ASGCT 26th Annual Meeting

May 16–20, 2023,
Los Angeles, CA

The longitudinal kinetics of AAV5 vector integration profiles in mice

Ashrafali Mohamed Ismail[1], Evan Witt[1], Taren Bouwman[1], Wyatt Clark[1], Bridget Yates[1], Matteo Franco[2], Sylvia Fong[1]

[1]BioMarin Pharmaceutical Inc., Novato, CA, USA; [2]ProtaGene CGT GmbH, Heidelberg, Germany, and ProtaGene Inc., Burlington, MA, USA

#65

BIOMARIN®

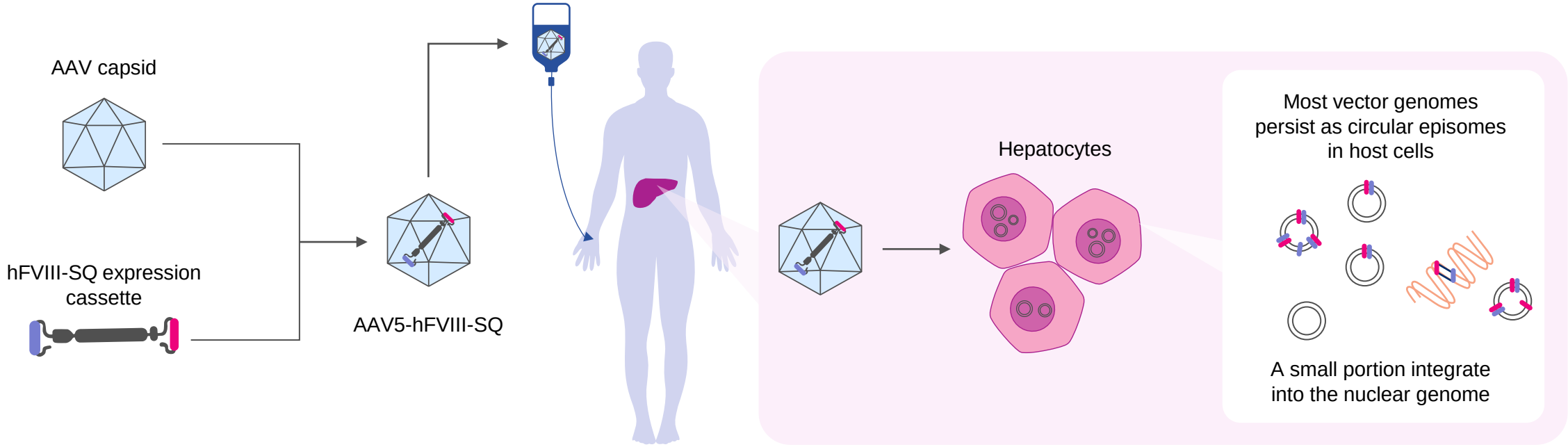
Disclosures

- Ashrafali Mohamed Ismail is an employee and stockholder of BioMarin Pharmaceutical Inc.

BIOMARIN®

Background

- While AAV vectors are regarded as safe and effective for gene therapy,[1] the theoretical risk of tumorigenesis may depend on vector regulatory features
- Valoctocogene roxaparvovec (AAV5-hFVIII-SQ) is a gene therapy for hemophilia A that uses an AAV5 vector to deliver a B-domain–deleted hFVIII coding sequence controlled by a liver-selective promoter[2]

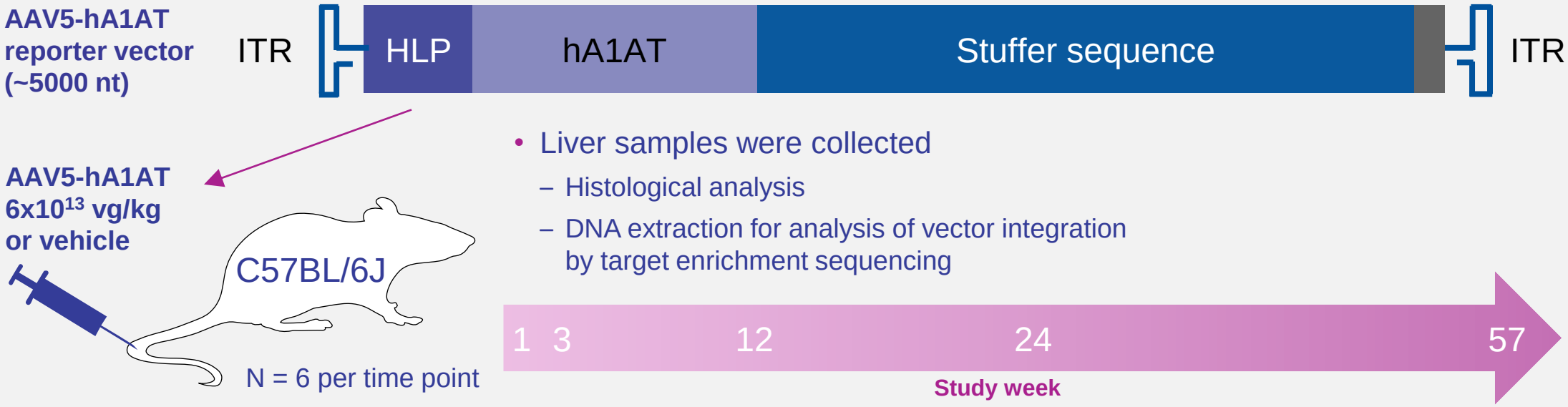


AAV, adeno-associated vector; AAV5, AAV serotype 5; hFVIII-SQ, human factor VIII, SQ variant.

1. Wang D, et al. *Nat Rev Drug Discov*. 2019;18(5):358-78. 2. Mahlangu J, et al. *N Engl J Med*. 2023;388(8):694-705.

Study objective and design

- To investigate the long-term kinetics and integration profiles of AAV5 vectors, we utilized a reporter vector that mimics key features of valoctocogene roxaparvovec in a mouse model
  - hA1AT is non-immunogenic[1]
  - Manufactured in HEK293 or *Sf* cells

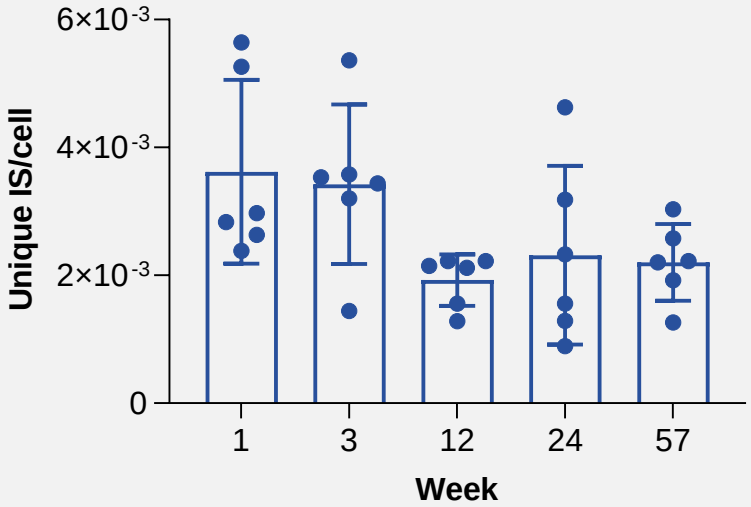


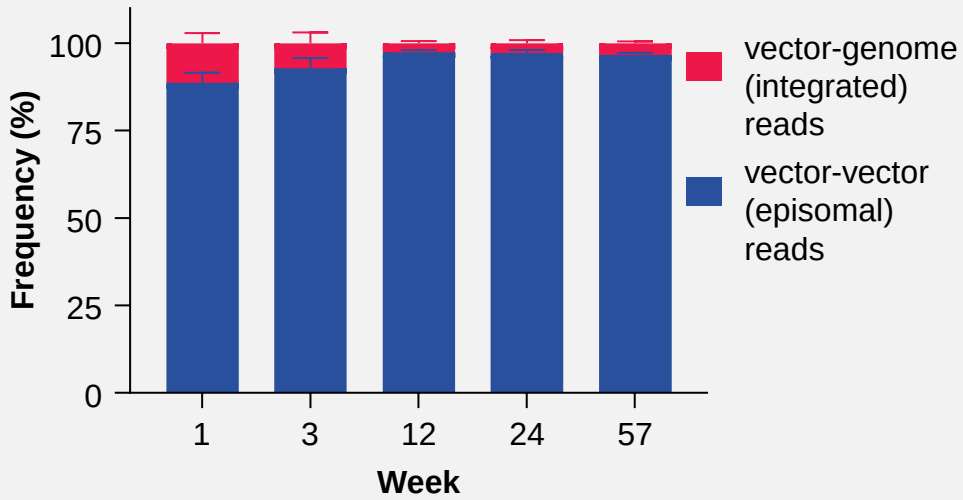
AAV5, adeno-associated virus serotype 5; hA1AT, human alpha-1 anti-trypsin; HLP, human liver-specific promoter; ITR, inverted terminal repeat; nt, nucleotide; *Sf, Spodoptera frugiperda*.

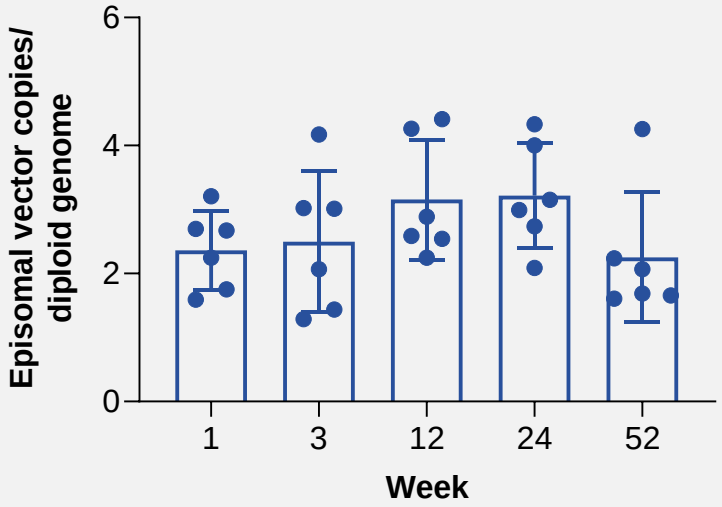
1. Zimmerer JM, et al. *J Immunol*. 2010;185(12):7285-92.

AAV5 vector integration occurs with low frequency and remains stable over time

- On average 2.70 ± 1.24 SD integration events occur per 1000 cells
- Based on estimations from the target enrichment sequencing results
  - Episomes were detected as early as week 1
  - Episomes remained stable after week 3, with ~97% of the vector genomes persisting in their episomal form[a]







[a]Target enrichment sequencing results were used to estimate vector genomes present as episomes (reads that contained a junction between 2 vector fragments) vs vector genomes that integrated into the host genome (a vector fragment followed by a host genome sequence).

AAV5, adeno-associated virus serotype 5; IS, integration site; SD, standard deviation.

AAV5 vector integrations exhibit poor targeting to specific genomic regions

- Common integration site analysis reveals integration preferences in the host genome and determines the clonal fate of the cell
- Most common integration sites demonstrated a random integration profile
  - The top 10 common integration sites are in highly expressed genes in the liver
  - Data suggest the vector integrations preferentially occur in regions of open chromatin

CIS rank	Order	Chr	Average position	Dimension (nt)	Gene	Contributing samples[a]
Top 1	33	17	39845964	5609	*Rn45s*	24 of 30
Top 2	27	18	12679116	126239	*Cabyr/Ttc39c*	17 of 30
Top 3	23	5	90470587	53459	*Alb/Afp*	14 of 30
Top 4	19	2	98664806	5061	*Lrrc4c*	12 of 30
Top 5	19	9	46037434	338841	*Sik3*	11 of 30
Top 6	17	9	121913038	38574	*1700048O20Rik*	11 of 30
Top 7	17	14	31127780	373509	*Dnah1*	14 of 30
Top 8	15	1	67200726	210088	*Cps1*	9 of 30
Top 9	15	2	26486672	274663	*Notch1*	12 of 30
Top 10	14	1	88231197	142390	*Trpm8*	10 of 30

Dimension is the distance between the most proximal and distal integration site within a specific CIS region. For normalized entropy, values of zero indicate that there is a single contributing sample to that CIS region, and values close to 1 indicate that all the different samples contributed equally

[a]Not including vehicle-treated controls.
AAV5, adeno-associated virus serotype 5; Chr, chromosome; CIS, common integration site; nt, nucleotide.

Most common integration sites within or near genes linked to severe adverse events were detected by a single sequence read

- Data suggest there were **no hotspots or clonal expansion** associated with *Sf*-produced vector and that the vector has a poor ability to target genes linked to severe adverse events[1-8]

Week	Sample	SAE gene	Distance to TSS [nt]	Chr	Total sequence count	Sequence count contribution
1	1	*Ccnd2*	14464	12	824	1
1	3	*Mecom*	23564	3	1833	1
1	5	*Mecom*	−30991	3	920	1
1	2	*Mn1*	71747	22	1033	1
1	1	*Hmga2*	2266	12	824	1
1	3	*Hmga2*	−77988	12	1833	1
1	3	*Lmo2*	53054	11	1833	1
3	4	*Lmo2*	8	11	1280	1
3	3	*Mecom*	−28837	3	1900	1
3	3	*Mecom*	−52519	3	1900	1
12	3	*Mecom*	3543	3	752	1
12	5	*Mecom*	−27072	3	792	1
24	3	*Mecom*	−32831	3	854	1
24	5	*Mecom*	58575	3	1706	1
24	6	*Mecom*	−23894	3	480	1
24	3	*Mn1*	−93904	22	854	1
57	4	*Mn1*	−892	22	834	1
57	5	*Ccnd2*	−5984	12	938	1

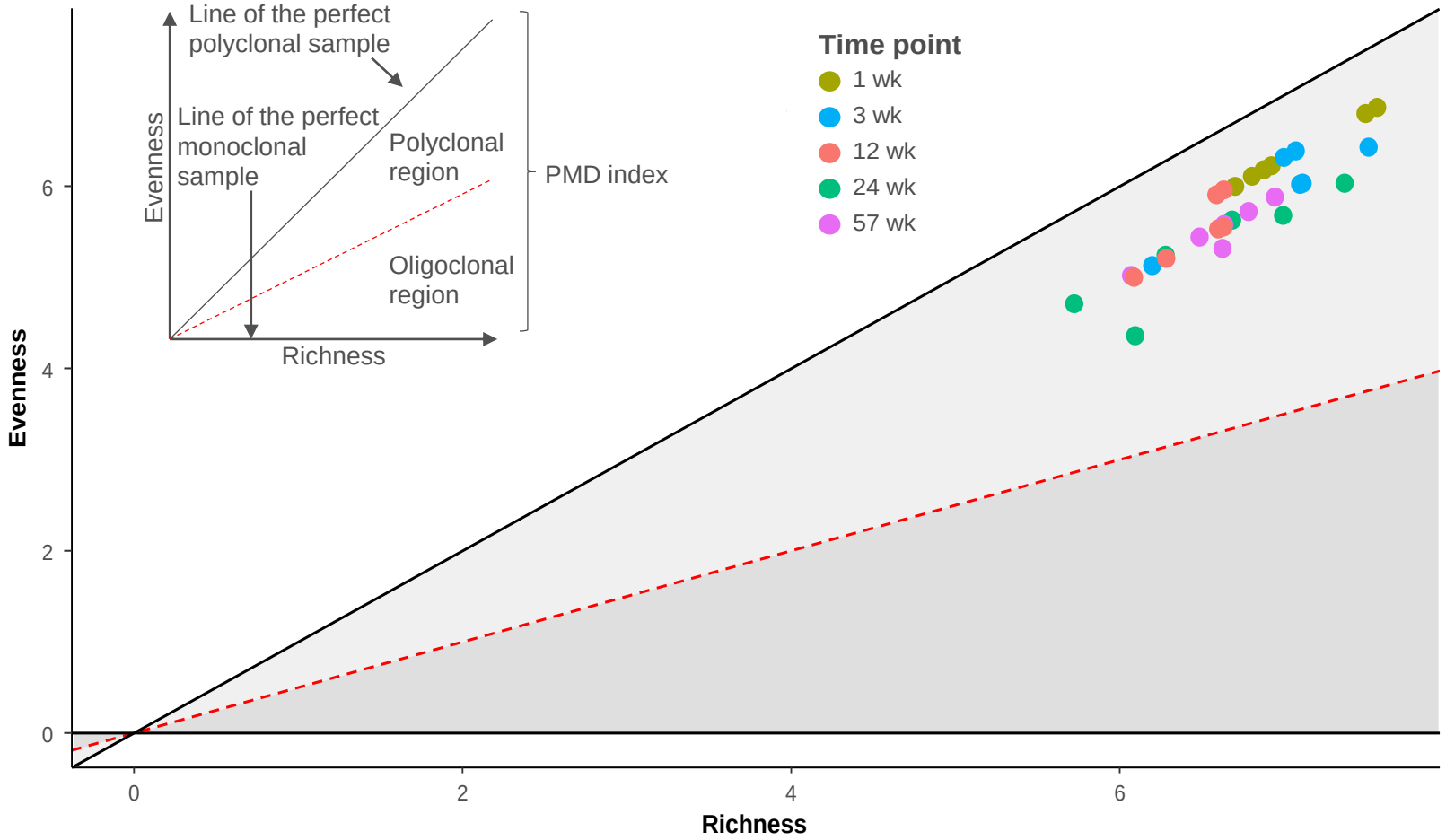
Chr, chromosome; nt, nucleotide; SAE, severe adverse event; *Sf, Spodoptera frugiperda*; TSS, transcription start site.

1. Braun CJ, et al. *Sci Transl Med*. 2014;6(227):227ra33. 2. Cavazzana-Calvo M, et al. *Nature*. 2010;467(7313):318-22. 3. Deichmann A, et al. *J Clin Invest*. 2007;117(8):2225-32. 4. Hacein-Bey-Abina S, et al. *J Clin Invest*. 2008;118(9):3132-42. 5. Hacein-Bey-Abina S, et al. *N Engl J Med*. 2003;348(3):255-6. 6. Hacein-Bey-Abina S, et al. *Science*. 2003;302(5644):415-9. 7. Howe SJ, et al. *J Clin Invest*. 2008;118(9):3143-50. 8. Ott MG, et al. *Nat Med*. 2006;12(4):401-9.

BIOMARIN®

AAV5 vector integration is not associated with clonal expansion

- A polyclonal-monoclonal distance index tool demonstrated the *Sf* vector–treated samples clustered near the theoretical maximum for polyclonality
  - A clonality plane was constructed based on 2 extreme components of diversity:
    - Richness (the total number of integration sites)
    - Evenness (the relative number of reads detecting each integration site)
  - The ratio of the distance from the theoretical threshold for maximal polyclonality and monoclonality defines the polyclonal-monoclonal index

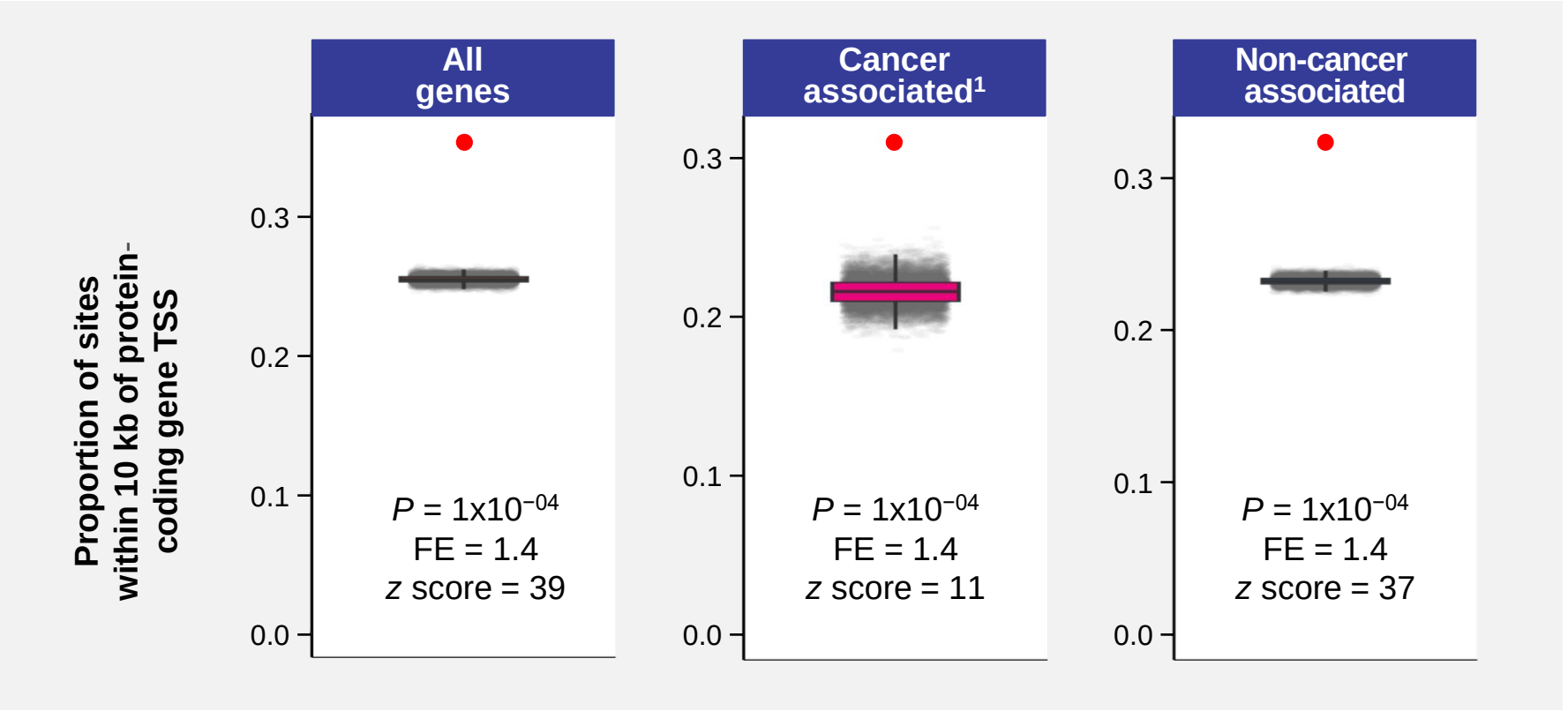


AAV5, adeno-associated virus serotype 5; PMD, polyclonal-monoclonal distance; *Sf, Spodoptera frugiperda*.

BIOMARIN

Vector integrations do not preferentially occur near cancer-associated genes

- Integration occurs more frequently near genes highly expressed in the liver



The red dot represents the proportion of observed integrations that fall within 10 kb of a protein-coding gene TSS, and the box plots represent the distribution of integrations that would be expected by chance based on the median simulated value. Genes with the highest expression profile in the liver (percentile greater than 90%) according to the GTEx liver data are classified as highly expressed. *P*-values represent 1 minus the percentile of the observed value within the expected distribution. FE indicates the magnitude of integration enrichment and *z* scores are a proxy for statistical enrichment.

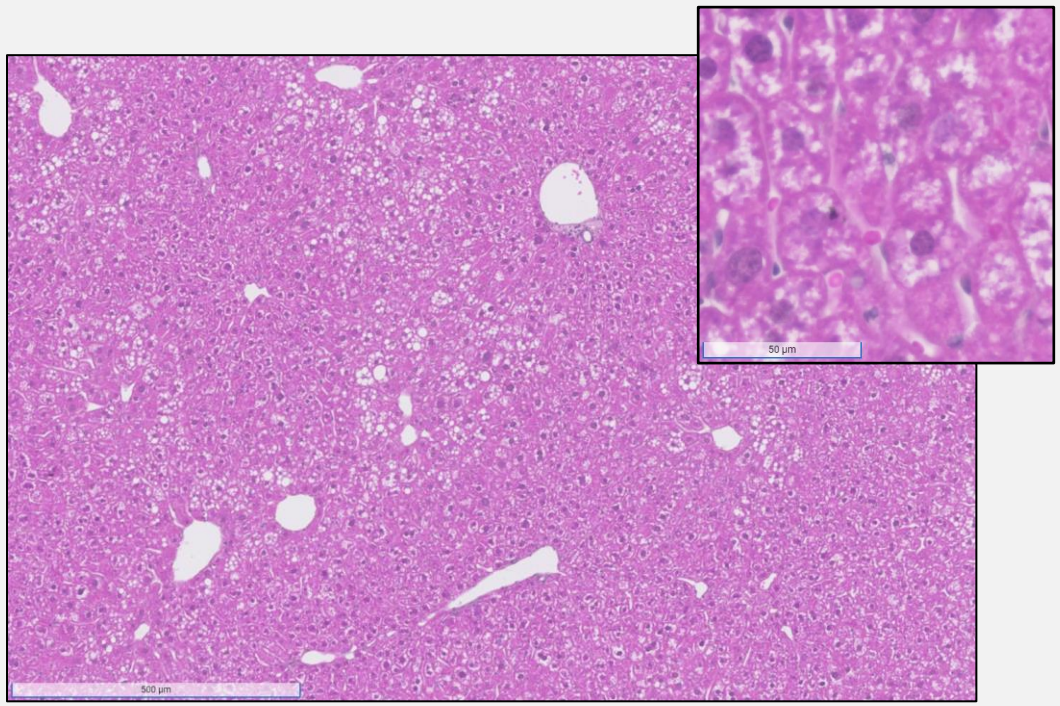
FE, fold enrichment; GTEx, genotype-tissue expression; TSS, transcription start site.

1. Sondka Z, et al. *Nat Rev Cancer*. 2018;18(11)696-705.

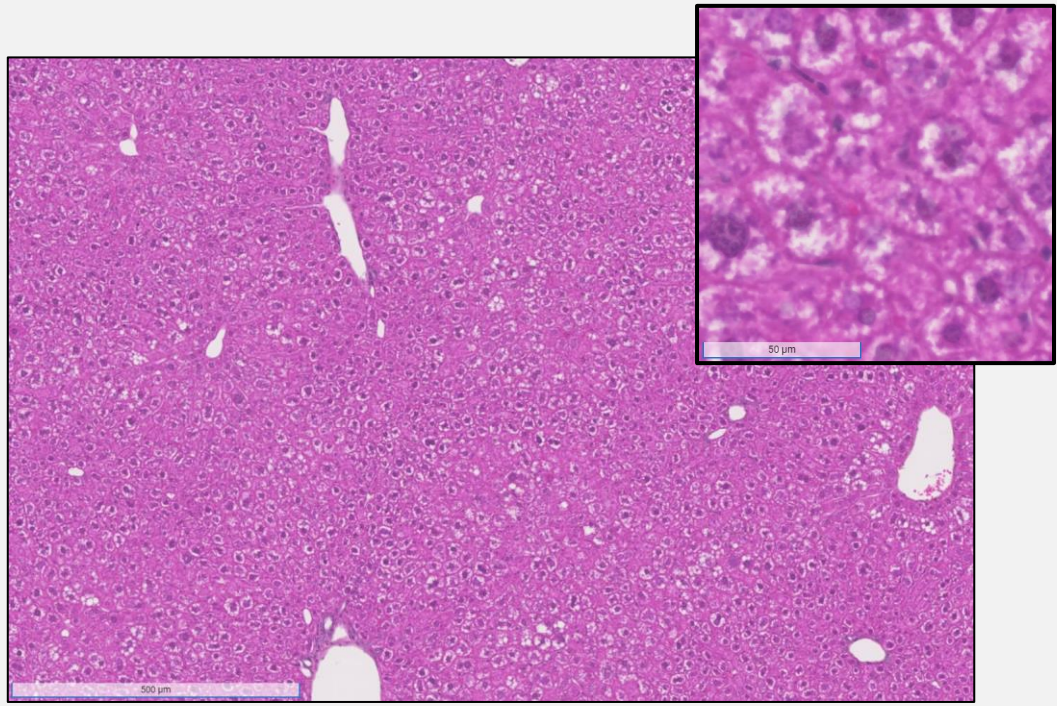
There were no signs of liver tumors throughout a 57-week study

- No signs of dysplasia, inflammation, fibrosis, or cellular stress were detected

Vehicle (57 weeks)



AAV5 (57 weeks)



AAV5, adeno-associated virus serotype 5.

Conclusions

- For an AAV5 vector containing the same regulatory elements as valoctocogene roxaparvovec:
  - The vector integrations occurred largely within the first week after vector administration, and integration frequencies did not increase with time
  - The predominant vector form was episomal (~97%), thus lowering the risk for tumorigenesis
  - The vector had low integration rates and integrated more frequently near transcriptionally active genes, suggesting the vector had a poor ability to target specific genomic regions
    - There was no preferential integration of the vector in or near cancer-associated genes
  - The longitudinal analysis in mice demonstrated no clonal expansion or tumorigenesis
- Results are presented for *Sf* vector–treated mice, but similar results were obtained with vectors produced from either manufacturing system

AAV5, adeno-associated virus serotype 5; *Sf, Spodoptera frugiperda*.

Acknowledgments

- Funding for this study was provided by BioMarin Pharmaceutical Inc.
- Medical writing support was provided by Tony Sallese, PhD, of AlphaBioCom, a Red Nucleus company, and funded by BioMarin Pharmaceutical Inc.

BIOMARIN®