Considerations for AAV Analytical Comparability Studies for Products with Low Batch Numbers

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Manufacturing changes are often implemented during the development of AAV gene therapies. These changes may be significant, for example changing the manufacturing platform to generate a scalable manufacturing process, or much smaller, such as transferring an existing process from one manufacturing site to another.

These manufacturing changes must be accompanied by comparability studies demonstrating that the post-change product has an equivalent safety and efficacy profile to the pre-change product. If analytical comparability can be demonstrated based on a good understanding of product critical quality attributes (CQAs) and using methods that can provide high assurance of safety and efficacy, then repetition of preclinical toxicity or human dose-finding and efficacy studies may not be needed.

Several guidance and draft guidance documents are available from various agencies to guide the comparability process.[1-3] Ideally, a large number of pre- and post-change batches should be compared to provide statistical assurance that the change(s) introduced do not affect product CQAs. However, since many AAV gene therapies are often produced for rare diseases with relatively low numbers of patients, and since batch manufacturing costs are high, a limited number of batches is normally available.

Comparability plans must therefore be tailored for AAV gene therapies yet be compatible with current guidance. CQA risk assessments must be conducted when changes are made to different parts of the process, e.g. upstream, downstream, or formulation and strategies established for generating data that provide sufficient statistical assurance of comparability using only a small number of pre- and post-change batches.

Ascend's process flow includes the following steps:

1. Identify the specific process change – improvement, scale up, etc.
2. Conduct a risk assessment to identity relevant CQAs and assess the risks of changes to each
3. Establish a comparability test plan, including specifying the number of batches and the inclusion of side-by-side testing. (Side-by-side testing of pre-and post-batches is recommended.1,2)
4. Determine acceptance criteria, including the use of appropriate statistical methods and the number of test occasions needed. A statistician should always be consulted when developing a comparability plan.
5. Use the results to assess the comparability and assess the risk to patients of any differences.

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CQA Risk Assessment

During the CQA risk assessment at Ascend, each attribute is assessed for the probability of being affected by the change, the potential severity of the impact(s) on safety and efficacy, and the risk of not detecting a change in the attribute. Each is then assigned a level of low, medium, or high (see Table 1). Based on the scores, the attributes are assigned to a tier, and each tier is assigned an appropriate assessment of comparability.

Table 1. Scoring of CQA Risk Assessments

Tier	Score	Probability	Severity	Detectability Risk	
3	Low	Unlikely (<2%)	Low potential to affect safety and efficacy	Methods reliably detect changes in the attribute with high precision and accuracy	Specification
2	Medium	Moderately likely (2-20%)	Safety or efficacy may be impacted – non- serious adverse events, small changes in efficacy	Methods are available but may suffer from limited sensitivity, precision, or accuracy	Quality Range
1	High	Highly likely (>20%)	Safety or efficacy may be significant changes in efficacy)	Methods are not available or do not have suitable sensitivity, precision, or accuracy	Significance Testing

Tier 1 CQAs, which present the highest risk, are subjected to significance testing.

For **Tier 2** CQAs, quality ranges must be defined within which the values for all post-change batches must fall to be considered comparable for those attributes. The range should typically be tighter than the specification limits and depends on severity of the CQA and on the precision of the method. For example, a range may be defined by the mean of the pre-change batches, +1.5 standard deviations.

Specification compliance is considered for **Tier 3** CQAs, which present the lowest risk.

Potential changes to stability and device compatibility are also risk-assessed. If they fall in **Tier 3**, the studies may not be needed. If they are classified as **Tier 1** or **Tier 2**, studies confirming stability and device compatibility should be performed.

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Examples of CQA Risk Assessments

Representative upstream, downstream, and formulation changes were subjected to risk assessments to demonstrate how these different changes are evaluated using Ascend's approach to comparability assessment. CQAs considered in these evaluations included packaged DNA impurities, post-translational modifications (PTMs), potency, process residuals, aggregation, and percent full capsids.

1 Upstream change: improved plasmid design

Using a different plasmid was assessed to have a high potential to impact three CQA; packaged DNA impurities, potency, and % full capsids were all determined to be **Tier 1** attributes. PTMs fell in **Tier 2**, while process residuals, aggregation and device compatibility and stability were placed in **Tier 3**.

2 Downstream change: addition of a virus filtration step

For this process change, no CQAs were assessed to fall in **Tier 1**. Potency, process residuals, and aggregation were determined to be **Tier 2** attributes. All remaining CQAs were identified as **Tier 3**.

3 Formulation change: addition of surfactant

For this change, potency and aggregation were assessed to be **Tier 1** and **Tier 2** CQAs, respectively. Ther remaining attributes fell in **Tier 3**. In this case, device compatibility and stability were also placed in **Tier 1**.

For **Tier 1** impurities, for example packaged DNA impurities, significance testing with an upper boundary can demonstrate that the mean levels of packaged impurities in the post-change process are not higher than those of the pre-change process with an acceptable margin (10%). However, because even at a lower level of impurities, products may still be considered not comparable as outlined in reference 1, a risk assessment will be required to justify a one-sided test.

For other **Tier 1** attributes, such as potency, the two one-sided t-test (TOST) procedure can demonstrate equivalence between the means of the pre- and post-change batches to within a defined margin (e.g., +30%) It is important to note that a quantitative potency assay with good precision is required to be able to determine comparability using this approach.

For **Tier 2** attributes, appropriate quality ranges, e.g. 1.5-2 standard deviations from the mean of the pre-change batches may be sufficient to demonstrate comparability.

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Sample savings with selected low-volume methods

Because comparability testing may require side-by-side testing and multiple measurements per sample to achieve statistical significance, the sample burden can be very high. Choosing methods which require low volumes of material can reduce that burden and allow a robust determination of comparability using relatively low amounts of sample. Some examples of low volume methods include:

- Mass photometry: >40x over analytical ultracentrifugation (AUC)
- Duplex ddPCR: 2x over singleplex ddPCR
- Backgrounded membrane imaging (BMI): >3x over light obscuration
- Gyros® automated ELISA: 4x over traditional ELISA
- Nanopore sequencing: 10X over traditional PacBio sequencing

Many of these methods also provide cost reductions and improvements in throughput.

Summary

If changes to upstream and/or downstream AAV manufacturing processes and/or product formulations are made, it is necessary to demonstrate that the pre- and post-change batches have comparable CQAs to ensure the changes do not affect the safety and efficacy of the gene therapy products.

Ascend assesses the risk of important CQAs being impacted by a change, and based on this evaluation classifies the attributes into different tiers. Different statistical approaches are then used commensurate to the risk posed by the CQA to assess the comparability for pre- and post-change batches/products

We also have established approaches for improving data quality when small numbers of batches are available, including the use of low-volume analytical methods that can enable side-by-side testing and testing of the same sample multiple times.

To learn more, download our ESGCT 2024 poster on comparability or email us.

References

1. *FDA: Manufacturing Changes and Comparability for Human and Cellular Gene Therapy Products. Draft Guidance for Industry July 2023*
2. *FDA: Demonstration of Comparability of Human Biological Products, Including Therapeutics Biotechnology-derived Products April 1996*
3. *ICH Q5E Comparability of Biotechnological/biological Products Subject to Changes in their Manufacturing Process*