Building the future of gene therapies together

Few batches, big questions: Navigating AAV analytical comparability

A CDMO built on decades of expertise in CGT, vaccines & immunotherapies

Ensuring a top-quality science led-service and deep understanding of product commercialization

Company creation to address challenges in competency & quality of AAV manufacturing

Set out to build a true end-to-end science-led CDMO by consolidating key talent and expertise in AAV development

Delivering service through established teams that have walked the path from bench to the clinic and beyond.

Acquisition of Freeline CMC team & facility following official launch after a >$130M Series A fundraise

- Pioneering AAV team having carried 3× clinical products
- Disruptive & scalable AAV manufacturing platform & IP
- Leading process & analytical development expertise

Acquisition of Beacon GMP facility and industry leading GMP manufacturing & quality team

- Cutting-edge newly licensed site for Phase I / II / III trials
- Highly experienced team in late-stage development

Merger with ABL Inc., bringing 30+ years manufacturing track-record & cross-modality expertise

- Immediately available early-phase GMP capacity
- End-to-end offering including Fill / Finish capabilities
- Broad modality experience, including vaccines, oncolytic viruses, immunotherapies and cell and gene therapy

ABINGWORTH · Monograph · PCC Bio · Petrichor Healthcare Capital Management · CATHAY HEALTH · 4BIO CAPITAL · AJINOMOTO · DIGITALIS · DEERFIELD · EW Healthcare Partners

Our global footprint enables tailored support across all stages of development

Ensuring a best-in-class service from pre-clinical development to commercial manufacturing

AAV Centre of Excellence & EMA GMP QC Hub
Munich, Bavaria - Germany

≈ 40 FTE

- 58'000 sq. ft
- Acquired Q2 2023
- Biosafety Level 1
- 0x GMP Suites

ascend *Capabilities*

- Process & Analytical Development
- Formulation Development
- Technology & Plasmid Innovation
- Non-GMP Manufacturing
- Suspension Systems – up to 200L)
- Adherent Systems – 8x iCELLis Nano
- GMP QC (EMA Jurisdiction)

Currently hold open capacity for pre-clinical development up to toxicology & GMP QC

Early-Phase GMP Centre of Excellence
Rockville, Maryland – United States of America

≈ 90 FTE

- 71'000 sq. ft
- Acquired Q2 2024
- Biosafety Level 2
- 6x GMP Suites

ABL inc. *Capabilities*

- Process & Analytical Development
- GMP Manufacturing – up to Phase II
- Multi-Product Compliant Facility
- Suspension Systems – up to 2x200L
- Adherent Systems – iCELLis Nano / 500
- Fill / Finish – up to Phase II
- GMP QC (FDA Jurisdiction)

Currently hold open GMP capacity available and F/F slots as of **Q1 2025**

Late-Phase GMP Centre of Excellence
Alachua, Florida – United State of America

≈ 70 FTE

- 50'000 sq. ft
- Acquired Q4 2024
- Biosafety Level 2+
- 7x GMP Suites

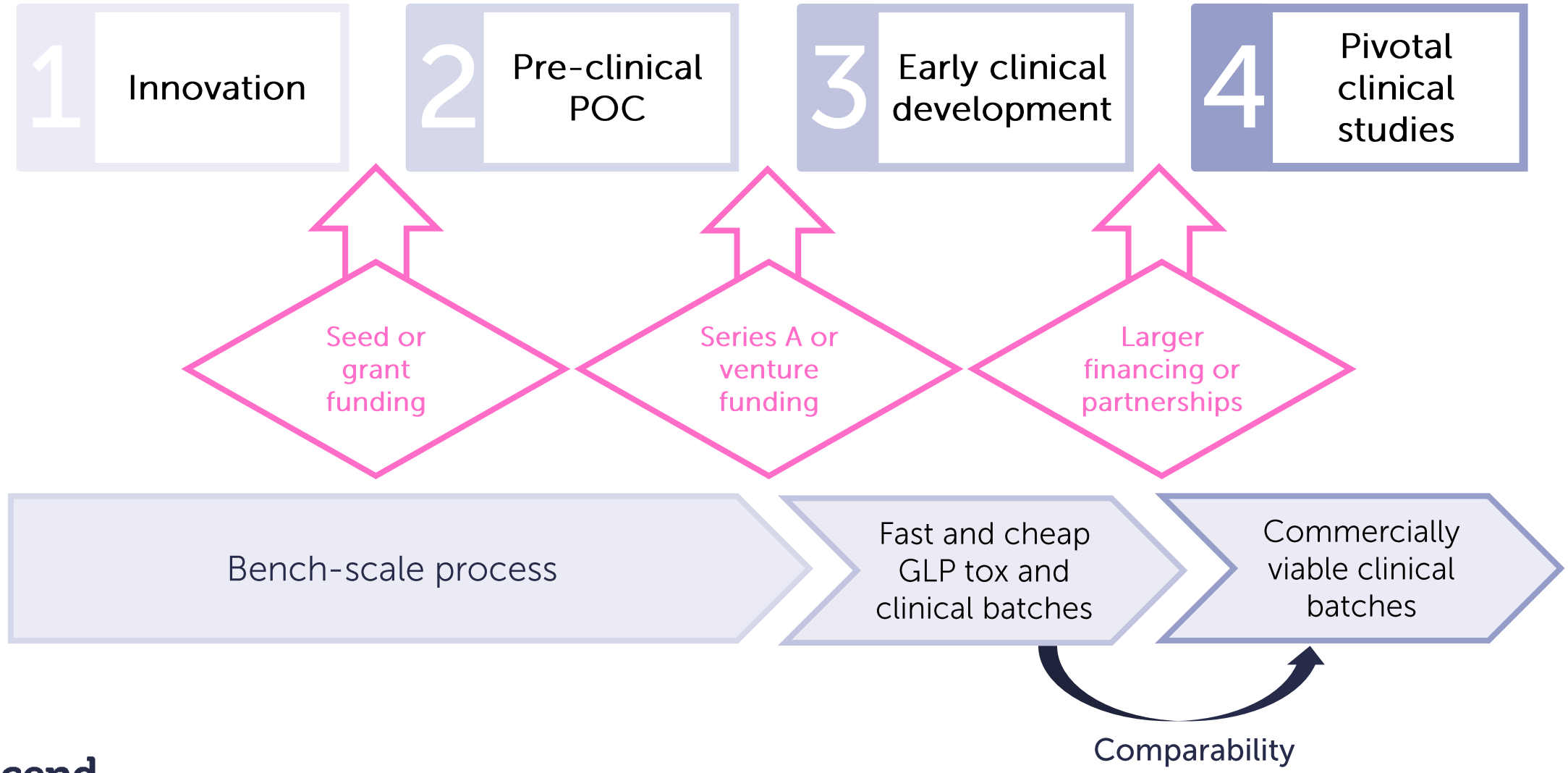
ascend *Capabilities*

- Process & Analytical Development
- GMP Manufacturing – up to Phase III and Commercial Ready **Q3 2025**
- Multi-Product Compliant Facility
- Suspension Systems – up to 200L
- Fill / Finish – Commercial Ready **Q1 2026**
- GMP QC (FDA Jurisdiction)

Currently manufacturing Ph3 Material in parallel to facility expansion – earliest slot **Q4 2025**

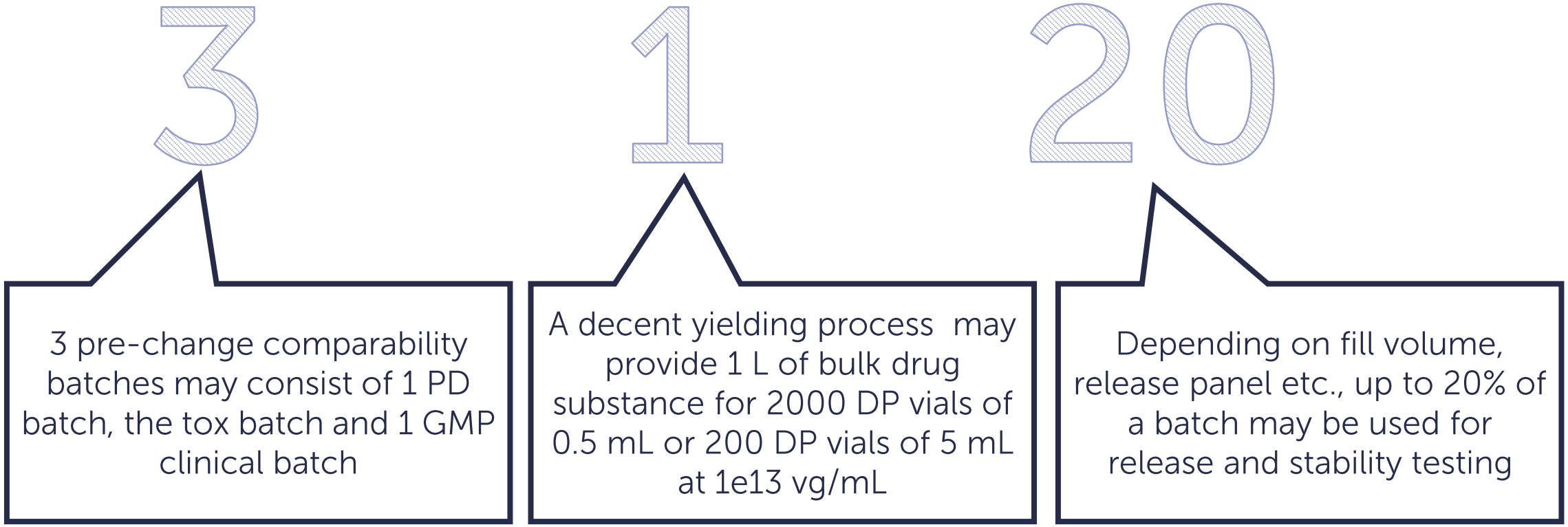
Comparability is inevitable in the development lifecycle for AAV products

Funding milestones drive the need for process changes in late clinical development



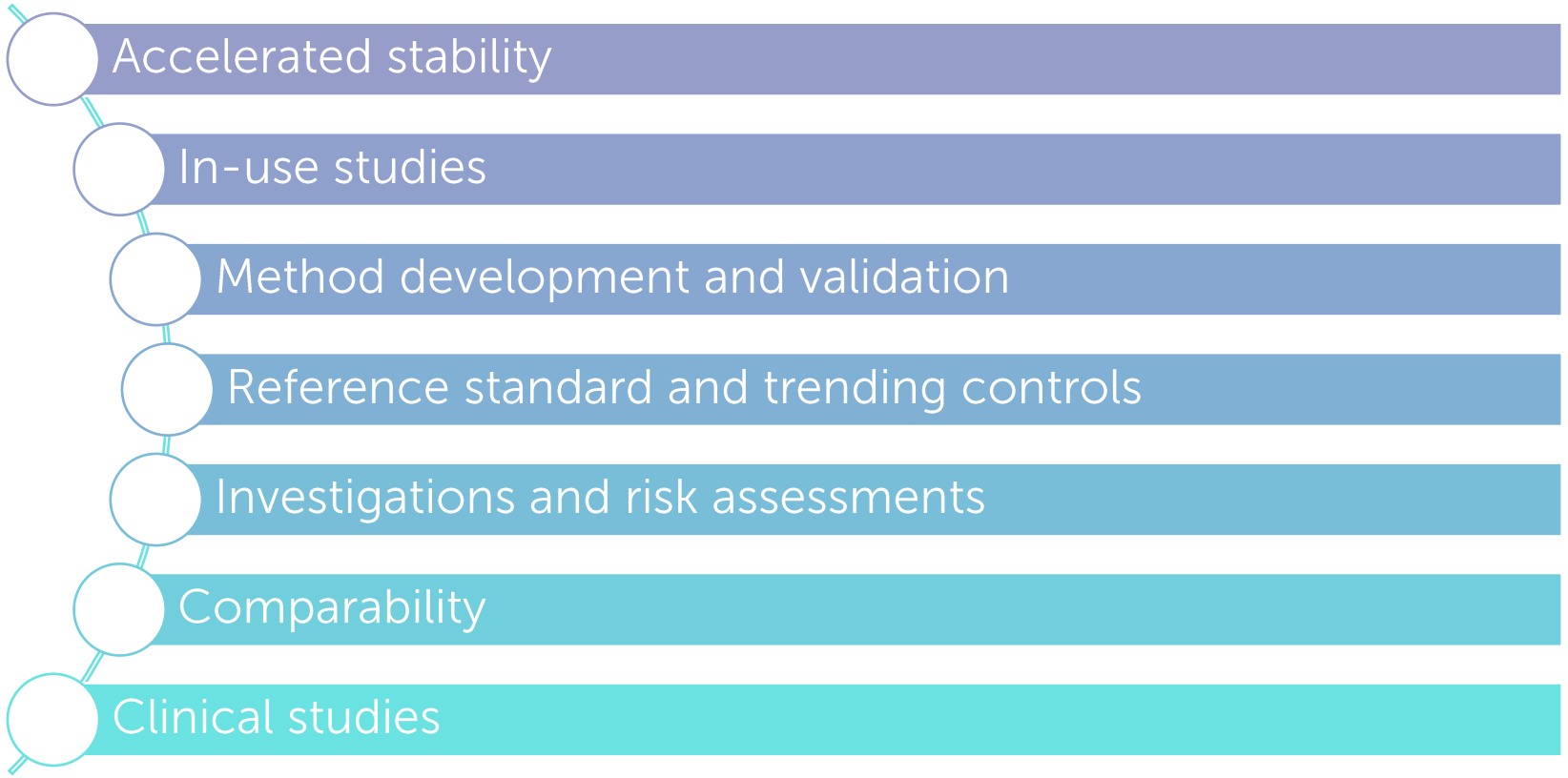
Early development is often done with a few small batches

Availability of AAV material by the numbers



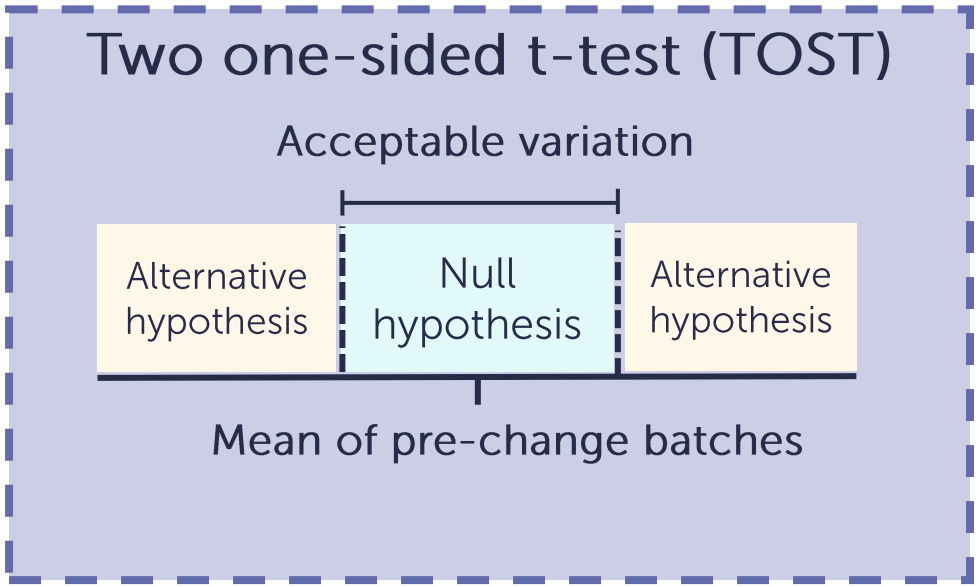
There are many competing demands on AAV material

Many studies need to be performed during early clinical development

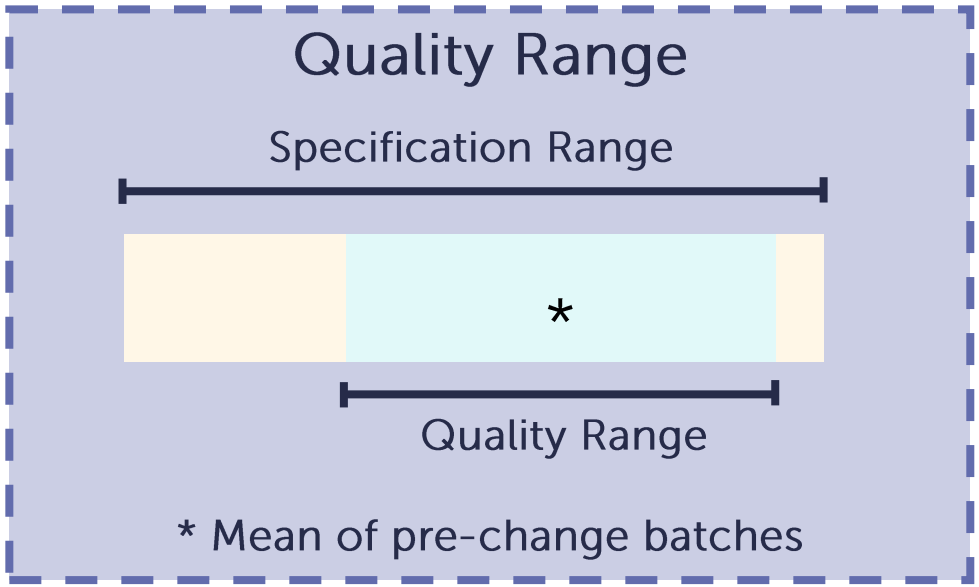


Statistical methods should be used to demonstrate comparability

The criticality assessment of each CQA helps guide the statistical method(s) chosen



Highest criticality

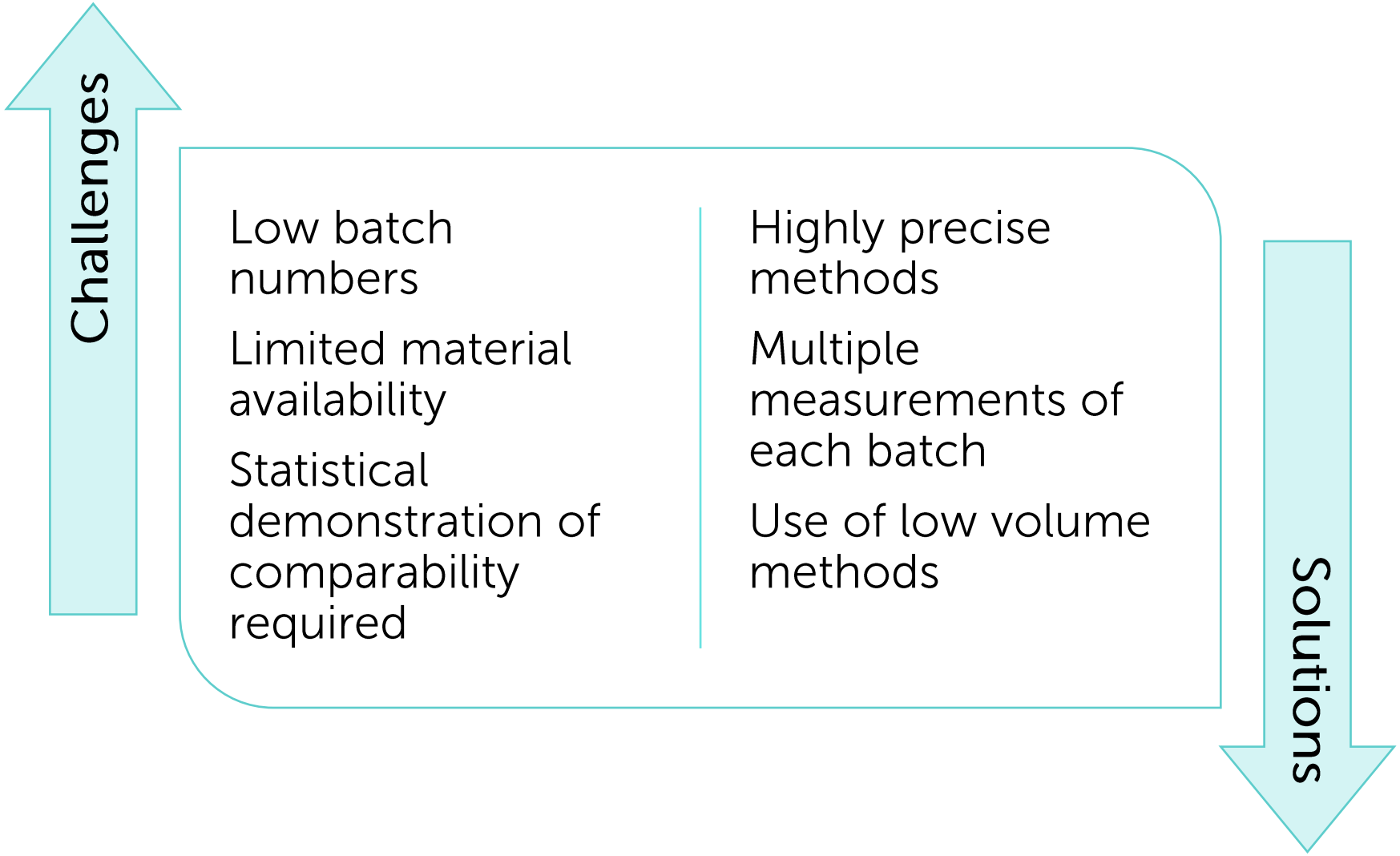


Mid-level criticality

Statistical methods can be applied with low batch numbers if analytical methods are sufficiently precise

AAV presents significant challenges to comparability

Solutions to those challenges include using better methods which use less product



Precise methods are required to statistically demonstrate comparability

Industry guidance emphasises the need for appropriate analytical methods

"The battery of tests for the comparability exercise should be carefully selected and optimised to maximise the potential for detecting relevant differences in the quality attributes"

ICH Q5E

"The manufacturer's ability to establish sensitive and validated assays for characterizing the product and biological activity and to evaluate the significance of differences noted in such assays can provide the basis for FDA to assess product comparability without the necessity of repeating clinical efficacy studies."

"Statistical methods should be applied to determine whether observed differences in quality attributes are statistically significant and whether they impact safety or efficacy."

U.S. Food and Drug Administration (FDA). (1996). Guidance for Industry: Demonstration of Comparability of Human Biological Products, Including Therapeutic Biotechnology-Derived Products.

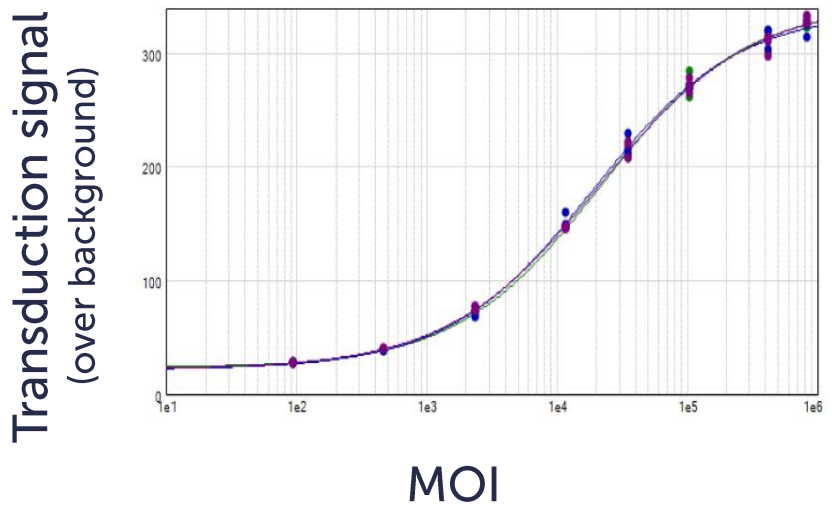
Excellent precision can be achieved for cell-based potency assays

All assay steps are optimized to minimize variability

Transduction of cells at various MOIs → Supernatant tested for protein activity (colorimetric assay) & protein amount (ELISA)

MOI	Average % CV of triplicates
8.40E+05	6.1
4.20E+05	6.0
1.05E+05	4.2
3.50E+04	4.4
1.17E+04	6.0
2.33E+03	10.0
4.67E+02	10.3
9.33E+01	14.9

6%
Intermediate Precision

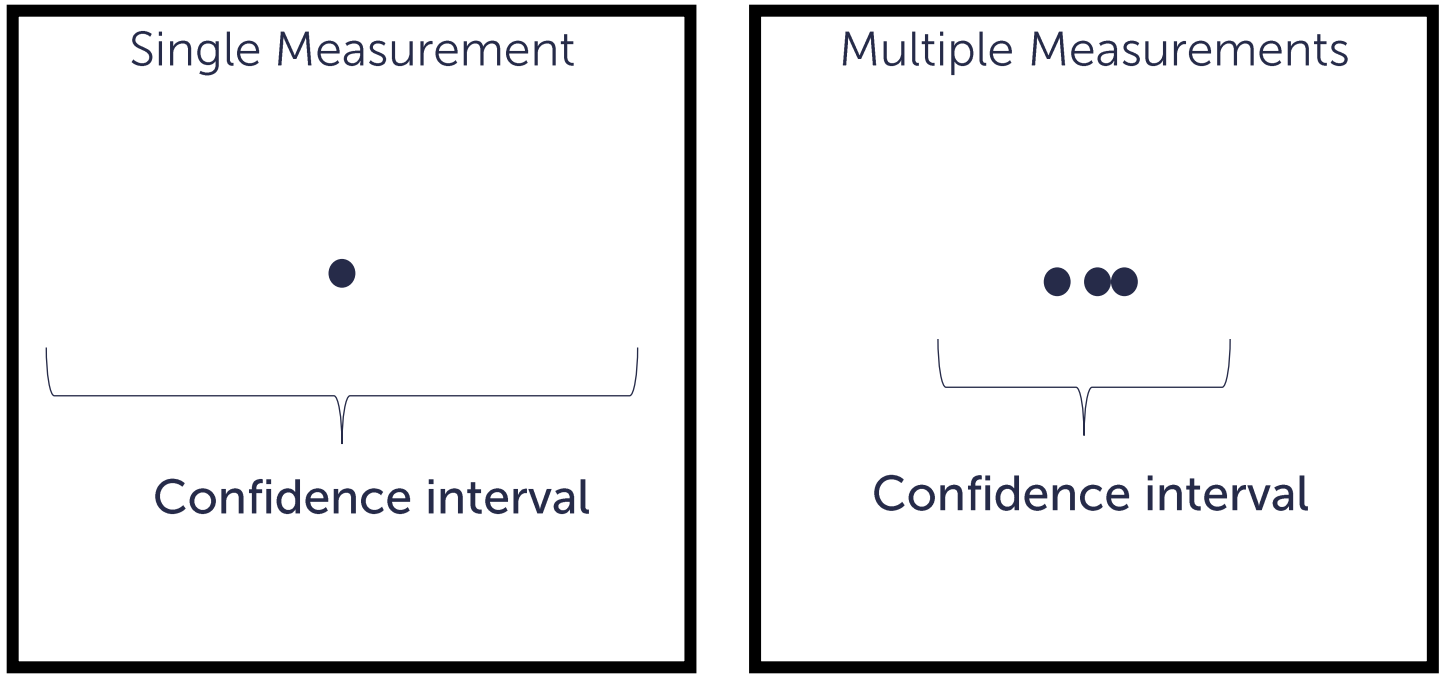


Optimal Precision Achieved by:

1. Optimising all aspects of cell culture and transduction conditions
2. Training, training, training

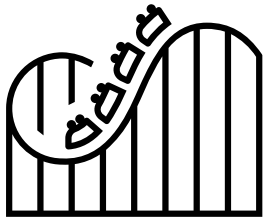
Further precision can be achieved by performing multiple measurements

Muliple measurements must be averaged and treated statistically as one batch

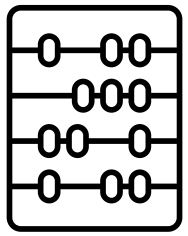


Low volume analytical methods enable more data to be generated

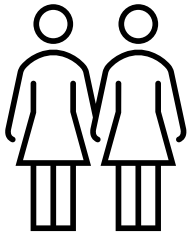
Comparability requires large amounts of data



Big changes mean many potential CQAs impacted and **many assays** included in comparability



Multiple repeats per assay may be required for statistical demonstration of comparability

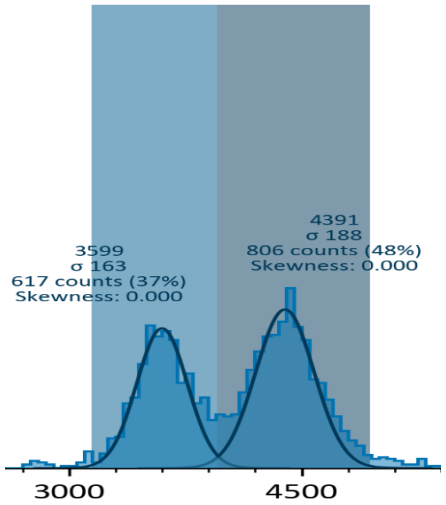


Side-by-side testing of pre- and post-change batches is recommended

Percentage of full capsids as a key CQA for AAV comparability

Mass photometry can lead to more than 40x sample savings compared to AUC

>40x sample savings

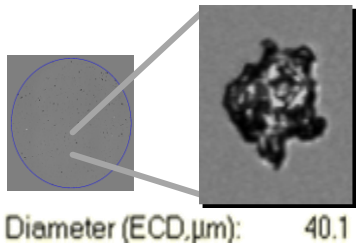


- ✓ Typically < 10 µL needed, compared to 400 µL/replicate on AUC
- ✓ 21CFR Part 11 compliant software, method suitable for validation/release testing
- ✓ Guidance on use and validation included in the British Pharmacopoeia

Subvisible particle testing typically requires large volumes

Backgrounded membrane imaging can lead to about 30x sample savings compared to light obscuration

~30x sample savings



- ✓ Typically ~ 150 µL required, compared to approx. 5 mL for light obscuration
- ✓ Particle quantification in the size range from 2 µm to 5 mm
- ✓ 21CFR Part 11 compliant software, method suitable for validation/release testing

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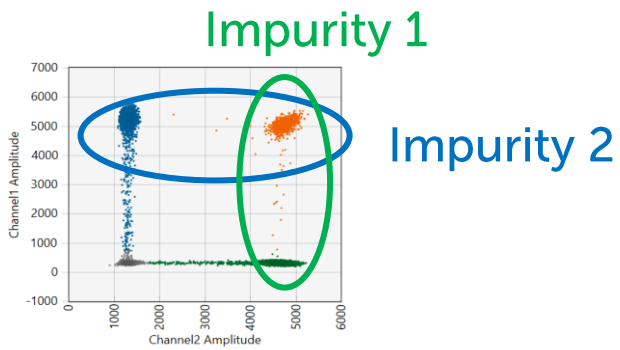
ddPCR is used for many quality attributes, including titer and DNA impurities

Duplex ddPCR can halve the amount of sample required compared to singleplex ddPCR

2x sample savings



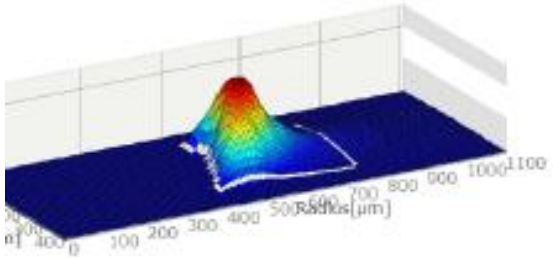
*** Validated at Ascend ***

- ✓ A single assay can quantify multiple impurities, limiting assay time and sample use
- ✓ 21CFR Part 11 compliant software, method suitable for validation/release testing

Immunoassays are used to monitor process impurities and capsid titer

Gyros immunoassays can lead to about 4x sample savings compared to conventional ELISA

~4x sample savings



*** Validated at Ascend ***

- ✓ For e.g. HEK293 HCP ELISA, 12 µL diluted sample are required as opposed to 50 µL for conventional ELISA
- ✓ 21CFR Part 11 compliant software, method suitable for validation/release testing
- ✓ Process residual assays (HCP, nuclease, residual column ligand) and capsid titer assays available

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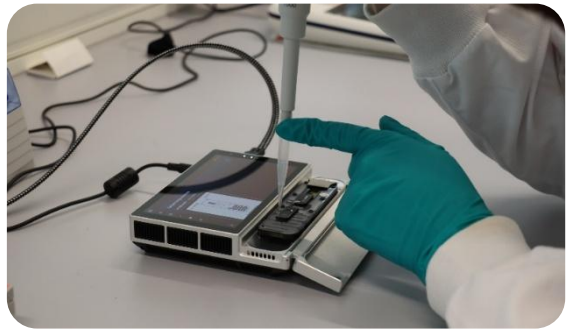
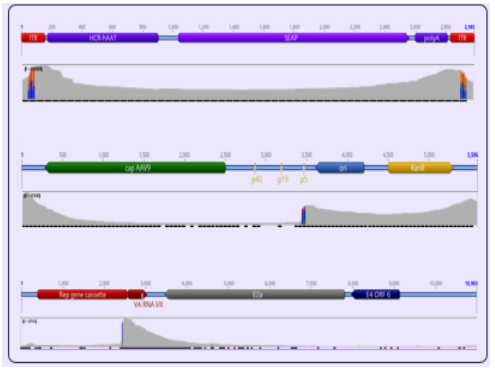
Long read NGS sequencing is a powerful characterisation tool for AAV

Nanopore sequencing uses about 10x less sample than PacBio

~10x sample savings





- ✓ Approx. 1e12 vg needed for Nanopore vs. 1E13 vg for PacBio
- ✓ 21CFR Part 11 compliant software
- ✓ Vector genome identity
- ✓ Extended characterisation of the vector genome integrity and DNA impurities (plasmid and host cell)

Dunker-Seidler F. et al. *Mol Ther Methods Clin Dev.* 2025 Jan 22;33(1):101417. doi:10.1016/j.omtm.2025.101417

Low volume methods significantly reduce use of AAV material

Enables multiple measurements and side-by-side testing with less sample than conventional methods

6.5 mL

Conventional release panel

- AUC for full/empty
- Light obscuration for subvisible particles
- 2x ddPCR for plasmid impurities
- 3x ELISA for process residuals
- ELISA for capsid titer

Extended characterisation

- PacBio for vector genome integrity and DNA impurities

330 µL

Adapted release panel

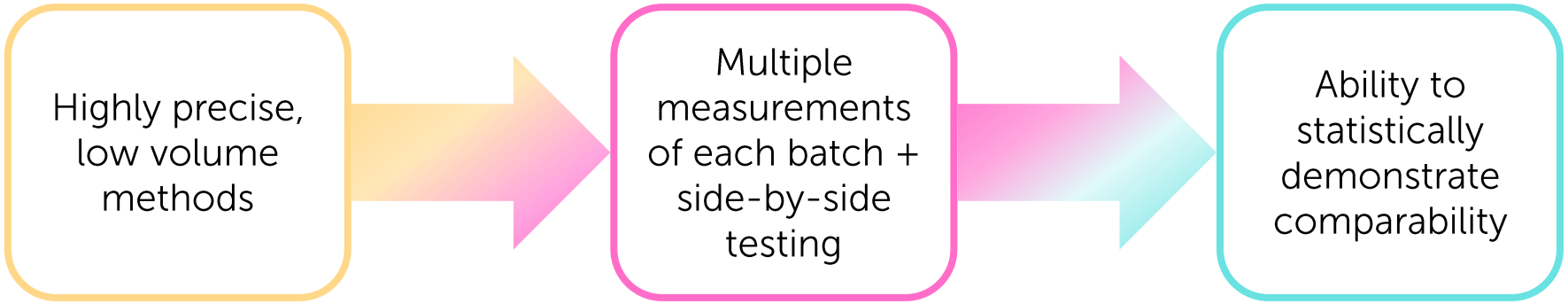
- Mass photometry for full/empty
- Backgrounded membrane imaging for subvisible particles
- 1x duplex ddPCR for plasmid impurities
- 3x Gyros for process residuals
- Gyros for capsid titer

Extended characterisation

- Nanopore NGS for vector genome integrity and DNA impurities

Few, small batches are a surmountable problem in AAV comparability

Highly precise low volume methods are significant assets



Thank you for your attention

Feel free to contact me at sonya.schermann@ascend-adv.com