BioPhorum
CELL & GENE THERAPY

Industry recommendations for phase appropriate approach for assay validation in Cell and Gene Therapies (CGTs)

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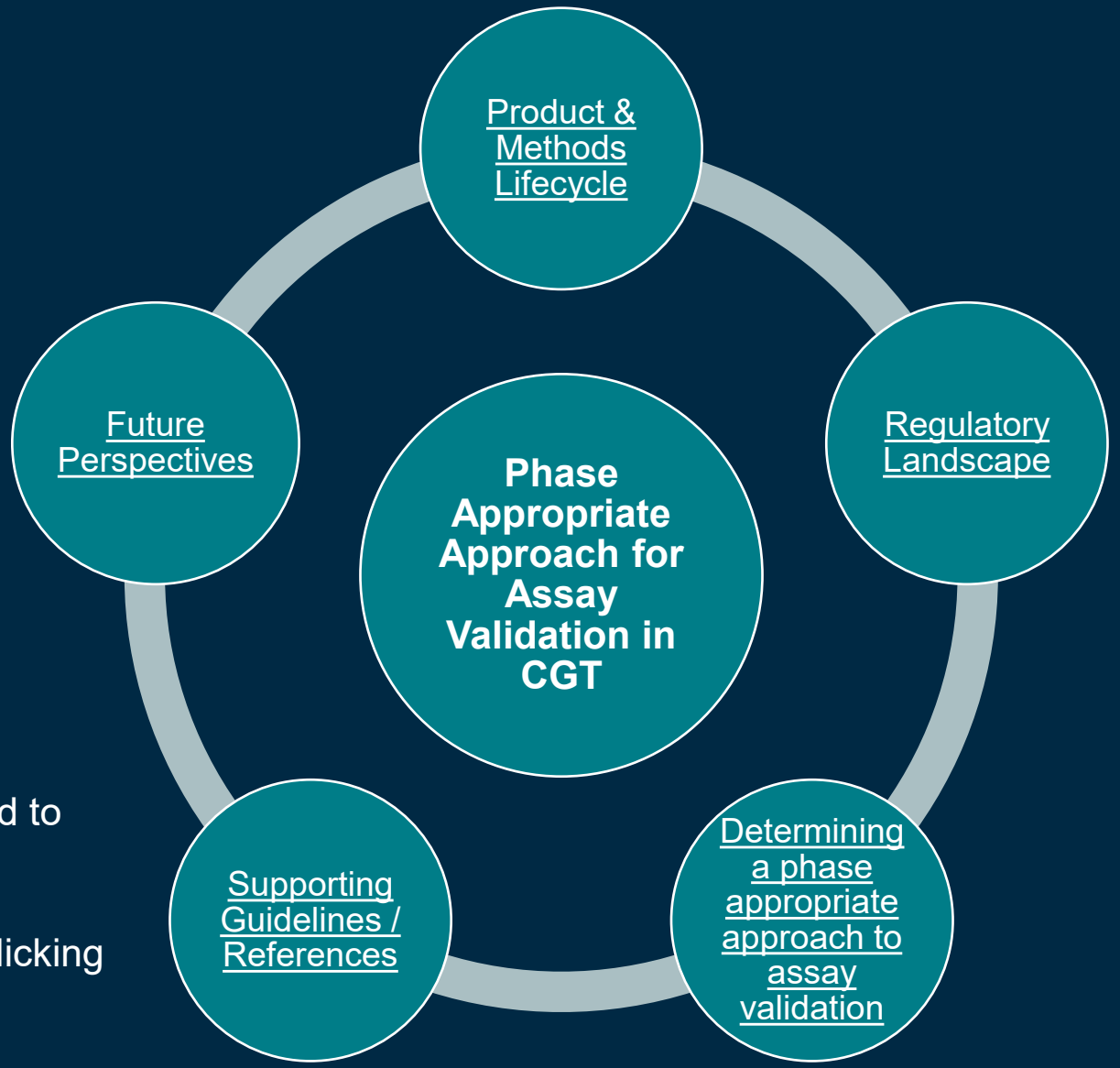
Navigation Hub



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Executive Summary

A phase-appropriate approach to assay validation continues to be a widely accepted and adopted strategy to support the clinical development of general biologics; cell and gene (CGT) therapies are no exception. However, current regulatory guidance is inadequate in regard to sufficiently guiding and supporting phase-appropriate readiness of analytical assays used in all phases of CGT clinical development and regulatory filing. This insufficient clarity has led to a lack of consensus within the cell and gene community for the phase-appropriate development and validation of the analytical assays utilized at all phases of the CGT product life cycle.

The result of having insufficient and/or inadequate data packages leads to an increased risk of delays in regulatory filing and approval of the clinical candidates. Given the often-accelerated pace of CGT clinical programs for therapies targeted for patients with unmet medical needs or where other traditional treatments may have been insufficient, any delays to the regulatory approval process could have a significant impact on the availability of drugs to patients.

In this presentation, members of BioPhorum's Cell & Gene Therapy Phorum present their consolidated opinions and recommendations with an aim to promote alignment on a common phase-appropriate approach to analytical assay validation with respect to the critical quality attributes of the most common CGT modalities. The team's recommendations are aimed towards providing a faster and more efficient route to CGT product development that is compliant with the regulatory standards.

Table of abbreviations

Abbreviation	Term
AAV	Adeno-Associated Virus
ATPs	Analytical Target Profiles
AUC	Analytical Ultracentrifugation
BLA	Biologics Licence Application
CAR-T	Chimeric Antigen Receptor T Cell Therapy
CDMO	Contract Development and Manufacturing Organization
CE-SDS	Capillary Electrophoresis Sodium Dodecyl Sulfate
CGTs	Cell and Gene Therapies
CI	Confidence Interval
CMC	Chemistry, Manufacturing and Controls
CFU	Colony-Forming Unit
cIEF	Capillary Isoelectric Focusing
Cryo-TEM	Cryogenic Transmission Electron Microscopy
CTA	Clinical Trials Application
CQAs	Critical Quality Attributes
ddPCR	Droplet digital Polymerase Chain Reaction
DL	Detection Limit
DOEs	Design of Experiments
DLS	Dynamic Light Scattering
ELISA	Enzyme-Linked Immunosorbent Assay

Abbreviation	Term
GMP	Good Manufacturing Practice
HPLC	High Performance Liquid Chromatography
LC-MS	Liquid Chromatography Mass Spectrometry
LoD	Limit of Detection
LoQ	Limit of Quantitation
MAA	Marketing Authorization Application
MFI	Multiplex Fluorescent Immunoassay
MoA	Mode or Mechanism of Action
MS	Mass Spectrometry
NGS	Next Generation Sequencing
NTA	Nanoparticle Tracking Analysis
pCQAs	Potential Critical Quality Attributes
PERT	Product-Enhanced Reverse Transcriptase
PPQ	Process Performance Qualification
QL	Quantitation Limit
qPCR	Quantitative Polymerase Chain Reaction
QTPP	Quality Target Product Profile
rAAV	Recombinant Adeno-Associated Virus
RT-PCR	Reverse transcription Polymerase Chain Reaction
RVV	Retroviral Vector

Abbreviation	Term
SDS-PAGE	Sodium Dodecyl Sulfate – Polyacrylamide Gel Electrophoresis
SEC	Size Exclusion Chromatography
SEC-MALS	Size Exclusion Chromatography with Multiangle Light Scattering
$TCID_{50}$	Tissue Culture Infectious Dose (50)
TEM	Transmission Electron Microscopy
TOST	Two One-Sided Test
TPPs	Target Product Profiles

Table of definitions

Term	BioPhorum definition	Timing
Early phase	Considered as a representative term applicable to product development during Phase I and or Phase II clinical trials.	N/A
Late phase	Considered as a representative term applicable to product development during Phase II Pivotal or Phase III clinical trials.	N/A
Commercial phase	Considered as a representative term applicable post-licensure approval.	N/A
Method Development*	The experimentally based selection of equipment, consumables & reagents and processes in order to create a non-GxP analytical method capable of measuring an intended attribute for an intended purpose. In general, method development will include technology selection, feasibility, optimization studies to assess critical assay parameters. It might also require a pre-qualification of the assay that will be subsequently qualified and eventually validated in the GMP setting. Robustness parameters are typically assessed providing initial information on what conditions are critical. Additional parameters ought to be assessed as part of trending (e.g., lot of reagents). The data collected during development and trending should be summarized in the method validation report (USP 1220), alongside any additional robustness parameter assessed during formal validation. Output manifested as "method development report“	This may refer to the initial assay development conducted ahead of assay qualification for phase I and for continuous improvement throughout assay lifecycle
Qualification	The documented activity of determining the performance capabilities of a method through laboratory studies such that an assessment of the method’s applicability for intended use can be made. Qualification studies typically follow the method development phase and precede validation studies. The method qualification is recommended to be conducted under a formal protocol with ‘proposed’ preliminary acceptance criteria. Analytical method qualifications will cover the same aspects, characteristics, and principles of the validation studies as outlined in ICH and USP guidelines, except for robustness and reproducibility. Parameters determined during qualification will include linearity and range, specificity, accuracy, quantitation limit (QL), detection limit (DL), and precision as applicable for the type of assay. In instances where the assay is to be performed across sites, reproducibility ought to be assessed, as well.	Intended for early phase product (phase I/II),
Matrix Qualification	Activity performed on specific sample matrices to understand a specific matrix interference when the method has been previously qualified or validated as a generic platform method. While a risk-based approach may be used to determine the appropriate attributes to be assessed, both product and process (i.e., the analytical method) should be evaluated jointly. The matrix qualification is protocol driven and should formally be recorded as an extension to the qualified/validated status of the method.	At time of change of matrix to support demonstrating qualified/validated status of the assay for specific matrix. Ahead of use on GMP samples
Validation	A validation is the process by which there is established documented evidence through defined tests and challenges that an analytical method meets the design criteria (i.e., pre-determined specifications established by a qualified method) and that an adequate control strategy is established to ensure that it will continue to produce reliable data that meets predetermined performance criteria. Typical parameters, as outlined in ICH and USP guidelines, should be aligned with the previously executed qualification studies whereby the equivalent attributes in the qualification studies of linearity and range, specificity, accuracy, quantitation limit (QL), detection limit (DL), and precision will be evaluated. This is a GMP activity to establish by laboratory studies that the performance characteristics of the analytical method meet the requirements for the intended use as a GMP lot release/stability testing method.	Generally, though not always, intended for late phase products - timing of assay validation activities will be dependent on the specific assay and the context which is it being used.
Platform Method	"A platform analytical procedure can be defined as a multi-product method suitable to test quality attributes of different products without significant change to its operational conditions, system suitability and reporting structure. This type of method would apply to molecules that are sufficiently alike with respect to the attributes that the platform method is intended to measure." *ICH Q2(R2)***.* The use of platform methods can reduce the method development time and allow method validation data to be leverage across various product reducing time and material requirements. The need for matrix verification (i.e., no adjustment of the compared process or method is needed to achieve results with acceptable accuracy to the original standard or process) should be formally risk assessed and documented, and if necessary, executed as a formal study in line with assay use. The assessment should consider any minor product specific handling or method adjustments within the scope of the platform method to determine the level of additional validation required and associated documentation.	N/A

***May also be termed "Feasibility Study" if method is transferred to CDMO from customer.**
**** In draft and likely to be finalized in Q4 2023**

Introduction

Cell and Gene Therapies (CGTs) are a new and evolving class of biopharmaceuticals that have tremendous potential in the treatment of disease. The approval of products such as Luxturna, Zolgensma, Yescarta and Hemgenix, to name a few, have provided an enticing path for the use of gene therapy as a one-time therapeutic approach for the treatment of monogenic diseases. Analytical methods play a critical role in successful clinical development of a drug product and commercial launch; the BioPhorum Cell and Gene Therapy (CGT) High Level Analytics workstream was established to unite analytical subject matter experts working in this rapidly expanding field to accelerate shared understanding and best practice approaches.

As is the case in the development of all medicinal products, it is expected that the associated analytical methods (as well as the manufacturing process, the final product presentation, and the control strategy) will evolve with increasing process and product knowledge as programs progress further deep into clinical development. However, owing to the relative inexperience with CGT modalities compared with more established platforms (e.g., protein biologics) that have greater regulatory precedence, coupled with significantly shorter development timelines and the added complexities of CGT manufacturing processes, developers of these advanced medicinal therapies face additional challenges to support assay development and validation activities than what is perhaps seen for other modalities.

However, it may yet be possible to draft a strategy that provides a foundation for sponsors of CGT modalities to address phase-appropriate development and validation of analytical methods for their CGT products.

This presentation documents the recommendations from a collaboration of CGT Industry experts and aims to promote Industry alignment on a common strategy for phase-appropriate approach to analytical method development and validation that is compliant, and that represents an efficient pathway towards each stage of filing. Considering the critical quality attributes (CQAs) of some of the most common, current CGT modalities (e.g., in vivo gene therapy (AAV) and ex vivo gene modified CT (e.g., CAR-T)), a series of tables outlining CQAs per product class, and methods that can be used for their measurement is presented. Together with recommendations on the timing and rigor of validation activities commensurate with each phase, this presentation is designed to help sponsors prioritize their assay validation efforts.

Product and Methods Lifecycle

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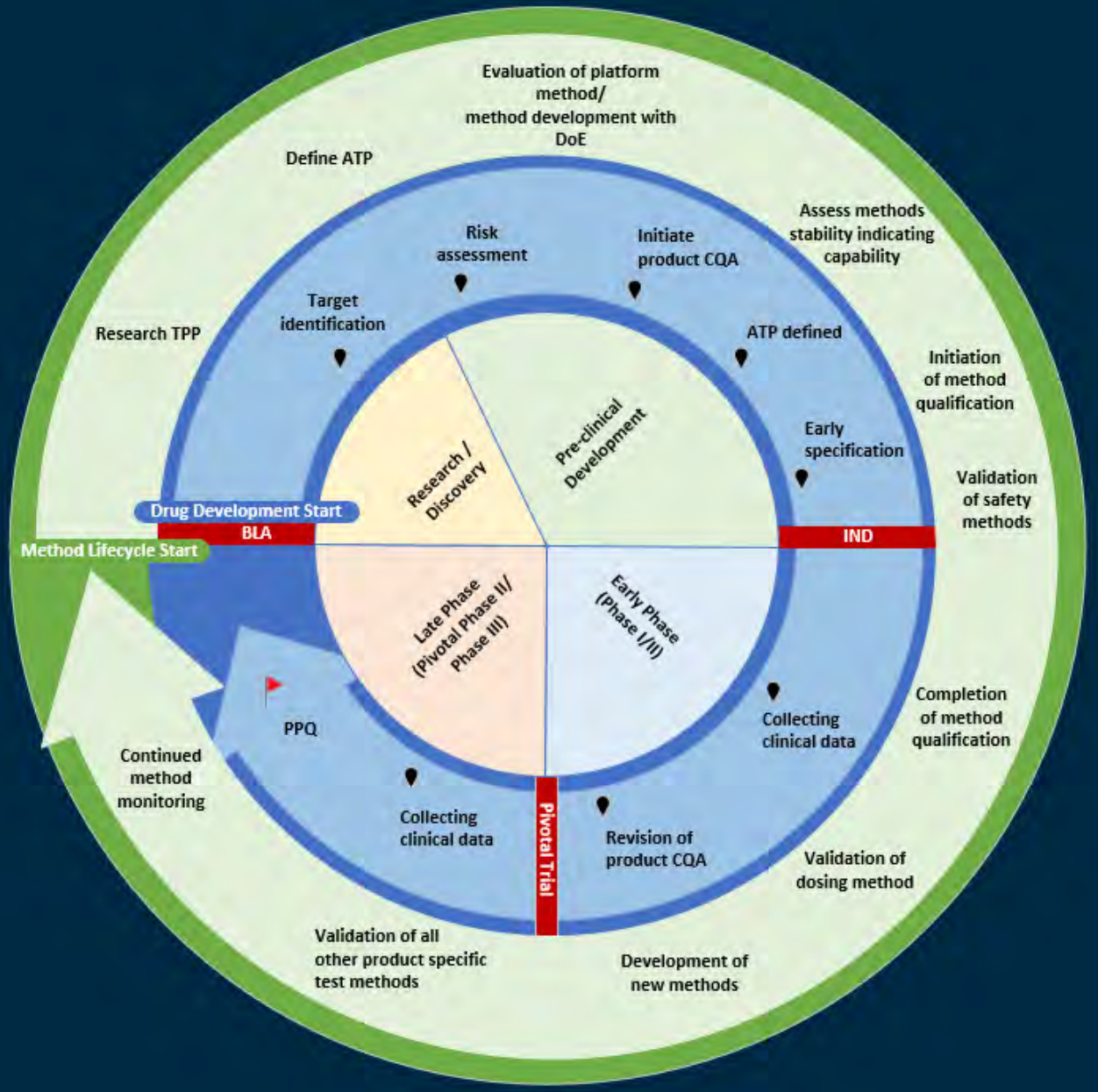
Product and Methods Lifecycle

CGT products typically aim to treat patients with rare, life limiting and/or threatening conditions where no other viable treatment or cure exists. To aid the sponsors' ability to bring these promising therapies to the marketplace as early as possible, regulatory agencies have established industry guidelines and frameworks to expediate the development and approval process. However, there is insufficient guidance detailing how CGT developers can accelerate chemistry, manufacturing and controls (CMC) development whilst ensuring the processes, and products are fully characterized and controlled. In addition, because CGT modalities are relatively new, many CQAs may not yet be known nor fully understood, and well-defined platform methods for each CQA may not exist.

Each CGT product class has a unique product profile, attribute, manufacturing process and control requirements. Therefore, each product class requires its own considerations for assay validation. This presentation outlines a framework for analytical method lifecycle management, and provides a phase-appropriate approach for method validation for typical CQAs across the differing CGT modalities

While not completely inclusive, the slides will provide an illustration of some of the key activities and milestones important for the development of medicinal products from R&D through to application for licensure. The schematic diagram as developed by BioPhorum members provides a high-level overview of the CMC efforts generally considered to be important in the drug product development life cycle (blue arrow) relative to a proposed analytical method life cycle approach for CGTs (the green arrow).

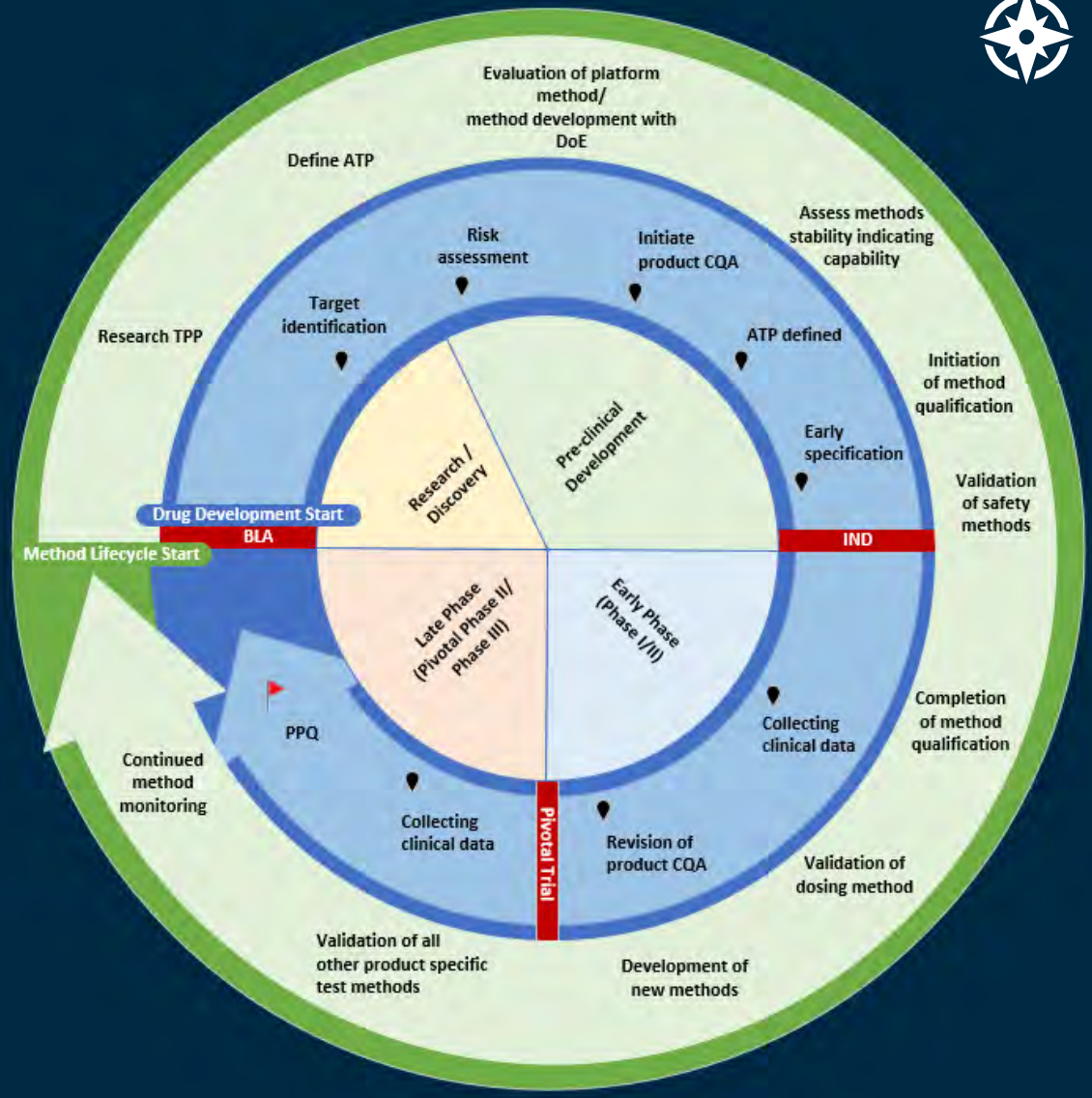
Product and Methods Lifecycle



Product and Methods Lifecycle

The life cycle of analytical methods is closely aligned to the product lifecycle

- Biological products are developed against the target product profile (TPP) which informs the desired attributes of the therapeutic product.
- During pre-clinical development, product quality risk assessments help inform the potential product critical quality attributes (pCQAs) that in turn will inform the phase appropriate analytical assay validation strategy.
- It is not unusual to have majority (if not all) of quality attributes considered as pCQAs at preclinical / early clinical development.
- Method development activities are performed to establish methods to address the pCQAs prior to initiating first in human studies, i.e., to meet the established ATP.
- Method summaries and any available method qualification data is included in the clinical trials application (CTA).
- Following successful CTA filing, the program moves into the early clinical phase to collect data to inform on the safety and efficacy of the therapeutic product.
- CQAs will be refined as new methods are developed/implemented and additional process/product knowledge is gained throughout the clinical development phases.
- As the products move into the pivotal trial phase & PPQ lots, method validation studies ensure analytical data is fit for purpose and robust to meet performance criteria defined in the Analytical Target Profile (ATP).
- Where products have accelerated approval pathways CMC development timelines may be reduced to support early pivotal trials. Additional agency interactions can be sought to agree development plans in line with approval timelines.
- Final methods and support data are submitted in the Biological Licence Application/Marketing Authorization Application (BLA/MAA) filing to move the program into commercial manufacturing.
- Continued method performance monitoring provides ongoing assurance throughout lifecycle.

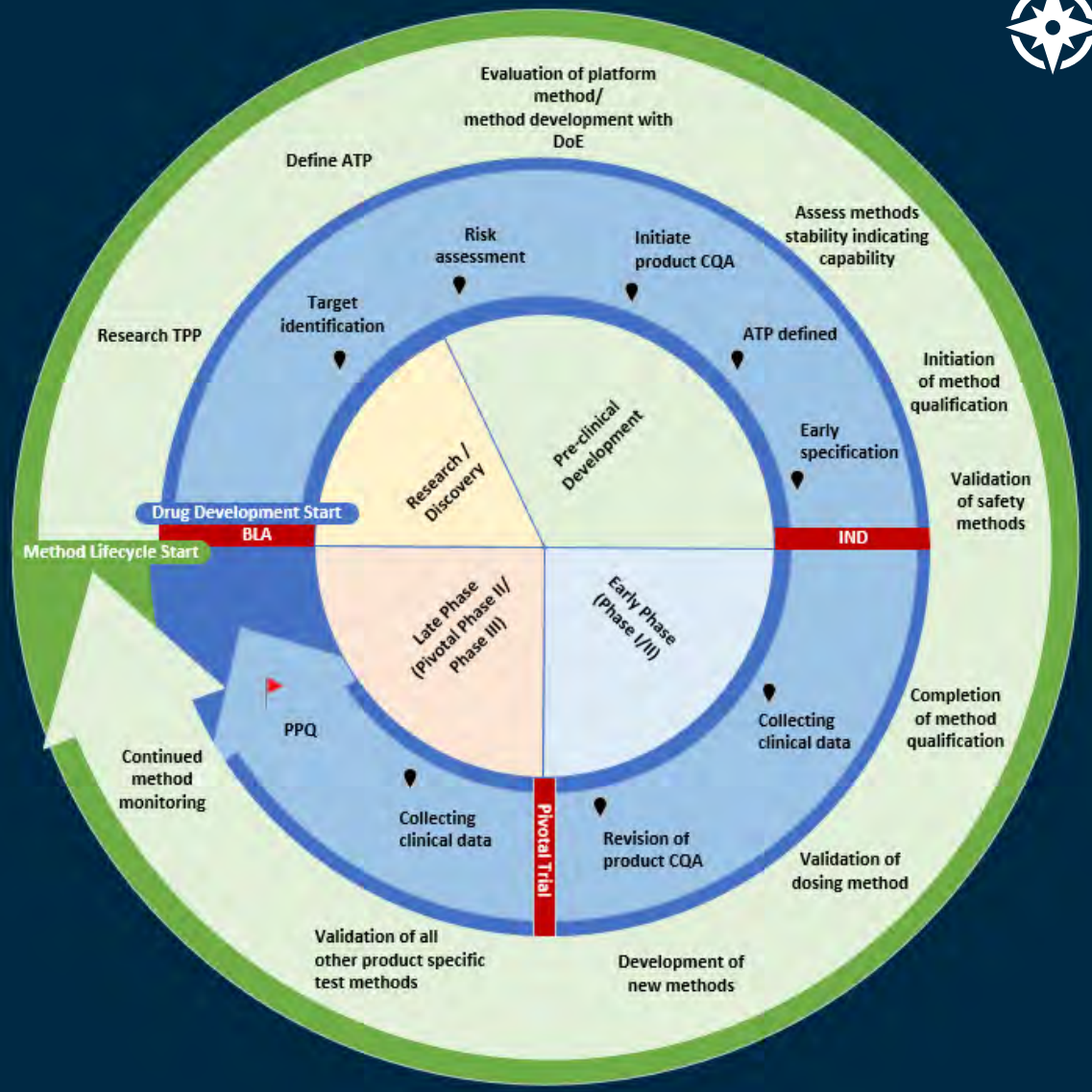


This sub team acknowledges that not all CGTs will necessarily follow linear development as depicted in the figure (e.g., for products where there is an unmet medical need, development activities need may be fast-tracked / compressed

Product and Methods Lifecycle

Analytical methods follow a risk-based method lifecycle to ensure they are suitable

- Throughout pre-clinical development, analytical method development is initiated, and the ATP is defined
- The ATP is a prospective, technology independent, description of the desired performance of an analytical procedure, and it defines the required quality of the reportable value produced by the procedure
- The ATP is based on the intended use for the procedure and should include target precision and accuracy (bias), serving as a basis for procedure qualification criteria, and a guide for monitoring of the procedure during its life cycle. When possible, the target performance should be based on process control strategy requirements
- Early feasibility assessments may determine the suitability of any pre-existing platform methods for new product, otherwise, new method(s) may be developed
- Method development should support the understanding of procedure parameters that may impact assay performances (e.g., sample preparation, number of replicates). Risk assessments are recommended to identify the procedure parameters to investigate
- Execution of DOEs define assay parameters for optimum performance, and should also evaluate which methods may be capable of assessing product stability
- As the product moves into early phase clinical development, analytical assay qualification activities are executed to demonstrate the methods are fit for the intended purpose, and performances in line with ATP.



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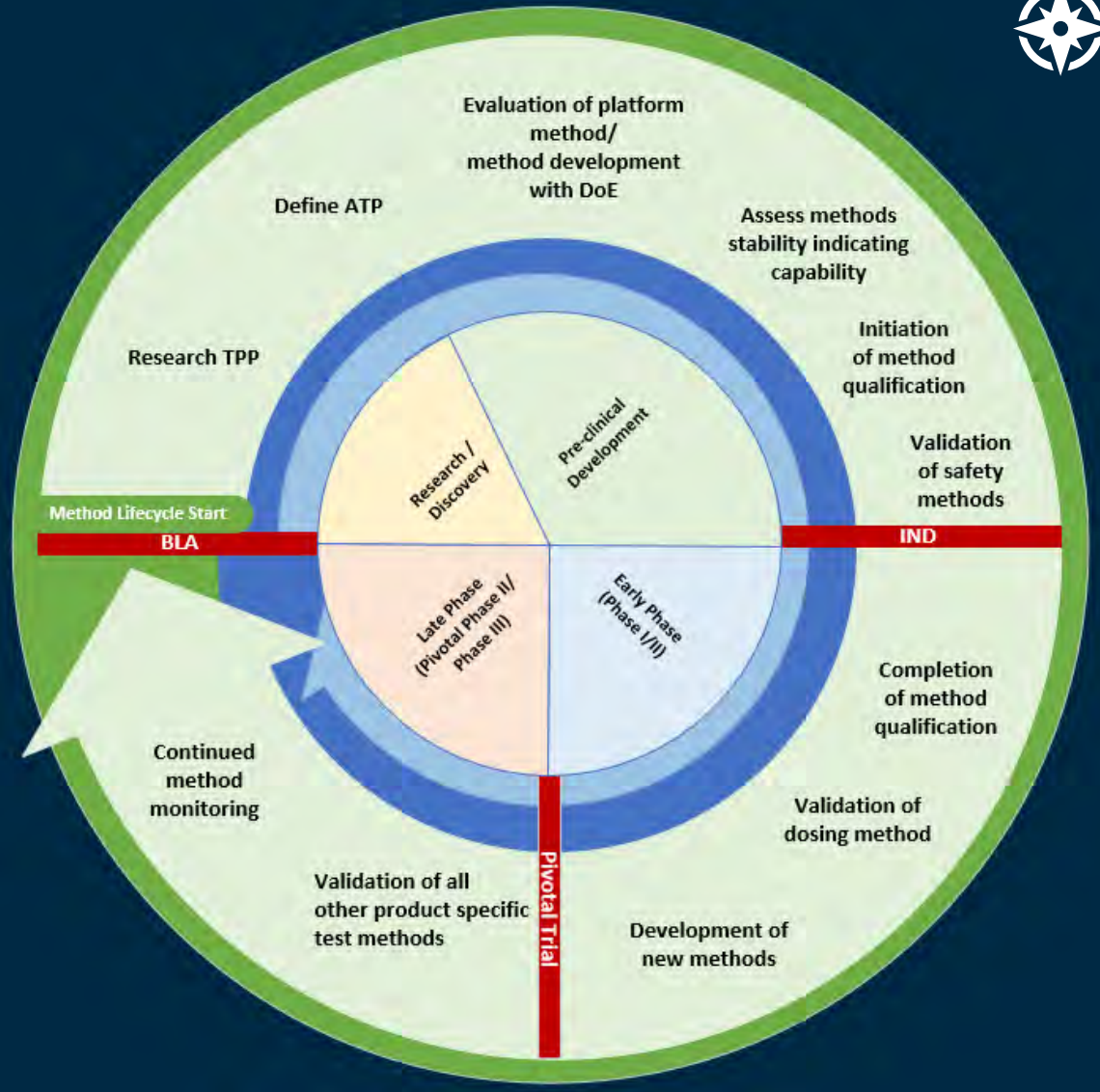
Product and Methods Lifecycle

Analytical methods follow a risk-based method lifecycle to ensure they are suitable

- Assay trending and monitoring, against ATP, should be leveraged to further understand assay performance and variability beyond what can be explored during assay qualification.
- Performance monitoring should be leveraged to understand the impact of process and product variation on assay performances beyond what can be explored during qualification.
- Throughout product development new analytical methods may be developed to build process understanding which are required to be qualified and validated as appropriate to the clinical phase.
- During late phase clinical development method validation activities are undertaken to formally demonstrate assay performance to appropriate criteria. Due to the rapid nature of CGT development, method validation may take place throughout phase III clinical trials.
- Where products have accelerated approval pathways, method validation activities may be required at earlier stages to support pivotal clinical trials.
- Where platform methods exist, prior method qualification/ validation data may be leveraged to reduce the level of assessment required at each stage.
- It is mandatory that by product registration (BLA/MAA) that all assays required for release and stability analysis are validated to ICH standards.
- Following PPQ and post approval, the methods will be continually assessed or verified as per life cycle and follow post approval change guidelines if any method needs to be changed.



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Analytical Method Bridging

When a new/revised method with improved robustness, sensitivity or accuracy and operational simplicity is developed to support clinical lot release and stability, replacing the existing method requires a bridging study. Bridging studies may also be required when previous method is no longer available (e.g., reagents, equipment, supplier).

At a minimum, bridging studies should be anchored to historical, well established and qualified or validated method. Typically, it follows on from the new method qualification and/or validation activity.

Based on ICH guideline Q14 on analytical procedure development (March 2022 Draft), the design and extent of the studies needed to support the change including an appropriate bridging strategy to establish the numerical relation between the reportable values of each of the methods, and its impact on the specification of the product.

Various approaches can be undertaken, and the selected bridging strategy should be risk-based dependant (including statistical support) on product development stage, ongoing studies, number of retention of historical batches etc.

Method Bridging Examples

Single Lot Between the Two Methods

- A protocol driven study design for a sample series with a single lot
- Testing between the two methods
- Compare the accuracy/precision
- Determine an appropriate confidence interval (CI) of the recovery difference using a TOST (two one-sided test) and compare the result to the equivalency bounds.

Multiple Retention Lots with the New Method

- A wide variety of samples (e.g., lot release, stability, stressed, critical isoforms) should be tested under a protocol driven study using the new analytical procedure.
- Determine an appropriate confidence interval (CI) of the difference between methods and compare the result to the equivalency bounds.

Regulatory Landscape

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Regulatory Landscape - Guidelines associated with analytical methods development and validation

Reliable analytical methods are required to have assurance of the quality of the therapeutic product with respect to its safety, identity, purity and strength, and/or potency. Sponsors are expected to provide sufficient information in their regulatory submissions relevant to the risk associated with the intended use of the analytical method. A development phase-appropriate approach to generate the data regarding the reliability of the method, also known as validation is allowed by regulatory agencies.

According to the US FDA's CMC guidance for investigational gene therapies, validation of analytical procedures is usually not required for original IND submissions for phase I studies; however, it should be demonstrated that test methods are appropriately controlled. In general, scientifically sound principles for assay performance should be applied (i.e., tests should be specific, sensitive, and reproducible and include appropriate controls or standards). They recommend the use of compendial methods when appropriate and qualify safety-related tests prior to initiation of clinical trials. To ensure safety of gene therapy products, the assays used to determine dose (e.g., vector genome titer by qPCR, transducing units, plaque forming units, transduced cells) should be qualified prior to initiating clinical studies. In addition, assays used to measure replication competent vectors should also meet current FDA recommendations for sensitivity at an early stage of development.

Although the EMA is similar in their requirements, they include higher expectations for safety tests. According to the GMP for ATMPs guidance from the European Commission,

- First-in-human and exploratory clinical trials: Sterility and microbial assays should be validated. In addition, other assays that are intended to ensure patient's safety should also be validated (e.g., when retroviral vectors are used, the analytical methods for testing for replication competent retrovirus should be validated).
- Throughout the clinical development, the suitability of analytical methods used to measure critical quality attributes (e.g., inactivation/removal of virus and/or other impurities of biological origin) should be established but full validation is not required. Potency assays are expected to be validated prior to pivotal clinical trials.
- Pivotal clinical trials: Validation of analytical methods for batch release and stability testing is expected.

It can be inferred from the above that more information is expected for product safety-related assay early in clinical development. For other assays, there should be sufficient information on suitability based on their intended use in the manufacturing process. Confusion exists as exact expectations for the different types of assay per phase are not clearly outlined. This presentation seeks to help eliminate the confusion and suggest phase-appropriate requirements for some commonly used gene therapy types.

Regulatory Landscape

Guidelines associated with analytical methods development and validation

Issuing body	Title	Date published
FDA	Chemistry, Manufacturing, and Control (CMC) Information for Human Gene Therapy Investigational New Drug Applications (INDs); Guidance for Industry.	2020
FDA	Considerations for the Development of Chimeric Antigen Receptor (CAR) T Cell Products; Draft Guidance for Industry.	2022
FDA	Chemistry, Manufacturing, and Control (CMC) Information for Human Somatic Cell Therapy Investigational New Drug Applications (INDs); Guidance for industry.	2008
FDA	Potency Tests for Cellular and Gene Therapy Products; Guidance for Industry.	2011
FDA	Analytical Procedures and Methods Validation for Drugs & Biologics; Guidance for Industry.	2014
EMA	Draft guideline on quality, non-clinical and clinical requirements for investigational advanced therapy medicinal products in clinical trials.	2018
EMA	Requirements for quality documentation concerning biological investigational medicinal products in clinical trials	2019
EMA	Guidelines on good manufacturing practice specific for advanced therapies.	2017
EMA	Quality, non-clinical and clinical aspects of medicinal products containing genetically modified cells.	2021
ICH Q2(R2)*	Validation of Analytical Procedures	2022
ICH Q14*	Analytical Procedure Development	2022
USP <1220>	Analytical Procedure Life Cycle	2022

*In draft and likely to be finalized in Q4 2023.

Note: this is not an exhaustive list

Determining a phase appropriate approach to assay validation

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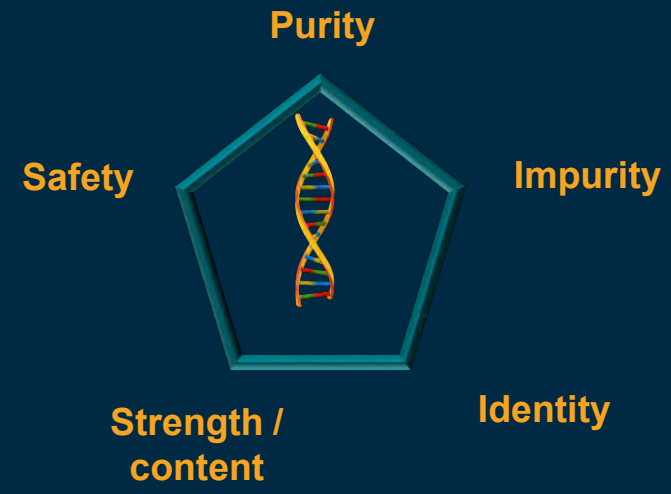
CGT Critical Quality Attributes

Pharmaceutical development, as described in ICH Q8(R2), requires quality attributes be assessed for their criticality to determine their impact on the quality of the final product. The CQAs may be a physical, chemical, biological or microbiological property or characteristic that should be within an appropriate limit, range or distribution to ensure the desired product quality. CQAs are generally associated with the drug substance, excipients, intermediates (in-process materials) and drug products. Where data are not available, ICH Q11 recognizes that the impact of different product variants and impurities on clinical efficacy, pharmacokinetics, and patient safety can be determined through a risk-based assessment.

As guided by ICH Q8(R2) and ICH Q11 a cascade of interacting elements is defined:

- Creation of target product profile (TPP) for the drug being developed
- Definition of quality target product profile (QTPP) based on the TPP
- Establishment of critical quality attributes (CQAs) by a risk-based assessment



Typically, product CQAs are generated at the preclinical stage and reviewed formally at each development phase and finalized before commercialization thus forming the basis for product specifications, and the product and process control strategy. As such, at preclinical and early development stages CQAs may be consider potential CQAs (or pCQAs) under further data and product knowledge is acquired.

The following slides detail examples of pCQAs for various CGT modalities which would be risk assessment for each product under development. Where product knowledge is limited, some attributes may be considered pCQAs and evaluated through product characterization studies however, where attributes are risk assessed as likely to have an impact on product safety and efficacy, these attributes should be controlled through product release testing.

Note: General pharmacopeia methods such as pH, appearance, sub-visible particles and volume in container undergo pharmacopeial method verification rather than validation, therefore are not detailed in the coming sections.

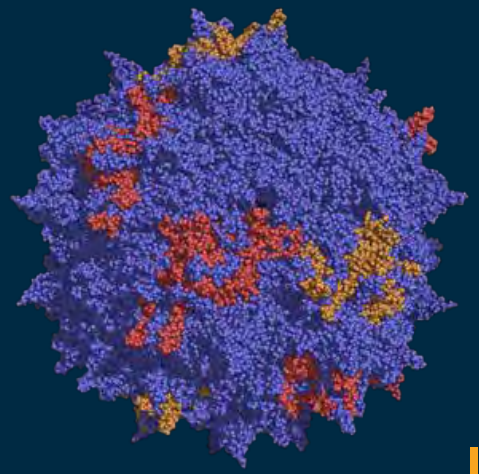
pCQAs – AAV

Purity

Capsid Purity
%Full Particles
DNA homogeneity
VP Ratio
Vector genome integrity

Safety

Sterility
Bioburden
Rhabdovirus
Adventitious agents
Replication competent virus
Mycoplasma
Endotoxin
Mycobacterium



Identity

Transgene (genome) identity
Capsid identity
Peptide mapping
Post-translational modifications

Strength/ Content

Dose (Vg Titer)
Capsid Titer
Potency (expression)
Infectious Titer
Potency (functional)

Impurity

Host cell protein
Host cell DNA & RNA
Particulates
Defective/ non-infective particles
Packaged host cell DNA
Residual Baculovirus titer
%Empty capsids
Residual plasmid
Aggregates

pCQAs – CAR-T

Purity

Cell marker expression
(e.g., CD3+, CD34+)

Safety

Sterility
Mycoplasma
Bioburden
Endotoxin
Adventitious agents
Replication competent virus
Cytotoxicity
Cytokine independent proliferation
(allogeneic products)
Oncogenesis

Identity

Transgene (genome) identity
Cell identity

Strength/ Content

Vector copy number
Cell number
% Viability
% Transduction
Potency
On-Target editing frequency

Impurity

Unintended cellular populations
Residual process additives

Phase appropriate status rAAV Gene Therapy Drug Product,
Quality attributes and assays for release

Quality Attribute Category	Quality Attribute	Example of Analytical Method / Technology (for release)	Recommended Phase Appropriate Status (Release Only)		
			Early Phase (Phase I)	Early Phase (Phase II)	Late Phase (Phase III / Commercial)
Safety	Sterility[1]	Compendial[3]	Verified		
	Mycoplasma[1]	Compendial[3] / PCR	Verified		
	Bioburden[1]	Compendial[3]	Verified		
	Endotoxin[1]	Compendial[3]	Verified		
	Adventitious virus[2]	Various (qPCR, Plaque, TEM, PERT)	Qualified		Validated
	Replication competent virus[2]	Cell-based assay	Qualified / Validated		Validated
	Rhabdovirus[4]	RTPCR	Qualified		
Strength / content	Dose (VG titer)	qPCR / ddPCR	Qualified	Validated[5]	
	Potency (functional)	Product-specific MOA - reflective relative potency	Qualified [6]		Validated
	Potency (expression)	Target expression (e.g., ELISA, qPCR)	Qualified		Validated
	Infectious titer / Relative infectivity	TCID50, CFU, ddPCR, Imaging	Qualified		Validated
	Capsid titer	ELISA, SEC	Qualified		Validated
	Excipient Content	Various techniques specific to the excipient(s)	Qualified		Validated
Purity	Capsid Purity (VP / CP ratio)	CE-SDS / SDS-PAGE	Qualified		Validated
	% full particles	HPLC, AUC, SEC-MALS	Qualified		Validated
Impurity	Host cell DNA & RNA	qPCR / ddPCR	Qualified		Validated
	Host cell protein	ELISA	Qualified		Validated
	% empty capsids	HPLC, AUC, SEC-MALS	Qualified		Validated
	Aggregates, particulates	SEC-MALS, AUC, DLS	Qualified		Validated
	Residual helper plasmid[7]	qPCR / ddPCR	Qualified		Validated
	Residual helper virus[7]	qPCR / ddPCR	Qualified		Validated
	Leachable affinity ligand	ELISA	Qualified		Validated
	Residual process additives (transfection reagents, Triton X-100, PEI, Polysorbate 20)	Various (HPLC, LC-MS, ELISA, Enzyme activity)	Qualified		Validated
Identity	Transgene (genome) identity	qPCR / ddPCR, ELISA, restriction digestion, Sequencing (Sanger)	Qualified		Validated
	Capsids identity	Serotype specific ELISA, LC-MS	Qualified		Validated

1. Testing should be performed at each stage of production at which contamination is most likely to be detected
2. Testing should be conducted on material collected at appropriate stage of manufacturing process
3. Analytical procedures different than those outlined in compendia e.g., rapid sterility tests or rapid mycoplasma tests, may be acceptable alternatives provided sufficient justification provided to the regulator
4. Specifically for insect cells, testing should be conducted as per regulatory guidelines on a selected number of batches.
5. Must be validated before registrational studies (PhII pivitol and/or PhIII)
6. If Mechanism of Action (MOA) is understood then expectation is that early phase should be at least qualified, if the MOA is not fully understood then the alternative potency assay i.e. target expression should be minimally qualified.
7. Specifically for HEK platform

Phase appropriate status CAR-T Gene Therapy Drug Product,
Quality attributes and assays for release

Quality Attribute Category	Quality Attribute	Example of Analytical Method / Technology (for release)	Recommended Phase Appropriate Status (Release Only)		
			Early Phase (Phase I)	Early Phase (Phase II)	Late Phase (Phase III / Commercial)
Safety	Sterility[1]	Compendial[3]	Verified		
	Mycoplasma[1]	Compendial[3]	Verified		
	Bioburden[1]	Compendial[3]	Verified		
	Endotoxin[1]	Compendial[3]	Verified		
	Adventitious virus	qPCR	Qualified		Validated
	Replication competent virus[2]	qPCR/ELISA	Qualified		Validated
	Cytotoxicity	Various (ELISA, Flow, Imaging)	Qualified		Validated
	Cytokine independent proliferation	Flow Cytometry	Qualified		Validated
	Vector Copy Number (VCN)	ddPCR / qPCR	Qualified		Validated
Strength / content	%Viability	Fluorescence Microscopy	Qualified		Validated
	Potency (functional, direct or indirect)	Cell Based Assay	Qualified		Validated
	% Transduction (expression)	Flow Cytometry	Qualified		Validated
	Cell number	Fluorescence Microscopy	Qualified		Validated
	On target editing	TIDE	Qualified		Validated
Purity	Cell marker expression	Flow Cytometry	Qualified		Validated
Impurity[4]	Unintended cell population	Flow Cytometry	Qualified		Validated
Identity	On target (genome) identity	TIDE, ddPCR / qPCR, ELISA	Qualified		Validated
	Cell identity	Flow Cytometry	Qualified		Validated

1. Testing should be performed at each stage of production at which contamination is most likely to be detected
2. Testing should be conducted on material collected at appropriate stage of manufacturing process
3. Analytical procedures different than those outlined in compendia e.g., rapid sterility tests or rapid mycoplasma tests, may be acceptable alternatives provided sufficient justification provided to the regulator. Those non-compendial methods usually require method qualification and validation instead of verification.
4. If needed, residual process additives as the impurity are tested for in process or process validation purpose, but not for DP release. Therefore, it is not listed in this table.

Recommendations for scope of method qualification

Analytical methods are required to be validated in accordance with ICH Q2 prior to MAA filing. Although method validation is not required as part of IND/IMPD submissions during clinical development, sponsors are required to demonstrate the suitability of the analytical methods for the intended purpose.

There is little regulatory guidance on these requirements beyond ICH Q2(R2)* which states "the scientific principles described in this guideline can be applied in a phase appropriate-manner during clinical development“.

Due to this, the industry has typically applied the term ‘method qualification’ to apply to studies performed to demonstrate the suitability of analytical methods prior to method validation. However, due to lack of regulatory guidance, practices may vary between laboratories.

This section will provide some guidance on the minimum expectations for method qualification and industry practices in comparison to method validation.

*in draft and likely to be finalized in Q4 2023.

Recommendations for scope of method qualification

Comparison of requirements for method qualification and validation

Requirements	Method Qualification	Method Validation
Compliance level	Performed with the principles of GMP applied however a full GMP setting is not required. As a minimum vendor IQ/OQ/PQ is expected with suitable level of data integrity controls in place	Full GMP setting with qualified instruments
Protocol	Company dependent, standardized protocols/procedures may be applied	Yes Where platform methods are applied generic validation protocols may be utilized with a specific details included in the validation report
Approval Responsibility	Technical (AD / QC) approval required with QA approval dependent on company procedures	Technical (AD / QC) and QA approvals required as a minimum.
Acceptance Criteria	Target criteria applied based on ATP	Acceptance criteria based on historical data, instrument capability, product target and ATP
Robustness	No, may be performed as part of method development and summarized in the qualification report (as per ICH Q14)	Typically performed pre-validation and summarized during validation report/ protocol. If key robustness study missing, should be included in validation.
Parameters	As per ICH Q2, accuracy assessments may be limited due to lack of suitable reference materials. As a minimum: specificity, precision and accuracy. LoD or LoQ for impurity tests.	As per ICH Q2
Reference/test samples used	Representative material should be used however this may be from small scale or non-GMP lots due to limited material availability.*	Primary reference standard should be used with a test sample/reference representative of the phase III/ commercial process.*
Leveraging platform data	Risk-based approach to leverage existing platform data reducing time and material requirements. Only critical parameters evaluated on product samples.	Performed on product to be approved, platform data can support validation criteria setting and assay design space.

*For stability indicating methods degraded samples or spiked impurities should be applied to demonstrate the method as stability indicating

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Future Perspectives

Besides recognizing the often-accelerated pace of CGT clinical programs for therapies that have the potential to be transformative to patients, there are several other aspects to be considered regarding of the assay validation. One of the common dilemma is about the very limited product yield of CGT programs compared to traditional biological products. Along with the appropriate phase approach to assay validation, minimizing the use of material and meeting the assay validation requirement and intention should be carefully balanced. The potential use of platform assay validation approach with product specific verification, utilizing representative material other than the final products for certain part of the assay validation, or validation without controls could be considered while developing the assay validation strategy. For potency assay, the appropriate phase approach can also be combined with the matrix approach for overall assay development and validation design.

Unlike traditional biological product, final drug substance and drug product are usually essentially the same material. For CGT products, there are sometimes a lot of critical components, such as vector, activating Abs, used to generate final DP. We should also consider the appropriate phase approach of the assay characterization, qualification or validation strategy associated with those components as they may not be treated as the same way as traditional DS/FDS.

Along with the innovative CGT medicines development, the supporting new technologies are also emerging. Some methods which were typically considered as characterization assay now implemented more in GxP labs as release assay, such as NGS, AUC, MS, etc. When assessing the technologies, software limitation, especially data integrity, should be evaluated as well as the method. Selection of the method for the assay release and the phase appropriate approach for assay validation could be considered together while setting up product specification.

With the increased number of approved CGT products on the market, authorities are developing more guidance regarding regulatory requirements. ICH Q14 has been developed to describe scientific principles and risk-based approaches for developing and maintaining suitable analytical procedures, while ICH Q2 provides guidelines for establishing, submitting, and maintaining evidence that an analytical procedure is fit for purpose, assuring drug quality. The EMA has drafted a guideline on quality, non-clinical and clinical requirements for investigational advanced therapy medicinal products in clinical trials to provide guidance on the structure and data requirements for a clinical trial application for exploratory and confirmatory trials with ATMPs. *Consideration for the development of CART Cell products guidance for industry* from the FDA provides CAR-T cell specific recommendations regarding CMC, pharmacology and toxicology and clinical study design. The phase-appropriate approach to assay validation should always use the most up to date regulatory guidance.

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