# Efficient, safe, and cost-effective production of potent AAV vectors using whole plants as bioreactors.

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CIRSIUM BIOSCIENCES

# Gene Therapy's Manufacturing Demand Challenge

"If (GTx manufacturers) can't **streamline manufacturing**.....gene therapies will become **increasingly inaccessible** to the **patients that rely on them**."

*- FDA CBER Director Peter Marks*

**Example** - Cost of 1 dose of Lenmeldy - $4.25 million and Hemgenix - $3.5 million
Cost of manufacturing 1 dose of Hemgenix is ~1-2 million

*DeGroot, L. (2023, November 6). "Pull every lever": Marks doubles down on urgency to improve gene therapy manufacturing. Endpoints News. https://endpts.com/pull-every-levermarks-doubles-down-on-urgency-to-improve-gene-therapy-manufacturing/)*

*Hosseini, M., Fath, S. ., Hosseini, M., & Fath, S. . (2023, July 6). Cutting the cost of gene therapy manufacturing. Roland Berger. https://www.rolandberger.com/en/Insights/Publications/Cutting-the-cost-of-genetherapy-manufacturing.html*

# Gene Therapy's Manufacturing Demand Challenge

## 2023 Bioreactor Capacity Demand Soared to 2 Billion Liters, **While the Average Batch Size Remained at Just 500L**

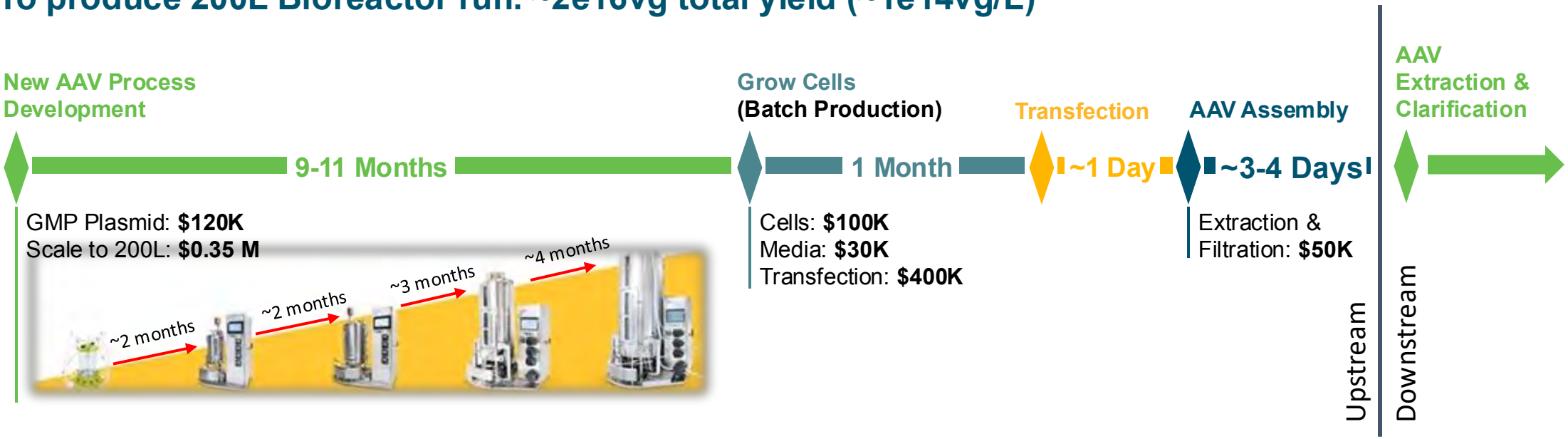
2 Billion Liters = ~800 Olympic swimming pools

500L Bioreactor

Source: Market research reports (LEK, McKinsey, 360i Research, Facts and Factors, Mordor Intelligence, Grandview Research)

# Gene Therapy's Manufacturing Demand Challenge

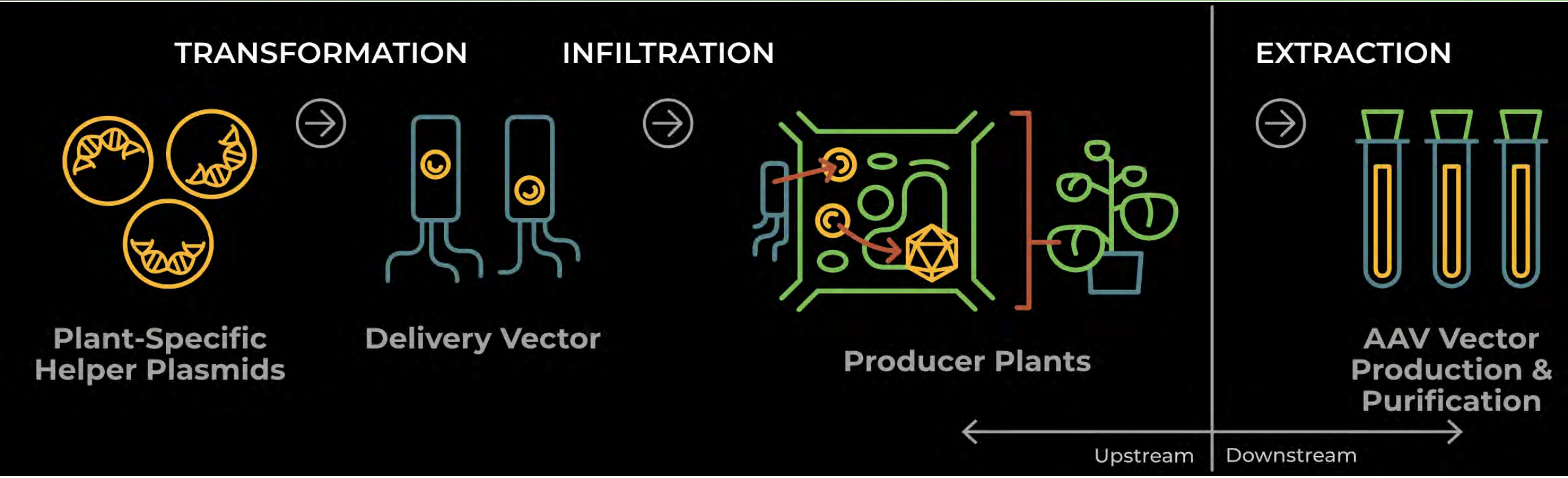
## To produce 200L Bioreactor run. ~2e16vg total yield (~1e14vg/L)




Upstream Process:
**10-12 Months For ~$1.1M**

Lyle et al., 2023 *Biotechnology Bioengineering* 2023;1-14.
DOI: 10.1002/bit.28402

# Cirsium's Plant-Based Solutions



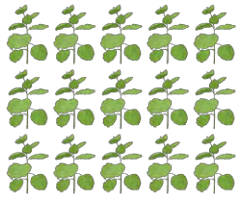

**With cutting-edge production technology, we utilize transient gene delivery in plants and high-density Controlled Environment Agriculture (CEA) to manufacture high-quality AAV vectors for genetic medicine.**

# Cirsium's Plant-Based Solutions

## Produce ~2e16vg of GMP Compatible Crude – eq. 200L Bioreactor Run (~1e14vg/L)

**New AAV Process Development** — 6-8 Weeks

Non-GMP Plasmids in MCB and WCB: **$5K**
Grow plants: **$15K**



**10-15 plants**
~1-2e14vg yield
1L bioreactor equivalent
~10x Upstream cost reduction

**Infiltration** (Batch Production) — < 1 Week

Grow Bacteria: **$1K**
Infiltration: **$1K**
*Ability to maintain standing capacity of plants to infiltrate every day

**AAV Assembly** — ~3-4 Days

Extraction & Filtration: **$50K**

**x 2,000**

**2000 plants**
~1-2e16vg yield
200L bioreactor equivalent
>10x Upstream cost reduction

**AAV Extraction & Clarification**

Upstream

Downstream

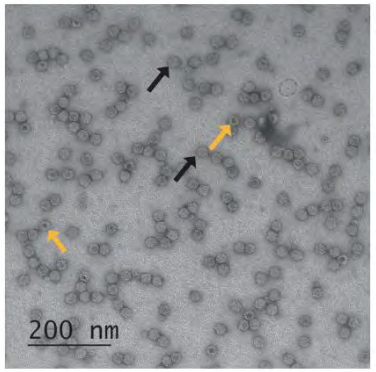
**Upstream Process:**
**2 Months For ~$72K**

# Plant-Produced AAVs Have Comparable Quality to HEK293T Cell-Produced AAVs

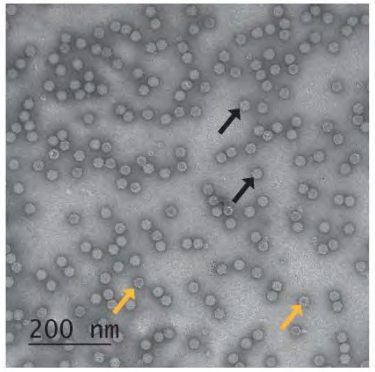
| Properties | Plant-AAV9-CMV-GFP | Mammalian-AAV9-CMV-GFP | Validation |
|---|---|---|---|
| % Full particles in the crude lysate | 31.87% | 29.62% | ELISA (in house) |
| Genomic titer (vg/mL) (post purification) | 2.5e12 vg/mL (qPCR)<br>2.5e12 vg/mL (dPCR) | 2.5e12 vg/mL (qPCR)<br>2.6e12 vg/mL (dPCR) | qPCR (in house)<br>dPCR(outsourced) |
| Endotoxin levels <USP 85> | <0.05EU/mL (Pass) | <0.05EU/mL (Pass) | LAL chromogenic test (outsourced) |
| Bacteriostasis and Fungistasis <USP 71> | Successfully validated for Sterility testing | Successfully validated for Sterility testing | Direct Transfer (outsourced) |
| Sterility Test <USP 71> | No growth observed (Pass) | No growth observed (Pass) | Direct Transfer (outsourced) |
| Mycoplasma Test | No mycoplasma detected | No mycoplasma detected | PCR based (in house)<br>Culture (outsourced) |

# High titer/quality AAV vectors can be produced by using plant based AAV manufacturing

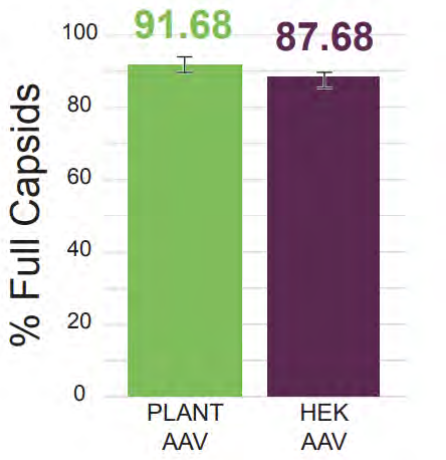
**HEK293-Produced AAV2-CMV-EGFP**




**Plant-Produced AAV2-CMV-EGFP**

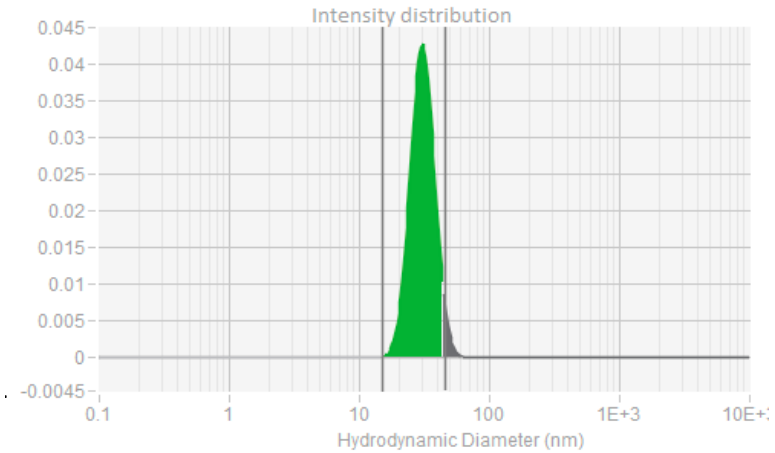



↗ = full ↗ = empty



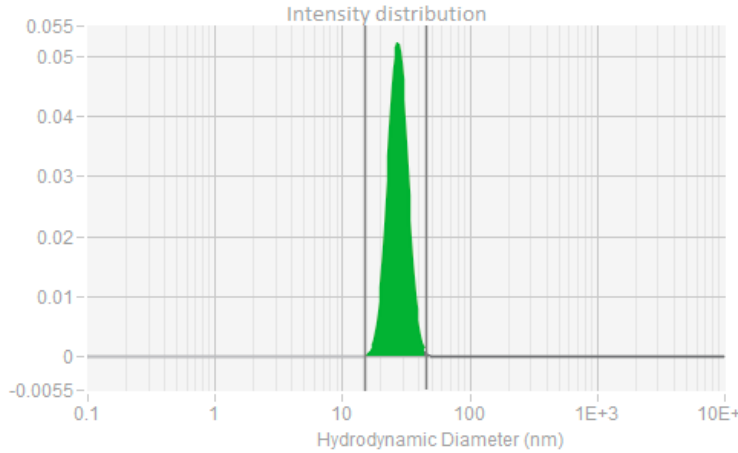

TEM Processing and Imaging performed by Salk Biophotonics Core Facility

Plant-AAV9




| Results | | |
|---|---|---|
| Aggregate intensity | 3.4 | % |
| Capsid intensity | 96.6 | % |
| Small particle intensity | 0 | % |
| Capsid diameter | 30.4 | nm |
| Total capsid titer | 2.56E+12 | cp/ml |

HEK-AAV9




| Results | | |
|---|---|---|
| Aggregate intensity | 0.2 | % |
| Capsid intensity | 99.8 | % |
| Small particle intensity | 0 | % |
| Capsid diameter | 27.0 | nm |
| Total capsid titer | 2.64E+12 | cp/ml |

**Unchained Labs Stunner data:**

- PDI is <0.1 for both material, suggesting no aggregation
- DLS shows comparable capsid diameter
- Capsid titer - ~2.5e12/mL

# Study design for comparability testing of plant-AAV with HEK293-AAV in non-human primates (NHP)

**Groups:** Vehicle (n=1)
Plant-AAV (n=3)
HEK293-AAV (n=3)

**RoA:** Intravenous (2.5e12vg/kg)
Intrathecal (2.5e12vg)

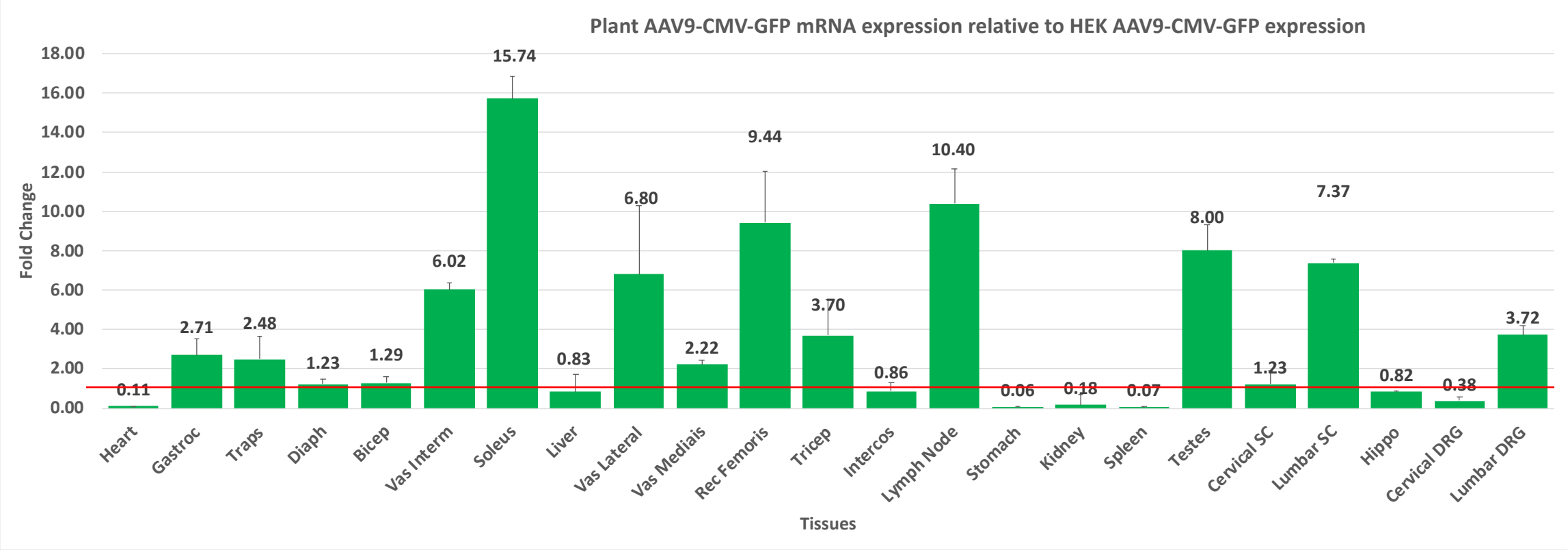
**In-life:** 45days

All animals had no/low AAV9-Nab at the time of dosing.

**Table 1: Animal Recruitment Summary**

| Group | Animal ID | Treatment | Route | Dose Volume | Dose (vg/kg) | Baseline Body Weight (kg) |
|---|---|---|---|---|---|---|
| 1 | E128 | Vehicle | IT | 1 mL/kg | $2.5 \times 10^{12}$ | 5.61 |
| | | | IV | 1 mL | | |
| 2 | E129 | PLANT-AAV9-CMV-EGFP | IT | 1 mL/kg | $2.5 \times 10^{12}$ | 5.44 |
| | | | IV | 1 mL | | |
| | E153 | | IT | 1 mL/kg | $2.5 \times 10^{12}$ | 6.34 |
| | | | IV | 1 mL | | |
| | E352 | | IT | 1 mL/kg | $2.5 \times 10^{12}$ | 6.11 |
| | | | IV | 1 mL | | |
| 3 | E402 | HEK293-AAV9-CMV-EGFP | IT | 1 mL/kg | $2.5 \times 10^{12}$ | 5.33 |
| | | | IV | 1 mL | | |
| | E409 | | IT | 1 mL/kg | $2.5 \times 10^{12}$ | 5.63 |
| | | | IV | 1 mL | | |
| | E413 | | IT | 1 mL/kg | $2.5 \times 10^{12}$ | 6.04 |
| | | | IV | 1 mL | | |

# AAV9-CMV GFP mRNA expression data in NHP tissue by qRT-PCR: fold change of plant AAV9 to HEK AAV9



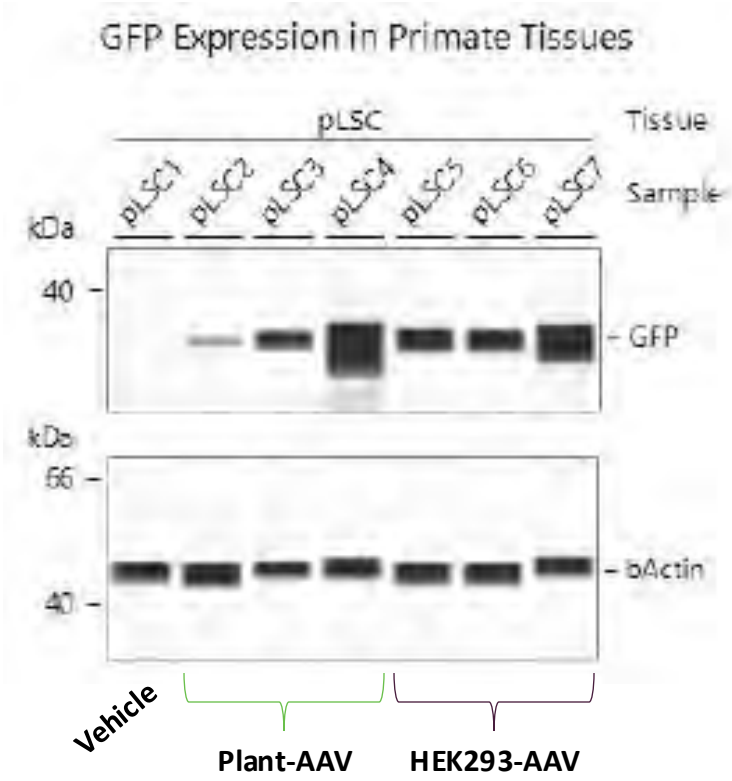

- Quantification of GFP expression across tissue samples indicates higher transgene expression for plant AAV9 vs HEK AAV9 in most skeletal muscles and lumber spinal cord, lymph nodes and testes.

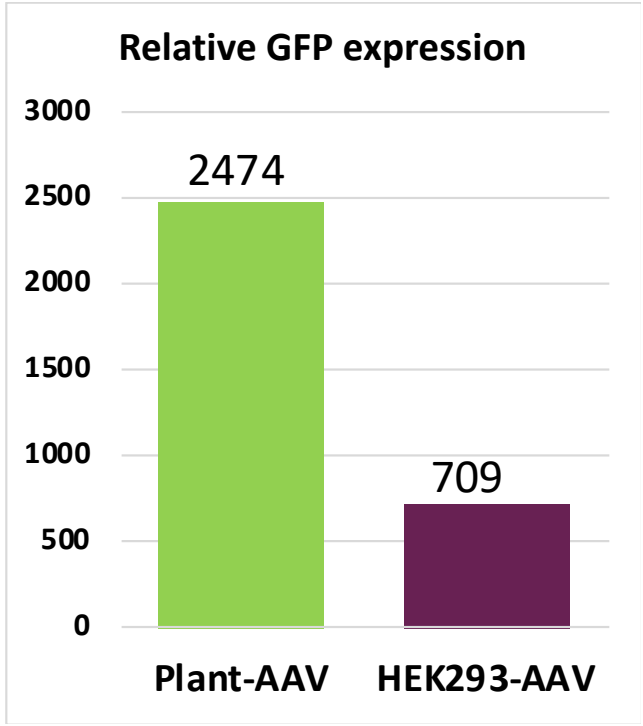
# Increased/comparable production of GFP Protein by plant-AAV relative to HEK293-AAV in the lumbar spinal cord tissue (NHP)

**Tissue:** Lumbar Spinal Cord

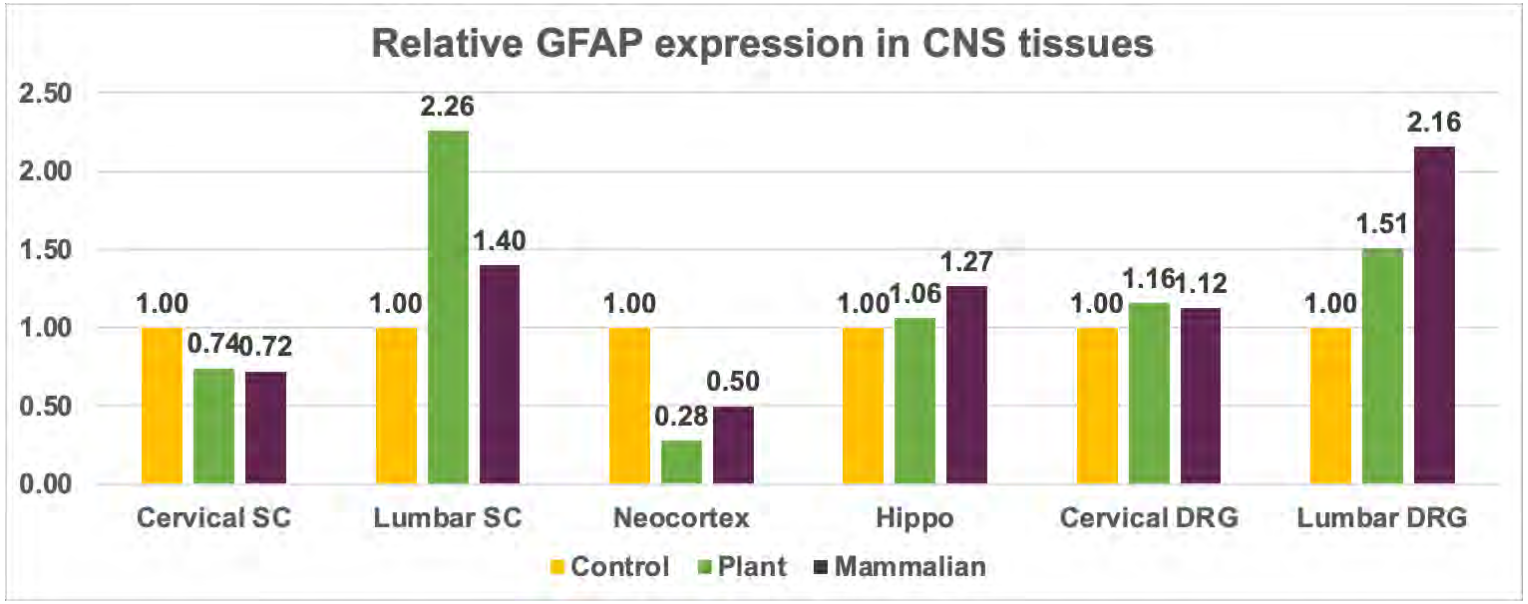
**Groups:** Vehicle (n=1)
Plant-AAV (n=3)
HEK293-AAV (n=3)

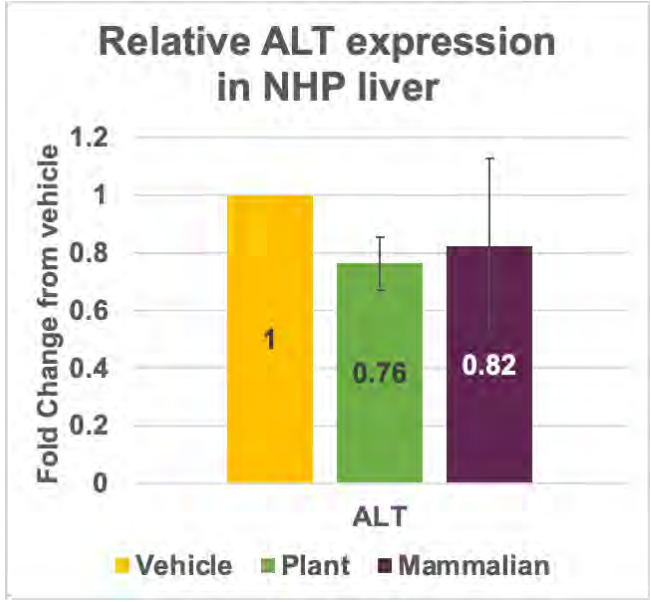
**In-life:** 45days



# Assessment of inflammatory markers GFAP and ALT in NHP tissues demonstrates excellent tolerability of plant AAV9



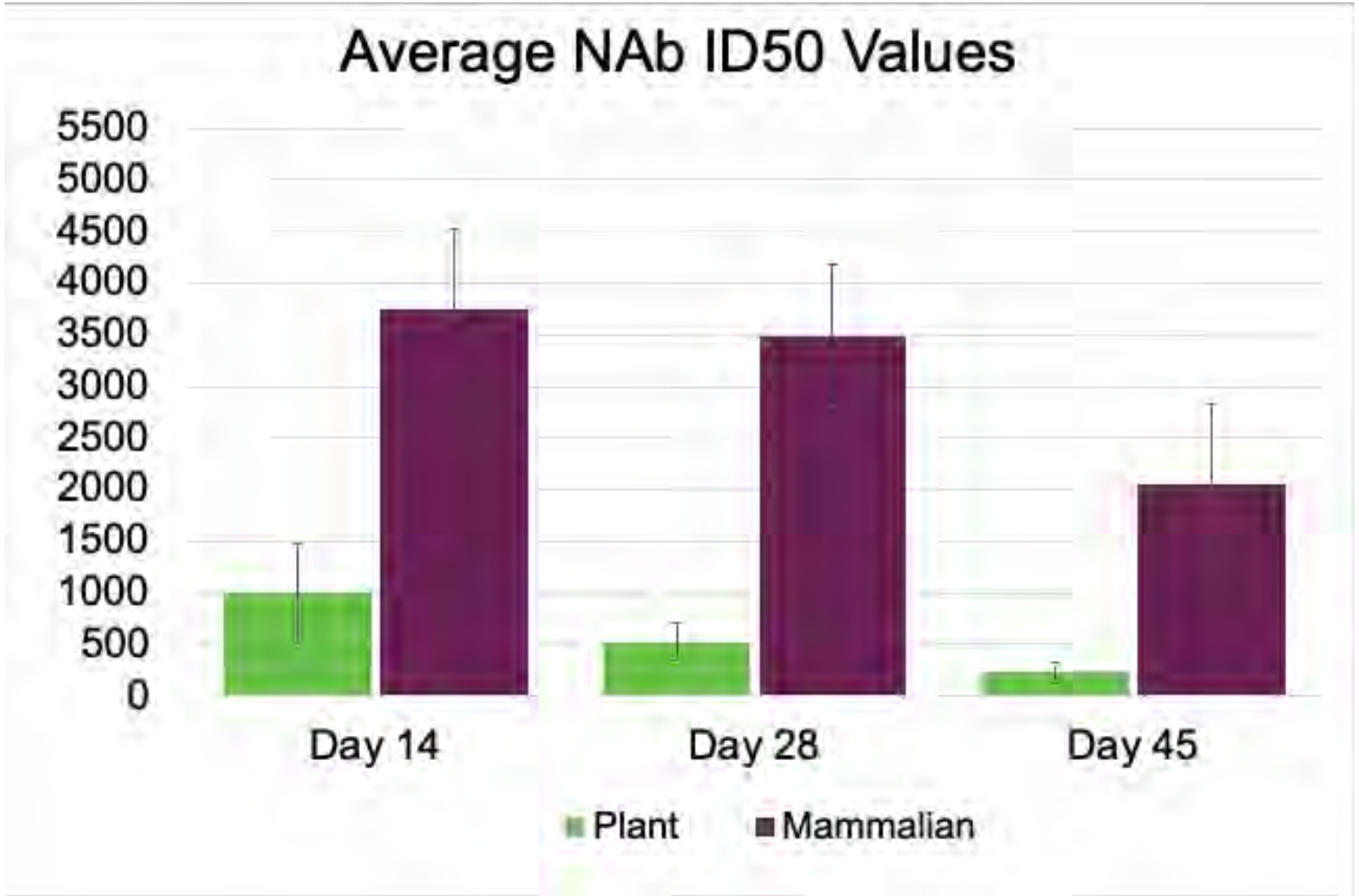




**Quantification of relative GFAP** expression, a marker of glial reactivity, indicates no significant difference in both treatment groups from vehicle only control group

**Quantification of relative ALT** expression, a marker of inflammatory liver damage, also indicated no significant difference in both treatment groups compared to vehicle only

**Conclusion**: Both plant and HEK produced AAV9-CMV-GFP vectors were well tolerated in the CNS and liver in treated NHPs

# Neutralizing Ab data (Luciferase assay) indicates significantly reduced antibody response to Plant-AAVs, compared to the HEK-AAVs



# Summary Slide: Plants are the Answer to Gene Manufacturing

- Manufacturing AAVs in plants can reduce the cost of manufacturing by >10X.
- Since plant produced AAVs are linearly scalable, it can significantly reduce the process development times.
- Since downstream processing for plant produced AAVs is identical to existing mammalian DSP, this platform can be easily adopted.
- Plant produced AAVs are high quality and pass the QC requirements.
- Plant produced AAVs can transduce the correct cell types in non-human primates and show comparable biodistribution as that of mammalian cell produced AAVs.

## Contact

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