Presentation #OR54

Vector genome loss and epigenetic modifications impact long-term transgene expression of AAV5 vectors produced in mammalian HEK293 and insect *Sf* cells

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Disclosures

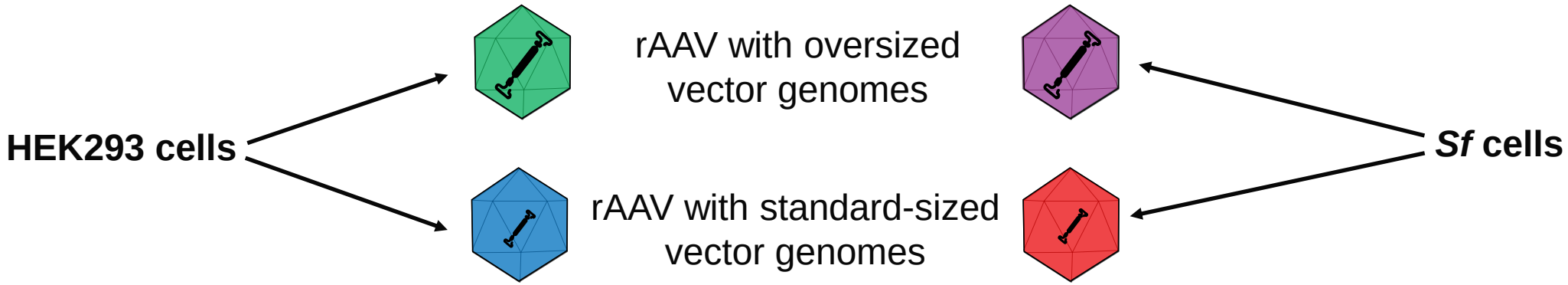
- Funding for this study was provided by BioMarin Pharmaceutical Inc.
- All authors are employees and stockholders of BioMarin Pharmaceutical Inc.

Introduction

- rAAV can be produced in human HEK293 cells or *Sf* insect-cell systems[1–4]
- Valoctocogene roxaparvovec (AAV5-hFVIII-SQ) is a gene therapy for the treatment of severe haemophilia A produced with *Sf* cells[5]
  - The vector genome slightly exceeds the optimal packaging capacity of AAV vectors[6]

Objective:

- To compare the long-term durability of expression in mice treated with “oversized” (~5000 nt) and “standard-sized” (4600 nt) rAAV5 vectors produced in HEK293 or *Sf* cells
- To investigate the mechanistic factors affecting long-term transgene expression

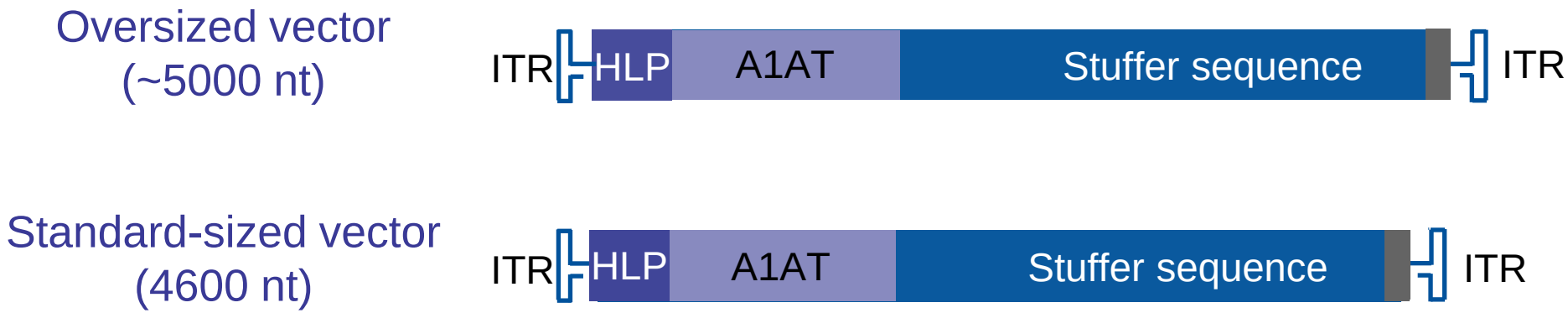


AAV, adeno-associated virus; AAV5, AAV serotype 5; HEK293, HEK293 cells; hFVIII-SQ, human factor VIII, SQ variant; rAAV, recombinant AAV; *Sf, Spodoptera frugiperda* cells.

1. Chahal PS, et al. *J Virol Methods*. 2014;196:163–73. 2. Kotin RM, et al. *Hum Gene Ther*. 2017;28(4):350–60. 3. Kondratov O, et al. *Mol Ther*. 2017;25(12):2661–75. 4. Kurasawa JH, et al. *Mol Ther Methods Clin Dev*. 2020;19:330–40. 5. Rangarajan S, et al. *N Engl J Med*. 2017;377(26):2519–30. 6. Bunting S, et al. *Mol Ther*. 2018;26(2):496–509.

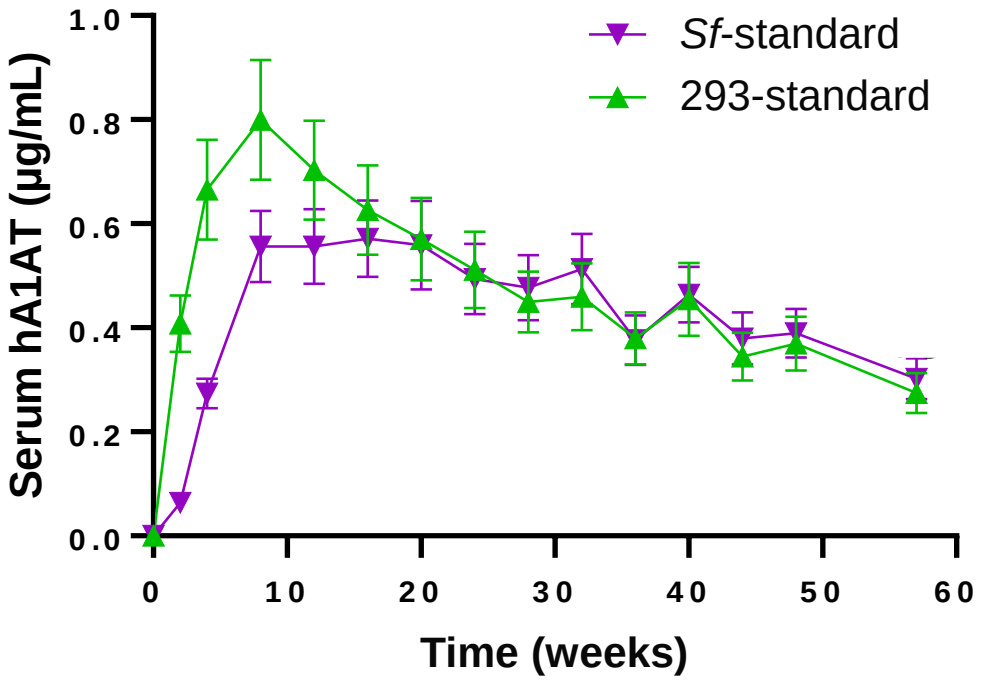
Study design

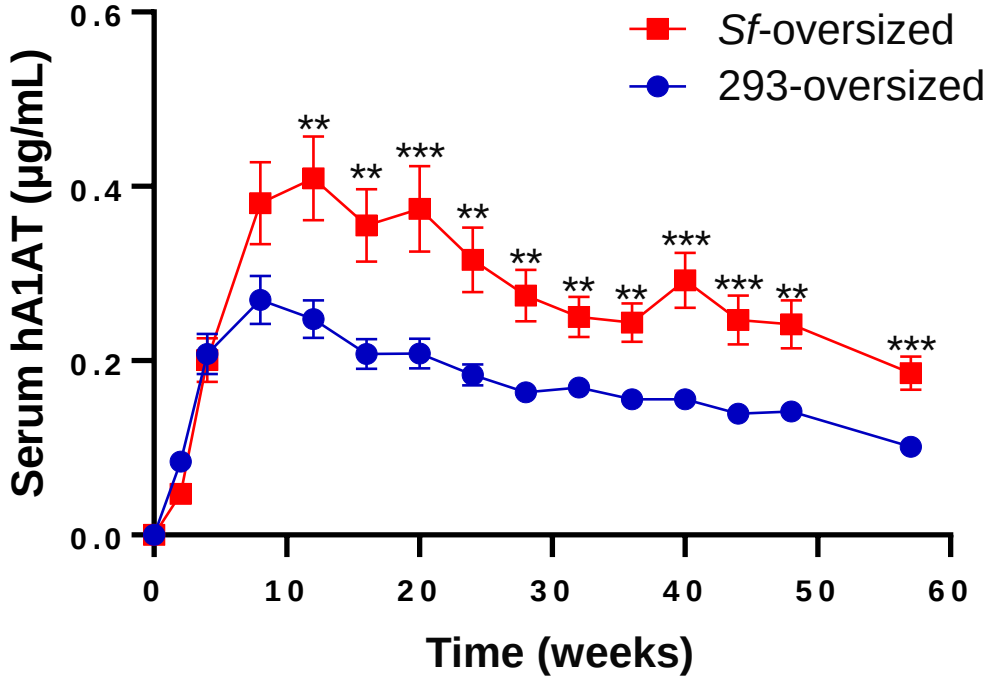
- C57BL/6 WT mice were administered an AAV5-hA1AT vector IV at a dose of $6x10^{13}$ vg/kg
  - Serial bleed cohort: blood was collected weekly during the first 4 weeks after dosing, then monthly through week 57
  - Takedown cohort: livers were collected for oversized vector genome analysis at weeks 1, 3, 12, 24, and 57



A1AT, alpha-1 antitrypsin; AAV5, adeno-associated virus serotype 5; FVIII, factor VIII; HLP, human liver-specific promoter; ITR, inverted terminal repeat; IV, intravenous; nt, nucleotide.

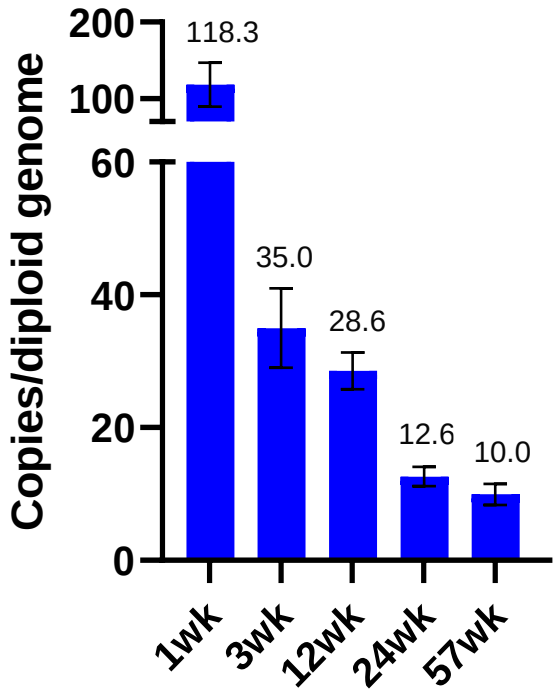
Oversized vectors produced in *Sf* cells demonstrated higher transgene expression long-term compared with 293

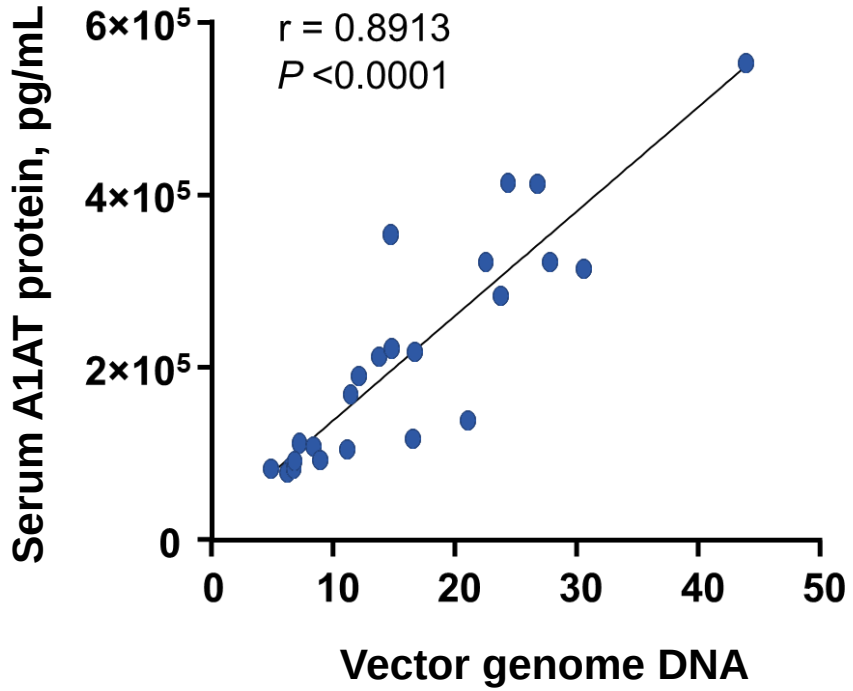




***P* <0.01; ****P* <0.001 using a repeated measure analysis on log transformed serum hA1AT. Data are mean ± SE.
293, human HEK293 cells; hA1AT, human alpha-1 antitrypsin; SE, standard error; *Sf, Spodoptera frugiperda* cells.

Degradation of the vector genome in the liver is the primary mechanism mediating decline in transgene expression with 293-produced vector





- Peak protein expression occurred at 12 weeks

Vector genome digested using KpnI restriction enzyme and quantified by ddPCR. Data are mean ± SE.
293, human HEK293 cells; A1AT, alpha-1 antitrypsin; ddPCR, droplet-digital polymerase chain reaction; SE, standard error; wk, week.

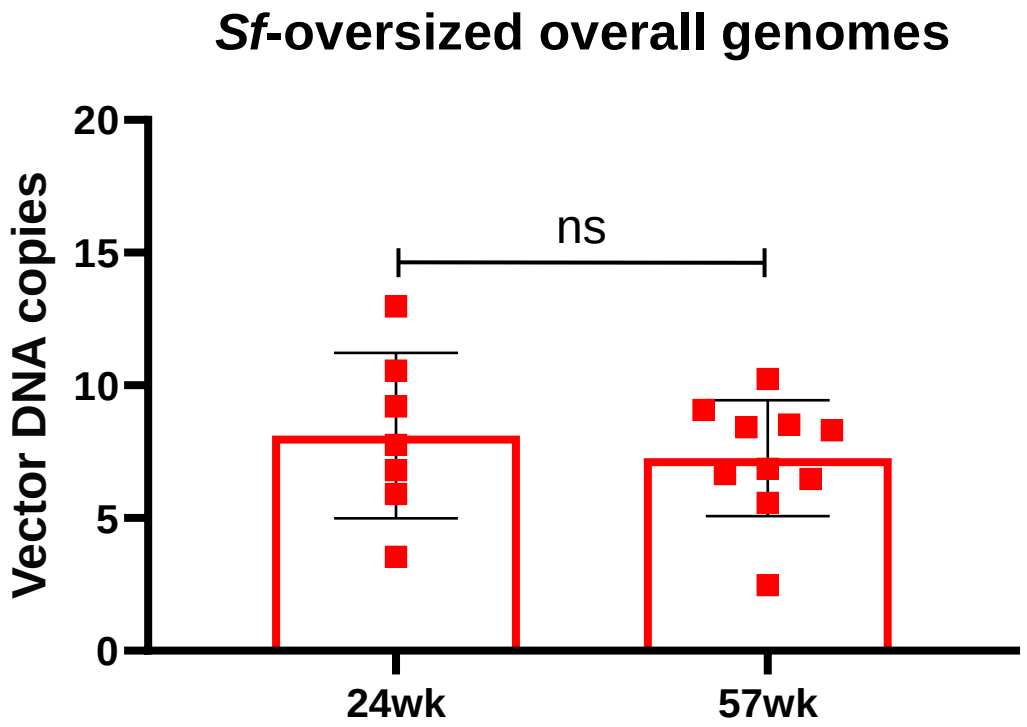
293-produced vectors elicit a heightened immune response compared with *Sf*-produced vectors

- RNA-seq analysis of liver tissue from mice at 12 weeks post-dose
  - Significantly upregulated pathways in 293-treated mice were associated with innate immune response
  - 293-treated mice also showed higher innate immune responses compared with the vehicle control

Top upregulated pathways	293- vs *Sf*-produced vector	
	***P* adj**	**NES**
TNFα signaling via NFκB	3.02E-08	2.09
G2m checkpoint	0.0416	1.37
Myc targets v2	0.0409	1.69
Myc targets v1	0.0043	1.60
Inflammatory response	0.0409	1.42
INFγ response	0.0071	1.57
TGFβ signaling	0.0409	1.58
Allograft rejection	0.0409	1.39
Apoptosis	0.0409	1.44
INFα response	0.0071	1.69
P53 pathway	0.0409	1.37

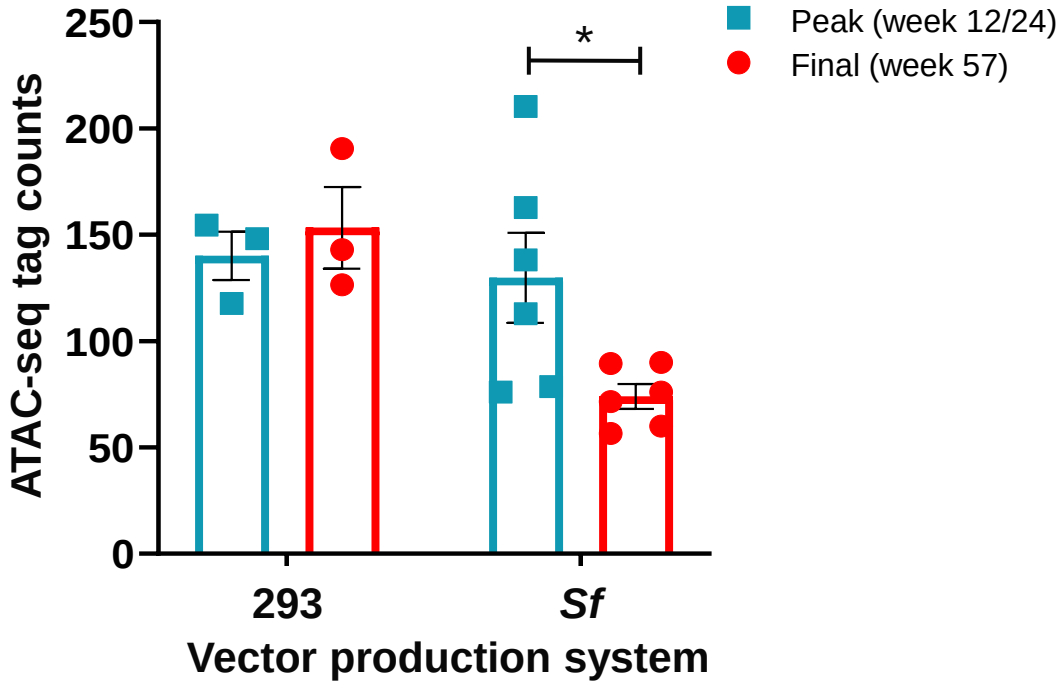
The Benjamini-Hochberg method was used to adjust *P*-values for differential expression and gene set enrichment analyses. NES determines whether a gene set is moving up (indicating that it is positively regulated) or down (indicating that it is negatively regulated) in the gene rankings for 293 vs *Sf* vector-treated mice (n = 7 per group).
293, human HEK293 cells; INFα, interferon-α; INFγ, interferon-γ; Myc targets v, Myc targets version; NES, normalised enrichment score; NFκB, nuclear factor kappa B; *P* adj, adjusted *P*-value; TGFβ, transforming growth factor-β; TNFα, tumour necrosis factor-α; *Sf, Spodoptera frugiperda* cells.

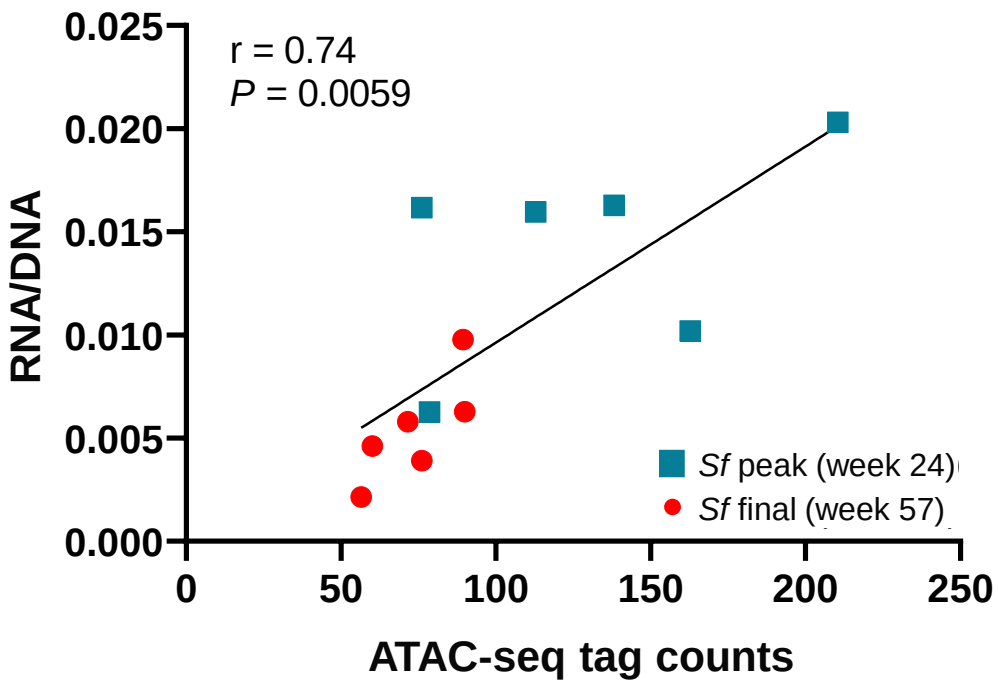
Copy numbers of *Sf*-produced vector genomes did not decrease from peak expression to the final time point



Ns, not significant using a Welch's t-test. Data are mean ± SE. Vector DNA copies are per diploid genome.
SE, standard error; *Sf, Spodoptera frugiperda* cells; wk, week.

A decrease in genome accessibility may impact the decline in transgene expression with *Sf*-produced vector

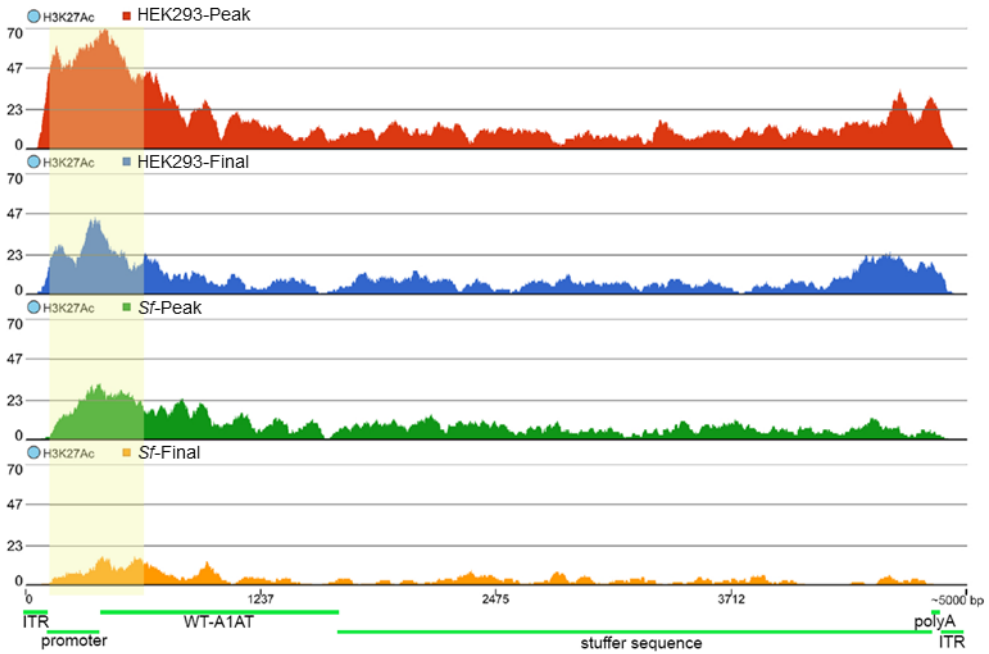


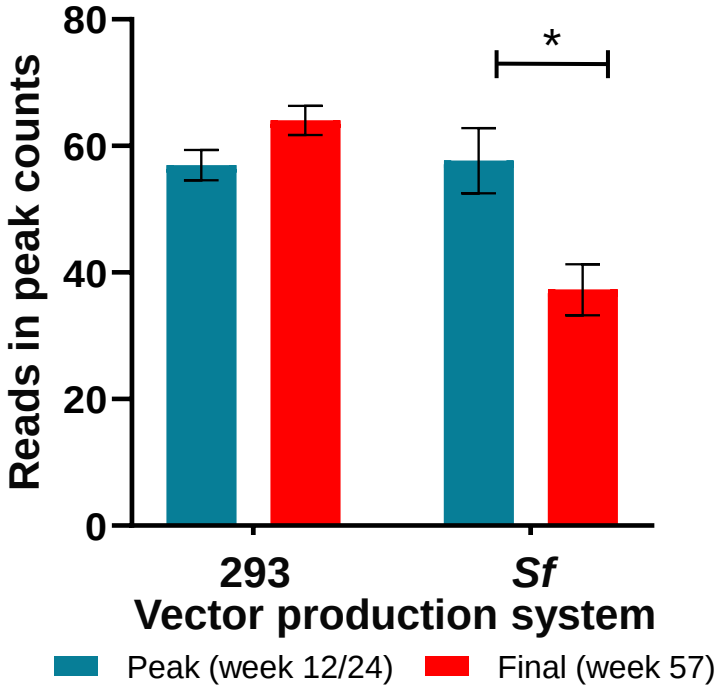


**P* <0.05 using a Welch's t-test on the log transformed ATAC-seq tag counts. Data are mean ± SE. Tag counts are ATAC-seq peak regions in the AAV5 promoter (1–660 bp) corrected for sequencing depth and normalised to vector genome copies.
293, human HEK293 cells; ATAC-seq, assay for transposase-accessible chromatin sequencing; SE, standard error; *Sf, Spodoptera frugiperda* cells.

Changes in chromatin and histone modifications mediate decrease in transgene expression for *Sf*-produced vectors

H3K27ac-based epigenetic modifications

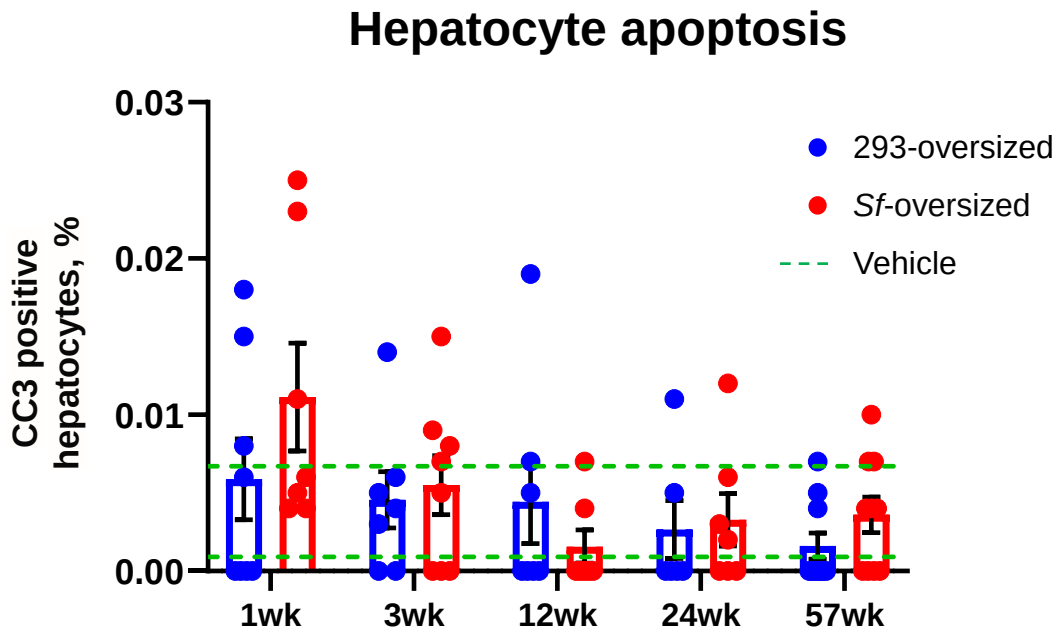


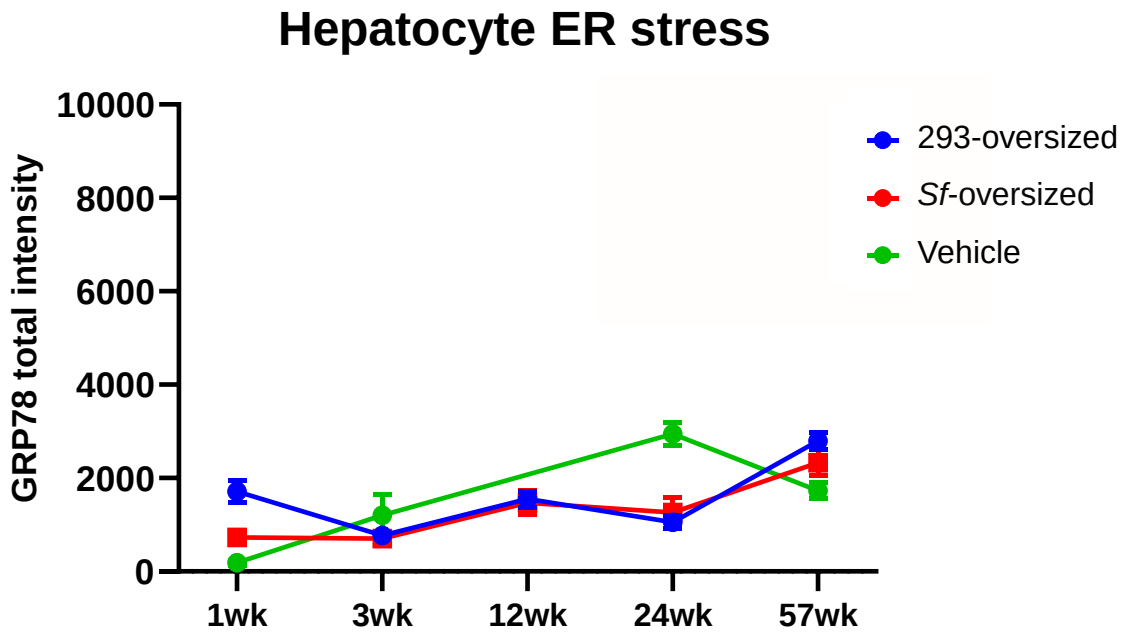


- H3K4me3, another active histone marker, had similar results
- No associations observed with the repressive histone marker H3K9me3

The highlighted yellow portion corresponds to the region where mean and SE peak counts were assessed as shown in the bar graph.
**P* <0.05 using a Student's t-test within each cell line. Data are mean ± SE.
293, human HEK293 cells; H3K27ac, acetylation of lysine 27 on histone H3; ITR, inverted terminal repeat; polyA, polyadenylation signal; SE, standard error; *Sf, Spodoptera frugiperda* cells; WT-A1AT, wild-type alpha-1 antitrypsin.

Liver histology evaluations showed minimal changes in hepatocyte apoptosis or ER stress





Data are mean ± SE.
293, human HEK293 cells; CC3, cleaved-caspase 3; ER, endoplasmic reticulum; GRP78, glucose regulatory protein 78; SE, standard error; *Sf, Spodoptera frugiperda* cells; wk, week.

Conclusions

- rAAV5 vectors produced in HEK293 and *Sf* cells showed similar long-term durability of expression in mice, despite distinct mechanisms contributing to the decline in transgene expression over time
- Oversized vectors produced in *Sf* cells may represent a viable alternative to vectors produced in HEK293 cells
- Genome metabolism and more pronounced innate immune responses may mediate the decline in transgene expression by 293-produced vector
- Dynamics of genome accessibility including transcription factors or histone binding and other epigenetic modifications, may distinctly impact durable transgene expression of *Sf*-produced vector
  - Additional analysis of epigenetic regulation of rAAV vectors would deepen our understanding of AAV-mediated transgene expression
- No liver tumours were observed after more than 1 year of follow-up in mice dosed with AAV5-hA1AT vectors produced in either manufacturing system

293, human HEK293 cells; AAV, adeno-associated virus; AAV5, AAV serotype 5; hA1AT, human alpha-1 antitrypsin; HEK293, HEK293 cells; rAAV, recombinant AAV; rAAV5, rAAV serotype 5; *Sf, Spodoptera frugiperda*.

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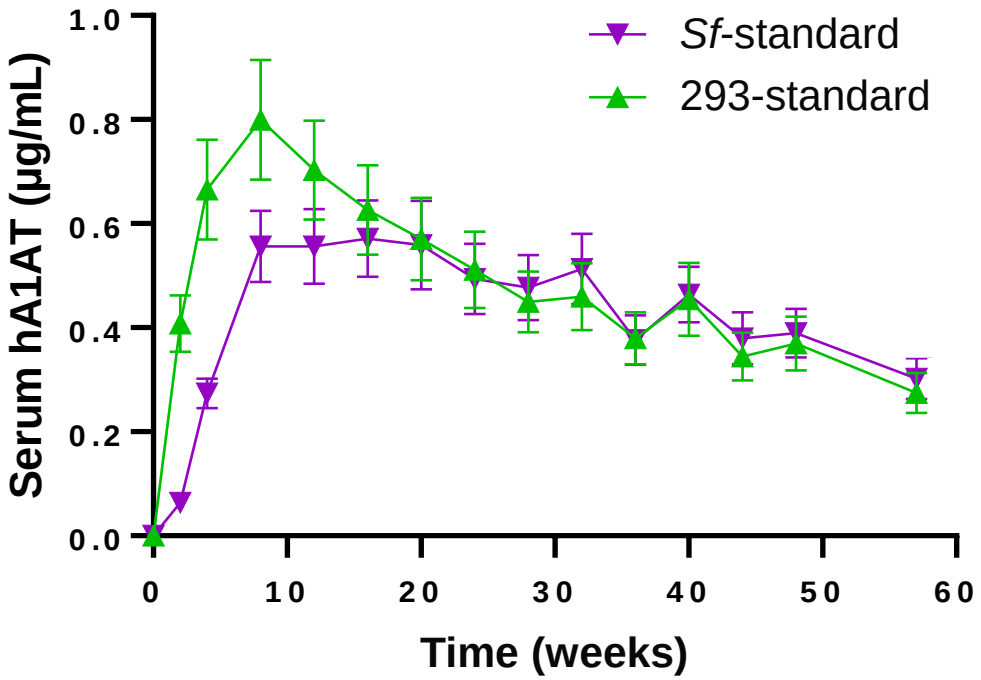
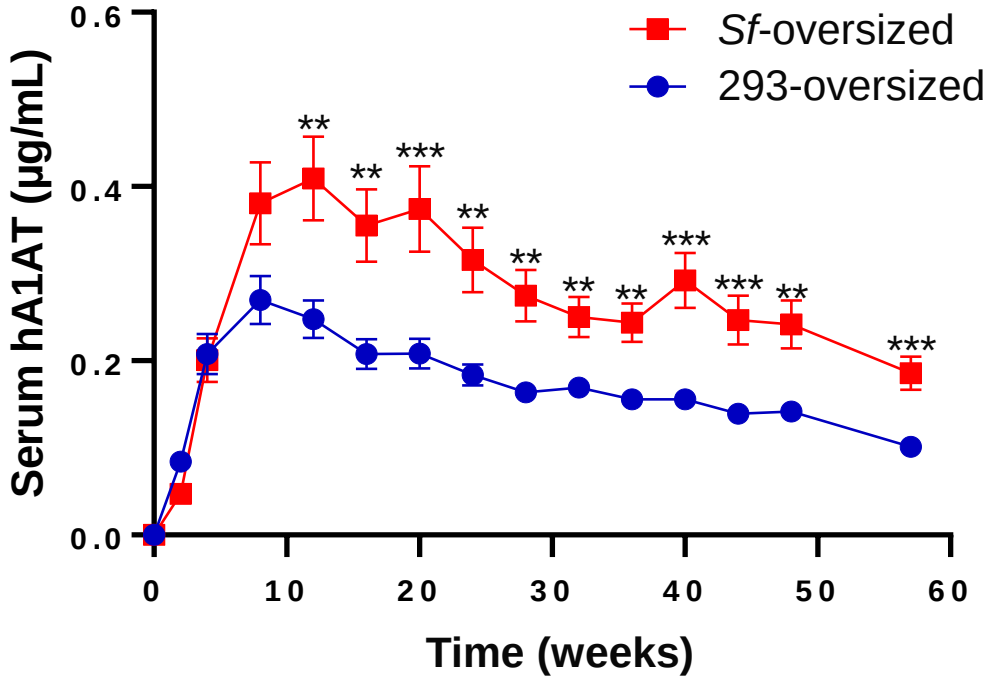
Question and answer session





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