CEPI

Comparability Template

Annex: Viral Vector illustrative example

1 Introduction

The example outlined in this annex is a hypothetical yet practical scenario of viral vector manufacturing, control, and development. Nevertheless, the approach illustrated through this recombinant adenovirus vaccine example could be extrapolated to various other types of viral vectors (e.g., MVA).

The content of this annex is not regulatory binding. Modification as appropriate for your organization is anticipated during the usage of the template.

1.1 Product Information / Problem Statement

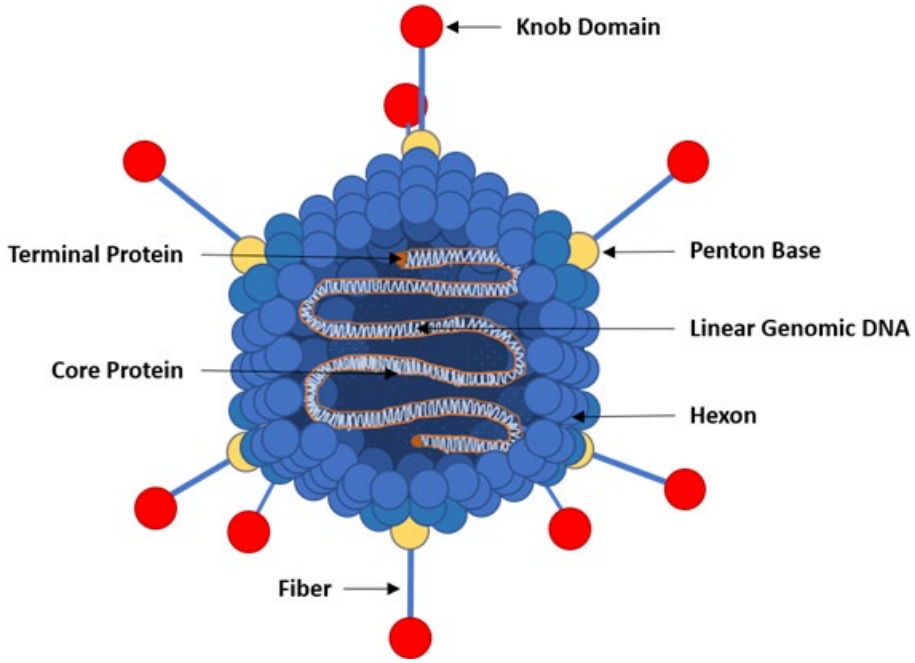
In this viral vector example, an adenovirus vector vaccine containing a SARS-CoV-2 spike protein transgene was developed to address global pandemic, and an analytical comparability assessment between pivotal clinical and PPQ materials was conducted. A list of Critical Quality Attributes (CQA) and the associated analytical testing control strategy serve as the basis of the comparability study design.

The recombinant adenovirus type 26 (Ad26) vaccine consists of a nonenveloped capsid which contains a single linear molecule of double--stranded DNA (dsDNA) of approximately 35 kilobase pairs (kbp). The adenovirus genome encodes the SARS-CoV-2 spike protein, as well as the adenoviral early and late-stage proteins, where the early-stage proteins are mainly nonstructural, regulatory proteins whereas the late-stage- proteins are mainly the adenovirus structural proteins. See **Figure 1** for the Ad26.COV2.S vector genome.

Figure 1: Schematic representation of the Ad26.COV2.S vector genome showing the early (E) and late (L) genes, the inverted terminal repeat (ITR) and the transgene (SARS-COV-2 Spike gene).

The dsDNA molecule is encapsulated by an icosahedral protein structure consisting of the structural proteins II (hexon), III (penton), IV (fiber), VI, VIII, IX and IIIa. Core proteins V, VII, X and the terminal protein are directly associated with the viral genome (refer to **Figure 2**). Vector particles have a diameter of approximately 90 nanometers from vertex to vertex (San Martin and Burnett).

Figure 2: Schematic Representation of the Structure of the adenovirus Virion



The recombinant adenoviral vector is replication-incompetent due to deletion of the E1 gene region (Δ E1A/E1B). The E1 deletion renders the vector replication-incompetent in non-complementing cells, such as human cells. In addition, (1) a part of the early region is removed to create sufficient space in the viral genome for insertion of foreign antigens (i.e., transgene protein), and (2) part of the E4 orf has been exchanged by the Ad5 homologue to allow production of replication-incompetent adenovirus vectors in the Ad5 E1 complementing cell line (i.e., PER.C6 TetR).

There is substantial non-clinical, clinical and commercial experience with adenovirus-based vaccines (both licensed and in development) that demonstrate their capacity to elicit strong humoral and cellular immune responses. The postulated mode of action is the induction of an immune response against the transgene protein, to prevent or limit infection of the virulent in question. To elicit an immune response against the transgene protein, the adenovirus vector needs to infect cells and mediate expression of the transgene protein followed by its presentation.

Key product specific considerations informing the comparability assessment are provided below:

There is extensive adenovirus vector platform prior knowledge from other products (both commercial and in development)

Multiple manufacturing processes were utilized to support the clinical program. The numbers of DS and DP lots produced by each clinical process, however, are limited.

To enable expedited global access, a large number of commercial DS and DP manufacturing sites are planned for commercial production concurrently, with an even greater number of different DS/DP nodes combinations

The DP manufacturing process involves only dilution and sterile fill operations as this is a solution for injection product

A risk-based- approach was undertaken, leveraging prior knowledge, for the comparability study design. The analytical comparability between pre- and post-change- materials were assessed. In cases where analytical differences were noticed, scientific justifications were provided. The totality of the analytical comparability data demonstrated comparability between the pre- and post-change (clinical vs. PPQ) materials.

1.2 Critical Quality Attributes and Analytical Testing Control Strategy

Product Critical Quality Attributes (CQA) and their associated control strategy serve as the basis of the comparability study design. The attributes considered critical to viral vector vaccine product and the analytical control strategy as an element of the overall control strategy to ensure product safety and efficacy are described in this section.

Table 1 summarizes the list of (p)CQA(s), for illustrative purposes, as well as the rationale for the criticality assignment.

Table 1 (p)CQAs for adenovirus based recombinant vectora

Quality Attributes	Criticality Assignment	DS/DP	Rationale
General physicochemical characteristics *Determination of the physicochemical properties of the product*			
Appearance: Color of solution	CQA	DS, DP	Change in color suggest a change in the product which may affect efficacy and safety
Appearance: Clarity	CQA	DP	Change in clarity suggest a change in the product which may affect efficacy and safety
Appearance: Visible particles	CQA	DP	Visible particles may pose a safety risk
Container Closure Integrity	CQA	DP	Container/closure is the protection between the product and the external environment, including microbial contaminants
pH	CQA	DS, DP	pH impacts product stability and therefore may impact efficacy and safety
Osmolality	CQA	DS, DP	Osmolality is an indirect measure of product formulation and related to potential pain upon injection
Volume	CQA	DP	Variations in extractable/deliverable volume can result in incorrect dosing, impacting efficacy and safety
Excipients *A constituent other than the active substance, added in the formulation for a specific purpose.*			
Example: Polysorbate	CQA	DS, DP	Polysorbate is important in enhancing viral product stability and therefore safety and efficacy
Content			

Quality Attributes	Criticality Assignment	DS/DP	Rationale
A measure of the amount of product required to determine the dose			
Viral product concentration	CQA	DS, DP	Viral particle and viral genome concentrations impact efficacy and safety
Potency *A measure of the biological activity based on the attribute(s) of the product which is linked to the relevant biological properties*			
Infectious titer	CQA	DS, DP	Infectivity of the adenovirus is a required aspect of the biological activity, according to its mechanism of action. Additionally, virus particles to infectious units ratio is indicative of the biological activity.
Transgene expression level	CQA	DS, DP	A certain level of transgene expression is a required aspect of the biological activity, according to its mechanism of action.
Identity			
Identity	CQA	DS, DP	Dosing with the wrong product would negatively affect efficacy and/or patient safety.
Genome sequence	CQA	DS	Changes in the sequence of the transgene and flanking region may lead to expression of an antigen that does not induce a protective immune response. Changes in the sequence of the backbone sequence may impact infectivity.
Product Related Substances and Impurities *Certain product variants, such as aggregates and viral protein degradation products, may have an impact on safety and efficacy*			

Quality Attributes	Criticality Assignment	DS/DP	Rationale
Adenovirus higher order structure	CQA	DS, DP	Correct expression of the adenovirus protein leads to the expected higher order structure. This in turn leads to proper assembly of the viral particle, including enclosure of the genome content, which is essential for its mechanism of action.
Virus particle aggregates	CQA	DS, DP	Presence of soluble virus particle aggregates may lead to decreased infectivity, may precipitate out of solution and could theoretically have a potential impact on immunogenicity.
Empty virus particles	CQA	DS, DP	Presence of undesired empty particles has an impact on clinical efficacy.
Process-related Impurities			
Host cell DNA	CQA	DS	Host cell DNA may cause safety risks (e.g., known oncogene) and need to meet WHO expectation
Host cell protein	CQA	DS	Host cell protein could be immunogenic and residual host cell protease may cause proteolytic degradation
Residual compound[b]	CQA	DS	Potential safety risk if above toxicological limit. CQA designation may be adjusted based on Safety Risk Assessment.
Contaminants			
Bacterial endotoxin	CQA	DS, DP	Microbiological contaminants pose a safety risk
Mycoplasma	CQA	DS	Mycoplasma poses a safety risk

Quality Attributes	Criticality Assignment	DS/DP	Rationale
Mycobacteria	CQA	DS	Mycobacteria poses a safety risk
Bioburden	CQA	DS	Microbiological contaminants pose a safety risk
Sterility	CQA	DP	Microbiological contaminants pose a safety risk
Adventitious viruses	CQA	DS	Viral contaminants pose a safety risk
Replication Competent Adenovirus	CQA	DS	Replication competent adenovirus poses a safety risk

[a] the list of CQA to be evaluated case by case especially in pandemic scenario

[b] If there is a change to the manufacturing process, the level of each residual compound needs to be assessed with respect to its toxicological limit. Based on this assessment it may be deemed a CQA.

As defined in ICH Q8(R2), the control strategy is a planned set of controls, derived from current product and process understanding, that ensures process performance and product quality. The goal of the control strategy is to ensure critical quality attributes (CQAs) of Drug Substance (DS) and Drug Product (DP) are within the acceptable ranges that maintain product safety and efficacy. The analytical testing component of the control strategy is described below.

The analytical testing control strategy is summarized in **Table 2**, which includes routine testing (in-process control/test, lot release and/or stability) and non-routine testing (PPQ only or characterization) to ensure each critical quality attribute is maintained within its acceptable range. For certain CQAs applicable to both DS and DP, testing at either DS or DP may be sufficient based on other control elements in place. It is worth noting that DS and/or DP CQAs may not require routine specification testing (release and/or stability), if adequately controlled by other elements of the holistic control strategy. For example, the quality attributes may be controlled via in-process testing if subsequent manufacturing process and long term storage are not expected to have any impact (e.g. Replication Competent Adenovirus); via at-scale or appropriate small-scale process characterization/validation studies that demonstrate robust and consistent control (e.g. endonuclease); via procedural controls and/or surrogate control through routine testing of related CQAs (e.g. pH and osmolality for buffer component and stabilizing agent in product formulation). Furthermore, the analytical testing control strategy can evolve as additional product and process understanding become available or process changes are introduced, which may justify re-classification of attribute criticality, or

addition/removal of the specification tests. As one element of the overall control strategy, the analytical comparability study may include only a subset of the analytical testing controls based on the product/process understanding available at the time of the process change.

Table 2 Analytical Testing Control Strategy including process and product characterization testing

Category	CQA	Analytical Method[a]	Drug Substance	Drug Product
General Physicochemical Characteristics	Appearance	Color of Solution	Release and Stability	Release and Stability
		Clarity	--	Release and Stability
	Container Closure Integrity	CCIT (Dye Ingress)	--	Stability
	Particulate Matter	Visibile Particles	--	Release and Stability
	Volume	Extractable or Deliverable Volume	--	Release
	pH	pH	IP, Release and Stability	Release and Stability
	Osmolality	Osmolality	Release[b]	Release[b]
Excipients	Polysorbate	Polysorbate (HPLC-ELSD)	Release[b]	Release[b]
Content	Viral Product Concentration	Viral Particle Concentration (AEX)	IP, Release and Stability	IP, Release and Stability
		Viral Genome Concentration (qPCR)	IP and Characterization	Characterization

Category	CQA	Analytical Method[a]	Drug Substance	Drug Product
Potency	Infectious titer	Infectivity	IP (PPQ only), Release and Stability	Release and Stability
		Virus particles to infectious units ratio. See 'VP concentration' and 'infectivity'	Characterization	Characterization
	Transgene Expression level	Transgene Expression (ELISA)	Release[e]	Release[e]
Identity	Identity	Identity (qPCR)	Release	Release
	Genome Sequence	Genome Integrity (restriction enzyme mapping)	Characterization	--
Product-related substances and impurities	Adenovirus Higher order structure	Viral Particle Size (NTA)	--	Characterization
		Viral Particle Molar Mass (FFF-MALS)	--	Characterization
	Empty virus particles	DNA: Protein ratio (A260/A280)	Release	Release
	Virus particle aggregates	A320	--	Characterization

Category	CQA	Analytical Method[a]	Drug Substance	Drug Product
Process-related impurities	Host cell DNA[c]	qPCR	IP (PPQ only) and Release	--
	Host cell protein[c]	HCP ELISA	IP (PPQ only) and Release	--
	Residual endonuclease[c]	endonuclease ELISA	IP (PPQ only) and Release	--
Contaminants	Bacterial Endotoxin	LAL	IP and Release	Release
	Mycoplasma	Mycoplasma	IP	--
	Mycobacteria	Mycobacteria	IP	--
	Bioburden	Bioburden	IP and Release	--
		Filter Integrity Test	IP	--
	Sterility	Sterility	--	Release[d]
	Adventitious viruses	Safety Testing	IP	--
	Replication Competent Adenovirus	Safety Testing	IP	--

**IP: meaning both In Process Controls or In Process Monitoring tests*

a. Test method(s) alternative to the examples listed in this table may be adopted as appropriate

b. Testing may be performed on either DS or DP if appropriately justified

c. The analytical testing control strategy may be adjusted based on the Safety Risk Assessment and/or additional process knowledge (e.g., impurity removal studies)

d. Usually tested at time 0 and end of shelf-life

e. Due to a potential long lead time of Transgene Expression assay development, a characterization assay may be appropriate, with post-marketing commitment of implementing a validated method for lot release

2 Description of the Changes & Justification

In this illustrative example, 3 clinical manufacturing processes were utilized to support the clinical development, and the 3rd clinical manufacturing process (pre-change process) was used for the production of pivotal clinical trial materials. A new manufacturing process (post-change process) was subsequently developed to provide commercial supply and is subjected to process performance qualification (PPQ). Besides site change, a number of process changes are introduced to implement a commercially viable process including process simplification and therefore enable accelerated technology transfer to the global manufacturing network, as well as improving scalability in order to meet the large commercial demand. A comparison of the clinical and commercial processes is summarized in **Table 3** for Drug Substance and **Table 4** for Drug Product. Justification for each type or step of the process change are also indicated.

Table 3 Comparison of clinical and commercial DS manufacturing processes

Change ID	Description of changes	Pre-Change (Pivotal clinical)	Post-Change (PPQ and Commercial)	Justification of the change
DS1	Site of manufacture	Pilot plant	Large scale facility	Larger scale to meet commercial demand
DS2	Scale	200L	2000L	Larger scale to meet commercial demand
DS3	Seed	MVS	WVS	To not deplete MVS based on commercial demand
DS4	Cell bank	MCB	WCB	To not deplete MCB based on commercial demand
DS5	Infection	MOI 10 on Day 4	MOI 0.1 on day 1	Reduce use of viral seed
DS6	Harvest	Centrifugation	In situ cell lysis	Process simplification and scalability

Change ID	Description of changes	Pre-Change (Pivotal clinical)	Post-Change (PPQ and Commercial)	Justification of the change
DS7	Harvest	No bioreactor cooling	Active bioreactor cooling	Reduce adenovirus particle degradation
D8	Clarification	Centrifugation	Filtration	Process simplification and scalability
DS9	Purification	Cesium chloride ultracentrifugation	Tangential flow filtration and anion exchange chromatography	Process improvement and scalability
DS10	Formulation	Dialysis	Tangential flow filtration	Process simplification and scalability
DS11	Storage	Refrigerated	Frozen	Improve stability
DS12	Storage	Small vessel (e.g., 100 mL)	Large vessel (e.g., 10 L)	Process improvement and scalability

Table 4 Comparison of clinical and commercial DP manufacturing processes

Change ID	Description of changes	Pre-Change (Pivotal clinical)	Post-Change (Commercial)	Justification of the Change
DP1	Site of manufacture	Pilot plant	Large scale facility	Larger scale to meet commercial demand
DP2	Scale	20 L	800 L	Larger scale to meet commercial demand
DP3	DS Thaw	NA	Thaw DS	Frozen source DS
DP4	DS batch pooling	One DS batch	Multiple DS batches	Less DS waste

Change ID	Description of changes	Pre-Change (Pivotal clinical)	Post-Change (Commercial)	Justification of the Change
DP5	DS site pooling	One DS site	Multiple DS sites	Less DS waste
DP6	Excipients	Formulation A	Formulation B	Improve stability, CoGs, and/or manufacturing process
DP7	Primary packaging components	Glass vial	Siliconized glass vial	Non adsorption
DP8	DP presentation	Vial	Pre-filled syringe	Improve in use handling
DP9	DP presentation	Single dose	Multi dose	Reduce material use
DP10	Filling Line	Peristaltic pump	Piston pump	Filling line change
DP11	Storage conditions	-20C	2-8C	Commercial demand and ease supply chain

3 Change Impact Assessment

CQAs potentially affected by process changes described in **Table 3** and **Table 4** are summarized in **Table 5** and **Table 6** for Drug Substance and Drug Product, respectively, along with associated justifications. The comparability assessment of potentially affected CQAs will be performed based on product/process specific knowledge and considers a minimum set of the analytical tests for inclusion in the comparability study design.

Table 5 Drug Substance - Change Impact Assessment on CQA

Change ID	DS CQA / Attributes potentially impacted	Justification
DS1 (Site of manufacture)	All except for genome sequence	Change in manufacturing facility may affect performance of all process steps and therefore associated CQAs
DS2 (Scale)	All except for genome sequence	Change in manufacturing scale at individual process steps may affect the process performance and therefore associated CQAs
DS3 (Seed)	All	Viral seed contains the genomic content. Change in viral seed may affect viral protein expression, viral particle assembly, and therefore performance of subsequent process steps
DS4 (Cell bank)	All	Major process change that could potentially affect process performance and all product quality attributes.
DS5 (Infection)	All	Major process change that could potentially affect process performance and all product quality attributes.
DS6 (Harvest)	content, potency, product-related substances and impurities, process-related impurities	Change in harvest step may affect viral product yield, degradation, infectivity, and level of process residues
DS7 (Harvest)	Potency, product-related substances	Reduction in temperature should have a positive impact on potency and product-related impurities
D8 (Clarification)	Content, potency, process-related impurities	Change in harvest step may affect viral product yield, infectivity, and level of process residues

Change ID	DS CQA / Attributes potentially impacted	Justification
DS9 (Purification)	Content, potency, product-related substances and impurities, process-related impurities, pH, Osmolality	Change in purification may affect viral product yield, degradation, infectivity, level of process residues, and DS composition
DS10 (Formulation)	Content, pH, Osmolality, excipients	Change in the formulation step may affect process yield and DS composition
DS11&DS12 (Storage)	Content, potency, product-related substances, process-related impurities, appearance	Change in storage condition may affect product stability
		additional assessments of Extractables and Leachable maybe required

Table 6 Drug Product - Change Impact Assessment on CQAs

Change ID	DP CQA / Attributes impacted	Justification
DP1 (Site of manufacture)	All	Change in manufacturing facility may affect performance of all process steps and therefore associated CQAs
DP2 (Scale)	Content, potency, product-related substances and impurities	Change in DP manufacturing scale may affect viral product yield, infectivity, and degradation

Change ID	DP CQA / Attributes impacted	Justification
DP3 (DS Thaw)	Potency, aggregation, empty viral particles	Freeze/thaw stress may induce aggregation, viral particle break down and change in potency
DP4 (DS batch pooling)	Potency, aggregation, empty viral particles	Mixing may cause shear stress and induce aggregation, viral particle break down and change in potency
DP5 (DS site pooling)	None	DS comparability studies demonstrated the DS material from different manufacturing processes is comparable. This should only be done when commercial and concurrent manufacturing.
DP6 (Excipients)	Content, potency, product-related substances, process-related impurities, excipients	Change in formulation may affect stability of the product and of process-related impurities.
DP7 (Primary packaging components)	Content, potency, product-related substances, process-related impurities	Change in product contact surface may impact the product and process-related impurities based on, e.g., stickiness.
DP8&DP9 (DP presentation)	All	Change of container may affect all CQA due to, e.g., other product contact surface, different container closure, syringe impurities.
DP10 (Filling line)	Potency, aggregation, empty viral particles	Filling line (and/or pump) change may affect potency, aggregation, and viral particle break down due to potential change in shear stress and contact surface
DP11 (Storage conditions)	All except contaminants	Increase in storage temperature may affect all CQA except contaminants due to, e.g., more energy causing aggregation.

4 Comparability Strategy

The comparability assessment includes process comparison as shown in Section 2, as well as analytical comparability studies. The analytical comparability assessment consists of release and characterization tests (Section 4.1), as well as stability studies (Section 4.2). These studies will be performed according to the analytical comparability protocol containing pre-defined comparability criteria (see Section 6.1 for the approaches of setting comparability criteria).

In the accelerated scenario of vaccine development to address global pandemic, a few key considerations below are incorporated in the comparability strategy. The underlined principles, however, may be applicable to a non-pandemic development program, provided that prior knowledge is sufficient to justify such approaches:

For attributes where extensive prior knowledge exists (e.g., attributes agnostic of the transgene), platform expectations (e.g., release specifications based on platform knowledge) are applied as comparability criteria.

For attributes where statistically derived criteria are deemed appropriate, lot testing results from all 3 clinical manufacturing processes and the product platform are included as the reference data set to derive a meaningful statistical assessment. This is justified as all clinical process materials have been demonstrated to be safe and efficacious in clinical studies, therefore representative of the entire clinical experience and a proper assessment has been made that determined the platform data were not impacted by the product-specific transgene. Additionally, comparability of each clinical process material to the previous non-clinical or clinical process material was demonstrated prior to their introduction into the clinical study. Of note, if there was no product platform knowledge available it would be up to the Sponsor to assess if a more complex statistical tool (than min-max) may be used to additionally support comparability (see also Part B1 for further information).

To accelerate global access to the vaccine product, multiple commercial DS and DP manufacturing sites are introduced to the manufacturing network during approximately the same time period, with a large number of DS/DP nodes combinations. DS and DP comparability for these commercial manufacturing processes are each demonstrated independently through comparison to the same set of clinical process lots.

Commercial process DP lot selection at each DP manufacturing site is independent of source DS to allow flexible commercial supply network with different combinations of DS and DP nodes. This is justified provided that the commercial DS from each site/scale is demonstrated to be comparable to the clinical DS. In other words, if the DS manufacturing processes have been demonstrated to be comparable, they can be interchangeably used as DS source for the DP manufacturing processes and no additional comparability study will have to be performed.

Comparative stability studies of DP under accelerated stability conditions are not needed to assess DP comparability, provided that the source commercial process DS is demonstrated to be comparable to the

clinical process DS through the comprehensive comparability assessment that includes comparative stability studies. This is justified as the commercial DP manufacturing process involves only dilution and sterile fill operations.

4.1 Release and Characterization Testing

The CQAs listed in **Table 1** represent the attributes that are potentially linked to the clinical performance. Consequently, these CQAs will be controlled as part of the manufacturing process and are the foundation of the analytical comparability assessment. A comprehensive set of comparative release and characterization testing included in the comparability studies is described in **Table 7** (the values denoted in **Table 7** are merely for illustrative purpose and are not related to any commercial product or product in development, except for guideline related values, e.g., subvisible particles). It is worth noting that the test selection in the analytical comparability assessment may be adjusted based on the product/process specific knowledge and control strategy and may be a subset of this comprehensive list based on the type(s) of process changes and potentially affected CQAs as described in **Table 5** and **Table 6.** Comparisons between commercial and clinical materials may either be made directly by comparative testing or indirectly by meeting compendial/regulatory requirements.

Table 7 Release and Characterization Testing

Attribute potentially impacted	**Which intermediate/DS/DP tested**	**Analytical Method**	**Comparability Acceptance Criteria**	**Justification**
Appearance – Color	DS, DP	Visual	≤ B2	Meet specification[a]
Appearance – Clarity	DP	Visual	≤ EP standard of opalescence	Meet specification[a]
Appearance – Visible Particles	DP	Visual	Practically free from visible particles	Meet specification[a]
pH	Process intermediate, DS, DP	pH	6.0 - 7.0	Meet specification[a]
Osmolality	DS[f], DP	Osmometer	320 – 480 mOsm/kg	Meet specification[a]

Attribute potentially impacted	Which intermediate/DS/DP tested	Analytical Method	Comparability Acceptance Criteria	Justification
Polysorbate	DS[f], DP	HPLC-ELSD	0.05 – 0.15% w/v	Meet specification[a]
Volume	DP	Volumetric	5 doses per vial at ≥ 0.5 mL	Meet specification[a]
Viral Particle Concentration	Process intermediate, DS, DP	AEX	Report results for process intermediate; ≥ 1.0×10^{11} vp/mL for DS; 0.6 – 1.4 $\times10^{11}$ vp/mL for DP	Viral product concentration related[b]
Viral Genome Concentration	Process intermediate, DS, DP	qPCR	Report results for process intermediate; Report results for DS; 0.3 – 1.7 $\times10^{11}$ vg/mL for DP	Viral product concentration related[b]
Infectivity	Viral seed, DS, DP	Infectious titer	Report results for process intermediate; ≥ 1.0 $\times10^{9}$ for DS; 0.6 – 2.2 $\times10^{9}$ for DP	Viral product concentration related[b]
Transgene Expression	DS, DP	ELISA	38 – 166%	Meet specification
Identity	Viral seed, DS	qPCR	Identity confirmed	Qualitative attribute
Genome Sequence	Viral seed, DS	Restriction Enzyme Mapping	Consistent with expected DNA fragment pattern	Qualitative attribute

Attribute potentially impacted	Which intermediate/DS/DP tested	Analytical Method	Comparability Acceptance Criteria	Justification
Viral Particle Size/Mass	DS[f], DP	NTA FFF-MALS	80 – 100 nm 75 – 150 MDa	Statistically derived criteria[d]
Aggregates	DS[f], DP	UV A320:A260	0.10 – 0.35	Statistically derived criteria[d]
Empty:Full ratio	DS, DP	DNA:Protein ratio by A260:A280	1.2 – 1.4	Meet specification
P:I Ratio	DS, DP	Viral Particle Concentration: Infectivity	≤ 170 vp/ifu	Meet specification
Residual Host Cell DNA	Process intermediate, DS	qPCR	≤ 10 ng/dose	Meet specification[e]
Residual Host Cell Protein	Process intermediate, DS	ELISA	≤ 200 ng/dose	Meet specification[e]
Residual Endonuclease	Process intermediate, DS	ELISA	≤ 10 ng/dose	Meet specification[e]

Associated with clinically inactive components or attributes that only need to be controlled within a range that ensures product stability, efficacy, and patient safety

Viral product concentration related attributes that just need to be sufficiently high in process intermediate(s) and DS to enable further processing; Meet specification for DP

Product-related substances/impurities and functional activity attributes with numerical output. Lower and/or upper tolerance intervals from statistical analysis of clinical process lot results are used to inform the comparability criteria

Product-related substances/impurities attributes that are primarily dependent on the viral vector and not expected to change significantly between different gene of interest. Prior knowledge of other vaccine products based on the same adenovirus vector platform are utilized to inform the comparability criteria, verified against the observed range of product specific clinical lot results.

Process-related impurities controlled to ensure patient safety

Comparability test may be performed for DP only if appropriately justified

4.2 Stability

Stability studies may supplement the release and characterization comparability analytical tool package by detecting potential differences, if any, that may otherwise not be detected by testing DS or DP at release. Since the DP manufacturing process involves only dilution and sterile fill operations, comparative stability at accelerated conditions were conducted for DS comparability only. Three pre-change DS lots, as well as 3 post-change DS lots from each DS manufacturing site, were placed at the accelerated stability conditions (25 °C, 60% Relative Humidity). Samples were pulled at 0, 1, 2, 3, and 4 week time points and analyzed using the primary stability indicating attribute, infectivity.

5 Lot Selection

A summary of the DS and DP lots included in an analytical comparability assessment is provided in

Table 8. The use of each lot in different comparability studies is also indicated.

Table 8 Analytical Comparability Lot Selection

Process	Lot Number	Release	Characterization	Stability (accelerated)
Clinical Process 1	Lot a	√	√	--
Clinical Process 2	Lot b	√	√	--
	Lot c	√	--	--
Clinical Process 3	Lot d	√	√	√
	Lot e	√	--	√
	Lot f	√	--	√
Commercial Process*	Lot g	√	√	√
	Lot h	√	√	√
	Lot i	√	√	√

* Analytical comparability of commercial process lots produced at different commercial manufacturing sites/scales will each be independently demonstrated following this comparability protocol.

6 Testing Methods

The analytical tool package applied to DS and DP evolves throughout the development history. Some analytical methods used to support release and stability testing of clinical process materials were either replaced with orthogonal methods or further optimized. For the purpose of the analytical comparability assessment, available clinical lots were tested by updated methods in order to facilitate direct comparison. Alternatively, appropriate method bridging studies have been carried out to demonstrate equivalence of the proposed commercial methods and the corresponding historical clinical methods.

6.1 Acceptance Criteria

The approaches to comparability acceptance criteria are detailed in the accompanying Comparability Template – Overview document (Principles and Tools). Commercial lot release specifications are applied as the comparability acceptance criteria for attributes that are:

Associated with clinically inactive components or attributes that only need to be controlled within a range that ensures product stability, efficacy, and patient safety (e.g. Appearance, pH, Osmolality, excipients, volume)

Process-related impurities controlled to ensure patient safety (e.g. residual host cell DNA, residual host cell protein)

DS concentration, which is not directly linked to clinical dosing since the DP concentration may be adjusted from that of the source DS, except for a minimum concentration requirement to enable DP manufacturing

For attributes which are impacted by the gene of interest (e.g., transgene expression)

For these attributes, release results meeting specification will ensure that the quality of the product meet the regulatory and compendial requirements, taking into account the extensive prior knowledge on other products of the same adenovirus vector platform.

Statistically derived criteria are applied as supportive information to comparability for attributes that are agnostic to the gene of interest (e.g., viral particle size/mass).For these attributes, tolerance intervals based on data from clinical process materials and platform knowledge data are used to inform the comparability acceptance criteria. Qualitative criteria are applied to Identity and Genome Sequence, where the testing results are qualitative in nature, as well as the degradation trend analysis, where slopes from linear regression analysis of the infectivity loss are compared.

7 Results and Analysis

Lot release and characterization testing results are evaluated against the comparability acceptance criteria listed in **Table 7**. Stability results are evaluated qualitatively by comparison of degradation trends.

The results of the analytical testing and an assessment of comparability will be documented in a report. The materials will be deemed comparable if the results meet the comparability acceptance criteria. Failure to meet one or more of the comparability criteria will be addressed through a root-cause investigation and, if the failure is confirmed, a risk assessment of the potential impact on the product safety and efficacy will be conducted. The overall analytical comparability can be concluded if the observed difference, if any, is not expected to have any negative impact on safety and efficacy based on the assessment. Otherwise a (non-)clinical study, or additional process development/optimization may be necessary.

Two potential outcomes of the analytical comparability studies are provided below as possible scenarios.

Scenario A

Release and characterization results of all post-change DS lots included in the comparability study meet the comparability acceptance criteria except for that described below.

One post-change DS lot has a DNA: Protein ratio (A260:A280 ratio) of 1.5, which is slightly higher than the upper limit of the comparability acceptance criteria (1.2 – 1.4). While a lower A260/A280 ratio may be indicative of higher abundance of empty viral particles, a slightly higher ratio is not expected to have any negative clinical impact. Furthermore, the A260:A280 ratio of the DP lot filled with this post-change DS lot, as well as those of other DS lots manufactured using the same post-change process, is within the comparability acceptance criteria. Since the DP manufacturing process is not expected to affect viral particle full: empty ratio, the apparent higher A260:A280 ratio of the post-change DS lot may be attributable to method variability.

Scenario B

In the DS3 study case a WVS was introduced, i.e., an extra viral passage. All the release and characterization criteria were met with exception of full genome sequencing. Both at the 5' and 3' ends of the genome (the inverted terminal repeat [IVR]) minor genetic changes were observed. Based on this observation a root cause investigation was initiated. An orthogonal sequencing method confirmed the observation. A study was then performed to assess if these genetic alterations had an impact on either the safety or potency of the product. Furthermore, genetic stability studies needed to confirm that these were the only genetic alterations. It was concluded based on these extensive studies that this change had no impact on infectivity, RCA (nor on other CQA) or manufacturing process impact, and thus not on quality, safety or potency. Therefore, the post-change was deemed comparable to the pre-change.

8 Conclusion

Scenario A

In conclusion, all available lot release, characterization, and stability test results from the DS comparability studies meet the pre-defined comparability assessment criteria or have justifiable minor differences that are not expected to have adverse impact on quality, safety and efficacy. The totality of the data demonstrates that the post-change DS manufacturing process is comparable to the pre-change DS manufacturing process.

Scenario B

In conclusion, all available lot release, characterization, and stability test results from the DS comparability studies meet the pre-defined comparability assessment criteria or have a justifiable difference that is not expected to have adverse impact on quality, safety and efficacy. The totality of the data demonstrates that the post-change DS manufacturing process is comparable to the pre-change DS manufacturing process.

9 References

N/A