Molecular Characterization of Recombinant AAV5 encoding FVIII after Human administration

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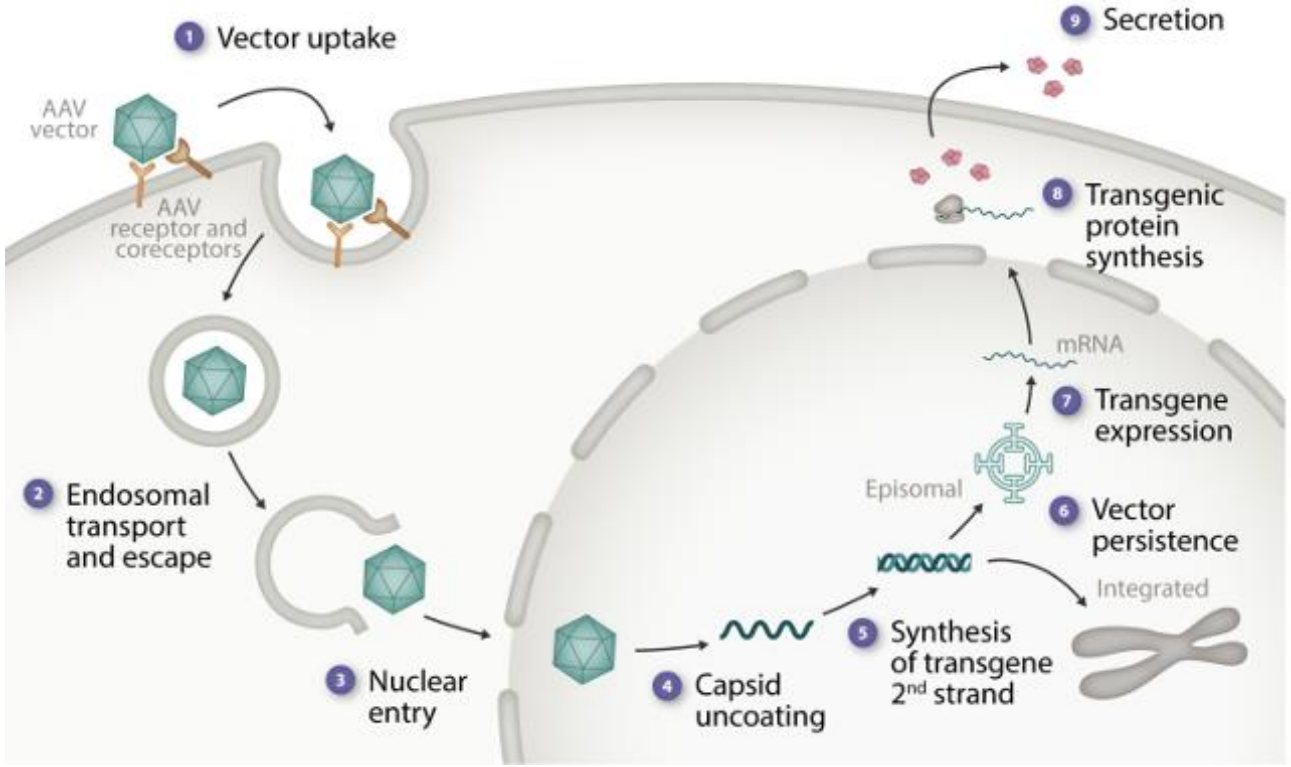
Disclosures

- Employee and shareholder of BioMarin Pharmaceutical Inc.

What factors may contribute to variability in FVIII gene therapy?

- **There are many steps between AAV-GT administration and successful expression**

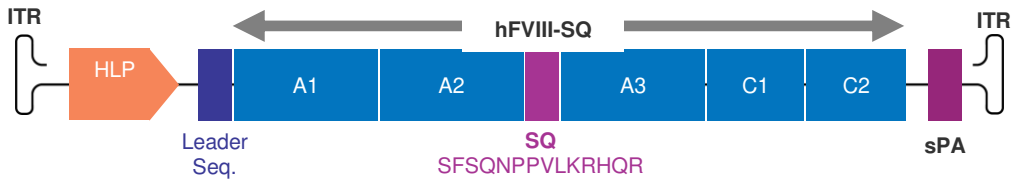


Key hypothesis: Genetic/Epigenetic differences/expression variations in host factors in steps affecting steps in AAV transduction, transcription and protein expression/secretion and post-dosing immune response impact on AAV GT outcomes

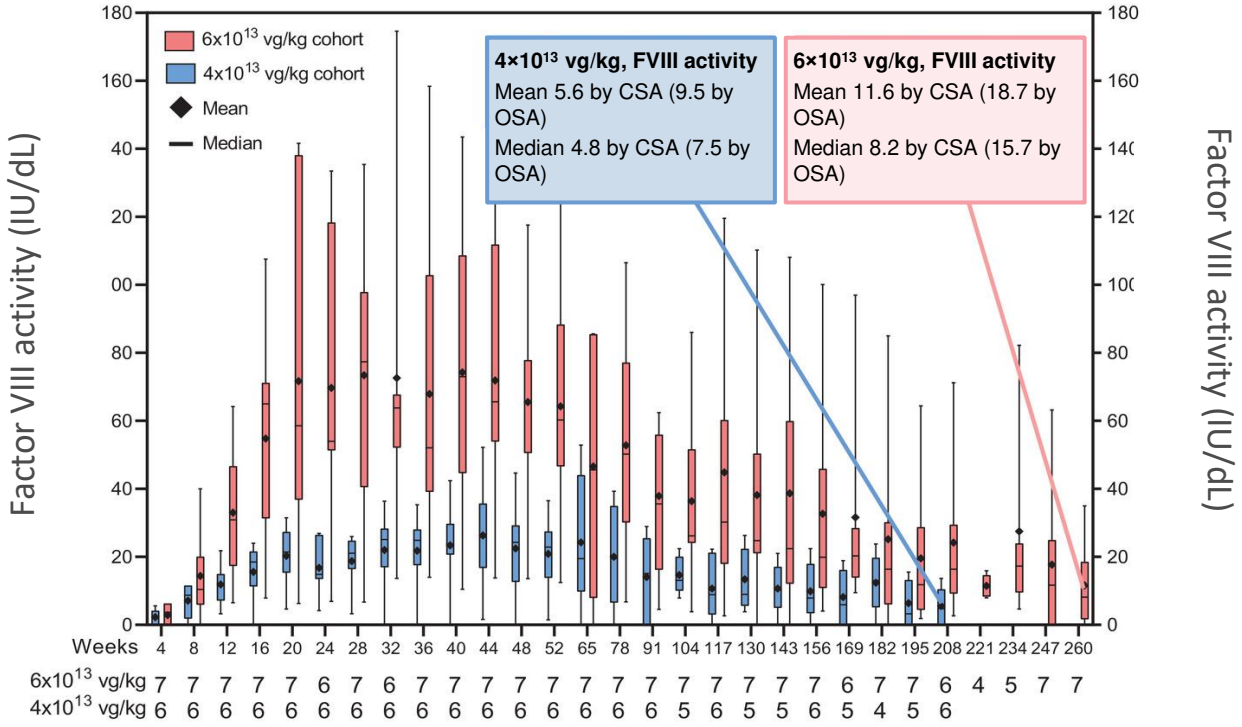
Batty P, Lillicrap D. Hemophilia Gene Therapy: Approaching the First Licensed Product. Hemasphere. 2021;5(3):e540.

Valoctocogene roxaparvovec (AAV5-hFVIII-SQ)

- Efficacy in severe hemophilia A
  - 5-year expression
  - Bleed reduction
  - Quality of life
- Safety
- Variability
  - Intra- and inter-study
- Durability



FVIII activity measured by the chromogenic substrate assay for participants in the 6 × 10^{13} and 4 × 10^{13} vg/kg cohorts over 5 and 4 years, respectively[a]



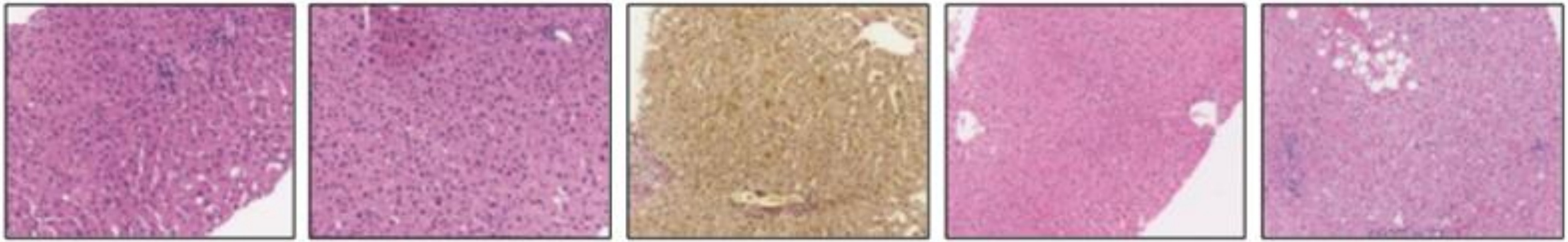
[a]FVIII activity levels taken within 72 h of exogenous FVIII administration were excluded. FVIII activity that fell below the lower limit of quantitation (<3.0 IU/dl) was imputed as 0 IU/dl. Whiskers represent the minimum and maximum values; boxes represent the 25th and 75th percentiles.

CSA, chromogenic substrate assay; FVIII, factor VIII; OSA, one-stage assay

1. Pasi KJ, et al. Haemophilia 2021;27(6):947–56

Liver biopsy study to understand transduction and liver health

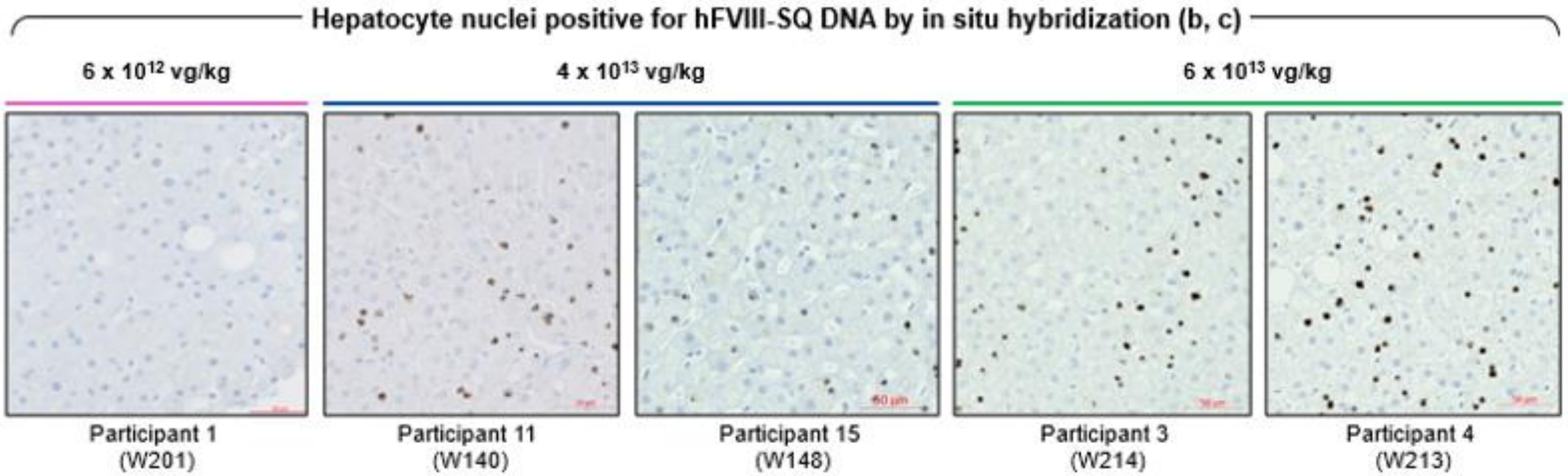
- **No liver injury observed 2.6-4.1 years following treatment**
- Histopath Results:
  - Mild steatosis
  - **No** fibrosis, necrosis, inflammation, tumors



Fong S, et al. Nat Med 2022

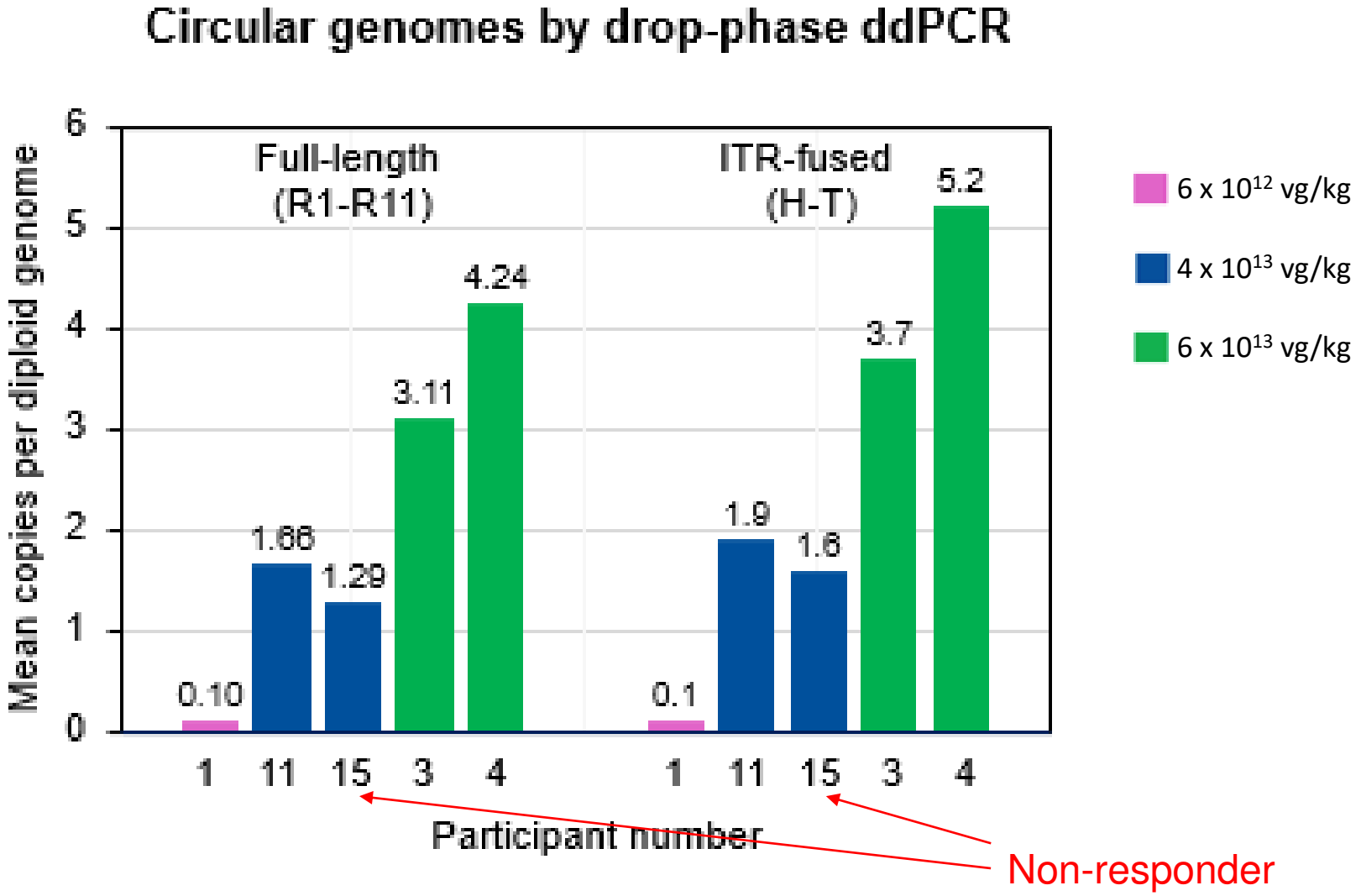
Liver biopsy study to understand transduction efficiency

- **Dose-dependent maintenance of FVIII transgene observed after 4 years**



Fong S, et al. Nat Med 2022

Full-length and processed AAV genomes also show stable dose response



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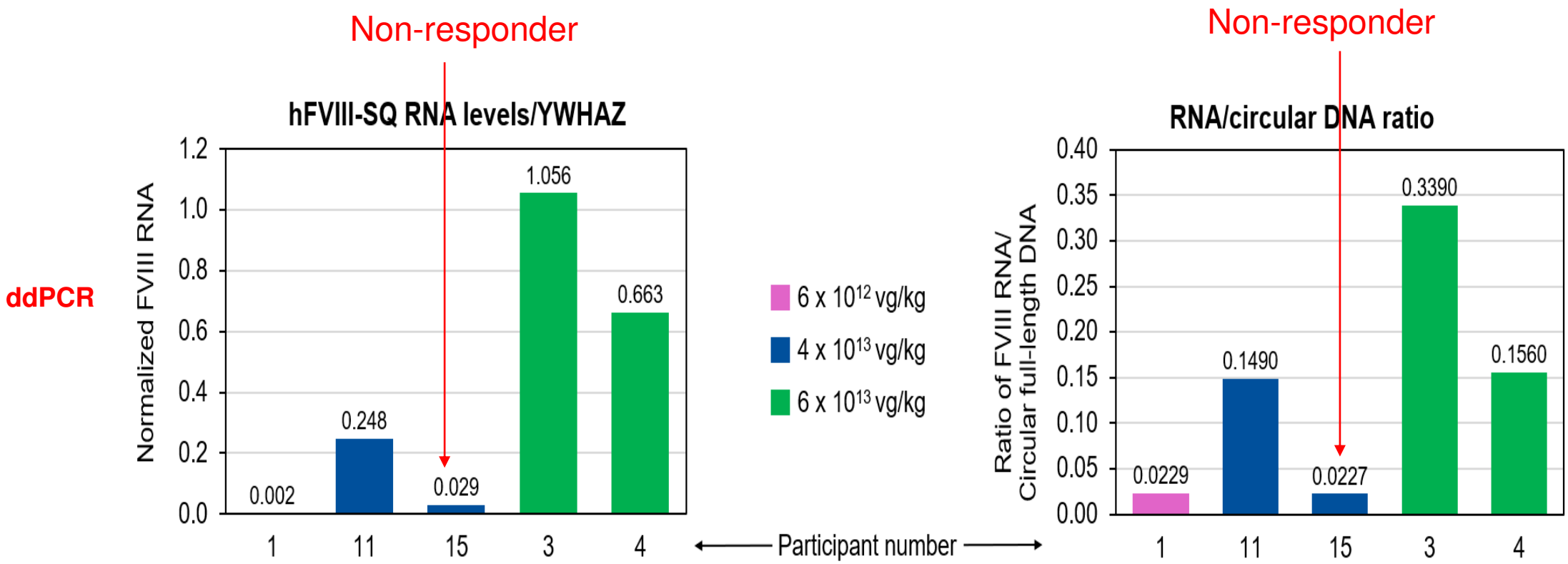
Low hFVIII-SQ RNA/DNA ratio found in low responder

- In general, dose-dependent FVIII-SQ RNA levels detected
- In Participant 15, low FVIII-SQ RNA observed with fewer RNA+ hepatocytes
- Something holding back RNA expression from DNA?



Fong S, et al. Nat Med 2022

Overview of integration analyses

- Genomic integration following gene therapy: what do we know today?
- Vector integration analyses
  - Healthy liver tissue
  - Parotid gland acinar cell carcinoma case
  - B-cell acute lymphoblastic leukemia case
- Long-term follow-up of study subjects and patients dosed in the real world

Background

- Genomic integration refers to insertion of genetic material into host chromosomes
- Viral vectors are commonly used in gene therapy research, as vehicles to deliver genetic material into target cells
- Different viral vectors have different characteristics[1,2,3]
  - **Retroviral vectors (including lentiviral vectors*)** integrate as part of their life cycle and are used in gene therapy to target and integrate into the genome of dividing cells (e.g. hematopoietic stem cells)[2]
  - **Adeno-associated viral (AAV) vectors** exhibits a low level of integration on a per cell basis and are used in gene therapy research for approaches targeting non-dividing cells or cells that do not actively divide such as liver and muscle[2]
- Theoretically, integration could increase the risk of unintended consequences, such as mutagenesis, genotoxicity and/or oncogenesis[1,2]

*Lentiviruses belong to the family Retroviridae (retrovirus)

1. Lundstorm K. Trends Biotechnol 2003;21:117-22; 2. Walther W & Stein U. Drugs 2000;60:249-71. 3. Lundstrom K. Diseases 2018 21;6:42

Recent reports of cancer diagnoses in patients previously treated with investigational gene therapy

- Retroviral gene therapy has been associated with oncogenesis attributed to vector integration[1,2,3]
  - Recently, Bluebird Bio and Orchard Therapeutics have each reported cases of leukemia
    - Causality of leukemia was not confirmed to be caused by vector integration in either case
    - Clonally expanded integrant observed and suspected to contribute with Stremvelis (Orchard)
    - AML case after BB305, clonally expanded vector near VAMP4 may not be related

- To date, AAV vector DNA integration has been observed without resulting in carcinogenesis[4]
  - uniQure announced a case of hepatocellular carcinoma (HCC) in a patient who received AAV gene therapy
    - Results of the investigation found that it was highly unlikely the HCC was caused by etranacogene dexaparvovec[5]
  - BioMarin has reported 2 cases of cancer in study subjects dosed >2 years ago with a 6 x 10^{13} vg/kg BMN 270 (valoctocogene roxaparvovec), both considered unrelated to the study drug:
    - 1 case of acinic cell carcinoma (AcCC) of the parotid gland, diagnosed in November 2021[6]
    - 1 case of B-cell acute lymphoblastic leukemia (B-ALL), reported in September 2022[7]

1. Strimvelis: EPAR (risk-management-plan-summary); 2. Zynteglo: EPAR (risk-management-plan-summary); 3. bluebird bio Announces Temporary Suspension on Phase 1/2 and Phase 3 Studies of LentiGlobin Gene Therapy for Sickle Cell Disease (bb1111) [press release]. February 16, 2021. 4. Nguyen GN et al. Nat Biotechnol 2021;39:47–55. 5. uniQure Announces Findings from Reported Case of Hepatocellular Carcinoma (HCC) in Hemophilia B Gene Therapy Program [press release] March 29, 2021.6. Presented at the World Federation of Hemophilia (WFH) Congress: 8-11 May 2022 (Montréal and virtual); 7. BioMarin 8K form reported 12 Sept 2022 to the Securities and Exchange Commission, Washington D.C. 20549

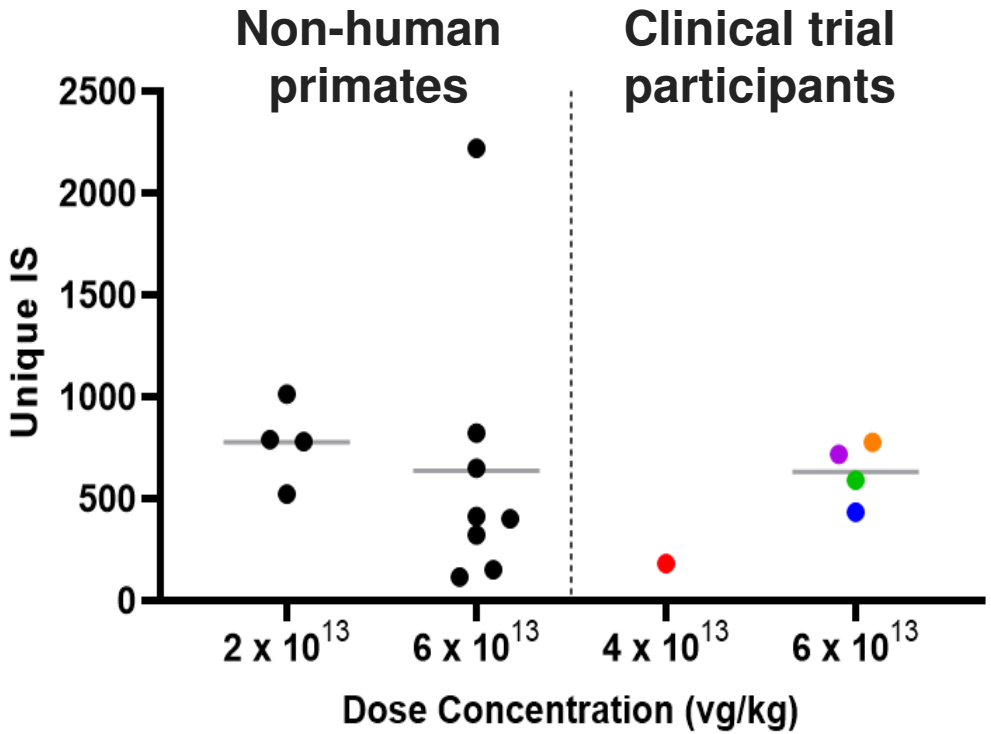
Current Investigational Work

Integration patterns and relative cell growth in healthy human liver tissue

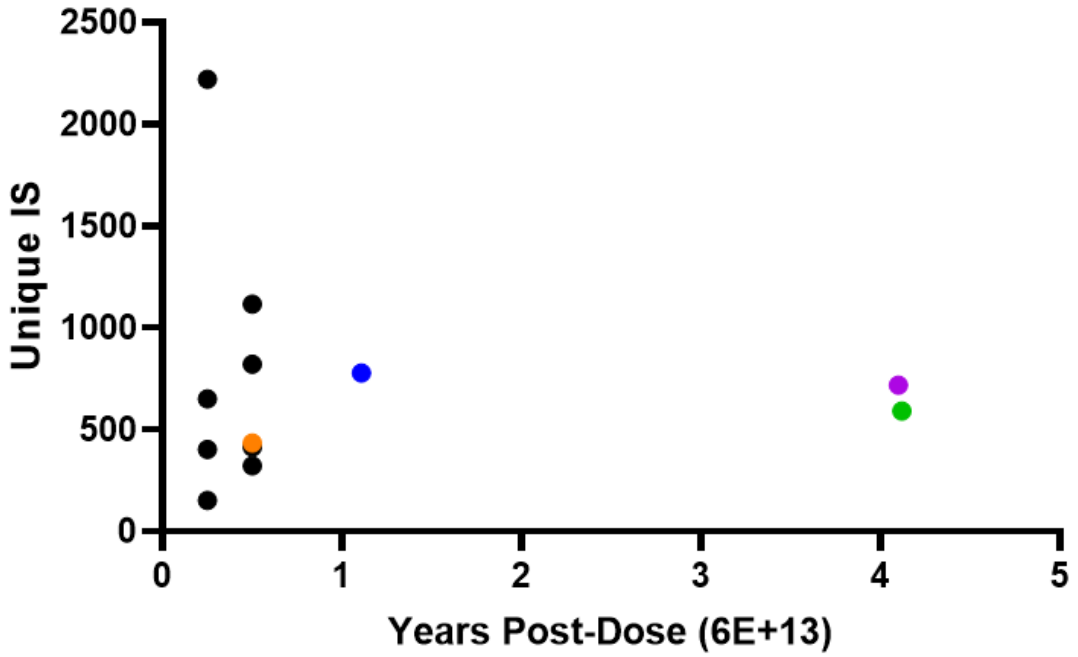
Healthy liver biopsies used for integration analyses

Study	270-303	270-301	270-201		
Participant	A	B	C	D	E
Dose, vg/kg	6×10^{13}	6×10^{13}	6×10^{13}	6×10^{13}	4×10^{13}
Time to Biopsy, years	0.50	1.11	4.12	4.10	2.85

Integration frequency consistent with non-clinical NHP study and no increase seen after 1 year



Number of integration sites identified in NHP range



No increase in integration after one year

IS: integration sites; NHP: non-human primates; Presented at the World Federation of Hemophilia (WFH) Congress: 8-11 May 2022 (Montréal and virtual)

Vector copy number and integration frequency

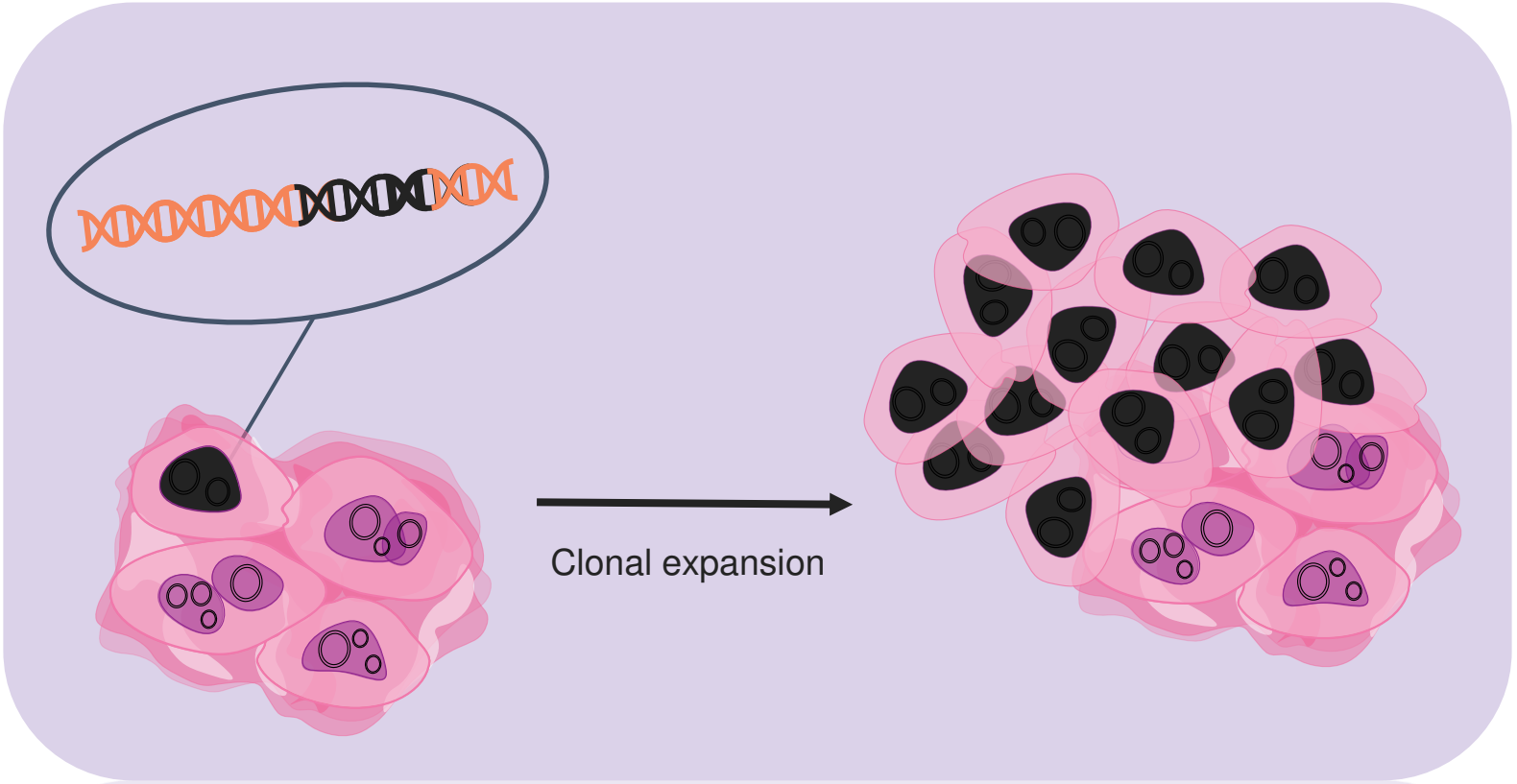
Participant	Dose level, vg/kg	Time to biopsy, years	VCN	Unique IS	IS/cell
A	6×10^{13}	0.5	3.7	435	3.19E-03
B	6×10^{13}	1.11	8.4	778	5.71E-03
C	6×10^{13}	4.12	5.9	592	4.34E-03
D	6×10^{13}	4.10	8.7	718	5.29E-03
E	4×10^{13}	2.85	1.3	182	1.33E-03
Average	-	**2.5**	**5.6**	**541**	**3.97E-03**

1-9 vector copies per cell; 1-6 integration events per 1,000 cells

- Comparable to rates of insertion observed in individuals previously infected with AAV2 or AAV2/13 (1 integration per 1,000 cells), which has not been epidemiologically associated with increased risk of HCC[1]
- Several orders of magnitude lower than the annual rate of natural mutations in humans (0.5 mutations per E9 base pairs, or approximately 3 mutations/diploid cell/year)[2]

HCC: hepatocellular carcinoma; VCN: vector copy number; Presented at the World Federation of Hemophilia (WFH) Congress: 8-11 May 2022 (Montréal and virtual); 1. Qin W et al. Oncogene 2021;40:3060-71; 2. Milholland B et al. Nat Comm 2017;8:15183

Using integration to study clonal expansion



Each integration event uniquely labels the cell in which it occurs, allowing the relative growth of that cell to be measured

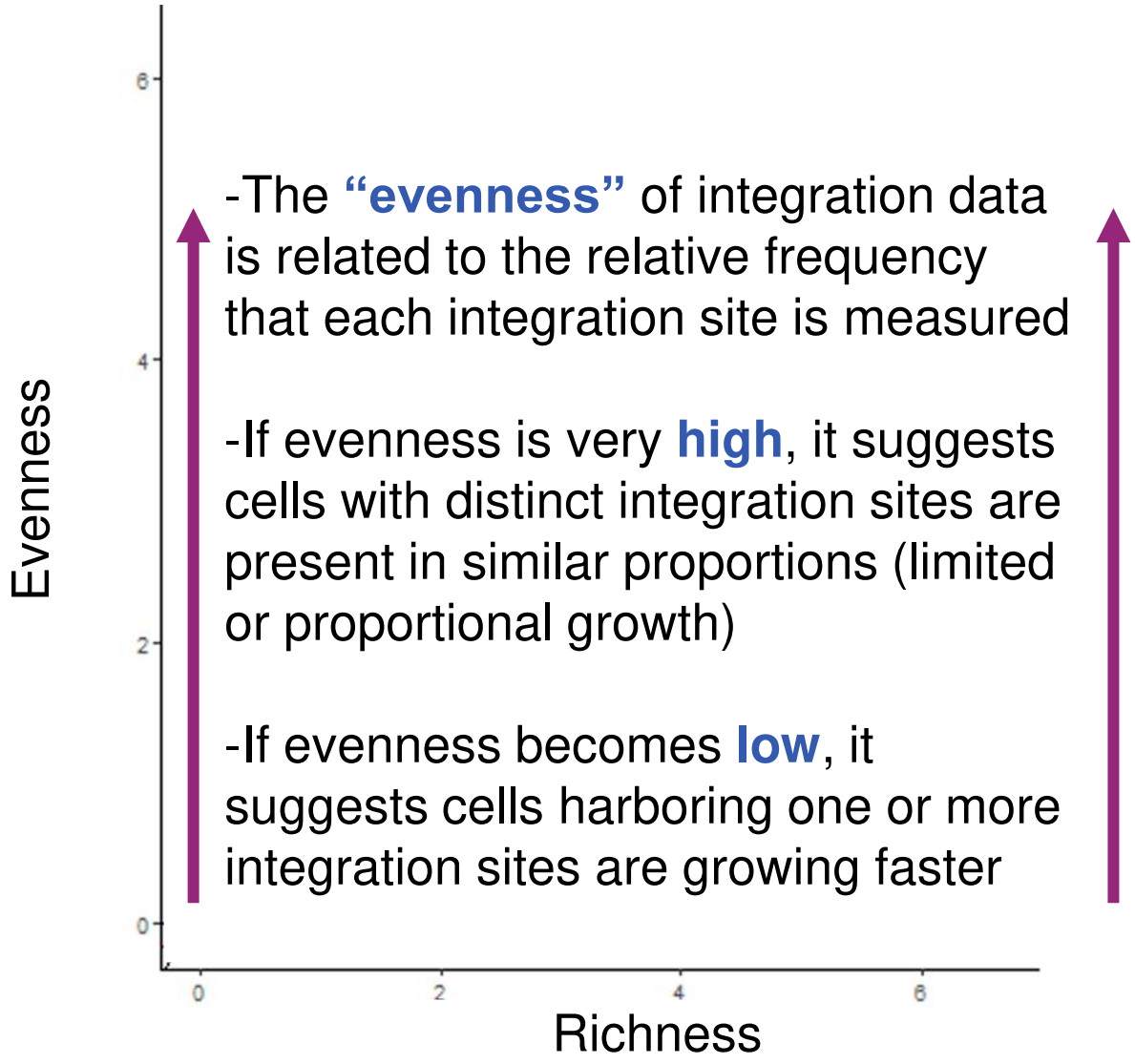
Presented at the World Federation of Hemophilia (WFH) Congress: 8-11 May 2022 (Montréal and virtual)

Using integration patterns to monitor relative growth

-The **“richness”** of an integration data set is related to the total number of distinct integration sites observed in it

-Some richness in integration sites needed if the relative impact of each integration event on cell growth is to be measured

Using integration patterns to monitor relative growth



-The **"evenness"** of integration data is related to the relative frequency that each integration site is measured

-If evenness is very **high**, it suggests cells with distinct integration sites are present in similar proportions (limited or proportional growth)

-If evenness becomes **low**, it suggests cells harboring one or more integration sites are growing faster

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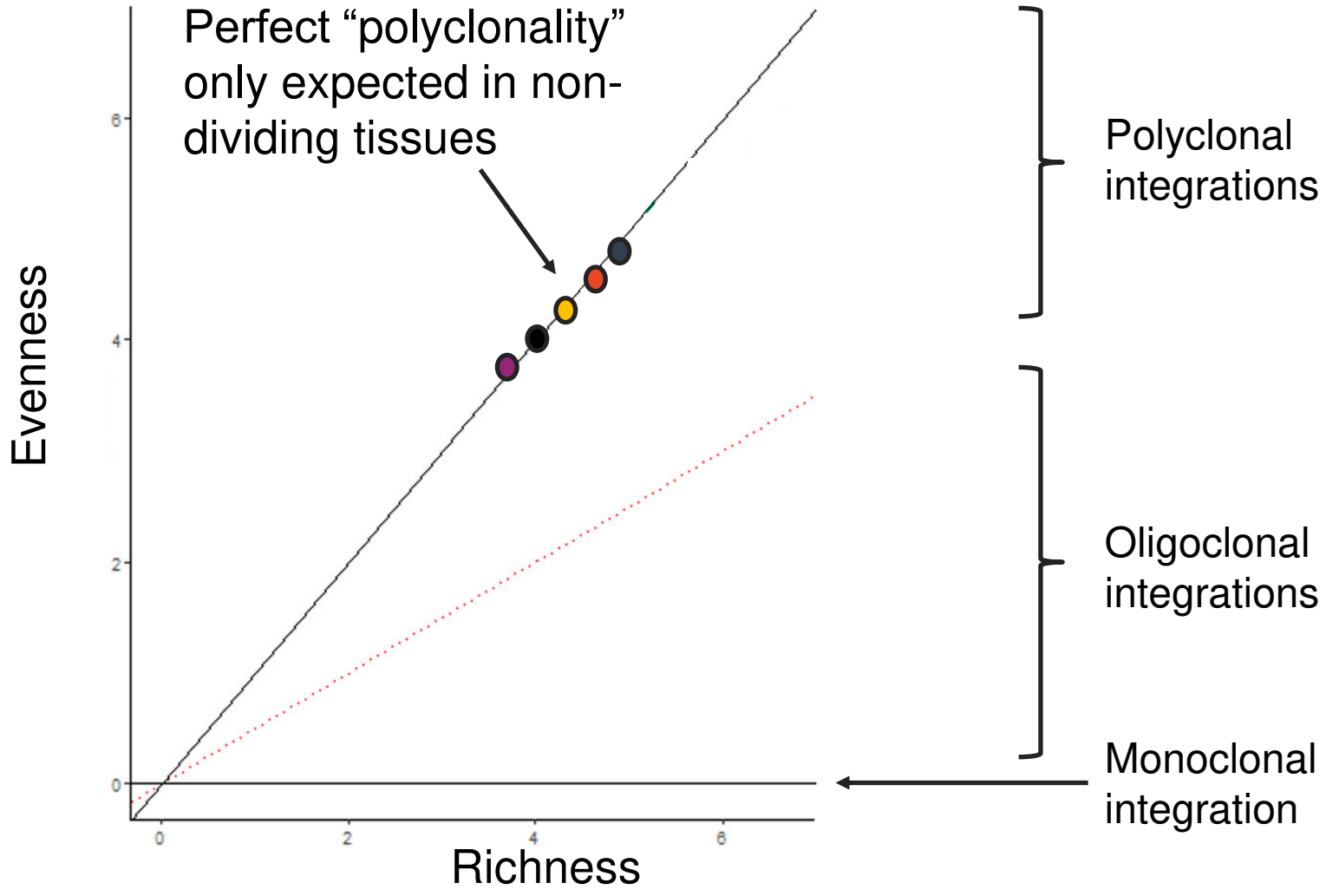
Theoretical example: No growth or even polyclonal growth



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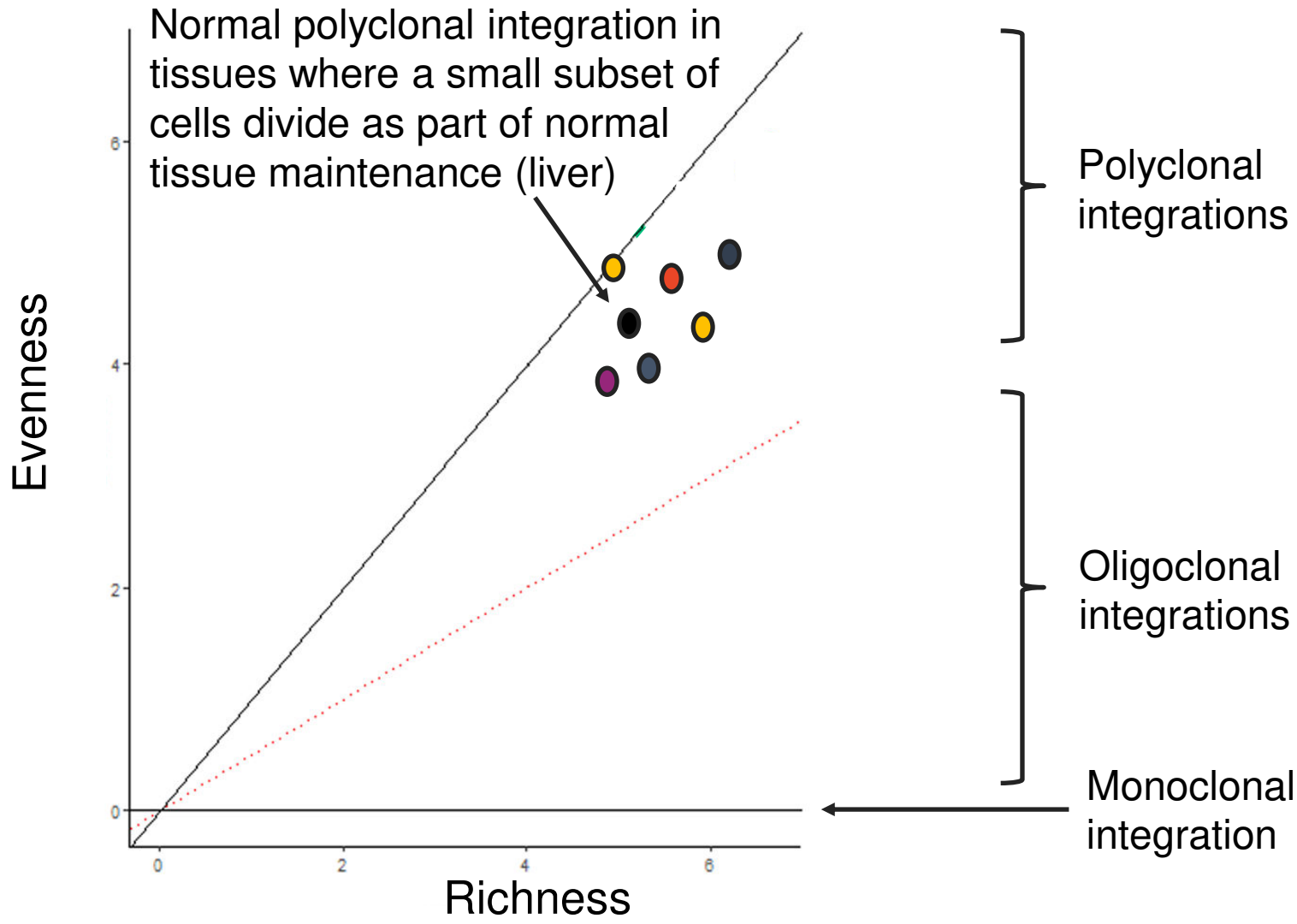
Theoretical example: Normal polyclonal growth with few cell divisions



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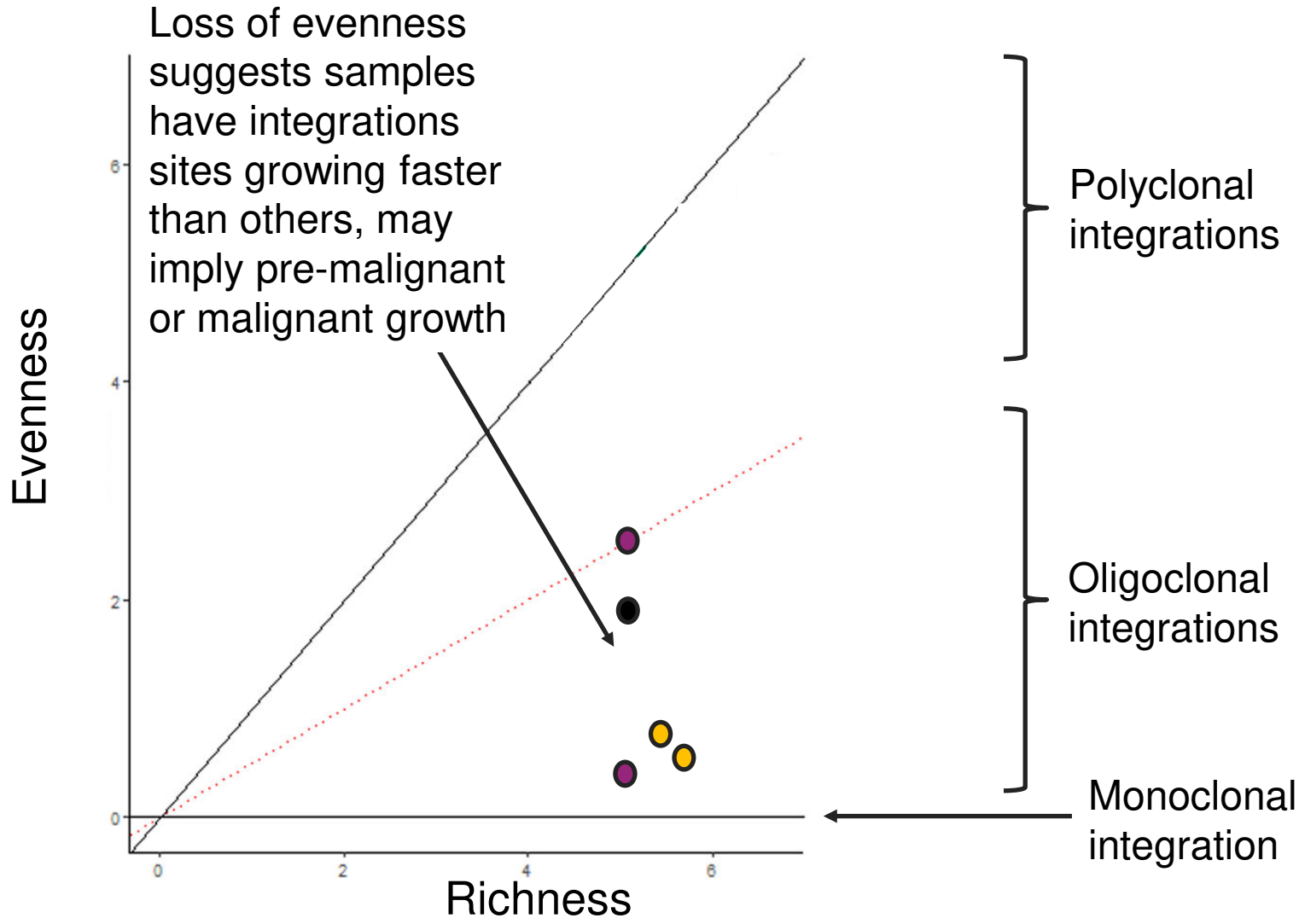
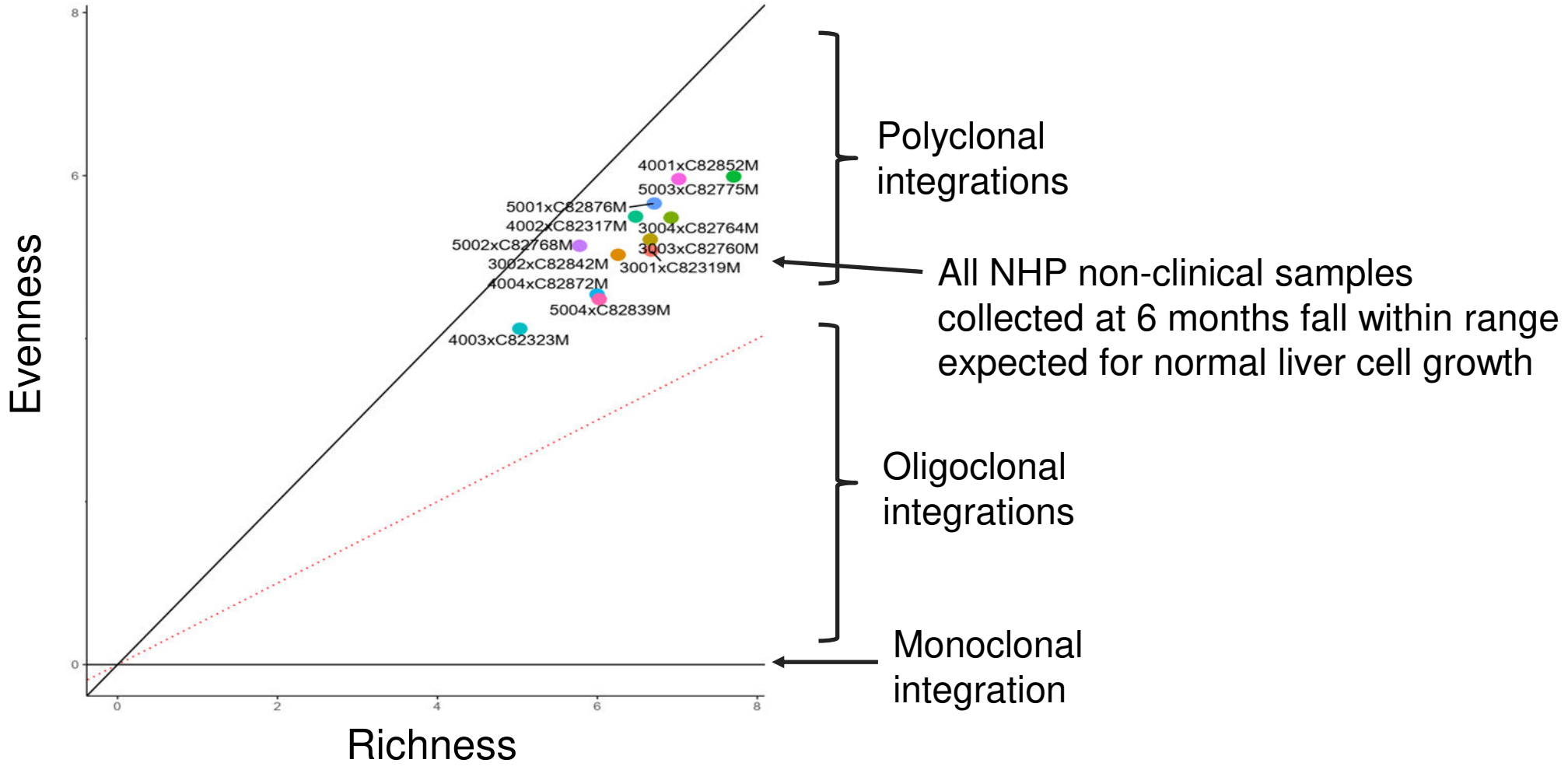
Theoretical example: Oligoclonal/clonal expansion by one or few cells



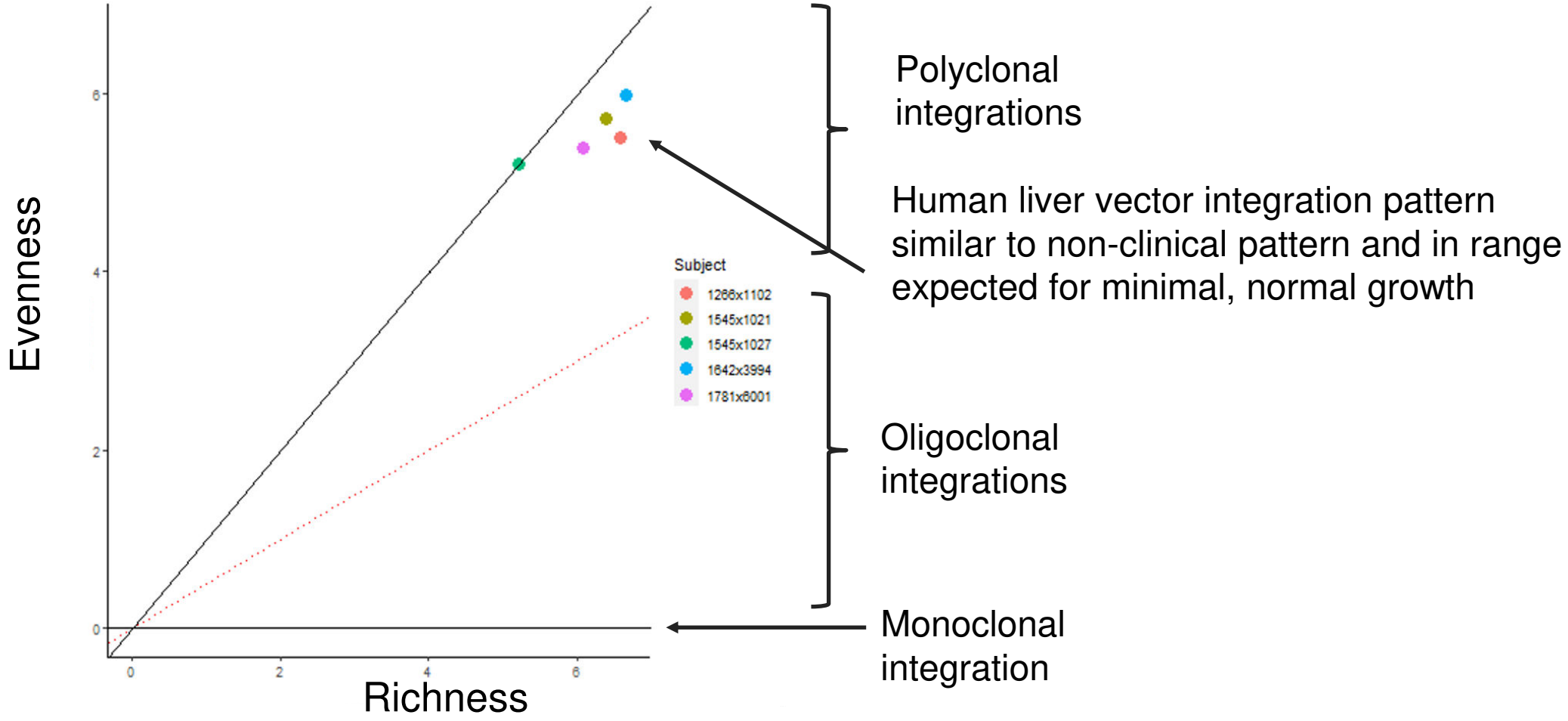
Presented at the World Federation of Hemophilia (WFH) Congress: 8-11 May 2022 (Montréal and virtual)

No evidence for biased growth after 270 integration detected in NHP



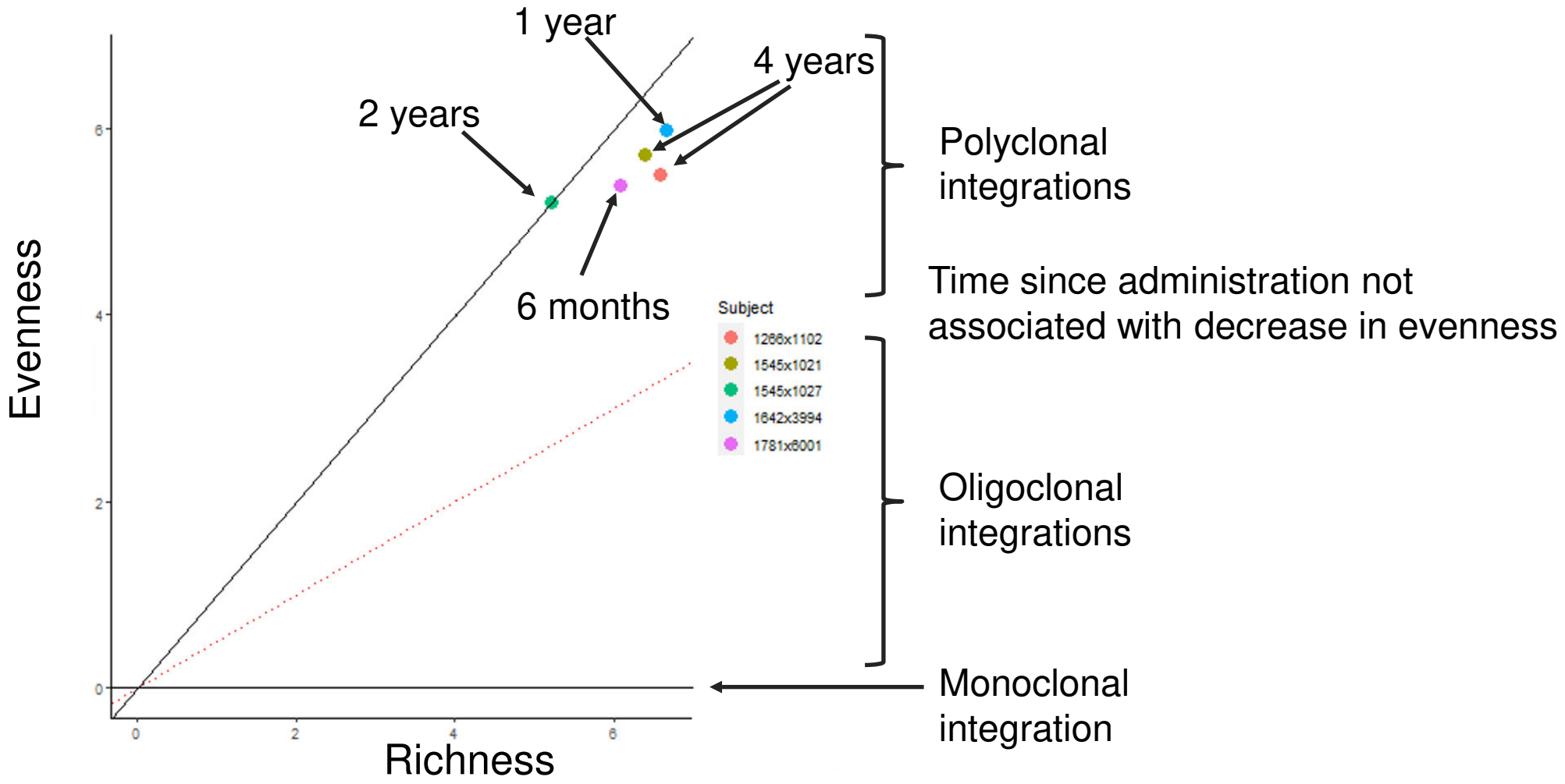
Presented at the World Federation of Hemophilia (WFH) Congress: 8-11 May 2022 (Montréal and virtual)

No evidence for biased growth after 270 insertion detected in healthy human liver



Presented at the World Federation of Hemophilia (WFH) Congress: 8-11 May 2022 (Montréal and virtual)

No evidence for biased growth after 270 insertion detected in healthy human liver



Presented at the World Federation of Hemophilia (WFH) Congress: 8-11 May 2022 (Montréal and virtual)

Limitations of our studies

- Can not rule out distinct integration patterns in regions we did not biopsy
- We have only analysed a modest number of individuals
- Total follow-up time still only 4 years
- Assay calibration suggests high sensitivity, but exact relationship between number of integration counts and number of cells with an insertion is not firmly established

Presented at the World Federation of Hemophilia (WFH) Congress: 8-11 May 2022 (Montréal and virtual)

Conclusions: Healthy human liver integration study

- Overall pattern of insertion very similar to non-clinical studies
- Low integration rates as expected from non-clinical studies
- Comparable to rates found in those exposed to natural AAV, where no epidemiological risk identified
- No evidence for malignant or pre-malignant expansion of cells harboring insertions

Liver biopsy summary:
Benign integration profile for valoctogene roxaparvovec

Presented at the World Federation of Hemophilia (WFH) Congress: 8-11 May 2022 (Montréal and virtual)

Integration site analysis of parotid gland tumor-containing and adjacent healthy tissue

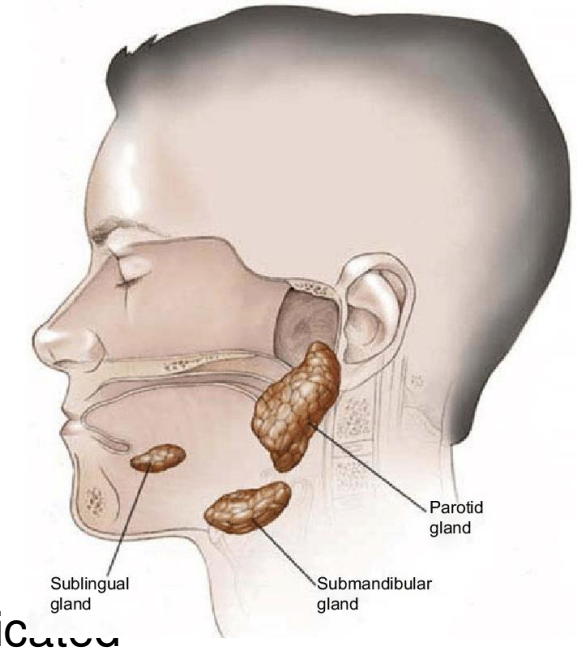
Case of acinic cell carcinoma in phase 1/2 participant

Background

- 47-year-old male with a past medical history of severe hemophilia A, bilateral ankle hemophilic arthropathy, and HCV
- Received 6 x 10^{13} vg/kg of valoctocogene roxaparvovec in April 2016 in phase 1/2 study
- No known AcCC risk factors

Diagnosis

- Reported a lump on the right side of the neck that had been present for about a year but was otherwise asymptomatic
- Following an ultrasound, an adenoma in the tail of the parotid gland was diagnosed
- Further work-up with fine needle aspirate cytology of the parotid gland was consistent with AcCC
  - Assessed as unrelated to valoctocogene roxaparvovec



Treatment

- Underwent right parotidectomy with neck dissection December 2021, uncomplicated
- Pathology confirmed diagnosis of low-grade AcCC with clean surgical margins
- Surgery was considered definitive treatment

AcCC: acinic cell carcinoma; HCV: hepatitis C virus; Garuti G et al. Degener Neurol Neuromuscul Dis 2019;9:19-26; Presented at the World Federation of Hemophilia (WFH) Congress: 8-11 May 2022 (Montréal and virtual)

Vector integration frequency in tumor and adjacent healthy tissue was comparable

Tumour sample		Healthy sample	
Pool 1C	5.72 E-05 IS/cell	Pool 1H	1.19 E-04 IS/cell
Pool 3C	7.26 E-05 IS/cell	Pool 3H	8.14 E-05 IS/cell
Average tumour	6.49 E-05 IS/cell	Average healthy	1.00 E-04 IS/cell
Overall Average in Parotid		**8.26 E-05 IS/cell**	

Average ~30 times lower integration frequency in parotid vs. liver; consistent with relative tropism of AAV5 capsid for these tissues

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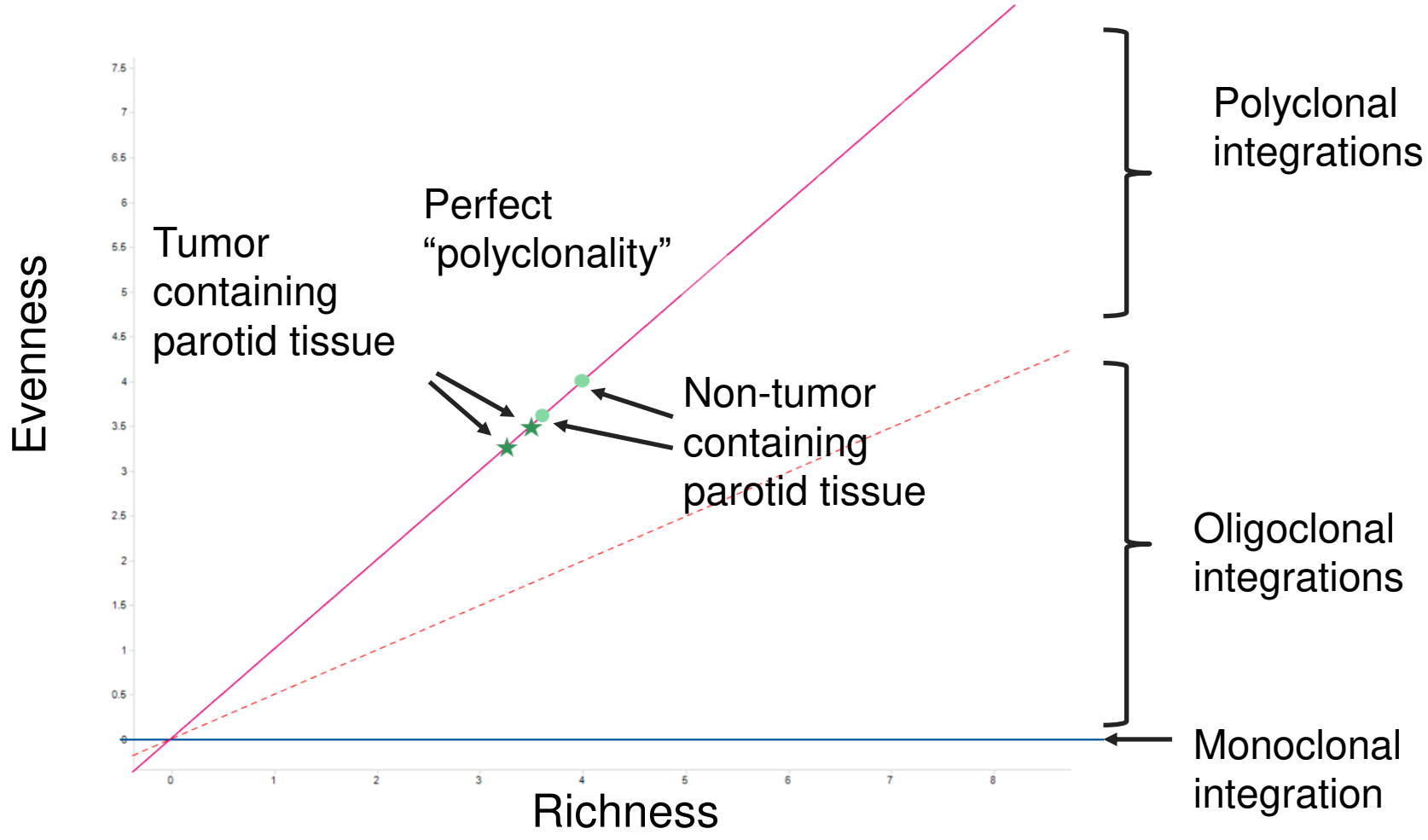
Tissue with tumor does not display increased clonality



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Examining integration events near cancer-associated genes

IS <100 kb from any cancer gene					Read number			
Cancer gene name	Distance to TSS (bp)	Chr	Integration locus	Nearest gene	Tumor pool 1C	Healthy pool 1H	Tumor pool 3C	Healthy pool 3H
NAB2	50,252	12	57142401	LRP1	1			
STAT6	10,262	12	57142401	LRP1	1			
CHD4	-233	12	6592333	CHD4	1			
ZNF384	-76,824	12	6592333	CHD4	1			
TAF15	50,465	17	35914401	LRRC37A8P			1	
EPAS1	-34,583	2	46259084	EPAS1		1		
TMEM127	22,015	2	96288009	SNRNP200		1		
TMEM127	22,062	2	96288056	SNRNP200		1		
KIT	96,162	4	54825454	KIT			1	
FGFR1OP	-1,899	6	167022891	FGFR1OP		1		

- No insertions observed in any samples within 100kb of AcCC driver gene
- No insertions within 100 kb of any cancer-associated genes overlap between tumor-containing samples, suggesting that detected insertions are unlikely to be present in tumor cells
- 1 insertion observed in one tumor sample near salivary-gland tumour associated gene (KIT), but this insertion was well down stream of gene and therefore unlikely to have upregulating influence
- WGS (less sensitive) found 4 insertions sites, none near cancer genes

Bp: base pairs; Chr: chromosome; kb: kilo bases; TSS: transcriptional start site; WGS: whole genome sequencing;
Presented at the World Federation of Hemophilia (WFH) Congress: 8-11 May 2022 (Montréal and virtual)

Conclusions: Parotid tumor study

- Tumor cells were only 2% of sample limiting our informative analyses to TES insertion site analysis
- No evidence for clonal expansion of vector insertion in parotid tumor tissue
- Overall background integration rates lower in parotid than liver, consistent with lower biodistribution in non-clinical study
- Results consistent with investigators and DMBs conclusion that this event is likely unrelated to valoctocogene roxaparvovec

No evidence for association between valoctogene roxaparvovec and tumor to date*

*The valoctocogene roxaparvovec SmPC reports a theoretical risk of malignancy - All occurring malignancies need to be reported

TES: Target Enrichment Sequencing; DMB: data monitoring board

Presented at the World Federation of Hemophilia (WFH) Congress: 8-11 May 2022 (Montréal and virtual)

Analyses of B-Cell Acute Lymphoblatic Leukemia Cells for Tumorogenic Mutations and Presence of Vector DNA

Case of B-cell acute lymphoblastic leukemia

Background

- Mid-20s male with a past medical history of severe hemophilia A
- Received 6 x 10^{13} vg/kg of valoctocogene roxaparvovec in 2020 in phase 3 study

Diagnosis

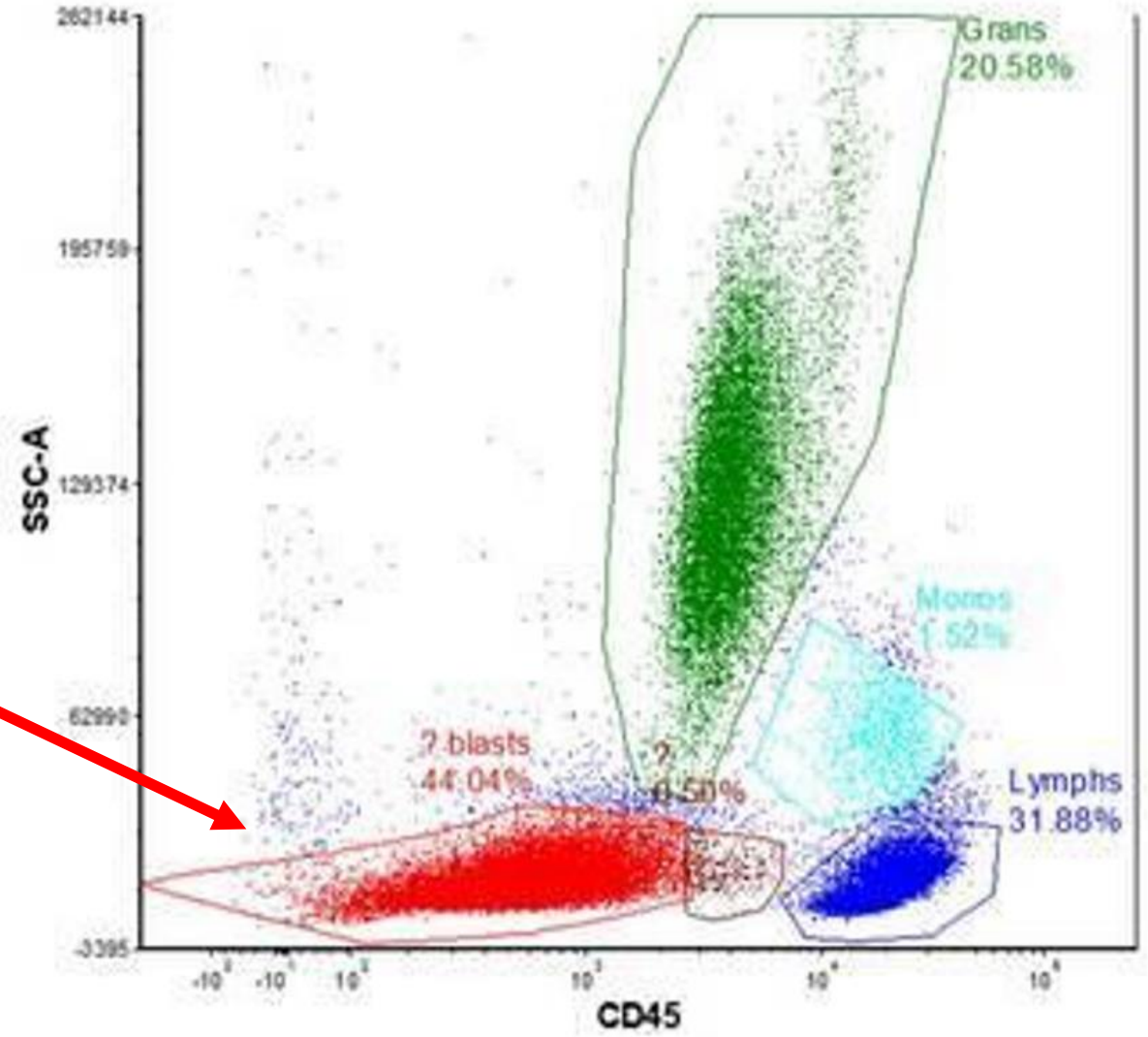
- Reported back pain & intermittent fevers, in October 2021, that was still not resolving in early 2022, and including some unintended weight loss
- Imaging (CT) and peripheral blood workup were unremarkable except for elevation of some inflammatory markers. Other autoimmune workup did not have significant findings
- Weight loss, anemia, and exacerbated back pain prompted bone marrow aspiration and biopsy, finding >80% lymphoblasts in the marrow
- CPT sample also collected from peripheral blood

Treatment

- Chemotherapy ongoing
- Molecular pathology confirmed diagnosis of B-ALL with Philadelphia-like rearrangement in CRLF2, mutations in JAK1 and IKZF1

Circulating lymphoblasts have distinct cell surface properties allowing them to be identified by flow cytometry

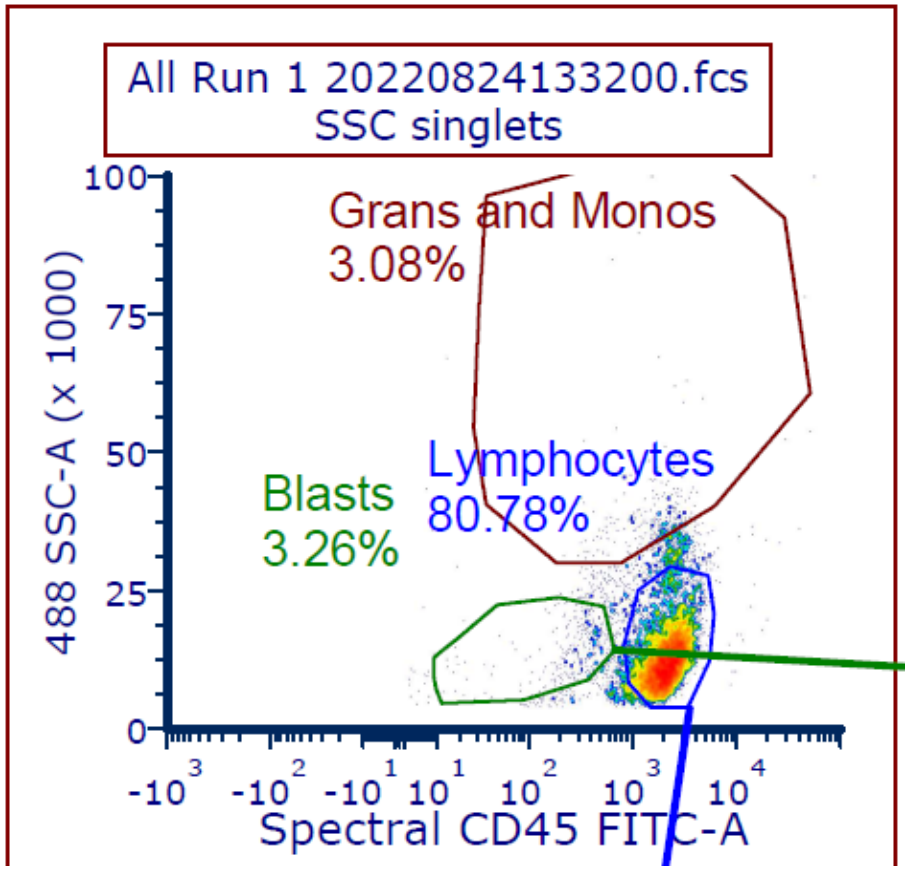
In classic B cell-ALL, a CD45-lo, SSC-lo, $CD3^-$, $CD19^+$ population of blasts can often be identified in the circulation that is not observed in healthy individuals



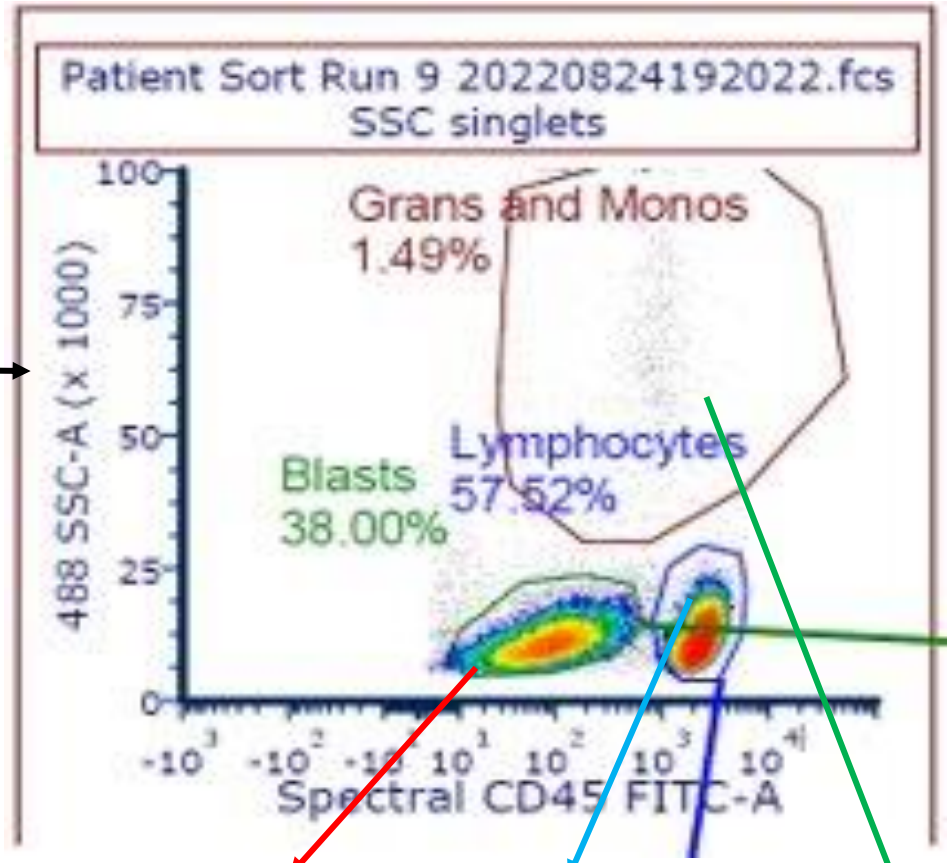
From Katherine Calvo, B cell ALL flow cytometry (hematology.org), http://imagebank.hematology.org/collection/60062

Flow cytometry used to identify presumptive blast cells in the circulation and to fractionate them from other blood cell types

- Healthy Donor

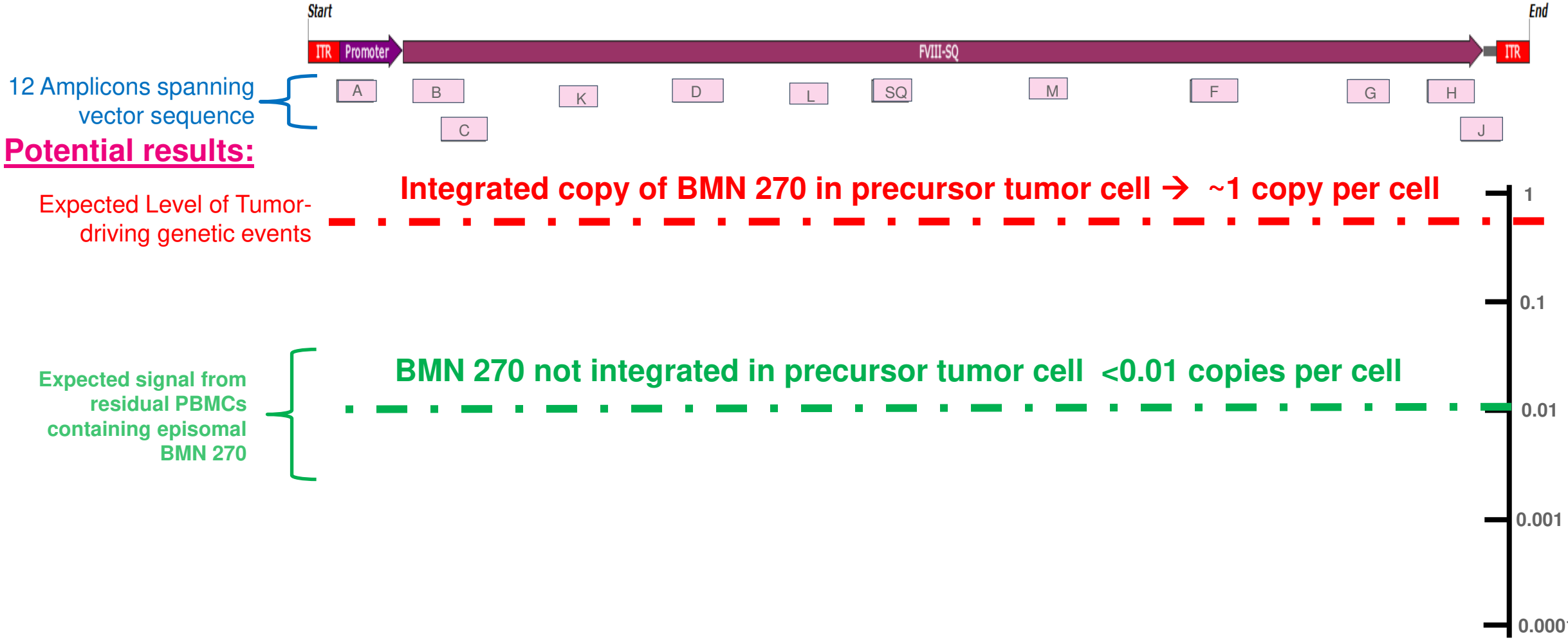


- B-ALL subject

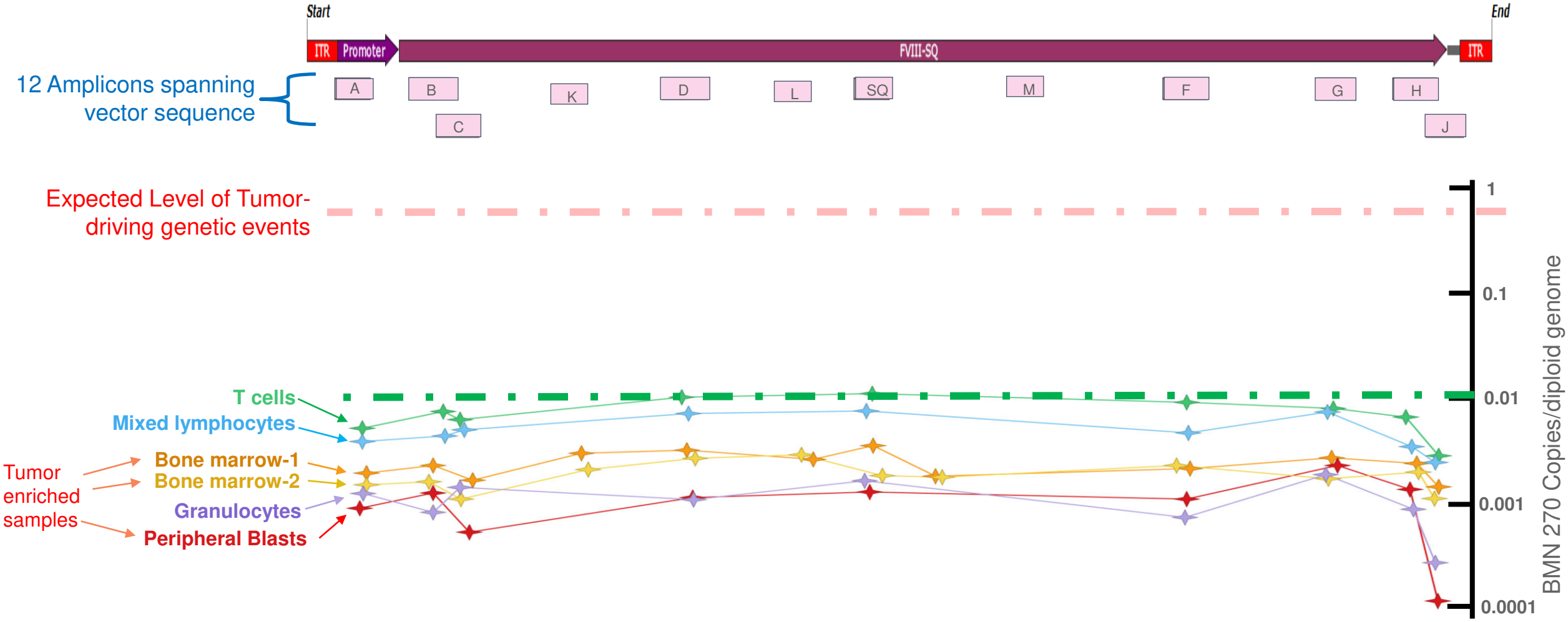


- Granulocytes harvested → DNA
- Tumor lymphoblast ~90% enriched → DNA
- Mixed lymphocytes → DNA
- T cell/Monos enriched → DNA

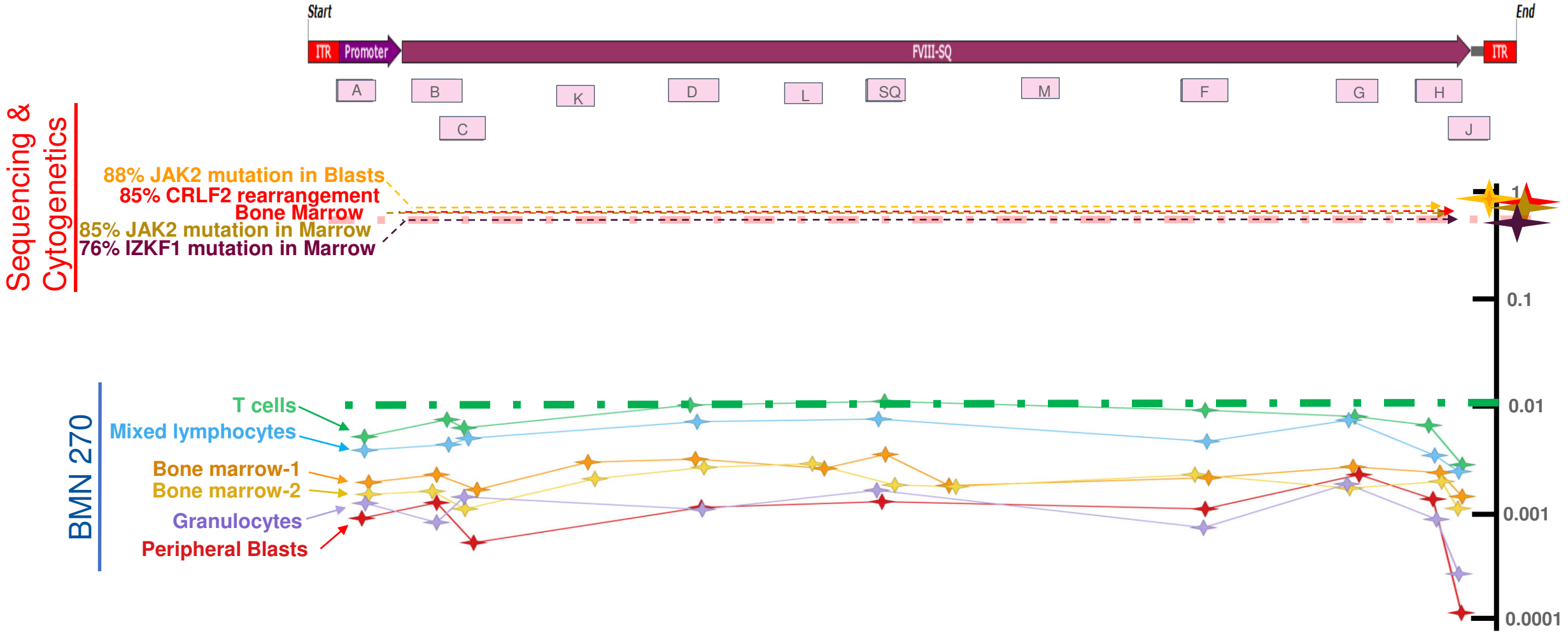
BMN 270 vector copy numbers per cell were assessed at 12 sites – >90% enrichment by FACS allowed clear predictions if vector integration were associated with clonal expansion of lymphoblasts



In samples with >90% tumor cells, BMN 270 vector copies per cell were <0.003 - Results incompatible with clonally expanded BMN 270 in tumor cells



Pathogenic JAK2 mutation associated with B-ALL found in marrow and FACS enriched Blast cells, but not in FACS enriched T-cell and mixed lymphocyte fractions. FISH on marrow identified CRLF2 rearrangement



Conclusions: B cell-ALL study

- Bone marrow and FACS enrichment of circulating blasts provided DNA from samples with ~90% tumor cells, enabling informative molecular investigations
- No evidence for clonal expansion of 270 vector DNA in tumor cells
- Enriching tumor cells by FACS depleted vector levels below the low levels found in circulating lymphocytes: consistent with absence of vector in tumor cells
- Mutations commonly associated with B-ALL in the young adult population were found in many tumor cells, consistent with their clonal expansion
- >100x whole genome sequencing results:
  - Somatic mutations readily identified (JAK2, IZKF1)
  - No BMN270 vector integration observed
- Results consistent with investigator and DMBs view that this event is likely unrelated to Roctavian

No evidence for association between Valoctogene Roxaparvovec and tumor

BioMarin is committed to long-term follow-up of study subjects and patients dosed in the real world

BioMarin is committed to long-term collaboration with the hemophilia community to study cancer risk in recipients of valoctocogene roxaparvovec

- Phase II and III study participants will be enrolled in a 15-year long-term extension study
  - This will facilitate potential detection of prominent safety signals, including risk of cancer
- Patients dosed in the real-world setting will also be asked/encouraged to participate in the WFH Gene Therapy Registry (WFH GTR) enabling additional systematic examinations of safety and efficacy
- In Europe (and proposed in US) valoctocogene roxaparvovec label directs patients and HCPs to contact BioMarin in the case of any malignancy, enabling a standardized molecular and genomics analysis to be carried out on any available samples

HCPs: healthcare practitioners

Continued studies of vector integration in tumor samples will require close collaborations between BioMarin, HCPs and hemophilia community

After BioMarin is contacted concerning a cancer case:

- Consent forms to obtain samples and perform genetic analysis will be sent to treatment center with sample collection kit and clear instructions
- Enough tumor and healthy adjacent tissue for genomic analysis will be requested – ideally flash frozen tissue, but FFPE fixed tissue will be acceptable
- Samples will be shipped to central lab for dissection/enrichment of tumour tissue and extraction of DNA from tumor and control samples
- Molecular and genomic analyses will be performed on tumor and control samples to explore molecular aetiology of each case. Currently we plan:
  - Quantification of vector abundance in tumor-enriched and adjacent control samples
  - Insertion site analysis by whole genome sequencing or target enrichment sequencing
  - Targeted sequencing of cancer gene panel to identify and quantify any acquired somatic variants associated with clonal expansion
  - Gene expression studies if warranted based on vector integration findings
- Timely communication of findings to relevant health authorities, with updates to clinical, scientific and patient community via conferences and publications as insights emerge

FFPE: Formalin-Fixed Paraffin-Embedded; BioMarin data on file

Looking to the future…

- We can expect to see further cases in valoctocogene roxaparvovec recipients:
  - The National Cancer Institute (US) estimates there are 442.4 new cancer cases per 100,000 individuals each year – This varies across countries[1]
  - Given most people with hemophilia are men we expect this to be higher, and two studies suggest that individuals with hemophilia may have an increased risk of cancer[2,3]
  - BioMarin has investigated 2 cases over 400 recipient-years[4,5]
  - Increased observation time and aging of treated patients will increase the reporting rate of malignancy cases
- While no contribution of vector DNA to first two cancer cases was detected:
  - Diligent, collaborative studies of both cancer rates and vector DNA will be needed to understand whether cancer risk is altered in our gene therapy recipients by either vector integration or by other factors

1. NCI data – Internet: https://www.cancer.gov/about-cancer/understanding/statistics; 2. Liu CJ et al. Haemophilia 2014;20:741–6; 3. Lövdahl S et al. Blood Coagul Fibrinolysis 2016;27:631-6; 4. Presented at the World Federation of Hemophilia (WFH) Congress: 8-11 May 2022 (Montréal and virtual); 5. BioMarin 8K form reported 12 Sept 2022 to the Securities and Exchange Commission, Washington D.C. 20549

THANK YOU