MGTA-145 in Combination with Plerixafor Mobilizes Large Numbers of Hematopoietic Stem Cells that Lead to Rapid Engraftment Following Autologous Transplantation in Nonhuman Primates

Patrick C. Falahee[1], Kevin A. Goncalves[2], Stefan Radtke[2], Sharon L. Hyzy[1], Hans-Peter Kiem[2,3], Anthony E. Boitano[1], Dwight M. Morrow[1], Michael P. Cooke[1]

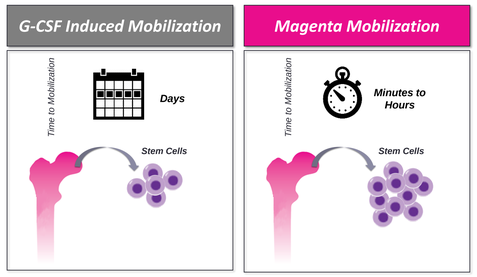
[1] Magenta Therapeutics, Cambridge, MA;

[2] Stem Cell and Gene Therapy Program, Fred Hutchison Cancer Research Center, Seattle, WA; [3] Department of Medicine, University of Washington, Seattle, WA

BACKGROUND

The majority of bone marrow transplants (BMT) utilize granulocyte-colony stimulating factor (G-CSF)-mobilized peripheral blood (mPB) as the source of hematopoietic stem cells (HSCs). However, CD34+ harvest with G-CSF is variable and frequently unpredictable, often requires multiple apheresis sessions and is associated with significant side effects. Identification of a novel first-line mobilization regimen that consistently produces high numbers of engraftable CD34+ cells 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 CD34+ cells in nonhuman primates (NHP; Blood 2017 130:1920). In the current study, we evaluated the ability of MGTA-145 + plerixafor to mobilize a PB graft sufficient to engraft in an autologous NHP transplant.



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 the mobilized graft

The number and frequency of various immune cell subsets in peripheral blood at 4 hours post dose administration was assessed by flow cytometry.

Leukocyte population	Phenotype
T cells	CD45+CD3+CD20-
CD4+ T cells	CD45+CD3+CD20-CD4+
CD8+ T cells	CD45+CD3+CD20-CD8+
Regulatory T cells (CD4+ Tregs)	CD45+CD3+CD20-CD4+CD25+CD127-
B cells	CD45+CD3-CD20+
Natural killer (NK) cells	CD45+CD3-CD20-CD8+
Myeloid Dendritic Cells (mDCs)	CD45+CD3-CD20-CD8- HLA-DR+CD11c+
Plasmacytoid Dendritic Cells (pDCs)	CD45+CD3-CD20-CD8- HLA-DR+CD11c-CD123+
$CD34^{dim}$ CD11b+ Cells	CD45+$CD34^{dim}$CD11b+

Table 1: Phenotypic characterization of immune cell subsets.
Flow cytometry gating strategy was adapted from Kean et al., *Blood*. 2011.

Autologous transplantation in NHP

Rhesus macaques were mobilized with a single injection of MGTA-145 + plerixafor or 5 daily injections of G-CSF, and peripheral blood was collected by leukapheresis beginning 1-2 hours post dose administration. The number and frequency of CD34+ and CD34+CD90+CD45RA- cells in mPB was assessed at multiple time points by flow cytometry.

Positively-selected CD34+ cells were gene-marked (by lentiviral or *CRISPR/Cas9* editing) in culture and autologously transplanted back into the rhesus macaques at 48 hours following split-dose total body irradiation (TBI; 1080 cGy).

Animals were monitored for 60 days following transplant and time to neutrophil (>500/µL) and platelet (>25,000/µL) recovery was measured. Multilineage reconstitution and clonality of gene-marked HSCs in peripheral blood and BM will be assessed (study ongoing).

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

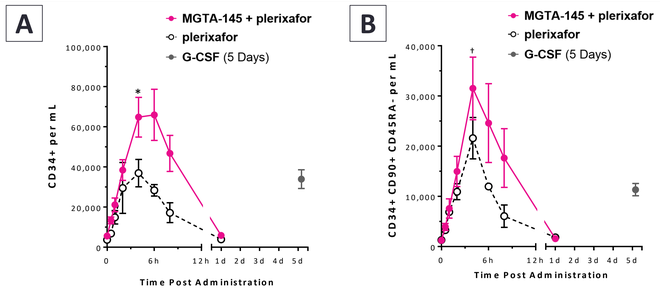


Figure 1: A single injection of MGTA-145 + plerixafor mobilizes higher numbers of CD34+ and CD34+ CD90+ CD45RA- cells compared to a multi-day regimen of G-CSF in nonhuman primates. Rhesus macaques received a single injection of plerixafor (1 mg/kg SC) alone or MGTA-145 (450 µg/kg IV) + plerixafor or 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 was determined by Student's *t* test. *p<0.05 for comparisons to plerixafor and † p<0.05 for comparisons to G-CSF, as shown.

GRAFT COMPOSITION

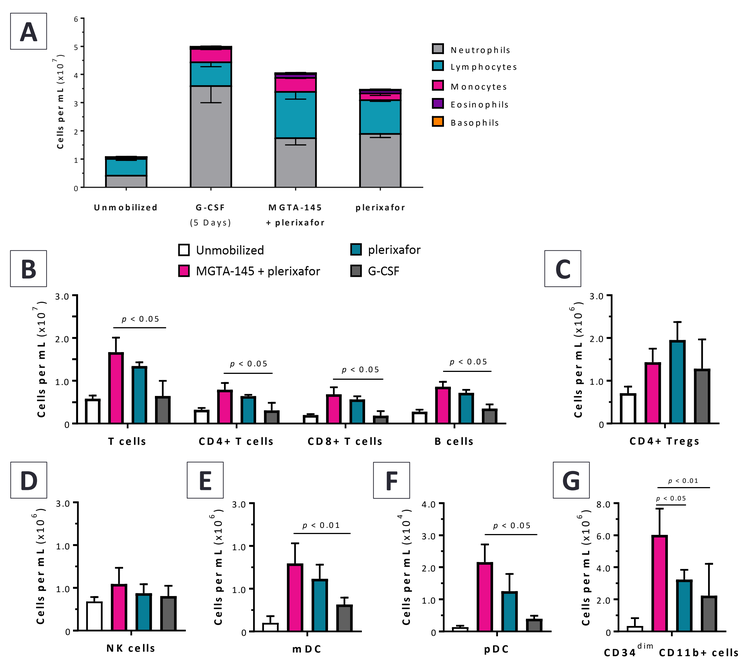
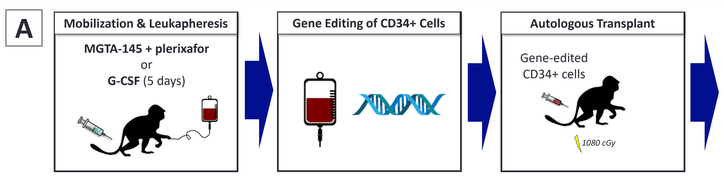


Figure 2: MGTA-145 + plerixafor-mobilized grafts contain higher numbers of T cells, B cells, Dendritic cells and $CD34^{dim}$ CD11b+ cells compared to G-CSF.
(A) CBC analysis of peripheral blood collected at 4 hours post dose administration of a single injection of MGTA-145 + plerixafor or plerixafor alone or 5 daily injections of G-CSF in rhesus macaques. Detailed immune phenotyping by flow cytometry at 4 hours post dose administration revealed that MGTA-145 + plerixafor mobilizes a unique graft in nonhuman primates. Differences in the number of **(B)** T and B cells, **(C)** CD4+ regulatory T cells, **(D)** NK cells, **(E)** myeloid dendritic cells (DCs), **(F)** plasmacytoid DCs and **(G)** $CD34^{dim}$ CD11b+ cells are shown. Data represent 3-13 animals per group and are expressed as mean +/- SEM. Statistical significance determined by Student's *t* test, as shown.

AUTOLOGOUS TRANSPLANTATION IN NHP

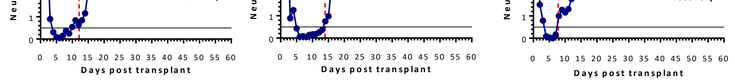


- Neutrophil recovery
- Platelet recovery
- Hematopoietic reconstitution in peripheral blood
- Clonality of gene-edited CD34+ cells from BM aspirates (Day 60)

B

	MGTA-145 + plerixafor		MGTA-145 + plerixafor		G-CSF		G-CSF	
	Cells Harvested	Infusion Product	Cells Harvested	Infusion Product	Cells Harvested	Infusion Product	Cells Harvested	Infusion Product
CD34+/kg	2.29 x 10^6	1.72 x 10^6	1.14 x 10^6	0.52 x 10^6	1.58 x 10^6	2.10 x 10^6	0.54 x10^6	N/A
CD90+/kg	0.91 x 10^6	0.78 x 10^6	0.58 x 10^6	0.23 x 10^6	0.43 x 10^6	0.77 x 10^6	0.15 x10^6	N/A
%CD90+	38%	45%	51%	45%	27%	38%	27%	N/A

C



Insufficient yield
Transplant halted

Figure 3: **Autologous transplant of MGTA-145 + plerixafor-mobilized CD34+ cells leads to rapid neutrophil recovery in nonhuman primates.**
(A) Schematic for a nonhuman primate model of autologous transplantation of gene-edited CD34+ cells. Rhesus macaques were mobilized with a single injection of MGTA-145 + plerixafor or 5 daily injections of G-CSF and mobilized peripheral blood was collected by leukapheresis starting at 1-2 hours post dose administration. Mobilized CD34+ cells were isolated by positive selection and gene edited in a 48 hour culture prior to autologous transplantation back into rhesus macaques following TBI. **(B)** Apheresis yields and **(C)** time to neutrophil recovery are shown for 2 animals transplanted with MGTA-145 + plerixafor-mobilized CD34+ cells compared to 1 animal transplanted with G-CSF-mobilized CD34+ cells. An additional animal mobilized with G-CSF had insufficient CD34+ and CD90+ yield and was not transplanted due to safety concerns. All animals received daily injections of G-CSF (10 µg/kg) post transplant as supportive care to treat neutropenia until neutrophil counts passed 500/µL. The second animal to receive MGTA-145 + plerixafor-mobilized CD34+ cells is at day 18 post transplant, and the animal that received G-CSF-mobilized CD34+ cells is at day 37 post transplant.

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
  - Allows same day mobilization and apheresis in NHP
- **MGTA-145 is differentiated from G-CSF**
  - Mobilizes more CD34+ CD90+ CD45RA- HSCs
  - 2/2 NHP successfully mobilized with MGTA-145 + plerixafor compared to 1/2 NHP mobilized with G-CSF
  - MGTA-145 + plerixafor-mobilized grafts contain higher numbers of $CD34^{dim}$ CD11b+ cells that may play an important role protecting against acute graft-versus-host disease following allogeneic transplantation (See our poster on Saturday evening, **Poster ID 427**).
- Studies to assess platelet recovery, hematopoietic reconstitution and the clonality of gene-edited CD34+ cells in peripheral blood and bone marrow are ongoing.

Next Step: Magenta plans to launch a phase 1 clinical study to evaluate MGTA-145 in 2019.