Impact of genome accessibility and long-term expression of adeno-associated virus 5 produced in mammalian (HEK293) and insect (*Sf*) cell lines

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Background

- Valoctocogene roxaparvovec (AAV5-hFVIII-SQ) is an investigational gene therapy in development for the treatment of severe hemophilia A
  - The vector expresses a B-domain deleted human factor VIII coding sequence driven by a liver-selective promoter
  - The genome of AAV5-hFVIII-SQ exceeds the optimal packaging capacity of AAV vectors
  - AAV5-hFVIII-SQ is produced using a baculovirus - *Spodoptera frugiperda* (*Sf*) insect-cell system
  - Human HEK293 cells are also used for rAAV vector production and may package larger genomes better, while *Sf*-based systems offer a more scalable alternative for commercialization

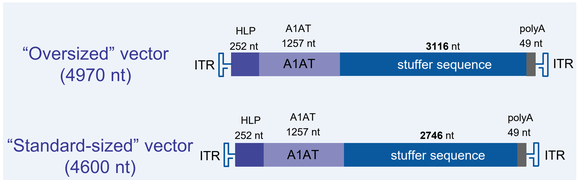
Objectives

- To compare the long-term durability of expression in mice treated with “oversized” (~4900 nt) and “standard-sized” (4600 nt) rAAV5 human alpha-1 antitrypsin (rAAV5-hA1AT) vectors produced in HEK293 or *Sf* cells and understand the mechanistic factors affecting long-term transgene expression

Study design

- C57BL/6 WT mice (8 weeks of age) were administered intravenously with vectors at 6x10^{13} vg/kg dose
  - **Serial bleed cohort:** blood was collected weekly during the first 4 weeks after dosing, then monthly through week 57 for serum hA1AT protein assay
  - **Take down cohort:** livers were collected for oversized vector genome analysis at weeks 1, 3, 12, 24, and 57
    - A1AT reporter vectors were used instead of AAV5-hFVIII-SQ to give sequence length flexibility and minimize variability from serial blood sampling using the tail nick method, which has been shown to activate the clotting cascade and lead to consumption of FVIII

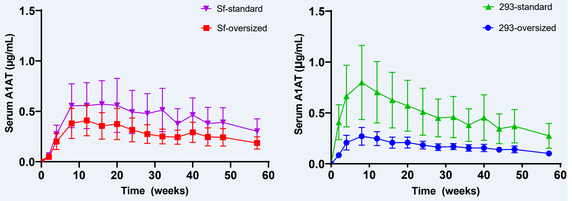
Figure 1. AAV5-HLP-hA1AT reporter vectors



A1AT, alpha-1 antitrypsin; HLP, human liver-specific promoter; ITR, inverted terminal repeat; nt, nucleotide; polyA, polyadenylation signal; *Sf*, *Spodoptera frugiperda*

Results

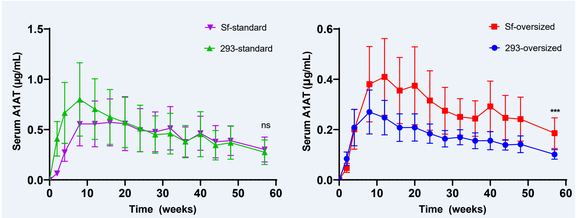
Figure 2. Standard-sized vector achieved higher expression levels compared to oversized vector regardless of production system



Data are mean ± SD, A1AT, alpha-1 antitrypsin; *Sf*, *Spodoptera frugiperda;* 293, human HEK293 cells

- Protein expression levels were significantly lower in mice dosed with oversized vector as compared to standard-sized vector
  - At week 57, the difference between oversized and standard-sized vectors was greater for 293-produced vectors (209% higher) than for *Sf*-produced vectors

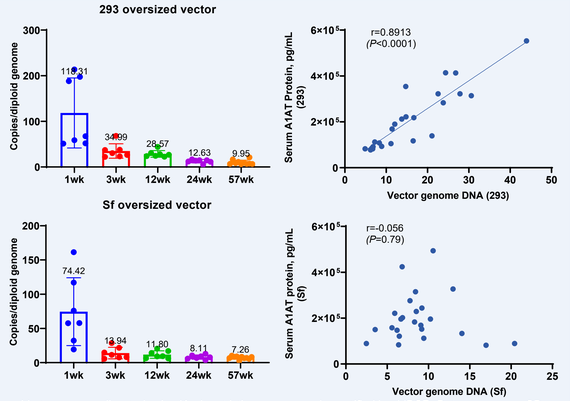
Figure 3. Vectors produced in *Sf* and 293 systems showed comparable long-term durability and similar pattern of expression profile



Data are mean ± SD; ns, not significant; *** *P*<0.001; A1AT, alpha-1 antitrypsin; *Sf, Spodoptera frugiperda;* 293, human HEK293 cells

- For all vectors, serum hA1AT protein levels peaked by 8-12 weeks post-dose followed by a decline (47-63%) to week 57
  - For standard-sized vectors, there was no significant difference in hA1AT expression between HEK293 and *Sf*-produced vectors from weeks 12-57
  - In contrast, mice dosed with *Sf*-produced oversized vector had slightly higher circulating hA1AT level from weeks 12-57 (77% higher level by week 57, *P*<0.0001)

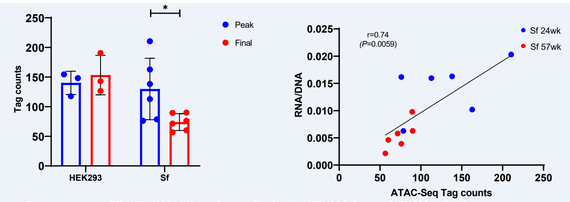
Figure 4. Degradation of liver vector genome mediates decline of transgene expression in mice dosed with 293-produced vector



Vector genome digested using KpnI restriction enzyme and quantified by ddPCR. Data are mean ± SD, *Sf, Spodoptera frugiperda;* 293, human HEK293 cells

- Liver vector genomes decreased continuously from week 1 through week 57 in mice dosed with 293-produced vector, and significantly correlated with circulating A1AT protein levels
- In contrary, changes in vector genome levels were minimal (week 3 through week 57) in mice dosed with *Sf*-produced vector and had no correlation with circulating A1AT protein levels.
- This suggests genome metabolism in hepatocytes is responsible for the decline of A1AT protein in circulation in mice dosed with 293-produced vector but not in mice dosed with *Sf*-produced vector

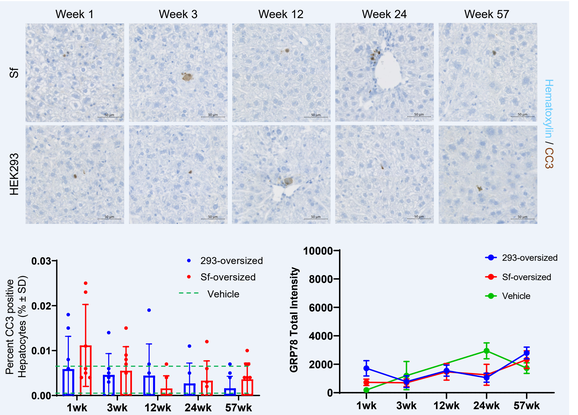
Figure 5. Decrease in genome accessibility impacts decline of transgene expression in mice dosed with *Sf*-produced vector



Data are mean ± SD, * *P*<0.05; *Sf, Spodoptera frugiperda;* HEK293, human HEK293 cells; Tag counts are ATAC-Seq peak regions in the AAV5 promoter (1-660 bp) corrected for sequencing depth and normalized to vector genome copies

- ATAC-Seq analysis showed a decrease in genome accessibility from peak (week 24) to final (week 57) time point in *Sf*-vector dosed mice (*P*=0.045) but not in HEK293-vector dosed mice

Figure 6. Liver evaluations showed no tumors and minimal changes in hepatocyte proliferation, apoptosis, or ER stress



CC3, cleaved-caspase 3; *Sf, Spodoptera frugiperda;* HEK293, human HEK293 cells; Data are mean ± SD

- No significant differences in cleaved-caspase 3 staining, ER stress sensor - GRP78, or cell proliferation (data not shown) in mice dosed with HEK293- and *Sf*-produced vectors

Conclusions

- rAAV5 vectors produced in HEK293 and *Sf* cells showed similar long-term durability of expression in mice
- Oversized vectors produced in *Sf* cells may represent a viable alternative to vectors produced in HEK293 cells
- Determinants and dynamics of genome accessibility in *Sf* cells, including transcription factor binding and epigenetic modifications, may have distinct impacts on the durability of transgene expression
  - Deeper analysis of vector genome methylation, histone marks, and transcription factors occupancy are in progress
- No liver tumors were observed after more than one year of follow up in mice dosed with AAV5-hA1AT vectors produced in either manufacturing systems

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Disclosures:
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