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Development and optimisation of an upstream manufacturing process for IKAR-001: a bicistronic AAV8 gene therapy for geographic atrophy

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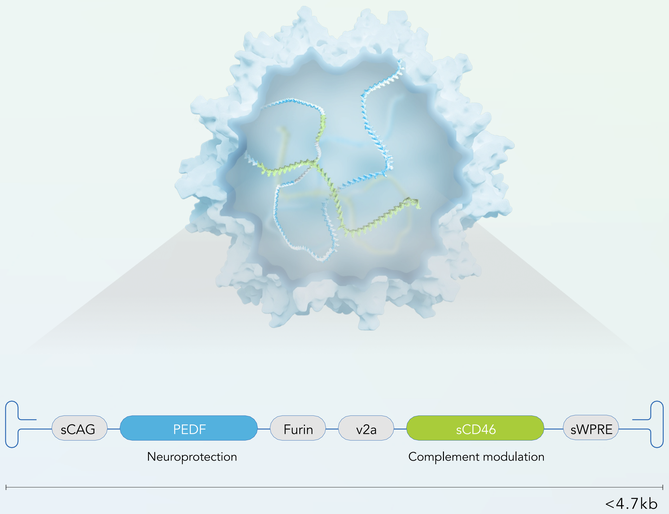
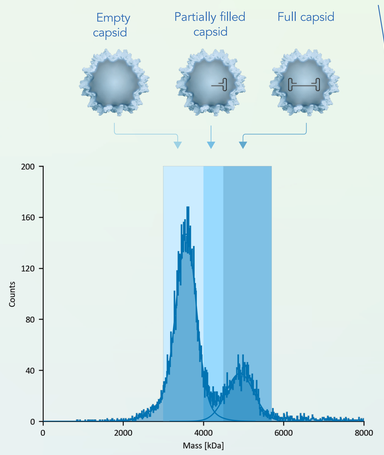
IKAR-001 is a bicistronic AAV gene therapy that co-expresses the complement-regulatory protein, CD46, and the neuroprotective protein pigment epithelium-derived factor (PEDF) for the treatment of geographic atrophy (GA). Platform AAV upstream process (USP) conditions often yield low-quality AAV product that complicates process scale-up to GMP. Here we present a rational, data-driven approach that enabled systematic in-house optimisation of upstream AAV production for IKAR-001

1 IKAR-001: A bicistronic AAV8 for GA



2 AAV manufacturing is intrinsically heterogenous

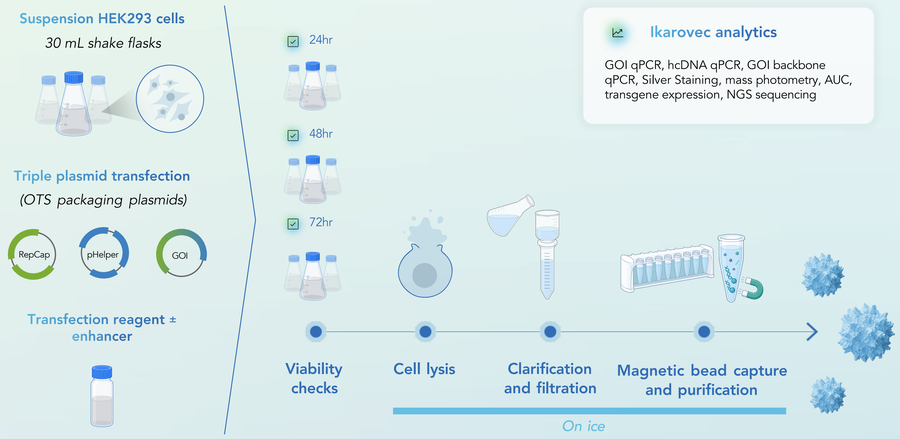


Conditions tested

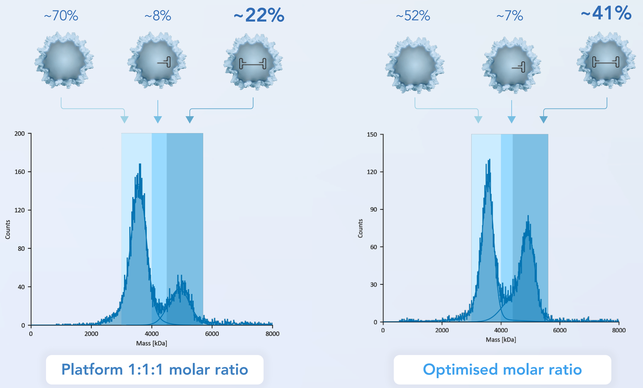
1. Transfection reagent selection
2. Plasmid molar ratio DoE
3. Addition of enhancers
4. VCD : pDNA DoE
5. Transfection reagent: pDNA DoE
6. Packaging plasmid selection

3 In-house USP optimisation workflow

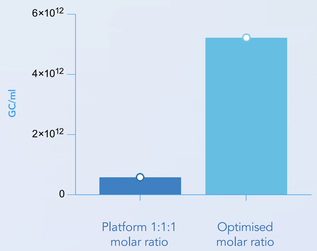
A screening approach to optimise the upstream IKAR-001 production was performed using suspension HEK293 cells in 125 mL shake flasks and a transient triple-plasmid transfection workflow. Vector product quality was then assessed for genomic titre (GOI-specific qPCR), encapsidation of impurities (qPCR), and empty: full ratio (mass photometry/AUC).

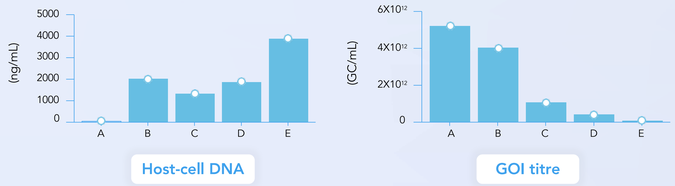


4 IKAR-001: A bicistronic AAV8 for GA



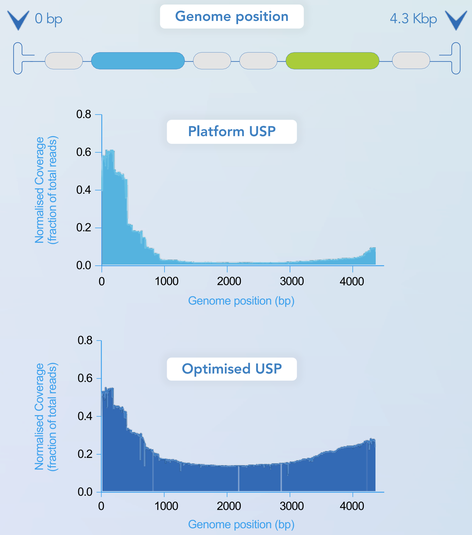
DoE-like studies identified a plasmid molar ratio that increased full capsid ratio and GOI titre in the absence of enhancers.





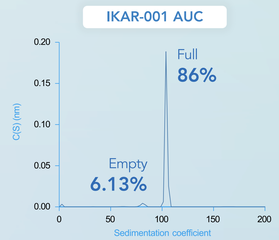
Packaging plasmid system A was selected to minimize encapsidation of impurities such as host-cell DNA, while maximising GOI titre.

5 Optimised USP conditions improve full-length transgene encapsidation



Long-read NGS coverage plots show 35% improvement in full-length transcript encapsidation following USP optimisation.

4 Scalable bicistronic AAV suspension process achieved



PEDF ddPCR (GC/mL)
2.60E+13

sCD46 ddPCR (GC/mL)
2.41E+13

- Successful removal of empty capsid population via two-step AEX process
- Full capsid population >85% at pilot scale
- Qualified multiplex ddPCR assay demonstrates comparable titres based on either transgene

7 Summary

- Systematic evaluation of USP conditions in-house provides a time- and cost-efficient approach to enhance AAV scalability to a GMP process.
- IKAR-001 product quality attributes were improved by optimising plasmid molar ratios and packaging plasmid system specifically.
- In-house USP was successfully technically transferred to a global CDMO.
- Global CDMO developed and optimised a two-step AEX downstream process to produce IKAR-001 at bioreactor scale with excellent critical quality attributes.

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