Re-Dosing of Liver-Targeted AAV Within and Across Clades in Mice: Effects of Neutralizing Antibodies and Vector-Specific Factors

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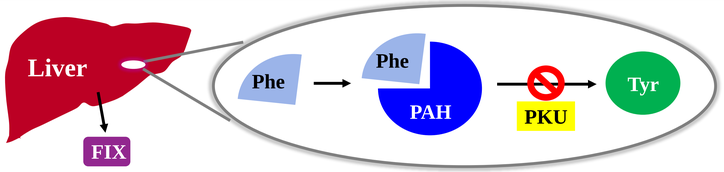
Introduction

Recombinant adeno-associated virus (rAAV)-based gene therapy is a promising approach for the treatment of inherited diseases. Since AAV vectors are non-replicating, episomal transgene expression may wane over time, either due to dilution as a result of organ growth in pediatric patients or in response to tissue injury or inflammation. The formation of neutralizing antibodies (nAb) and memory T cells in response to the initial AAV vector treatment may limit the ability to re-dose. Here, we investigated the AAV re-dosing potential using different AAV clades to deliver liver-specific genes, coagulation factor IX (*FIX*) and phenylalanine hydroxylase (*PAH*), in the pre-clinical *Pah*^{enu2} mouse model of phenylketonuria, which has elevated serum phenylalanine (Phe).

Liver Regulates Blood Clotting and Protein Metabolism



PAH: Phenylalanine hydroxylase; Tyr: Tyrosine; PKU: Phenylketonuria; FIX: Coagulation factor IX

Study Design

Study Arm A

	1st Dose: 5E+13 vg/kg (Day 0)	2nd Dose: 5E+13 vg/kg (Day 116)
Group 1	AAV5_ssLP1-FIX (AAV5 Clone)	AAVHSC15_ssDnG-PAH (Clade F)
Group 2	AAV6_ssLP1-FIX (Clade A)	AAVHSC15_ssDnG-PAH (Clade F)
Group 3	AAV8_ssLP1-FIX (Clade E)	AAVHSC15_ssDnG-PAH (Clade F)
Group 4	AAV9_ssLP1-FIX (Clade F)	AAVHSC15_ssDnG-PAH (Clade F)
Group 5	AAVHSC15_ssLP1-FIX (Clade F)	AAVHSC15_ssDnG-PAH (Clade F)
Group 6	AAVHSC17_ssLP1-FIX (Clade F)	AAVHSC15_ssDnG-PAH (Clade F)
Group 7	Formulation Buffer	Formulation Buffer

Study Day	-14	0	3	21	31	51	66	99	116	119	137	161	183	203
Peripheral immune cell profile	✓		✓	✓	✓	✓	✓	✓		✓	✓	✓	✓	✓
Peripheral cytokine profile	✓		✓	✓	✓	✓	✓	✓		✓	✓	✓	✓	✓
nAb								✓						✓
Liver vg														✓
Blood vg			✓	✓	✓	✓	✓	✓		✓	✓	✓	✓	✓
Serum Phe/Tyr	✓		✓	✓	✓	✓	✓	✓		✓	✓	✓	✓	✓
Plasma FIX	✓		✓	✓	✓	✓	✓	✓		✓	✓	✓	✓	✓

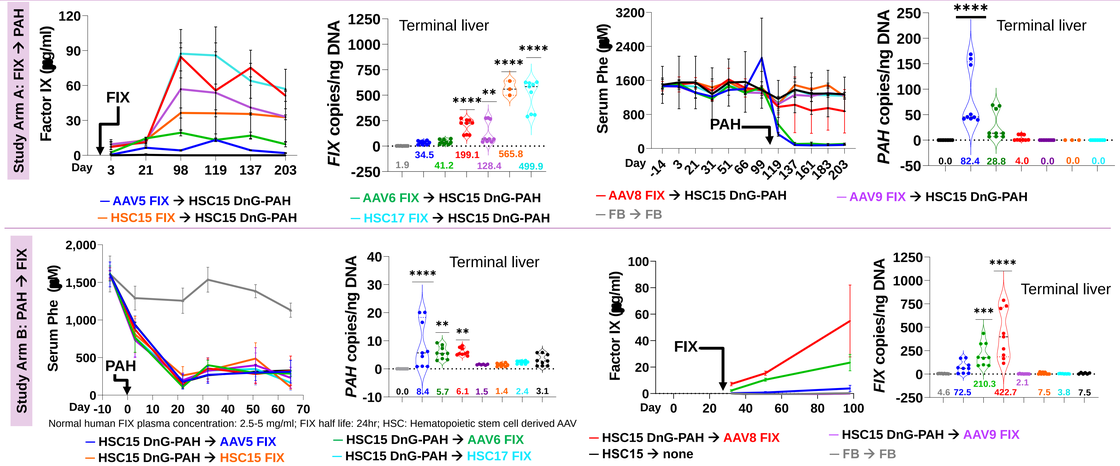
Study Arm B

	1st Dose: 5E+12 vg/kg (Day 0)	2nd Dose: 5E+13 vg/kg (Day 29)
Group 1	AAVHSC15_ssDnG-PAH (Clade F)	AAV5_ssLP1-FIX (AAV5 Clone)
Group 2	AAVHSC15_ssDnG-PAH (Clade F)	AAV6_ssLP1-FIX (Clade A)
Group 3	AAVHSC15_ssDnG-PAH (Clade F)	AAV8_ssLP1-FIX (Clade E)
Group 4	AAVHSC15_ssDnG-PAH (Clade F)	AAV9_ssLP1-FIX (Clade F)
Group 5	AAVHSC15_ssDnG-PAH (Clade F)	AAVHSC15_ssLP1-FIX (Clade F)
Group 6	AAVHSC15_ssDnG-PAH (Clade F)	AAVHSC17_ssLP1-FIX (Clade F)
Group 7	AAVHSC15_ssDnG-PAH (Clade F)	None
Group 8	Formulation Buffer	Formulation Buffer

Study Day	-14	0	3	22	29	32	51	65	98
Peripheral immune cell profile	✓		✓	✓		✓	✓	✓	✓
Peripheral cytokine profile	✓		✓	✓		✓	✓	✓	✓
nAb				✓					✓
Liver vg									✓
Blood vg			✓	✓		✓	✓	✓	✓
Serum Phe/Tyr	✓		✓	✓		✓	✓	✓	✓
Plasma FIX	✓		✓	✓		✓	✓	✓	✓

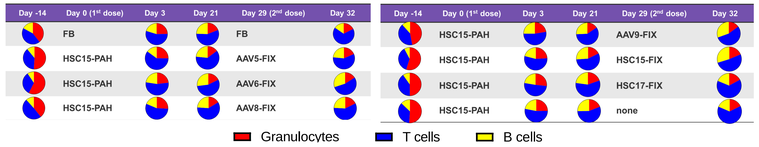
Results

Re-Dosing Across Different AAV Clades and Clones is Successful; However, Pre-Existing Vector Genome (vg) and Other Factors in the Target Tissue Could Inhibit Successful Re-Dosing



- Animals dosed with AAV5, AAV6, or AAV8 then followed by AAVHSC15, or vice versa showed successful transduction and gene expression, as shown by FIX expression and Phe reduction.
- Animals could not be re-dosed with the same AAV Clades: e.g., AAV9 ↔ AAVHSC15.
- The interval between two doses and liver capacity may determine the success of the second dose, even between different clades and clones of AAV
  - In the study arm A, only one mouse in the [AAV8-FIX 5E+13 vg/kg → AAVHSC15-PAH 5E+13 vg/kg] group had *PAH* copy number at 11.3/ng DNA, whereas two other mice had 0.38 *PAH* copies/ng DNA. Consistently, the mouse with vg 11.3 *PAH* copies/ng DNA had successful serum Phe reduction, whereas the other two did not have changes in serum Phe level.
  - On the contrary, In the study arm B, every mouse in the [AAVHSC15-PAH 5E+12 vg/kg → AAV8-FIX 5E+13 vg/kg] group showed successful reduction of serum Phe level and FIX expression. *PAH* copy number per ng DNA ranged between 1.4 and 8.4.
  - These data indicate that the presence of a high level of vg residing in the cells from the first dose in a given timeframe (study arm A) and/or other liver factors could itself inhibit the transduction of the second dose, which is an additional mechanism beyond the presence of nAb.

Immune Response is Not Significantly Different Between Treatment Groups



- Major immune cell types including CD4$^+$ T cells, CD8$^+$ T cells, B cells, monocytes, granulocytes, and dendritic cells were analyzed by using flow cytometry.
- Cytokines including IL-1β, IL-6, IFN-γ, IFN-β, IL-12p70, TNF-α were analyzed by using Meso Scale Discovery.
- There was little to no difference in the immune response between treatment groups in both study arms (data on study arm B is not shown).

Conclusions

- This pre-clinical mouse study demonstrates that re-dosing across different AAV clades/clones is possible.
- Our data points to the importance of the transduction level and other target tissue factors in driving successful AAV re-dosing.
- Further studies are warranted for translation into human patients.