Table 6: Manufacturing, Characterization and Testing for Bispecific Antibody

Facilitators –

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Scope:

Bispecific antibodies are designed to recognize two different antigens or two different epitopes of the same antigen, and this dual specificity is being utilized for a number of applications. There are many bispecifics existing in different formats and platforms currently in development, predominantly in oncology indications. With the diversity of bispecific formats comes a diversity in novel variants. This roundtable will focus on the unique challenges in developing bispecific antibodies from the perspective of designing a robust manufacturing process, developing analytical tools for characterization and release testing, and setting specifications.

Questions for Discussion:

1. What are the challenges around the manufacturing process for product and process-related impurities and their respective control strategies?
2. How is the strategy for process validation the same or different from a typical mAb?
3. Have there been any challenges in developing or validating methods for detecting unique and novel variants (e.g., homodimers, other specific variants associated with protein engineering and manufacturing platform (i.e., light chain mispairing for 1-cell process))?
4. What types of potency assays are utilized?
5. How have Sponsors set clinically relevant specifications for new variants, such as homodimers as related to new MOAs, with limited or no prior knowledge? How much is non-clinical data leveraged?
6. What types of dosing schemes are being used for these molecules? Do they present challenges for CMC development (e.g., multiple vial configurations or formulations, in-use strategies, etc.)?

Discussion Notes:

January 25 –

1. Challenges around manufacturing process for product and process-related impurities, control strategies?
    a. Single cell line vs redox/re-formation of bispecific
        i. Considerations, amount material needed, impurity profile
    b. How much extra time is needed for development, for example in purification, for bispecifics over traditional mAbs?
        i. Approximately 2 years for bispecifics vs 12-18 months for traditional mAbs
2. How is the strategy for PV the same or different than a typical mAb?
    a. In 2-cell line bispecifics, how much are the homodimers characterized? What about the redox step, is this highly characterized or just an 'intermediate' step - mostly viewed as an intermediate step not fully characterized
3. Challenges in developing/validating methods for unique and novel variants over traditional mAb platform?
    a. Question asked regarding developing novel methods and what technologies are used for this?
    b. What about the potency of each arm, is it tuned specifically, and how are assays developed for this?
        i. Someone mentioned challenge in developing bioassay, depending on what you are trying to engage and downstream affect
        ii. If one target is engaged, is there a downstream affect that affects the other arm binding?
    c. Someone mentioned unique quality attribute requiring a few months assay development time, but traditional platform mAb knowledge can be used as a starting point for most assays
    d. Total number of assays is larger with a bispecific, and that's true for both characterization and release. For example homodimer monitoring and other attributes not present for a mAb
4. Potency assays and how utilized?
    a. Assay detecting the 2 molecules at the same time
    b. Depends on MOA of molecule, how does binding occur with respect to how the molecule acts?
    c. ADCC and other downstream effectors need to be considered if part of the MOA
    d. Do the homodimer impurities have any clinical effects? Do their levels need strict control because of this?
    e. Assays developed for each target antigen, but have a dual-affinity assay to detect 'realistic' mechanism of action, like an cell-based bioassay or ELISA monitoring both arm binding
    f. At what stage do the cell-based bioassays need to be implemented? Discussion on product acceleration and how sometimes it isn't ready until almost to PV
5. How have sponsors met clinically relevant specs for new variants, such as homodimers, with limited or no prior knowledge. How much non-clinical data leveraged?

a. Leverage all available data, both development and clinical batches as well as what your process is capable of maintaining. Any literature available to get an idea of toxicity? Several mentioned leveraging all of this data to set specifications
b. Question regarding how wide to set the specs, are there any 'rules' on this? Someone mentioned leveraging process capability, assay capability, looking at development history. Start with wider specs and tighten as you go. Sometimes you don't have a lot of prior knowledge and need to make best judgement on where to set these specs.

6. Dosing schemes for these molecules? Challenges for CMC development?
    a. Somone mentioned trying to find less-invasive dosing like SubQ. Make sure clinical team can dose both IV and SubQ. High potent compounds generally
        i. Using same formulation for both delivery methods, actively trying to do both? Yes - to maximize options for dosing but big push for SubQ, gives product differentiation
    b. Low dosing due to potency? Yes, need to cover a wide range of doses because of this.
        i. Multiple vial configurations to cover low and high end of dose range, also may need to increase scale of manufacturing to compensate and this may impact development timelines
            1. Is this observed early enough to make cell line changes for additional titer? This is possible, but may not be worth the effort to go back and bridge new cell lines
        ii. Does this pose a challenge for analytical methods? Yes, the concentration can be very low and typical analytical methods may not work
    c. What about clinically relevant specs, when you only have a handful of batches in these low-dose molecules?
        i. Someone mentioned similar to ADC programs where the dose is very low, only a few lots available for spec setting. Set initial spec, and commit to re-evaluating it post-approval and adjust as necessary. Used 30 batches in this example

Other questions:

With low-dose, high potent molecules, any need for changes to cleaning validation?

Yes, previous methods for cleaning validation not sensitive enough and have had to develop new techniques to perform cleaning validation

Propensity for aggregation? Higher in bispecific vs mAb?

Not aware of example that shows bispecifics might aggregate more than mAb

Molecular assessment approach in discovery to weed out any candidates that have potential issues

Charge variants?

What happens when the bispecific forms, and how the charge variants compare to the homodimer molecules. When you form the bispecific, are the acidic/main/basic predictable based on the starting homodimer molecules? - Not really, it's not a linear relationship. There is a mixing that occurs and it isn't as clear cut as you might predict.

Evolving release strategy for bispecifics?

Had to implement assays like LC-MS for release for bispecifics? Not yet, but sometimes assays like peptide map can be used for release

In the future LCMS may need to be implemented for release, depending on the properties of each molecule

CMC Reviewer: what things do they look for specifically?

Potency. Aggregation, charge variants, aspects may be unique to bispecifics.

Potency assay that reflects the MOA as early as possible

Reason for going with a regular mAb over a bispecific?

Not necessarily, depends on disease indication. mAbs still work well for so many disease indications

January 27 –

1. What are the challenges around the manufacturing process for product and process-related impurities and their respective control strategies?

The challenges depend on the type of format used for the manufacturing of the bispecific antibody (one cell line vs. two cell line process, for example). If using a two cell line process, may have different process-related impurities to consider.

Bispecific antibodies have unique product-related impurities (e.g. homodimers). It is possible additional purification steps could be required to separate these species. If the activity of these impurities is significantly different than the bispecific antibody, they may need to be controlled as part of the specifications. Therefore, the manufacturing process for bispecific antibodies can include more controls than typical mAbs.

2. How is the strategy for process validation the same or different from a typical mAb?

For two cell line processes, PV studies are doubled for the upstream processes (cell culture) so this should be considered. Additional CQAs that are not applicable to typical mAbs may also

need to be monitored during PV to determine whether they are impacted by process variation. In general, however, the PV is very similar as for typical mAbs.

3. Have there been any challenges in developing or validating methods for detecting unique and novel variants (e.g., homodimers, other specific variants associated with protein engineering and manufacturing platform (i.e., light chain mispairing for 1-cell process))?

The development of methods can be challenging given that many of the characteristics (e.g. size) of the bispecific antibodies are similar to the homodimer impurity. Assays need to be able to sufficiently resolve the different species, which may require additional optimization. Since the CQAs can differ for bispecific antibodies, it may be necessary to introduce additional characterization assays to address novel impurities.

4. What types of potency assays are utilized?

Binding assays are generally used early on in clinical trials, with cell-based assays used later. For sponsors with several bispecific antibodies with some overlapping targets, the cell-based potency assays may be introduced earlier as they have been established for products with similar targets. Ideally the cell-based assays are introduced as early as possible. The type of potency assay is really dependent on the MoA of the bispecific antibody so there is a very wide range of types of potency assays used for these products.

5. How have Sponsors set clinically relevant specifications for new variants, such as homodimers as related to new MOAs, with limited or no prior knowledge? How much is non-clinical data leveraged?

In some cases, the safety teams can assist with setting specifications based on non-clinical studies of the different variants. In many cases, however, given that there can be limited manufacturing experience, scientific justifications are used to support the proposed specifications.

Certain types of homodimers are well-characterized and clinical safety data are available.

6. What types of dosing schemes are being used for these molecules? Do they present challenges for CMC development (e.g., multiple vial configurations or formulations, inuse strategies, etc.)?

A step-up dosing approach can be used given that the bispecific antibodies can be especially potent so starting at lower doses is necessary. This type of strategy can require multiple vial configurations which can potentially add additional PV requirements. The sponsors can discuss with regulators how many PV batches would be required for each vial configuration if they only differ in fill volume, for example.

February 2 –

- Question 1 from agenda responses:

- In many cases we are doing 2 cell cultures with common purification and some that are single cell cultures.
  - The two cell culture brings the challenge that you have 2 different processes involved. How to figure out how to understand which components come from which part of the culture because you can't see that until after.
- Had same issues.
  - Invested a lot in the process development when scaled up
  - Still have work to do on the redox reaction
  - Some progress on single culture methods but two cell lines can add ~3 weeks
  - There are pros and cons to each one
- Lower volume items can be experimented on
- High volume items – speed is a necessity
- Question: If clean up is done after first protein A column?
- Answer from team – yes.
- Variations in response as to how to target clean up of host cell proteins
- We have a very tactical approach to monoclonals
  - Group that screens in advance so we get good ones from them
  - Testing is straight forward but purification is not.
- Question: Do you have a model section for each specific antibody?
- each cell culture piece would be separate yes.
- Question: Do you find you are having to invest a lot in the formulation development process? We have had mixed experience. Many of our platforms have excipients. Depending on how clean the biospecific is coming out we are getting good stability as long as we can purify out through fragmentation or other methods. We are getting good stability at average storage conditions.
- Biospecifics have to keep an eye out for preclinical warning signs
- Question: Is there a general rule of thumb for max amountt of homodimers?
- For some mechanisms, homodimers may pose safety issues in which case you must keep them low (based on invivo & invtro models). For those that aren't safety concerns, they can be looked at as inactive variants unless they have homogenicity risk.

- Makes one think about discussions around drug conjugates. There are antibiodies that have no drug compatibility and may be targeted as an impurity
- We refer to things as CQAs or non-CQAs instead of impurities and all Antibodies are CQAs
- Different configurations beyond homodimers. There are small amts of fragments occurring that aren't proteolysis, caused by molecule not forming correctly in single cell line. Odd chromatographic patterns. We can do more characterization to identify. Need to focus on products of interest.

• Question: In cases of fragmentation. Are there any specifics you look for in the process?

- I have seen discussions about fragmentation or molecule not forming.
  - These are areas we tend to focus on
- We have some biospecifics which were engineered to improved their purification or detection variants.

• Jumped to Question 6 from agenda:

- Subcutaneous vs. iv infusion
- Because early dosing is such a low dose.
- It is hard to get a controlled dose with all the dilutions.
- It does make for a real challenge
  - Even later in clin studies they may use different dosing schemes that can add complications
- Quite often our med teams get excited about alternative delivery/devices. Trying to get them to invest that upfront is a challenge instead of waiting til phase 3
- We are seeing it earlier and earlier in development now.
  - Most programs don't put much thought/activity into it before inhuman
  - Then there is the challenge that clin teams are always ewaiting for data as to what the final product should look like but the formulators want that earlier
  - Need to develop 2 or 3 options in parallel
- We know have introduced a customer facing meeting before phase 1.
  - Get us better planning/funding before starting
  - Broader asset team to lay out potential options.

- Gets revisited a year later after idea is submitted
- These are important discussions
  - This is what target product profile discussion is important. So many want to old discussion until after phase 2 and I am the one stating it is needed earlier.
- This should be a written document in each company from which the TPP flows

- Question around formulation: onbody product late in process microbial challenge discussions were raise, what has been your experience around that?
  - We used to try to do all those studies and doses in a 4-hour window to perform specific studies
- I am not in that area anymore, so I am not sure if that is still the process.

- Question 4 from agenda on Potency assays
  - Where both arms are needed started assays at phase 1 looking for activities of both arms of antibody
- Phase 1 used 2 different binding elisas
  - Even when your molecule is bringing 2 items together
- Does it have to be a biological assay?
  - For phase 1 we have used binding elisas
- For commercial there is expectation of a cell-based assay
  - Teams often complain these are quite difficult to conduct.
- That doesn't fly anymore
- It is done now as a routine QC test
  - Cell based assays are still tough but necessary

- Question 5:
  - Is a certain aspect of biospecifics that can be leveraged across?
  - Single cell we can rely on traditional platforms and prior knowledge
  - For single cell the entire drug is produce in on, but for a two cell you need a linkage or conjugate?
  - Usually in protein engineering ther is some design aspects to make sure heavy and light changes link together.

- Has the there been any redesign with CRISPR and other techniques?
- Not that I know of for biospecifics. I don't know of any ting through cell line engineering
- For biospecific FABs is there an issue with getting those secreted?
- Seems like it would be a real problem getting those out of the cell
- Where do you think this field is going John?
- i am seeing more different constructs. Traditional aas oo have 2 different fab arms but now the fabs are stacked or c terminus tht is different. A more liberal use of protein engineering
- More war head, antimicrobials antifungals being stuck on/ surgical precision of design. Frankensteined molecules.
- Some in engineering group are conservative s to what might pose a immunogenicity risk or not.