BioPhorum

RAW MATERIALS

A proposal to align release standards for transfection reagents

CONNECT
COLLABORATE
ACCELERATE™

Contents

List of figures

List of tables

About BioPhorum

BioPhorum's mission is to create environments where the global biopharmaceutical and device industry can collaborate and accelerate its rate of progress, for the benefit of all.

Since its inception in 2004, BioPhorum has become the open and trusted environment where senior leaders of the biopharmaceutical industry come together to openly share and discuss the emerging trends and challenges facing their industry.

Growing from an end-user group in 2008, BioPhorum's membership now comprises top leaders and subject matter experts from global biopharmaceutical manufacturers and suppliers, working in both long-established and new Phorums. They articulate the industry's technology roadmap, define the supply partner practices of the future, and develop and adopt best practices in drug substance, fill finish, process development and manufacturing IT.

In each of these Phorums, BioPhorum facilitators bring leaders together to create future visions, mobilize teams of experts on the opportunities, create partnerships that enable change and provide the quickest route to implementation, so that the industry shares, learns and builds the best solutions together.

©BioPhorum Operations Group Ltd | November 2023

Authors

Eurofins BioPharma Product Testing
Andrew Schaefer

Janssen
Allison Finch
Ephrath Tesfaye

Regeneron
Vedangi Sample
John Trasatti

Roche
Julia Emmenecker
Ulrike Strauss

UCB
Nic Preyat

Contributors

Members that supported by joining discussions, responding to survey/email questions and reviewing content.

AbbVie
Reshma Sundar

FUJIFILM Irvine Scientific
Amanda Cinquin

Roche
Annette Peceny

Sanofi
Ying Liu

BioPhorum
Victoria Mwanza
Nicholas Ainsworth

Abstract

Cell and gene therapy (CGT) represents a novel and growing class of innovative products that often have complex manufacturing processes. In many cases, genetic material is introduced into eukaryotic cells via transient transfection, a critical manufacturing step. Due to inherent complexity and criticality, there is an acute need for a standardized approach for chemical transfection reagents' certifications of analysis (CoAs) used in GMP manufacturing processes.

Herein, a quality by design (QbD) approach was employed to derive manufacturing process- and product-impactful raw material and/or ancillary material attributes. Although polyethyleneimine (PEI), a cationic polymer, is explored as an exemplar chemical transfection reagent, this approach is also applicable to other transfection reagents used in CGT manufacturing processes. The analysis and learnings discussed may be extrapolated to other transfection materials providing a platform for discussion with supplier and ideally enabling uniform and meaningful material attributes to be reported on these CoAs.

1.0

Introduction and scope

CGT products are disruptive modalities that were introduced to complement medical solutions offered by small molecules and classical biologics. CGT represents a class of novel and innovative products that has the potential to cure orphan diseases and genetic disorders with limited, or no pre-existing treatments. CGT products often have complex manufacturing processes, some of which involve the transfer of genetic material of eukaryotic cells via a transient transfection.

The transfer of free genetic material into a cell does not occur naturally. Transient transfection requires the presence of particular physical (e.g. membrane disruption through electroporation) or chemical conditions to enable entry of the nucleic acid through the plasma membrane. Cationic polymers and lipid nanoparticles are the preferred classes of transfection reagents in the industry to achieve large-scale transfection for both adherent and suspension cells. The absence of standard practices for the characterization and testing of these transfection reagents raises quality concerns that may hinder product development and increase costs for CGT product developers.

The scope of this stimulus article will focus on the cationic polymer, polyethyleneimine, commonly known as PEI. Using the QbD approach, the target material profile of PEI will be assessed as to its intended use, quality, safety and the regulatory criteria needed to manufacture a cell and gene therapy drug substance (DS), intermediate and/or drug product (DP). A set of material attributes will be defined to understand the performance and predicted outcome of the raw material from both a scientific and risk strategy. The goal of this article is to establish a control strategy for PEI and harmonize information across suppliers and recommend test methods that contribute to robust CGT process manufacturing.

2.0

Regulatory landscape

Table 1 looks at each regulation and highlights how it either supports the CGT specification space or provides challenge.

Table 1: Regulatory landscape review

Guideline	Focus
USP-NF <1043> Ancillary Materials for Cell, Gene and Tissue-Engineered Products	Development of the appropriate material qualification programs for CGT products.
ISO 20399 Biotechnology—Ancillary materials present during the production of cellular therapeutic products (2018)	Part 2 is targeted at ancillary material (AM) suppliers on best practice to ensure consistent manufacture of AM products. It describes the information that should be obtained and provided to the AM user to demonstrate lot-to-lot consistency of the AM product with respect to characteristics, quality attributes, biosafety and performance.
ICH M7 Assessment and control of DNA reactive (mutagenic) impurities in pharmaceuticals to limit potential carcinogenic risk—Scientific guideline	ICH M7 page 13 gives acceptable daily intake of an unknown DP elemental impurity. Currently there is no impurity limit for polyethyleneimine.

The following additional points should be considered:

- The USP general chapter <1043> describes different types of AM and risk categories for these. Per USP <1043>, AMs are raw materials that are not intended to be in the final therapeutic product. ISO 20399 is similarly targeted at AMs (defined as materials introduced during the manufacture of cellular therapeutic products) used in CGT manufacture but is further targeted at suppliers on best practice to ensure consistent manufacture of AMs and describes the information that should be obtained and provided to the material user to demonstrate lot-to-lot consistency with respect to characteristics, quality attributes, biosafety and performance.
- From the manufacturer's perspective, USP <1043> would expect product safety including screening, qualifying, documenting sources of animal-derived components as free of suspected adventitious agents, and validating inactivation and testing of initial raw material and final purified human-/animal-derived components for the presence of such agents. However, there are no definitions of animal-derived component-free levels in USP general chapter <1043>. ISO 20399 describes levels of animal-derived component-free and guides testing approach to demonstrate lot-to-lot consistency with respect to composition, including identity, quantity and purity of components. Testing requirements include microbial and viral contamination, and non-biological contamination. If a claim is made regarding consistency, especially of performance, functional assay should support claims as well as performance testing.

- Relevant to polymer-based transfection reagents, suppliers typically describe the grades of materials used in CGT manufacturing as research use only (RUO) or research grade, good manufacturing practice (GMP) 'grade' or research for further manufacturing use (RMF)
- It is the responsibility of the CGT product manufacturer to qualify a given AM for their application and to ascertain the material's labeling, essential features, quality characteristics and suitability for use. Fundamentally, the user must consider the impact of material quality on product quality, taking a QbD approach to the development process in accordance with ICH Q8 (R2) and a risk management approach consistent with ICH Q9 (step 4) Quality Risk Management, ICH Q11 (step 4) Development and Manufacture of Drug Substances
- Further consideration from CGT product manufacturers on the final DS and/or DP is to ensure proper clearance of all elemental impurities. In the case of PEI, there is currently no safe limit specified by USP. The ICH M7 guidance defines a conservative acceptable limit for low-risk chemicals that have not yet been studied.

3.0

Material characteristics and test requirements

3.1 Overview of QbD approach

Transfection reagents are a heterogenous group of complex, non-compendial raw materials that play integral roles in CGT manufacturing (e.g. triple transfection step in lentiviral vectors (LVV) or adeno-associated virus (AAV) production). Due to the complexity, variety and often proprietary nature of these reagents, identifying key material attributes and avenues of controls can be challenging for end-users. Current control strategies often rely on reviewing supplier CoAs.

However, supplier CoAs are inherently DP/DS-agnostic and may not capture the most meaningful material attributes for a specific CGT application. Furthermore, CoAs often vary between suppliers, making the evaluation of transfection reagents across suppliers difficult without additional criteria.

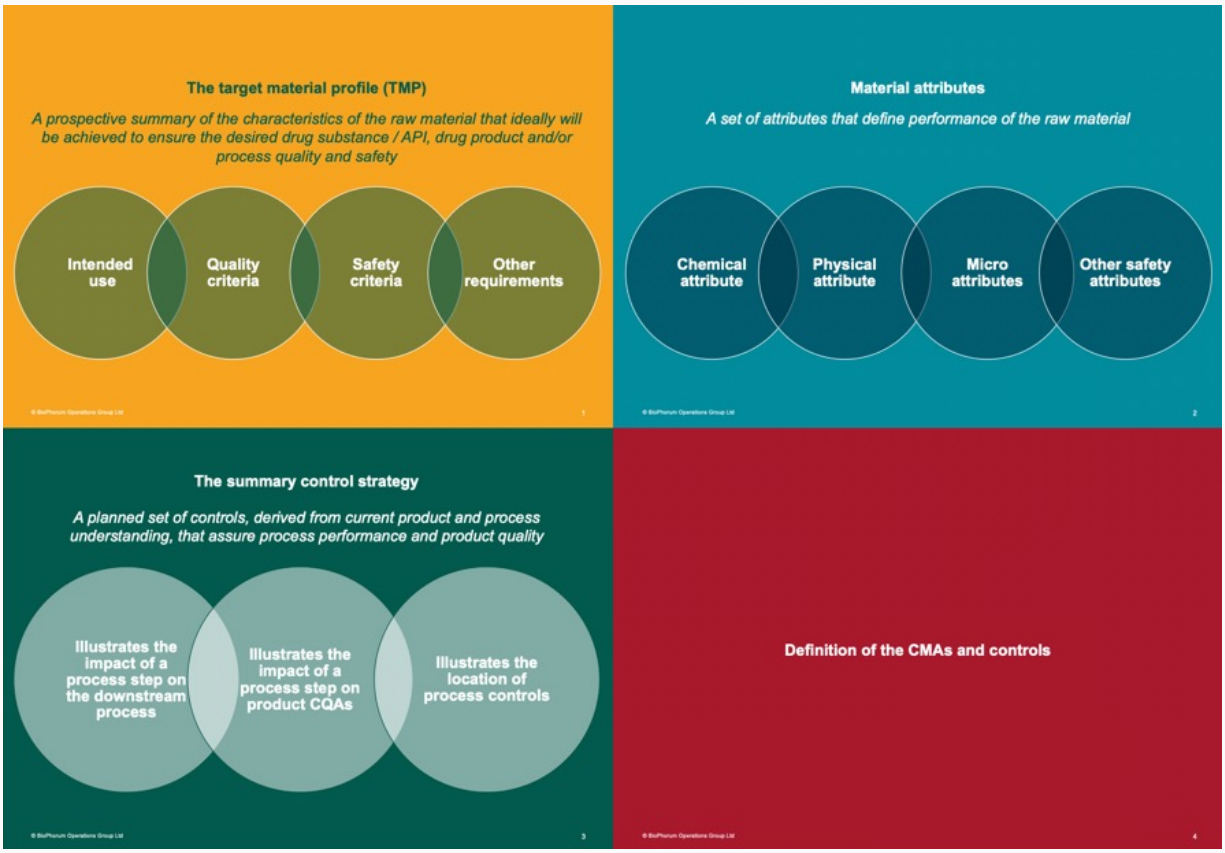
The aims of this paper are two-fold:

1. To build a deeper technical understanding of the material characteristics most critical for transfection performance
2. To better understand how these material attributes fit into the broader control strategy of an example CGT process.

To achieve these goals the *BioPhorum approach to the registration of innovative raw materials using quality by design principles*[2] was implemented. The original document was developed as a guide for describing complex raw materials based on identified critical material attributes (CMAs). Although the original focus was to enable greater supply chain flexibility (e.g. dual sourcing) and streamline comparability assessments (regulatory submissions), the framework it presents for systematically collecting and integrating material, process and product knowledge is of broader value.

The BioPhorum approach is a four-step process based on QbD principles. These four steps are:

Figure 1: Outline QbD process



The QbD approach is a mature quality approach to the definition of product quality, as described in ICH Q8. The mature quality approach is defined as a control strategy based on the definition of the quality target product profile (QTPP), of the product critical quality attributes (CQAs), of linking material attributes and process parameters to the DP CQAs.

Sections 3.2 to 3.6 describe how this process was applied to PEI-based transfection reagents used in lentiviral triple transfection.

3.2 Defining the quality target material profile (QTMP) and material attributes

This paper focuses on the cationic polymer, PEI, as the transfection reagent. PEI is available in either powder or liquid form from multiple suppliers. It is important to understand the material profile and attributes to ensure successful use of the material and meet the quality requirements for GMP manufacturing. When working with PEI at manufacturing scale, four groups of attributes need to be addressed by the supplier to ensure that the raw material is suitable for use; those attributes are as follows: physical, chemical, microbial and safety.

Physical attributes of the PEI raw material such as molecular weight, polymer chemistry (degree of branching) and osmolality contribute to the overall function of PEI in regard to the polydispersity and Zeta potential of the raw material.

Chemical attributes of the PEI raw material such as pH, optimal buffers for formulation of the raw material, complexation media and cell culture media also contribute to the polydispersity and Zeta potential of PEI. These chemical attributes allow for binding and condensing of DNA into small particles that are delivered to the cell membrane. pH and Zeta potential of the raw material directly affect the surface charge of the PEI and how well it will bind and condense DNA, contributing to the size of the final complex. Polydispersity of the raw material and the final complex will dictate the transfection efficiency or how well the particles transfect the majority of the cells. Due to the nature of PEI used for CGT, the raw material should have minimal cytotoxicity with an understanding of the molecular weight along with any degree of side branching.

The microbial and safety attributes of the PEI raw material are maintained by manufacturing in accordance with current good manufacturing practice (cGMP) guidelines under robust quality systems (e.g. ISO, ICH7) to ensure requirements are met under EMA/USP/ICH. A supplier process validation package containing the minimum safety information as follows: sterility or low bioburden (powder form), animal origin-free (AOF) manufacturing, a supplier stability study (ideally with 24 months of data) and current manufacturing facility in accordance with harmonized GMP requirements for ATMPs from USP 1043, ICH Q11 and EudraLex Volume 4 Part IV.

Table 2: Quality target material profile (QTMP) and material attributes

			Material attributes																						
			Physical attributes									Chemical attributes				Microbial attributes			Other safety attributes				Built into process design and overall manufacturing capability
			Appearance	Molecular weight	Polymer chemistry—structural modifications	Polymer chemistry—degree of branching	Osmolality	pH	Buffer additives/reagent formulation	Identity assay	Polydispersity	Complexation media components	Cell culture media components	Elemental impurities	Monomer content and polymerizing agents	Reagent surface charge density (Zeta potential at a given pH and temp)	Sterility or low bioburden for powder products	Low endotoxin	Mycoplasma free	Animal-origin free manufacturing	Supplier stability study minimum 24 months	Manufacturing facility in accordance with USP <1043> or equivalent such as ICH Q11, EudraLex Volume 4 Part IV: GMP requirements for ATMPs	Supplier process validation package	
Quality target material profile (QTMP)	Intended use	The reagent must enable the transfer of the DNA through the cell membrane into the cell		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓			✓									
	Quality criteria	The reagent has to electrostatically bind the negatively charged DNA and the condense DNA into small particles		✓	✓	✓	✓	✓	✓			✓				✓									
		The reagent has to buffer the endosome to enable the release of the DNA into the cytoplasm and avoid intracellular degradation		✓	✓	✓		✓			✓					✓									
		The reagent has to enable the nuclear delivery of the DNA		✓	✓	✓		✓								✓									
		The reagent-DNA complexes should be within optimal size range		✓	✓	✓		✓			✓					✓									✓
		The reagent should have well-understood and well-controlled polydispersity		✓							✓														
		For reagents made of branched polymers, degree of branching should be well understood and well controlled				✓																			
		The reagent should have well-understood and well-controlled molecular weight		✓							✓														
		The reagent should have minimal cytotoxity		✓	✓	✓			✓		✓														
	Safety criteria	The reagent should be manufactured in accordance with cGMP and under robust quality systems (e.g. ISO) Suggest to harmonize language with USP <1043>; e.g. "the [Ancillary Material] meets the necessary functional, quality, and documentation requirements demanded by the relevant regulatory authorities". Current requirements for materials as per EMA/USP/ICH:"	✓																			✓			
		Raw materials 1. USP<1047> 2. USP<1046> 3. EMA Part IV of the Annex to directive 2001/83/EC EP 5.2.12		**Ancillary materials** Referred to as 'raw material in EU' USP<1043> specific for cell and gene therapy		**Starting materials** 1. ICH Q7 2. ICH Q3A 3. EMA Part I of the Annex to Annex to directive 2001/83/EC																			

Table 2: Quality target material profile (QTMP) and material attributes (continued)

			Material attributes																					
			Physical attributes									Chemical attributes					Microbial attributes			Other safety attributes				Built into process design and overall manufacturing capability
			Appearance	Molecular weight	Polymer chemistry—structural modifications	Polymer chemistry—degree of branching	Osmolality	pH	Buffer additives/reagent formulation	Identity assay	Polydispersity	Complexation media components	Cell culture media components	Elemental impurities	Monomer content and polymerizing agents	Reagent surface charge density (Zeta potential at a given pH and temp)	Sterility or low bioburden for powder products	Low endotoxin	Mycoplasma free	Animal-origin free manufacturing	Supplier stability study minimum 24 months	Manufacturing facility in accordance with USP <1043> or equivalent such as ICH Q11, EudraLex Volume 4 Part IV: GMP requirements for ATMPs	Supplier process validation package	
Quality target material profile (QTMP)	Safety criteria	The reagent should be animal-origin free with an animal-derived component free certificate or Bovine Spongiform Encephalopathy (BSE) Transmissible Spongiform Encephalopathy (TSE) statements																		✓				
		Clearance of transfection reagent												✓	✓									As part of process characterization understand impurities limits in drug products
		The reagent should have nitrosamine, halal, melamine statements																						
		If bought in a powder format, it would be expected to have a low bioburden if in semi-finished state, i.e. liquid format sterility assurance required by notified body															✓	✓	✓					
	Manufacturability—other requirement	The reagent should perform well at small- and large-scale processes—scalability																						Note physical and chemical attributes need to remain consistent regardless of volume Process characterisation study
		The reagent should have demonstrated stability over proposed shelf life. Shelf life based on understanding of stability-indicating attributes (proposed checked off). Minimum shelf life of 24 months		✓	✓	✓				✓	✓						✓	✓	✓		✓		✓	Requirement= stability study (supplier or internal)
		The reagent must have a defined process- and product-related impurity profile or documented risk assessment												✓	✓								✓	
		Compatibility with other process components																						As part of supplier selection and process validation
		Stability of the intermediate, i.e. transfection reagent and plasmid in unique process environment																						Process characterisation study
		Pack size and configuration should be carpetable with current and future state of the process																						As part of the supplier selection and process characterisation studies

3.3 Defining the product control strategy

The product control strategy is based on process and product understanding. The product control strategy assures process performance and product quality by using a planned set of controls. The PEI control strategy defines the risks associated with the current CGT process steps and the product quality or CQAs that are directly impacted by PEI. Twelve CQAs have been defined against seven CGT process steps and the control measures that influence the CQA at the intermediate, purified DS and/or final DP stage.

Table 3 shows the product control strategy for PEI. The green boxes across the top represent each step in a CGT process. The yellow/orange boxes represent the CQA or release specification test for an intermediate, purified DS or a final DP. The 'green checkmarks' represent the process step(s) that affect the CQA. The writing underneath the 'green checkmark' details the steps involved and potential control measures to take. The 'red boxed X' represent the process step(s) that do not affect the CQA.

Table 3: Product control strategy or process

Preculture and expansion	Bioreactor production—Transfection complex prep	Bioreactor production—Add transfection complexes onto the cells in the BRX	Bioreactor production—Incubate cells with transfection complexes	Bioreactor production—Harvest and clarification	Downstream purification and formulation	Final fill finish	CQA: Release spec test for end product/ intermediate Purified DS
✓ 1. Viability and cell density 2. Metobolic profile 3. Type of reactor 4. Reactor conditions	✓ 1. Amount 2. Transfection reagent ID 3. DNA amount 4. Ratio of DNA vs cell 5. Ratio of DNA vs transfection reagent 6. Volume media 7. Complexation time 8. Mixing speed 9. Plasmid sequence	✓ 1. Viable cell density 2. Transfection complex 3. Volume 4. Method of addition pump vs gravity 5. Reactor condition 6. Scale up/geometry of vessel (mixing condition/ speed of addition) 7. Shear stress applied to the complex	✓ Reactor condition, i.e. pH, osmolality, agitation, temperature	✓ 1. Harvest time 2. Harvest cell density 3. Cell viability 4. Depth filtration trefoil factor 1 (TFF1) 5. Buffer exchange 6. Lysis of the cell	✓ 1. Load and flow rate, dependent on actor 2. Load parameters are driven by capsid or VG titer 3. Buffer pH, osmolality 4. Type of resins 5. Key factors to remove	✓ 1. Temperature 2. Final filtration	Physical infectious and capsid titer
x	✓ 1. Plasmid quality 2. Transgene sequence	✓ 1. Plasmid quality 2. Transgene sequence	x	x	x	x	Insert sequence (ID)
✓ 1. Viability and cell density 2. Metobolic profile 3. Type of reactor 4. Reactor conditions	✓ 1. Amount 2. Transfection reagent ID 3. DNA amount 4. Ratio of DNA vs cell 5. Ratio of DNA vs transfection reagent 6. Volume media 7. Complexation time 8. Mixing speed 9. Plasmid sequence	✓ 1. Viable cell density 2. Transfection complex 3. Volume 4. Method of addition pump vs gravity 5. Reactor condition 6. Scale up/geometry of vessel (mixing condition/speed of addition) 7. Shear stress applied to the complex	✓ Reactor condition, i.e. pH, osmolality, agitation, temperature	✓ 1. Harvest conditions (temp/ reagent/timing) 2. Nuclease used	x	x	% transgene expression (potency)
x	✓ 1. Amount 2. Transfection reagent ID 3. DNA amount 4. Ratio of DNA vs cell 5. Ratio of DNA vs transfection reagent 6. Volume media 7. Complexation time 8. Mixing speed 9. Plasmid sequence	✓ 1. Viable cell density 2. Transfection complex 3. Volume 4. Method of addition pump vs gravity 5. Reactor condition 6. Scale up/geometry of vessel (mixing condition/ speed of addition) 7. Shear stress applied to the complex	✓ Reactor condition, i.e. pH, osmolality, agitation, temperature	✓ 1. Harvest conditions (temp/ reagent/timing) 2. Nuclease used 3. Clarification technology 4. Filtration method	✓ 1. Load and flow rate, dependent on actor 2. Load parameters are driven by capsid or VG titer 3. Buffer pH, osmolality 4. Type of resins	x	Sub-phenotype expression (impurity)
✓ Raw materials endotoxin level	✓ Raw materials endotoxin level	✓ Raw materials endotoxin level	✓ Raw materials endotoxin level	✓ Raw materials endotoxin level	✓ 1. Raw materials endotoxin level 2. Buffer pH 3. Osmolality 4. Type of resins	✓ Raw materials endotoxin level	Endotoxin
✓ Raw materials endotoxin level	✓ Raw materials endotoxin level	✓ Raw materials endotoxin level	✓ Raw materials endotoxin level	✓ Raw materials endotoxin level	✓ Raw materials bioburden level Buffer pH, osmolality, type of resin	✓ Raw materials bioburden level	Bioburden

Table 3: Product control strategy or process (continued)

Preculture and expansion	Bioreactor production—Transfection complex prep	Bioreactor production—Add transfection complexes onto the cells in the BRX	Bioreactor production—Incubate cells with transfection complexes	Bioreactor production—Harvest and clarification	Downstream purification and formulation	Final fill finish	CQA: Release spec test for end product/ intermediate Purified DS
✓ Raw materials mycoplasma level	✓ Raw materials mycoplasma level	✓ Raw materials mycoplasma level	✓ Raw materials mycoplasma level	✓ Raw materials mycoplasma level	✓ Raw materials mycoplasma level	✓ Raw materials mycoplasma level	Mycoplasma
✓ Raw materials adventitious agents level	✓ Raw materials adventitious agents level	✓ Raw materials adventitious agents level	✓ Raw materials adventitious agents level	✓ Raw materials adventitious agents level	✓ Raw materials adventitious agents level	✓ Raw materials adventitious agents level	Adventitious agents
x	x	✓ Type of cell line, viable cell density, transfection conditions, host cell DNA test	✓ Viable cell density	✓ Total cell density, DNase activity/ time filtration, lysis	✓ Resin, pH	x	Host cell DNA
x	x	x	✓ Cell line, viable cell density	✓ Total cell density, filtration, lysis	✓ Resin, pH, hold time, filter type, residuals host cell protien (HCP) test.	x	Host cell protein
x	✓ Plasmid design and plasmid amount. Reagent to DNA ratio. Transfection reagent type/profile	x	✓ Cell line, viable cell density, plasmid design	✓ Total cell density, DNase activity/ time filtration, lysis	✓ Resin, pH	x	Residual plasmid
x	✓ 1. Amount 2. Transfection reagent 3. DNA amount 4. Ratio of DNA vs cell 5. Ratio of DNA vs transfection reagent 6. Volume media 7. Complexation time 8. Mixing speed 9. Plasmid sequence 10. Complexation volume	✓ 1. Viable cell density 2. Transfection complex 3. Volume 4. Method of addition pump vs gravity 5. Rector condition 6. Scale up/geometry of vessel (mixing condition/ speed of addition) 7. Shear stress applied to the complex	✓ Reactor condition, i.e. pH, osmolality, agitation, temperature	x	x	x	Transgene packaging
x	✓ 1. Amount 2. Transfection reagent 3. DNA amount 4. Ratio of DNA vs cell 5. Ratio of DNA vs transfection reagent 6. Volume media 7. Complexation time 8. Mixing speed 9. Plasmid sequence 10. Complexation volume	✓ 1. Viable cell density 2. Transfection complex 3. Volume 4. Method of addition pump vs gravity 5. Rector condition 6. Scale up/geometry of vessel (mixing condition/ speed of addition) 7. Shear stress applied to the complex	✓ Reactor condition, i.e. pH, osmolality, agitation, temperature	x	x	x	Non-transgene packaging
x	✓ Amount of transfection reagent vs number of cells Size of transfection reagent	x	x	✓ Size of transfection reagent	✓ Resin and pH	x	Residual for transfection reagent

3.4 Defining the critical material attributes (CMAs)

A systematic risk assessment approach was used to define CMAs and respective control strategies for the material attributes of PEI-based transfection reagents (defined in Section 3.2). While both the solid PEI and solution preparations are discussed, the attributes were evaluated based on the liquid formulation of the reagent. Adequate steps must be taken to solubilize the solid PEI material prior to transfection steps. Although both solution and solid material are discussed interchangeably in this document, additional evaluation of material attributes and control strategy may be necessary for the solid form.

Scoring was performed in three categories: (1) **Impact** on product quality and process performance, as outlined in the QTMP and product control strategy, (2) supplier **variability** and (3) **detection**. The results of the scoring, the rationale for each score and the respective control strategies are summarized in Table 4.

CMAs are defined as attributes that have a high impact on product quality or process performance. To ensure the desired quality of the output material, controls such as appropriate limits, ranges or distributions must be defined for these material attributes.

While medium-impact attributes are not considered critical, they still require control as they may affect product quality and process performance when combined with other attributes. The necessity of controls for these attributes is determined by scoring of the variability and detection.

The scores in each category were defined according to the BioPhorum approach[2] **BioPhorum's QbD approach to registering complex raw materials as guidance**. Tables can be found in Appendix 1.

Table 4: Definition of critical material attributes

Physical attributes		
Appearance		
Impact—Medium	Variability—Medium	Detection—Low
Rationale	• Impact is scored as medium: appearance of material can be visually inspected to identify any issues; the impact of these issues could vary • Variability is scored as medium: appearance may be dependent on the manufacturing process or the chemical composition of the transfection reagent, which may vary between suppliers • Detection is scored as low: appearance is included in the incoming raw material testing; testing for the degree of coloration and for particulates should also be included in the CoA.	
Control strategy	Include degree of coloration and particulates in the CoA. Include appearance of the transfection reagent in the incoming raw material testing.	
Molecular weight (MW)		
Impact—High	Variability—High	Detection—Low
Rationale	• Impact is scored as high: MW of PEI-based transfection reagents impacts their performance and cytotoxicity • Variability is scored as high: MW may depend on the product and distinguish one product from another • Detection is scored as low: well-defined methods to determine MW, such as gel permeation chromatography (GPC), are in place.	
Control strategy	MW should be included in the CoA.	
Polymer chemistry—structural modifications		
Impact—High	Variability—High	Detection—Low
Rationale	• Impact is scored as high: structural modifications could change the surface charge of the polymer and affect the performance of the transfection reagent, i.e. the transfection efficiency • Variability is scored as high: structural modifications of the PEI distinguish one product from another • Detection is scored as low: intended structural modifications should be easy to detect with an in-house test panel specified by the end-user. It should also be included in the CoA. Unintentional structural modifications may be much harder to detect.	
Control strategy	Structural modifications should be included in the CoA. Additionally, an in-house test panel may be set up to test for structural modifications.	

Table 4: Definition of critical material attributes (continued)

Physical attributes	
Polymer chemistry—degree of branching	
Impact—High	Variability—High / Detection—Low
Rationale	• Impact is scored as high: the degree of branching could change the surface charge of the polymer and affect the performance of the transfection reagent, i.e. the transfection efficiency • Variability is scored as high: the degree of branching distinguishes one product from another • Detecting is scored as low: the degree of branching should be easy to detect with an in-house test panel specified by the end-user. It should be included in the CoA.
Control strategy	Degree of branching should be included in the CoA. Additionally, an in-house test panel may be set up to test for the degree of branching of the polymer.
Osmolality	
Impact—High	Variability—High / Detection—Low
Rationale	• Impact is scored as high: the osmolality indirectly impacts the concentration of the PEI-based transfection reagent • Variability is scored as high: the reagent concentration may vary between suppliers and so does the osmolality • Detection is scored as low: the osmolality can be easily determined by freezing-point depression. Additionally, the osmolality should be included in the CoA.
Control strategy	Osmolality should be included in the CoA. The proposed analysis method is freezing-point depression.
Chemical attributes	
pH	
Impact—Medium	Variability—High / Detection—Medium
Rationale	• Impact is scored as medium: the pH of a PEI-based transfection reagent does not necessarily indicate the reagent's performance but is rather indicative of its composition. Furthermore, the pH might affect the reagent's stability. It also contributes to the pH of the transfection complex solution, which is an important parameter in the transfection unit operation and can affect process performance. The pH of the transfection complex solution can also be modulated by other factors such as supplements. Eventually, the pH of the transfection reagent might affect the pH inside the production bioreactor, which is an important process parameter that can affect process performance. However, the pH inside the bioreactor is measured and can be controlled. Furthermore, the amount of transfection reagent in the production bioreactor is relatively small • Variability is scored as high: the transfection reagent pH values vary between products. Additionally, some of the suppliers have very wide pH specification ranges or do not include the pH in the product specification • Detection is scored as medium: the pH of the transfection reagents can be measured easily. However, pH measurement is not commonly performed in-house and should be provided in the CoA.
Control strategy	pH should be included in the CoA with narrow specification ranges. For the transfection reagent a pH below 6.0 is recommended. In the production process, the pH of the complexation solution should be between pH 7.0 and 9.5[1] RESEARCH ARTICLE Unusual Salt and pH Induced Changes in Polyethyleneimine Solutions.
Buffer additives/reagent formulation	
Impact—High	Variability—High / Detection—High
Rationale	• Impact is scored as high: certain substances can enhance or impede the performance of PEI-based transfection reagents • Variability is scored as high: there is a lack of knowledge on the buffer formulation • Detection is scored as high: buffer formulation and additive specifications are not included in the CoA. Analysis of the transfection reagent formulation is not usually performed by the end-user.
Control strategy	<50mM NaCl (support information: RESEARCH ARTICLE Unusual Salt and pH Induced Changes in Polyethyleneimine Solutions)[1]. Reagent formulation is analyzed by the supplier and included in the CoA. In-house testing could be performed (e.g. ICP, MS, OES, IC, titration, etc.)
Identity assay	
Impact—High	Variability—High / Detection—Low
Rationale	• Impact is scored as high: the reagent's identity determines its performance, i.e. the reagent's ability to transfer DNA into the cell • Variability is scored as high: the identity of the transfection reagent is supplier IP and can vary between suppliers • Detection is scored as low: the identity assay is part of the supplier release specification and partially included in the CoA.
Control strategy	ID testing is part of the CoA. Methods could include HPLC, NMR, FT-IR, SEC.

Table 4: Definition of critical material attributes (continued)

Polydispersity			
Impact—High	Variability—High	Detection—High	
Rationale	• Impact is scored as high: intra-lot variability in the transfection reagent's polydispersity may impact complex formation and contribute to lot-to-lot variability in virus productions. Additionally, the introduction of PEI molecules of various sizes and/or molecular weights into cells may result in unpredictable cytotoxicity. Eventually, polydispersity may be a stability-indicating attribute • Variability is scored as high: there is a lack of knowledge • Detection is scored as high: polydispersity may be measured and sufficiently controlled at the supplier but is not included in the CoA. In-house measurements are not usually performed.		
Control strategy	Include polydispersity of the reagent in CoA. Methods could include DLS, GPC-RI.		
Complexation media components			
Impact—High	Variability—High	Detection—High	
Rationale	• Impact is scored as high: certain media components may enhance or impede the performance of PEI-based transfection reagents and media vendor proprietary recipes do not disclose specific additives in the media formulation • Variability is scored as high: there is a lack of knowledge from supplier to supplier • Detection is scored as high: the complexation media may comprise a broad range of components that may interact with the reagent.		
Control strategy	Supplier to provide list of components that may interfere with PEI-based transfection. User should perform design of experiment studies to understand the impact to the process.		
Cell culture media components			
Impact—High	Variability—High	Detection—High	
Rationale	• Impact is scored as high: certain media components may enhance or impede the performance of PEI-based transfection reagents and media vendor proprietary recipes do not disclose specific additives in the media formulation • Variability is scored as high: there is a lack of knowledge • Detection is scored as high: the complexation media may comprise a broad range of components that may interact with the reagent.		
Control strategy	Supplier to provide list of components that may interfere with PEI-based transfection. User should perform design of experiment studies to understand the impact to the process.		
Elemental impurities			
Impact—High	Variability—Medium	Detection—High	
Rationale	• Impact is scored as high: elemental impurities could impact the transfection efficiency and cell culture performance. Both may impact process performance and may result in lot-to-lot variability • Variability is scored as medium: inter-lot variability of elemental impurities is low. However, changes between suppliers can vary • Detection is scored as high: there is a lack of knowledge. It is currently not clear whether elemental impurities are sufficiently reported in the CoA and which elemental impurity profile is acceptable and does not interfere with process performance.		
Control strategy	The reagent must have a defined process and product-related impurity profile or documented risk assessment. Elemental impurities should be considered in transfection reagent specification and be included in the CoA. The recommended analytical method is ICP-MS.		
Monomer content and polymerizing agents			
Impact—High	Variability—Low	Detection—Low	
Rationale	• Impact is scored as high: other species like leftover monomers or polymerizing agents included in the transfection reagent may impact the performance of the transfection reagent • Variability is scored as low, as testing for monomers or polymerizing agents is part of the suppliers' product purity testing and results are included in the CoA • Detection is scored as low, as the monomer content and the concentration of polymerizing agents are included in the CoA. Based on the specifications and proposed test method provided by the supplier, monomer content and polymerizing agents could also be measured in-house.		
Control strategy	Monomer content and polymerizing agents should be included in the suppliers' product purity testing. Specification should be zero or at a level that is known to not affect transfection efficiency.		

Table 4: Definition of critical material attributes (continued)

Reagent surface charge density (Zeta potential at a given pH and temp)			
Impact—High	Variability—High	Detection—High	
Rationale	• Impact is scored as high: the surface charge density of a PEI-based transfection reagent influences the electrostatic interactions between the transfection reagent and DNA. Therefore, heterogeneity in the surface charge density may impact the performance of the transfection reagent at multiple steps of the transfection process, including complex formation and intracellular DNA release • The variability is scored as high: there is a lack of knowledge. The surface charge density emerges from structural features of the PEI molecule (e.g. polymer length, functional groups, side chains, modifications), which may be supplier intellectual property (IP) and therefore may vary between suppliers • Detection is scored as high: the reagent surface charge density must be measured and sufficiently understood and controlled by the end-user.		
Control strategy	Engage with suppliers to understand how surface charge is controlled and measured in the manufacturing process. User should perform design of experiment studies to understand the impact to the process.		
Microbial attributes			
Sterility or low bioburden for powder products			
Impact—High	Variability—Low	Detection—Low	
Rationale	• Impact is scored as high: microbial growth will have an impact on the cell culture process • Variability is scored as low: process steps for manufacture are validated • Detection is scored as low: sterility testing/bioburden testing is included in the CoA and performed in-house within incoming raw material testing.		
Control strategy	Sterility or bioburden testing should be included in the CoA. Sterility testing should be performed in accordance with USP <71> or an alternative recognized test method. Bioburden testing should be performed in accordance with USP <61> / USP <62> or an alternative recognized test method.		
Low endotoxin			
Impact—High	Variability—Low	Detection—Low	
Rationale	• Impact is scored as high: the level of endotoxin will have an impact on the cell culture process • Variability is scored as low: process steps for manufacture are validated • Detection is scored as low: endotoxin testing is included in the CoA and performed in-house within the incoming raw material testing.		
Control strategy	Endotoxin testing should be performed in accordance with USP <85> or an alternative recognized test method and be included in the CoA.		
Mycoplasma free			
Impact—High	Variability—Low	Detection—Low	
Rationale	• Impact is scored as high: the presence of mycoplasma will have an impact on the cell culture process • Variability is scored as low: process steps for manufacture are validated • Detection is scored as low: mycoplasma testing is included in the CoA and performed in-house within the incoming raw material testing.		
Control strategy	Mycoplasma testing should be performed in accordance with USP <63> or an alternative recognized test method and be included in the CoA.		
Other safety attributes			
Animal-origin free manufacturing			
Impact—Medium	Variability—Low	Detection—Low	
Rationale	• Impact is scored as medium: AOF transfection reagents are preferred, as animal-derived components might induce inter-lot variabilities and require BSE/TSE statements to ensure product safety. For AOF transfection reagents, an AOF manufacturing process must be ensured, as the unintentional presence of animal-derived components poses a high safety risk. Animal-derived components could be detected in the transfection reagent but are not routinely tested for in-house controls by the end-user • Variability is scored as low: PEI-based transfection reagents are synthetically manufactured • Detection is scored as low: the presence of animal-derived components can be identified through CoA and other related supplier documentation.		
Control strategy	Animal origin/BSE/TSE statement by supplier, confirming the non-animal origin of all materials used in the manufacturing process.		

Table 4: Definition of critical material attributes (continued)

Supplier stability study minimum 24 months			
Impact—Medium	Variability—Low	Detection—Low	
Rationale	• Impact is scored as medium: may have an impact on the performance of the transfection reagent if the material is out of specification. For the supplier stability study a minimum of 24 months is requested • Variability is scored as low: the material is stable in nature • Detection is scored as low: the material is supplied with a CoA confirming the product shelf life.		
Control strategy	Expiration date is included in the CoA and supported by the supplier stability study.		
Manufacturing facility in accordance with ICH Q11, EudraLex Volume 4 Part IV: GMP requirements for ATMPs, or equivalent. USP <1043>			
Impact—High	Variability—Medium	Detection—Medium	
Rationale	• Impact is scored as high: the guidelines ensure that the manufacturing facility is designed and operated in a manner that ensures consistent quality and meets the requirements of the intended use • Variability is scored as medium: the application of the regulatory guidelines can vary between suppliers. Therefore, a supplier audit should be included in the supplier selection/qualification process • Detection is scored as medium: in the supplier qualification process it will be assured that manufacturing facilities have a certified accreditation. Supplier monitoring and audits should be performed regularly.		
Control strategy	Assure that the material is supplied only by manufacturing facilities with certified accreditation. Supplier monitoring and audits are performed regularly.		
Supplier process validation package			
Impact—High	Variability—Medium	Detection—Low	
Rationale	• Impact is scored as high: you would lose assurance the material is manufactured in a control manner • Variability is scored as medium: validation packages can vary between supplier • Detection score is low: the drug master files or equivalent are an established quality process accepted by regularity agencies. Accreditations or certifications by applicable GMP standards further support a low score.		
Control strategy	Filing validation package with regulatory bodies and supplier qualification.		

3.5 Defining the material characteristics and test requirements based on QbD approach

Based on the knowledge acquired through the definition of the QTMP, the product control strategy and the CMAs, specifications were defined for the material attributes with high or medium impact on product quality and process performance. This included recommended test methods that could be used to harmonize the evaluation of these attributes.

Together with the control strategy defined in Section 3.4, Table 5 summarizes the recommended test methods, and specifications for the high- and medium-impact material attributes of PEI-based transfection reagents.

Table 5: Recommended raw material specification for polymer-based transfection reagents

		Proposed testing requirements		
		Proposed test method	**Proposed acceptance criteria**	**Control strategy for attribute**
Physical attributes	Appearance	Degree of coloration and particulates	Clear, colorless solution, free of particulates	Check CoA/part of incoming raw material testing
	Molecular weight	Gel permeation chromatography (GPC)	Agreed range with vendor—pass/fail	Ask supplier to include in CoA as part of identity testing/ determine internally via e.g. GPC
	Polymer chemistry—structural modifications	IR/NMR	Agreed range with vendor—pass/fail	Ask supplier to include in CoA as part of identity testing/ determine internally via test panel
	Polymer chemistry—degree of branching	GPC + IR/NMR	Agreed range with vendor—pass/fail	Ask supplier to include in CoA as part of identity testing/ determine internally via test panel
	Osmolality	Freezing-point depression	<50mM NaCl	Ask supplier to include in CoA, incoming raw material check
Chemical attributes	pH	pH	Acidic solution of pH<6.0	Based on the manufacturing process when in buffer solution the pH should be 7–9.5pH
	Buffer additives/reagent formulation	ICP, MS, OES, IC, titration	<50mM NaCl	Definition of composition—high level Fingerprint testing annual/quarterly Low salt to promote high interchain repulsion so that PEI will be in a favorable linear state
	Identity assay	Assay/ID test, e.g. HPLC, NMR, FT-IR, SEC	Pass	CoA check
	Polydispersity	DLS, GPC-RI	Monodispersed	Ask supplier to have on CoA
	Elemental impurities	ICP—MS	Drug product profile—pass	The reagent must have a defined process- and product-related impurity profile or documented risk assessment
	Monomer content and polymerizing agents	Agree as part of supplier purity testing	Zero or at a level that is known not to affect transfection efficiency	CoA
	Reagent surface charge density (Zeta potential at a given pH and temp)	To be agreed with vendor	Agreed range with vendor—pass/fail	Assay as final DS release
Microbial attributes	Sterility or low bioburden for powder products	USP <71> or alternative recognized test such as PCR	No growth detected or equivalent (example no PCR signal)	Check CoA/part of incoming raw material testing
	Low endotoxin	USP <85> or alternative recognized validated test	≤0.5EU/mL	Check CoA/part of incoming raw material testing
	Mycoplasma free	USP <63> or alternative recognized validated test	None detected	Check CoA/part of incoming raw material testing
Other safety attributes	AOF manufacturing	In line with internationally recognized regulatory standard	Material is synthetic; supplier to confirm as part of CoA material has not been contaminated with material of animal origin	Statement on CoA
	Supplier stability study minimum 24 months	In line with internationally recognized regulatory standard	Shelf life statement—expiry on CoA	CoA expiration date

3.6 Manufacturing process consideration

Attributes with high and medium impact on product quality and process performance that are not directly ascribed to the transfection reagent itself, but rather to the transfection reagent manufacturing process, were assessed comparably to the CMAs of the transfection reagent. Specifications and appropriate test methods and control strategies for these attributes were discussed and defined by the team. The recommended manufacturing process and material qualification controls and specifications are summarized in Table 6.

Table 6: Recommended manufacturing and material qualification controls for polymer-based transfection reagents

		Proposed testing requirements		
		Proposed test method	**Proposed acceptance criteria**	**Control strategy for attribute**
Chemical attributes	Polydispersity	DLS	Monodispersed	Ask supplier to have on CoA if sold as a solution. Shelf life
	Complexation media components	Supplier qualification	User should perform design of experiment studies in order to understand the impact to the process	Supplier to provide list of components that may interfere with PEI-based transfection User should perform design of experiment studies in order to understand the impact to the process
	Cell culture media components	Supplier qualification	User should perform design of experiment studies in order to understand the impact to the process	Supplier to provide list of components that may interfere with PEI-based transfection User should perform design of experiment studies in order to understand the impact to the process
	Elemental impurities	ICP—MS	Drug product profile—pass	The reagent must have a defined process- and product-related impurity profile or documented risk assessment
	Reagent surface charge density (Zeta potential at a given pH and temp)	To be agreed with vendor	Agreed range with vendor—pass/fail	User should perform design of experiment studies to understand the impact to the process Assay as final DS release
Other safety attributes	AOF manufacturing	In line with internationally recognized regulatory standard	Material is synthetic—supplier to confirm as part of CoA material has not been contaminated with material of animal origin	BSE/TSE statement
	Supplier stability study minimum 24 months	In line with internationally recognized regulatory standard	Material is synthetic, manufactured and stable in nature. CoA would confirm shelf life	CoA expiration date and supplier stability study to support
	Manufacturing facility in accordance with USP <1043> or equivalent such as ICH Q11, EudraLex Volume 4 Part IV: GMP requirements for ATMPs	Supplier qualification	Assuring material is supplied by manufacturing facility with certified accreditation and regular supplier monitoring and audits	Supplier qualification
	Supplier process validation package	Supplier qualification	Filing validation packaging with regulatory bodies it is assumed process is stable and manufacturing material of the required quality requirements	Supplier qualification

4.0

Proposal

The CGT space represents new challenges in manufacturing a treatment that is efficacious while being safe for the patient. Each raw material added to the CGT process needs to be scrutinized in regard to safety, and its planned purpose and use. The introduction of target genetic material via transient transfection is a critical step in CGT manufacturing. The reagents required for transfection have broad market differences in manufacturing quality and attributes tested. This paper highlights critical PEI quality material attributes for a successful CGT transient transfection manufacturing process: physical, chemical, microbial and safety. To remedy the current lack of standardization, the QbD approach was used to determine the target material profile of PEI. The QbD approach enabled better understanding of the material characteristics that are most critical for transfection performance, and better understanding of how these material attributes fit into the broader control strategy of a CGT process.

Table 4 demonstrates that microbial and safety attributes are better understood and characterized for release-testing requirements, whereas physical and chemical attributes are significantly tied to the suppliers' proprietary information and process know how. Four of the five physical attributes and eight of the nine chemical attributes were scored with a high impact and high variability, suggesting, with a scientific rationale, that these are critical for the success of a CGT process. This paper outlines the impact and variability scored high due to a gap in knowledge from the field and or suppliers' proprietary information. Seven of the nine chemical attributes were scored with a high or medium detectable score. There is an opportunity for improvement by working with the suppliers on standardizing a PEI transfection reagent CoA and/or collaborating on a set of appropriate assays needed for regulatory approval. The cumulation of this work is summarized in Table 7.

Clearly, there is an opportunity for improvement by working with suppliers on standardizing a PEI transfection reagent CoA and collaborating on appropriate assays needed for regulatory approval. While this paper outlines a framework for that standardization, it also demonstrates a practical means to extrapolate to other transfection materials prevalent in the CGT industry. With this, industry can build a deeper technical knowledge of these materials and agree to greater standardization in attributes testing.

Table 7: Summary of material attributes and proposed specification

			Material attributes																				
			Physical attributes									**Chemical attributes**					**Microbial attributes**			**Other safety attributes**			
			Appearance	Molecular weight	Polymer chemistry—structural modifications	Polymer chemistry—degree of branching	Osmolality	pH	Buffer additives/reagent formulation	Identity assay	Polydispersity	Complexation media components	Cell culture media components	Elemental impurities	Monomer content and polymerizing agents	Reagent surface charge density (Zeta potential at a given pH and temp)	Sterility or low bioburden for powder products	Low endotoxin	Mycoplasma free	AOF manufacturing	Supplier stability study minimum 24 months	Manufacturing facility in accordance with USP <1043> or equivalent such as ICH Q11, EuroAxx Volume 4 Part 1 & 2 GMP requirements for ATMPs	Supplier process validation package
Quality target material profile (QTMP)	Intended use	The reagent must enable the transfer of the DNA through the cell membrane into the cell		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓			✓								
	Quality criteria	The reagent has to electrostatically bind the negatively charged DNA and the condense DNA into small particles		✓	✓	✓	✓	✓	✓			✓				✓								
		The reagent has to buffer the endosome to enable the release of the DNA into the cytoplasm and avoid intracellular degradation		✓	✓	✓		✓			✓					✓								
		The reagent has to enable the nuclear delivery of the DNA		✓	✓	✓		✓								✓								
		The reagent-DNA complexes should be within optimal size range		✓	✓	✓		✓			✓					✓								
		The reagent should have well-understood and well-controlled polydispersity		✓							✓													
		For reagents made of branched polymers, degree of branching should be well understood and well controlled				✓																		
		The reagent should have well-understood and well-controlled molecular weight		✓							✓													
		The reagent should have minimal cytotoxity		✓	✓	✓			✓		✓													
	Safety criteria	The reagent should be manufactured in accordance with cGMP and under robust quality systems (e.g. ISO). Suggest to harmonize language with USP <1043>; e.g. "the [Ancillary Material] meets the necessary functional, quality, and documentation requirements demanded by the relevant regulatory authorities". Current requirements for materials as per EMA/USP/ICH:* **Raw materials** 1. USP<1047> 2. USP<1046> 3. EMA Part IV of the Annex to directive 2001/83/EC EP 5.2.12 **Ancillary materials** Referred to as 'raw material in EU' USP<1043> specific for cell and gene therapy **Starting materials** 1. ICH Q7 2. ICH Q3A 3. EMA Part I of the Annex to Annex to directive 2001/83/EC	✓																			✓		
	Safety criteria	The reagent should be AOF with an animal-derived component free certificate or BSE/TSE statements																		✓			
		Clearance of transfection reagent												✓	✓									
		The reagent should have nitrosamine, halal, melamine statements																						
		If bought in a powder format, it would be expected to have a low bioburden if in semi-finished state, i.e. liquid format sterility assurance required by notified body															✓	✓	✓				
	Manufacturability—other requirement	The reagent should perform well at small- and large-scale processes—scalability																						
		The reagent should have demonstrated stability over proposed shelf life. Shelf life based on understanding of stability-indicating attributes (proposed checked off). Minimum shelf life of 24 months		✓	✓	✓				✓	✓						✓	✓	✓		✓		✓
		The reagent must have a defined process- and product-related impurity profile or documented risk assessment												✓	✓								✓
		Compatibility with other process components																						
		Stability of the intermediate, i.e. transfection reagent and plasmid in unique process environment																						
		Pack size and configuration should be carpetable with current and future state of the process																						

Proposed testing requirements	**Proposed test method**	Degree of coloration + particulates	Gel permeation chromatography (GPC)	IR/NMR	GPC + IR/NMR	Freezing-point depression	pH	RAMAN/IR/NMR-fingerprint/DLS for size	Assay/ID test e.g. HPLC, NMR, FT-IR, SEC	DLS/GPC-RI	Supplier qualification	Supplier qualification	ICP—MS	Agree as part of supplier purity testing	To be agreed with vendor	USP <71> or alternative recognized test such as PCR	USP <85> or alternative recognized validated test	USP <63> or alternative recognized validated test	In line with internationally recognized regulatory standard	In line with internationally recognized regulatory standard	Supplier qualification	Supplier qualification
	Proposed acceptance criteria	Clear, colorless solution, free of particulates	Agreed range with vendor—pass/fail	Agreed range with vendor—pass/fail	Agreed range with vendor—pass/fail	<50mM NaCl	Acidic solution of pH <6.0	<50mM NaCl	Pass	Monodispersed	User should perform design of experiment studies in order to understand the impact to the process	User should perform design of experiment studies in order to understand the impact to the process	Drug product profile—pass	Agreed range with vendor—pass/fail	Engage with suppliers to understand how surface charge is controlled and measured in the manufacturing process	No growth detected or equivalent (example no PCR signal)	≤0.5 EU/mL	None detected	Animal origin/BSE/TSE statement by supplier, confirming the non-animal origin of all materials used in the manufacturing process	CoA would confirm shelf life	Assuring material is supplied by manufacturing facility with certified accreditation and regular supplier monitoring and audits	Filing validation packaging with regulatory bodies it is assumed process is stable and manufacturing material of the required quality requirements
	Control strategy for attribute	Check CoA/part of incoming raw material testing	Ask supplier to include in CoA as part of identity testing/determine internally via e.g. GPC	Ask supplier to include in CoA as part of identity testing/determine internally via test panel (tbd)	Ask supplier to include in CoA as part of identity testing/determine internally via test panel (tbd)	Ask supplier to include in CoA, incoming raw material check	Based on the manufacturing process when in buffer solution the pH should be between 7–9.5pH	<50mM NaCl Reagent formulation is analyzed by the supplier and included in the CoA. In-house testing could be performed (e.g. ICP, MS, OES, IC, titration etc.)	ID testing is part of the CoA. Methods could include HPLC, NMR, FT-IR, SEC	Ask supplier to have on CoA	Supplier to provide list of components that may interfere with PEI-based transfection. User should perform design of experiment studies in order to understand the impact to the process	Supplier to provide list of components that may interfere with PEI-based transfection. User should perform design of experiment studies in order to understand the impact to the process	The reagent must have a defined process and product-related impurity profile or documented risk assessment	CoA	User should perform design of experiment studies to understand the impact to the process	Check CoA/part of incoming raw material testing	Check CoA/part of incoming raw material testing	Check CoA/part of incoming raw material testing	BSE/TSE statement on CoA	CoA expiration date and supplier stability study to support	Supplier qualification	Supplier qualification
	Should be part of CoA or part of manufacturing controls, i.e. supplier approval or validation work		CoA check	CoA check	CoA check	CoA check	CoA	CoA	CoA check	CoA check	Manufacturing controls	Manufacturing controls	Manufacturing controls and CoA	CoA check	Manufacturing controls	CoA check	CoA check	CoA check	Manufacturing controls and CoA	Manufacturing controls and CoA	Manufacturing controls	Manufacturing controls

5.0

Feedback and responding to the content of the article

Readers are invited to comment on the specific standards and tests where possible, in addition to the overall proposed approach. Feedback may be provided by completing this form.

Specification testing:

1. What experience do you have of supplier ID methods being unable to discriminate similar molecules in their facility? What have you done to address this?
2. What has your organization developed for testing polymer-based transfection reagent identity?
3. Have you used nuclear magnetic resonance (NMR), fourier transform infrared (FT-IR) or other methods for testing polymer-based transfection reagent identity?
4. If yes, could you share more details of your method?
5. What other testing and/or methodology would you recommend?
6. Do you agree with the testing proposed in Table 5?
7. What release specifications do you use: vendor-specified criteria on CoAs, or user/process-defined CQAs, or other?
8. What is your experience with polymer-based transfection reagents of other origins?
9. What marketed grades of material are you using in your process?
10. What phase of the approval process are you in?

Regulatory:

11. Are there relevant references other than USP <1043> or ISO 20399 that should be evaluated from a regulatory perspective that may dictate the use of these transfection reagents?
12. Would you be interested in helping to write a pharmacopeia entry for transfection reagents?

The team leveraged multiple discussions and undertook a blinded survey to enable sharing of data and opinions. The final proposals are a combination of thoughts, suggestions and questions. The objective of this paper is to solicit feedback on the proposed universal standards and testing for polymer-based transfection reagents. Table 5 sets out an industry best practice which could be used as a basis for a chapter.

Readers are invited to comment on the specific standards and tests where possible, in addition to the overall proposed approach. Feedback may be provided by completing the following **form**.

The team recognizes that the proposed approach is a work in progress. It understands that many users are doing some innovative work. This is an opportunity for you to use your voice to inform a standardized approach, a baseline of tests and agreed methods that should be followed for acceptable specification ranges for polymer-based transfection reagents.

6.0

Benefits

An agreed framework for release testing polymer-based transfection reagents has many benefits. It will provide confidence that your actions are aligned with those of your peers. The alignment will bring reliability and consistency to the manufacturing process as everyone is meeting the same standard for a particular material.

It will also mean that manufacturers, suppliers and clients can use the same language and refer to an agreed reference table and testing. Importantly, when you come to file with regulatory authorities, you will have a data pack that covers what they will expect to see and that demonstrates you are managing and controlling that material appropriately.

Nobody has tried to define the polymer-based transfection reagent release testing needed for CGT processes, so the BioPhorum approach is an industry first. With the explosive growth of the CGT industry, the need for these release specifications is loud and clear. What do you think?

Appendix

Risk assessment tables used to define the CMAs

Definition—Impact	
Low	Based on our product and process knowledge and understanding, the attribute does not contribute in itself to the QTMP or the control strategy associated with the raw material and its use. Control of product quality or fulfilling of the QTMP may be achieved in different ways. ***No more characterization required.***
Medium	Based on our product and process knowledge and understanding, the attribute may contribute to the QTMP or the control strategy associated with the raw material and its use. This attribute has an impact on product and process quality when combined with others. A material attribute of medium impact may require controls.
High	Based on our product and process knowledge and understanding, the attribute contributes directly to the QTMP or the control strategy associated with the raw material and its use. **Critical material attribute:** "A physical, chemical, biological or microbiological property or characteristic of an input material that should be within an appropriate limit, range, or distribution to ensure the desired quality of output material."

Definition—Variability	
Low	Based on our product and process knowledge and understanding, the attribute is robust; it presents low variability.
Medium	Based on our product and process knowledge and understanding, the attribute may demonstrate some variability.
High	Based on our product and process knowledge and understanding, the attribute cannot be described as robust, it presents a high variability.

Definition—Detection	
Low	The attribute is adequately measured/Failure of the attribute can be detected before it is added to the manufacturing process.
Medium	The attribute is measured; however, some variability may occur undetected/failure of the attribute can be detected before product release.
High	The analytical method is not appropriate/Failure of the attribute cannot be detected at product release.

Glossary

Term	Definition
AAV	Adeno-associated virus
AM	Ancillary material
AOF	Animal-origin free
BSE	Bovine Spongiform Encephalopathy
CGT	Cell and gene therapy
CMA	Critical material attribute
CoA	Certificate of analysis
CQA	Critical quality attribute
DLS	Dynamic light scattering
DP	Drug product
DS	Drug substance
EMA	European Medicines Agency
FT-IR	Fourier transform infrared
GMP	Good manufacturing practice
GPC	Gel permeation chromatography
HPLC	High-performance liquid chromatography
ICP-MS	Inductively coupled plasma mass spectrometry

Term	Definition
IP	Intellectual property
IR	Infrared
ISO	International Organization for Standardization
LC-MS	Liquid chromatography—mass spectrometry
LVV	Lentiviral vectors
MW	Molecular weight
NMR	Nuclear magnetic resonance
PEI	Polyethyleneimine
QbD	Quality by design
QTMP	Quality target method profile
RMF	For research use or further manufacturing
RUO	Research use only
SEC	Size exclusion chromatography
TMP	Target material profile
TSE	Transmissible Spongiform Encephalopathy
USP	United States Pharmacopeia

Reference summary

Reference	Title
USP <61>	Microbiological Examination of Nonsterile Products: Microbial Enumeration Tests
USP <62>	Microbiological Examination of Nonsterile Products: Test for Specified Microorganisms
USP <63>	Mycoplasma Tests: A New Regulation for Mycoplasma Testing
USP <71>	Sterility Tests
USP <85>	Bacterial Endotoxins Test General Chapter
USP <1043> general and USP-NF <1043>	Ancillary Materials for Cell, Gene, and Tissue-Engineered Products
USP <1046>	Cell-Based Advanced Therapies and Tissue-based Products
USP-NF <1047>	Gene Therapy Products
ISO 20399	Biotechnology—Ancillary materials present during the production of cellular therapeutic products and gene therapy products
ICH M7	Assessment and Control of DNA Reactive (Mutagenic) Impurities in Pharmaceuticals to Limit Potential Carcinogenic Risk
ICH Q8 (R2)	Pharmaceutical development
ICH Q9	Quality risk management
ICH Q11	Development and manufacture of drug substances (chemical entities and biotechnological/biological entities)
EudraLex Volume 4 Part IV	EU Guidelines for Good Manufacturing Practice (GMP) for Medicinal Products for Human and Veterinary Use

References

1 RESEARCH ARTICLE Unusual Salt and pH Induced Changes in Polyethyleneimine Solutions Kimberly A. Curtis, Danielle Miller, Paul Millard, Saswati Basu, Ferenc Horkay, Preethi L Chandran, Department of Chemical Engineering, Howard University, Washington, DC, United States of America, 2 Section on Quantitative Imaging and Tissue Sciences, Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, Maryland, 20892, United States of America.

2 **BioPhorum's QbD approach to registering complex raw materials as guidance.**

Permission to use

The contents of this report may be used unaltered as long as the copyright is acknowledged appropriately with correct source citation, as follows 'Entity, Author(s), Editor, Title, Location: Year'

https://doi.org/10.46220/2023CGT015

Disclaimer

This document represents a consensus view, and as such it does not represent fully the internal policies of the contributing companies.

Neither BioPhorum nor any of the contributing companies accept any liability to any person arising from their use of this document.

The views and opinions contained herein are that of the individual authors and should not be attributed to the authors' employers.

CONNECT COLLABORATE ACCELERATE
is a trademark of BioPhorum Operations Group.