AWARD NUMBER: W81XWH-15-1-0234

TITLE: Establishment of Donor Chimerism Using Allogeneic Bone Marrow with AMP Cell Coinfusion

PRINCIPAL INVESTIGATOR: Megan Sykes, MD

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

REPORT DATE: NOVEMBER 2018

TYPE OF REPORT: Final report

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	**2. REPORT TYPE**	**3. DATES COVERED**
NOVEMBER 2018	Final	15 AUG 2015–14 AUG 2018

4. TITLE AND SUBTITLE

Establishment of Donor Chimerism Using Allogeneic Bone Marrow with AMP Cell Coinfusion

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-15-1-0234

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

Megan Sykes, MD

E-Mail: ms3976@columbia.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

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

Columbia University Medical Center, New York, NY, 10032-3702

8. PERFORMING ORGANIZATION REPORT

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Materiel 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

While composite tissue reconstruction in the form of composite tissue allografts represents a therapeutic option in the treatment of congenital abnormalities, oncologic surgery and traumatic injuries, immunological rejection of the allograft 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. Induction of durable mixed allogeneic hematopoietic chimerism via hematopoietic cell transplant (HCT) has been shown to be a powerful approach to inducing tolerance to allografts in rodents. However, it is much more difficult to achieve durable mixed chimerism in large animals (Non-human primates) and humans. Human 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. We have investigated whether co-transplantation of human AMP cells can promote the induction of durable mixed allogeneic chimerism in a highly clinically relevant non-human primate HCT model. Our results show that intravenous injection of high dose (100million/kg) of AMP cells did not prolong mixed chimerism or facilitate induction of tolerance to an allograft, in association with lack of persistence of AMP cells in vivo. We detected anti-AMP cell natural antibodies in recipient monkeys, which may explain the lack of persistence of AMP cells in vivo. We then screened candidate animals to select those with low anti-AMP cell natural antibodies as transplant recipients and co-transplanted AMP cells via intrabone injection, hoping that direct delivery of AMP cells to their effector sites could facilitate exertion of their immunosuppressive effects. Among the two animals successfully performed using recipients with low anti-AMP cell natural antibodies and intrabone injection of AMP cells, one animal showed prolonged mixed chimerism, while the other did not. We conclude that co-transplantation of human AMP cells is not able to reliably prolong mixed allogeneic chimerism in our non-human primate HCT model.

15. SUBJECT TERMS

Mixed allogeneic chimerism, hematopoietic cell transplantation, tolerance, amnion-derived multipotent progenitor (AMP) cells, non-human primate, composite tissue allograft, intrabone injection, anti-AMP cell natural antibodies

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
					USAMRMC
a. REPORT	b. ABSTRACT	c. THIS PAGE			19b. TELEPHONE NUMBER *(include area code)*
U	Unclassified	Unclassified	Unclassified	31	

Table of Contents

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, hematopoietic cell transplantation, tolerance, amnion-derived multipotent progenitor (AMP) cells, non-human primate, composite tissue allograft, intra-bone injection, natural antibodies

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 30 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:

In the three-year reporting period, we aimed to determine whether co-transplantation of a high dose (100million/kg) of AMP cells would promote the induction of durable mixed allogeneic chimerism in non-human primates. To this end, we have co-transplanted high doses of AMP cells via intravenous and intra-bone injection.

3b-2. Major Activities:

We used a highly clinically relevant non-human primate HCT model to investigate whether co-transplantation of high dose of human AMP cells could promote induction of durable mixed allogeneic chimerism. In this model, the HCT recipients are conditioned with horse ATG, anti-CD40L, total body (two doses of 1.25 Gy) and thymic (7 Gy) irradiation. Rapamycin was administered for 30 days and tapered down for the following 21 days. On

Day 0, the animal received donor bone marrow cells, with or without AMP cells (100 million/kg, i.v. or intra-bone). 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. We first investigated the effects of intravenous injection of high dose of AMP cells (100million/kg) on chimerism and tolerance in two cynomolgus monkeys (BF585G and AL792C). The animals were conditioned and received donor bone marrow cells, with AMP cells (100 million/kg) intravenously. Donor skin was grafted to animal AL792C on Day 90. Anti-donor and anti-AMP antibodies in serum of animal AL792C were analyzed by complement-dependent cytotoxicity assay or indirect flow cytometry using anti-human IgM and anti-human IgG secondary antibodies. Mixed lymphocyte reactions were performed to determine the responses of host animals to donor alloantigens using peripheral blood mononuclear cells (PBMCs) as responder cells. The presence of AMP cells in tissues was determined by PCR. Concurrently, we performed two control animals (AT468G and BY648F) without the co-transplantation of AMP cells. The control animals received the same conditioning regimen. Results from these animals showed that co-transplantation of high dose of AMP cells was unable to promote induction of durable mixed chimerism or of tolerance to a donor skin graft, in association with lack of persistence of AMP cells in vivo. In addition, we found that anti-AMP cell natural antibodies existed in HCT recipients, which may cause rapid destruction of the infused AMP cells.

Thus in the ensuing experiments, we employed two approaches to solving these problems. First, we used a complement-dependent cytotoxicity assay to screen the candidate HCT recipients to select animals with low or no anti-AMP cell natural antibodies for transplantation. Second, we co-transplanted AMP cells via intra-bone injection, as we reasoned that direct delivery of AMP cells to their effector sites would allow them to exert their immunosuppressive effects to promote mixed chimerism induction. Animal AN620D was conditioned and received donor bone marrow cells and intra-bone injection of a high dose of AMP cells (100million/kg) on Day 0. However, this animal had difficulty recovering from anesthesia following intra-bone injection because the matrigel used in intra-bone injection caused embolism to the heart and pulmonary arteries. The animal was euthanized 24 hours following transplantation and we revised our intra-bone injection to avoid this adverse effect in the following experiments. After obtaining approval of the modified protocol from IACUC at Columbia University and the USAMRMC Animal Care and Use Review Office (ACURO), we then performed one experiment in which animal BF869D underwent hematopoietic cell transplant IV with intra-bone injection of AMP cells at the dose of 85million/kg. Results showed that mixed allogeneic chimerism of this animal was prolonged compared to that in control animals, suggesting that intra-bone injection of a high dose of AMP cells is able to promote mixed allogeneic chimerism. Therefore, we sought to determine whether this finding was reproducible by performing one more experiment in animal AB990I. This animal received intra-bone injection of AMP cells at the dose of 105million/kg. However, this animal was euthanized 24 days post-transplant due to worsening clinical condition. As this experiment was not complete, animal H891 was selected and an additional transplant was performed. This animal received intra-bone injection of AMP cells at the dose of 100milliom/kg. Unlike animal BF869D, mixed chimerism was not prolonged in this animal. Table 1 summarizes the HCT recipients in this project.

ID of recipients	Time of transplantation and observation	Duration of mixed chimerism	Dose and route of AMP cell injection	Notes	Time of report
AT468G	2016	>49 days	No AMP cells	This animal died on Day 49 post-transplant due to renal failure	1st annual report submitted in Aug 2016
BF585G	2015-2016	41 days	100million/kg via intravenous injection		1st annual report submitted in Aug 2016
AL792C	2016	48 days	100million/kg via intravenous injection		1st annual report submitted in Aug 2016
BY648F	2016	43 days	No AMP cells		2nd annual report submitted in Aug 2017
AN620D	2016	NA	100million/kg via intra-bone injection	This animal was euthanized 24 hours post-transplant due to adverse effects from AMP intra-bone injection	2nd annual report submitted in Aug 2017
BF869D	2017	83 days	85million/kg via intra-bone injection		2nd annual report submitted in Aug 2017
AB990I	2018	>23 days	105 million/kg via intra-bone injection	This animal was euthanized 24 days post-transplant due to worsening clinical condition	3nd annual report (final report) submitted in Nov 2018
H891	2018	45 days	100million/kg via intra-bone injection		3nd annual report (final report) submitted in Nov 2018

3b-3. Significant Results and conclusions:

- Transient mixed chimerism in control animals
  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 animal underwent BMT with the same conditioning regimen and immunosuppression but no AMP cells to serve as a control for animals that received AMP cells, After conditioning, the animal was infused with donor bone marrow cells (9.16×108 total BM cells/kg;

7.9×106 CD34+ cells/kg) without AMP cells on Mar 24, 2016. Following transplantation, donor chimerism in multiple lineages was detectable as usual within the first week. Donor chimerism in monocytes and neutrophils steadily increased over time and peaked by around Day 21, while chimerism in T, B and NK cells was detectable at much lower levels. The donor chimerism of this control animal plateaued in all lineages from Day 21 to Day 40, then began to decline (Figure 1A). CMV reactivation was first detected on Day 10 at a low level and viremia peaked from Day 22 to Day 30. Anti-viral therapy controlled the CMV and brought the viremia down to low levels in peripheral blood on Day 34 (Figure 1B). During the reactivation of CMV, mixed chimerism in all lineages did not show a significant decrease (Figure 1A). While donor chimerism remained stable, this animal demonstrated persistent pancytopenia requiring repeated blood transfusions. Its general condition deteriorated, which led to its death on Day 49. Within the last week before its death, donor chimerism declined, especially in monocytes and neutrophils, although it remained high at the time of death (Figure 1A).

We performed transplantion in the second control animal (BY648F) on July 28, 2016 under the same conditions as the previous control (AT468G). Following conditioning, the animal was infused with donor bone marrow cells (10.89×108 total BM cells/kg; 9.8×106 CD34+ cells/kg) without AMP 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 1C). 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 1D). 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 skin, was grafted to this animal. 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 accurate interpretation of the results, although the macroscopic assessment of the grafts supported the lack of tolerance in this animal. Thus, the duration of mixed chimerism in this control animal was used for comparison to that of animals receiving AMP cells.

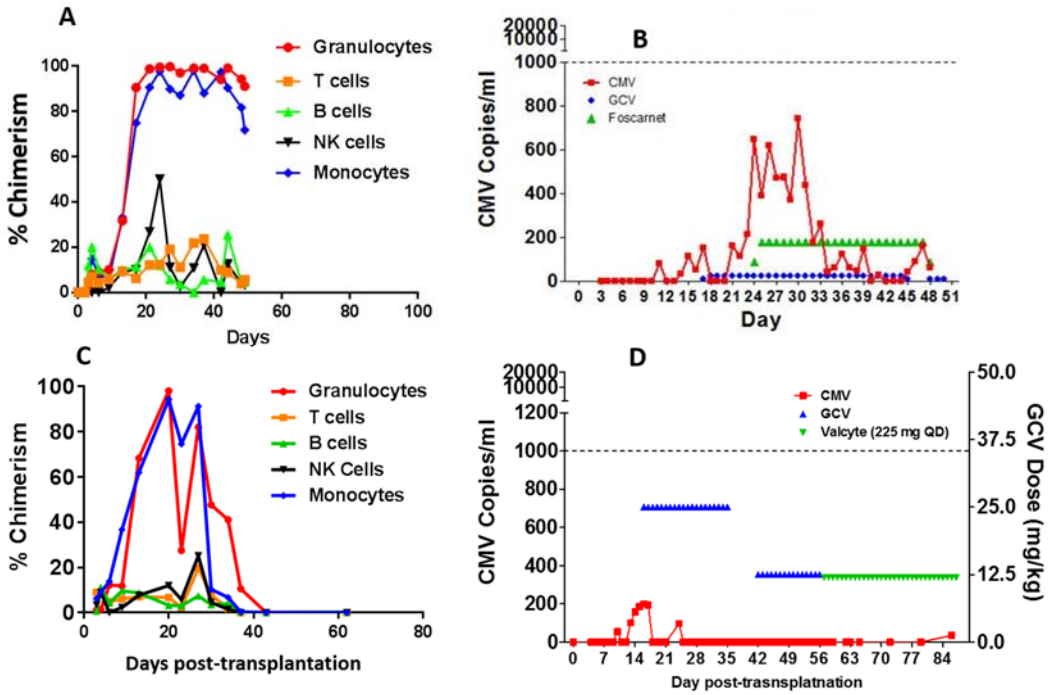


Figure 1. Donor chimerism of the control animals not receiving AMP cells in all lineages following transplantation. A. Animal AT468G. C. Animal BY648F. Reactivation of CMV following transplantation in control animals not receiving AMP cells. B. Animal AT468G. D. Animal BY648F.

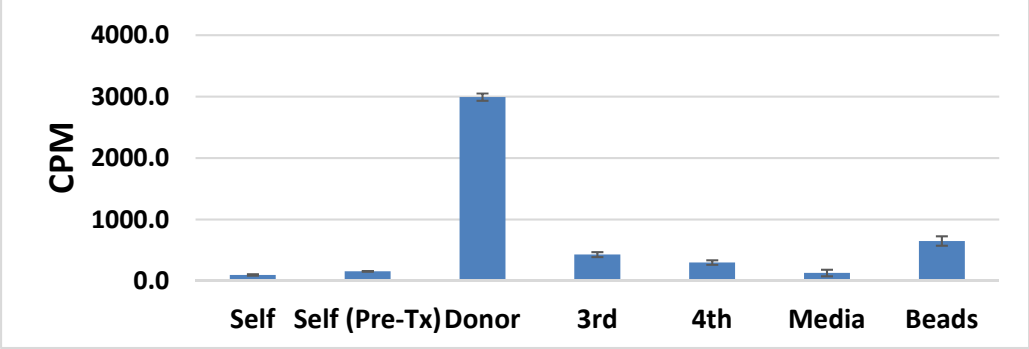


Figure 2. Mixed lymphocyte reaction to determine the responses from BY648F on Day 62 to the donor following transplantation. Recipient PBMCs were used as responder cells. Stimulator cells include recipient PBMCs harvested on Day 62 and prior to transplantation (designated as Self and Self (Pre-Tx) respectively),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

- Co-transplantation of high dose of AMP cells via intravenous injection did not prolong mixed chimerism

  Two animals (BF585G and AL792C) were transplanted with high dose (100million/kg) of AMP cells injected intravenously. The animal BF585G 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 5 days. On the transplant day, the animal was infused with donor bone marrow cells (461.25x10^6 total BM cells/kg; 15.7x10^6 CD34+ cells/kg) and AMP cells (100 million/kg) on November 5, 2015. The second animal receiving AMP cells (AL792C) was conditioned in late January 2016 and infusion of donor marrow cells (1400.45x10^6 total BM cells/kg; 15.74x10^6 CD34+ cells/kg) and AMP cells (100million/kg) was performed on February 4, 2016. No adverse effects associated with intravenous injection of high dose of AMP cells were seen in either animal.

  We monitored the donor chimerism in multiple hematopoietic cell lineages of animals receiving AMP cells following transplantation. As shown in Figure 3, donor chimerism in lymphocytes, including T, B and NK cells, was much lower than that in monocytes and neutrophils in both animals receiving AMP cells (Figure 3A and 3B). Donor chimerism in monocytes and neutrophils could be detected within the first week post-transplantation and increased over time, peaking on about Day 21 post-transplantation, while donor chimerism in lymphocytes remained low throughout the whole observation period. Donor chimerism in monocytes and neutrophils then started to decline gradually and completely disappeared on Day 41 (the first animal receiving AMP cells, BF585G) and on Day 48 (the second animal receiving AMP cells, AL792C) respectively, when chimerism in lymphocyte lineages also disappeared. Reactivation of CMV, a common complication seen in our model, was detected in the first AMP animal (BF585G) on Day 17 post-transplant. Therapy with ganciclovir was initiated on Day 27 and foscarnet was added on Day 38 as CMV was still detected, although at a low level (Figure 4A). The CMV was well controlled thereafter. Since CMV viremia was low during the loss of chimerism in this animal, it was unlikely that the reactivation of CMV was responsible for the loss of chimerism. Similarly, the correlation between loss of chimerism and reactivation of CMV in the second animal receiving AMP cells was also weak. Reactivation of CMV was detected on Days 17 and 31 with a low level of viremia, which was well controlled by anti-viral therapy. CMV viremia only lasted for a few days before coming under control (Figure 4B). Thus the complete loss of chimerism did not seem to be triggered by CMV reactivation in this animal. Compared to the control animals, both animals receiving intravenous injection of a high dose of AMP cells did not show prolonged mixed allogeneic chimerism. Taken together, these results indicate that intravenous injection of a high dose of AMP cells alone was not able to facilitate the induction of more durable mixed allogeneic chimerism in this model.

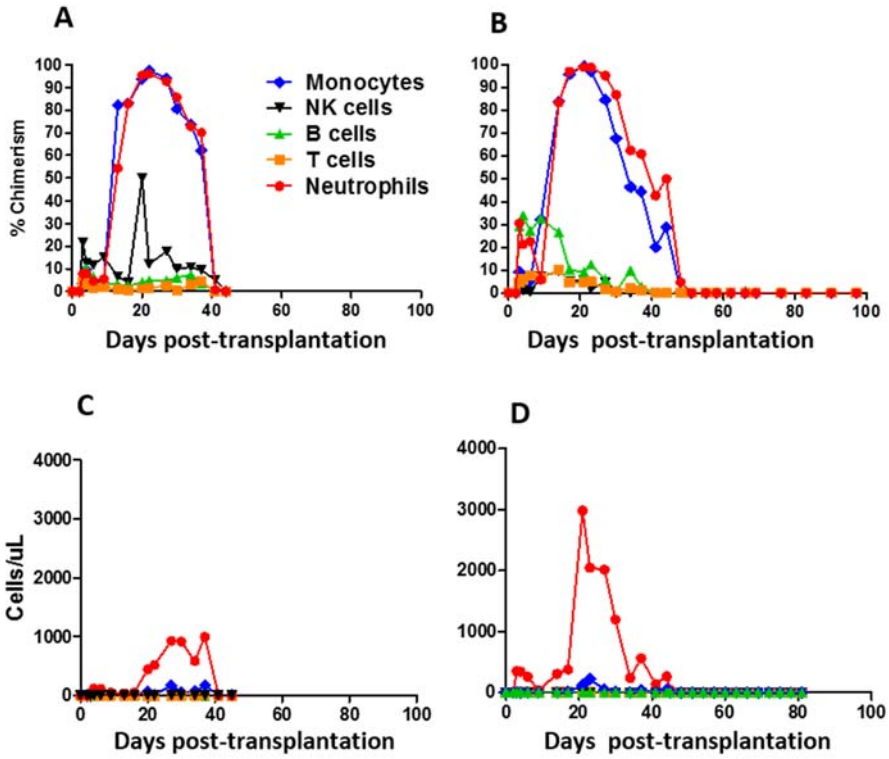


Figure 3. Donor chimerism expressed in percentages (upper panel, A and B) and absolute numbers (lower panel, C and D) in multiple lineages of animals receiving AMP cells in peripheral blood following transplantation. (A, C) The first animal receiving AMP cells (BF585G). (B, D) The second animal receiving AMP cells (AL792C).

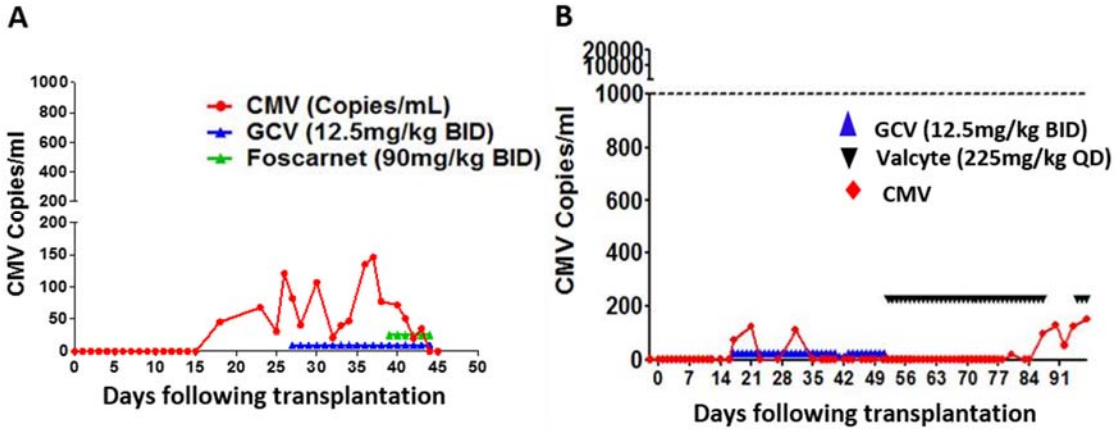


Figure 4. CMV levels in peripheral blood of animals receiving AMP cells following transplantation. (A) The first animal receiving AMP cells (BF585G). (B) The second animal receiving AMP cells (AL792C).

- Co-transplantation of a high dose of AMP cells via intravenous injection did not facilitate induction of tolerance
  In non-human primates and human patients, combined bone marrow and kidney transplantation resulted in tolerance to MHC-mismatched donor kidney allografts despite the transience of mixed chimerism. Although co-transplantation of a high dose of AMP cells via intravenous injection did not lead to prolonged mixed chimerism as shown in Figure 3, it remained possible that these cells could promote tolerance induction to donor alloantigens. The first animal (BF585G) receiving AMP cells was euthanized on Day 45 post-transplantation, after complete loss of donor chimerism. Mixed lymphocyte reactions using its frozen pre-transplant PBMCs and those harvested at the time of euthanasia as responders confirmed that tolerance to donor alloantigens had not been induced (Figure 5). A donor skin allograft, along with autologous and 3rd party skin allografts, was transplanted to the second AMP animal (AL792C) on Day 90 post-transplant, after donor chimerism had disappeared. However, all the skin grafts were lost due to technical issues. This animal was euthanized on Day 167 post-transplant. Mixed lymphocyte reaction was performed to determine the recipient T cell responses to donor alloantigens. Anti-donor antibodies in the serum were also assayed by complement-dependent cytotoxicity assay or flow cytometry using anti-human IgM and anti-human IgG secondary antibodies to assess humoral responses to the donor. As shown in Figure 6, mixed lymphocyte reaction using PBMCs harvested pre-transplantation and on Day 90 post-transplant demonstrated robust responses to donor stimulators, indicating that recipient T cells were not tolerized by the co-transplantation of AMP cells and donor bone marrow. In addition to T cell responses, anti-donor antibodies, mainly IgG, were detected on Day 91 post-transplant and later time points (Figure 7), and these were able to mediate potent cytotoxicity against donor PBMCs in vitro (Figure 8). Thus, a humoral response to donor alloantigens had occurred in vivo. Collectively, these data demonstrate that co-transplantation of AMP cells did not lead to the induction of tolerance to donor alloantigens.

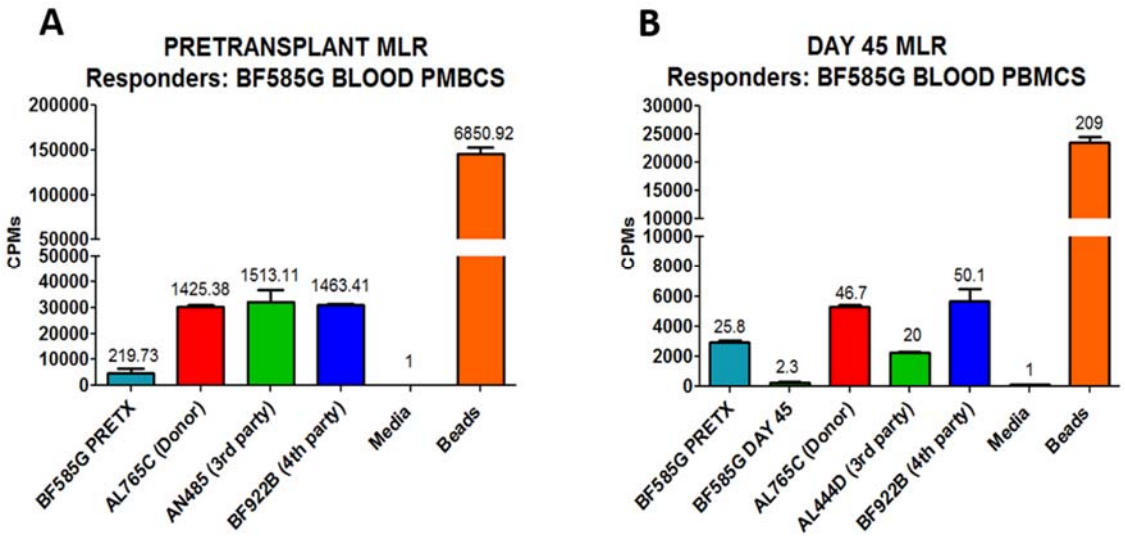


Figure 5. Responses of the first animal (BF585G) receiving AMP cells to donor alloantigens. Mixed lymphocyte reaction was performed prior to transplantation (A) and on Day 45 following transplantation (B) when the recipient was euthanized. PBMCs from the recipients were either unstimulated (Media), or stimulated with autologous, donor (AL765C), 3rd party (AN485), 4th party (BF922B) PBMCs and beads. Proliferation was determined by 3H incorporation. As shown, response of recipient PBMCs to donor stimulators was comparable to that to the 3rd party and 4th party stimulators, indicating that recipient T cells were not tolerant to the donor alloantigens. Numbers above each bar indicate stimulation index (cpm against stimulators/cpm with no stimulators)

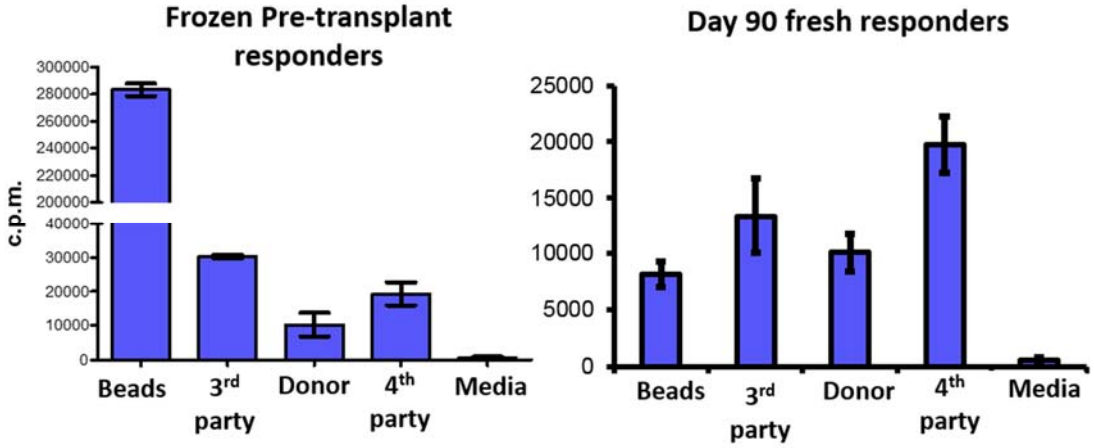


Figure 6. Responses to donor alloantigensof the second animal (AL792C) receiving AMP cells. Mixed lymphocyte reaction was performed using frozen pre-transplant PBMCs (A) and freshly isolated PBMCs (B) on Day 90 following transplantation as responders. PBMCs from the recipients were either unstimulated (Media), or stimulated with donor, 3rd party, 4th party PBMCs and beads. Proliferation was determined by ^{3}H incorporation. As shown, response of recipient PBMCs to donor stimulators was comparable to that to the 3rd party and 4th party stimulators, indicating that recipient T cells were not tolerant to the donor alloantigens.

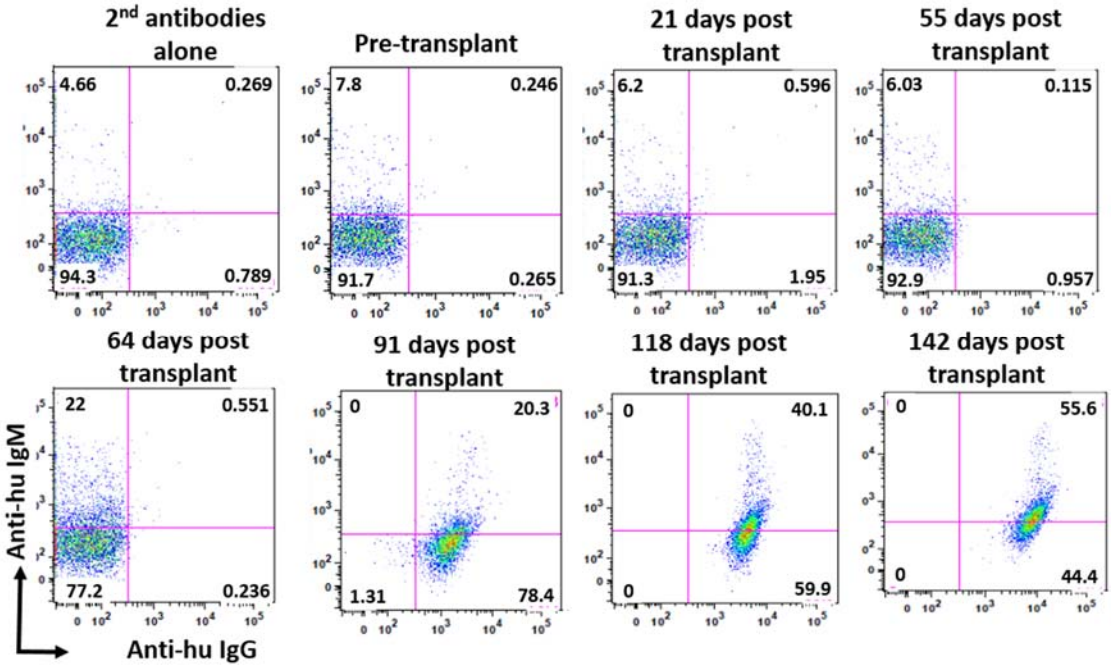


Figure 7. Anti-donor antibodies in the second animal (AL792C) receiving AMP cells. To determine if anti-donor antibodies were produced by the second animal receiving AMP cells, donor PBMCs were first incubated with serum from the recipient at different time points followed by incubation with anti-human IgM and anti-human IgG secondary antibodies and analyzed by flow cytometry. Donor PBMCs incubated with secondary antibodies but not serum served as control. No significant anti-donor antibodies were detected up to Day 55 post-transplant when the donor chimerism in peripheral blood was no longer detectable. On Day 64, significant amount of anti-donor IgM antibodies were detected and on Day 91 both anti-donor IgM and IgG antibodies were evident. These anti-donor antibodies were maintained afterwards. These data indicated that anti-donor humoral responses had been mounted to the donor and no tolerance of the recipient had been induced.

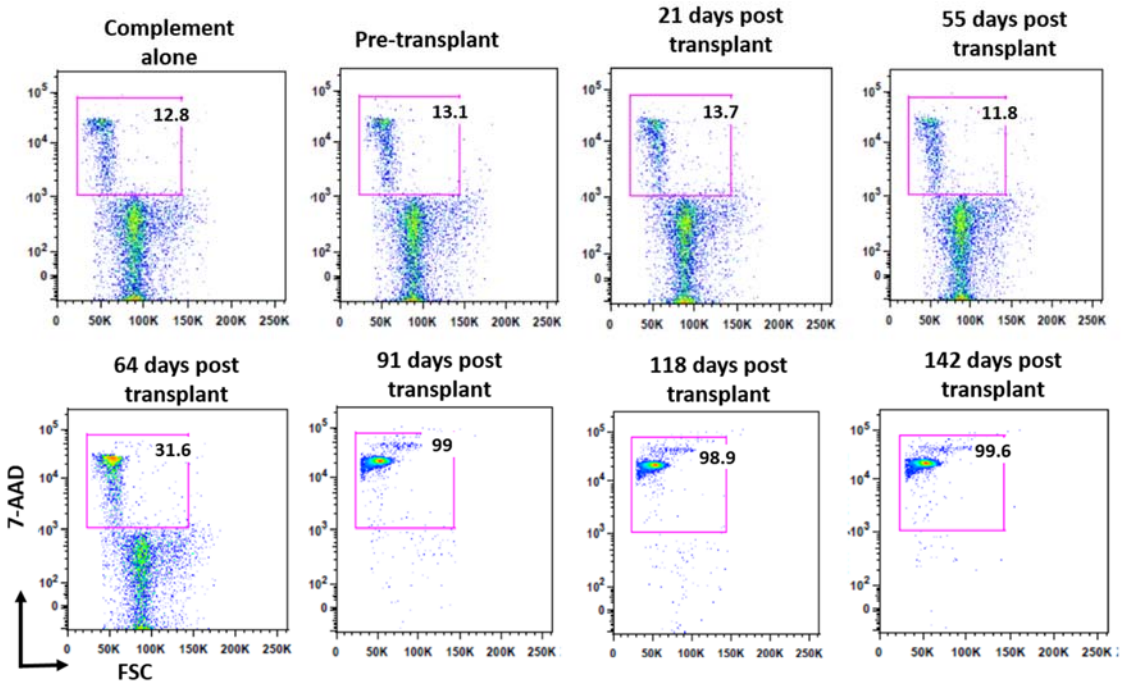


Figure 8. Anti-donor antibodies in the second animal (AL792C) receiving AMP cells determined by complement-dependent cytotoxicity assay. Donor PBMCs were first incubated with serum from the recipient at different time points followed by incubation with rabbit complement. 7-AAD was then added and analyzed by flow cytometry for 7AAD+ dead cells. Donor PBMCs incubated with complement without serum served as control. No significant anti-donor antibody-induced cell death was detected up to Day 55 post-transplant when the donor chimerism in peripheral blood was no longer detectable. On Day 64, significant amount of anti-donor antibody-induced cell death was detected and on Day 91 potent cell death was evident. Anti-donor antibody-induced cell death was maintained afterwards. Consistent with data in Figure 7, these data indicated that anti-donor humoral responses had been mounted to the donor and no tolerance of the recipient had been induced.

- Lack of persistence of intravenously injected AMP cells in vivo and presence of anti-AMP cell natural antibodies
  We tested whether or not the human AMP cells were able to travel to and/or persist in recipient tissues. When we tested for the presence of human DNA, we detected no evidence for AMP cells in bone marrow on Day 37 following infusion or in peripheral blood at multiple time points in the second animal receiving AMP cells intravenously (Figure 9), suggesting that the injected AMP cells were unable to travel to and/or persist in target tissues. In light of these data, we looked for factors that might lead to the destruction of AMP cells. We considered whether anti-AMP cell antibodies existed pre-transplantation or were induced at later time points post-transplant in the serum of the second animal receiving AMP cells. Indeed, anti-AMP cell antibodies, predominantly IgM, were detected in serum of this animal prior to transplantation. The levels of these anti-AMP cell antibodies remained constant throughout the observation period and no anti-AMP cell IgG antibodies were detected, indicating that the recipient animal was not primed by AMP cell-derived antigens (Figure 10 and 11). These results suggest that pre-existing anti-AMP cell antibodies might be a factor promoting rapid destruction of AMP cells and contributing to their lack of biological effect. Thus, our data suggest that approaches to delivering AMP cells to and enhancing their persistence in target tissues may be needed to facilitate their immunosuppressive effects in monkeys.

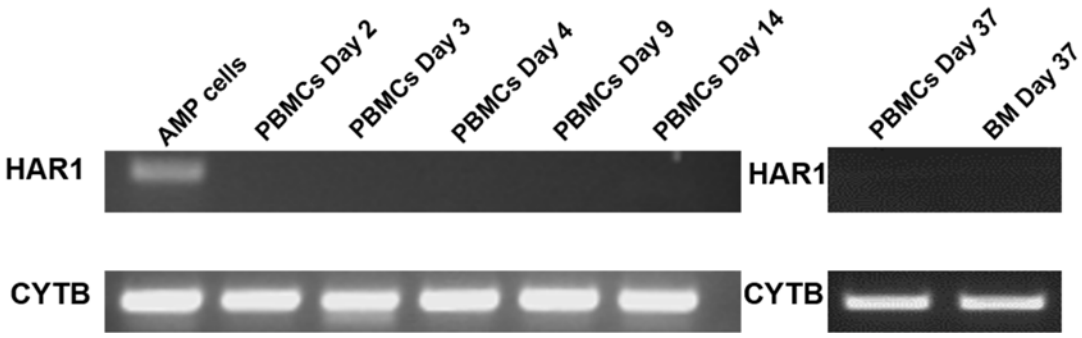


Figure 9. Absence of AMP cells in peripheral blood and bone marrow. PCR was performed to detect AMP cells in peripheral blood (PBMCs) at multiple time points and in bone marrow (BM) on Day 37 in the 2nd animal receiving AMP cells (AL792C). AMP cells were not detected in these tissues any time point. HAR1: human accelerated region 1. CYTB: cytochrome b (present in the mitochondrial genome). CYTB is used as a housekeeping gene. Primers for HAR1 were specific for human sequence. This PCR was able to detect DNA from a minimum of 4000 human cells. 60,000 to 180,000 PBMCs and 780,000 bone marrow cells were used for PCR.

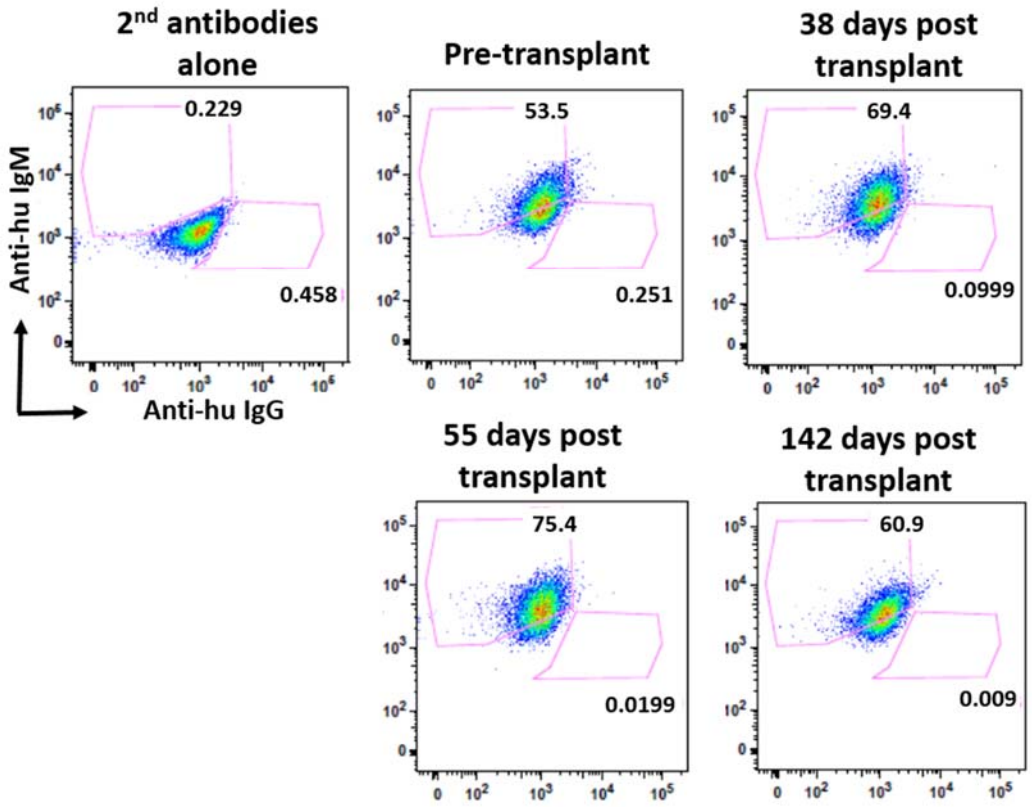


Figure 10. Anti-AMP cell antibodies in the second animal receiving AMP cells (AL792C). To determine if anti-AMP cell antibodies were produced by the second animal receiving AMP cells, AMP cells were first incubated with serum from the recipient at different time points followed by incubation with anti-human IgM and anti-human IgG secondary antibodies and analyzed by flow cytometry. Donor PBMCs incubated with secondary antibodies but not serum served as control. Anti-AMP cell antibodies, predominantly IgM were detected prior to transplant and at different time points post-transplant. These data demonstrated the presence of pre-existing anti-AMP cell antibodies and suggested that the recipient was not primed by the AMP cell-derived antigens.

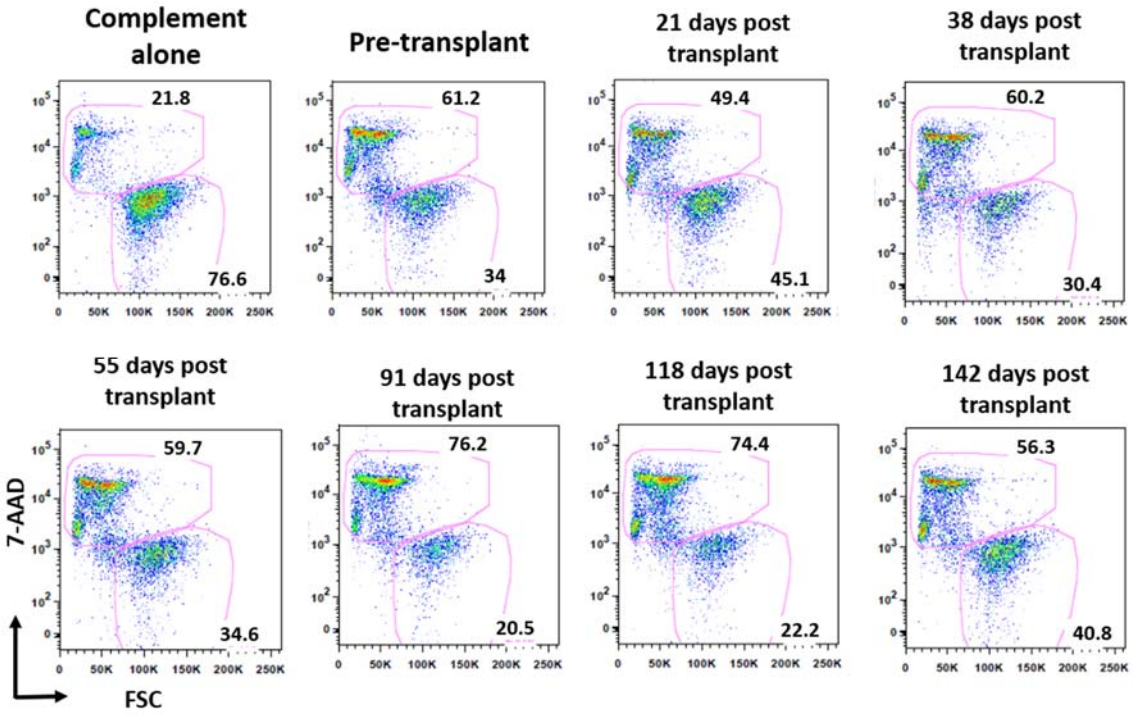


Figure 11. Anti-AMP cell antibodies in the second animal (AL792C) receiving AMP cells detected by complement-dependent cytotoxicity assay. AMP cells were first incubated with serum from the recipient at different time points followed by incubation with rabbit complement. 7-AAD was added and 7-AAD+ dead cells were analyzed by flow cytometry. AMP cells incubated with complement without serum served as control. Consistent with data in Figure 10, anti-AMP cell antibodies were detected prior to transplant and at different time points post-transplant. These data demonstrated pre-existing cytotoxic anti-AMP cell antibodies and suggested that the recipient was not primed by the AMP cell-derived antigens.

- Screening of candidate animals for serum anti-AMP cell natural antibodies allows selection of optimal transplant recipients
  Our data above demonstrating the presence of anti-AMP cell natural antibodies in the recipient suggest that these antibodies may 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 candidate animals for 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 12, 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 one of the animals (BF896D) receiving introbone injection of AMP cells described below.

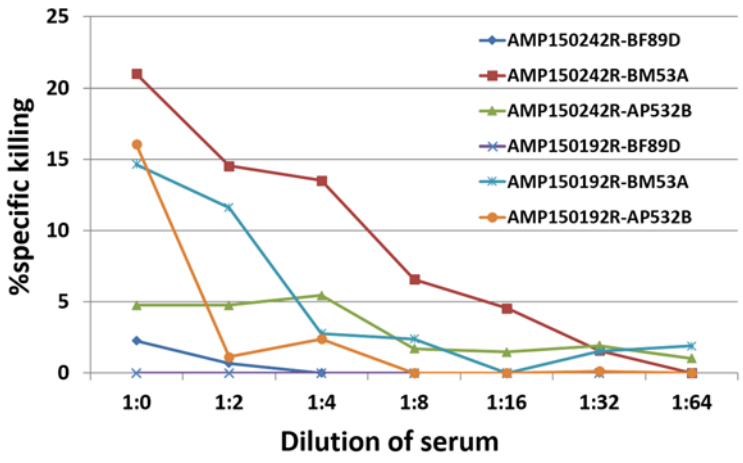


Figure 12. Levels of anti-AMP cell natural antibodies in potential transplant recipients. The serum levels of anti-AMP cell natural antibodies from three candidate animals (BF89D, BM53A and AP532B) against two lots of AMP cells (AMP150242R and AMP150192R) were determined by complement-dependent cytotoxicity assay. As shown, animal BF89D demonstrated undetectable levels of anti-AMP cell natural antibodies against AMP cell lot AMP150192R.

- Intra-bone injection of AMP cells was associated with prolonged mixed allogeneic chimerism

  Our data (Figure 9) demonstrated that intravenous infusion of AMP cells resulted in lack of AMP cells in peripheral blood and bone marrow, suggesting that they might be promptly eliminated by hematopoietic cells and/or 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 intra-bone injection on Sept 15, 2016. We hypothesized that direct delivery of AMP cells to the recipient bone marrow by intra-bone injection would 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 matrigel in 20 mL and injected into the bilateral tibias of the recipient. However, this animal had difficulty recovering from anesthesia following intra-bone 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 13). However, since it was not possible to detect cells in the blot clot, qPCR detecting human DNA was used to address whether there were AMP cells in the blot clot and other tissues. As shown in Table 2, 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 matrigel in the intra-bone injection and only AMP cells, not together with donor bone marrow cells, were injected intra-bone. The volume of cell suspension injected into each bone was also reduced. After obtaining approval of the modified protocol from the 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 transplantion IV with intra-bone 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 (8.86×108 total BM cells/kg; 15×106 CD34+ cells/kg, 38.5×106 CD3 T cells/kg). AMP cell lots 150192R and 150242R were thawed, with 2.7×108 and 4.4×108 AMP cells recovered respectively. Each lot of AMP cells were resuspended in 5ml of plasmalyte and injected to one tibia of the recipient via intra-bone injection. The dose of AMP cells was 85million/kg. The intra-bone 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 in lymphocytes. Unlike the control animal, in which donor chimerism started to decline 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 14). Similar to the control animal, only mild reactivation of CMV was detected in the fourth week with a low level of CMV viremia, which was well controlled by anti-viral therapy over the whole observation period (Figure 15). In vitro, the response against the donor on Day 77 was decreased compared to that to the third party stimulators (Figure 16). Skin grafts were performed on Day 90 on this animal to determine whether or not the recipient developed tolerance to the donor. Results from macroscopic assessment of the grafts showed that survival of the donor graft was not prolonged compared to the 3rd party graft. 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.

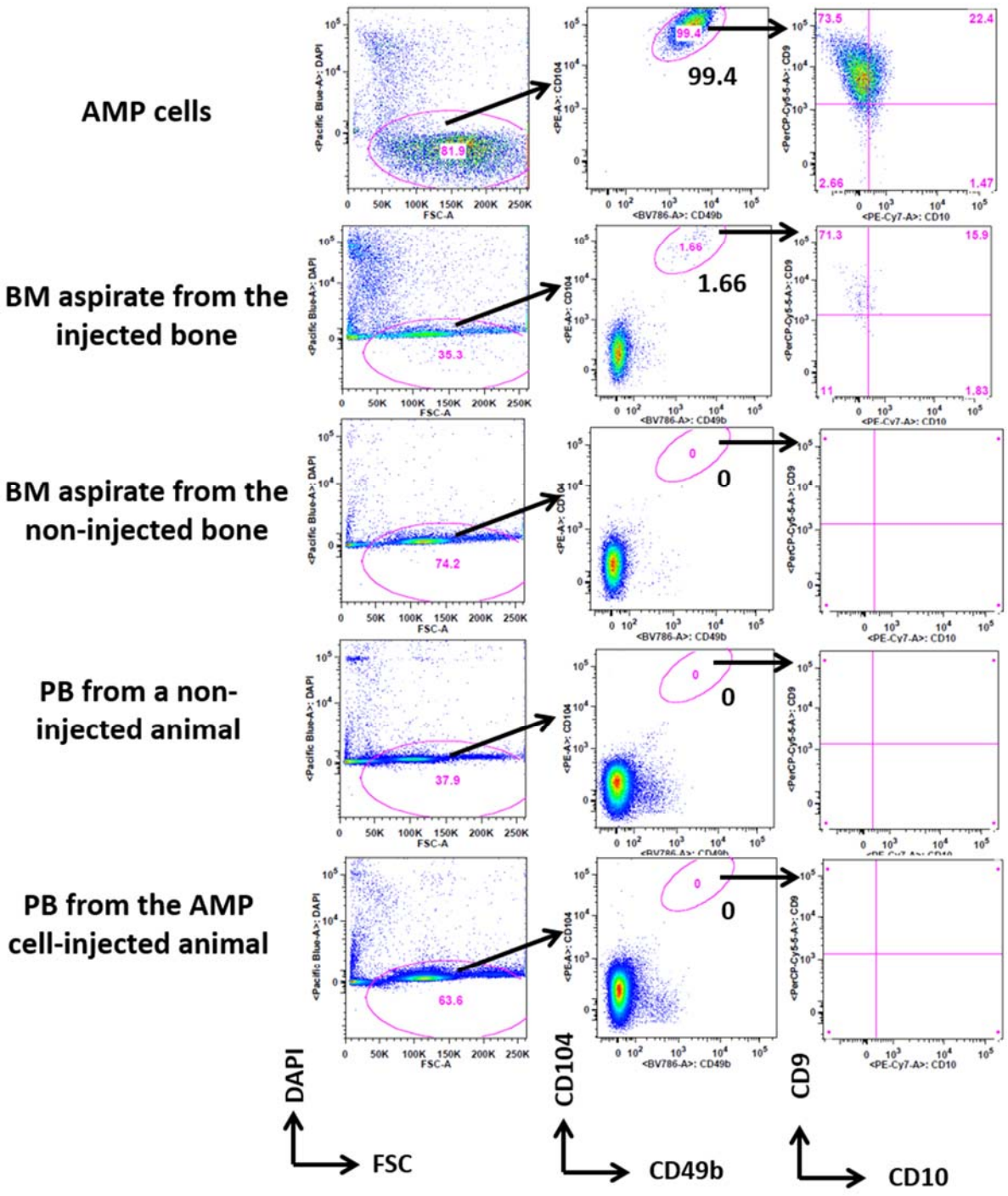


Figure 13. Detection of injected AMP cells in the bone marrow aspirate of animal AN620D from the injection site by flow cytometry. AMP cells have a phenotype of CD49b+CD104+CD10+CD9+. Cells with this phenotype were only seen in the BM aspirate from the AMP cell intrabone injection site, but not in other 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 2. Detection of human DNA in tissues by qPCR. DNA was purified from AMP cells and tissues taken from the animal (AN620D) 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.

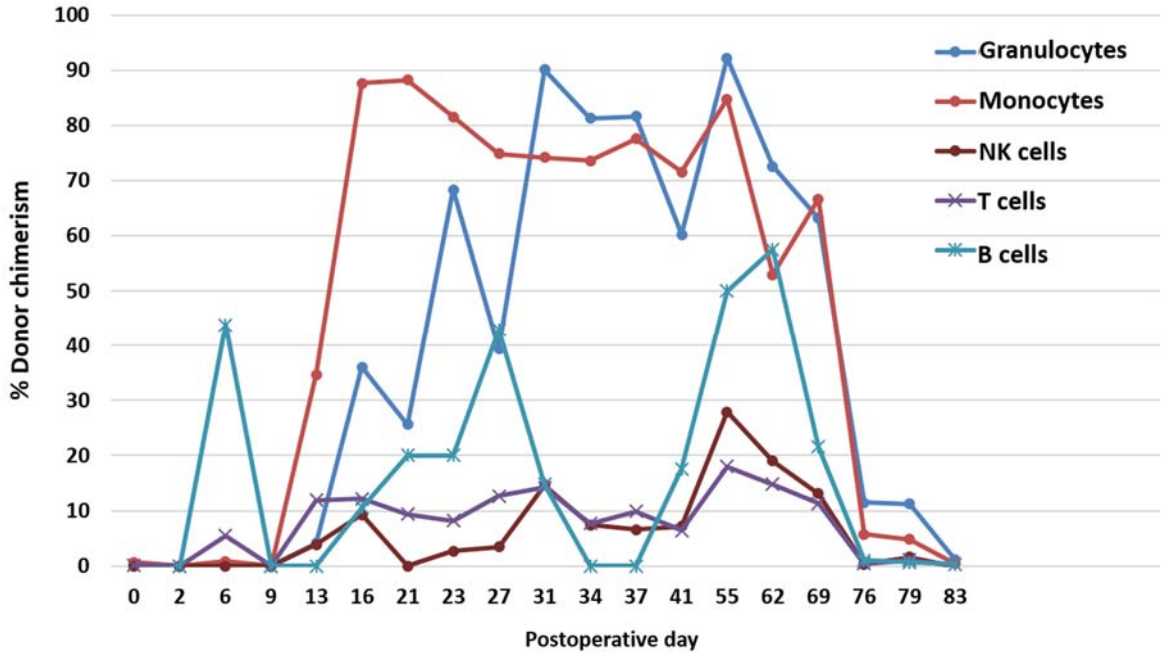


Figure 14. Donor chimerism of the animal (BF869D) receiving AMP cells via intrabone injection in all lineages following transplantation.

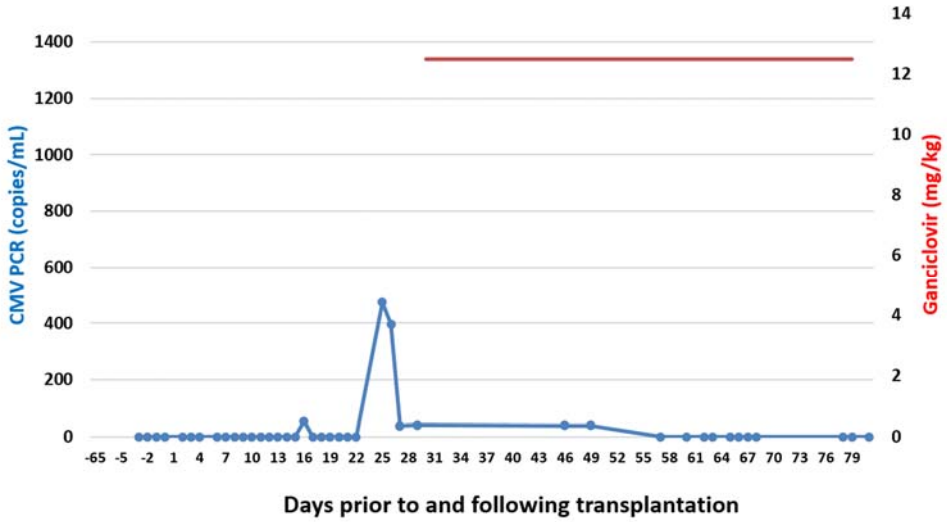


Figure 15. Reactivation of CMV following transplantation in the animal (BF869D) receiving AMP cells via intrabone injection.

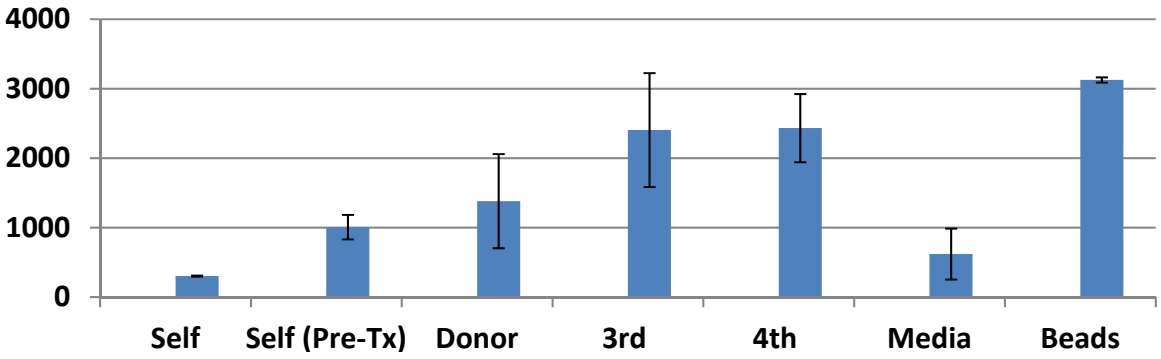


Figure 16. Mixed lymphocyte reaction to determine the responses from BF869D to the donor on Day 77 following transplantation. Recipient PBMCs were used as responder cells. Stimulator cells include recipient PBMCs harvested on Day 62 and prior to transplantation (designated as Self and Self (Pre-Tx) respectively), Donor, 3rd and 4th party PBMCs. Recipient PBMCs cultured with media served as negative control and with beads as positive control.

- Prolongation of mixed chimerism by intra-bone injection of high dose of AMP cells was not reproducible

  To confirm the finding in animal BF869D that intra-bone injection of a high dose of AMP cells may prolong mixed chimerism, a recipient animal AB990I was chosen by screening serum anti-AMP cell natural antibodies (Table 3). This animal (AB990I) underwent hematopoietic cell transplantion with intra-bone injection of AMP cells on Jan 24, 2018. This animal was conditioned with horse ATG (50mg/kg on day -2, -1, 0), anti-CD40L (20mg/kg on day 0, 2 followed by 10mg/kg on day 5, 7, 9, 12), total body (1.25 Gy on day -6 and -5) and thymic irradiation (7 Gy on day -1). In addition, rapamycin was

administered for a course of 30 days and tapered down for the following 3 weeks. Donor bone marrow cells from both the iliac crest and vertebral bones (6.67×10^8 total BM cells/kg; 17×10^6 CD34+ cells/kg, 36×10^6 CD3+ T cells/kg) were infused intravenously into the recipient. Intra-bone injection of AMP cells (105 million/kg) was successfully performed without any complication observed clinically. Although donor chimerism was detected in all lineages following transplantation (Figure 17), 20 days post-transplant the recipient was found to be azotemic with hyperkalemia and was treated for possible sepsis based on the clinical features. Despite the treatment, edema developed the next day that only initially responded to treatment. The clinical condition of the recipient continued to deteriorate as medications failed to correct edema and anuria. On day 23 post-transplant, the recipient was euthanized as his clinical condition had met the criteria for euthanasia based on the protocol. Histopathology of the lungs of this animal showed infarction, probably caused by pulmonary embolism, multifocal pneumonia and interstitial lung disease with multifocal interstitial fibrosis. This pathology is likely the cause of the deteriorated clinical condition leading to its euthanasia.

We then performed another experiment in which animal H891 underwent hematopoietic cell transplant IV with intra-bone injection of AMP cells on June 28, 2018. This recipient was screened for low levels of anti-AMP cell antibodies prior to the transplant (Table 4). 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 (8×10^8 total BM cells/kg; 12.6×10^6 CD34+ cells/kg, 52.6×10^6 CD3+ T cells/kg). AMP cells from lots (FE150256R) and (RF15011) were thawed, with 4.8×10^8 and 3.5×10^8 AMP cells recovered respectively. Each lot of AMP cells was resuspended in 5ml of plasmalyte and injected to one tibia of the recipient via intra-bone injection. The dose of AMP cells was 133 million/kg. The intra-bone injection of AMP cells was successfully completed without any complication observed clinically.

Donor chimerism in multiple lineages was detectable in the second week, peaking at week 3 following transplantation, with a much higher percentage of donor cells in the granulocyte and monocyte lineages compared to lymphocytes. Donor chimerism remained relatively stable from Day 23 to Day 34, with chimerism in lymphocytes, including T, B and NK cells, showing a trend of slow increase over time (Figure 18). Donor chimerism was detected in bone marrow cells aspirated on Day 34 in all lineages, including CD34+ hematopoietic/ progenitor cells (Figure 19). However, donor chimerism in peripheral blood started to decline on Day 37 and was totally lost by Day 45 (Figure 18).

Mild reactivation of CMV was detected in the second week (Days 13-19 post-transplant) with low level of CMV viremia. A second wave of CMV reactivation was detected from Day 23-37 with increasing levels of CMV viremia (reaching >10,000 copies/mL). CMV reactivation was controlled by anti-viral therapy and no CMV was detected beyond Day 38 (Figure 20). Although the second wave of CMV reactivation coincided with the decline of donor chimerism, it is unclear whether or not CMV reactivation might have played a role in triggering or promoting the loss of donor chimerism. To further determine if this recipient was tolerant to the donor alloantigens or not, mixed lymphocyte reaction was performed on Day 56 post-transplant to measure the response of

recipient PBMCs to donor alloantigen stimulation. IFN-γ production by and proliferation of recipient PBMCs were assayed by ELISpot and H^3 incorporation respectively. As shown in Figure 21, significantly higher IFN-γ production by and proliferation of recipient PBMCs were detected when stimulated with donor cells compared to autologous cells, indicating that this recipient did not develop tolerance to the donor.

In conclusion, as shown in the data above, the kinetics of mixed chimerism in this animal (H891) were similar to that in the control animal BY648F not receiving AMP cells and these results did not reproduce those seen in our previous experiments where intra-bone injection of high dose of AMP cells led to the prolongation of mixed chimerism (animal BF89D).

Taken together, we conclude that co-transplantation of donor bone marrow with intravenously-infused AMP cells does not substantially promote mixed allogeneic chimerism in our non-human primate transplant model compared to control recipients that did not receive AMP cells. One out of four BMT recipients that received intra-bone AMP administration achieved prolonged chimerism compared to the controls. We hypothesize that CMV viremia could have interfered with bone marrow engraftment and the development of durable mixed chimerism in this model.

ID number of recipient animal	Lot number of AMP cells	Cytotoxicity on AMP cells mediated by serum of recipient animal
BF16F	FD160126	12.88%
BF16F	FC160117	26.07%
BF16F	RDF15095	17.40%
BF16F	RDF15067	5.87%
AB990I	FD160126	1.06%
AB990I	FC160117	9.47%
AB990I	RDF15095	4.95%
AB990I	RDF15067	0.00%
AK482J	FD160126	13.09%
AK482J	FC160117	17.14%
AK482J	RDF15095	11.00%
AK482J	RDF15067	0.84%
AR570E	FD160126	8.92%
AR570E	FC160117	21.30%
AR570E	RDF15095	16.90%
AR570E	RDF15067	0.00%

Table 3. Levels of anti-AMP cell natural antibodies in potential transplant recipients. The serum levels of anti-AMP cell natural antibodies from four candidate animals (BF16F, AB990I, AK482J and AR570E) against two lots of AMP cells (FD160126, FC160117, RDF15095 and RDF15067) were determined by complement-dependent cytotoxicity assay. As shown, animal AB990I demonstrated undetectable or extremely low levels of anti-AMP cell natural antibodies against AMP cell lot RDF15067 and FD160126 respectively.

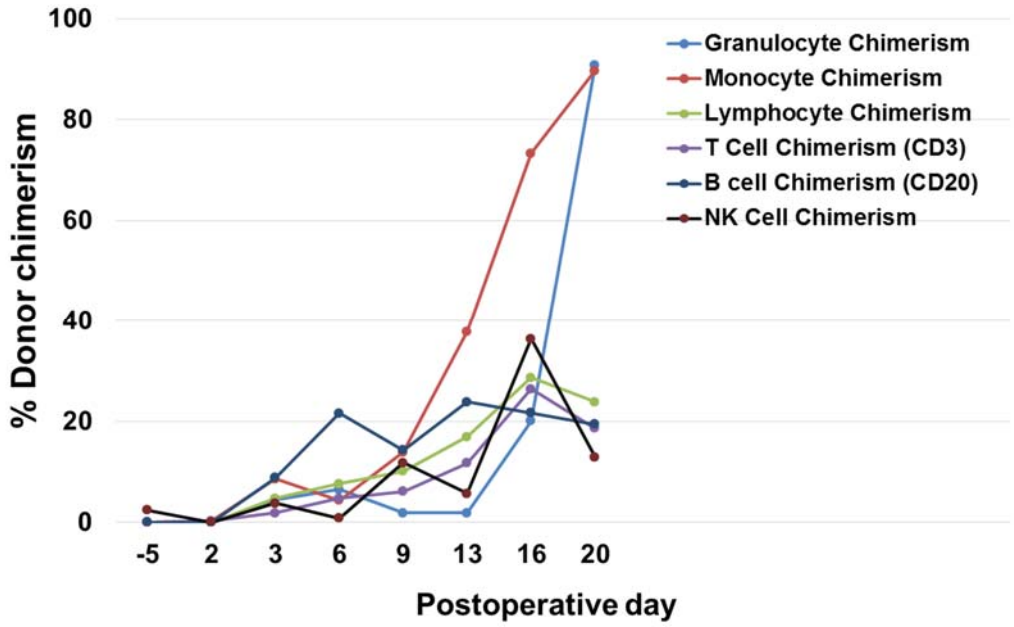


Figure 17. Kinetics of donor chimerism in peripheral blood of AMP cell recipient AB990I following bone marrow transplant. Multi-lineage mixed chimerism was detected in peripheral blood.

AMP cell lot number	ID number of recipient animal	Cytotoxicity on AMP cells mediated by serum of recipient animal
RF15011	H530	15.66%
RF15011	BF16E	19.21%
RF15011	AD776F	12.63%
RF15011	H577	22.76%
RF15011	H891	6.45%
RDF15095	H530	12.06%
RDF15095	BF16E	12.40%
RDF15095	AD776F	9.76%
RDF15095	H577	15.73%
RDF15095	H891	8.96%
FE150256R	H530	16.69%
FE150256R	BF16E	21.32%
FE150256R	AD776F	9.87%
FE150256R	H577	19.85%
FE150256R	H891	7.55%
FC160117	H530	33.94%
FC160117	BF16E	27.68%
FC160117	AD776F	20.67%
FC160117	H577	25.31%
FC160117	H891	22.39%

Table 4. Levels of anti-AMP cell natural antibodies in potential transplant recipients. The serum levels of anti-AMP cell natural antibodies from six candidate animals (H530, BF16E, AD776F, H577 and H891) against four lots of AMP cells (RF15011, RDF15095, FE150256R and FC160117) were determined by complement-dependent cytotoxicity assay. As shown, animal H891 demonstrated the lowest levels of anti-AMP cells against AMP cell lots RF15011, RDF15095 and FE150256R. H891 was chosen as HCT recipient of AMP cell lots RF15011, RDF15095 and FE150256R.

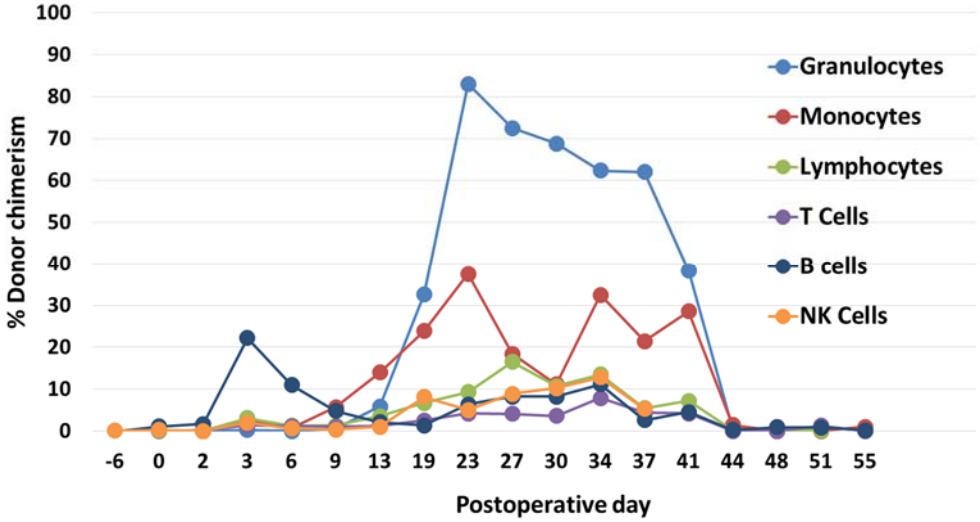


Figure 18. Donor chimerism of the animal (H891) receiving AMP cells via intra-bone injection in all lineages following transplantation.

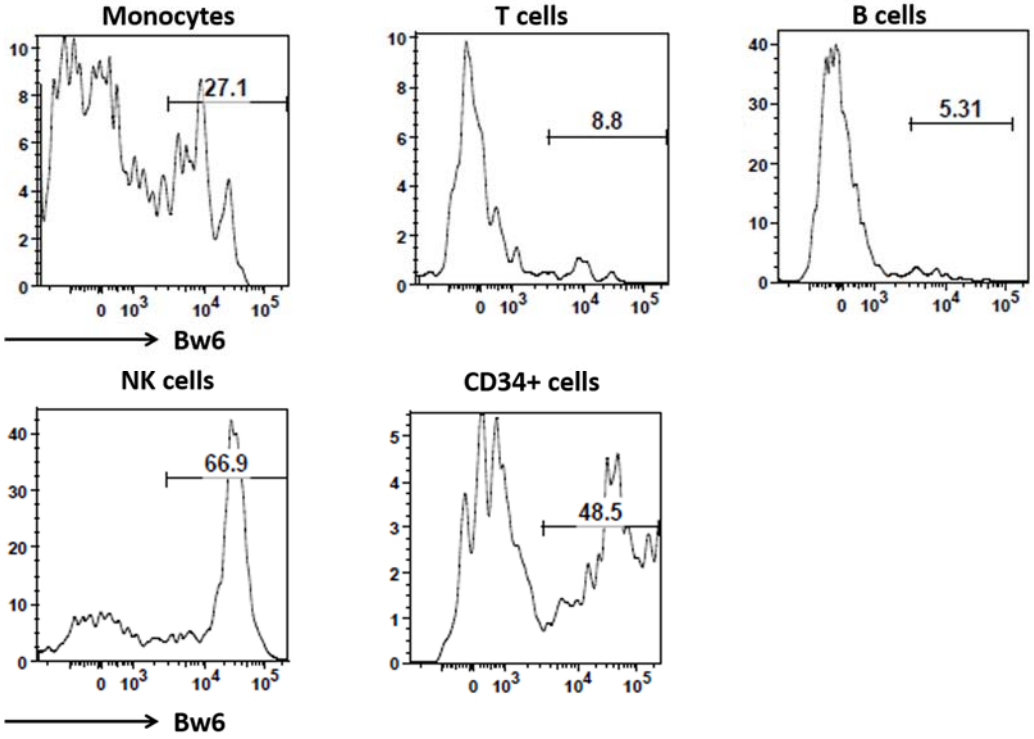


Figure 19. Donor chimerism in bone marrow aspirate on Day 34 following transplant from animal H891. Bone marrow aspirate of the recipient was analyzed by flow cytometry and donor cells were identified as Bw6+ cells. As shown, donor chimerism in multiple lineages and CD34+ hematopoietic stem/progenitor cells was detected.

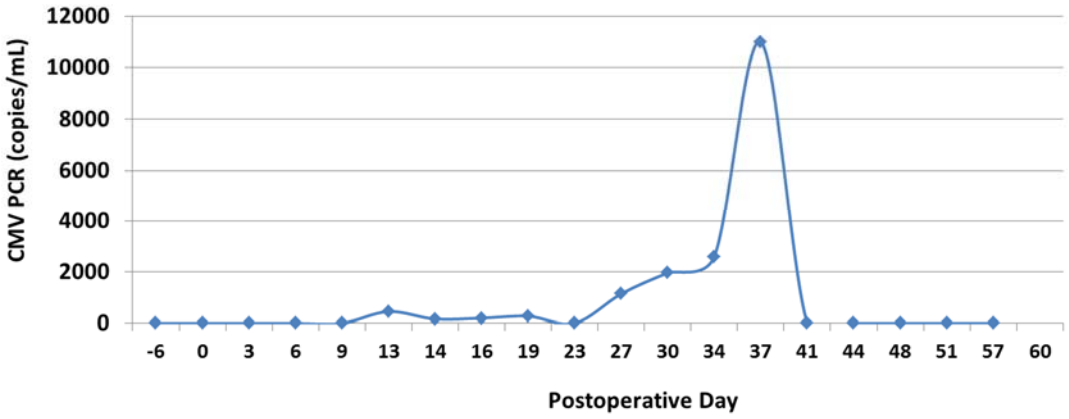


Figure 20. Reactivation of CMV following transplantation in the animal (H891) receiving AMP cells via intrabone injection.

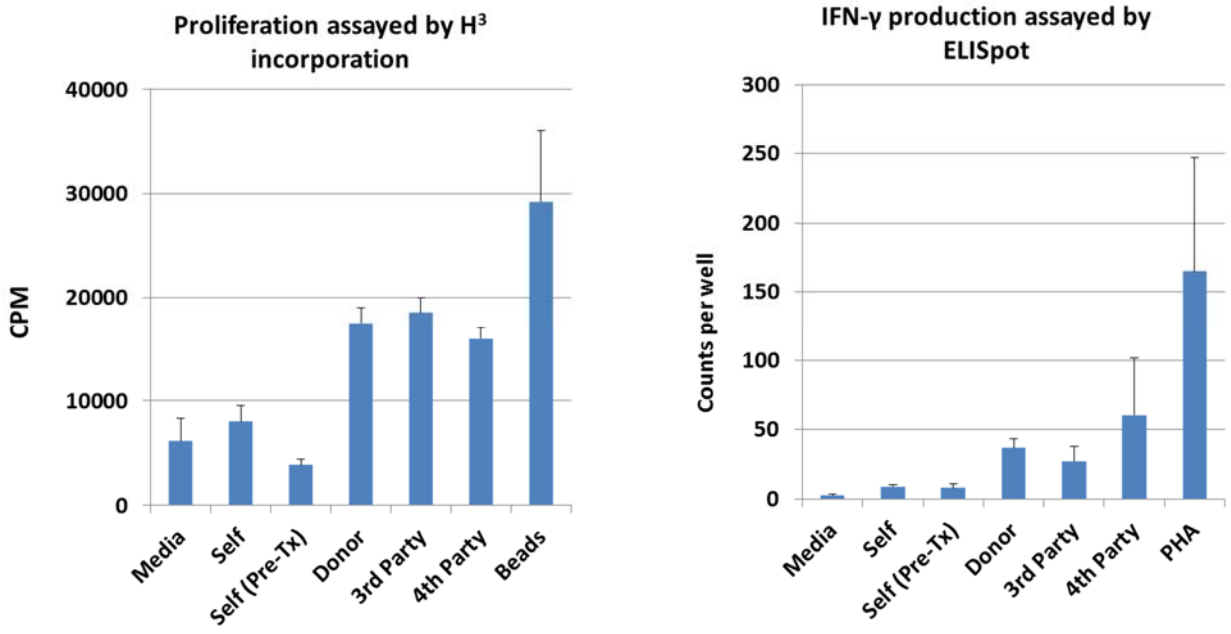


Figure 21. Mixed lymphocyte reaction to determine the responses of recipient from H891 to the donor on Day 56 following transplantation. Mixed lymphocyte reaction was set up using recipient PBMCs as responder cells. Stimulator cells included recipient PBMCs harvested on Day 56 and prior to transplantation (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 or PHA as positive control. H^3 incorporation was used to determine the proliferation and ELISpot was used to determine the production of IFN-γ.

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

This project has provided training opportunities for Paula Alonso Guallart, who is a graduate student.

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?

This is the last reporting period.

4. IMPACT

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

While immunosuppressive effects of AMP cells on alloresponses have been demonstrated in *in vitro* studies and in mouse models, our data demonstrated that co-transplantation of a high dose of AMP cells intravenously did not facilitate induction of durable mixed chimerism in our non-human primate model. Although co-transplantation of high dose of AMP cells via intra-bone injection led to prolongation of mixed chimerism in one animal, this finding was not readily reproducible. In addition, the transient mixed chimerism in all animals receiving AMP cells did not lead to tolerance of the recipients to the donors. As our non-human primate animal model is highly clinical relevant, these results do not support clinical trials of AMP cells in hematopoietic cell **transplantation** for mixed chimerism induction.

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

In this project, we have demonstrated that anti-human natural antibodies existed in monkeys and this 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 with complement-dependent cytotoxicity is feasible and should be used to identify the optimal recipient.

4C. What was the impact on technology transfer?

We have shared ourn results with Noveome Biotherapeutics (formerly Stemnion Inc.), who 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

Nothing to report

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

Nothing to report

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

Nothing to report

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: PI Researcher Identifier (UNI of Columbia University): MS3976 Nearest person month worked: 2% effort = < 1 calendar months Contribution to Project: Dr. Sykes oversees the whole project
Name: Adam Griesemer (No change) Project Role: Co-Investigator Researcher Identifier (UNI of Columbia University): ADG2101 Nearest person month worked: 3% effort = < 1 calendar months Contribution to Project: Dr. Griesemer oversees and aids in transplantation procedures performed on animals and participates in data interpretation.
Name: Paula Alonso Guallart (No change) Project Role: Postdoctoral Research Scientist

Researcher Identifier (UNI of Columbia University): PA2396 Nearest person month worked: 6 calendar months Contribution to Project: Dr. Guallart is responsible for performing all clinical procedures on animals in this project and data analysis and interpretation.
Name: Dil Ekanayake-Alper Project Role: Veterinarian Researcher Researcher Identifier (UNI of Columbia University): DKE2107 Nearest person month worked: as needed Contribution to Project: monitor animals clinically
Name: Hao Wei Li (No change) Project Role: Co-Investigator Researcher Identifier (UNI of Columbia University): HL2591 Nearest person month worked: 4 calendar months Contribution to Project: Dr. Li oversees and performs in-vitro procedures in this project, including preparation of AMP cells for transfusion, immunological assays, etc. and data analysis and interpretation.

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?

Nothing to report

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

W81XWH-15-1-0234

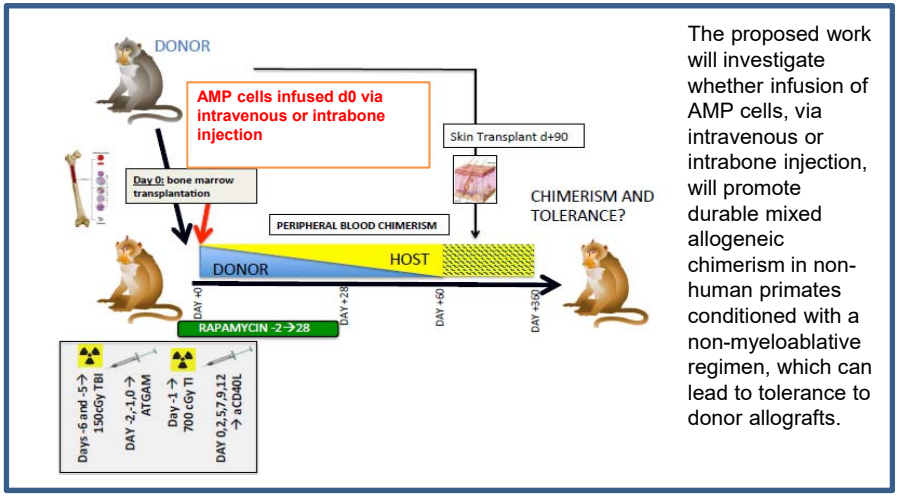
PI: Megan Sykes, MD **Org:** Columbia University **Award Amount:** $1,200,000 total cost dollars

Study/Product Aim(s)

• Determine whether co-transplantation of amnion-derived multipotent progenitor (AMP) cells, via intravenous or intrabone injection, can promote durable mixed chimerism and induce tolerance

Approach

AMP cells were co-transfused via intravenous or intrabone injection with allogeneic bone marrow cells to non-human primates conditioned by a non-myeloablative regimen to induce mixed hematopoietic chimerism. Recipients were screened to have low anti-AMP cell natural antibodies. Donor chimerism were followed and tolerance will be determined by in vitro assays and skin graft.



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

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: (1/15/19)

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

Actual Expenditure: $1,200,000 total cost