Table 23: Bispecific Antibody

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SCOPE:

The therapeutic success of monoclonal antibodies over the past 3 decades has spawned the development of next generation molecules including bispecific antibodies (BsAbs). Currently, there are 2 BsAbs (Blincyto® and Removab®) commercially available in the market. BsAbs can bind to two different antigens or two different epitopes on the same antigen and are classified in a variety of different formats. The mechanism of action for BsAbs can vary from re-directing T-cells to kill tumor cells (cell-cell bridging) to binding two different cell surface receptors, soluble ligands, or other proteins to modulate disease progression. FDA issued an April 2019 guidance that includes general considerations and recommendations for BsAb development programs, as well as regulatory, quality, nonclinical, and clinical considerations. The development of BsAbs presents new challenges and strategies compared to conventional monoclonal antibodies. This roundtable intends to discuss some of these key issues and how they are handled across the industry.

QUESTIONS FOR DISCUSSION:

1. How is BsAb process development different from standard mAbs?
2. How does the impurity profile change for BsAbs?Is residual homodimer considered a process or product impurity?Is it a stability-indicating CQA?
3. How is light-chain swapping monitored?
4. What additional CQAs are expected for a BsAb compared to a standard mAb?
5. How are these controlled (by process, testing, or both)?
6. Can they be validated out during PPQ?
7. How does the CQA identification approach change for a BsAb?
8. What is the potency assay strategy for a BsAb: That requires cell-cell bridging (i.e. T-cell re-direction)? Does not require cell bridging (i.e. binding soluble antigens)? What is the strategy during early clinical development vs. commercialization?
9. What is the strategy for the parental homodimers, if applicable?

DISCUSSION NOTES:

1. How is BsAb process development different from standard mAbs?
    a. How does the impurity profile change for BsAbs? Is residual homodimer considered a process or product impurity? Is it a stability-indicating CQA?
    b. How is light-chain swapping monitored?

Need to show that the structure is correct, often reporting the percent that is different from a desired form. Particular interest in homodimer, aggregates and combined effects (e.g., homo-aggregated forms). Have seen up to 5% mis-paired forms. One company introduced Fc changes that only allow one type of homodimer, enabling focus on removing that form.

Use mass spec to assess quality, can be used for clone screening as well. Rapid cIEF is useful for screening. Rarely have a “platform” for these molecules. Analytical development is an iterative process, adding or modifying mAb-based methods. Asymmetric formats, e.g., when one half has an extra Fab

motif, can make it easier to find variants because the homodimer masses are very different from the intended form.

Cell culture conditions can affect light chain pairing / mispairing.

If using a "two cell" system (different cell lines and cultures for each half), you can store the common half as a Protein A eluate pool for later development.

In the IND, be sure to describe the intended form and how it can be confirmed.

Is there an industry view about shared issues? Unclear if this is feasible now.

2. What additional CQAs are expected for a BsAb compared to a standard mAb?
    a. How are these controlled (by process, testing, or both)?
    b. Can they be validated out during PPQ?
    c. How does the CQA identification approach change for a BsAb?

Reduction step may introduce impurities that need to be cleared downstream.

Homodimers are a particular concern for bispecifics that target T cells. Use physico-chem assays, not cell-based assays, for batch release; can use cell-based (including PBMC-based) for characterization. May need stability tests if HD can re-form. Consider studying *in vivo* rearrangement.

Light chain swaps are hard to measure if they result in the same mass as the intended form. May be able to use non-reduced peptide maps. Not an issue if both halves use the same light chain.

During clone screening, product quality is more important than titer. Doses are often low enough that titer is not critical.

3. What is the potency assay strategy for a BsAb:
    a. That requires cell-cell bridging (i.e. T-cell re-direction)?
    b. Does not require cell bridging (i.e. binding soluble antigens)?
    c. What is the strategy during early clinical development vs. commercialization?
    d. What is the strategy for the parental homodimers, if applicable?

Binding assays are used early in development for most participants; one participant described using a cell-based assay from the start. Participants have used SPR and AlphaLISA for binding assays. Describe why potency losses have occurred.

Characterization of the half-antibody intermediates is often limited to just measuring aggregated forms.

Parental half-mAb stability is also important; instability is a supply issue. Generally, stability is similar for bispecifics that have a mAb structure; other constructs can be less stable.

Low dose means low protein content in drug products, which make variants and instability harder to detect, and there will be challenges with surface adsorption for the low dose products (one participant mentioned using syringe infusion pumps).

The EpiVax tool can be used for immunogenicity screening. No information yet about anti-drug antibody incidence vs. magnitude of introduced sequence changes. Homodimer safety remains a credible risk, but there is no evidence yet that homodimers have caused safety problems such as target-independent T cell activation.