Potency Assessment of Active Immunotherapy Products

Draft Guidance for Industry

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U.S. Department of Health and Human Services
Food and Drug Administration
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Table of Contents

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Draft Guidance for Industry

This draft guidance, when finalized, will represent the current thinking of the Food and Drug Administration (FDA or Agency) on this topic. It does not establish any rights for any person and is not binding on FDA or the public. You can use an alternative approach if it satisfies the requirements of the applicable statutes and regulations. To discuss an alternative approach, contact the FDA staff responsible for this guidance as listed on the title page.

I. INTRODUCTION

This guidance document provides recommendations for developing assays[1] to assess potency[2] as a part of a potency assurance strategy for active immunotherapy products (ACTIMPs)[3]. Active immunotherapies aim to treat a pre-existing disease or condition by inducing, stimulating, or modulating immune effector cells through introduction of an antigen (or antigens) associated with that disease or condition to the immune system. This guidance does not apply to preventive vaccines for infectious disease indications, bacteriophage products for infectious diseases, live biotherapeutic products, fecal microbiota for transplantation (FMT) products or allergenic products. Considerations related to potential safety concerns associated with ACTIMP product design are outside the scope of this guidance. Sponsors are therefore encouraged to consult with the appropriate product office regarding such matters.

When finalized, this guidance will describe the FDA's current thinking regarding design, validation, and evaluation of potency tests for ACTIMPs. This guidance is intended to supplement the guidance for industry, when finalized, on *Potency Assurance for Cellular and Gene Therapy Products* (Ref. 1) with additional advice and considerations specifically for ACTIMPs, including peptide- and-protein based ACTIMPs that are not cellular or gene therapy products (Ref. 2).

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

[1] For the purposes of this guidance document, the term *assay* is synonymous with the terms *test* and *analytical procedures*.

[2] As defined in 21 CFR 600.3(s), *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."

[3] Active immunotherapy refers to the process of inducing an immune-mediated antigen-specific therapeutic effect in the human body. Active immunotherapy products activate or modulate the immune system to treat preexisting conditions. The effects of active immunotherapies on the immune system may persist even after the product is no longer detectable in the body.

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

This guidance is limited to providing recommendations to assure the potency of ACTIMPs regulated as biological products under section 351 of the Public Health Service Act (PHS Act) (42 U.S.C. § 262 (a)). Preventive vaccines for infectious disease indications, bacteriophage products for infectious diseases, live biotherapeutic products, fecal microbiota for transplantation (FMT) products and allergenic products are beyond the scope of this guidance.

The goal of active immunotherapies is to treat a pre-existing disease or condition by inducing, stimulating, or modulating immune effector cells by introducing an antigen (or antigens) associated with that disease or condition to the immune system. ACTIMPs may be vectors that express antigens,[4] peptide or protein antigens,[5] or cells that present or express antigens (e.g., cell lysates or antigen-pulsed antigen presenting cells (APCs)). A subset of ACTIMPs are intended to treat cancer by activating immune cells that target tumor-specific antigens and/or tumor-associated antigens (TAAs), including neoantigens and viral antigens expressed by virus-transformed cancer cells. Recent advances in genomics and new knowledge regarding mutation load in tumor cells have led to rapid improvements in ACTIMPs intended to treat cancer through induction of anti-cancer immune responses. ACTIMPs also include products intended to treat autoimmune disease by inducing immune tolerance against specific antigens associated with that autoimmune disease.

Potency assays for cell and gene therapy products often focus on direct evaluation of biological activity of the product, for example by evaluating biological activity of expressed proteins, activity of recombinant protein-expressing cells, or a function of the cells themselves. However, assessment of ACTIMP's potency is challenging because the biological activity relies on host immune responses. The challenge can be even greater for individualized (personalized) therapeutic ACTIMPs, where the use of patient-specific neoantigens raises unique considerations for designing potency assays, including limited availability of specific reagents for each product lot (e.g., where there is no readily available antibody to measure expressed antigen). In this guidance, we discuss a science- and risk-based approach (Ref. 3) to developing suitable potency assays for ACTIMPs. We also provide specific examples of assays that can help assure the potency of various types of ACTIMPs.

[4] For purposes of this guidance, the term *vectored ACTIMP* refers to a product that uses a virus, microbe (typically a bacterium), DNA, or RNA to deliver nucleotide sequences that express target antigens. *Vector* refers to the virus, microbe, DNA or RNA used as the antigen delivery system.

[5] Peptide and protein antigen-based ACTIMPs are biological products.

III. REGULATORY FRAMEWORK

All biological products regulated under section 351 of the PHS Act must be evaluated to assure that the product is safe, pure, and potent,[6] that the continued safety, purity, and potency is assured,[7] and that quality is intact.[8] Potency assays are critical in ensuring that a product is capable of functioning as intended and are performed during lot release testing, comparability studies (Refs. 4 - 5), and stability testing (Ref. 6). Potency assays help assure that only product lots that meet defined criteria (Ref. 7) are administered during clinical investigations and after marketing authorization.

- **Potency Expectations for Licensed ACTIMPs:** Biologics license applications (BLAs) for products regulated under section 351 of the PHS Act must contain, among other things, data demonstrating that the product is potent, and that the continued potency of the product is assured.[9] FDA regulations allow for considerable flexibility in determining the appropriate measurement(s) of potency for each product. Potency refers to the ability or capacity of the product to achieve the intended therapeutic effect; therefore, the suitability of assays to measure potency is evaluated on a case-by-case basis to account for product-specific attributes. All potency assays used for lot release testing of licensed biological products must comply with applicable regulations, including applicable Biological Product and Current Good Manufacturing Practice (CGMP) regulations.[10, 11] Further, such assays must be validated.[12] Potency assay(s) should be used as a part of the stability determining tests to establish product expiration date for licensure.

- **Potency Expectations for Investigational ACTIMPs:** In early-phase clinical investigations, it may not be possible to meet all of the requirements for potency described above for licensed biological products (Refs. 8 - 10). Nonetheless, sponsors must submit as part of the Investigational New Drug Application (IND) data to assure the identity, quality, purity and strength, as well as stability, of products used during all phases of clinical study.[13] "[T]he amount of information needed to make that assurance will vary with the phase of the investigation, the proposed duration of the investigation, the dosage form, and the amount of information otherwise available."[14]. To facilitate the development of ACTIMPs, we recommend a progressively iterative approach to potency

[6] Section 351(a)(2)(C)(i) of the Public Health Service Act (PHS Act) (42 U.S.C. 262(a)(2)(C)(i)) and 21 CFR 601.2(a).
[7] 21 CFR 601.2(d).
[8] See e.g., section 351(a) of the PHS Act and 21 CFR Part 312.
[9] See 42 U.S.C. 262(a)(2)(C)(i) and 21 CFR 601.2(a), § 601.2(d), § 601.12(a)(2) and § 601.20(c).
[10] See 21 CFR 600.3(s), §610.1, §610.10.
[11] For products regulated under section 351 of the PHS Act, conformance testing (21 CFR 601.20(a)) and control of the manufacturing process (21 CFR 601.20(c)) are required to comply with FDA's Current Good Manufacturing Practice (CGMP) For Finished Pharmaceuticals regulations (21 CFR Parts 210 and 211) as well as applicable biologics regulations in 21 CFR Part 600.
[12] See 21 CFR 211.165(e) and § 211.194(a)(2).
[13] 21 CFR 312.42(b)(1)-(2) (clinical holds) and § 312.23(a)(7)(i-ii) (IND content and format).
[14] 21 CFR 312.23(a)(7)(i).

assurance and potency assay development. The guidance for industry *Potency Assurance for Cellular and Gene Therapy Products,* when finalized, will describe such an approach and, when final, will represent FDA's current thinking. Though this recommended approach to potency assurance allows substantial flexibility, especially during early product development, investigations may be placed on clinical hold if product potency is inadequate to assess the risks to subjects or if subjects would be exposed to an unreasonable and significant risk of illness or injury.[15] We recommend timely discussions with your CBER review team[16] as you design, evaluate, and validate your potency assay(s) (Ref. 11).

IV. GENERAL RECOMMENDATIONS ON POTENCY ASSAYS FOR ACTIVE IMMUNOTHERAPY PRODUCTS

A. Assessing Potency of ACTIMPs

- A thorough understanding of the ACTIMP's active ingredient(s) and mechanism of action (MOA) should be used to identify potency-related critical quality attributes (CQAs) that should be measured for lot release, stability, and comparability. The relationship between MOA (e.g., stimulation of an antigen-specific T cell response) and potency-related CQAs (e.g., epitope properties) should be supported (e.g., by published medical or scientific information that is generally accepted by experts qualified by scientific training and experience in the relevant field including FDA experts, product characterization studies, nonclinical studies, or clinical studies.)[17] Additional knowledge about the product's MOA and CQAs can also be gained through monitoring immune responses in study subjects who have received the ACTIMP.

- Potency assays should be quantitative and should have adequate precision to reliably discriminate between a product that is sufficiently active to achieve its intended therapeutic effect and a product that has insufficient activity, to identify sub-potent lots and allow for their rejection.

- For ACTIMPs, potency assays should directly or indirectly measure the ability or capacity of the product to mediate immune responses to achieve the intended therapeutic effect.

[15] 21 CFR 312.42(b)(1)-(2).

[16] These discussions can be in the form of IND amendments or meetings. See 21 CFR 312.31(b)(3) and the draft guidance for industry *Formal Meetings Between the FDA and Sponsors or Applicants of PDUFA Products - Guidance for Industry* (September 2023) at https://www.fda.gov/regulatory-information/search-fda-guidance-documents/formal-meetings-between-fda-and-sponsors-or-applicants-pdufa-products, which when final, will represent FDA's current thinking on the topic.

[17] For further information about generally accepted scientific knowledge, see Guidance for Industry: *Generally Accepted Scientific Knowledge in Application for Drug and Biological Products: Nonclinical Information*; May 2023, https://www.fda.gov/media/168408/download**.** When finalized, it will represent FDA's current thinking on the topic.

- The potency-related CQAs of the product should be measured during lot release testing, and appropriate acceptance criteria should be established to ensure consistent performance of ACTIMP lots. 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.

- Some ACTIMPs may contain many antigens that are expected to contribute to the potency of the product and thus should be considered potency-related CQAs. Other ACTIMPs may have unidentified antigens (such as in tumor lysates). In both cases, it may not be feasible to fully assess the contribution of each antigen to product potency. We have the following recommendations for these situations:

  - When an ACTIMP consists of cells or cell lysates and the contribution of each antigen to the overall activity of the product is either unknown or is dependent upon the contribution of other components in the product, then a single potency assay may be appropriate to assess potency of the product.

  - When an ACTIMP consists of a defined combination of different peptides or proteins, a single potency assay that measures the immunological potential of the product may be appropriate. However, such assays should also be combined with measurement of the quantities of individual peptides or proteins to ensure a comprehensive assessment of the ACTIMP's potency.

- All assay reagents necessary for potency assays should be generated or a source for their supply identified early in clinical development. These reagents should be qualified as suitable for their intended purposes, to facilitate early assay development and ensure assay consistency.

- As a part of the potency assay development process sponsors should discuss their potency assurance strategy and potency assay development plans with the CBER review team assigned to their Investigational New Drug (IND) application (Ref. 11).

- Some ACTIMPs may contain one or more substances commonly referred to in the scientific literature as an *adjuvant*. An adjuvant must not be introduced into a licensed product unless there is satisfactory evidence that it does not adversely affect the safety or the potency of the product.[18] If an adjuvant is used in an ACTIMP, we recommend that sponsors consult with their CBER review team for guidance on the appropriate control strategy for the adjuvant as a part of the overall potency assurance strategy.

[18] 21 CFR 610.15(a).

B. Establishing Potency-Related CQAs for ACTIMPs

- An understanding of how the product mediates a therapeutic effect is important for identifying the product's potency-related CQAs. We recommend that sponsors evaluate data from various sources, including preclinical or proof-of-concept data (in vitro or in vivo), previous human clinical trial experience (your prior knowledge), and in vitro cellular or biochemical characterization studies.

- Potency-related CQAs should be identified for each product by considering the following:

  - Knowledge of the ACTIMP's MOA;
  - Product characterization data;
  - How the CQA(s) affect the product's biological activity i.e., by assessing the relationship between candidate potency-related CQAs and the product's ability or capacity to achieve the intended immune response; and
  - Data from studies of candidate potency-related CQAs.

C. Approaches for Potency Measurement of ACTIMPs

- **Bioassays:** The most direct assessment of ACTIMP potency is a quantitative bioassay that measures the product's effect on the immune system using living cells, tissues, or animals. Bioassays can help mitigate risks to a product's potency that may not be fully addressed through physicochemical assays alone. Bioassays may be either in vitro or in vivo assays or a combination of these methods. We encourage methods that replace or reduce the use of animals whenever possible.[19] Examples of in vitro bioassays relevant for measuring ACTIMP potency include, but are not limited to, cell culture-based assays that quantitate the ability of the ACTIMP to stimulate antigen-specific T cell responses such as proliferation, cytokine production, or cytotoxicity. Examples of in vivo assays include induction of an antigen-specific immune response in appropriate animal models, collection of samples from animals, and measurements that reflect the proposed MOA, such as tetramer staining, intracellular cytokine staining, Enzyme-Linked Immunospot (ELISPOT), or antibody responses.

- **Physicochemical assays:** If you have established that the potency of an ACTIMP can be adequately assured without a bioassay, you can perform lot release testing of potency-related CQAs using physicochemical assays alone. Physicochemical

[19] 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. We support the principles of the "3Rs," to reduce, refine, and replace animal use in testing when feasible.

assays may be used to assess immunochemical, biochemical, and/or molecular attributes of the product that are related to potency.

- **Multiple measures:** For products that have multiple potency-related CQAs that cannot be adequately evaluated using a single assay, you should develop multiple complementary assays that measure attributes associated with the product's function. This strategy may include a combination of different bioassays and/or physicochemical tests.

V. POTENCY ASSAYS FOR SPECIFIC TYPES OF ACTIVE IMMUNOTHERAPY PRODUCTS

The following recommendations are listed by type of ACTIMP:

A. Non-personalized[20] Peptide- and Protein-based ACTIMPs

- In the case of ACTIMPs where each product batch contains the same peptide(s), peptide concatemers, purified proteins, protein conjugates, or recombinant protein(s), we recommend that the potency assay and the necessary assay-specific reagents (e.g., assay specific cell lines, antibodies, tetramers) be developed and qualified during early product development to ensure assay consistency throughout the product life cycle.

- When the physicochemical attributes of a non-personalized protein or peptide product have been established as sufficient to assess the product's ability or capacity to achieve the intended immunological response, the physicochemical attributes alone may be used to evaluate the product's potency for lot release testing and stability studies.

- When physicochemical tests alone are not sufficient to define the product's ability or capacity to achieve the intended immunological response, a bioassay may be needed to assure the product's immunological activity. For example, a bioassay may be needed to assess potency if the product consists of multiple proteins that cannot be adequately quantitated through physicochemical assays alone, or if the product is susceptible to chemical or structural changes that may affect antigen processing or epitope presentation and these changes cannot be reliably quantitated using physicochemical tests.

- If you are designing a bioassay for a product that consists of multiple proteins or multiple antigenic epitopes concatemerized into a single polypeptide chain, it may be sufficient for the bioassay to quantitate the activity of one or more individual

[20] The term "non-personalized" is used in this guidance to refer to ACTIMPs that contain the same active and inactive components for all subjects or patients, as opposed to personalized ACTIMPs that may contain components that are specific for an individual subject or patient.

antigenic epitopes in the product or concatemer, rather than assessing all of the epitopes.

- When a bioassay is used to determine potency based on the activity of a limited number of antigens in a product or concatemer, additional physicochemical tests should also be performed to confirm sequence identity of the polypeptide chain and to quantitate the protein(s) concentration.

- Some non-personalized peptide- and protein-based ACTIMPs are supplied in multiple vials for the drug product (DP) that are each formulated to contain a subset of the DP peptides/proteins in the ACTIMP (e.g., when an ACTIMP consists of multiple synthetic peptide epitopes that are formulated separately in order to prevent precipitation or aggregation and are mixed together with a suitable adjuvant at the clinical site prior to use). For such products, potency release testing should be performed separately for each vialed subset of the DP.

B. Non-personalized Vectored ACTIMPs

- Vectored ACTIMPs, including those based on viral vectors, bacterial vectors, DNA, or RNA, depend on two biological activities to induce their intended effect: the ability to transfer a genetic sequence to a cell, and the intended biological effect of the expressed gene sequence. Therefore, potency assays for these types of ACTIMPs should incorporate a quantitative potency bioassay either through a protein expression assay (when justified by evidence of a direct relationship between the level of expressed protein and its immunological activity), or through a bioassay that measures immune responses to the expressed protein (for example, the ability of vector-transduced APCs to activate target antigen-specific T cells).

- Because of concerns related to induction of off-target immune responses and the possibility of antigenic competition and reduction of the therapeutic effect, we do not recommend the introduction and use of reporter genes or irrelevant epitopes in the vector, unless their functional role in rendering a therapeutic benefit has been demonstrated in proof-of-concept studies.[21]

C. Personalized Peptide- and Protein-based ACTIMPs

For personalized peptide- and protein-based ACTIMPs, that depend on bioinformatics pipelines[22] to identify patient specific epitopes (such as those identified as being present in a tumor), the development of a product specific bioassay for lot release of each

[21] The use of reporter genes for vector selection and product tracking are outside the scope of this guidance document.

[22] A bioinformatics pipeline is a set of bioinformatics software tools that work together to achieve a desired effect. Examples of software tools include, but are not limited to, sequence alignment software, software to identify the unique genetic sequence that determines a neoantigenic epitope, and software that determines optimal sequences of neoantigens for individual patients.

patient's product may not be practical. In these cases, physicochemical methods may be utilized to assess the product's potency-related CQAs for lot release if the manufacturing process is adequately controlled to mitigate risks to product potency. To facilitate the use of physicochemical assays, we recommend that you qualify the entire manufacturing process,[23] including justification for the use of a specific bioinformatics pipeline,[24] which provides sufficient assurance of immunological potential of the predicted epitopes. Once the entire manufacturing process is qualified, it may be acceptable to use physicochemical assays that measure potency-related CQAs for DP lot release and stability testing. Potency verification of neoantigens manufactured based on the use of bioinformatics pipeline should follow a stepwise approach as described below:

- For Phase 1 clinical studies, potency of the neoantigen peptides/proteins may be inferred based on sequence confirmation, supported by scientific justification of the bioinformatics pipeline. The scientific justification may be based on data from in silico studies and existing prior knowledge (from preclinical studies and from published, peer reviewed medical and scientific literature that is generally accepted by experts qualified by scientific training and experience in the relevant fields) about the ability to predict relevant epitopes.

    - For later phase studies, when the neoantigen epitopes are manufactured using a qualified manufacturing process, including use of a justified bioinformatics pipeline, measurements of potency-related CQAs of the neoantigenic epitopes based on physicochemical testing may be sufficient to confirm product potency for lot release, without the need to develop potency-related bioassays for each personalized ACTIMP lot.

- If changes are made to the bioinformatics pipeline[25] or to other aspects of the manufacturing process that may lead to changes in the predicted epitopes or potency-related CQAs, then additional studies may be necessary to ensure that the changes do not adversely affect the potency of the product relating to the safety and effectiveness of the product. Note that a change to the bioinformatics pipeline can affect a product's safety and efficacy, and is subject to reporting

[23] For recommendations on manufacturing process qualification, refer to FDA's guidance Process Validation: General Principles and Practices (January 2011), available at: https://www.fda.gov/regulatory-information/search-fda-guidance-documents/process-validation-general-principles-and-practices.

[24] A complete description of bioinformatics pipeline qualification is outside the scope of this guidance document. In general, bioinformatics pipeline qualification for ACTIMPs is the demonstrated ability of the software algorithms incorporated in the bioinformatics pipeline to accurately and reproducibly predict neoantigenic epitopes that can stimulate the desired tumor-specific immune responses in the target patient population in a human leukocyte antigen (HLA)-specific manner. Supporting data justifying the use of a qualified bioinformatics pipeline in the manufacturing process may be obtained through prior knowledge from scientific literature, proof of concept studies (POC), in vitro or in vivo laboratory studies, in-silico analysis, data from prior clinical studies, or a combination of these.

[25] Assessing changes to the bioinformatics pipeline is beyond the scope of this guidance. We recommend that you assess the changes and discuss them with the bioinformatics review team for your IND.

requirements for essential information that must be reported to the Agency.[26, 27] The need for additional assessments, including comparability of product potency, to support introduction of manufacturing changes, including changes to the bioinformatics pipeline, should be determined based on a comprehensive risk assessment. Managing changes to the manufacturing process and determining comparability following manufacturing changes are beyond the scope of this guidance.[28]

D. Personalized Vectored ACTIMPs

- For personalized vectored ACTIMPs, we recommend that you evaluate potency-related CQAs of the vector using a bioassay (section IV. B of this guidance). The activity of the vector expressed personalized target epitopes derived using a bioinformatics pipeline may be assessed using a similar strategy as that described for personalized peptide/protein-based ACTIMP (section IV.C of this guidance).

- Once the entire manufacturing process has been qualified including justification for a specific bioinformatics pipeline (section V.C of this guidance), physicochemical testing (such as confirmation of vector sequence) may be sufficient to assure the activity of personalized target epitopes included in the ACTIMP lots. However, if the bioinformatics pipeline or manufacturing process is changed, additional qualification studies using lots generated by the new process may be needed to assure product potency.

- When multiple patient specific target epitopes are combined in a single reading frame to generate the coding sequence for delivery by a vector, the effect of the order of epitopes (including any repeated epitope sequences) in the coding sequence should be evaluated and justified. To provide assurance of similar levels of potency for different patient specific product lots, we recommend that

[26] The Chemistry, Manufacturing and Controls (CMC) information in an IND should describe a sponsor's commitment to perform manufacturing and testing of the investigational product as stated in the IND or in a cross-referenced IND or master file. If a manufacturing change could affect product quality, we consider the manufacturing change essential information that must be submitted in an information amendment to the IND under 21 CFR 312.31(a)(1).

[27] For licensed products, an applicant must inform FDA about each change in the product, production process, quality controls, equipment, facilities, responsible personnel, or labeling established in the approved license application(s); certain changes require submission to and approval by the agency prior to distribution of the product made using the change. 21 CFR § 601.12(a)(1), (b), and (c). When implementing a manufacturing change for a licensed product, an applicant must assess the effect of the change and demonstrate the lack of adverse effect of the change on potency as it relates to the safety or effectiveness of the product before distributing the post-change product. 21 CFR 601.12(a)(2).

[28] For proposed recommendations on the principles of determining comparability after manufacturing changes, refer to FDA's Draft guidance for Industry "Manufacturing Changes and Comparability for Human Cellular and Gene Therapy Products (2023)": https://www.fda.gov/regulatory-information/search-fda-guidance-documents/manufacturing-changes-and-comparability-human-cellular-and-gene-therapy-products. When final, the guidance will represent FDA's current thinking.

the rationale for the order in which the epitopes are arranged in the coding sequence be kept constant to the extent feasible.

- As with non-patient specific vectored ACTIMPs, we do not recommend the introduction and use of reporter genes or irrelevant epitopes in the vector, unless their functional role in rendering a therapeutic benefit has been demonstrated in proof-of-concept studies.

E. Cell-based ACTIMPs

Examples of cell-based ACTIMPs include products composed of living cells (e.g., antigen-pulsed dendritic cells), irradiated cells (e.g., non-dividing cancer cells), or cell-derived products (e.g., tumor lysates) that are used to induce antigen-specific immune responses in vivo or to modulate immune responses in the patient. The cells may be genetically modified or unmodified cells.

- Potency assays for live cell-based ACTIMPs should measure an activity of the cellular product that can be used to evaluate its ability to perform as intended.

- The potency of cell-based ACTIMPs that express known antigens and/or immunomodulatory molecules (e.g., cytokines) should be assessed by evaluating potency-related CQAs such as the expressed antigen and/or immunomodulator along with additional potency-related CQAs of the cells (e.g., assessment of co-stimulatory molecule expression by the cell).

- Cell viability is an important potency-related CQA and should be a part of the overall potency assessment.

- For ACTIMPs consisting of different cell types, where the contributions of each cell type to the activity of the ACTIMP are either unknown or are interdependent with other cell types in the product, it may be appropriate to assess potency on the product as a whole.

- When two or more cell populations contribute to product function via the same MOA (e.g., two types of APCs or a product containing two autologous cell lysates), a combined potency assay that measures the total biological activity may be sufficient.

- When two or more cell populations are intended to contribute to product activity via distinct and independent MOAs, the contribution of each cell population to the potency of the product should be evaluated independently or in a combined assay that can measure all distinct and independent contributions.

Antigen-pulsed APC-based ACTIMP

- When an APC is pulsed with an antigen or multiple antigens, the quality of the antigen(s) used to pulse APC is critical to the activity of the product. We have the following recommendations for assessing potency of an antigen-pulsed APC:

  - Potency of antigen-pulsed APCs should be assessed by measuring potency-related CQAs of the APC. In this example, the potency-related CQAs may include assessments of cell viability, upregulation of HLA or other activation markers on the APC surface, cytokine production, or a relevant biological activity (e.g., the ability to activate T cells). Therefore, to ensure a comprehensive assessment of the relevant potency-related CQAs of the product, we recommend the use of orthogonal methods to adequately determine APC function.

  - The critical attributes of the individual peptides and tumor lysates used to pulse APCs should be assessed as part of the control strategy for critical materials, and specifications for these attributes should be included in the overall potency assurance strategy. The quality of the antigen(s) should be assessed as part of vendor qualification, material acceptance criteria, and stability.[29] Some of these material quality measures might include bioassays or physicochemical testing that could also relate to the activity of the material.

Irradiated tumor cell-based ACTIMPs

- Irradiated tumor cells include autologous tumor-derived primary cells, allogeneic primary cells, tumor-derived cells that are cultured ex vivo for a short period of time (heterogenous cells), or homogenous tumor cell lines. These cells are sometimes genetically modified to express an immunomodulatory protein (e.g., a cytokine such as the human granulocyte macrophage colony stimulating factor [GM-CSF]). The cells are irradiated to prevent growth in vivo while preserving their ability to express the full spectrum of immune-stimulating proteins and antigens. Irradiated cells are expected to survive for a short period of time in vivo to provide their biological effect. The level of biological activity may vary depending on the extent of irradiation and whether the cells are still viable. We therefore recommend that you determine the percentage of live cells (membrane-intact cells), unless the potency of the product does not depend on the presence of membrane-intact cells. Depending on the type of irradiated cell product, additional types of potency assays may be appropriate. We recommend a

[29] Each lot of components, drug product containers, and closures shall be withheld from use until the lot has been sampled, tested, or examined, as appropriate, and released for use by the quality control unit (21 CFR 211.84(a))

risk-based approach to determine suitable potency assays to help assure product quality. The examples below are for illustrative purposes only.

- Example 1: If the irradiated cellular product is not genetically modified and the product's activity is not dependent on the continued production of a cellular protein, it may be sufficient to assess potency using physicochemical assays for relevant potency-related CQAs (e.g., amount of antigen or specific protein patterns).

- Example 2: If the irradiated cell is genetically modified in such a way to enable continued production of an immunomodulatory molecule, such as GM-CSF, an assay that provides a quantitative measure of GM-CSF protein expression and its biological function should be included as a potency assay.

Tumor lysate-based ACTIMPs

- Tumor lysates may be derived from autologous or allogeneic primary tumors or from tumor-derived cell lines. Loss or degradation of essential antigens during the manufacturing process, during storage, or after a manufacturing change could significantly reduce product potency. In order to reduce variability arising from the manufacturing process and the variability in each tumor isolate, we recommend that you determine the quantity or quantities of relevant protein or proteins in the lysate(s) during characterization studies and use this information to develop lot release acceptance criteria to ensure lot-to-lot consistency.

VI. REFERENCES

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