Potency Assurance for Cellular and Gene Therapy Products

Draft Guidance for Industry

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I. INTRODUCTION

This draft guidance provides recommendations for developing a science- and risk-based strategy to help assure the potency[1] of a human cellular therapy[2] or gene therapy[3] (CGT) product. A potency assurance strategy is a multifaceted approach that reduces risks[4] to the potency of a product through manufacturing process design, manufacturing process control, material control, in-process testing, and potency lot release assays.[5] The goal of a potency assurance strategy is to ensure that every lot of a product released will have the specific ability or capacity to achieve the intended therapeutic effect.

This draft guidance document, when finalized, will supersede the document entitled "Guidance for Industry: Potency Tests for Cellular and Gene Therapy Products," dated January 2011 (January 2011 guidance).[6] When finalized, this guidance will describe FDA's recommendations for potency assays for CGT products and for a comprehensive approach to potency assurance

[1] As defined in 21 CFR 600.3(s), the word *potency* is interpreted to mean the specific ability or capacity of the product, as indicated by appropriate laboratory tests or by adequately controlled clinical data obtained through the administration of the product in the manner intended, to effect a given result.

[2] For the purposes of this guidance, "cellular therapy products" include tissue-engineered medical products regulated under section 351 of the Public Health Service Act (PHS Act) (42 U.S.C. 262).

[3] Human gene therapy seeks to modify or manipulate the expression of a gene or to alter the biological properties of living cells for therapeutic use. FDA generally considers human gene therapy products to include all products that mediate their effects by transcription or translation of transferred genetic material, or by specifically altering host (human) genetic sequences. Some examples of gene therapy products include nucleic acids, genetically modified microorganisms (e.g., viruses, bacteria, fungi), engineered site-specific nucleases used for human genome editing, and ex vivo genetically modified human cells.

[4] Risks are factors that may adversely affect product quality, including product potency. Sources of risk to the potency of a product include, but are not limited to, inadequately designed or poorly controlled manufacturing processes, variable materials, and undetected changes in the potency-related attributes of the product.

[5] For the purposes of this guidance document, the term *assay* is synonymous with the terms *test* and *analytical procedure*. Many CGT products undergo release testing using multiple potency assays; the January 2011 guidance expresses this concept of multiple assays using the term *assay matrix*.

[6] See https://www.fda.gov/media/79856/download.

that is grounded in quality risk management.[7] Potency assays remain an important part of assuring the potency of CGT products, but the comprehensive strategy described in this draft guidance document also includes complementary approaches to help assure potency.

In general, FDA's guidance documents, including this guidance, do not establish legally enforceable responsibilities. Instead, guidances describe the FDA's current thinking on a topic and should be viewed only as recommendations, unless specific regulatory or statutory requirements are cited. The use of the word should in FDA's guidances means that something is suggested or recommended, but not required.

II. BACKGROUND

The scope of this guidance document is limited to assuring the potency of CGT products that are regulated as biological products under section 351 of the Public Health Service Act (PHS Act) (42 U.S.C. 262).[8, 9]

This guidance document includes recommendations for helping to assure the potency of CGT products at all stages of the product lifecycle. For investigational products, we describe how to progressively implement a strategy for potency assurance during product development, and we provide additional considerations for assuring the potency of products that are undergoing rapid clinical development. For licensed products, we describe requirements for potency assurance, including testing required for lot release.

Developing assays that measure the potency of CGT products can be challenging. In this guidance document, we emphasize that potency assays and their corresponding acceptance criteria should be designed to make meaningful contributions to potency assurance by reducing risks to product potency. We provide illustrative examples of approaches to potency assay development that are grounded in quality risk management. Due to the diversity of CGT products and the product-specific nature of potency assays, the recommendations in this guidance document regarding the selection and design of potency assays are necessarily general. FDA may issue additional guidance documents that provide further advice about potency assays for specific classes of CGT products.

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[7] Quality risk management is a systematic process for the assessment, control, communication, and review of risks to the quality of the drug product across the product lifecycle. See Guidance for Industry: *Q9(R1) Quality Risk Management*; May 2023, https://www.fda.gov/media/167721/download.

[8] Cellular and gene therapy products meet the definition of "biological product" in section 351(i) of the PHS Act (42 U.S.C. 262(i)) when such products are applicable to the prevention, treatment, or cure of a disease or condition of human beings (see Federal Register Notice: Application of Current Statutory Authorities to Human Somatic Cell Therapy Products and Gene Therapy Products (58 FR 53248, October 14, 1993), https://www.fda.gov/media/76647/download).

[9] This guidance does not apply to vaccines for infectious disease indications, bacteriophage products, live biotherapeutic products, fecal microbiota for transplantation (FMT) products and allergenic products.

III. REGULATORY FRAMEWORK

A. Licensed CGT Products

To obtain a biologics license, a biologics license application (BLA) must contain data demonstrating that the product is safe, pure, and potent, and the continued safety, purity, and potency of the product must be assured.[10] Additional potency-related requirements for licensed products are as follows:

- Each lot of product must be tested for potency, and potency assays must be performed on a sample that is taken after completing all manufacturing steps that may affect potency.[11] The Center for Biologics Evaluation and Research (CBER) may permit an alternative approach to the requirements for lot release testing for potency in Title 21 Code of Federal Regulations (CFR) 610.1 and 21 CFR 610.10, but only if you demonstrate that the alternative approach will provide assurance of potency that is equal to or greater than the assurance of potency that would be provided by following the requirements in 21 CFR 610.1 and 21 CFR 610.10.[12]

- Before introducing a change to the manufacturing or testing of an approved biologic, you must assess the effects of the change, and you must demonstrate that the change does not adversely affect the potency of the product as it may relate to the safety or effectiveness of the product.[13]

B. Investigational CGT Products

You should describe your strategy for potency assurance in your investigational new drug application (IND). Your IND must contain sufficient chemistry, manufacturing and control information to assure the proper identification, quality, purity and strength[14] of the investigational drug, although the amount of information needed will vary depending on the phase of the investigation.[15] The amount of information that must be submitted to the IND will increase as you expand the scope of clinical investigations.[16] Accordingly, the degree of potency assurance for a product should be appropriate for the phase of clinical investigations and should progressively increase during the course of clinical development, as described in more detail in section IV.G of this guidance.

[10] See 42 U.S.C. 262(a)(2)(C)(i), 21 CFR 601.2(a), 21 CFR 601.2(d), and 21 CFR 601.20(c).
[11] See 21 CFR 610.1 and 21 CFR 610.10.
[12] See 21 CFR 610.9.
[13] See 21 CFR 601.12(a)(2).
[14] In this guidance document, the term *strength* is interpreted to include both the concentration and potency of a product.
[15] See 21 CFR 312.23(a)(7)(i).
[16] See 21 CFR 312.23(a)(7)(ii) - (iii).

During all phases of clinical investigation, your IND must contain sufficient data to support the stability of the drug substance (DS) and drug product (DP) during planned clinical investigations.[17] Stability studies should include assessments of potency, as described in more detail in section V.A of this guidance.

FDA may place investigations on clinical hold at any phase if, among other reasons, subjects would be exposed to an unreasonable and significant risk of illness or injury or if FDA finds there is insufficient information to assess whether the risks to subjects are reasonable.[18] FDA may place a study on clinical hold on such grounds if the potency of the product to be administered in an investigation is not adequately assured, or the information in the IND is not adequate to assure the potency of the product to be administered in the study.[19]

FDA's review of INDs for phase 2 and 3 investigations includes assessing "the likelihood that the investigations will yield data capable of meeting statutory standards for marketing approval,"[20] and FDA may place a phase 2 or 3 investigation on clinical hold if "the plan or protocol for the investigation is clearly deficient in design to meet its stated objectives."[21] If the lots of product that the sponsor plans to administer in such an investigation are not consistently potent, then some lots may not have the capacity to achieve the intended therapeutic effect in subjects, and therefore the investigation may have reduced statistical power to detect an effect of the product. In addition, an investigation conducted with product lots that have unknown or inadequately-controlled potency may be unable to provide information for ensuring the continued potency of the product after licensure[22] because it may be unclear whether the potency of the licensed product will be similar to the potency of the lots that were administered in the investigation. Therefore, if an IND does not provide adequate assurance of product potency, a phase 2 or 3 investigation that is intended to provide substantial evidence of effectiveness for a marketing application may be considered clearly deficient in design to meet its stated objectives and placed on clinical hold.

[17] See 21 CFR 312.23(a)(7)(ii) and 21 CFR 312.23(a)(7)(iv)(a) - (b).
[18] See 21 CFR 312.42(b)(1)(i),(iv) and 21 CFR 312.42(b)(2)(i).
[19] See 21 CFR 312.23(a)(7)(i), 21 CFR 312.42(b)(1)(iv), and 21 CFR 312.42(b)(2)(i).
[20] See 21 CFR 312.22(a).
[21] See 21 CFR 312.42(b)(2)(ii).
[22] See 21 CFR 601.2(d).

C. Current Good Manufacturing Practice

The facilities and methods used for manufacturing CGT products must comply with current good manufacturing practice (CGMP),[23] and many aspects of CGMP help to assure product potency.[24] The following recommendations for compliance with CGMP can also contribute to potency assurance. These recommendations are discussed in more detail in the sections of this guidance that follow:

- The manufacturing process should be designed and qualified to assure potency of the product and uniformity of the product from lot to lot.
- The materials used for manufacturing may affect the product's potency. Materials should meet suitable specifications before being used in the manufacturing process.
- Containers, closures, and product-contact equipment should be evaluated for potential adverse effects on product potency.
- Manufacturing process controls and in-process testing should be adequate to help assure potency of the product.
- Potency assays used for lot release should be verified to be suitable for their intended purpose (able to measure potency with sufficient specificity, accuracy and/or precision over the reportable range of the assay). Potency assay performance characteristics should be established under actual conditions of use and documented during assay qualification and validation.
- Phase-appropriate assays and acceptance criteria for potency should be established, and lots that fail to meet acceptance criteria should be rejected.

To further facilitate compliance with CGMP, you should develop an effective pharmaceutical quality system.[25] Your overall aim should be to establish a

[23] Manufacturing for investigational and licensed drugs (including biological products) must comply with CGMP, as required by section 501(a)(2)(B) of the Federal Food, Drug, and Cosmetic Act (FD&C Act) (21 U.S.C. 351(a)(2)(B) and (j)). DP manufacturing must also comply with FDA's CGMP regulations for finished pharmaceuticals in 21 CFR part 211, except that most phase I investigational drugs are exempt from the requirement to comply with part 211. See 21 CFR 210.2(c) and Guidance for Industry: *CGMP for Phase I Investigational Drugs*; July 2008, https://www.fda.gov/media/70975/download.

[24] As defined in 21 CFR 210.3(b)(16), the term *strength* encompasses the term *potency* when the term *strength* is used in the CGMP regulations for finished pharmaceuticals in part 211.

[25] See Guidance for Industry: *Quality Systems Approach to Pharmaceutical CGMP Regulations*; September 2006, https://www.fda.gov/media/71023/download and Guidance for Industry: *Q10 Pharmaceutical Quality System*; April 2009, https://www.fda.gov/media/71553/download.

manufacturing process that is in a state of control,[26] which will help to assure that the product will consistently be potent.

IV. DEVELOPING A POTENCY ASSURANCE STRATEGY

A potency assurance strategy is a comprehensive approach to help ensure that every lot of a product will have the potency necessary to achieve the intended therapeutic effect. The foundation of an effective potency assurance strategy is a manufacturing process that is designed to consistently produce a potent product. Potency assurance strategies should also reduce risks to potency by controlling aspects of the manufacturing process that may affect potency, which should include controls on material quality, control or monitoring of manufacturing process parameters, and in-process testing. Finally, potency assurance strategies should include lot release testing that confirms that potency-related quality attributes meet appropriate acceptance criteria. Lot release testing for most CGT products should include at least one bioassay[27] that measures a biological activity related to the intended therapeutic effect of the product, as described in more detail in section V of this guidance.

In this section, we provide recommendations for using quality risk management to develop and refine a potency assurance strategy. Your potency assurance strategy should evolve during product development as you gain manufacturing experience and product knowledge.

A. Quality Risk Management and Assurance of Potency

At all stages of the product lifecycle, you should use quality risk management to assess risks to product potency and to reduce those risks to acceptable levels. We recommend that you consider the following concepts[28] when designing a potency assurance strategy for your product:

- **Quality target product profile (QTPP).** A QTPP should include a summary of the potency-related characteristics of the product. The QTPP should be developed based on your understanding of the product's mechanism of action (MOA), the intended clinical indication, and the route of administration.

[26] A state of control is a condition in which the set of controls consistently provide assurance of continued process performance and product quality. See Guidance for Industry: *Q10 Pharmaceutical Quality System*; April 2009, https://www.fda.gov/media/71553/download.

[27] The term *bioassay* generally means an assay that measures the effect of a test article on living cells, tissues, or animals. For the purpose of this guidance document, when discussing products that are themselves composed of living cells or tissues, we use the term *bioassay* more broadly to also include assays that measure a biological activity of the living cells or tissues in the product itself. Additionally, for the purpose of this guidance document, assays that are not bioassays are referred to as *physicochemical assays*.

[28] See Guidance for Industry: *Q8(R2) Pharmaceutical Development*; November 2009, https://www.fda.gov/media/71535/download and Guidance for Industry: *Q9(R1) Quality Risk Management*; May 2023, https://www.fda.gov/media/167721/download.

- **Control strategy.** Manufacturing process controls and product quality controls play vital roles in potency assurance, as described in more detail in section IV.F of this guidance. These controls include process parameters, in-process testing, material testing or examination, lot release tests, and associated acceptance criteria.

- **Critical quality attribute (CQA).** Potency-related CQAs are attributes of the product that are important for achieving the intended therapeutic effect. You should identify the potency-related CQAs of your product to the extent needed to establish a phase-appropriate control strategy. Your manufacturing process should consistently produce lots that have all CQAs within appropriate pre-determined limits.

- **Critical process parameter (CPP).** CPPs are manufacturing process parameters that can influence CQAs. You should identify CPPs that may affect potency-related CQAs, and you should monitor or control these CPPs within appropriate pre-determined limits.

- **Risk assessment.** You should identify risks to potency-related CQAs, analyze the probability and severity of these risks, and evaluate their significance. You should assess risks not only when initially designing the manufacturing process and control strategy, but also throughout the product lifecycle.

- **Risk reduction.** Any risks to potency-related CQAs that are unacceptably high should either be avoided or reduced to acceptable levels by appropriately designing the manufacturing process and control strategy.

B. Applying Prior Knowledge and Experience

When designing a potency assurance strategy, we recommend that you utilize any relevant information that is available, including experience from manufacturing and testing a similar product, published information, and established scientific principles. Prior knowledge and experience with a specific product class can also help you to identify potency-related CQAs and assays to measure and control these CQAs.

Although prior knowledge and experience are valuable when initially designing a product's potency assurance strategy, manufacturers should consider that differences between products (e.g., MOA and intended therapeutic effect), differences in manufacturing processes, or differences in starting materials may affect potency in unexpected ways. For an autologous cell therapy product, for example, the level of the product's potency may be altered when cellular starting materials have been affected by disease or treatment history. Therefore, you should perform characterization studies and risk assessments for your specific product and manufacturing process rather than relying solely on prior knowledge and experience.

C. Gaining Product and Process Understanding

A thorough understanding of the product and the manufacturing process is important for developing an effective potency assurance strategy. We recommend that you consider the following advice at all stages of product development:

- **Mechanism of action.** An understanding of a product's MOA is important when identifying the product's potency-related CQAs and when developing a potency assurance strategy. If you do not fully understand your product's MOA, you should continue to seek such understanding during development and then update the product's potency-related CQAs and potency assurance strategy accordingly. In addition, CGT products often have multiple activities, and the specific activities that are most relevant to the therapeutic effect may depend on the targeted disease or condition. You should therefore consider the intended clinical indication when determining which quality attributes are relevant to the MOA and critical for product potency.

- **Nonclinical studies.** Nonclinical studies conducted early in development may be useful for learning about your product's MOA and for identifying connections between product attributes and the product's potential effect on a disease or condition. If available, information from nonclinical studies should be used to inform your initial potency assurance strategy, including selecting potency-related CQAs and identifying appropriate acceptance criteria.

- **Product characterization and identifying CQAs.** Product characterization refers to assessing a broad range of product attributes to understand the properties of the product more completely. Starting from the earliest stages of product development, we recommend that you conduct product characterization studies to better understand your product's MOA and to help identify product attributes that may be potency-related CQAs. We also recommend that you use characterization data when assessing manufacturing changes. Assays used in characterization studies do not necessarily need to be qualified, but they should be scientifically sound and fit for their intended purpose, be sufficiently precise to detect meaningful differences in product attributes, and provide results that are reliable.

- **Establishing a relationship between CQAs and potency.** Potency-related CQAs should ideally have a clear relationship to the product's MOA, and this relationship should be supported by prior knowledge (such as peer-reviewed literature), product characterization studies, nonclinical studies, or clinical studies. For products that have MOAs that are not fully understood, evidence of a statistical relationship between a product attribute and nonclinical or clinical outcomes may suggest that the attribute is relevant to potency. However, a statistical relationship alone cannot establish a mechanistic relationship between an attribute and potency. If needed, you should perform additional experiments or

studies with the product to determine whether there is a mechanistic relationship between a candidate potency-related attribute and potency.

- **Impact of material quality on potency.** During manufacturing process development, you should determine whether the attributes of the materials used during manufacturing may affect the product's potency-related CQAs. This information is valuable for developing material specifications, for performing supplier qualification, and for managing supply chain risk.

- **Process characterization and identifying CPPs**. You should perform process characterization studies to identify CPPs in your manufacturing process that affect potency-related CQAs, and you should mitigate risks to product potency by monitoring or controlling these CPPs. You may adjust CPPs as you gain an increased understanding of the product and the manufacturing process, but you should ensure that such changes to CPPs do not increase risks to product potency.

D. Risk Assessment

As part of quality risk management, you should use formal risk assessment tools to assess risks to potency comprehensively.[29] Risk assessments should start with identifying what might go wrong: what are the factors that might adversely affect the potency of your product both during and after manufacturing? The process of identifying risks to potency will be most effective when the product's MOA and potency-related CQAs are well understood and the risks to potency-related CQAs can be determined with confidence. Your risk assessment should include not only factors that may affect potency at the time of lot release, but also factors that may affect potency after lot release, such as the container closure, delivery devices, conditions for drug storage, shipping or handling, and conditions for thawing or preparing the drug for administration.

Analyzing and evaluating risks to potency can be challenging if assays used to measure potency-related CQAs have not been qualified to determine whether they have adequate performance. Using unqualified assays may decrease your ability to analyze risks to potency, due to a potential for inconsistent assay performance or uncertainty about the ability of the assay to detect clinically relevant changes in product potency.

Risks to potency should be reassessed as you increase your understanding of your product and manufacturing process. Before implementing manufacturing changes

[29] Risk assessment is a process for identifying hazards (e.g., failure modes of a manufacturing process, sources of variability), followed by analyzing and evaluating the risk that these hazards might harm product quality. See Guidance for Industry: *Q9(R1) Quality Risk Management*; May 2023, https://www.fda.gov/media/167721/download.

(including changes to materials), you should assess the risks that the changes may pose to product potency.[30]

Following evaluation of risks, any risks to potency that are unacceptably high should be mitigated or avoided through the design of the manufacturing process and the control strategy, as discussed in the following sections of this guidance.

E. Design of the Manufacturing Process

You should design your manufacturing process with the goal of consistently producing potent lots. We recommend that you use prior knowledge and experience to help develop a manufacturing process that minimizes known or likely risks to potency. Process development studies and process design do not need to be performed under CGMP conditions.

A major contributor to the variability of cellular products is the inherent variability of cellular starting materials, and manufacturers should assess the impact of such starting material variability on product potency. For many cellular products, some degree of variability in potency-related CQAs is unavoidable. When feasible, risks to potency caused by material variability should be mitigated by designing a manufacturing process with adaptive steps that compensate for variations in the material.[31]

F. Control Strategy

Your control strategy should mitigate any unacceptable risks to product potency. We recommend that your control strategy include the following elements, as applicable for the stage of the product lifecycle:

- **Control of materials.** If a link between a material attribute and product potency is known or suspected, this attribute should be controlled in the material's specification by examination of the supplier's test results and/or acceptance testing for each lot of the material. For example, if a manufacturing process for a cellular product includes a growth factor, the potential influence of the growth factor on the potency of the DP should be assessed. If necessary to reduce risks to product potency, the growth factor's biological activity[32] should be controlled in the material specification using a bioassay and an appropriate acceptance criterion.

[30] When implementing a manufacturing change for a licensed product, an assessment of the effect of the change on potency is required before distributing the post-change product. See 21 CFR 601.12(a)(2).

[31] See Guidance for Industry: *Q8(R2) Pharmaceutical Development*; November 2009, https://www.fda.gov/media/71535/download.

[32] For materials, we use the terms *biological activity* and *bioassay* instead of the terms *potency* and *potency assay*. Potency is a property associated with DS and DP, but not materials.

- **Process parameters.** When determining the operating ranges for process parameters, you should assess whether variation in the parameter has the potential to affect product potency. When manufacturing some cellular products, for example, a longer time in culture may decrease potency because of increased cell death or differentiation. In such cases, the duration of the culturing step is a CPP that should be assigned a limit based on prior knowledge and/or data from process development studies, process characterization studies, or process performance qualification studies.

- **In-process testing.** In-process samples should be tested to monitor quality attributes that may influence or predict product potency. For cellular products, for example, we recommend measuring viability, growth rate, and/or phenotype at relevant stages during manufacturing.

- **Lot release testing.** Potency release assays and their acceptance criteria are essential elements of a potency assurance strategy. As described in more detail in section V.B of this guidance, risks to potency-related CQAs often cannot adequately be mitigated by other aspects of the control strategy or process design. For potency testing of licensed products, potency release assays must be performed using a sample collected after completion of all manufacturing steps that may affect potency.[33] For example, if cryopreservation of a cellular product poses a high risk to the product's potency, then this risk should be mitigated by performing the potency assay on a sample taken after cryopreservation. For products such as tissue-engineered medical products that are not amenable to destructive sampling, we recommend that you conduct potency release testing on an additional unit of the lot that is manufactured in parallel for the specific purpose of providing a representative sample.

- **Continued process verification.** During manufacturing of a licensed product, you should routinely collect and analyze product and process data to verify that the manufacturing process remains in a state of control that assures potency.[34] These analyses may suggest potential opportunities to improve potency assurance through adjustments to the manufacturing process or control strategy. In certain cases, potency assurance may also be improved by including additional testing as part of continued process verification. For products that have an extremely short shelf life with insufficient time to complete a potency bioassay before lot release, it should be possible to perform lot release testing for potency using physicochemical assays. In such cases, we recommend that you also initiate one or more potency bioassays immediately after manufacturing the DP and evaluate the results when they become available post-release. For both investigational and

[33] See 21 CFR 610.1 and 21 CFR 610.10.

[34] See Guidance for Industry: *Process Validation: General Principles and Practices*; January 2011, https://www.fda.gov/media/71021/download.

licensed products, such post-release testing will help to verify that the manufacturing process is continuously capable of producing potent lots. The appropriateness and frequency of such post-release testing should be based on a risk assessment.

If one aspect of the potency assurance strategy cannot adequately mitigate a risk to product potency, then you should mitigate the risk by strengthening other aspects of the potency assurance strategy. For example, lot release testing may not be able to fully confirm potency if a product's potency-related CQAs are poorly understood or difficult to quantitate, or if a product has an extremely short shelf life that does not allow enough time to perform a bioassay. In these cases, other aspects of the potency assurance strategy (such as process design and process control) will take on increased importance and should therefore be more stringent and extensive.

G. Progressive Implementation of a Potency Assurance Strategy

You should have a defined potency assurance strategy throughout all stages of the product lifecycle, but during the early stages of product development some aspects of your strategy may not be fully mature. As you accumulate manufacturing experience and clinical data, you should progressively refine your risk assessments, manufacturing process, and control strategy, with the goals of maintaining product potency and strengthening potency assurance.

Before beginning clinical investigations, you should identify initial potency-related CQAs for your product, and you should perform a risk assessment and develop a strategy for reducing risks to these CQAs. To document that the potency assurance strategy will ensure an adequate level of potency for conducting early-phase clinical investigations and to obtain feedback on your plans for strengthening potency assurance, you should include the following information about your potency assurance strategy in Module 3 of the Common Technical Document (CTD) of your initial IND submission, and you should summarize this information in Module 2 of the CTD submission:

- Your product's MOA and QTPP, a list of your product's initial CQAs, and an explanation of how potency-related CQAs were identified.

- A description and justification of your potency assurance strategy, including risk assessments for potency-related CQAs and an explanation of how your process design and control strategy reduce risks to these CQAs. If your control strategy includes potency testing for lot release, you should provide a description of potency assays, assay performance characteristics, and justifications for acceptance criteria. If your control strategy does not include potency testing for lot release, you should explain how other aspects of your process design and control strategy provide adequate potency assurance for a product in early-phase clinical investigations.

- General descriptions of your plans for additional product characterization, plans for potency assay development, and plans for further strengthening your potency assurance strategy during product development.

Throughout early-phase clinical investigations, you should reassess and refine your product's QTPP, CQAs, CPPs, and potency assurance strategy. By later stages of clinical development, you should have developed a comprehensive potency assurance strategy that includes potency assays with appropriate acceptance criteria.[35]

As discussed in section III.B of this guidance, FDA may place certain investigations on clinical hold if the potency of the product is not adequately assured. Before beginning clinical investigations that involve significant risk or clinical investigations that are intended to provide substantial evidence of effectiveness to support a marketing application, the manufacturing process and the control strategy should provide phase-appropriate assurance that each lot of the product will be potent. Your control strategy for a product used in such investigations should include at least one physicochemical assay or bioassay that is performed on a suitable sample for lot release and that quantitates a potency-related CQA. Your control strategy should include acceptance criteria that are appropriate for the phase of investigation and that will result in rejection of sub-potent lots. Potency assays for products used in these types of clinical investigations should be qualified to demonstrate that the performance characteristics of the assays are fit for the intended purpose of the assay. Additionally, you should have evidence that potency-related CQAs are stable during storage and during preparation of the product for administration.

Before submitting a BLA, you should use all available product quality data and clinical data to reassess and refine your potency assurance strategy. Assays used for lot release and in-process testing must be validated.[36] You should describe the potency assays and reference materials that will be used for the licensed product, and you should explain and justify the impact of any differences from the potency assays and reference materials that were used during the clinical investigations that provide the primary evidence of effectiveness.

The potency assurance strategy in a BLA should be designed using knowledge gathered throughout development. For products in rapid clinical development programs, however, it can be challenging to gather this knowledge quickly. If you anticipate a compressed development timeline, we recommend that you thoroughly characterize the product and manufacturing process to help you rapidly establish a well-controlled manufacturing process that consistently yields a potent product. We also recommend that you develop, qualify, and implement potency assays before the initiation of clinical investigations. Implementing potency assays will allow you to confirm product potency and to collect

[35] Final acceptance criteria for the DS and DP are not expected until the end of clinical development. See 21 CFR 312.23(a)(7)(i).

[36] See 21 CFR 211.165(e) and 21 CFR 211.194(a)(2).

reliable potency data during even the earliest stages of clinical development. To increase the likelihood that the potency assays you develop will be usable for release of a licensed product, we recommend developing multiple assays that measure known or potential potency-related CQAs. We recommend that you evaluate the utility of these assays in parallel during early clinical investigations. Assays that are redundant may be discontinued later in development, as described in section V.B.1 of this guidance.

H. Requesting FDA Advice on a Potency Assurance Strategy

You should engage CBER early in development for feedback on your potency assurance strategy and your plans for developing potency assays. You should provide a detailed assessment of the risks to the potency of your product and explain how your potency assurance strategy reduces each of the identified risks to levels that are acceptable for the product's stage of clinical development. We also recommend that you consult CBER before making major changes to your potency assurance strategy.

We recommend that you request feedback either by asking CBER specific questions during meetings or by submitting an amendment to your IND that provides relevant background information and asks questions.[37] Your questions should be specific, rather than general or open-ended. During a meeting, you should limit discussion to the questions that you asked in the briefing materials; CBER cannot provide substantive feedback on new data or questions that you did not include in the briefing materials.

When asking for feedback on a potency assay, you should:

- Explain how the attribute measured by the assay is relevant to the product's MOA and the desired therapeutic effect. You should include supporting data, if available.

- Provide a clear description of the assay (e.g., reagents, reference materials, number of replicates, controls, method of analysis) and justification for the assay design. Assay descriptions should include sufficient detail to understand the assay, yet should be written concisely. We do not recommend that you submit assay protocols in meeting packages, unless specifically requested to do so.

- Provide a summary of any available information about the performance characteristics of the assay. We recommend that you also provide an assay qualification or validation report, if available.

- Describe any limitations of the potency assay and explain why the assay is suitable for its intended purpose, despite these limitations.

[37] See 21 CFR 312.31(b)(3).

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V. POTENCY ASSAYS AND ACCEPTANCE CRITERIA

A. Uses of Potency Assays

Assays measuring potency-related CQAs are critical for developing an effective potency assurance strategy and should be used in several ways throughout the product lifecycle.

- **Lot release testing.** Although the potency of a product cannot adequately be assured through release testing alone, release testing should be a key component of your potency assurance strategy. Meeting potency acceptance criteria at the time of lot release helps to confirm that the lot released will be acceptably potent. One or more potency assays are required for lot release of licensed biologics.[38]

- **Stability evaluation.** When feasible, we recommend that you identify potency-related CQAs that are stability-indicating by using forced degradation studies, real-time studies, or prior knowledge and experience. Stability studies should include assays that quantitate these stability-indicating CQAs, and you should evaluate potency data from product stored at the relevant long-term condition when establishing a shelf life for your product. If justified, acceptance criteria for potency-related CQAs in stability studies may be different from acceptance criteria used for lot release, but stability acceptance criteria for potency should still reflect the range of potency that is needed to mediate the intended therapeutic effect.

- **In-use studies and delivery device compatibility studies.** You should perform studies to evaluate whether your product's potency will remain acceptable during preparation of the product and during administration through delivery devices. If you anticipate a variety of delivery devices or in-use conditions, these studies should encompass or bracket the entire range of delivery devices or conditions, including the anticipated worst-case conditions.

- **Comparability studies.** If you change a product's manufacturing process, this change should be supported by risk assessments and studies that demonstrate that the change does not adversely affect the potency of the product.[39, 40] Manufacturing changes typically pose different risks to product quality than the risks encountered during routine manufacturing, and therefore you should assess the risk that manufacturing changes may affect potency-related attributes that are not evaluated by routine lot release tests. Comparability studies should include

[38] See 21 CFR 610.10.

[39] See Draft Guidance Document: *Manufacturing Changes and Comparability for Human Cellular and Gene Therapy Products*; July 2023, https://www.fda.gov/media/170198/download. When final, this guidance will represent the FDA's current thinking on this topic.

[40] For a licensed product, such assessments and studies are required before distributing the post-change product. See 21 CFR 601.12(a)(2).

analyzing data from the potency assays that are used for lot release and, if necessary, performing additional characterization studies of potency-related attributes that are at risk from the manufacturing change.

- **Manufacturing process studies.** An understanding of how manufacturing steps affect product potency is crucial for designing a manufacturing process and control strategy that assure potency adequately. We recommend that you use potency assays during process development studies, process characterization studies, process qualification studies, and continued process verification studies. Data from these studies should be used in risk assessments to identify steps in the manufacturing process that should be adjusted, monitored, or controlled to improve potency assurance.

B. Assay Selection and Design

Because CGT products usually have multiple potency-related CQAs that cannot be controlled adequately without release testing, your potency assurance strategy should typically include multiple release assays, each of which quantitates a potency-related CQA that is at risk. These assays may include physicochemical assays and/or bioassays. However, some potency-related CQAs that are related to a CGT product's biological activity can only be measured effectively with a bioassay, and if so we recommend that your potency assurance strategy include at least one bioassay. The central purpose of the bioassay should be to quantitate a potency-related CQA that is at risk, and it is not essential for the bioassay to mimic the product's MOA. Rather, your understanding of the MOA should help to drive selection of the product's potency-related CQAs.

Some CGT products consist of multiple active ingredients.[41, 42] For products that are subject to the requirements of 21 CFR part 211, there must be lot release testing to assess the strength of each active ingredient in the DP, which requires measuring the concentration or potency of each of the active ingredients.[43] For some CGT products that consist of multiple active ingredients, one bioassay may be sufficient to assess the potency of all of the active ingredients together (i.e., additional bioassays would not be needed to address risks to potency-related CQAs). In such cases, there should also be additional physicochemical assays to measure the concentration of each of the individual active ingredients.

[41] See 21 CFR 210.3(b)(7).

[42] Note that a CGT product that includes cells of multiple types does not necessarily have multiple active ingredients. For example, some CGT products consist of a complex mixture of different cell types, where the contribution of each cell type to the activity of the product as a whole is either unknown or is intertwined with the contribution of other cell types in the mixture. In such cases, the activity of the product is based on the totality of the cells in the mixture, and therefore the mixture of cells would be considered to be a single active ingredient.

[43] See 21 CFR 210.3(b)(16) and 21 CFR 211.165(a).

We recommend using risk assessment and prior knowledge and experience to identify how assay design, reagents, and parameters affect assay performance, and we recommend that you mitigate any unacceptable risks to assay performance through the design of the assay and its control strategy.[44]

1. Desirable Characteristics of Potency Assays

- **The assay should mitigate a risk to product potency.** Potency lot release assays reduce risk by detecting problems with potency-related CQAs, ideally leading to rejection of lots with unacceptable potency. You should implement lot release assays for potency-related CQAs that are at risk. If you demonstrate that other aspects of the process design or control strategy adequately ensure that a particular potency-related CQA will remain within acceptable limits, then a lot release assay for that CQA may not be needed. As noted in section V.D of this guidance, each lot release assay should have an appropriate quantitative acceptance criterion that mitigates risk to the potency-related CQA.

- **The assay should be precise.** Using an assay that has poor precision (high standard deviation, relative to the width of the acceptance criterion) increases the likelihood that a potent lot will be rejected or that a sub-potent lot will fail to be rejected. Bioassays may have substantial variability that can be difficult to eliminate. In such cases, we recommend that potency bioassays be designed to quantitate potency relative to a reference material, which will increase the precision of the reportable value for the bioassay. If assay precision cannot be sufficiently improved by changing the design of the assay, then we recommend that you reduce the standard uncertainty of the measurement by routinely performing multiple independent assay runs for each sample and reporting the mean value.[45] The number of runs should be pre-specified in the assay protocol.

- **The assay should be accurate.** An inaccurate assay will produce biased results that do not closely match expected values. The assay should have adequate precision and accuracy across the reportable range of the assay.

- **The assay should be specific.** Specificity should be demonstrated by testing non-potent product samples during assay qualification. When feasible, we recommend that specificity be evaluated using a very similar product (or an

[44] See Draft Guidance Document: *Q14 Analytical Procedure Development*; August 2022, https://www.fda.gov/media/161202/download. When final, this guidance will represent the FDA's current thinking on this topic.

[45] For a normally-distributed variable x with a standard deviation of s, if n independent measurements of x are acquired, then the standard uncertainty μ of the mean value $\bar{x}$ can be estimated as $\mu_{\bar{x}} = \frac{s}{\sqrt{n}}$.

altered version of the product) that does not possess the potency-related attribute that is detected by the assay. In addition, specificity should be demonstrated by showing lack of interference from relevant product-related impurities and sample matrices.

- **The assay should be robust.** If not, assay results may be unreliable and there may be frequent invalid assay runs. You should build robustness into the assay using a quality risk management approach by identifying the potential sources of assay unreliability and either eliminating them or mitigating their impact.

- **Minimize assay redundancy.** A potency assay that measures one quality attribute may mitigate risks to other related quality attributes. For example:

  - The MOA for a CGT product often depends on a stepwise chain of biological activities that occur after administration. The process design and the control strategy should provide assurance that each lot of the product can carry out these biological activities, but it may not be necessary to test each of the activities directly. For example, if a later step in the chain of biological activities is completely dependent on the earlier steps, then a bioassay at the later step that adequately ensures the product's biological activity at that step will typically be sufficient to also ensure the biological activities at the earlier steps.

  - Some active ingredients have multiple linked biological activities that each contribute to the efficacy of the product. In such cases, we recommend that you evaluate whether a bioassay that adequately controls one of these biological activities might also mitigate risks to the other linked biological activities, potentially in conjunction with relevant physicochemical assays. If so, a separate bioassay to measure each biological activity may not be necessary for assuring potency of the active ingredient.

- **Minimize the use of animals in potency assays.** We encourage replacement, reduction, or refinement of animal usage in assays.[46] We recommend that you use in vitro bioassays instead of animal-based bioassays when it is possible to do so without compromising potency assurance.

2. Approaches to Potency Assay Selection and Design

[46] For further information about FDA's approach to alternative methods, see https://www.fda.gov/science-research/about-science-research-fda/advancing-alternative-methods-fda.

Examples of recommended approaches to potency assay selection are listed below. These examples are not intended to cover all situations, and we emphasize that alternative approaches may also be acceptable.

- **For a cellular product:** A potency assay should measure a product attribute that is relevant to the product's intended therapeutic effect. However, identifying such attributes for cellular products can be challenging if the MOA is complex or poorly-defined. We recommend that you use nonclinical data and published scientific studies when identifying candidate potency-related CQAs and that you assess a broad range of attributes early in development during product characterization studies. Such data or studies may reveal certain protein expression patterns or other attributes that are associated with the product's biological activity. If a mechanistic relationship between an attribute and the product's biological activity can be established, this attribute may be a potency-related CQA. If risks to a potency-related CQA cannot be adequately mitigated through other aspects of your potency assurance strategy, then you should include an assay for this CQA as one of the potency assays in the product's lot release specification.

- **For a product with an extremely short shelf life:** There may not be sufficient time to perform a bioassay before the release of a short-lived product, such as a non-cryopreserved cellular therapy product. Therefore, in addition to one or more physicochemical potency assays that are performed on a sample of the DP for lot release, your strategy for assuring the potency of such a product should incorporate sufficient in-process testing for attributes that predict product potency. In addition, for investigational products with an extremely short shelf life, you should initiate one or more potency bioassays immediately after manufacturing the DP and evaluate the results when they become available post-release, with the goal of confirming product potency and manufacturing process reliability. Post-release potency bioassays should also be part of potency assurance for licensed products that have an extremely short shelf life, if the bioassays add value to continued process verification and reduce risks to potency.[47]

- **For a viral gene therapy vector intended for direct administration:** Even at the earliest stage of product development, release testing for a gene therapy vector that expresses a transgene should generally include a potency assay that quantitates transgene mRNA or protein in transduced cells. During further development of the product, you should also

[47] If a released lot of a licensed product is discovered to have unacceptable potency after distribution, you must submit a biological product deviation report to CBER. See 21 CFR 600.14(b).

comprehensively evaluate risks to the potency-related CQAs of the vector particles and risks to the vector's nucleic acids. For example, risks to the vector particles might compromise their structural integrity or ability to deliver nucleic acids to cells. Risks to the nucleic acids might compromise their length, sequence, or activity. For any risks to potency-related CQAs that are not adequately mitigated by your manufacturing process design or control strategy, you should reduce the remaining risks to acceptable levels by implementing additional potency assays with appropriate quantitative acceptance criteria.

- **For vector-transduced patient-specific cellular products:** When products are manufactured on demand for individual patients, the failure of a vector-transduced cellular DP lot to meet specifications may significantly delay patient treatment while another lot is manufactured. To reduce the risk of manufacturing a sub-potent lot of cellular DP, you should demonstrate that each vector lot has adequate biological activity before it is used for manufacturing cellular DP. Therefore, your strategy for assuring potency of the cellular DP should include not only a potency assay and quantitative acceptance criterion for DP lot release, but also a bioassay and quantitative acceptance criterion for release of each vector lot.

- **For a tissue-engineered medical product:** The potency of tissue-engineered medical products can depend on a wide range of physical, structural, and biological factors. Therefore, we recommend collecting a comprehensive set of characterization data from cells, scaffolds, or both (as applicable), using non-destructive and destructive assays to characterize physical, biomolecular, biochemical, immunological, and other biological properties. These characterization data may reveal biological, chemical, biomechanical, or physiological attributes that may be mechanistically related to the product's biological activity and may predict the potency of the tissue-engineered medical product. If such an attribute is a potency-related CQA and a risk assessment determines that other aspects of your potency assurance strategy cannot adequately mitigate risks to this CQA, then you should include an assay for this CQA as one of the potency assays in the product's lot release specification.

C. Assay Control and Change Management

1. Suitability

Potency assay protocols should include pre-defined acceptance criteria for sample suitability and system suitability.[48] Sample and system suitability criteria should be established based on risk assessment of the assay, and these suitability criteria should be designed to detect when the assay fails to perform properly. An assay run should be invalidated if suitability criteria are not met.

Typical sample suitability assessments for potency assays should include acceptance criteria for the sample response curve and limits on variability among sample replicates. Typical system suitability tests should include verifying that reference materials, positive controls, and negative controls meet pre-defined acceptance criteria. In addition to establishing these suitability acceptance criteria, we recommend that you use control chart analysis[49] of control sample data to detect any adverse trends in potency assay performance over time, as part of lifecycle management of the assay.[50]

2. Reference Materials

Many potency assays are bioassays that are calibrated relative to a reference material that has been assigned an arbitrary potency value (e.g., 100%). For CGT products, there may be no compendial standard or otherwise-recognized standard that is relevant to assessing the potency of your product. In such cases, you should develop an in-house reference material. It is often appropriate to designate a well-characterized lot of DP as a reference material.

You should establish a protocol for qualifying reference material lots, including replacement reference material lots. We recommend that you thoroughly qualify reference material lots using both routine release assays and in-depth characterization studies. Reference material lots should also be monitored to evaluate their stability. Before exhausting the supply of your current lot of reference material, you should evaluate the potency of a replacement lot using multiple independent assays run against the current lot, and you should use these data and pre-specified statistical procedures to assign a potency value to the replacement reference material lot.

In addition to the reference material that is used as a calibrator in each assay run, we recommend that each assay run also include a separate control material for use as a system suitability test, unless risk assessment of the assay indicates that such

[48] See Draft Guidance Document: *Q14 Analytical Procedure Development*; August 2022, https://www.fda.gov/media/161202/download. When final, this guidance will represent the FDA's current thinking on this topic.
[49] See Guidance for Industry: *Q9(R1) Quality Risk Management*; May 2023, https://www.fda.gov/media/167721/download.
[50] See Guidance for Industry: *Analytical Procedures and Methods Validation for Drugs and Biologics*; July 2015, https://www.fda.gov/media/87801/download.

a system suitability test is not necessary to assure the proper performance of the assay. Any new lot of control material should be qualified using multiple potency assay runs, and you should use the data from these runs to assign an expected potency range to the lot of control material for the system suitability test.

3. Qualification and Validation

Assay qualification involves determining the assay's performance characteristics (e.g., accuracy, precision, specificity, and sensitivity). Qualifying a potency assay allows one to determine whether assay performance is adequate for the intended purpose of helping to assure product potency, or whether assay performance instead needs to be further optimized. Potency assays should be qualified as soon as feasible, and no later than the initiation of clinical investigations that are intended to provide substantial evidence of safety and effectiveness for a marketing application.

DP release assays for a licensed product must be validated.[51] Assay validation should confirm the performance characteristics of the fully-optimized assay by comparing assay performance during the validation study to appropriate pre-specified acceptance criteria for accuracy, precision, specificity, and other relevant performance characteristics.[52] If robustness was not thoroughly evaluated and documented during assay development or qualification (or if there were post-qualification changes to the assay that might make it less robust), then robustness should be evaluated during assay validation to confirm that you have adequate understanding and control of the conditions and parameters that affect assay performance.

Many potency assays are bioassays, and bioassays are susceptible to numerous difficult-to-control sources of variability, including variability among instruments, variability among analysts running the assay, and variability among the lots of cells or other biological reagents used in the bioassay. We recommend that you identify potential sources of variability that pose risks to assay performance, and you should evaluate the effect of these sources of variability on performance characteristics such as precision and accuracy. If unacceptable risks are identified, you should reduce these risks to acceptable levels by either changing the design of the assay or improving control of the assay, for example by including additional control materials.

4. Assay Changes and Transfers

[51] See 21 CFR 211.165(e) and 21 CFR 211.194(a)(2).

[52] See Guidance for Industry: *Analytical Procedures and Methods Validation for Drugs and Biologics*; July 2015, https://www.fda.gov/media/87801/download and *Q2(R1) Validation of Analytical Procedures: Text and Methodology Guidance for Industry*; September 2021, https://www.fda.gov/media/152208/download.

When replacing or changing a validated potency assay, you should validate any new assay or perform partial revalidation of any changed assay, with the goal of achieving at least the same degree of control of the potency-related attribute as with the original assay. When transferring a potency assay to a new laboratory, you should perform a risk assessment and prospectively design an assay transfer study that has sufficient statistical power to evaluate assay reproducibility between the original and the new laboratories.[53] We recommend using equivalence testing to evaluate whether results from the new potency assay or new laboratory are sufficiently similar to results from the original assay or original laboratory.

D. Acceptance Criteria

Assays for potency-related CQAs should include quantitative acceptance criteria that contribute to potency assurance by mitigating risks to the potency-related CQAs. You should use a quality risk management approach to determine initial acceptance criteria, and you should refine the acceptance criteria based on additional risk assessments as you gain manufacturing experience and product knowledge. We do not recommend acceptance criteria of "report" or "for information only" for release assays, because such acceptance criteria do not add to potency assurance.

The acceptance criteria for a potency assay should include an appropriate quantitative lower limit to confirm that each lot has an adequate ability or capacity to mediate the intended therapeutic effect. If your product has biological activities that pose potential safety risks (or if it is unclear whether a product with high potency will be safe), you should also use available manufacturing data, nonclinical studies, and/or clinical experience to set an appropriate quantitative upper limit to confirm that the potency of each lot will not be in a potentially unsafe range.

For cellular products that have high inherent variability, acceptance criteria for potency release assays may be relatively permissive in early development, if justified in your IND. However, the acceptance criteria should ensure that lots will be rejected if their potency is outside of the expected range, as guided by available manufacturing data, nonclinical studies, and/or clinical experience.

For a licensed product, acceptance criteria for potency release assays should link product potency to evidence of clinical effectiveness from clinical investigations. Specifically, the acceptance criteria should be designed to ensure that the potency of the lots distributed under the license will be consistent with the potency of the lots that were administered to subjects in the clinical investigations that provided the primary evidence of the product's effectiveness.

[53] See Guidance for Industry: *Analytical Procedures and Methods Validation for Drugs and Biologics*; July 2015, https://www.fda.gov/media/87801/download.

As noted in section V.A of this guidance, potency assays have important uses beyond controlling potency for lot release, including assessing product stability, delivery device compatibility, and product comparability after a manufacturing change. The acceptance criteria for these types of assessments should be selected using a quality risk management approach, and they may differ from acceptance criteria for lot release.