Table 22: Vaccine Potency Assays

Facilitators –
Brian Nunnally, *Seqirus, A CSL Company*
Jyothy Pillai, *Merck & Co., Inc.*
Varnika Roy, *GlaxoSmithKline*

Scope:

Potency is defined as a quantitative measure of biological activity based on the attribute of the product which can be linked to relevant biological properties. The assay to measure the vaccine potency is used throughout the vaccine lifecycle to support its research and development and is a regulatory requirement for the release and stability of clinical materials. Historically, potency assays based on *in vivo* models (e.g., challenge with the pathogens after vaccination) have been used to measure the activity of the product related to its specific ability or capacity to achieve a defined biological affect. However, the immune response in an animal model may not be predictive of what will ultimately occur in humans. In addition, with the variability observed in animal experiments and in conjunction with 3R (Reduction, Refinement and Replacement) policies *in vitro* tests should be considered as an alternative to provide biological activity measurement by relating structural conformity/integrity to functional activity of the antigen and demonstrating consistency of lot to lot production.

Questions for Discussion:

1. Do you use in vitro relative potency methods for potency release assays vs in vivo?
2. Do you have experiences showing in vitro to in vivo concordance what were the hurdles? Did you do this in Phase 1 or Phase II?
3. Experience of moving immuno based potency assays to new technologies such as Octet or Multiplex Bio-plex vs a traditional ELISA- What were the challenges?
4. Do you focus the Potency assay on showing Consistency Lot to Lot vs Mode of Action demonstration?
5. DP- Drug Product vs Reconstituted Vaccine: Release potency assay on the DP vs Characterization on the Reconstituted vaccine- What panel would you use?
6. What about replacing an in vivo assay with in vitro for a legacy product? Share any examples?
7. What kind of comparability studies would be needed to support to a new potency assay? Are clinical studies always necessary?

Discussion Notes:
January 26 and 28, February 2 and 4, *combined* –

Historically bioactivity of vaccines assessed in vivo, shift towards in vitro models will (1) reduce variability, (2) support the reduction of use of live animals (3Rs principle), promote lot to lot consistency in structural integrity assessment and mode of action

How to facilitate the move from in vivo assays to in vitro assays?

- Typical development for new products starts with in vivo assays with a transition to in vitro assays
- In vivo assays support assessment of immunogenicity, but are less sensitive
  - May not be the best mechanism to show stability
- Assess sensitivity of an assay based on analysis of degraded products
  - Show changes in structure or difference in a targeted attribute, evaluate samples with varying attributes, eg, hypothetical sample with high aggregates, how is the potency of that sample impacted? Can the same result be correlated between IVRP vs. in vivo assay?
  - Typically in vitro assays are typically more sensitive in demonstrating impact to potency though specific to the example, aggregation could illicit an immune response
- Tom: Avoid using the language 'assay' when describing in vivo methods, also rather than correlation use 'concordance'
- 3Rs may not be sufficient justification for switch to in vivo, justification needs to be based on the superiority of an in vitro assay for assessing potency

- What is the design space for potency assays? – It must be understood
  - Potency assays are not a replacement for characterization of product attributes wrt to clinical significance
  - Can a correlation between with pre-clinical studies be demonstrated with potency?
  - Be sure to take into account all available data, and do not lose site with data produced in very early development
  - Can potency/function be controlled through manufacturing? Some protein based vaccines have implemented this approach

- How have HAs responded to the use of in vitro assays?
  - Examples: HPV vaccine has been successful in developing and gaining initial approval without an in vivo assay on the specification. The Hep B vaccine has been successful in switching from an in vivo assay to an in vitro assay in the post approval space in some countries
  - Other countries have long standing requirements for in vivo assays, eg, JPN example for the mouse potency assay for HepB vaccine. Multiple submissions and interactions with HA to discuss transition to an in vivo assay. Strategy started with submission to add IVRP to the specification alongside in vivo assay, with the intention to remove the in vivo assay at a later stage.

However, the third party manufacturer of the assay kit discontinued manufacture, which will likely force sponsor as well as in-country labs to switch to using an in vitro assay only and drop the in vivo assay

- Is there benefit to selecting primary cell lines vs. a transformed cell line?
  - T: cells are simply a substrate to show relative potency to a standard, an assessment of relative potency reduces variability introduced by a given cell line
  - Varnika: Primary cell lines are not required. Appropriate sourcing and maintenance of the cell line is more important, transformed cell lines are more straightforward. Appropriate reference standards are the most important.
  - Once clinical data are available, establishment of an early clinical batch as a reference standard becomes the basis for establishing relative potency for subsequent batches

Approach to setting specifications?:

- Expectation is that specification acceptance criteria is set pre-PPQ
- Often difficult to avoid wide AC due to the variable nature of ELISA/cell based assays
- Dose ranging studies should be considered
  - Ideally dose is in the middle of the plateau of the mab response
  - statistical analysis of quality of attributes of a batch wrt the patient response.
- What is the approach w/o dose ranging?
  - Pool data from a prospective number of lots and set specs based on statistical analysis
  - This approach can lead to tighter AC if all batches used in the clinic have potency that is within a narrow range. Often batch potency is assessed at release only
  - Suggestions to support wider AC:
- Assessment of product dosed at the end of shelf life or dose of degraded product helpful in assessing
- Consider dosing patients with batches that are at the end of shelf life, or a batch that has been intentionally degraded to pre-set potency criteria. Once batch is degraded to this pre-set potency, freeze it, and dose with it.
- Also consider planning patient dosing as material ages to support an assessment of patient response as a product ages. Eg, dose a patient when a given batch is 6 mos., 9 mos., 12 mos., etc., with corresponding assessments of potency via in vitro / in vivo assay would also be evaluated during stability studies.

Small doses:

- Clinical dose has to be max ½ mL volume and some early clinical studies requiring very small dosages – 0.2, 0.5, 1 and 2 µg
  - Low doses especially true for RNA vaccines
  - Evaluation of range of low doses establishes whether materials at low target doses, with

less than ideal potency, show a response. This wiggle room should support a sider specification later in development.

- How to design manufacturing to accommodate low volume? Eg, 1 ug DP, single dose vial?
  - CMC needs to work closely with clinical and analytical folks
- Analysis of such low dose is challenging, assuming variability of 30% in your potency assay, could lead to insufficient dose,
  - Example: 1 ug target dose, 70-130% AC at release, batch close to lower end of AC when tested at release (could be as low as 70%), expected degradation over time. Likely to be significantly different the 1ug target dose.

Health authority expectation is concordance with an animal model be established

- Animal models, and in vivo potency assays, are expensive and difficult to develop. Large number of animals required
- Pfizer showed 'lots' of primate model data in support of the COVID vaccination

How are potency assays developed for mRNA vaccines?

- do they show antigen expression in the cell, immune response?
- The appropriate sequence should equal efficacy but may not show expression
- New mRNA vaccines evaluated as platforms

How are inactivated virus vaccines assessed for potency? Can degradation be shown?

- No experience at the table.

Multiplexed products – how to show potency with multiple antigens?

- Bioplex / multiplex assays - illuminescent assays are becoming more prevalent over the last 2 years rather than having an separate ELISA for each antigen
- Need to be adopted into the GMP\QC enviroment

Protein based vaccines that are diluted in the clinic with either a diluent or an adjuvant:

- V: Approach to having a potency assay on release panel for filled drug product. Then characterize reconstituted drug product and compare back to DP, but do not have them as release assays for the reconstituted DP. Any experience?
  - L - Seen both situations w/ potency on release for reconstituted DP, and also as a characterization assay