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TITLE: Establishment of donor Chimerism Using Allogeneic Bone Marrow with AMP Cell Co-infusion

PRINCIPAL INVESTIGATOR: Megan Sykes

CONTRACTING ORGANIZATION:
Columbia University Medical Center, New York, NY 10032

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6. AUTHOR(S)
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14. ABSTRACT
Composite tissue allografts (CTA) have been more and more frequently used to treat combat-associated injuries, however, rejection remains to be the major barrier that could not be solved by potent non-specific immunosuppression. Induction of tolerance to the CTA is the ideal solution. Combined mixed allogeneic chimerism induction and kidney transplantation has been shown to induce robust tolerance to the kidney allograft despite transient mixed chimerism in non-human primates and humans. Evidence suggests that durable mixed chimerism may be required for tolerance induction of all types of allografts. In this study, we investigate whether co-infusion of amnion-derived multipotent progenitor (AMP) cells, which have unique immunomodulatory properties, can promote durable mixed allogeneic chimerism induction in a non-human primate model. Results suggest that intravenous infusion of high dose of 3rd party AMP cells did not lead to prolonged mixed chimerism, which was associated with the inability of AMP cells to traffic to or persist in the target organs. We will determine whether intrabone injection of AMP cells will allow these cells to exert their immunosuppressive activities to promote durable mixed chimerism induction.

15. SUBJECT TERMS
Nothing listed

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
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1. INTRODUCTION

Composite tissue reconstruction in the form of composite tissue allografts (CTA) represents a therapeutic option in the treatment of congenital abnormalities, oncologic surgery and traumatic injuries. Immunological rejection remains the major barrier for this therapy and its avoidance requires life-long potent immunosuppression, which itself has life-threatening adverse effects. Induction of tolerance to allografts is the ideal solution. Mixed chimerism induction via hematopoietic cell transplantation (HCT) has been shown to facilitate tolerance induction to kidney allografts in non-human primates and humans despite the transience of donor chimerism. However, evidence indicates that durable mixed chimerism may be required for tolerance induction to tissues or organs other than kidney. Amnion-derived multipotent progenitor (AMP) cells possess a unique immune phenotype and low immunogenicity and demonstrate immunosuppressive activities in vitro and in vivo in mouse models. In this project, we are investigating whether co-transplantation of AMP cells can promote the induction of durable mixed allogeneic chimerism in a highly clinically relevant non-human primate HCT model.

2. KEY WORDS

Mixed allogeneic chimerism, tolerance, amnion-derived multipotent progenitor (AMP) cells, non-human primate, composite tissue allograft, intrabone injection

3. ACCOMPLISHMENTS

3a. Goal of this project

The major goal of this project is to develop an optimal regimen combining non-myeloablative conditioning, infusion of AMP cells and transient post-transplant immunosuppression for the induction of durable mixed allogeneic chimerism in cynomolgus monkeys so that tolerance will be applicable to CTA and all donor organ types.

We aim to extend and improve upon the transient chimerism achieved using the established non-myeloablative regimen consisting of low dose TBI, co-stimulatory blockade and 28 days of post-transplant immunosuppression with either cyclosporine or rapamycin. We will address the hypothesis that permanent, multilineage mixed chimerism will be achieved and this will be associated with robust donor-specific tolerance when AMP cells are given in the early post-BMT period.

3b. Accomplishments under these goals

3b-1. Specific Objective: Within this annual reporting period, we aimed to determine whether co-transplantation of the highest dose of AMP cells via intrabone injection would promote the induction of durable mixed allogeneic chimerism in non-human primates. To this end, we have performed bone marrow transplantation in two animals with a high dose of AMP cells (85-100 million/kg) via intrabone injection (AN620D and BF869D) and a similar transplant in one animal (BY648F) that did not receive AMP cells. We have monitored the chimerism of these animals and performed in vitro assays to monitor their responses to donor alloantigens.

3b-2. Major Activities: To investigate the effects of the highest dose of AMP cells on the induction of durable mixed allogeneic chimerism in cynomolgus monkeys, the animals were conditioned with horse ATG, anti-CD40L, total body (two doses of 1.25Gy) and thymic irradiation (7Gy). In addition, rapamycin was administered for 30 days and tapered to 0 over

the subsequent 21 days. One animal received intra-bone donor bone marrow and AMP cell injections (80-100 million/kg) on Day 2, one received IV bone marrow and intra-bone AMP cells on Day 2, and one control received only IV bone marrow on Day 2. For one of the animals receiving AMP cells (BF869D), we screened the candidate animals for the serum levels of anti-AMP cell natural antibodies to select an animal with the lowest levels as the hematopoietic cell transplant recipient. Following BMT, we monitored donor chimerism in peripheral blood by flow cytometric analysis twice a week and monitored the CMV levels in peripheral blood by PCR twice a week. Levels of rapamycin in blood were also monitored. In one animal that received AMP cells (BF869D) and the control animal (BY648F), mixed lymphocyte reactions and skin grafts were performed to determine the responses of host animals to donor alloantigens. One animal receiving intrabone injection of AMP cells (BF869D) is still being monitored at time of submission of this report.

3b-3. Significant Results and conclusions: Data from these animals lead to the following major findings:

- ***Transient mixed chimerism in the absence of overt CMV activation in the control animal***

  To determine whether co-transplantation of AMP cells can enhance induction of mixed allogeneic chimerism, the duration of mixed chimerism in animals receiving AMP cells needs to be compared to that of control animals not receiving AMP cells. In the first year of this project, we performed one transplant in a control animal (AT468G). This control animal needed to be euthanized unexpectedly due to clinical deterioration at week 7 post-transplantation. Thus, the actual duration of mixed chimerism in the absence of any interfering factors was unknown. In this reporting period, we obtained results from the second control animal (BY648F). This animal was transplanted under the same conditions as the previous control (AT468G). Donor chimerism monitoring results demonstrated the engraftment of donor hematopoietic cells with mixed chimerism in multiple lineages, including granulocytes, monocytes, NK, B and T cells. Donor chimerism in all non-lymphocyte lineages (granulocytes and monocytes) peaked at about 3 weeks post-transplantation and then started to drop, while donor chimerism in lymphocyte lineages (T, B and NK cells) remained persistently low. Chimerism in all lineages became undetectable by Day 43 and afterwards (Figure 1A). Only mild reactivation of CMV was detected on around Day 14 and with low levels of CMV viremia, which was well controlled by anti-viral therapy over the whole observation period (Figure 1B). Mixed lymphocyte reaction on Day 62 showed robust responses of the recipient to the donor stimulators, confirming that no tolerance was induced to the donor alloantigens in this animal (Figure 2). On Day 90, donor skin, along with a third party and an autologous recipient skin, was grafted to this animal. Results showed that the recipient rejected both the donor and the third party skin allografts around the same time between day 14 and 17. Unfortunately, histologically both grafts showed severe epithelial damage potentially from the cryopreservation process that hindered the interpretation of the results, although the macroscopic assessment of the grafts support the previous findings. In addition, minimal epithelial damage and dermal inflammatory infiltrates were observed in the self-graft. Thus, the duration of mixed chimerism in this control animal was used for comparison to that of animal receiving AMP cells in the following experiments.

- ***Screening of candidate animals for serum anti-AMP cell natural antibodies leads to selection of optimal transplant recipient***

  Our previous data demonstrated the presence of anti-AMP cell natural antibodies in the recipient, suggesting that these antibodies contribute to the prompt disappearance of AMP cells following infusion and thus may be partially responsible for the lack of immunosuppressive effects mediated by AMP cells on mixed chimerism recipients. To solve this issue, we screened the candidate animals for the serum levels of anti-AMP cell natural antibodies so that we could select an animal with the lowest levels as the hematopoietic cell transplant recipient in the next experiment. Serum from three animals was isolated and AMP cell samples from two lots reserved for our experiment were obtained from Noveome Biotherapeutics, Inc. Complement-dependent cytotoxicity assay was performed to determine the levels of anti-AMP cell natural antibodies in these animals. As shown in Figure 3, serum from the animal BF869D demonstrated undetectable and extremely low levels of antibodies against AMP cells lot numbers 150192R and 150242R, respectively. Meanwhile, the other two candidates demonstrated much higher levels of antibodies against both lots of AMP cells. Based on these results, BF869D was chosen as the next BMT and AMP cell recipient receiving AMP cells from lots 150192R and 150242R. The inclusion of AMP cell lot 150242R in the experiment, despite the extremely low but detectable anti-AMP cell antibodies in serum of BF869D, was because the cell number from a single lot of AMP cells (150192R) was too low to achieve the desired dose/kg. These data indicate that the anti-AMP cell natural antibodies varied in different animals and selection of animals with low anti-AMP cell natural antibodies was warranted and was supported by the favorable results in the second animal receiving introbone injection of AMP cells described below.

- ***Intrabone injection of AMP cells was associated with prolonged mixed allogeneic chimerism***

  Our previous data demonstrated that intravenous infusion of AMP cells resulted in the accumulation of AMP cells in the lungs, where they might be promptly eliminated by hematopoietic cells and destroyed by pre-existing natural anti-AMP cell antibodies. These results led us to hypothesize that the lack of impact of human AMP cells on mixed chimerism might be due to their inability to travel to and/or persist in the target cell tissues where they could exert their immunosuppressive effects. To address this issue, we performed one transplant (AN620D) with AMP cells administered by intrabone injection on Sept 15, 2016. We hypothesize that direct delivery of AMP cells to the recipient bone marrow by intrabone injection will enable AMP cells to exert their immunosuppressive effects to protect the engrafted donor bone marrow, thus promoting the induction of durable mixed chimerism. This animal was conditioned with horse ATG, anti-CD40L, total body and thymic irradiation. On Sept 15, 2016, 900 million AMP cells (at the dose of 100 million/kg), together with donor bone marrow cells (8.08×108 total BM cells/kg), were mixed with a matrigel in 20 mL and injected into the bilateral tibias of the recipient. However, this animal had difficulty recovering from anesthesia following intrabone injection. Ultrasound examination suggested embolism to the heart and pulmonary arteries. With the inability to wean the animal from anesthesia, we decided to euthanize this animal on Sept 16, 2016. Autopsy confirmed the presence of embolism in the pulmonary arteries and right ventricle. Tissues, including bone marrow aspirates, peripheral blood and the blood clots were taken and sent for pathological analysis. In the right ventricular clot, bone marrow was found with tri-lineage

hematopoiesis and 3-4% clear vacuoles, probably adipocytes/fat from bone marrow (normal bone marrow constituent). Some of the vacuoles were larger than typical adipocytes (differential: disrupted adipose tissue, air, or occasionally a foreign substance such a silicone may give the same histological effect). Trichrome staining highlighted a patchy fine peri-cellular meshwork (stroma), which supported the finding that the clot represented bone marrow tissue, not just cells of bone marrow origin that migrated in the circulation.

To address whether the AMP cells or other factors caused this adverse effect, we attempted to detect AMP cells in the tissues taken from this animal by flow cytometry and qPCR. Our results showed that AMP cells could only be detected in the bone marrow aspirate from the injection site, but not other tissues (Figure 4). However, since it was not possible to detect cells in the blot clot, qPCR detecting the human DNA was used to address whether there were AMP cells in the blot clot and other tissues. As shown in Table 1, consistent with the flow cytometry data, human DNA was detected in the bone marrow aspirate from the injection site, confirming the presence of AMP cells. However, human DNA was also detected in peripheral blood and bone marrow aspirate from the non-injection site at lower levels. The presence of human DNA in these tissues suggested that AMP cells were lysed following injection and human DNA thus entered the systemic circulation. We did not detect any human DNA in the blood clot and this result did not support the possibility that the injected AMP cells triggered the embolism and was consistent with our previous observation that intravenous injection of AMP cells did not result in embolism formation. Collectively, the data suggested that the collagen in the matrigel and/or disruption of autologous bone marrow stroma triggered the embolism.

To avoid the adverse effects described above, we modified our protocol to exclude the matrigel in the intrabone injection and only AMP cells, not together with donor bone marrow cells, were injected via intrabone injection. The volume of cell suspension injected into each bone was also reduced. After obtaining approval of the modified protocol from IACUC at Columbia University and the USAMRMC Animal Care and Use Review Office (ACURO), we performed one experiment in which animal BF869D underwent hematopoietic cell transplant IV with intrabone injection of AMP cells on May 11, 2017. This recipient was screened to have extremely low levels of anti-AMP cell antibodies against the AMP cells it would receive via intraobone injection prior to this transplant. This animal was conditioned with horse ATG, anti-CD40L, total body and thymic irradiation. In addition, rapamycin was administered for a course of 30 days and tapered down for the following 21 days. The animal was infused intravenously with donor bone marrow cells (8.86×10^8 total BM cells/kg; 15×10^6 CD34+ cells/kg, 38.5×10^6 CD3 T cells/kg). AMP cell lots 150192R and 150242R were thawed, with 2.7×10^8 and 4.4×10^8 AMP cells recovered respectively. Each lot of AMP cells were resuspended in 5ml of plasmalyte and injected to one tibia of the recipient via intrabone injection. The dose of AMP cells was 85million/kg. The intrabone injection of AMP cells was successfully completed without any complication observed clinically.

As in the control animal (BY648F) not receiving AMP cells, BF869D's donor chimerism in multiple lineages was detectable in the second week and peaked at week 3 following transplantation, with donor chimerism much higher in granulocytes and monocytes than that in lymphocytes. Unlike the control animal, in which donor chimerism started to

declined once it reached its peak, donor chimerism in this animal persisted at similar levels in all lineages, although chimerism in B cells first showed a decline at week 3 and week 4 and later reappeared around week 6. Donor chimerism persisted to Day 76 following transplant and then started to decline and became undetectable after Day 83 post-transplant (Figure 5). Similar to the control animal, only mild reactivation of CMV was detected in the fourth week with low level of CMV viremia, which was well controlled by anti-viral therapy over the whole observation period (Figure 6). *In vitro*, the response against the donor on Day 77 was decreased compared to the third party stimulators (Figure 7). Skin grafts were performed on Day 90 on this animal to determine whether or not the recipient developed tolerance to the donor. Evaluation of the skin grafts is ongoing. Thus, compared to the control animal not receiving AMP cells, the AMP cell recipient demonstrated prolonged donor chimerism, suggesting that AMP cells were able to mediate immunosuppressive effects in vivo on anti-donor alloresponses. These results warrant further studies to confirm these findings.

3C. Opportunities for training and professional development provided by this project

This project has provided training opportunities for Paula Alonso Guallart, who is poised to achieve her PhD.

3D. How were the results disseminated to communities of interest?

They have not yet been reported.

3E. What do you plan to do during the next reporting period to accomplish the goals?

The results described above suggest that AMP cells when administered by intrabone injection are able to mediate immunosuppressive effects to prolong mixed allogeneic chimerism. Based on the results described above, we plan to repeat this experiment twice to confirm these findings in the next reporting period.

Figures

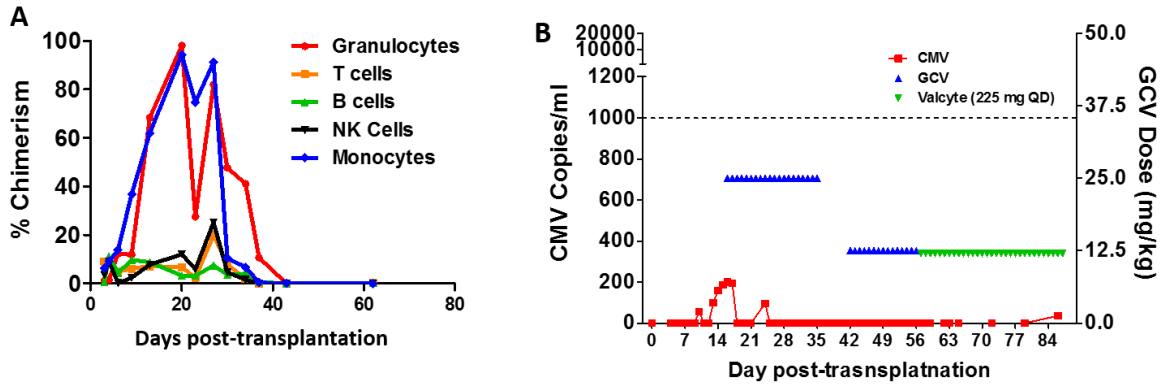


Figure 1. A. Donor chimerism of the control animal not receiving AMP cells in all lineages following transplantation. B. Reactivation of CMV following transplantation in the control animal not receiving AMP cells.

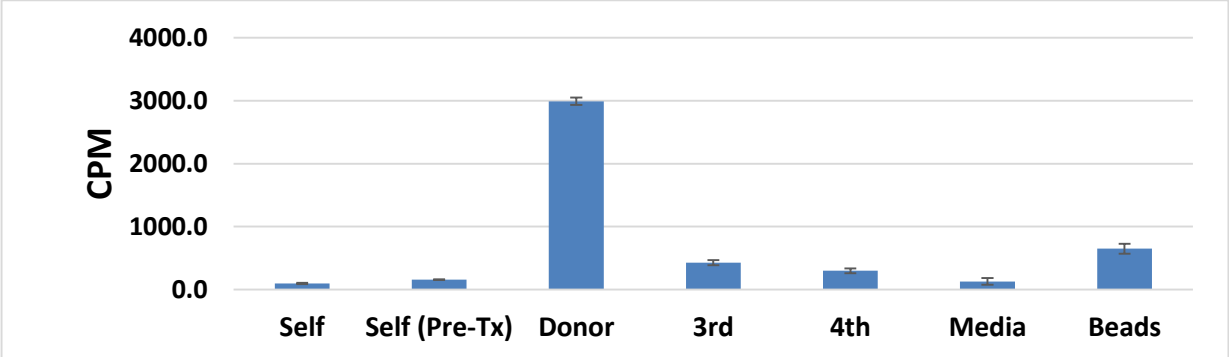


Figure 2. Mixed lymphocyte reaction to determine the responses from BY648F on Day 62 to the donor following transplantation. Recipient PBMCs were used as responder cells. Stimulator cells include recipient PBMCs harvested on Day 62 and prior to transplantation (designated as Self and Self (Pre-Tx) respectively),Donor, 3rd and 4th party PBMCs. Recipient PBMCs cultured with media served as negative control and with beads as positive control.

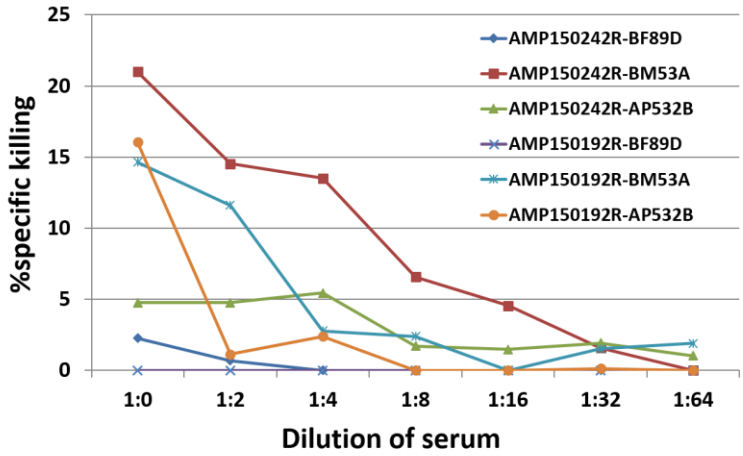


Figure 3. Levels of anti-AMP cell natural antibodies in potential transplant recipients. The serum levels of anti-AMP cell natural antibodies from three candidate animals (BF89D, BM53A and AP532B) against two lots of AMP cells (AMP150242R and AMP150192R) were determined by complement-dependent cytotoxicity assay. As shown, animal BF89D demonstrated undetectable levels of anti-AMP cell natural antibodies against AMP cell lot AMP150192R.

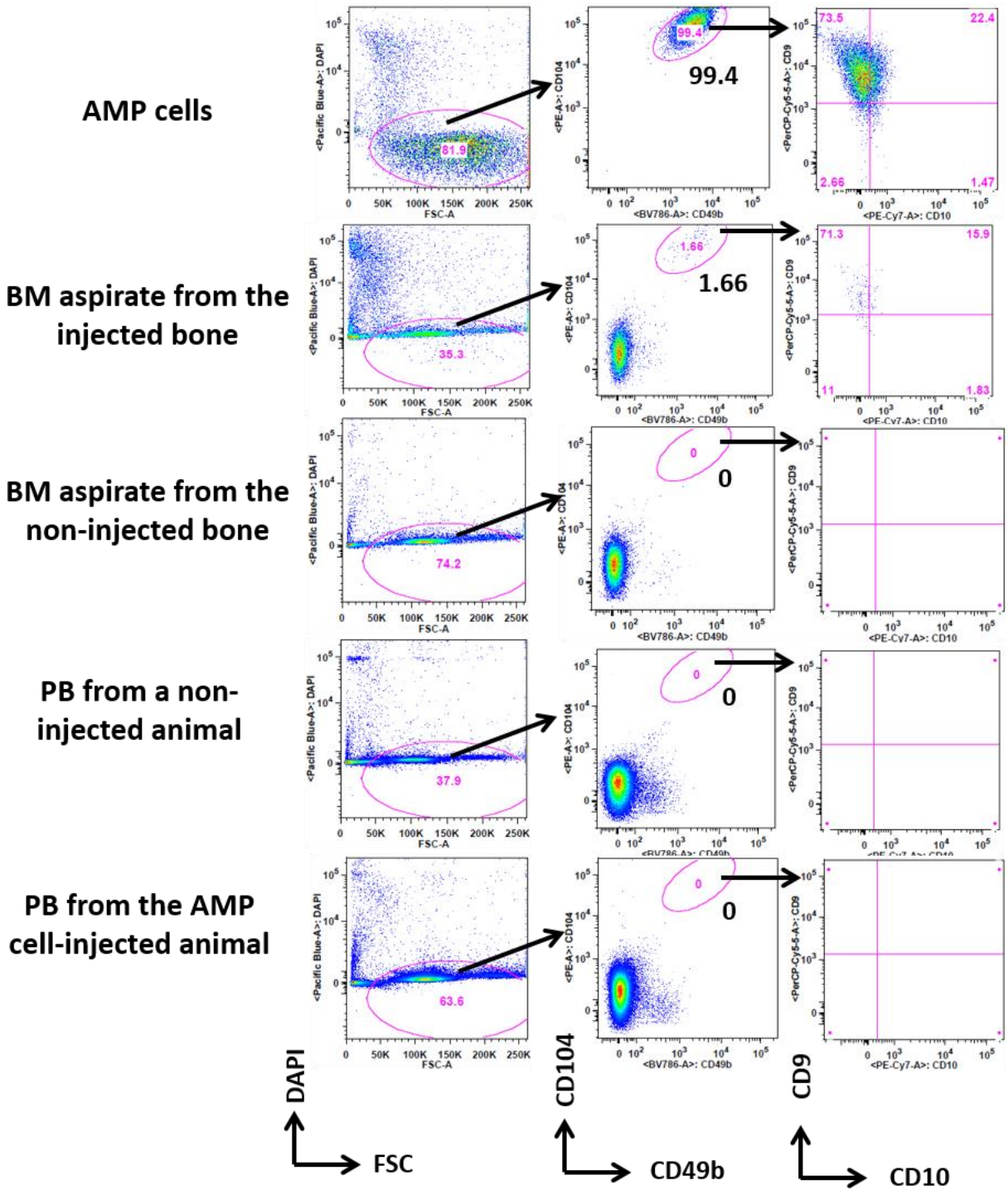


Figure 4. Detection of injected AMP cells in the bone marrow aspirate from the injection site by flow cytometry. AMP cells have a phenotype of CD49b+CD104+CD10+CD9+. Cells with this phenotype were only seen in the BM aspirate from the AMP cell intrabone injection site, but not in other tissues.

	Human DNA concentration (ng/μL)
AMP cells	**57.24**
Peripheral blood from a non-injected animal	**0**
Bone marrow aspirate from injection site	**0.73**
Bone marrow aspirate from non-injection site	**0.09**
Peripheral blood from the injected animal	**0.44**
Blood clot	**0**

Table 1. Detection of human DNA in tissues by qPCR. DNA was purified from AMP cells and tissues taken from the animal receiving intrabone injection of AMP cells and one animal without injection. Quantitative PCR (qPCR) was performed to quantify the amount of human DNA in these samples.

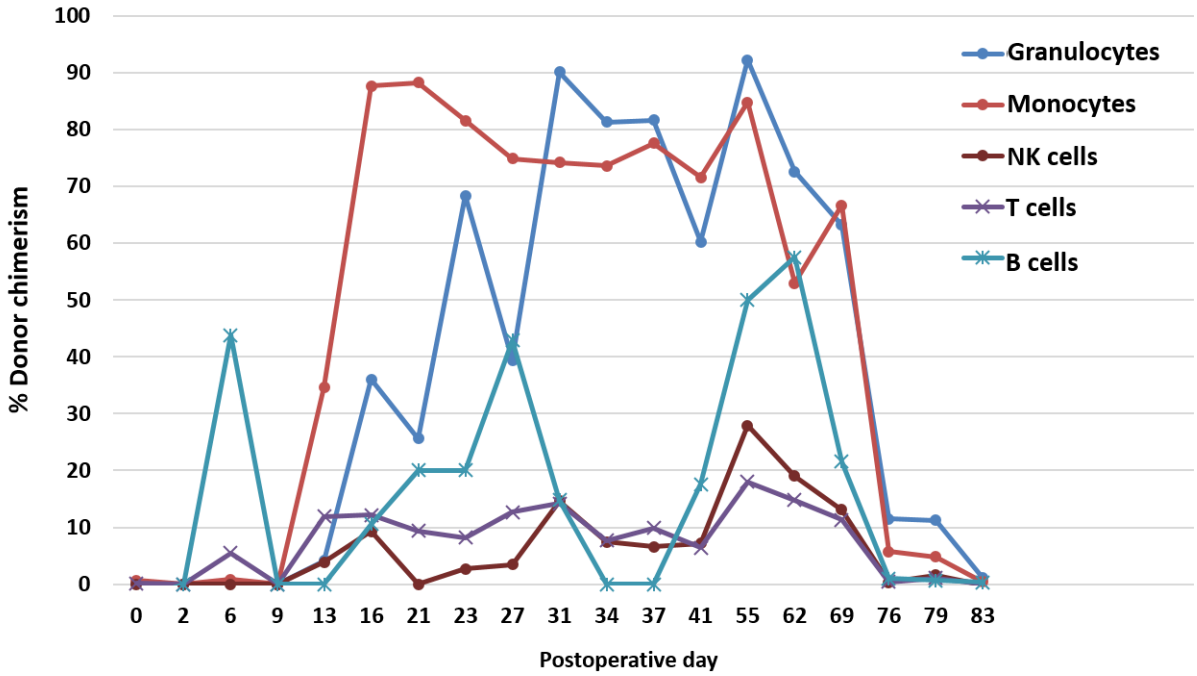


Figure 5. Donor chimerism of the animal receiving AMP cells via intrabone injection in all lineages following transplantation.

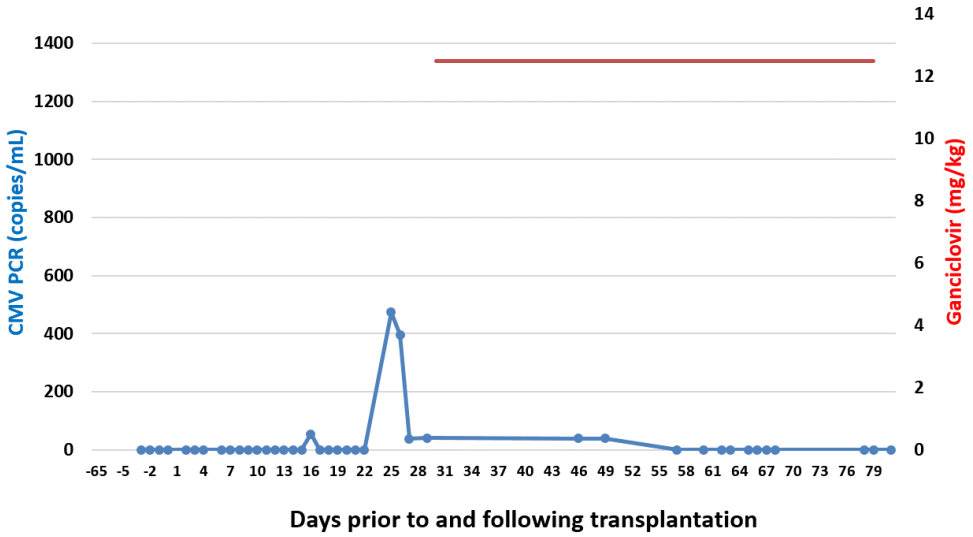


Figure 6. Reactivation of CMV following transplantation in the animal receiving AMP cells via intrabone injection.

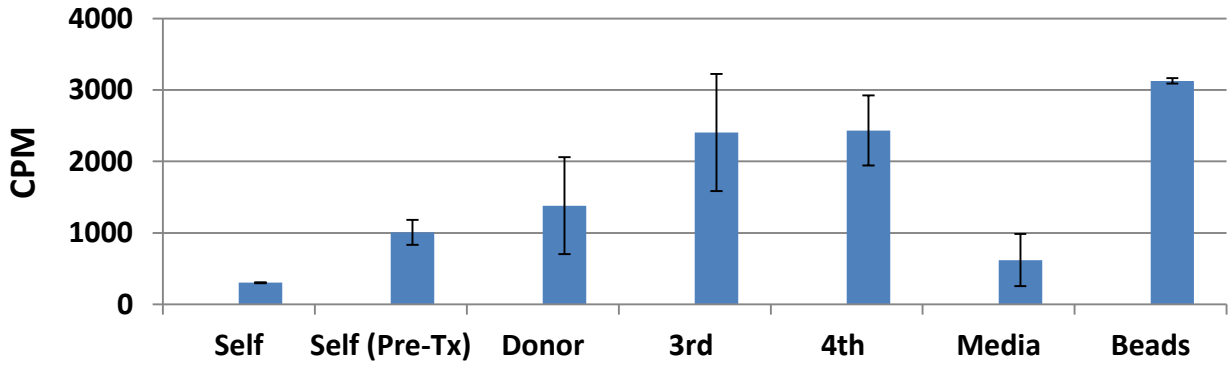


Figure 7. Mixed lymphocyte reaction to determine the responses from BF869D to the donor on Day 77 following transplantation. Recipient PBMCs were used as responder cells. Stimulator cells include recipient PBMCs harvested on Day 62 and prior to transplantation (designated as Self and Self (Pre-Tx) respectively), Donor, 3rd and 4th party PBMCs. Recipient PBMCs cultured with media served as negative control and with beads as positive control.

4. IMPACT

4A. What was the impact on the development of the principle discipline(s) of the project?

Our data showing that intrabone injection of high dose of human AMP cells prolonged mixed allogeneic chimerism in non-human primate are consistent with and verify the immunosuppressive effects of human AMP cells shown in mouse models. If confirmed to be reproducible, these results will support the clinical trial of using these cells to promote mixed chimerism induction in human patients undergoing hematopoietic cell transplant for induction of tolerance to transplanted organs and tissues.

4B. What was the impact on other discipline(s)?

Our previous demonstration of monkey anti-human natural antibodies has implications for the testing of other human cell types in monkey models. We further showed that screening the candidate recipient animals for low anti-human natural antibodies is feasible and should be used to identify the optimal recipient.

4C. What was the impact on technology transfer?

We have shared on results with Noveome Biotherapeutic (formerly Stemnion Inc.), who has provided the AMP cells for the study.

4D. What was the impact on society beyond science and technology?

None so far.

5. CHANGES/PROBLEMS

5A. Changes in approach and reasons for change

There will be two major changes in approach. As we have found that monkeys have anti-AMP cell natural antibodies, which may lead to rapid destruction of the AMP cells, we have screened the candidate animals for the levels of anti-AMP cell natural antibodies to choose a recipient with extremely low levels of anti-AMP cell antibodies prior to this transplant. The inability of AMP cells to travel to and persist in target tissues may have hampered their immunosuppressive effects. Instead of intravenous injection, we have performed intraosseus injection of AMP cells so that they can directly exert their immunosuppressive effects in bone marrow.

5B. Actual or anticipated problems or delays and actions or plans to resolve them

Noveome Biotherapeutic has reduced the manufacture of AMP cells, which may decrease our chance to obtain a recipient with low anti-AMP cell natural antibodies for the AMP cells it will receive. We will discuss with the company about maximizing their production of this cell type for this project.

5C. Changes that had a significant impact on expenditures

Nothing to report

5D. Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

After we encountered an adverse effect in the first intrabone injection of AMP cells to an animal, we report this event and submitted revision of our intrabone injection protocol to the IACUC at Columbia University and the USAMRMC Animal Care and Use Review Office (ACURO). Approval of the protocol was obtained from both institutions before we performed intrabone injection in the second animal in which no adverse effects were seen following intrabone injection. We will follow the current protocol in our future studies.

6. PRODUCTS:

6A. Publications, conference papers, and presentations

Nothing to report

6B. Journal publications

Nothing to report

6C. Books or other non-periodical, one-time publications

Nothing to report

6D. Other publications, conference papers, and presentations

Nothing to report

6E. Website(s) or other Internet site(s)

Nothing to report

6F. Technologies or techniques

Nothing to report

6G. Inventions, patent applications, and/or licenses

Nothing to report

6H. Other Products

Nothing to report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

7A. What individuals have worked on the project?

Name:	Megan Sykes (No change)
Project Role:	
Researcher Identifier (UNI of Columbia University):	
Nearest person month worked:	
Contribution to Project:	
Name:	Adam Griesemer (No change)
Project Role:	
Researcher Identifier (UNI of Columbia University):	
Nearest person month worked:	
Contribution to Project:	
Name:	Paula Alonso Guallart (No change)
Project Role:	
Researcher Identifier (UNI of Columbia University):	
Nearest person month worked:	
Contribution to Project:	
Name:	Dil Ekanayake-Alper (No change)
Project Role:	
Researcher Identifier (UNI of Columbia University):	
Nearest person month worked:	
Contribution to Project:	
Name:	Hao Wei Li (No change)
Project Role:	
Researcher Identifier (UNI of Columbia University):	
Nearest person month worked:	
Contribution to Project:	

Others with nominal effort:
Chengshie Wuu, radiation physicist <1 month effort in supervising radiations; Marcus Pereira, infectious disease clinician educator <1 month effort providing infectious disease expertise as a collaborator on the cynomolgus monkey studies; also two technicians <1 month effort covering animal care and procedures.

7B. Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Yes, see attached

7C. What other organizations were involved as partners?

Noveome Biotherapeutic (formerly Stemnion Inc.)

8. SPECIAL REPORTING REQUIREMENTS

8A. Collaborative awards

Nothing to report

8B. Quad charts

Attached

8C. Appendices

None

9. Appendix

None

Establishment of donor chimerism in non-human primates using allogeneic bone marrow with AMP cell co-infusion.

Insert ERMS/Log Number and Task Title Here
Insert Award Number Here

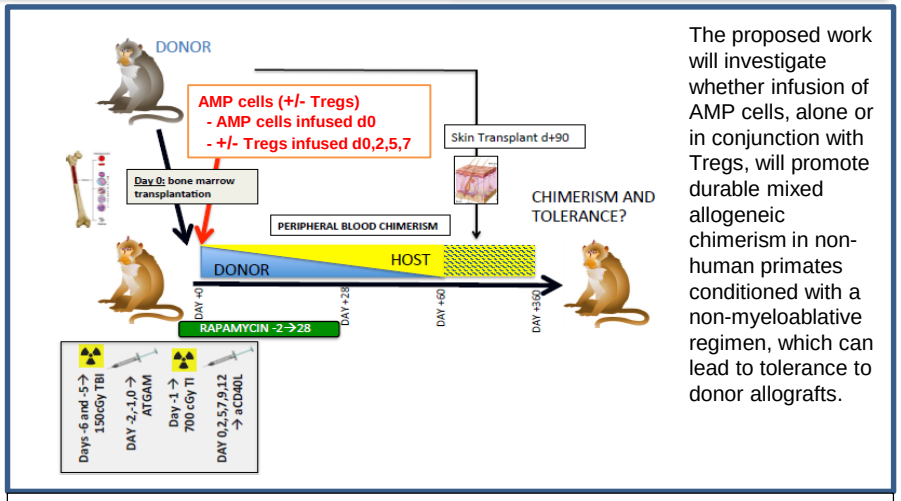
PI: Megan Sykes, MD **Org:** Columbia University **Award Amount:** $1,200,000 total cost dollars

Study/Product Aim(s)

- Determine whether co-transplantation of amnion-derived multipotent progenitor (AMP) cells, alone or with regulatory T cells (Tregs), can promote durable mixed chimerism and induce tolerance
- Modification of bone marrow transplantation protocol for clinical cadaveric organ transplantation

Approach

AMP cells, alone or together with Tregs, will be co-transfused via intrabone injection with allogeneic bone marrow cells to non-human primates conditioned by a non-myeloablative regimen to induce mixed hematopoietic chimerism. Donor chimerism will be followed and tolerance will be determined by skin graft.



The proposed work will investigate whether infusion of AMP cells, alone or in conjunction with Tregs, will promote durable mixed allogeneic chimerism in non-human primates conditioned with a non-myeloablative regimen, which can lead to tolerance to donor allografts.

Accomplishment: Demonstrated that expanded recipient Tregs can enhance and prolong chimerism and prolong donor skin graft survival

Timeline and Direct Cost Dollars

Activities CY	15	16	17	18
Determine effects of AMP cells alone via intravenous infusion	→	→		
Determine duration of mixed chimerism in animals without AMP cell infusion	→	→		
Determine effects of AMP cells alone via intrabone infusion		→	→	
Estimated Budget (direct $)	$378,434	$371,566		

Updated: (9/13/17)

Goals/Milestones

CY15 Goal – Determine the effects of AMP cells infused i.v. on mixed chimerism induction

- ❑ Start studies with infusion of the highest dose of AMP cells

CY16 Goals – Determine the effects of AMP cells infused i.v. and intrabone on mixed chimerism induction

- ❑ Complete studies with infusion of the highest dose of AMP cells infused i.v.
- ❑ Start studies with infusion of AMP cells via intrabone injection

CY17 and CY18 Goal – Determine the effects of AMP cells infused intrabone on mixed chimerism induction

- ❑ Complete studies with infusion of AMP cells via intrabone injection

Budget Expenditure to Date

Projected Expenditure: $1,200,000 total cost (two years)
Actual Expenditure: $1,014,523 total cost (end of second year)

Other Support, Megan Sykes, MD

ACTIVE

P01 AI045897 (PI: Sykes/Sachs) 08/17/17 – 07/31/21 1.8 calendar months
NIH/NIAID

A Tolerance Approach to Xenotransplantation; Proj 3: Tolerance of Adaptive and Innate Human Anti-Pig Immune Responses in Humanized Mice (Proj. Leader Sykes); Admin Core leader

The overall goal of this application is to use a humanized mouse, pig and primate models to optimize immune tolerance induction to avoid graft rejection while assuring a well-functioning immune system and optimizing the function of transplanted pig organs.

U19 AI131474 (PI: Sykes) 8/18/17 - 07/31/22 2.64 calendar months NIH-NIAID

Regulatory T cells to promote mixed chimerism for tolerance to islets and kidneys from deceased and living donors

The major goal of the studies in this proposal is overcome limitations to successful islet and kidney transplantation for both deceased donor (Project 1) and living donor (Project 2) transplantation of donor blood-forming cells to induce tolerance through mixed chimerism facilitated by a recipient regulatory cell population.

P01 AI106697 (PI: Farber) 6/1/13 - 05/31/18 1.20 calendar months
NIH/NIAID

Tissue compartmentalization of human lymphocytes; Proj 4: Lymphocyte reconstitution and responses in intestinal transplantation (Proj. Leader: Sykes)

The goal of this project is to investigate the causes of intestinal graft rejection, leading to future development of markers that predict rejection and acceptance intestinal and ultimately other types of transplants.

UC4DK104207 (PI: Sykes) 9/25/14 – 06/30/19 1.80 calendar months
NIH/NIDDK

Mice with autologous human T1D-derived immune systems and iPSC-derived beta cells

The major goal of the studies in this proposal is to use a unique “Personalized Immune” mouse model to establish models for understanding how Type 1 diabetes develops using insulin-producing β cells made from the patient’s own stem cells.

P01HL018646 (PI: Madsen) 11/14/14 – 10/31/19 0.06 calendar months
NIH/NHLBI

New Approaches to Cardiothoracic Tolerance Induction (Co I: Sykes)

The unifying goal of this program project is to combine mixed chimerism with novel strategies designed to amplify the contributions of Tregs in order develop a clinical tolerance protocol that can be rapidly translated to human recipients of heart and lung allografts.

R01 DK103585-01A1 (PI: Sykes) 04/01/15 – 03/31/19 0.96 calendar months
NIH/NIDDK/OD

Immune response to iPSC-derived beta cells in Type 1 diabetes

The major goal of the studies in this proposal is to use a unique “Personalized Immune” mouse model to establish models for understanding how Type 1 diabetes develops and how stem cell-derived β cells can best be used to replace the insulin-producing cells that are destroyed in diabetes.

R01 OD017949 (Sykes) 07/01/15 – 03/31/20 0.96 calendar months
NIH/OD

Robust allograft tolerance in non-human primates (plus equipment supplement)

The major goal of the studies in this proposal is to explore a strategy for achieving durable mixed chimerism across MHC barriers in primates with minimal toxicity, achieving tolerance that would be relevant for all types of organ and tissue allografts.

W81XWH-15-1-0234 (PI: Sykes) 08/15/15 – 08/14/17 0.24 calendar months
DoD Reconstructive Transplantation Research

Establishment of donor chimerism using allogeneic bone marrow with AMP cell co-infusion
The goal of this to project is to determine if the addition of AMP cells can permit the achievement of durable chimerism in a well characterized cynomolgus macaque bone marrow transplant (BMT) model that otherwise achieves only transient chimerism without GVHD across MHC barriers.

Spons. Proj. Agreement (PI: Sykes/Sachs) 08/01/15 – 07/31/18 1.2 calendar months United Therapeutics/Lung Biotechnology
Collaboration on Xenograft Lung Tolerance
The goal of this sponsored research is to establish lung xenograft tolerance in preclinical, large animal models.

Spons. Research Agreement (PI: Sykes) 02/18/16-01/17/18 0.06 calendar months CELLDEX Therapeutics
Combined use of Flt3L and rapamycin to promote durable mixed allogeneic chimersim
The goal of this sponsored research is to determine if the addition of Flt3L and rapamycin to a non-myeloablative conditioning regimen will promote sustained, rather than temporary engraftment of HLA-disparate hematopoietic cell transplants in humanized mice.

U01AI063594-13 (PI: Heeger) 10/1/16 – 8/31/17 0.24 calendar months NIH/NIAID
Effects of inhibiting early inflammation in kidney transplant patients
The goal of this 1 year ancillary pilot study is to assess the feasibility of working with samples collected from the CTOT-19 multicenter clinical trial, and to determine whether alterations in TCR repertoires over time can be detected and are informative.

Sponsored Research Project (PI: Sykes) 02/14/17-08/13/2017 0.12 calendar months ITB-MED
Effect of Siplizumab on natural Tregs and effector T cells
The goal of this study is to use healthy donor PBMCs to determine the impact of siplizumab on retention/expansion of Tregs vs. naïve and effector T cells and on induction of Tregs from effector T cells.

PENDING
R34AI120911 (PI: Kato/Sykes/Lavine) 12/01/17-11/30/18 0.6 calendar months NIH/NIAID
Study of Intestinal Transplantation with Emphasis on Rejection
The goal of the study is to improve outcomes in patients undergoing intestinal transplantation through a better understanding of intestinal graft rejection, and to study the immune mechanisms behind intestinal rejection, which will hopefully lead to methods of inducing immune tolerance.

R21TR002279 (PI: Sykes) 11/01/17-10/31/19 0.6 calendar months NIH/NCATS
TCR and BCR deep sequencing to distinguish autoimmune recurrence from allograft rejection
The goal of this project is to distinguish rejection from recurrent autoimmune liver disease after liver transplantation by using a sequencing approach to identify and track the B and T cell clones that cause autoimmunity and rejection, and to better understand the interplay between alloimmune and autoimmune responses causing liver transplants to fail and ultimately provide better treatment to patients needing transplants for these diseases.

R01 (Multi-PI: Yamada/Sykes) 4/1/18-3/31/23 0.6 calendar months NIH/NIAID
Intestinal allograft tolerance in large animals
The overall goal of this proposal is to develop a large animal preclinical model, for tolerance induction following intestinal transplantation (ITx) and to develop a conditioning protocol appropriate for tolerance induction in parent to child (living donor LD) ITx.

OVERLAP

None of these projects overlap. However, efforts will be adjusted as to not exceed 12 person months should pending proposals be awarded.

Other Support, Adam Griesemer, MD

ACTIVE

U19 AI131474 (PI: Sykes) 8/18/17 - 07/31/22 1.92 calendar months NIH-NIAID Co-Investigator ***Regulatory T cells to promote mixed chimerism for tolerance to islets and kidneys from deceased and living donors***

The major goal of the studies in this proposal is overcome limitations to successful islet and kidney transplantation for both deceased donor (Project 1) and living donor (Project 2) transplantation of donor blood-forming cells to induce tolerance through mixed chimerism facilitated by a recipient regulatory cell population.

R01 OD017949 (Sykes) 7/1/15 – 6/30/20 0.96 calendar months NIH-NIAID Co-Investigator

Robust allograft tolerance in non-human primates

The major goal of this study is to use *in vitro*-expanded recipient regulatory T cells to induce permanent mixed chimerism and tolerance following MHC-mismatched bone marrow transplantation in a translational nonhuman primate model.

W81XWH-15-1-0234 (Sykes) 08/15/15 – 08/14/17 0.36 calendar months DOD Co-Investigator ***Establishment of donor chimerism using allogeneic bone marrow with AMP cell co-infusion***

The goal of this project is to enhance donor bone marrow engraftment and promote durable mixed chimerism and tolerance by utilizing co-infusion of amnion-derived multipotent progenitor cells along with MHC-mismatched bone marrow transplantation in nonhuman primates.

P01 AI045897 (multi PI: Sykes/Sachs) 8/17/17 - 7/31/21 0.96 calendar months NIH/NIAID Co-Investigator

A Tolerance Approach1 to Xenotransplantation; Proj 2: Achieving Xenograft Tolerance through Mixed Chimerism

The overall goal of this application is to use a humanized mouse, pig and primate models to optimize immune tolerance induction to avoid graft rejection while assuring a well-functioning immune system and optimizing the function of transplanted pig organs.

Spons. Proj. Agreement (Sykes/Sachs) 8/1/15 – 7/31/18 1.2 calendar months United Therapeutics/Lung Biotechnology Project Leader ***Collaboration on Xenograft Lung Tolerance***

The goal of this sponsored research is to establish lung xenograft tolerance in preclinical, large animal models.

U19AI128949 (Farber) 1/1/17-12/31/21 0.48 calendar months NIH/NIAID Co-Investigator

Human anti-viral immune responses in tissues and circulation

The overall goal of this research program in human immunity is to obtain in-depth profiles of how human innate and adaptive immune cells in tissues respond to viral infection, with a focus on the globally pervasive herpesvirus, Cytomegalovirus (CMV). There are three projects to profile macrophage, Dendritic cell (DC) and T cell responses to viruses in different sites using single cell transcriptional and proteomics approaches.

R56AI031046-26 (Sachs) 02/08/17-1/31/18 0.48 calendar months NIH/NIAID

Co-Investigator ***Tolerance to Vascularized Allografts in Miniswine***

The goal of these studies is to develop an understanding of the mechanisms by which allograft tolerance is induced and maintained in this large-animal model, in order to permit development of appropriate protocols for induction of tolerance to organ allografts in the clinic.

Pilot Application (Chen/Yang) 3/1/17 - 02/28/18 0.36 calendar months JDRF Co-Investigator

Improving engraftment and survival of pig islet xenografts through transgenic expression of human CD47
The major goal of this one-year pilot research project is to demonstrate proof-of-concept that the transgenic expression of human CD47 (hCD47), a cell-surface “marker of self”, will prevent phagocytosis and improve porcine islet engraftment and survival in xenotransplantation.

U01AI100119 (Farber) 08/01/17-07/31/2 2 0.48 calendar months NIH/ NIAID Co-Investigator ***Development of lung T cell responses in infant respiratory immunity***
The goal of these studies is to elucidate mechanisms for the distinct properties of infant T cell responses, with a focus on investigating in situ priming of infant immune responses in tissues.

PENDING
None

OVERLAP
None

Other Support – Haowei Li, PhD

ACTIVE

W81XWH-15-1-0234 (PI: Sykes) 08/15/15 – 08/14/17 3.6 calendar months
DoD Reconstructive Transplantation Research $375,000
Establishment of donor chimerism using allogeneic bone marrow with AMP cell co-infusion
The goal of this project is to determine if the addition of AMP cells can permit the achievement of durable chimerism in a well characterized cynomolgus macaque bone marrow transplant (BMT) model that otherwise achieves only transient chimerism without GVHD across MHC barriers.
Role: Co-Investigator

P01HL018646 (PI: Madsen) 11/14/14 – 10/31/19 0.6 calendar months
NIH/NHLBI $64,078
New Approaches to Cardiothoracic Tolerance Induction (Co I: Sykes)
The unifying goal of this program project is to combine mixed chimerism with novel strategies designed to amplify the contributions of Tregs in order develop a clinical tolerance protocol that can be rapidly translated to human recipients of heart and lung allografts.
Role: Co-Investigator

Spons. Research Agreement (PI: Sykes) 02/18/16-01/17/18 1.8 calendar months
CELLDEX Therapeutics $63,334
Combined use of Flt3L and rapamycin to promote durable mixed allogeneic chimersim
The goal of this sponsored research is to determine if the addition of Flt3L and rapamycin to a non-myeloablative conditioning regimen will promote sustained, rather than temporary engraftment of HLA-disparate hematopoietic cell transplants in humanized mice.
Role: Co-Investigator

Noveome (fka Stemnion) research (PI: Sykes) 01/20/15-12/31/17 0.6 calendar months
Noveome, Inc. $53,078
Facilitating mixed allogeneic chimerism induction by co-transplantation of Amnion-derived Multipotent Progenitor Cells (AMP cells) in humanized mice with established immune system
The major goal of the studies in this proposal is to determine whether co- transplantation of amnion-derived multipotent progenitor cells (AMP cells) can promote induction of mixed allogeneic hematopoietic chimerism in humanized mice with established immune system.
Role: Co-Investigator

P01 AI045897 (PI: Sykes/Sachs) 08/01/17 – 07/31/21 6 calendar months
NIH/NIAID $1,712,717
A Tolerance Approach to Xenotransplantation; Proj 3: Tolerance of Adaptive and Innate Human Anti-Pig Immune Responses in Humanized Mice
The overall goal of this application is to use a humanized mouse, pig and primate models to optimize immune tolerance induction to avoid graft rejection while assuring a well-functioning immune system and optimizing the function of transplanted pig organs.

PENDING

None

OVERLAP

None.