Table 11: Replacement of In Vivo Assays

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SCOPE:

Historically, assessment of potency and safety for biologics has relied heavily on in vivo testing. The necessity of animal testing is being increasingly challenged for ethical reasons, which in and of itself is becoming a significant constraint in certain markets. The need for higher throughput, lower variability, and reduced cost, further drives away from animal-based approaches. As knowledge of immunity and mechanism of action grows, and as technologies advance, a well-characterized and a well-controlled development strategy may become possible with minimal animal testing. While in vitro - in vivo correlation may not always be possible to establish, it may potentially be unnecessary, for instance, where no sufficiently relevant animal models exist. Confidence in the suitability of the in vitro approach relies on establishing relevance and reliability. Complexity of mechanism dictates distinct considerations for multicomponent vaccines, gene therapy and gene editing, cell therapy. We would like to discuss approaches to animal testing reduction: what has worked and why.

QUESTIONS FOR DISCUSSION:

1. When is in vivo testing the superior approach? When is it essential?
2. What limitations of in vitro tests limit regulatory acceptability?
3. Is In vivo potency testing necessary for late stage viral immunotherapy vaccines or is in vitro potency sufficient for BLA? How does this vary based on whether all relevant protective epitopes are mapped versus partially known?
4. Where in vitro methods are sensitive to forced degradation and measure reduced potency, is there need for in vivo testing and why?
5. For adjuvanted formulations, where adjuvant is demonstrated to be stable, and in vitro methods measure stable potency, i.e. stable epitopes, is this package sufficient or is in vivo testing warranted and why?
6. For viral vector-based therapeutics, where infectivity and expression in vitro methods are in place, is in vivo immunogenicity required as well?

DISCUSSION NOTES:

- Discussion focused on two types of in vivo assays for vaccines: Safety vs Potency assays
    - Examples were discussed encompassing different platforms e.g. bacterial vs viral
    - There are many different FDA offices covering vaccines; it is advisable to be knowledgeable about which office the sponsor is working with and to solicit feedback from the most appropriate Health Agency Office
- Take home messages
    - Understand your product's mechanism of action
        - Choose the appropriate epitopes to monitor and be able to justify why they are critical/relevant; one epitope per antigen is can be acceptable

        - Characterize your epitope: conformational (preferred) or linear (may not be stability indicating)
    - Select relevant/appropriate methods and understand what the methods are specifically evaluating. If a sponsor is looking to substitute/replace an in vivo method with an in vitro method, certain criteria need to be met
        - to support a substitution or replacement of the in vivo method with the in vitro method, both methods need to be measuring same attribute if justification is based on in vivo to in vitro correlation
        - The expectation is that the in vitro method provides equal or superior sensitivity when compared to the in vivo method
        - Value added superior sensitivity must be for relevant attribute
        - The methods should be stability-indicating and sensitive to degradants
        - Superior precision and superior sensitivity of the in vitro assay do not guarantee replacement without relevance and stability indication
    - An adjuvant present with a vaccine should not necessitate in vivo testing although in vitro testing in presence of adjuvant is expected.

- If …
    - MoA is understood and the relevant epitopes are known and characterized
    - the in vitro method is (a) sensitive to degradants (b) stability indicating (c) measuring the same attribute as the in vivo method (d) performing well,

…then there should not be hurdles to substitute it for the in vivo method. However, if hurdles persist, it is recommended to keep having the discussion with the right office. Science and data should drive the discussion.

- Reference: monograph 5214