Roundtable Discussions Session 1 and 2 - Table 1: Potency Assays

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Abstract:

The development of potency assays meeting regulatory expectations for CGT products represent a major challenge for developers. As biologics, CGT products must comply with applicable biologics and cGMP regulations. The potency assays need to provide an accurate, reliable, and consistent demonstration of the biological activity of the product, need to relate to the mechanism of action as well as be able to detect subpotent batches. Besides providing results for product release and stability, the potency assay is crucial for the assessment of comparability when developers introduce manufacturing changes.

This round table discussion will focus on potency assays for GT products where the MoA is based on a cascade of events like infection, transduction, mRNA expression, and protein expression leading ultimately to the intended biological function. Based on a case study we will discuss major questions for the development of a potency assay testing strategy in a matrix approach during the product development life cycle.

Outcome: develop a suitable, phase-appropriate potency assay control strategy for an AAV-based GT product).

Discussion Notes:

Questions discussed based on case study as described below:

- Potency matrix proposed for an AAV GT product with a dual MoA (downregulation of mutated gene and wt gene introduction)
- Phase 1: potency matrix including testing of infectivity and expression (downregulation of mutated target protein and protein expression of wt protein) for release and stability; no functional assay mirroring MoA established but early research data available, e.g POC, disease model showing expected clinical outcome
- Phase 3: quantitative expression assays as above; characterization testing includes a functional assay in a relevant cell line; a correlation study was performed showing that functional assay is correlated with protein expression

Phase 1

- **Is an expression assay (i.e. ELISA) sufficient for Ph1 release and stability?**
  - Table generally agreed that an ELISA measuring protein expression of target down regulation and wt transgene expression is sufficient for Phase 1.
  - Example from a CT product based on 3 different transgenes was shared: initially only transgene expression was measured and based on FDA feedback, also protein expression was measured resulting in phase 1 release to include gene and protein expression (ELISA) for 6 total quantitative expressions.

- Some products don't have commercially available antibodies to measure protein expression. Protein expression can be replaced with a functional assay combined with mRNA expression if the mRNA is correlated to the functional assay.
- It is noted that measurement of a down regulated mutant could be very hard analytically, and thus potency for this MOA could be difficult to assess.

- **As protein expression is linked to mRNA expression, what is acceptability of not measuring mRNA expression in the potency matrix?**
  - Agreement this is acceptable, although preferable to have both mRNA and protein expression; mRNA expression could be included in the characterization panel
  - An example was provided of experience with having only protein expression (mRNA expression was not developed) and had favorable feedback from FDA on acceptability at early phase.
  - Measuring mRNA expression alone will not suffice. Generally, EU doesn't accept mRNA alone in place of protein expression, but this could be case-by-case, if justified

Phase 3:

- **Is an expression assay adequate for release and stability testing provided that correlation is with a functional assay which is qualified used for characterization?**
  - Acceptability of approach is dependent on the correlation package
  - A hypothetical case of an imaging functional assay was discussed:
    - An image assay can provide more certainty on the potency of the substance
    - The functional assay needs to be at least semi-quantitative and a descriptive correlation can be established, e.g.
    - Stress samples (heat treatment) or samples with different qualities (e.g. different full/ empty ratio) from different process versions to be tested in the assays to be correlated
    - The functional potency imaging assay should be maintained as a characterization tool to be used as needed for comparability assessments
  - The general expectation is that a functional assay has been developed for Phase 3, however sponsors will have limited experience from batch testing at this point in time. Also, the functional assay is not validated by then. One sponsor shared experience with FDA advice received to attempt to justify replacing functional assay at the BLA stage and collecting data until then.
  - Communication with FDA & EMA is needed on the intended potency assay strategy before the start of pivotal studies; share plans to receive HA consent to ensure that sufficient data is available at the time of BLA/ MAA submission.
  - A specific sponsor case was discussed:
    - This product has similar MOA to a vaccine where the therapy is highly dependent upon the patient's response
    - A functional potency assay was successfully established and qualified, but it is very complex and displays a very high variability
    - A quantitative, less variable expression assay is under development with the intent to correlate to functional assay
    - Table discussed to characterize the product in preclinical / characterization assays from different patient cells; data would not necessarily be submitted in the M3

- **What are points to consider for successful correlation?**
  - Amount of data was not necessarily agreed but there was consensus that you need to discuss plans with authorities

- Use development studies: try to correlate functional assay with development data using degraded samples
- Correlation can be difficult to demonstrate if the assays are variable. If you can't show a strong correlation for this reason, a justification based on the assay variability can be acceptable it can be acceptable

- **Acceptability of removal of infectivity assay from release control in a Phase 3 potency matrix?**
  - Regulators (EMA) requests for the ratio of vector particles to infectious particles; in accordance with current general EP chapter ratio of full to empty capsid needs to be consistent
  - Although protein expression is reliant on infectivity, regulators want a clear measure of infectivity based on a specific, self standing assay; infectivity and expression do not necessarily correlate because there is variability to both the infectivity part and expression part of the assay and thus it is better to have a separate measure of infectivity.
  - Caveat: current infectivity assays are based on HeLa cells and thus not representative of infectivity of the target tissue/ cells
  - Protein expression is relative to a reference standard and not absolute measure
  - Potentially, a comprehensive characterization and understanding of the impact of empty and partially full vectors on infectivity could be used to justify the removal of the infectivity assay from the release control testing
    - Some companies are doing empty capsid spiking and testing in mice to understand if in-vitro has any impact in vivo.
- FDA vs EU: it was mentioned that EU still wants to see the 3 levels of potency assay- infectivity, expression, functional. Infectivity assay is still expected to be tested in spite of knowledge on the highly variability; the value of the infectivity assay in light when having a reasonable variable functional assay was discussed
- A potential justification to remove the infectivity assay from the control system based on the lower variability of the expression assay was discussed; the expression could become a surrogate for the infectivity assay; however, it is questionable if this can circumvent the EP requirement
- Experience was shared that requests were received from EH HAs (Germany, France) on inclusion of parameter for ratio of infectivity to particles.
- Luxturna example (see also presentation) was shared:
  - No quantitative potency method even in Phase 3 they
  - Quantitative assay implemented after commercialization
  - Table agreed this approach would not be acceptable today with expectation being to develop functional assays much earlier
  - Infectivity is generally used as an early potency assay. Expression is the next step in the matrix with the challenge of relying on commercial antibodies. Last is the functional assay for characterization with the qualification at a later phase. All 3 assays are used until BLA at least, as there is not enough data to justify removal from the control system. Although infectivity is considered an older type of method, it is useful for process changes in looking at infectivity to vg ratio for consistency.
- Conclusion: removal of infectivity assay is not in the immediate future, but could be considered after marketing authorization. Regulators don't have the confidence yet in AAVs to remove this assay