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Journey to Commercialisation: CMC Comparability Strategy for a Lentiviral Vector-Transduced Autologous $CD34^+$ Cell Therapy in Rare Disease

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PrimeRA Pharma Partners is proud to have collaborated with Fondazione Telethon on the regulatory development of Waskyra™, supporting its path to marketing authorisation in both the EU and the US. This collaboration reflects our shared commitment to turning innovative gene therapies into approved treatments for patients with rare diseases. We value our close working relationship with Fondazione Telethon and the opportunity to contribute regulatory and CMC expertise to this milestone in gene therapy development.

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Waskyra™: the first cell-based gene therapy for Wiskott–Aldrich syndrome

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- **Waskyra™ (etuvetidigene autotemcel)** is indicated for paediatric patients aged six months and older and adults with Wiskott–Aldrich syndrome (WAS) who have a mutation in the *WAS* gene, for whom haematopoietic stem cell transplantation (HSCT) is appropriate, and for whom no suitable HLA-matched related stem cell donor is available.
- **Waskyra™ received EU EU CHMP Positive Opinion and US biologics licence approval in 2025** through the Collaboration on Gene Therapies Global Pilot.
- **Development of Waskyra™ began in 2008**, when the first WAS lentiviral vector (LVV) was generated; the first patient was treated in a clinical trial in 2010. The nearly two-decade journey involved three sponsors spanning academia, industry and charitable foundations.
- **As a rare-disease therapy, Waskyra™ had a small clinical manufacturing history:** only 34 drug product batches were manufactured over 16 years of clinical development, despite extensive process changes to both the WAS LVV and the cellular drug product.
- **The programme navigated a rapidly evolving regulatory landscape from 2008 to 2025**, facing increasingly stringent expectations for CMC development, process characterisation, analytical comparability, manufacturing consistency and long-term safety as it progressed from early clinical development to commercial approval.

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Incremental clinical optimisation, then commercial-readiness changes to DS and DP

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- Approximately 37 process changes were introduced and justified across the lentiviral vector (LVV), drug substance (DS) and drug product (DP) manufacturing processes during clinical development.

Lentiviral vector (LVV)

Two manufacturing scales, 6 process versions, 23 changes in total:
Cell expansion, raw materials and reagents, introduction of downstream purification, harvest collection, sterile filtration, scale-up and formulation

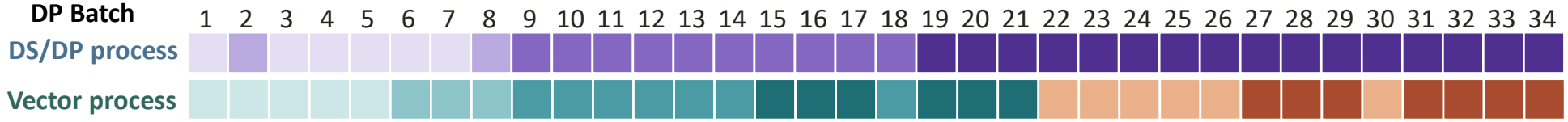
Comparability read-outs
Vector CQAs: titre, impurities, safety and stability, plus DS/DP CQAs

Drug substance & drug product (DS/DP)

4 process versions, 14 changes in total:
DS: raw materials, cellular source, cell enrichment process and storage conditions
DP: additional washing, formulation, excipient source, filling and container closure, and manufacturing site

Comparability read-outs
DS CQAs: viability, purity, cell growth and expansion, vector copy number, transduction efficiency, and clinical outcomes

- Of the 34 DP batches submitted for review, only 7 (21%) were made with the commercial process, which made process consistency and comparability harder to demonstrate across the development history.



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Risk-based impact assessment determines the evidence needed for each change

- As a programme moves towards licensure, the cost of a non-comparable change rises and regulatory flexibility narrows.
- Risk also depends on how directly a change can affect the final drug product.
- Several small changes can add up to a significant cumulative risk.

Example changes in Waskyra™	Phase	Assessed impact	Evidence generated
New plasmid suppliers for LVV	Pivotal	LVV starting material; low impact on all CQAs	CoA comparison and justification, with small-scale LVV manufacturing
Human serum albumin source for DP formulation	Pivotal	Excipient change; medium impact	Small-scale healthy-donor (HD) side-by-side studies and DP stability (long-term and in-use)
Cellular source change (BM → mPB) for DS manufacturing	Phase II	Key source material; medium impact	HD split-batch comparison, retrospective clinical batch comparison and statistical analysis
Fresh to cryopreserved DP, with manufacturing site change	Phase II	Major formulation change; high impact	Non-clinical (NSG, in vitro), split HD batches, 3-way clinical batch comparison and clinical follow-up
New LVV MCB and manufacturing room change	Post-PPQ / commercial	Medium impact	1 PPQ-confirmatory LVV batch + 1 successful DP batch

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Five layered evidence types, escalating from analytical data to clinical outcomes

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1	**Retrospective impact assessment**	Early clinical phase: each change scored against pre-PPQ CQAs, leveraging platform knowledge from similar products
2	**Historical GMP batch analysis**	Release data trended across process versions, with statistical analysis to justify equivalence in early development
3	**Prospective side-by-side studies**	Classic approach, e.g. split healthy-donor designs to remove donor variability; fresh vs cryopreserved DP from one mPB collection at two sites
4	**Stability comparability**	Usually part of prospective comparability: regression/ANCOVA for stability trending, or predefined acceptance criteria for in-use stability
5	**Clinical bridging**	When prospective comparability is inconclusive or not feasible, e.g. up to 4-year follow-up for formulation and cell source changes

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Reliable analytical methods, especially potency assays, are the cornerstone of comparability

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Analytical method

- Reliable, fit-for-purpose evidence across identity, purity, safety and potency
- Assess method changes and bridge legacy results

Functional Potency Assay

- Mechanism-relevant biological activity
- Links product quality to clinical outcome

Statistical Analysis

- Is the observed difference meaningful?
- Predefine margins, endpoints and analysis plan
- Quantify variability, uncertainty and trends

Comparability Assessment

- Analytical reliability
- Biological relevance
- Quantitative interpretation

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A quantitative, MOA-relevant potency measure is essential to a credible comparability conclusion

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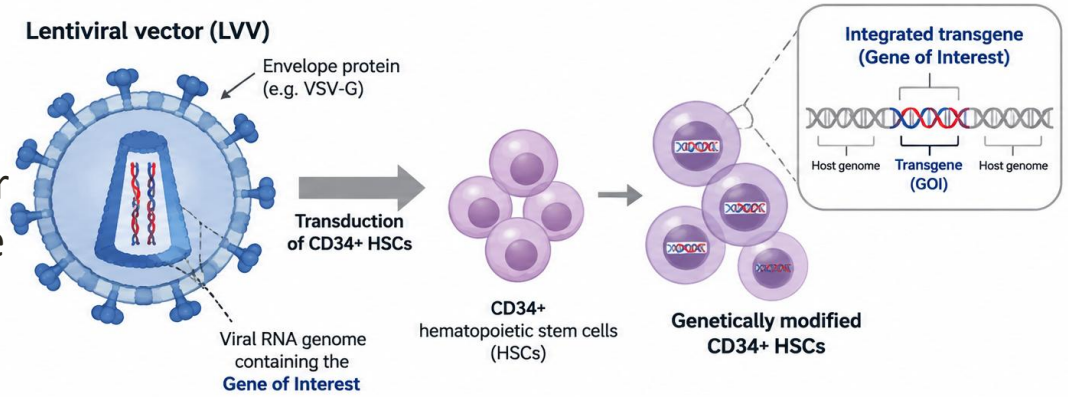
- For CGT products, potency covers not only proof of gene transfer but, more critically, the biological effect of the expressed gene (**functional potency**) – for both the LVV and the DP.

- Potency links the DP lots used in pivotal trials to the commercial product, so the clinical evidence carries over.

- Transgene-related assays such as transduction efficiency and vector copy number do not directly measure the biological function of the therapeutic protein. They are therefore not functional potency assays, and cannot be considered efficacy-indicating unless correlated with clinical efficacy data.



***"FDA permitted a potency assurance strategy based on WASP expression assay and an interim analysis demonstrating correlation of WASP expression values in the final product with increases in platelet count between baseline and Day 180, a relevant clinical outcome, rather than a drug product (DP) functional potency assay."*– FDA, Waskyra Summary Basis for Regulatory Action**

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Invest in functional potency assays early to maximise later comparability success

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Retain samples from every lot	"…we recommend that samples be retained from all lots to facilitate future analysis of potency to support comparability."
Run candidate assays in parallel	Develop several assays for known or potential potency-related CQAs and evaluate them during early trials
Build an assay matrix	Combine complementary assays for gene transfer and transgene function, correlated with biological activity
Add orthogonal support	Use animal models or extended characterisation to support comparable biological activity
Qualify before relying on it	"Using unqualified assays may decrease your ability to analyze risks to potency…"
Engage regulators early	Request a meeting before significant changes, especially late in development

2026 flexibility: scope and limits

FDA may accept limited comparability data for changes it considers minor and low-risk to product quality, if quality attributes are met and the change is submitted before dosing (CMC Flexibilities 2026).

CBER will not expect "overly stringent and onerous comparability data" for minor changes (Jan 2026 statement).

This flexibility covers minor changes only; major changes (site, scale, vector process, formulation) still need comparability data that include potency.

Source: Mfg Changes & Comparability draft (2023); Potency Assurance draft (2023); CMC Flexibilities (2026); FDA statement (Jan 2026)

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Analytical method changes during clinical development complicate comparability

- **Late-stage method validation:** Potency and impurity methods are often fully validated only late in development. Later changes may follow method optimisation, reagent discontinuation or technology transfer between sites.
- **Risk of analytical bias:** Without method bridging studies, a difference in results may reflect a genuine change in product quality or simply a change in the method.
- **Impact on statistical comparability:** The power and reliability of a comparability assessment depend on consistent analytical data generated with comparable methods.
- **Waskyra™ experience:** By BLA/MAA submission, 9 LVV and 7 DP analytical method changes had been made, with a further 4 implemented post-approval.

Key lessons for future development

- **Qualify and validate critical assays early:** Prioritise dose-determining and potency assays to expose limitations early and avoid changes during pivotal development.
- **Establish impurity methods before pivotal trials:** Develop and qualify or validate impurity assays before pivotal studies begin; their sensitivity requirements make optimisation and validation particularly challenging.
- **Plan sample retention proactively:** Retain representative samples from early batches, especially ahead of site transfers or significant process changes, to enable retrospective method bridging and comparability assessment.

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Key takeaways: comparability is built across the lifecycle, not proven at the end

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With few batches and many changes, comparability rests on early planning, potency and retained samples.

1	**Plan for change from day one**	~37 changes but only 34 batches, 7 on the commercial process: every batch and retained sample counts.
2	**Scale evidence to risk and phase**	Risk-based impact assessment sets the evidence per change; the bar rises towards licensure.
3	**Layer the evidence**	Analytical, side-by-side and stability data first; clinical bridging only when these are inconclusive.
4	**Make potency the bridge**	MOA-relevant potency links pivotal lots to commercial product; Waskyra™ correlated potency matrix with clinical outcome.
5	**Lock methods early, engage regulators**	Qualify potency and impurity assays before pivotal studies, bridge method changes, discuss major changes early.