CEPI

Comparability Best Practices

Annex: Protein Subunit vaccines illustrative example

1 Introduction

This document presents an illustrative example of how to utilize the comparability blank template as applied to a protein subunit vaccine.

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

An adjuvanted protein subunit vaccine is under development to address a viral threat rapidly spreading several world regions; the vaccine is entering Phase 3 Clinical Trials (CT) after successful Phase 1/2 results, showing a good safety profile and allowing to select a safe vaccine dose higher than the minimum active. Due to the heavily accelerated timelines, the Phase 1/2 Clinical Trial was executed with a lyophilized antigen, re-suspended with adjuvant before administration. While recognizing that a liquid formulation could be easier to manufacture, supply and administer, the producer selected an initial lyophilized formulation, due to the extremely short time to assess product stability, the limited prior knowledge and the premature analytical characterization of adjuvanted liquid formulation at the onset of development. In parallel to CMC and clinical activities for Phase 1/2, liquid formulation feasibility was assessed, and was selected for Phase 3 studies, based on the following evidence:

- Critical quality attributes of the new formulated product have been identified,
- Analytical strategy has been developed for liquid formulation based on lyophilized formulation,
- Stress and accelerated stability studies on antigen and on candidate liquid formulations have been performed, allowing (i) the selection of the formulation components ensuring best structural preservation of the antigen; (ii) the demonstration of suitability of analytical methods to detect changes; (iii) the assessment of statistical modelling strategies to support stability prediction/shelf- life definition of the liquid formulation without completion of real time studies, which was not possible due to the emergency,

- Real time stability was started and the stability data is going to be provided to the relevant Authorities as available.

1.2 Critical Quality Attributes & Related Analytical Strategy

Table 1 summarizes a guideline of illustrative (p)CQA(s), as well as the rationale for the criticality assignment. If sufficient product knowledge has been accumulated a (p)CQA in this list could become a non critical Quality Attribute (QA). The comparability exercise focused only on the CQAs impacted by the changes, as clarified in the subsequent sections.

Table 1 some (p)CQAs for the protein subunit vaccine (DP focus)*

Quality Attributes	**Criticality Assignment**	**DS/DP**	**Rationale**
General physicochemical characteristics *Determination of the physicochemical properties of the product*			
Appearance: Color of solution	CQA	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, to exclude microbial contaminants
pH	CQA	DP	pH variations may lead to product degradation and hence impact efficacy;

Quality Attributes	Criticality Assignment	DS/DP	Rationale
Osmolality	CQA	DP	Osmolality is an indirect measure of product formulation and related to potential pain upon injection
Extractable Volume	CQA	DP	Variations in extractable/deliverable volume can result in incorrect dosing, impacting efficacy and safety
Excipients (Adjuvant) *A constituent other than the active substance, added in the formulation for a specific purpose.*			
Adjuvant content	CQA	DP	Critical to ensure the desired stimulation of the immune response
Adjuvant identity	CQA	DP	Using the wrong adjuvant would negatively affect efficacy and/or patient safety.
Antigen Content *A measure of the amount of product required to determine the dose*			
Protein content	CQA	DP	Critical to ensure that the expected amount of antigen is administered (DP);
Potency *A measure of the biological activity based on the attribute(s) of the product which is linked to the relevant biological properties*			
Confirmation of the right protein conformation	CQA	DS/DP	Critical to support biological activity
Potency test	CQA	DP	Critical for confirmation of biological activity
Identity			

Quality Attributes	Criticality Assignment	DS/DP	Rationale
Antigen identity	CQA	DP	Dosing with the wrong product would negatively affect efficacy and/or patient safety.
Product Related Substances and Impurities *Certain product variants, such as aggregates and viral protein degradation products, may have an impact on safety and efficacy*			
Protein degradation	CQA	DP	Impact on efficacy considering epitopes preservation issues
Aggregation	CQA	DP	Observed loss in potency upon inducing aggregation in stress studies
Process-related impurities			
Residual host cell DNA	CQA	DS/DP	
Residual host cell protein	CQA	DS/DP	
Contaminants			
Endotoxins/ pyrogens	CQA	DP	Increased risk of adverse events for patients.

*This list is illustrative and not intended to be exhaustive (all the mandatory tests should be performed according to the regional requirements)

Table 2 summarizes a guideline of illustrative analytical testing strategy.

Table 2 Analytical Testing Strategy

Category	CQA	Analytical Method	Drug Substance	Drug Product
	Attribute	Method ID	Release/stability/IP*	Release/stability/IP*

Category	CQA	Analytical Method	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 (e.g., Dye Ingress)	--	Stability
	Particulate Matter	Visibile Particles	--	Release and Stability
	Extractable Volume	Extractable or Deliverable Volume	--	Release
	pH	pH	IP, Release and Stability	Release and Stability
	Osmolality	Osmolality	Release	Release
Excipients (Adjuvant)	Content	Aluminium content (for alum based adjuvant)/ spectrofotometry (for DNA based adjuvant)	--	Release
Antigen Content	Protein content	Colorimetric	Release	Release
Protein Conformation	Confirmation of the right protein conformation	Circular Dichroism	Characterization	Characterization

Category	CQA	Analytical Method	Drug Substance	Drug Product
Potency	Potency test	IVRP	Characterization	Release, stability
Identity	Binding to specific antibody	ELISA	Release, stability	Release, stability
Product-related substances and impurities	degradant	SDS-PAGE, HPLC	Characterization	Characterization
Process-related impurities	Residual host DNA	PCR	Release	--
	Residual host protein	ELISA	Release	--
Contaminants	Endotoxin	LAL	Release	Release

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

2 Description of the Changes & Justification

This example focuses on comparability assessment between lyophilized product used in Phase 1/2 and the liquid formulation considered for Phase 3, to support Phase3 start without repeating Phase 1/2 studies. Further context of the change is provided in the Introduction. A list of changes impacting the drug product with its justification is reported in **Table 3** below.

Table 3 Changes to Drug Product

Change ID	**Description of changes**	**Pre-Change** (Toxicology/Early phase clinical trial)	**Post-Change** (Phase 3 clinical trial)	**Justification of the Change**
DP1	Site of Manufacture for DP/ scale	Pilot Plant, Lyo manufacturing	Phase 3 scale facility,	Volume needs for clinical studies
DP2	Formulation process	Lyo manufacturing for antigen, liquid formulation for adjuvant	Antigen- Adjuvant liquid formulation process	Change in presentation to simplify manufacturing, supply and administration
DP3	Excipients	Lyo formulation requiring bulking agent	Optimized formulation composition to maximize stability in liquid formulation	Change in presentation to simplify manufacturing, supply and administration
DP4	Physical state for antigen	Lyophilized	Liquid, adjuvanted	Change in presentation to simplify manufacturing, supply and administration
DP5	Specifications	*Lyo product*	*Liquid product*	Change in presentation to simplify manufacturing, supply and administration
		Bulking agent content (eg sucrose, mannitol, ...)	*No bulking agent*	Bulking agent is not needed for liquid formulation

Change ID	Description of changes	Pre-Change (Toxicology/Early phase clinical trial)	Post-Change (Phase 3 clinical trial)	Justification of the Change
		Residual moisture	*Not applicable*	Residual moisture is not a quality attribute of liquid formulation
		No adsorption test needed	*Adsorption %*	Percentage of Adsorption is not measured at release of the lyo product, since adjuvant and antigen are in contact just before administration
		Testing upon reconstitution with water	*Liquid formulation implies difference in analytical testing (eg sample prep)*	Buffer change; adjuvant and antigen in solution
DP6	Filling Line	*Lyo process*	*Liquid process*	Change in presentation to simplify manufacturing, supply and administration
	Storage conditions	*No change (2-8°C)*		

2.1 Change Impact Assessment

Change impact assessment on Critical Quality Attributes are reported in **Table 4** below.

Table 4 Drug Product - Change Impact Assessment on CQAs

Change ID	CQA / Attributes impacted	Justification
DP1 Site of Manufacture for DP/ scale	N/A	The change under evaluation and impact assessment is driven by formulation change-see below
DP2 Formulation process	See formulation components/ physical state discussions	See formulation components/ physical status discussions
DP3 Excipients	Bulking agent pre-change	Not present in liquid formulation
	Buffer components	Changed after buffer adaptation to liquid formulation- could have an impact on stability profiles (see adjuvant and physical state)
DP4 Physical state for antigen	Antigen degradation	Liquid formulation may imply a different stability profile for antigen vs lyophilized product. This could be detected through testing of the listed CQAs
	Antigen aggregation	
	Potency	
DP5 Specifications	See changes DP2-4	Some specifications test changed due to the change in formulation/ physical state
DP6 Filling line	See formulation components/ physical state discussions	See formulation components/ physical status discussions

3 Comparability Strategy

The comparability strategy between lyophilized and liquid formulation is grounded on analytical characterization, focusing on the CQAs which are known to be potentially impacted by the change. This side-by-side comparison is justified considering the stage of development of the product and the limited historical data/prior knowledge and is deemed appropriate as it is aligned with ICH Q5E principles, highlighting relevance of similarity of product quality expectations.

The analytical panel includes both release and additional characterization tests, as appropriate.

The analytical methods used for the impacted CQAs were the same for both formulations, as applicable, with verification of matrix change effect as part of the Phase 3 method qualification. All methods were qualified, and a commitment was taken to complete validation of specification methods (robustness assessment) after manufacturing of PPQ lots [**Ref 1**]. Prior knowledge was used to simplify and optimize method validation experimental activities.

To support demonstration of suitability of the selected analytical methods to detect changes in the CQAs, stress studies were performed on the liquid formulation, and the samples were tested according to analytical comparability panel, to confirm the ability of the analytical methods to monitor modifications.

In this context, stress and accelerated stability data, on both liquid formulation and (reconstituted) lyophilized vaccine, were used to optimize liquid formulation composition and to have a basis to compare stability profiles. Advanced stability modeling approaches were developed to support shelf-life estimation for the new formulation, in preparation of Phase 3 campaign and to support supply plans across different regions involved in Phase 3 and for future commercialization of the vaccine [**Ref 2 and Ref 3**]. Potency acceptance criteria were based on clinical evidence (upper limit based on potential safety concern, lower limit based on minimum active dose).

Phase 1/2 clinical lots (corresponding to the selected dose for Phase 3) were used as reference standards for Phase 3 potency testing of liquid formulation, to further support comparability.

For tests in release/ stability panel, acceptance criteria for comparability were those reported for specifications. For additional characterization tests, acceptance criteria were set considering the observed difference between stressed and un-stressed samples, based on the analytical method suitability studies reported above.

Table 5 Comparability strategy

Change ID	Affected Step	Affected Attributes	Analytical Method	Comparability Acceptance Criteria	Justification
DP4 Physical state for antigen DP5 Specifications	N/A	Antigen degradation	UPLC	Specifications	In this lifecycle state and context, reliance on the product quality criteria for comparability is appropriate as no other data are available.
		Antigen aggregation	SEC-MALLS	Specifications	
		Potency	IVRP	Specifications	
		Antigen conformation	Circular Dichroism	Comparable profile and outcome of stress studies	No alteration of conformation observed
		% of adsorption	UPLC after desorption	Liquid formulation specifications (also for Lyo reconstituted with adjuvant)	In this lifecycle state and context, reliance on the product quality criteria for comparability is appropriate as no other data are available.

4 Results and Analysis

The analytical characterization of the compared lots demonstrated no relevant impact on CQAs due to the formulation change, at release as reported in **Table 6**.

Table 6 Analytical characterization of the compared lots

Quality Attributes	**Pre-Change**				**Post-Change**		
	Ph1 Lot1	**Ph1 Lot2**	**Ph2 Lot1**	**Ph2 Lot 2**	**Ph3 Lot 1**	**Ph3 Lot 2**	**Ph3 Lot 3**
Antigen degradation	<5%	<5%	<5%	<5%	<5%	<5%	<5%
Antigen aggregation	8%	9%	9%	10%	<5%	<5%	<5%
Potency	0.8	0.9	0.8	0.8	0.9	0.9	0.8
Antigen conformation	Conform	Conform	Conform	Conform	Conform	Conform	Conform
% adsorption	>90%	>90%	>90%	>90%	>90%	>90%	>90%

Accelerated stability results (3 months), combined to predictive modelling assessment, highlighted different profiles between lyophilized and liquid formulations, as expected due to the different physical state, but confirmed CQAs values within specifications for liquid formulation up to the end of shelf life, proposed to be 2 years. Commitment was taken to continue real time stability studies and share timely updates with the relevant authorities Provided that there are no safety concerns, the selection of a dose higher than the minimum active provides further support to the proposed strategy for comparability and stability assessment, considering the fact that a certain level of degradation could be considered acceptable, if resulting in an effective dose within the minimum and the selected dose.

5 Conclusion

Based on the reported analytical comparability, it was concluded that changing from lyophilized to liquid adjuvanted formulation for the vaccine under study did not have a significant impact on product quality, and Phase 3 progression was proposed, with an expected shelf life of 2 years for the liquid formulation. Due to the heavily accelerated scenario, the following risk- based approaches were considered:

Use of analytical comparability, with focus on potentially impacted CQAs, identified through appropriate stress and accelerated stability studies

Reliance on modelling strategies to support stability assessment as part of the comparability exercise and for shelf-life proposal

Smart use of dose- finding, implying the selection of a safe dose higher than the minimum active, to support potency limits and stability assessment strategy

Risk- based analytical method validation, using prior knowledge to support simplification/ optimization of validation activities and deferring some evaluations (eg robustness assessment).

Early discussion with relevant Authorities was ensured to confirm suitability of the proposed approaches.

6 References

Table 7 References

Ref N°	Title/ link
Ref 1	*https://www.ema.europa.eu/en/documents/assessment-report/spikevax-previously-covid-19-vaccine-moderna-epar-public-assessment-report_en.pdf*
Ref 2	*https://media.tghn.org/medialibrary/2020/12/20201209_COVAX_Storage_temperature_and_shelf_life_workshop_presentation.pdf*
Ref 3	*Campa C, Pronce T, Paludi M, Weusten J, Conway L, Savery J, Richards C, Clénet D. Use of Stability Modeling to Support Accelerated Vaccine Development and Supply. Vaccines (Basel). 2021 Sep 30;9(10):1114*