De-risking Analytical Comparability a Manufacturing Process Change for a scAAV8 Therapy in Late Development

CASSS, Cell and Gene Therapy Products Summit

27 June 2023

Taro Fujimori, Ultragenyx

Outline

- Process Changes and Development History
- Key considerations: Control Strategy for Titer
  - FDA expectations for dosing based on nominal titer
  - Structure-Function for Self-Complementary AAV scAAV
  - qPCR to ddPCR analytical assay change for titer
  - Process B1 Control Strategy
  - Benchmarking analysis
- Process B2 Control Strategy for Titer
- Summary

Late-Stage Process Changes

- New DS and DP manufacturing sites: commercially viable
- New MCB/WCB: assurance of monoclonality
- Introduction of viral clearance step
- AEX collection procedure optimized to ***ensure product quality: full, intermediate, and empty capsids***
- New assay for DS/DP titer (qPCR to ddPCR) ***improved assay variability***
- Tighter acceptance criteria for DP titer: ***enable dosing by nominal titer***
- Revised DP target titer and extractable volume: ***ensure ≤30 vials per dose***

Upstream Process Development Overview

	Process A	Process B1	Process B2 (Commercial)
Uses	Phase 1/2	Phase 1/2, Phase 3	GMP batches not yet manufactured.
DS Manufacturing Site	DS Site A	DS Site B	DS Site C
Master Cell Bank / Working Cell Bank	Adherent, serum-dependent	Suspension, serum-independent	Suspension, serum-independent, monoclonal
Upstream Scale	84L	400 L	1000 L
Media	Serum containing	Chemically defined, no animal derived components	
Benzonase	w/ salt treatment	No salt treatment	
Harvest TFF (Tangential Flow Filtration)	No change in TFF pore size, Retentate is stored frozen prior to downstream processing		

- *Key objectives for Commercial Upstream Process Development:*
  - *Commercially viable drug substance manufacturing site and scale*
  - *MCB/WCB: assurance of monoclonality, no serum or other animal derived components, and suspension culture*

Downstream Process Development Overview

	Process A	Process B1	Process B2 (Commercial)
Downstream Scale	400 L	800 L	2000 L
Affinity	AAV8 Affinity Chromatography		
Anion Exchange	1 CV fractions collected and pooled		Single product pool fraction
Viral Filtration	NA		Viral filtration
In-Process Control Test (Formulation TFF Load)	Titer by qPCR (used to calculate Formulation TFF retentate volume to achieve target DS titer)		
Formulation TFF	No Change in TFF pore size or material of construction		
Formulation TFF Retentate	Target titer: 1.5 x 10^{13} GC/mL (qPCR)	Target titer: 3.0 x 10^{13} GC/mL (qPCR)	
DS Release testing	qPCR	ddPCR	

- *Key objectives for Commercial Downstream Process Development:*
  - *Implementation of viral filtration step*
  - *Anion Exchange collection criteria optimized to ensure product quality (%Full, %Intermediate, %Empty Capsids)*

Drug Product Development Overview

	Process A	Process B1	Process B2 (Commercial)
DP Manufacturing Site	DP Site A	DP Site B	DP Site C
Dilution of Thawed Drug Substance	Not performed		Target titer: 2.0E13 GC/mL (ddPCR)
Extractable Volume	1.0 mL		3.4 mL
DP titer, GC/mL Min – Max	qPCR 53% to 147% (n = 4)	ddPCR 65% to 135% (n = 11 batches)	ddPCR 85% to 115% (target range)
Dosing	Dosing based on measured titer		Dosing based on nominal titer
DP Release testing	qPCR	ddPCR	

- *Key objectives for Commercial Drug Product Development:*
  - *New DP manufacturing site: Commercially viable*
  - *Narrow limits for titer: enable dosing based on nominal titer*
  - *Greater than 5.1e13 GC / vial: ensure less than 30 vials per dose*

Process B2 Titer Control Strategy: Key Considerations

- *FDA expectations for dosing based on nominal titer*
- Structure-Function for Self-Complementary AAV: potency of full and intermediate capsids
- qPCR to ddPCR analytical assay change for titer: off-set correlated with the ratio of full and intermediate capsids
- Process B1 Control Strategy: qPCR as an IPC for formulation TFF and ddPCR as a release test
- Benchmarking commercial AAV products

FDA OTAT Town Hall meeting 9/29/22: Dosing Based on Nominal Titer for AAV Gene Therapies

- Hosted by the Office of Tissues and Advanced Therapies (OTAT) within the Center for Biologics – GT CMC Evaluation and Research.
- Q2. ***What is the must have and good to have CMC information for a gene therapy product going into phase three studies or late phase studies that intend to support a marketing approval?***
- ...***For an AAV vector, the product should be formulated using a nominal titre so you can gain experience with the nominal dosing strategy and that can be part of the determination of the efficacy assessment.*** Overall, we recommend that you move to the expected commercial configuration prior to conducting this pivotal study and this will reduce the risk in your developmental process. This includes using intended commercial manufacturing process at the intended manufacturing facility and using the expected testing strategy. This will position you to have the maximum data at your exposure to use for your license application and it's also going to reduce the complications related to comparability assessments that may occur during the review.

Process B1 Dosing Based on Measured Titer

- Dose Calculation Reference Sheets (separate table for each lot)
- If more than 1 product lot is needed for subject dosing, you will be contacted and provided further instructions.

Patient Weight Band (kg)	Dose (GC/kg) [redacted] E+13 [redacted] Concentration (GC/mL): [redacted] E+13 Lot#: [redacted]	
	Dose Volume (ml)	# of Vials
20 - 24	6	6
25 - 29	8	8
30 - 34	9	9
35 - 39	11	11
40 - 44	13	13
45 - 49	14	14
50 - 54	16	16
55 - 59	17	17
60 - 64	19	19
65 - 69	20	20
70 - 74	22	22
75 - 79	24	24
80 - 84	25	25
84 - 89	27	27
90+	28	28

Patient Weight Band (kg)	Dose (GC/kg) [redacted] E+13 [redacted] Concentration (GC/mL): [redacted] E+13 Lot#: [redacted]	
	Dose Volume (ml)	# of Vials
20 - 24	10	10
25 - 29	13	13
30 - 34	15	15
35 - 39	18	18
40 - 44	21	21
45 - 49	23	23
50 - 54	26	26
55 - 59	28	28
60 - 64	31	31
65 - 69	33	33
70 - 74	36	36
75 - 79	39	39
80 - 84	41	41
84 - 89	44	44
90+	46	46

Patient Weight Band	Dose (GC/kg) [redacted] E+13 [redacted] Concentration (GC/mL): [redacted] E+13	
	[redacted]	# of Vials
	7	7
	9	9
	11	11
	12	12
	14	14
	16	16
	18	18
	19	19
	21	21
	23	23
	25	25
	27	27
	28	28
	30	30
	32	32

Dosing Based on Nominal Titer: FDA Expectation for Commercial AAV Products

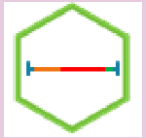
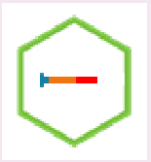
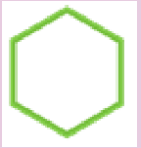
- Nominal titer: 2.0e13 GC/mL
- Extractable volume: 3.4 mL*
- Dose: 1.7e13 GC/kg
- Vials per dose: Patient body weight in kg divided by 4*
- Maximum number of vials per dose (90 kg patient*): 22 vials
- *Simple calculation for number of vials per dose that is applicable for all DP batches*

***Extractable volume, dosing calculation are provided for illustrative purposes. The company is evaluating appropriate highest body weight to use in calculating the optimal extractable volume per vial.**

Process B2 Titer Control Strategy: Key Considerations

- FDA expectations for dosing based on nominal titer
- ***Structure-Function for Self-Complementary AAV: potency of full and intermediate capsids***
- qPCR to ddPCR analytical assay change for titer: off-set correlated with the ratio of full and intermediate capsids
- Process B1 Control Strategy: qPCR as an IPC for formulation TFF and ddPCR as a release test
- Benchmarking commercial AAV products

Potency for Self-Complementary AAV (scAAV) Product Related Species and Impurities

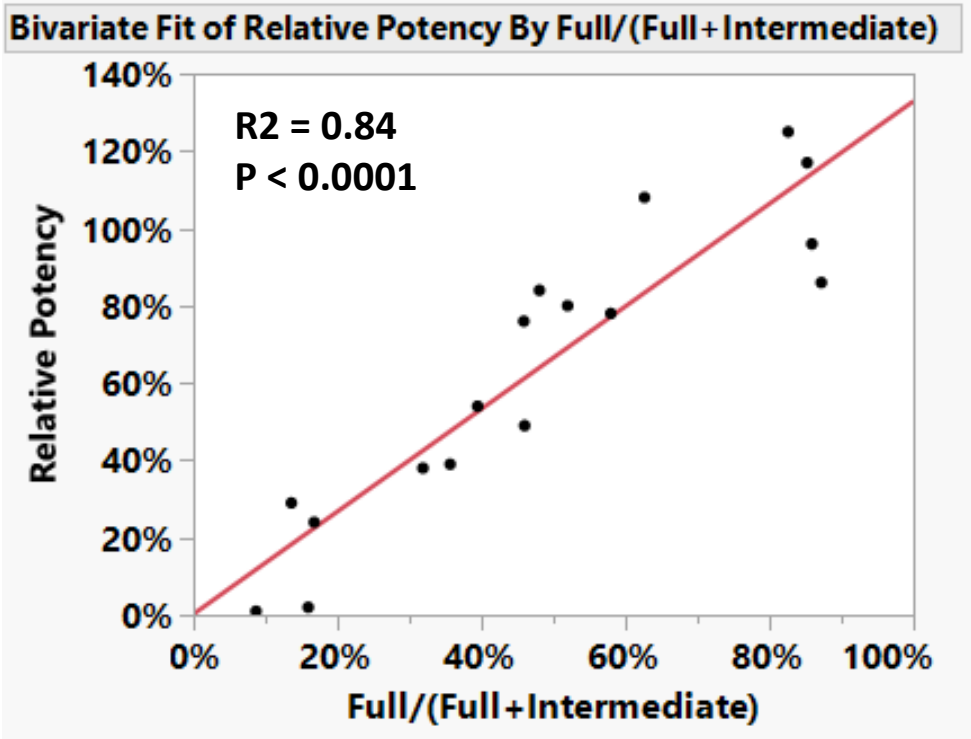
AAV Species	Genome	Potency*
 Full Capsid	Double Stranded (scAAV)	1X Potency*
 Intermediate Capsid	Single Stranded (ssAAV)	<0.05X Potency*
 Empty Capsid	No DNA	0 Potency

- Full Capsid (scAAV)
  - Product related species
  - Double stranded Gene of Interest
  - 20 to 100-fold higher activity relative to ssAAV (Wu et al 2008*)
- Intermediate Capsid
  - Product related impurity
  - Single stranded Gene of Interest (ssAAV)
  - The truncated form of DNA may be defective and inactive.
- Empty Capsid
  - Product related impurity
  - No DNA
  - No desired biological activity

*Wu 2008 Molecular Therapy, Vol. 16 No. 2, 280–289

ultragenyx

Linear Relationship Between Potency and %Full Capsid / (%Full Capsid + %Intermediate Capsid)



- 2 x B1 and 2 x B2 DS samples, Fractionated samples (CsCl) enriched for Full, Intermediate, and Empty Capsids
- Linear relationship between potency and %Full / (%Full + %Intermediate)
- Confirms that Potency for Full Capsids is much greater than Potency for Intermediate Capsids
- Ratio of %Full and %Intermediate capsids can be used as a surrogate measure for potency during process development
  - In-process samples: %Full and %Intermediate by AUC
  - Drug substance and drug product samples: potency assay

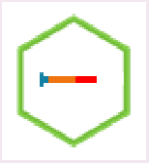
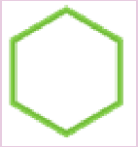
- *Key objective for B2 process development is to maintain a comparable ratio of %Full and %Intermediate capsids*

ultragenyx

Process B2 Titer Control Strategy: Key Considerations

- FDA expectations for dosing based on nominal titer
- Structure-Function for Self-Complementary AAV: potency of full and intermediate capsids
- ***qPCR to ddPCR analytical assay change for titer: off-set correlated with the ratio of full and intermediate capsids***
- Process B1 Control Strategy: qPCR as an IPC for formulation TFF and ddPCR as a release test
- Benchmarking commercial AAV products

Self-Complementary AAV: qPCR and ddPCR Response

AAV Species	Genome	qPCR Response	ddPCR Response
 Full Capsid	Double Stranded	1X Response	1X Response
 Intermediate Capsid	Single Stranded	0.5X Response	1X Response
 Empty Capsid	No DNA	0 Response	0 Response

- Basis of qPCR quantification:
  - Comparison between the signal from the samples and a series of plasmid standards
  - For scAAV, the genome copy number is calculated based on double stranded genomes.
  - ***The intermediate species (single-stranded) is counted as half a genome.***
- Basis of ddPCR quantification:
  - Partition of AAV capsids in tens of thousands of droplets.
  - Droplets with either a double stranded genome or a single stranded genome will be counted as positive.
  - ***The full and intermediate species are counted equally.***

qPCR to ddPCR Conversion Factor for Process A and Process B1

AAV Species	Genome	qPCR Response	ddPCR Response
Full Capsid	Double Stranded	1X Response	1X Response
Intermediate Capsid	Single Stranded	0.5X Response	1X Response

Process	%Full Capsid / %Intermediate Capsid	Titer by qPCR (defined as 1)	Titer by ddPCR (Conversion Factor)
A	0.4	1	2.5
B1	1.2	1	1.7

- The presence of intermediate capsid in scAAV products will cause the ddPCR titer to be off-set and higher than the qPCR titer
- The qPCR to ddPCR conversion factor is correlated to the ratio of %full and %intermediate capsids
- The qPCR to ddPCR conversion factor for Process A and Process B1 were empirically determined

- *Key objective for Process B2 development is a comparable ratio of %Full and %Intermediate Capsids relative to Process B1.*
- *Ratio of %Full and %Intermediate Capsids has potential impact on potency and titer.*

Process B2 Titer Control Strategy: Key Considerations

- FDA expectations for dosing based on nominal titer
- Structure-Function for Self-Complementary AAV: potency of full and intermediate capsids
- qPCR to ddPCR analytical assay change for titer: off-set correlated with the ratio of full and intermediate capsids
- ***Process B1 Control Strategy:***
  - ***Wide limits for titer***
  - ***Dosing based on measured titer***
- Benchmarking commercial AAV products

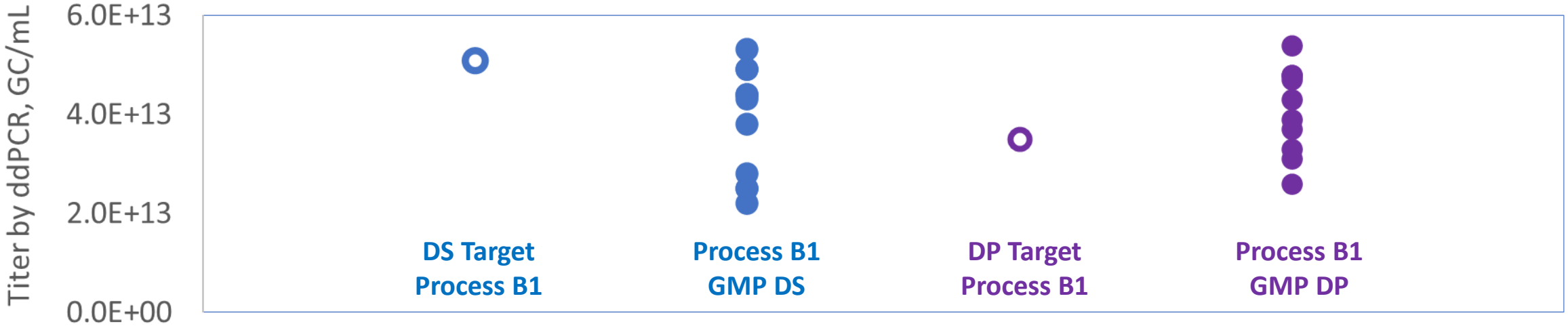
Process B1 Titer Control Strategy

Inputs into FTFF operations:

- FTFF Load (titer by qPCR and volume)
- qPCR to ddPCR conversion factor
- Target DS titer by ddPCR

Inputs into DP operations:

- DS (titer by ddPCR and volume)
- Target DP titer by ddPCR
- Optional dilution step (not performed for B1)



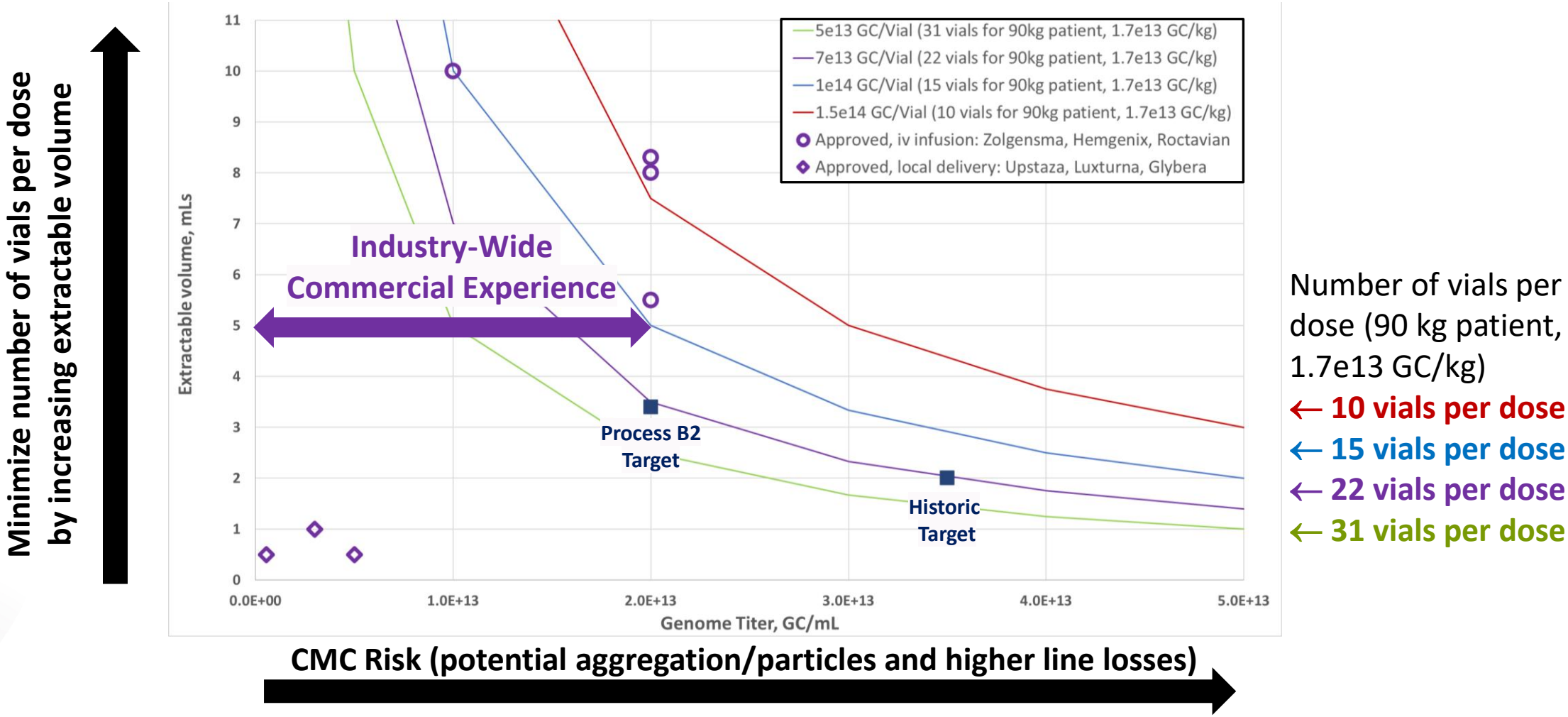
- Process B1: Wide Acceptance criteria for Titer (titer by ddPCR), limits not shown in graph
- Process B1: Dosed based on measured titer

ultragenyx

Process B2 Titer Control Strategy: Key Considerations

- FDA expectations for dosing based on nominal titer
- Structure-Function for Self-Complementary AAV: potency of full and intermediate capsids
- qPCR to ddPCR analytical assay change for titer: off-set correlated with the ratio of full and intermediate capsids
- Process B1 Control Strategy: qPCR as an IPC for formulation TFF and ddPCR as a release test
- ***Benchmarking Analysis: decrease target DP titer and increase DP extractable volume***

Benchmarking Drug Product Titer and Volume with Commercial iv Infusion GT Products



Number of vials per dose (90 kg patient, 1.7e13 GC/kg)

← **10 vials per dose**

← **15 vials per dose**

← **22 vials per dose**

← **31 vials per dose**

✓ ***Plan to stay within commercial experience for titer (≤2e13 GC/mL) to minimize CMC risk (aggregation/particles) and increase extractable volume to minimize vials per dose***

Process B2 Control Strategy for Titer

Process B2 will have comparable %Full and %Intermediate Capsids: impact on potency and qPCR to ddPCR conversion factor

Process B2 to be dosed based on nominal titer (ddPCR)

Process B2 will have narrow acceptance criteria for titer by ddPCR to enable dosing based on nominal titer

ultragenyx

Process B2 Control Strategy for Titer

Inputs into FTFF operations:

- **FTFF Load (titer by qPCR and volume)**
- **qPCR to ddPCR conversion factor (1.7)**
- **Target DS titer by ddPCR (5.1e13)**

Inputs into DP operations:

- **DS (titer by ddPCR and volume)**
- **Target DP titer by ddPCR (2.0e13)**
- **Dilution step**

- *Process B2 DS manufacturing: No planned changes to FTFF operations for titer control strategy*
- *Process B2 DS acceptance criteria: Set lower limit as target DP titer; same upper limit as for Process B1*
- *Process B2 DP manufacturing: Dilution step to achieve target DP titer (2.0e13 by ddPCR)*
- *Process B2 DP acceptance criteria: Narrow limits to enable dosing based on nominal titer*
- *Process B2 DP extractable volume: 3.4 mLs* to minimize number of vials per dose*
- *Process B2 DP potency acceptance criteria: to be evaluated*

*The company is evaluating the optimal extractable volume per vial.

ultragenyx

Summary

- **Late-stage process changes:**
  - New DS and DP manufacturing sites
  - New MCB/WCB
  - Introduction of viral clearance step
  - Optimize AEX collection procedure
  - New assay for DS/DP titer (qPCR to ddPCR)
  - Tighter acceptance criteria for DP titer to enable dosing by nominal titer
  - Proposed revision to target titer (titer by ddPCR) in QTPP
- **De-risking analytical comparability with:**
  - Product knowledge: structure-function studies, Potency versus %Full/% Intermediate Capsids
  - Process knowledge:
    - Minimize changes where possible: no changes to FTFF operations for titer control strategy
    - Optimize steps where needed:
      - AEX collection criteria for product quality (%Full and %Intermediate Capsids)
      - DP dilution step for tighter control of DP titer
  - Analytical method bridging studies to understand off-sets for in-process versus release methods
  - Benchmarking analysis for DP titers and volumes

ultragenyx

Acknowledgments

- Wei Zhang
- Xiaohui Lu
- Michael Alexa
- Kosta Kourembanas
- Hanson Zhou
- Lisa Pieri
- Megan Croshier
- Amy Medeiros
- Jack Ren
- Dainan Mao
- Rahul Manmode
- Kevin Gong
- Chao Huang
- Steve Stephanski
- Matt Marrichi
- James Warren
- Shannon Holmes

Q&A During Panel Discussion