# Co-Administration of MGTA-145 and Plerixafor Rapidly Mobilizes High Numbers of Hematopoietic Stem Cells and Graft-Versus-Host Disease Inhibiting Monocytic Cells in Nonhuman Primates

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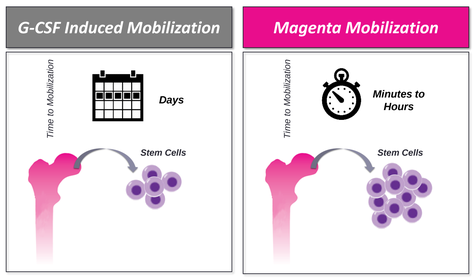
## BACKGROUND

The majority of bone marrow transplants (BMTs) utilize granulocyte-colony stimulating factor (G-CSF) mobilized peripheral blood (mPB) as the source of hematopoietic stem cells (HSCs). Mobilization with G-CSF requires a multi-day dosing regimen and fails to mobilize a large proportion of CD34+CD90+CD45RA- HSCs, the cell type responsible for hematopoietic engraftment. Moreover, up to 80% of allogenic recipients will experience significant side effects such as acute graft-versus-host disease (GvHD), even though G-CSF mPB contains a small but variable number of immunosuppressive monocytes. Identification of a mobilizing regimen that consistently and rapidly produces high numbers of HSCs and immunosuppressive monocytes without the need for G-CSF would be ideal. We previously reported that MGTA-145, a CXCR2 agonist, when combined with the CXCR4 inhibitor, plerixafor, rapidly mobilizes engraftable HSCs that lead to durable neutrophil and platelet recovery following autologous transplantation in nonhuman primates (NHP). As the risk of GvHD remains a significant clinical problem in the allogeneic setting, MGTA-145 plus plerixafor may rapidly mobilize an advantageous graft relative to the standard of care, G-CSF, since MGTA-145 plus plerixafor results in significantly higher numbers of both engraftable HSCs and highly immunosuppressive monocytes.




**Benefits of a novel first-line mobilization regimen:**

- Mobilize more HSCs
- Shorten time required for mobilization
- Fewer adverse events

## METHODS

**HSC Mobilization**

Rhesus macaques received a single injection of MGTA-145 + plerixafor, plerixafor alone or 5 daily injections of G-CSF and peripheral blood was assessed at multiple time points. White blood cells were enumerated on a HESKA Hematology Analyzer. Mobilization of CD34+ and CD34+ CD90+ CD45RA- cells was enumerated by flow cytometry.

**Characterization of CD34$^{dim}$ cells**

CD34$^{dim}$ cells present in mPB were characterized by flow cytometry. To evaluate the ability of CD34$^{dim}$ cells to suppress T cell proliferation *in vitro*, CD3+ T cells were sorted from PBMCs isolated at 4 hours post treatment with MGTA-145 + plerixafor and labeled with carboxyfluorescein succinimidyl ester (CFSE). T cells (50,000 per well) were stimulated *in vitro* with anti-CD2, -CD3 and -CD28-coated beads (10,000 per well) in the presence or absence of autologous CD34$^{dim}$ CD11b$^{+}$ cells (25,000 per well). T cell proliferation was measured by quantifying the number of CFSE$^{dim}$ T cells by flow cytometry following 4 days in culture at 37°C.

**Xenotransplantation in NSG mice**

To model acute GvHD, 6x10$^6$ peripheral blood mononuclear cells (PBMCs) isolated from peripheral blood of nonhuman primates treated with MGTA-145 + plerixafor, plerixafor alone or G-CSF were infused into sublethally irradiated NSG mice. Percent survival was measured. The number of donor-derived T cells in peripheral blood was assessed at Day 14 post transplant.

To evaluate the role of CD34$^{dim}$ cells in mediating protection from acute GvHD, a separate cohort of mice received 6x10$^6$ MGTA-145 + plerixafor-mobilized PBMCs following FACS-depletion of CD34$^{dim}$ CD11b+ cells.

*All in vivo research was conducted in accordance with the Guide for the Care and Use of Laboratory Animals published by the National Research Council of the National Academies and under the approval of the Institutional Animal Care and Use Committee.*

## HEMATOPOIETIC STEM CELL MOBILIZATION

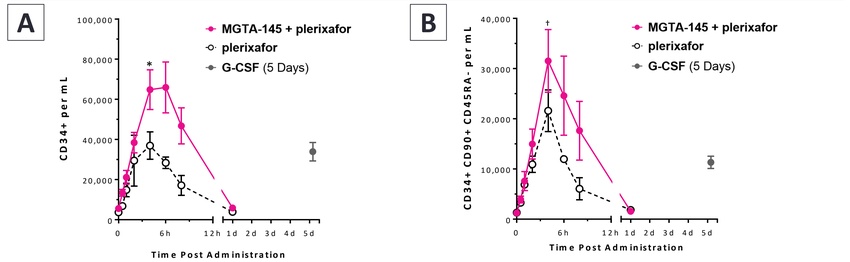



**Figure 1**: **A single injection of MGTA-145 + plerixafor mobilizes higher numbers of CD34+ and CD34+ CD90+ CD45RA- cells compared to a multi-day regimen of G-CSF in nonhuman primates.**
Rhesus macaques received a single injection of plerixafor (1 mg/kg SC) alone or MGTA-145 (450 µg/kg IV) + plerixafor or five daily injections of G-CSF (50 µg/kg SC). **(A)** Treatment with MGTA-145 + plerixafor leads to robust CD34+ cell mobilization that peaks at 4-6 hours post dose administration. **(B)** MGTA-145 + plerixafor mobilizes 2-3 fold higher numbers of CD34+ CD90+ CD45RA- cells, which are the cell type responsible for engraftment in nonhuman primates, compared to a multi-day regimen of G-CSF. Data represent 3-13 animals per group and are expressed as mean +/- SEM. Statistical significance between MGTA-145 + plerixafor versus plerixafor alone was determined by Student's *t* test. * $p < 0.05$.

## CHARACTERIZATION OF CD34$^{DIM}$ CELLS

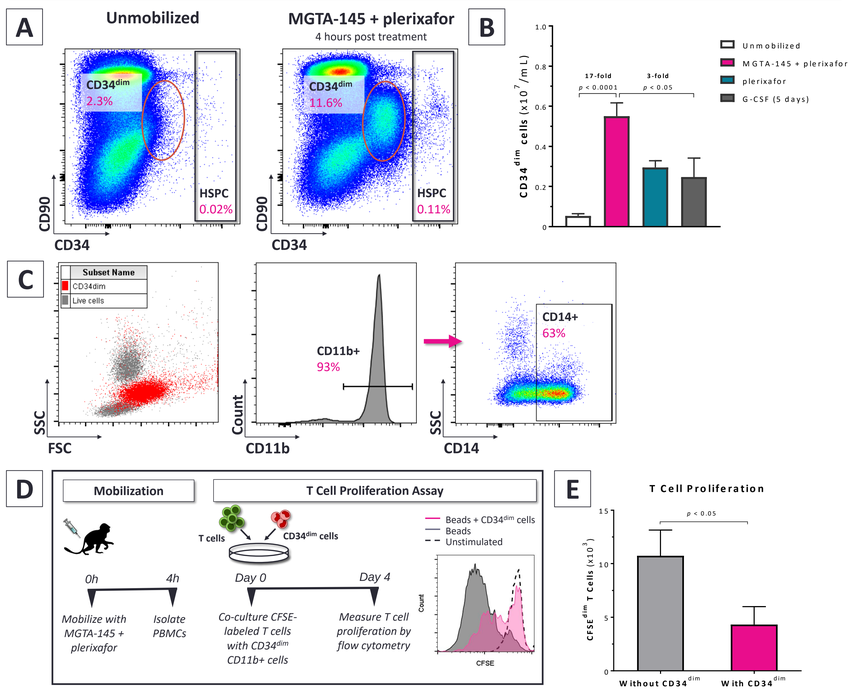



**Figure 2: MGTA-145 + plerixafor mobilizes higher numbers of immunosuppressive CD34$^{dim}$ monocytes in nonhuman primates.**
**(A)** Enumeration of mobilized hematopoietic stem and progenitor cells by flow cytometry revealed a significant increase in the number of CD34$^{dim}$ cells present at 4 hours post treatment with MGTA-145 + plerixafor. **(B)** MGTA-145 + plerixafor mobilized higher numbers of CD34$^{dim}$ cells compared to G-CSF or plerixafor alone. **(C)** Characterization of CD34$^{dim}$ cells by flow cytometry revealed that a majority are SSC$^{low}$ CD14+ CD11b+ monocytes. **(D)** Schematic for T cell proliferation assay using MGTA-145 + plerixafor-mobilized peripheral blood. **(E)** Co-culture of mobilized CD34$^{dim}$ cells with autologous, bead-activated T cells revealed that these cells are capable of suppressing CFSE-labeled T cell proliferation *in vitro*. Data represent 3-13 animals per group and are expressed as mean +/- SEM. Statistical significance determined by Student's *t* test, as shown.

## GRAFT COMPOSITION

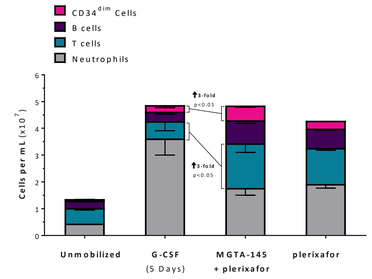



**Figure 3**: **MGTA-145 + plerixafor-mobilized grafts contains higher numbers of T cells than G-CSF-mobilized grafts.**
Rhesus macaques mobilized with a single injection of MGTA-145 + plerixafor had significantly increased numbers of CD34$^{dim}$ cells and T cells in peripheral blood at 4 hours post treatment compared to animals treated with 5 daily injections of G-CSF. Data represent 3-14 animals per treatment group and are expressed as mean +/- SEM. Statistical significance between G-CSF and MGTA-145 + plerixafor was determined by Student's *t* test.

## XENOTRANSPLANTATION IN NSG MICE

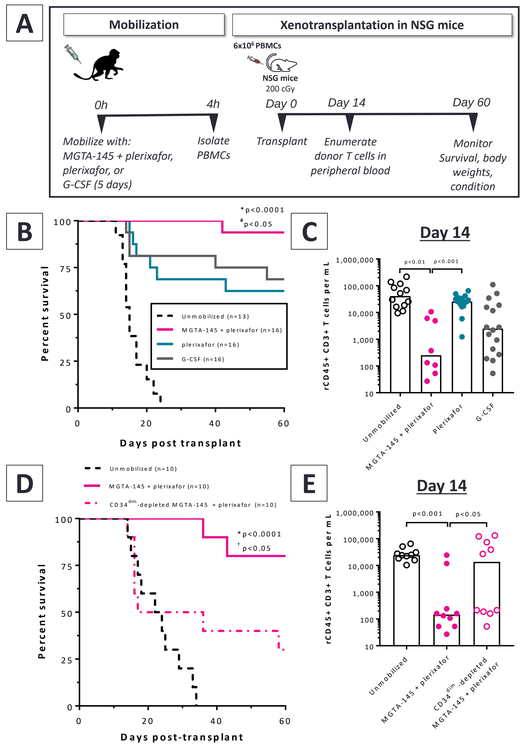



**Figure 4**: **MGTA-145 + plerixafor mobilizes CD34$^{dim}$ cells capable of suppressing Graft-versus-host disease in a mouse xeno-transplantation model.**
**(A)** Schematic for a mouse xeno-transplantation model to assess the development of acute GvHD following mPB transplantation is shown. **(B)** Percent survival of NSG mice transplanted with unmobilized versus MGTA-145 + plerixafor-, plerixafor- or G-CSF-mobilized PBMCs is shown. **(C)** The number of donor-derived T cells in peripheral blood at Day 14 post transplant is shown for each treatment group. A separate cohort of mice received CD34$^{dim}$-depleted PBMCs from MGTA-145 + plerixafor-mobilized NHP. **(D)** Percent survival and **(E)** Day 14 T cell numbers are shown. Data represent 8-16 animals per group. Statistical significance was determined by Log rank test (**B**,**D**) and Student's *t* test (**C**,**E**). * for comparisons to unmobilized, # for comparisons to plerixafor and † for comparisons to CD34$^{dim}$-depleted, as indicated.

## CONCLUSIONS

- **MGTA-145 is a potential new first line mobilization agent for use in combination with plerixafor**
- Robust mobilization of large numbers of HSCs in NHP
- Peak HSC mobilization corresponded with a 17-fold increase in the number of CD34$^{dim}$ cells in peripheral blood. Further characterization revealed that these cells express monocyte markers and are capable of suppressing T cell proliferation *in vitro*.
- MGTA-145 + plerixafor-mobilized CD34$^{dim}$ cells mediate protection against acute GvHD *in vivo*, suggesting that MGTA-145 + plerixafor grafts may be suitable for allogeneic as well as autologous transplantation.
- **Next Step**: Magenta plans to launch a phase 1 clinical study to evaluate MGTA-145 in 2019.