Table 9 and Table 27: Potency Assays and Use of Structure Function Models: Replacing in-vivo with in-vitro Potency Assays at Warp Speed

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Key Words:

potency, mechanism of Action, structure - function relationship

Scope:

The complexity of investigational product is exponentially increasing within the biologics space, where the intended product has multiples MoA (e.g. bispecific/trispecific antibody, Antibody-drug conjugates). The correlation between the mechanism of action of an investigational drug and the pathophysiology of disease is often not direct/clear, for example for mAbs that interfere with a signaling pathway three/four steps upstream of the physiological response needed. In the cell and gene therapy space, we often encounter situations in which the drug substance(s) have different MoAs than the ultimate drug product. At this table we will reflect on the available guidances for potency assay development, and reflect upon how to best address the need of demonstrating potency of the investigational product while balancing speed to clinic/speed to market and phase appropriate understanding of products.

Questions for Discussion/ Discussion Notes:

1. How can structure/function models be used to enhance understanding of the MoA, supplement/justify the potency assay, or provide data where potency assays cannot?

For mAbs, besides binding, need to understand effector function as part of the mode of action. After years of study, understanding of the correlation between Fc N-glycans, such as fucose, galactose, with the effector functions such as ADCC, CDC and ADCP is now available and may be used to establish models, which can be used to supplement binding assays or justify potential replacement of potency assays with models. These models can account for the majority of the effect, but there is some degree of unknown contribution. Some companies shared challenging experience with convincing agency of similar approach in a biosimilar program filing.

2. More complex biologics (i.e., mRNA vaccines) are less likely to rely on structure function models because of limited understanding of structure-activity relationships. When is the use of binding methods over cell-based methods justified for use as the potency assay?

Justification has to be based on extensive data and comparability. For well-understood biologics such as IgG1 mAbs, the extensive understanding of mAb Fc effector functions with N-glycan features can potentially be leveraged to justify the use of binding assays or biochemical assay and models to replace cell-based Fc effector functions. However, this approach may also not be acceptable by world-wide agencies. This will make no difference for the sponsor when only some agencies accept the model or binding assay replacement of cell-based assays.

3. Cell-based assays early vs late: balancing speed to clinic/market and phase appropriate understanding

There are some general accepted approaches. For mAbs: binding assay are recommended for Phase 1 release control, cell based bioassay is added at later phases. An exception was the COVID Ab cocktails, for which the FDA requested a neutralization cell-based assay in Phase 1, but this could be limited to infectious disease treatments. For ADCs, besides the binding assay, a bioassay is generally required at Phase 1, unless justified by a structure/function model and supplemented by a biochemical assay

4. Matrix of potency assays: how to pick release vs characterization?

Beginning in early development, assess possible potency assays as they are created and over development phases, decide which ones are indicative of clinical performance and selected for control at time of licensure. At this time, can provide argument to drop some assays. Several assays can be evaluated as part of characterization, not necessarily added to release/stability specs. The validation of what assay to utilize for release can relate to the mechanisms of action and what is indicative of Phase 3 clinical results. Finally, the number of assays required depends on target and modalities: new biologics tend to require more assays. For gene therapy, may need to evaluate functionality and not only show the correct ORF and protein expression. A large number of assays are utilized for gene therapy characterization and release including the various assays for DS and the DP.

5. Potency assays for products with multiple MoAs/synergistic MoAs/coformulated products - lessons learned and challenges?

In general, for co-forumlated mAbs, need to show function by each mAb and then one bioassay for the mAb cocktail

Coformulated products can present a challenge if they react in vivo, i.e., IgG4 can split and recombine in serum. Need to show how to adjust dose accounting for this effect

6. Potency assays where DS and DP have independent MoAs (e.g. CRISPR components encapsulated in LNP) - creative ideas and lessons learned?

   For CRISPR therapies, each DS has its own set of release specs (and bioassay), and then the combination is evaluated as part of the DP control strategy (and bioassay). Multiple release potency assays are typically required for CRISPR therapies.

Additional comments:

1. Given the large variability of in vivo assays, why aren't in vitro assays becoming more acceptable by all agencies?
2. In general terms, there is no uniform requirement for what bioassays are required globally, therefore, sponsors end up developing bioassays to meet all expectations
3. It is apparent that NGS will eventually become a QC release test for certain biologic modalities, etc, but still too costly
4. The use of ready-to-use cells in the cell-basedbioassays is becoming more standard practice. WCB and MCBs can be established for the cells used in potency assays.
5. In general, spec ranges are set wide for bioassays, and sometimes they stay that way thru later phases and beyond.

6. Do CMC people talk to non-clinical/clinical people? Sometimes, agency sees some potency assays in Module 4, which may also be used for CMC development. Some responded that communication between Research and CMC development is commonly present in the industry. The assays developed for early-stage discovery usually need to be modified into more robust formats for CMC development use. These research assays however are the foundation for binding or potency assays used in CMC development.
7. What is the purpose of potency assay, for process consistency or for indication of clinical outcome? This is a question well discussed at the round table. The difficulty of associating the potency assays directly with clinical study results presents questions about the value of some potency assays especially with those of large variability. Some think the potency assays reflecting as closely as possible the understood MoAs are used to monitor process and product quality consistency.