Acute safety and biodistribution profile of HSC-targeting virus-like particles based on helper-dependent adenovirus serotype 5/35++ in non-human primates

Patrick Au, Tiffany Vo, Robert Peters
Ensoma, Inc. Boston, MA USA

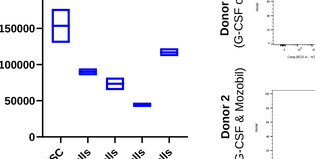
Abstract

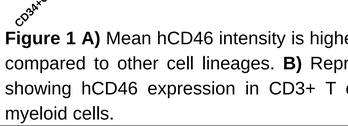
Ensoma has developed a novel platform for *in vivo* HSC gene therapy that uses virus-like particles (VLP) based on helper-dependent adenovirus serotype 5/35++ (HDAd5/35++). VLPs preferentially transduce hematopoietic stem cells (HSC) due to the use of CD46 for cell entry. The acute safety, biodistribution, and transgene expression profile of VLPs (up to 9.25E12 gc/kg) following IV administration in cynomolgus macaques was evaluated. VLPs exhibit a favorable tolerability profile and all animals survived to scheduled terminal euthanasia on Day 8. There were no VLP-related impacts on liver toxicity or histopathology. VLPs were associated with a transient elevation in cytokines and complement that resolved by 72 h. The biodistribution and transduction profiles were consistent with published data with highest VCN detected in liver, spleen, and lung; and minimal VCN and transgene expression in gonads. VLPs show promise for *in vivo* gene therapy.

Test species	Cyno, 3M, 4F Pre-screened negative for NAb / TAb
Test article	HDAd5/35++ EF1p-MGMT-EGFP HDAd5/35++ SB/Flp 1:1 ratio
Mobilization	G-CSF (Day -4, -3, -2, -1, 1) / Plerixafor (Day -1)
Immune prophylaxis	Dexamethasone: q.d. on Day -1 and b.i.d. on Day 1 Tocilizumab: b.i.d on Day 1 Anakinra: b.i.d. on Day 1
Study duration	7 days

The Ensoma VLP platform for *in vivo* HSC engineering

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





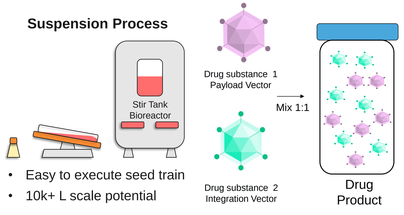
Engineered capsid
Adenoviral vector built on evolved, high efficiency gene delivery vectors

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

Scalable manufacturing of drug substance and drug product



Suspension-based manufacturing
Stir tank bioreactor improves per cell yield

Highly scalable
Demonstrated 2L, 10L, and 200L production

Low manufacturing cost

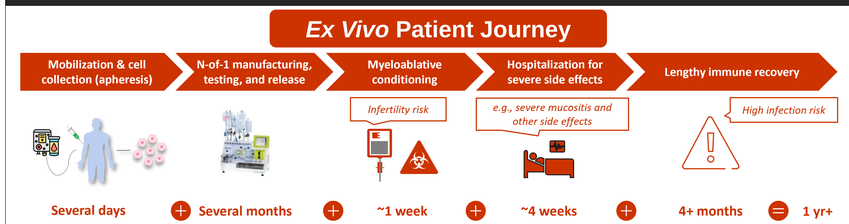
See Poster 2016: D. Pano … C. Wright et al. Development and Scale-up of a Novel Adenovirus Production Process

Ensoma's VLPs target CD46 receptor on HSCs for entry

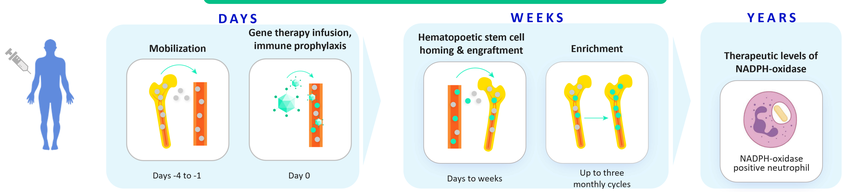
Figure 1 A) Mean hCD46 intensity is highest in human CD34+CD90+ primitive HSC population, compared to other cell lineages. **B)** Representative histograms of 2 mobilized PBMC donors showing hCD46 expression in CD3+ T cells, CD19+ B cells, CD56+ NK cells and CD33+ myeloid cells.

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

Ex Vivo Patient Journey



Our Vision: *In Vivo* Patient Journey



Ensoma is developing EN-374, an *in vivo* HSC-targeted therapy for chronic granulomatous disease (CGD). Following HSC mobilization, EN-374 (HDAd5/35++ DP consisting of 1:1 CYBB and MGMT-P140K therapeutic vector and Sleeping Beauty-Flp integration vector) is infused intravenously to transduce the mobilized HSCs. 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 temozolomide.

Biodistribution and gene expression profile

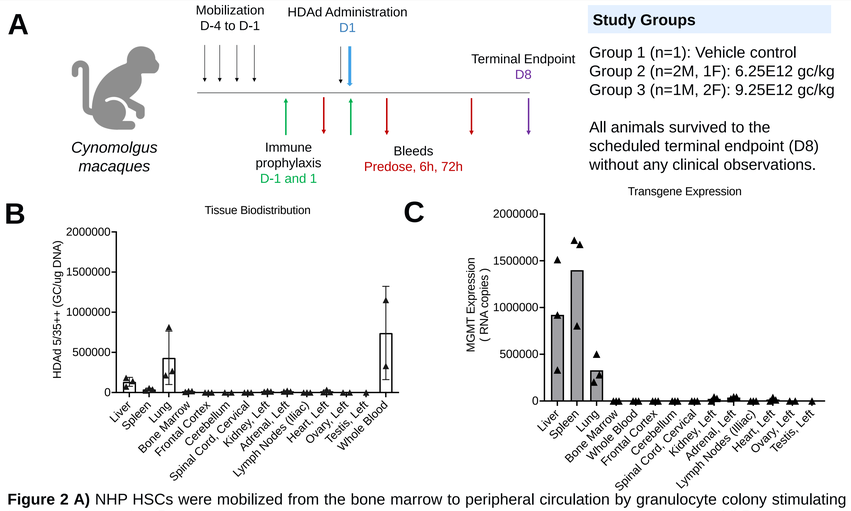


Figure 2 A) NHP HSCs were mobilized from the bone marrow to peripheral circulation by granulocyte colony stimulating factor and plerixafor. Dexamethasone, tocilizumab, and anakinra were given as immune prophylaxis. VLP drug product was administered at the peak of HSC mobilization. Bleeds were obtained predose, 6h, 72h, and at study end. **B)** Tissue biodistribution of HDAd5/35++ was measured in blood and perfused tissued by qPCR on Day 8 and was highest in the blood. **C)** Gene expression of MGMT in tissues was measured by RT-qPCR on Day 8. Vector genomes and gene expression were not observed in ovary and testis, suggesting minimal risk for germline transmission.

Histopathology findings

- There were no HDAd5/35++-related microscopic findings or gross changes.
- Myeloid hyperplasia and mixed or mononuclear inflammatory cells infiltration observed in different tissues were consistent with the administration of mobilization premedication.
- All other microscopic findings were considered incidental/commonly reported in cynomolgus monkeys and were considered unrelated to the administration of pre- or post-treatment medication and HDAd5/35++.

Clinical chemistry, hematology, and complement analysis

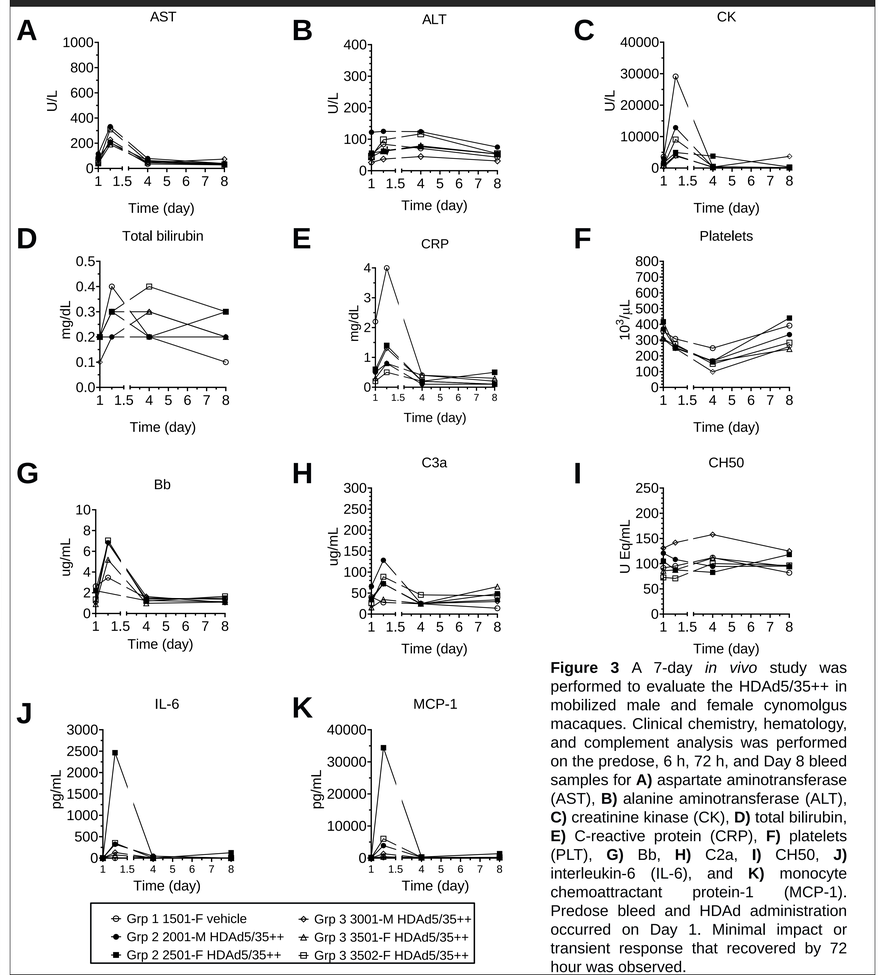


Figure 3 A 7-day *in vivo* study was performed to evaluate the HDAd5/35++ in mobilized male and female cynomolgus macaques. Clinical chemistry, hematology, and complement analysis was performed on the predose, 6 h, 72 h, and Day 8 bleed samples for **A)** aspartate aminotransferase (AST), **B)** alanine aminotransferase (ALT), **C)** creatinine kinase (CK), **D)** total bilirubin, **E)** C-reactive protein (CRP), **F)** platelets (PLT), **G)** Bb, **H)** C2a, **I)** CH50, **J)** interleukin-6 (IL-6), and **K)** monocyte chemoattractant protein-1 (MCP-1). Predose bleed and HDAd administration occurred on Day 1. Minimal impact or transient response that recovered by 72 hour was observed.

Conclusions

- Ensoma has developed a scalable, low-cost, *in vivo* HSC gene therapy platform for durable gene correction as an alternative to allogeneic transplant and *ex vivo* gene therapy.
- No detectable HDAd biodistribution or transgene expression was observed in gonads (ovaries and testes) by Day 8, suggesting minimal risk for germline transmission.
- HDAd administration resulted in minimal impact on liver enzymes (AST, ALT), transient increase in complement activation markers (Bb, C3a), and cytokines (IL-6, MCP-1) and transient reduction in PLT that resolves by 72 hours.
- IV administration of HDAd5/35++ vector up to 9.25E12 gc/kg was well tolerated in NHP and the data support the further development of HDAd5/35++ vector for *in vivo* HSC gene therapy.

Key founder publications and related posters

1. Adair et al. JCI, 2014.
2. Jansen et al. Cancer Gene Ther, 2002
3. Li et al. Mol Ther Methods Clin Dev, 2021.
4. Richter et al. Blood, 2016.
5. Wang et al Exp Hematol, 2008.
6. Wang et al Mol Ther Methods Clin Dev, 2018.

Poster 1780: S. Kattula et al. Novel In Vivo Gene Therapy Approach to Hematopoietic Stem Cell (HSC) Engineering Creates Durable HSC-Derived Neutrophils to Treat X-Linked Chronic Granulomatous Disease.
Poster 1783: C. Chokshi et al. In Vivo Engineering of Hematopoietic Stem Cells with Virus-Like Particles to Generate Multi-Lineage CAR Immune Cell Therapy for Cancer