Poster #4770

Single Dose of tGroβ (EN-145)/Plerixafor Safely and Effectively Mobilizes Primitive HSCs in Mouse Models for *In Vivo* Gene Therapy of Sickle Cell Disease

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Introduction

Background

Successful granulocyte colony-stimulating factor (G-CSF)-free mobilization is crucial for HSC gene therapies for sickle cell disease (SCD). Since G-CSF is contraindicated in SCD patients, single-agent plerixafor (P) mobilization is utilized. However, P alone does not reliably yield optimal hematopoietic stem (HSC) and progenitor (HSPC) cell numbers for *ex vivo* gene therapies (Leonard and Weiss, *Curr Opin Hematol.*, 2024). A single dose of CXCR2 agonist truncated GROβ (tGROβ) in combination with P has mobilized 3 to 4-fold more primitive HSCs compared to either multi-dose G-CSF or P alone in a Phase 1 healthy volunteer study (Magenta Therapeutics-Goncalves et al., *TCT*, 2021). This approach may enable G-CSF free mobilization for SCD, improving efficiency of *ex vivo* gene therapies, as well as Ensoma's *in vivo* HSC gene therapy, which intravenously delivers payload via a virus-like particle (VLP) derived from helper-dependent adenovirus (HDAd5/35++) to HSPC.

Goal

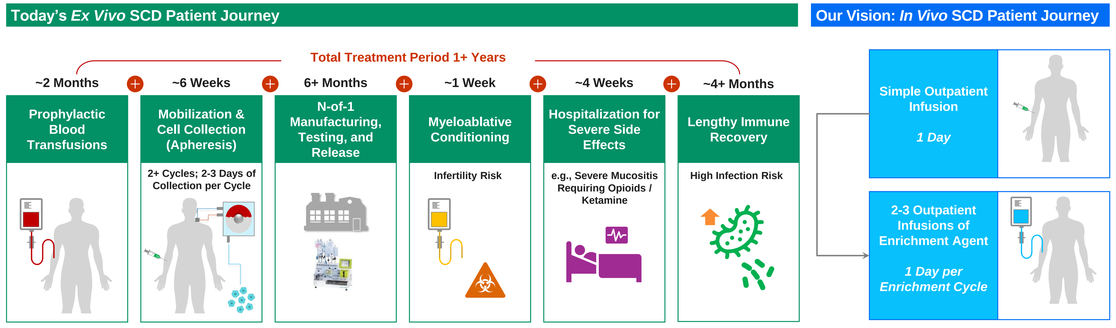
To evaluate the safety and efficacy of Ensoma's tGROβ: EN-145, formerly MGTA-145, in combination with P in our relevant SCD mouse models.

1) hCD46 transgenic mice (wild-type mice expressing human CD46 receptor making them susceptible to HDAd5/35++ transduction): Assess mobilization of LSKs and LT-HSCs with co-administered EN-145+P, as well as gene marking 3-days post mobilization and VLP delivery

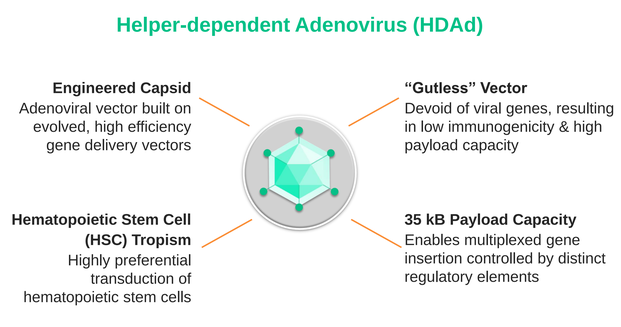
2) Townes Sickle Cell Disease Model (hCD46tgxTownes SS (CD46/SS): Assess safety and efficacy of EN145+P mobilization in mouse model of sickle cell disease

3) Humanized NBSGW mice: Determine efficacy of simultaneous EN145+P in mobilization of CD34+ cells and primitive HSCs in humanized NBSGW mice, and assess gene marking at 3- and 7-days post VLP

SCD | How Ensoma Will Solve *Ex Vivo* Challenges (Figure 1)

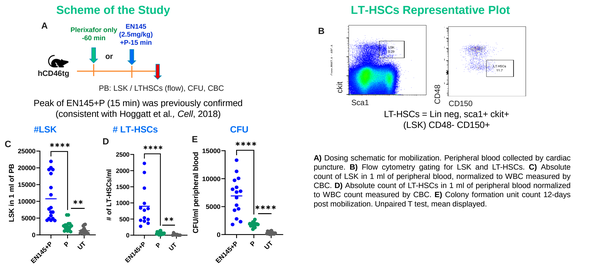


Ensoma's VLP Platform for *In Vivo* HSC Engineering (Figure 2)



Results

EN145+P Enables Robust LSK and LT-HSCs Mobilization in CD46tg Mice (Fig. 3)



A) Dosing schematic for mobilization. Peripheral blood collected by cardiac puncture. **B)** Flow cytometry gating for LSK and LT-HSCs. **C)** Absolute count of LSK in 1 ml of peripheral blood, normalized to WBC measured by CBC. **D)** Absolute count of LT-HSCs in 1 ml of peripheral blood normalized to WBC count measured by CBC. **E)** Colony formation unit count 12-days post mobilization. Unpaired T test, mean displayed.

Conclusions:
Co-administered EN-145+P improved mobilization of both mouse LSK (Lin-Sca1+cKit+ HSPCs) and long-term HSCs (LT-HSC, LSK CD48-CD150+) compared to P only

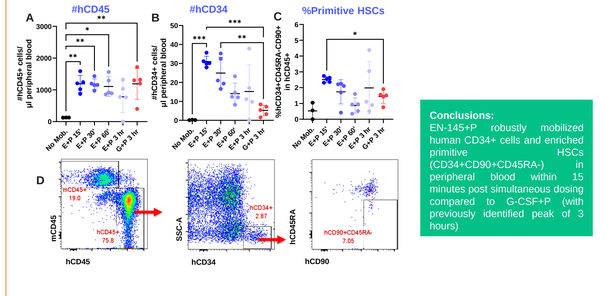
EN-145+P is Well-tolerated in CD46/SS Mice and Mobilizes More LT-HSCs than P Only (Fig. 5)

Significant Mortality Observed post-GCSF Dosing

A) Mortality observed in CD46/SS mice following dosing with G-CSF. **B)** Dosing schematic for mobilization. Peripheral blood collected by cardiac puncture to assess mobilization 60- or 15-minutes post plerixafor or EN-145+Plerixafor, respectively. To assess safety, cardiac puncture was performed at 6 hours post mobilization. **C)** WBC counts measured by CBC 6-hours post-mobilization. **D)** Absolute LSK measured 15- or 60-minutes post-mobilization, calculated by %LSK in CD45+ cells by flow cytometry and normalized to WBC measured by CBC. **E)** Absolute count of LT-HSCs in 1 ml of peripheral blood, calculated by %LT-HSC in CD45+ cells by flow cytometry and normalized to WBC count measured by CBC. **F)** Comparing absolute count of LSK in 1 ml of peripheral blood between CD46 and CD46/SS mice. Results from multiple studies - Unpaired T test , Kolmogorov-Smirnov.

Conclusions:
- 15% mortality of CD46/SS mice observed during G-CSF+P mobilization regimen
- No observed mortality or leukocytosis associated with EN145+P mobilization regimen
- EN145+P mobilized 6-fold more LT-HSC than P only
- Higher absolute LSKs were observed in peripheral blood of CD46/SS compared to CD46 mice in both EN145+P and P groups, likely due to compromised SCD BM niche

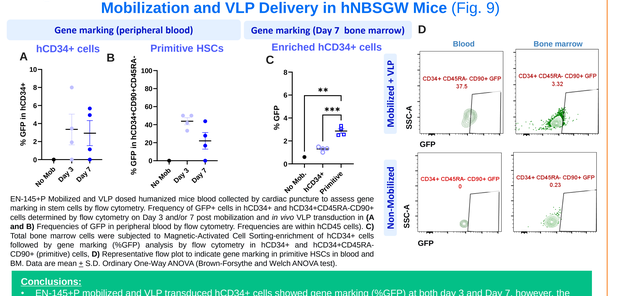
EN145+P Results in Robust Mobilization of hCD34+ and Primitive HSCs (Fig. 7)



Humanized NBSGW mice mobilized with G-CSF (250 µg/Kg) or EN-145 (2.5 mg/Kg) with Plerixafor (5 mg/Kg) by subcutaneous route were bled at 15, 30, 60 minutes and 3 hours. Total number of **(A)** hCD45+ cells **(B)** hCD34+ cells and **(C)** Frequency of hCD34+CD45RA-CD90+ (Primitive) stem cells in peripheral blood. **(D)** Representative flow plot gating for the human CD34+ and CD34+CD45RA-CD90+ (Primitive) stem cell analysis. Total number of hCD45+ cells per µl calculated by multiplying % human chimerism with WBC counts (K/µl) obtained from CBC and total number of hCD34+ cells per µl calculated by multiplying % hCD34+ cells of hCD45+ with total number of hCD45+ cells/µl of blood. Data is representative of two independent studies. Data are mean ± S.D. Ordinary One-Way ANOVA (Brown-Forsythe and Welch ANOVA test).

Conclusions:
EN-145+P robustly mobilized human CD34+ cells and enriched primitive HSCs (CD34+CD90+CD45RA-) in peripheral blood within 15 minutes post simultaneous dosing compared to G-CSF+P (with previously identified peak of 3 hours)

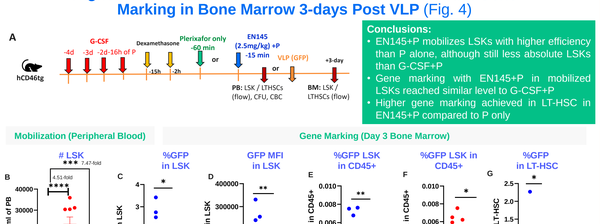
Significant Gene Marking in CD34+ and Primitive HSCs Following EN145+P Mobilization and VLP Delivery in hNBSGW Mice (Fig. 9)



EN-145+P Mobilized and VLP dosed humanized mice blood collected by cardiac puncture to assess gene marking in stem cells by flow cytometry. Frequency of GFP+ cells in hCD34+ and hCD34+CD45RA-CD90+ cells determined by flow cytometry on Day 3 and/or 7 post mobilization and *in vivo* VLP transduction in **(A and B)** Frequencies of GFP in peripheral blood by flow cytometry. Frequencies are within hCD45 cells). **C)** Total bone marrow cells were subjected to Magnetic-Activated Cell Sorting-enrichment of hCD34+ cells followed by gene marking (%GFP) analysis by flow cytometry in hCD34+ and hCD34+CD45RA-CD90+ (primitive) cells. **D)** Representative flow plot to indicate gene marking in primitive HSCs in blood and BM. Data are mean ± S.D. Ordinary One-Way ANOVA (Brown-Forsythe and Welch ANOVA test).

Conclusions:
- EN-145+P mobilized and VLP transduced hCD34+ cells showed gene marking (%GFP) at both day 3 and Day 7, however, the frequency of GFP decreased in primitive cells on Day 7 potentially indicating gene marked cells homing back to bone marrow.
- MACS-enriched hCD34+ cells from bone marrow on Day 7 indicated significant gene marking in both hCD34+ and hCD34+CD45RA-CD90+ (Primitive) cells.
- No gene marking in bone marrow observed in humanized NBSGW mice mobilized with G-CSF+P followed by VLP transduction.

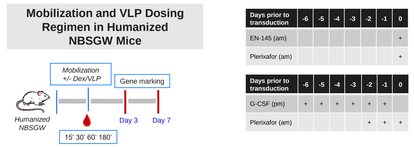
Higher HSC Mobilization with EN145+P Enables Robust Gene Marking in Bone Marrow 3-days Post VLP (Fig. 4)



Conclusions:
- EN145+P mobilizes LSKs with higher efficiency than P alone, although still less absolute LSKs than G-CSF+P
- Gene marking with EN145+P in mobilized LSKs reached similar level to G-CSF+P
- Higher gene marking achieved in LT-HSC in EN145+P compared to P only

A) Dosing schematic for mobilization and VLP. Peripheral blood collected by cardiac puncture to assess mobilization or mice were injected with VLP and harvested 3-days post-VLP to assess gene marking. **B)** Absolute count of LSK in 1 ml of peripheral blood, normalized to WBC measured by CBC. **C)** %GFP+ cells in LSK. **D)** The Mean fluorescence intensity of GFP within LSK cells. **E** and **F)** Frequency of %GFP LSK within CD45+ cells. **G)** %GFP in LT-HSC. Unpaired t-Test, mean displayed.

Mobilization and VLP Dosing Regimen in Humanized NBSGW Mice (Fig. 6)



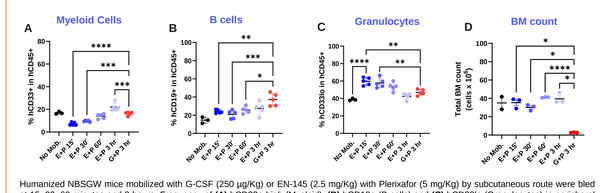
Days prior to transduction	-6	-5	-4	-3	-2	-1	0
EN-145 (am)							+
Plerixafor (am)							+

Days prior to transduction	-6	-5	-4	-3	-2	-1	0
G-CSF (pm)	+	+	+	+	+	+	
Plerixafor (am)					+	+	+

Schematic of Mobilization and VLP dosing in NBSGW mice:

- G+P mobilized CD34+ cells were transplanted into NBSGW mice (1x10^6 cells/mouse) . 7-weeks later, human chimerism is confirmed by flow cytometry in peripheral blood, at which time, mobilization and VLP delivery were performed as follows:
- Humanized NBSGW mice were mobilized with G-CSF (250 µg/Kg) or EN-145 (2.5 mg/Kg) with Plerixafor (5 mg/Kg) by subcutaneous route.
- Dexamethasone (10 mg/Kg) was dosed by intraperitoneal route in mice that received virus like protein (VLP).
- Mobilization kinetic timepoints for EN-145 + Plerixafor were 15 min, 30 min, 60 min and 3 hours.
- Gene marking by GFP level was determined by flow cytometry in bone marrow cells on Day 3 and Day 7 post mobilization and VLP dosing.

EN-145+P Results in Less Mobilization of Human Myeloid/B lymphoid cells than G-CSF+P While Maintaining the BM Cellularity (Fig. 8)



Humanized NBSGW mice mobilized with G-CSF (250 µg/Kg) or EN-145 (2.5 mg/Kg) with Plerixafor (5 mg/Kg) by subcutaneous route were bled at 15, 30, 60 minutes and 3 hours. Frequency of **(A)** hCD33+ high (Myeloid), **(B)** hCD19+ (B-cells) and **(C)** hCD33lo (Granulocytes) in peripheral blood. **(D)** Total BM count obtained on Day 3 post mobilization. Data is representative of two independent studies. Data are mean ± S.D. Ordinary One-Way ANOVA (Brown-Forsythe and Welch ANOVA test).

Conclusions:
- G-CSF+P mobilized more lineage- commited cells like myeloid (hCD33+) and B-cells (hCD19+) in peripheral blood post its peak mobilization of 3 hours (previously identified) than EN-145+P.
- EN-145+P instead released more granulocyte cells (hCD33lo) in peripheral blood at its peak mobilization of 15 min.
- EN-145+P regimen allowed maintenance of bone marrow (BM) cellularity 3 days post mobilization compared to G+P regimen

Summary:

Our comprehensive mouse studies add compelling support for the *in vivo* safety and efficacy of single-dose EN-145+P as a robust alternative to G-CSF for primitive HSC mobilization in SCD. These data support the potential therapeutic value of EN-145+P for *ex vivo* and Ensoma's *in vivo* VLP-based gene therapies in SCD.

References:

Choo et al, *Blood Advances*, 2024, Falahee et al., *ASTCT*, 2018, Hoggatt et al., *Cell*, 2018, Javed et al., *Cell*, 2022, Goncalves et al., *TCT*, 2021, Leonard and Weiss, *Curr Opin Hematol.*, 2024, Li et al., *Blood*, 2023, Li et al., *Blood Adv*. 2021, McIntosh et al., *Stem cell report*, 2015

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