vivo experiments as related to and outlined by the SOW.
Giorgio Raimondi, Ph.D.	Project Role: Co-I Nearest person month worked: 1 Contribution to Project: Dr. Raimondi participated in data collection, data interpretation, and supervision of the post doc fellow involved in this project.
Damon Cooney, M.D., Ph.D.	Project Role: Co-I Nearest person month worked: 1 Contribution to Project: Dr. Cooney participates in the large animal surgeries, data interpretation, as well as preparation and maintenance of the animal protocol.
Yongchn Wang, M.D., Ph.D.	Project Role: Research Associate Nearest person month worked: 1 Contribution to Project: Performs in vitro experiments as part of Dr. Sun's group

B. Changes in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period

Name (First and Last)	Current Support Changes
Gerald Brandacher	Change: Closed *Phase II: Novel Super-cooling of Genitourinary Cells and Tissues for Transplant* Sponsor: X-Therma / Prime: DOD Award: W81XWH-16-C-0212 Role: Co- I Date: 10/01/2018 - 05/24/2020
Gerald Brandacher	Change: Closed *Phase II: Non-Toxic, Highly-Effective Bioinspired Cryoprotectants* Sponsor: X-Therma

	Role: PI Date: 11/01/2019 – 10/31/2020
Gerald Brandacher	Change: Closed *Engineering a Hybrid Thymus to Unravel the Tolerogenic Properties of Vascularize* Sponsor:: CDMRP Award: W81XWH-16-1-0708 Role: Co-I Date 09/30/2016 – 06/29/2020
Gerald Brandacher	Change: Received *Human iPSC-derived EGFR+ functional Schwann Cells to Enhance Nerve Regeneration* Sponsor:TEDCO Award: 2020-MSCRFL-5414 Role: Co-I Date: 06/30/2020 – 06/29/2022

C. Other organizations involved as partners

Nothing to Report

8. Special Reporting Requirements
Nothing to Report

9. Appendices

AMR Antibody Mediated Rejection
DSA Donor Specific Antibody
HLA Human Leukocyte Antigen
HSCT Hematopoietic Stem Cell Transplantation
NCE No Cost Extension
SLA Swine Leukocyte Antigen
SOT Solid Organ Transplantation
VCA Vascular Composite Allotransplantation