

GlobeImmune Comments on Draft Guidance for Industry – Potency Tests for Cellular and Gene Therapy Products

[Docket No. FDA-2008-D-0520]

In general we are in agreement with the Draft Guidance on Potency Tests for Cellular and Gene Therapy Products. However, because it is intended to address potency for a number of types of products, it does not specifically address individual product types that are regulated by the Office of Cellular, Tissue and Gene Therapies. Products intended to stimulate cellular immunity present unique challenges with regard to the development of potency assays, and the following comments are intended to address these specific challenges.

Release and potency assays are essential for establishing and maintaining strict product quality control. The mechanism of action of product-mediated cellular immune activation involves many intermediate steps carried out by a variety of different cell types, so that assay characterization of any particular step may not adequately reflect the entire process. We thus concur with the proposed industry guidance that multiple potency assays may best be employed to define the product characteristics needed for a clinically meaningful immune response. Particular attributes of individual assays (eg, availability of reagents, precision, stability- and consistency-indicating properties, etc) will factor into whether a specific assay is a candidate for inclusion in release testing, stability testing or other uses such as comparability or characterization studies. Some specific examples are provided in this response to help identify additional complexities for reproducing elements of immune activation with different types of immunotherapeutic products. We look forward to working with the Agency on the iterative development of bioassays to quantify elements of immune activation needed for product potency and assurance of clinical benefit.

Innate and adaptive immune responses

Many different types of products are in development as cancer vaccines intended to stimulate innate and/or adaptive immune responses.. The innate response is characterized as the host recognition of danger signals, which consists of receptor recognition of molecular patterns, such as those found on foreign microbial organisms, by receptors on immune cells, including macrophages and dendritic cells. Agonists of immune activation under development include simple or complex mixtures of synthesized lipids, carbohydrates, nucleic acids or peptide-based conjugates as exogenous (artificial) adjuvants, or vectors such as viral, bacterial, yeast or transfected mammalian cells whose endogenous adjuvant properties trigger an innate response to the danger signal created by recognition patterns on the vectors. The danger signals that trigger the innate immune response may be further subdivided into danger detection (e.g. TLR agonists) and those that trigger additional downstream responses (e.g. phagocytic receptor agonists) of enhanced phagocytosis, upregulation of the antigen processing machinery, such as protein and peptide degradation, secretory pathway activation and surface receptor upregulation.

Activation of antigen-specific (adaptive) immune responses usually does not occur at the site of product administration (e.g. subcutaneous, intradermal, intravenous, etc). Hence, it is important to quantify not only the delivery system and the target antigen, but also the requisite downstream activities, many of which are not reproduced by *in vitro* potency assays. Immune activation involves trafficking of activated antigen-presenting cells (APCs) from the site of product administration to lymphoid tissue (e.g. draining lymph nodes) where the cells of the immune system (e.g. T, B and NK cells) are recruited and activated. The source of antigen for adaptive immune

responses is provided through direct delivery of the pre-synthesized target peptide antigen, or is generated upon alteration of host APCs by the vectors. Nevertheless, the clinical benefits resulting from antigen-specific T cell activation are usually achieved at sites remote from their place of activation in lymphoid tissue (e.g. at primary and metastatic tumor sites). The (re-) introduction of modified immune cells, such as *ex vivo* activated dendritic cells (DCs) or expanded T cell repertoires, does not necessarily translate to clinical benefit because their ability to migrate from the site of administration to site of function is maybe impaired or lost during *ex vivo* treatments. Thus, many assays of T cell function *in vitro* are not accurate representations of immune potency in humans since they do not reflect the spatial progress of relevant immune cells to achieve the desired response.

This industry guidance document is intended to guide the development of bioassays which quantify the potency of biologic products including those designed to elicit immune responses. Besides the multiple steps described for activation of the immune response, another set of elements that are not easily reproduced by *in vitro* assays involves immune regulation. Natural regulatory steps in the immune system prevent premature activation of the immune response until certain obstacles or thresholds are overcome (intrinsic regulation). Beyond the intrinsic thresholds for immune activation, control of the amplification of the immune response prevents runaway responses (extrinsic regulation). The engagement of numerous intracellular and extracellular regulatory steps means that there is not a simple (linear) correlation between the amount of a particular product or element in the product and the boosting of the immune response in the presence of extrinsic regulation. With multiple assays, though, establishing ‘fingerprints’ of immunotherapeutic product activities are more likely to succeed for correlating product potency to clinical benefit.

Considerations on specific examples in guidance document

Table 1 (Page 4):

Challenges to potency assay development for CGT products:	Examples:
Complex mechanism of action(s)	<p><i>Add the following bullet point:</i></p> <ul style="list-style-type: none"> • Relative biological activities include spectrum of immune response and stages of immune activation
Multiple active ingredients	<p><i>Add the following bullet point:</i></p> <ul style="list-style-type: none"> • Relative biological activities include spectrum of immune response and stages of immune activation
<i>In vivo</i> fate of product	<p><i>Add the following bullet point:</i></p> <ul style="list-style-type: none"> • Complex spectrum of outcomes from product interactions with phagocytic cells • Spectrum of cells that interact with product at local site • Recognition of <i>in vivo</i> processing of the product and transport of processed product from the site of administration to the location of interaction with other immune system components

<p><i>Add the following challenge:</i></p> <p>Inherent variability of assessing biological responses</p>	<ul style="list-style-type: none"> • Threshold (lower limit) and non-linearity of immune response
<p><i>Add the following challenge:</i></p> <p>Identification of the full spectrum of antigen-specific responses</p>	<ul style="list-style-type: none"> • Variability of individual MHC receptor profiles and immune response profiles • Determination of representative immune responses as a practical goal.

1) Complex mechanism of action (but may also fit in section for multiple active ingredients)

If the product contains elements that are designed to trigger both innate and adaptive immune responses, then potency assays should address the relative biological activities associated with the spectrum of the immune response.

2) *In vivo* fate of product

Following administration, the interactions between the product and phagocytic cells of the immune system are complex and difficult to reconstruct with potency assays. Product fate, including migration from the site of administration, is dependent on the dose of administration and the number of phagocytic cells that access the product at the site of administration. Product interactions with any one of several candidates of phagocytic cells depend on which cell takes up the product for downstream fates. For instance, neutrophils and macrophages phagocytose and process vectors/antigens at the site of administration, concomitant with local inflammatory responses. However, myeloid-derived DCs phagocytose immediately (at site of encounter with the product) yet delay antigen processing and presentation until the activated DCs reach the draining lymph nodes. Thus, the relationship between the site of administration and the product dose influences the overall outcome of immune activation.

3) Inherent variability of assessing biological responses - *Not listed*

As mentioned above, the contributions of intrinsic and extrinsic regulation influence the linearity of the immune response. Beyond surpassing the (lower) threshold needed to initiate the response, the non-linearity of the response due to regulatory elements suggests that establishing reproducible and precise (especially upper) ranges in a potency assay response is difficult.

4) Identifying the full spectrum of antigen-specific responses - *Not listed*

Because the antigen-specific immune response is directly related to the MHC (HLA) receptor type, and the variability between individual MHC receptor profiles is tremendous, identifying elements of the immune response that are pertinent for all potential patients may be a staggering task. Therefore, it should be recognized that the detection and quantification of any single product-mediated antigen-specific immune response in an assay does not represent the (spectrum of) immune responses that may influence clinical benefit.

Considerations on progressive potency assay implementation in guidance document

Page 9, Section III.E.2.

The draft guidance recommends that the potency assay design and acceptance criteria be sufficient to assure that a well-characterized, consistently manufactured product was administered during pivotal studies. This statement implies that the potency assay must be defined, developed and used prior to the initiation of pivotal clinical studies since product used on those studies is recently manufactured. In addition, since the term “well-characterized” has been used to define a standard for analytical characterization of biotechnology products that may not be achieved for CGT products, we recommend using a term such as “relatively well defined” or “relatively well understood”.

Development of a potency assay requires assessment of clinical relevance and relationship to efficacy. Since potency is required before entering pivotal studies, the appropriateness of the assay itself will not be known until the end of a pivotal study. The guidance suggests that multiple assays be developed and run on clinical samples, in order to determine which is the most informative. This approach is expensive and may pose logistical problems since multiple assays of unknown relevance will require establishment of acceptance criteria of unproven significance. Since the risks associated with this approach are likely lower for assays, such as analytical assays, that are more quantitative and precise, the guidance should suggest that a suite of assays designed to establish potency be weighted to analytical assays, and include an assay that demonstrates a biological effect, but need not include biological assays for each step of a complex mechanism of action. One approach has been suggested by J. Petricciani et al. (1) for whole cell cancer vaccines where potency is assessed by viable cells and quantitative antigen expression as part of batch release and developing an alternate bioassay to be used for comparability or manufacturing changes.

- There are important ideas and suggestions in the above referenced article specific to a new class of products that should be referenced in the guidance.
- Will FDA provide more clarity regarding timing for potency assays and expectations for products where neither cellular associated makers nor *in vivo* biological responses correlated with efficacy have been identified?

¹Petricciani, J. et al. (2007) Potency assays for therapeutic live whole cell cancer vaccines. *Biologicals* **35**, 107-113.