Novel *In Vivo* Gene Therapy Approach to Hematopoietic Stem Cell (HSC) Engineering Creates Durable HSC-Derived Neutrophils to Treat X-Linked Chronic Granulomatous Disease

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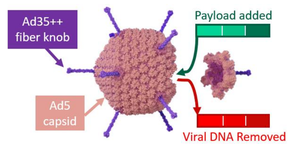
The Ensoma VLP Platform for *in vivo* HSC engineering

Helper-dependent Adenovirus (HDAd) 5/35++



Engineered capsid
Adenoviral vector built on evolved, high efficiency gene delivery vectors

Hematopoietic Stem Cell (HSC) Tropism
Highly preferential transduction of primitive hematopoietic stem cells through Ad35++ fiber knob targeting human CD46 receptor

"Gutless" vector
Devoid of viral genes, resulting in low immunogenicity & high payload capacity

35 kB Payload Capacity
Enables multiplexed gene insertion controlled by distinct regulatory elements

Ensoma's VLPs preferentially transduce primitive long-term HSCs by binding to the CD46 receptor

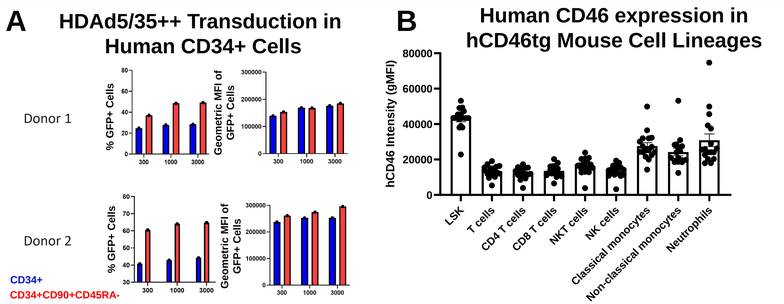


Figure 1 A) HDAd5/35-GFP transduction of CD34+ and primitive HSC populations (CD34+CD90+CD45RA-) in plerixafor mobilized human CD34+ cells from 2 donors, at MOI 300, 1000, and 3000 and cultured for 80 hours. **B)** Human CD46 expression is higher in Lin-Sca-1+c-Kit+ population, compared to other lineages in granulocyte colony stimulating factor and plerixafor mobilized hCD46 transgenic mice (hCD46tg) used in the current study.

X-linked chronic granulomatous disease (X-CGD) is a rare primary immune deficiency disorder

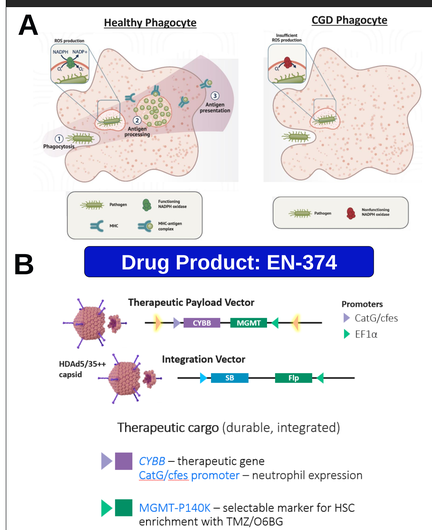
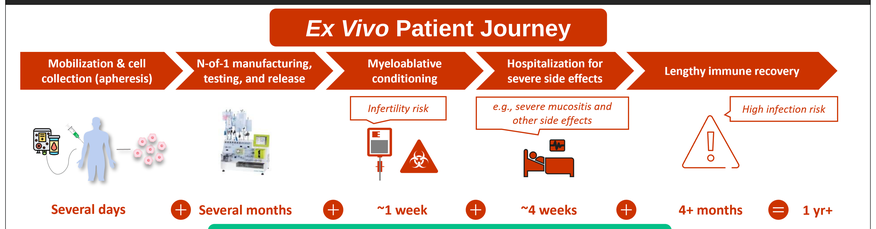


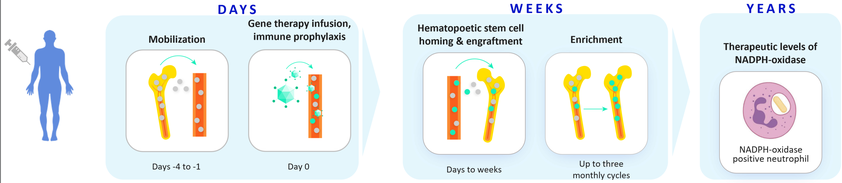
Figure 2 A) CGD is a rare primary immune deficiency disorder caused by a defect in the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase enzyme complex, with the most common mutations being in the X-linked gene, *CYBB,* affecting approximately 65-70% of patients with CGD. The inability to produce NADPH oxidase complex impairs the ability of phagocytic cells to eliminate bacterial and fungal pathogens with reactive oxygen species (1) **B)** EN-374 consists of two HDAd vectors. The therapeutic payload vector expresses a functional *CYBB* gene under the control of Cathepsin G/c-fes promoter, previously shown to regulate tissue-specific transgene expression and function in neutrophils (2, 3) and MGMT^{P140K}, a selective marker, under the control of a ubiquitous promoter, EF1α, to confer resistance to a regimen of O6BG and Temozolomide (TMZ). The integration vector contains encoding Sleeping Beauty (SB)100x transposase and Flippase (Flp) recombinase . In cells co-transduced by both vectors, the transposon is excised and integrated into the genomic DNA.

Ensoma's *in vivo* gene therapy patient journey for X-CGD

Ex Vivo Patient Journey



Our Vision: *In Vivo* Patient Journey



HSCs are mobilized to the peripheral circulation to facilitate VLP transduction. At the peak of HSC mobilization, the clinical product, EN-374, is infused intravenously to transduce the mobilized HSCs. Prior to EN-374 administration, an immune prophylaxis regimen is given to attenuate potential innate immune activation to HDAd. Gene-modified HSCs in the peripheral circulation then home back to the bone marrow shortly after transduction, leading to long-term engraftment. Gene-modified HSCs are then enriched to attain therapeutic levels by administration of O6BG and TMZ.

In vivo HSC engineering with EN-374 and O6BG/TMZ enrichment results in functional HSC-derived neutrophils

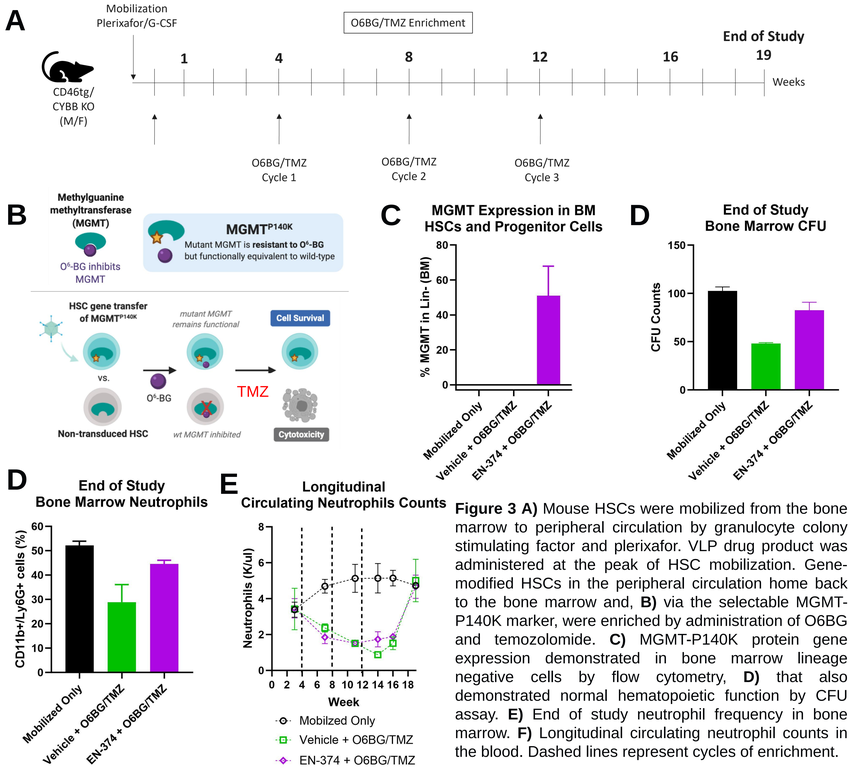


Figure 3 A) Mouse HSCs were mobilized from the bone marrow to peripheral circulation by granulocyte colony stimulating factor and plerixafor. VLP drug product was administered at the peak of HSC mobilization. Gene-modified HSCs in the peripheral circulation home back to the bone marrow and, **B)** via the selectable MGMT-P140K marker, were enriched by administration of O6BG and temozolomide. **C)** MGMT-P140K protein gene expression demonstrated in bone marrow lineage negative cells by flow cytometry, **D)** that also demonstrated normal hematopoietic function by CFU assay. **E)** End of study neutrophil frequency in bone marrow. **F)** Longitudinal circulating neutrophil counts in the blood. Dashed lines represent cycles of enrichment.

EN-374 restores CYBB expression and NADPH oxidase activity in neutrophils of X-CGD disease model mice

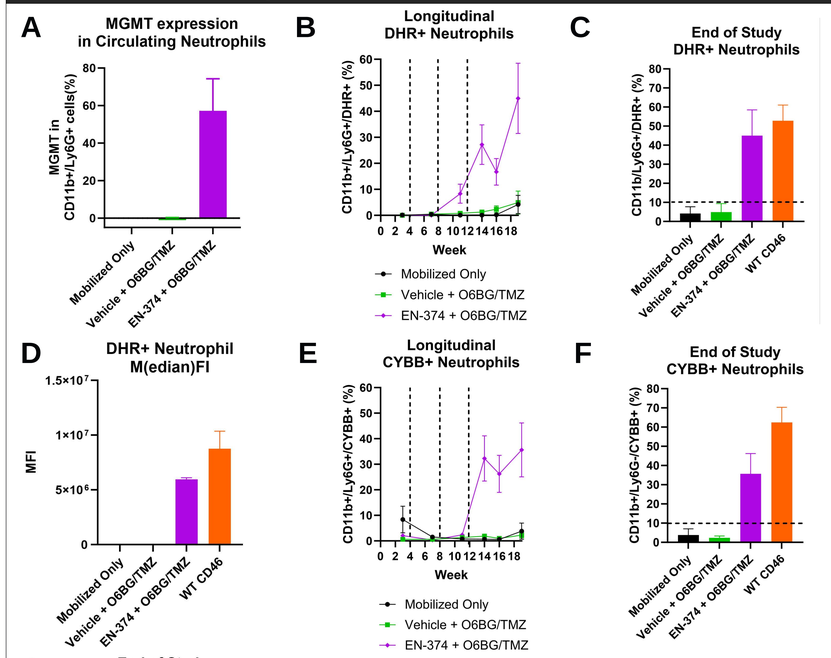


Figure 4 A 19-week *in vivo* study was performed to evaluate the EN-374 vector in mobilized male and female CD46tg/CYBB KO mice. **A)** Using an anti-human MGMT specific antibody, expression of MGMT-P140K protein gene marking in circulating neutrophils is assessed by flow cytometry end of study. Dihydrorhodamine (DHR) assay is a flow cytometry-based method to measure NADPH oxidase activity. **B)** Longitudinal and **C)** end of study DHR activity and **D)** DHR median fluorescent intensity in circulating peripheral neutrophils. **E)** Longitudinal and **F)** end of study CYBB protein in circulating neutrophils. **F)** End of study vector copy number (VCN) of payload in bulk bone marrow. These results together demonstrate that EN-374 is able to modify HSCs *in vivo* and exceed a therapeutic threshold (10%) of neutrophils with functional CYBB following O6BG/TMZ enrichment in CD46tg/CYBB KO mice. Vertical dashed lines indicate cycles of enrichment. Horizontal dashed line indicates 10% therapeutic threshold.

Conclusions

- The development candidate, EN-374, is intended to modify HSCs *in vivo* to express the functional *CYBB* gene and restore ≥10% of neutrophils with NADPH oxidase activity to confer clinically meaningful improvements in infection outcomes for X-CGD patients. Our data demonstrates compelling proof-of-concept for efficient *in vivo* HSC engineering that leads to the expression of functional CYBB in neutrophils at therapeutic levels in X-CGD mice.
- This study sets the foundation for the first *in vivo* HSC gene insertion therapy to reach the clinic and can be applied to a range of genetic disorders which thus far have only been addressed with *ex vivo* gene therapy or allogenic HSCT that involve significant patient burden and manufacturing limitations.

References

1) https://www.cgdpathways.com
2) Santilli et al. Mol Ther, 2011.
3) Kohn et al. Nat Med, 2020.

Related Ensoma Posters

- **Poster 1779 –** Acute safety and biodistribution profile of HSC-targeting virus-like particles based on helper-dependent adenovirus serotype 5/35++ in non-human primates
- **Poster 1783 –** In Vivo Engineering of Hematopoietic Stem Cells with Virus-Like Particles to Generate Multi-Lineage CAR Immune Cell Therapy for Cancer
- **Poster 2016 –** Development and Scale-up of a Novel Adenovirus Production Process