Demonstrated comparability between adherent HEK293 and Sf9 baculovirus suspension AAVrh.10 clinical manufacturing processes

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Disclosures

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Background

An AAVrh.10 gene therapy vector for use in a Phase I/II clinical study was manufactured using an adherent HEK293 manufacturing platform (the **HEK293 process**)

To support late-stage clinical development and future commercialization, a scalable, cost-efficient, and high-yielding Sf9-baculovirus production system (the **Sf9 process**) was developed and implemented into GMP manufacturing (**Poster by Kalu N, et al. Abstract ID: 3231**)

Because this manufacturing shift occurred during ongoing clinical development, a comprehensive analytical comparability assessment was conducted to ensure that drug product quality, potency, safety, and overall molecular identity were comparable[1]

Here, we present key data from the analytical comparability assessment between the **HEK293 process** and the **Sf9 process**; the full comparability package was submitted to FDA

AAVrh.10, adeno-associated virus serotype rh.10; FDA, US Food and Drug Administration; GMP, good manufacturing practice.

1. FDA. Guidance document: manufacturing changes and comparability for human cellular and gene therapy products. Available from: https://www.fda.gov/regulatory-information/search-fda-guidance-documents/manufacturing-changes-and-comparability-human-cellular-and-gene-therapy-products (Accessed April 22, 2026).

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Study design and analytical comparability strategy (1)

- A 3X3 representative-lot matrix across the two processes was used
  - 3x **HEK293**-derived drug product lots and 3x Sf9-derived drug substance lots were evaluated
- Two of the three Sf9 lots included in the comparability evaluation were at the 10 L scale
  - Supporting studies showed that the 10 L (small) scale is representative of the 200 L (full) scale) (**Poster by Kalu N, et al. Abstract ID: 3231**)

Process	Lot number	Scale	Historical or planned use
HEK293	1	5 sub-lots*	Stability, clinical†
	2	10 sub-lots	Clinical
	3	4 sub-lots	Reference standard
Sf9	1	10 L bioreactor	Reference standard
	2	10 L bioreactor	Optional supportive stability
	3	200 L bioreactor	Reference standard; stability

*One sub-lot is equivalent to eight 10-layer culture chambers. †Lot 1 release and stability data could be used by Lexeo Therapeutics to establish comparability acceptance criteria and evaluate comparability from release test data.

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Study design and analytical comparability strategy (2)

- **HEK293**-derived drug product lots and Sf9-derived drug substance lots were evaluated for **strength**, **potency**, **infectivity, identity**, **purity**, **impurity**, and **structural characterization** attributes

Not all attributes were treated the same:

- Some were tested side by side
- Some leveraged qualified release data
- Some required platform-appropriate re-evaluation

TIER 1

CQA most directly linked to clinical safety and efficacy

Attribute	Analytical method
Vector genome concentration (strength)*	GOI-specific ddPCR/ qPCR
Infectious titer*	$TCID_{50}$ cell-based infectivity assay
Quantitative expression-based potency*	ICW transgene expression assay
Vector genome: infectivity ratio	Calculated from ddPCR and $TCID_{50}$

TIER 2

CQA providing orthogonal structural and purity information

Attribute	Analytical method
Capsid identity	Peptide mapping by LC-MS/MS
Capsid protein composition (VP1/VP2/VP3)	CE-SDS/SDS-PAGE
Full/empty capsid content	SV-AUC
Aggregates	SEC-HPLC
Residual host cell DNA	qPCR (platform-appropriate assay)
Residual HCP	ELISA/ MS-based characterization

TIER 3

CQA further evaluating characteristics specific to the gene therapy vector

Attribute	Analytical method
Vector genome sequence identity and integrity	NGS
Encapsidated DNA composition	NGS (insert vs contaminating DNA species)
Capsid intact mass	Native/intact mass spectrometry
PTMs	LC-MS/MS PTM analysis
Mechanism-based potency (exploratory)	*Under development*
VP ratio characterization	LC-MS–based capsid quantitation

NGS data were presented on **Thursday, May 14**, by **Timothy Fenn.** Title: **Integrating sequencing into product release and comparability testing**

*Attributes tested side by side.

CE-SDS, capillary electrophoresis-sodium dodecyl sulfate; CQA, critical quality attribute; ddPCR, droplet digital polymerase chain reaction; ELISA, enzyme-linked immunosorbent assay; GOI, gene of interest; HCP, host cell protein; ICW, in-cell western; LC-MS/MS, liquid chromatography-tandem mass spectrometry; MS, mass spectrometry; NGS, next-generation sequencing; PTM, post-translational modification; qPCR, quantitative polymerase chain reaction; SDS-PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis; SEC-HPLC, size exclusion chromatography-high performance liquid chromatography; SV-AUC, sedimentation velocity analytical ultracentrifugation; $TCID_{50}$, 50% tissue culture infectious dose; VP, viral protein.

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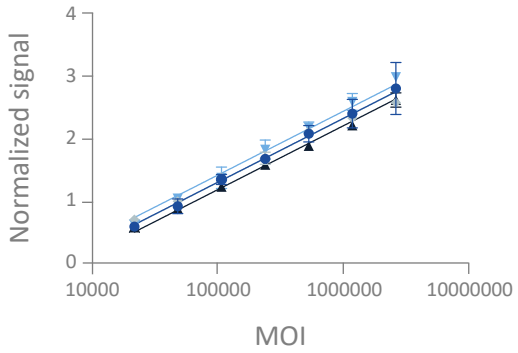
Expression-based potency demonstrated strong comparability between the HEK293 and Sf9 processes

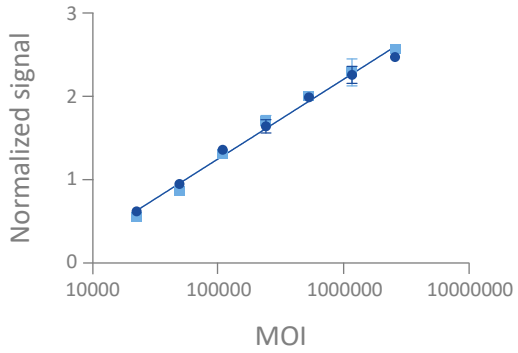
Relative potency by ICW

Plate 1



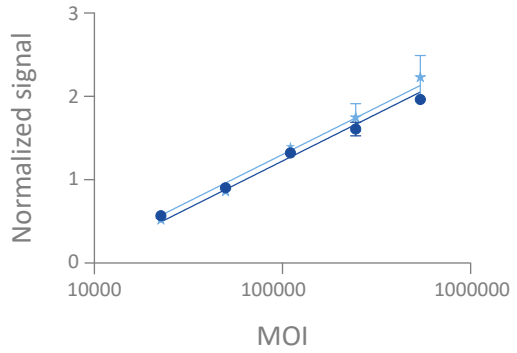
HEK293 process lot 3 (RS) — HEK293 process lot 2 — HEK293 process lot 1 — Sf9 process lot 1

Plate 2a



HEK293 process lot 3 (RS) — Sf9 process lot 2

Plate 2b



HEK293 process lot 3 (RS) — Sf9 process lot 3

- Acceptance criterion*: $\bar{x}_{(Sf9)} = \leq \bar{x}_{(HEK293)} \pm 3\sigma_{(HEK293)}$
- All lots met the predefined ICW acceptance criteria, with average relative potency of:
  - 92.0% for **HEK293**
  - 115.6% for Sf9
- Relative to **HEK293**, Sf9 process materials demonstrated **overlapping dose response** and **comparable relative potency**
- These data supported **no** *in vitro* **changes** in protein expression between the processes

*Predefined and agency aligned
MOI, multiplicity-of-infection; RS, reference standard

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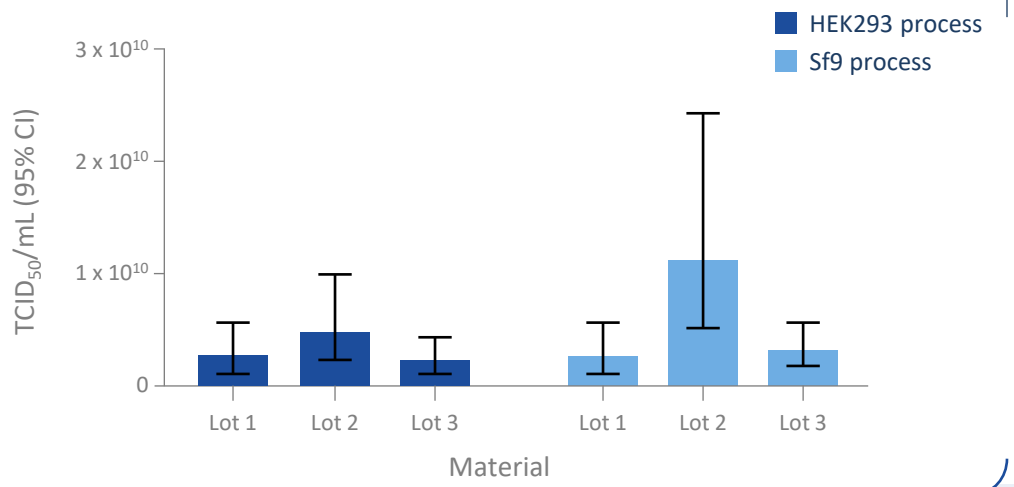
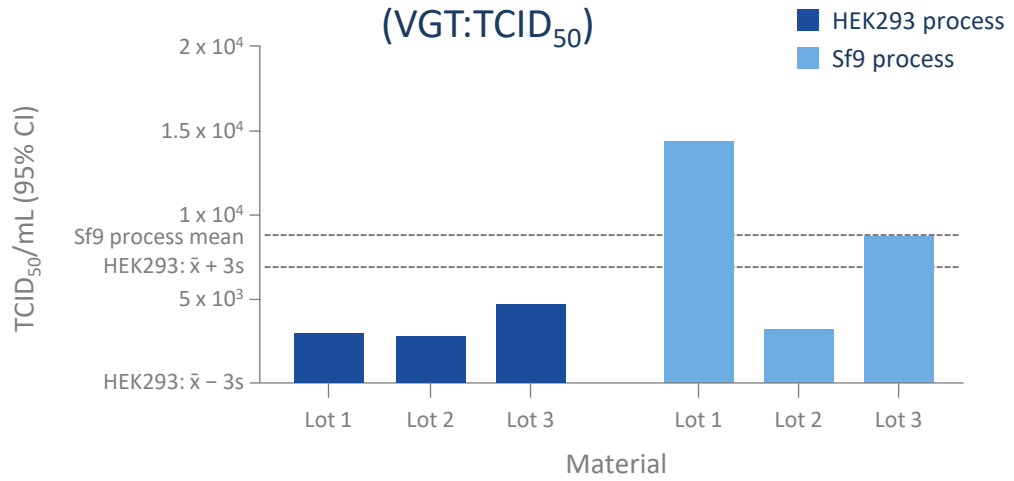
Functional infectivity was comparable between the HEK293 and Sf9 processes

Infectious titer



- Acceptance criterion*: $\bar{x}_{(Sf9)} = \leq \bar{x}_{(HEK293)} \pm 3\sigma_{(HEK293)}$
- Infectious titers were comparable across the **HEK293** and Sf9 processes
- After transfer of the test method to a new vendor, infectivity testing was performed in a side-by-side format

Infectivity ratio (VGT:$TCID_{50}$)



- Acceptance criterion*: $\bar{x}_{(Sf9)} = \leq \bar{x}_{(HEK293)} \pm 3\sigma_{(HEK293)}$
- Mean VGT:$TCID_{50}$ for the Sf9 process exceeded the predefined acceptance criterion
  - However, range of variation between the **HEK293** and Sf9 processes overlapped (p=0.2327; Welch's t-test)
  - Despite the known method variation and limited data points, these data indicate no differences in product quality between the **HEK293** and Sf9 processes

*Predefined and agency aligned.
CI, confidence interval; VGT, viral genome titer.

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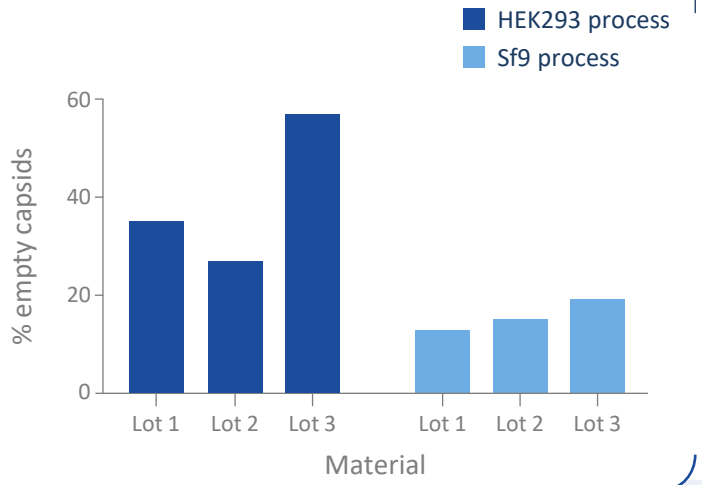
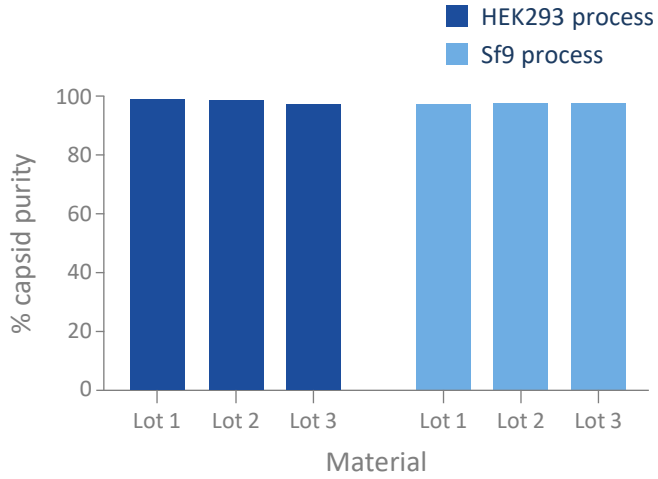
The Sf9 process exhibited lower average empty capsids compared with the HEK293 process

Empty capsids*



Capsid purity



VP capsid protein ratios (VP1:VP2:VP3)

Lot number	HEK293 process (normalized to VP3)	Sf9 process (normalized to VP3)
1	2.6: 4.2: 10.0	2.0: 1.4: 10.0
2	2.7: 4.3: 10.0	2.2: 1.5: 10.0
3	2.9: 4.9: 10.0	2.0: 1.4: 10.0

- Acceptance criterion†: **6–40% empty capsids**
- All **Sf9** process lots were within acceptance criterion range
- Compared with **HEK293**, **Sf9** process materials showed comparable or lower empty capsids (mean 39.5% vs 15.7% empty)
- For purity attributes, capsid purity and VP1 ratios were highly consistent across **HEK293** and **Sf9** processes

*Triplicate sedimentation coefficient distributions are plotted, and each capsid species is calculated from the average of the integrated area below the sedimentation range.
†Predefined and agency aligned.

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Conclusion

Strength, potency, infectivity, identity, structural integrity, purity, and reduced impurity burden of the material generated from the **Sf9** process were analytically comparable to the **HEK293** process, with no adverse impact to quality or safety

The improved scalability, higher yield, and impurity profile of the **Sf9** process support its use for ongoing clinical development and future commercial supply, without the need for dose conversion

These data collectively justify the manufacturing platform transition and provide a robust foundation for uninterrupted clinical supply

The full comparability package was submitted to FDA, which reviewed and accepted the conclusions, thereby enabling late-stage clinical development

Taken together, these data demonstrate how risk-based analytics, orthogonal characterization, and hypothesis-driven design can enable manufacturing evolution during clinical development

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