A Comparative Analysis of Engineered Liver Tropic Capsids Across Different Species: Towards Cross-Species Translation

487

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ABSTRACT

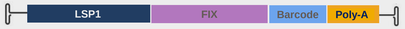
Advancements in genomic medicine emphasize the need for improved delivery systems, especially for translating research from rodents to humans. This study focuses on evaluating liver-targeting capsids for their effectiveness in different models, including human liver cells, WT mice, humanized mice, and non-human primates (NHPs), to assess their potential for medical applications.

Using various techniques, such as measuring RNA and protein levels, the study assessed how well different capsids delivered genes in each model. Consistent results were found across humanized mouse models and human liver cells, suggesting promising candidates for further research. Notably, the top-performing capsid in humanized models also worked well in mice, indicating its potential for preclinical and clinical studies. Moreover, the leading capsid identified in mouse and primary human hepatocyte studies remained effective in NHPs, which are more relevant to humans.

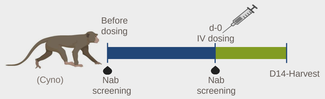
These findings not only enhance our understanding of capsid behavior but also establish a standardized screening method for evaluating capsids across different species. Identifying a common capsid with consistent performance across various models represents a significant advancement in genomic medicine.

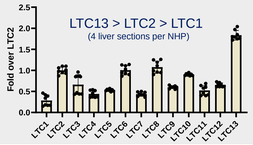
LIVER TROPIC CAPSID (LTC) LTC13 EMERGED AS TOP PERFORMER IN NAÏVE NHP

Construct design



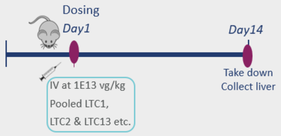
Group	Animal number	Dosage (vg/kg)	Capsids	Duration	Route	Readout
1	3	~1.5E12 for each vector (2E13vg/kg as total dosage)	10 VR-I variants, LTC1, LTC2 and LTC13	14 days	IV	1. RNA and DNA from liver Biodistribution DNA/RNA in different tissues

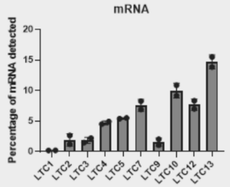




- LTC13 exhibited the highest potency in mRNA expression among the tested capsids in promoting transgene hFIX mRNA expression in non-human primates (NHP).

LTC13 DEMONSTRATED HIGHER RNA EXPRESSION IN WILD TYPE MICE



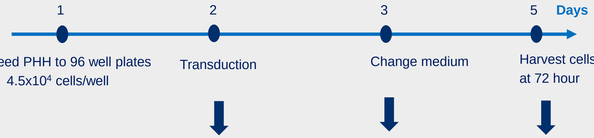


- In wild-type (WT) mice, mRNA analysis indicated that LTC13 was more effective in delivering the transgene to the nucleus for expression.

All animal procedures were reviewed and approved by the Institutional Animal Care and Use Committee (IACUC).

LTC13 EXHIBITED INCREASED TRANSDUCTION EFFICIENCY IN PRIMARY HUMAN HEPATOCYTES (PHH)

Ex-vivo transduction assay design

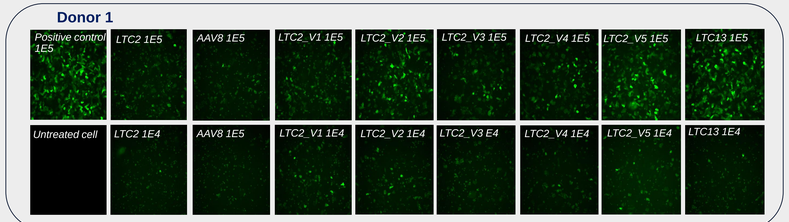


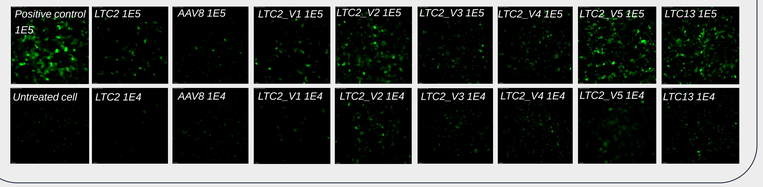
- MOI*: 1e5, 1e4 vg/cell
  - LTC1
  - LTC2
  - LTC13
  - Individual variants

*MOI: multiplicity of infection

- GFP observation
- GFP - image

Ranking tropism of LTC variants by GFP visualization in PHH





LTC13 > LTC2 > LTC2-V3 or V4 etc.

- Ranking of the transduction efficiency in both donor types consistently positioned the LTC13 as the top performer among tested capsids, aligning with the findings observed in non-human primates, wild-type mice, and humanized FRG and PXB mice.

ELEVATED HEPATOCYTE TROPISM WITH LTC13 IN HUMANIZED MICE

Humanized FRG vs. PXB mice

FRG (Fah-/- Rag2-/- Il2rg-/-)

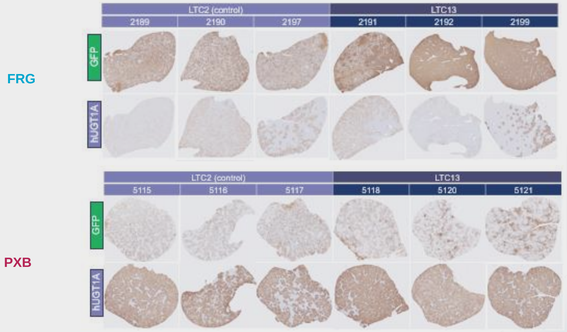
- Created and derived from a combination of three genetic modifications (Fah, Rag2, and Il2rg), knocking out these genes severely deficient the mice in producing their own functional hepatocytes, immune cells, and certain cytokines.
- Lack T, B and NK cells, tolerate human hepatocyte transplantation.

PXB (uPA & SCID)

- Created and derived from a combination of two genetic modifications (uPA and SCID), by inserting human hepatocytes into mice genetically modified to produce lower levels of mouse liver enzymes.
- Lack B, T cells, still have some NK cells, showed severely comprised.

LTC13 and LTC2 capsid variant comparison by GFP immunostaining in the liver

LTC2 and LTC13 vectors were IV administered at 1E13 vg/kg to humanized hFRG and PXB mice for two weeks



According to the human marker hUGT1A1:

- approximately 50% of human hepatocytes population in FRG mice
- approximately 90% of human hepatocytes population in PXB mice

- FRG and PXB contain a heterogeneous population of mouse and human hepatocytes
- LTC13 resulted in elevated transgene expression throughout the liver compared to LTC2

CONCLUSIONS

- LTC13 demonstrated superior potency in promoting transgene expression in both non-human primates and wild-type mice, with enhanced liver tropism observed.
- Consistent ranking across human donor types positioned LTC13 as the top performer in transduction efficiency among tested capsids, aligning with findings from various animal models.
- The identification of LTC13 as a common capsid with tropism and transduction capabilities across various species is a significant advancement in genomic medicine. Its consistent performance in promoting gene delivery simplifies research efforts and offers a versatile tool for studying genomic medicine efficacy and disease modeling across various animal models.