Double Duty Bioassays: A Regulatory Perspective on Bispecific Antibody Bioassay Development

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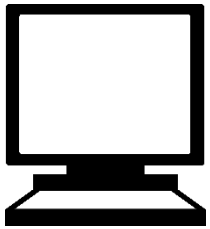
Pharmaceutical Quality

A quality product of any kind consistently meets the expectations of the user.



Pharmaceutical Quality

A quality product of any kind consistently meets the expectations of the user.

Drugs are no different.


FDA
Patients expect safe and effective medicine with every dose they take.

Pharmaceutical quality is

assuring *every* dose is safe and effective, free of contamination and defects.


It is what gives patients confidence in their *next* dose of medicine.

Disclaimer

This presentation reflects the views of the author and should not be construed to represent FDA's views or policies.

Outline

- Bispecific antibody submissions to FDA
- FDA draft guidance for bispecific antibody programs
- Trends in bispecifics submissions
- Bispecific bioassay challenges
- Examples of bispecific bioassay considerations over the course of development
- Examples of cell-based bioassays for bispecifics
- General bioassay expectations/considerations
- Conclusions

There can be a strong scientific rationale to engage more than one target

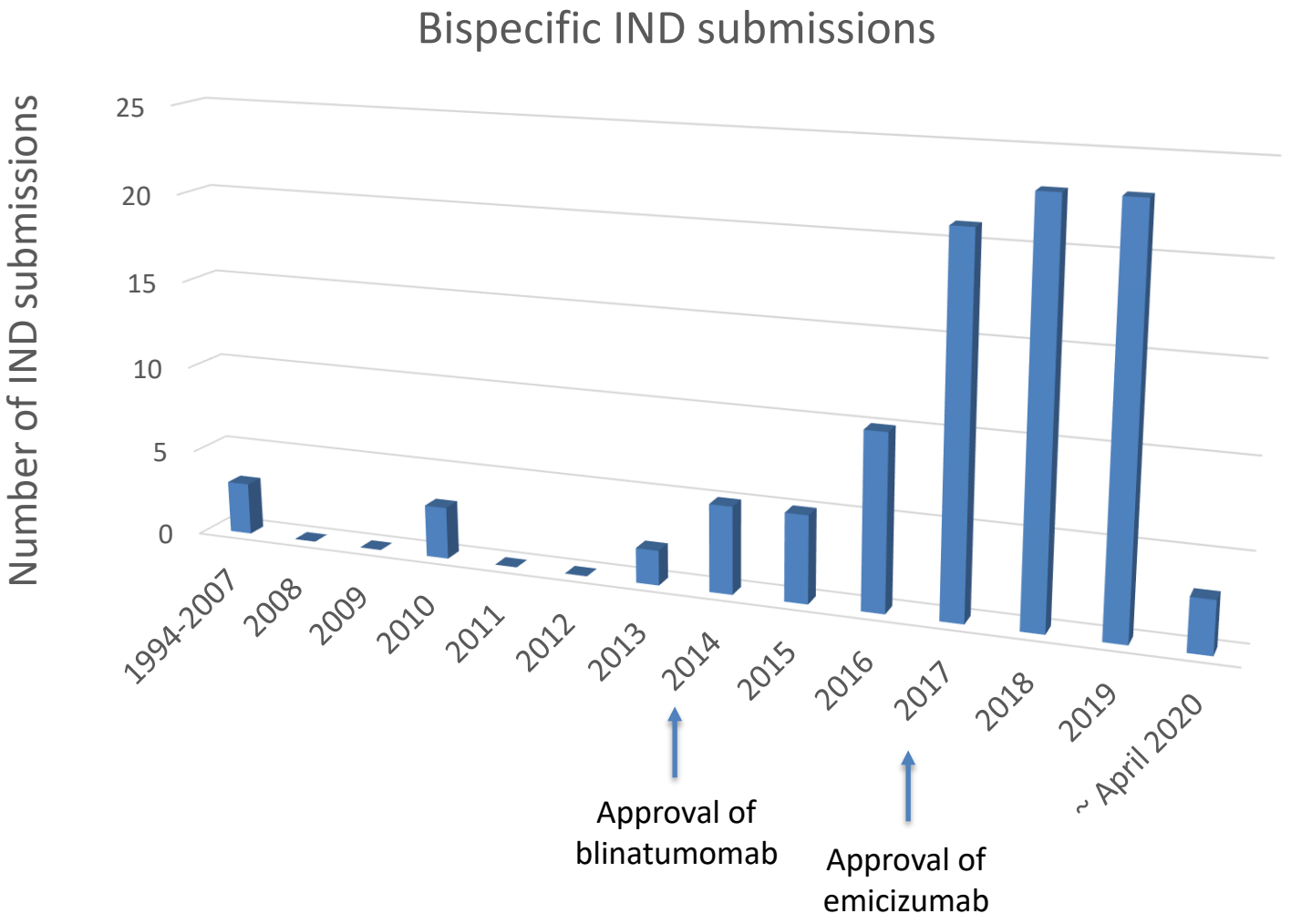
Examples of Multi-target biologic therapies:

- **Antibody Cocktails**
  - Individual mAbs pooled during DS/DP manufacture
  - May not be feasible to test separately
  - A fixed dose combination is administered
- **Combinations of different antibodies**
  - Separate manufacture of DS/DP
  - May be co-developed or developed individually; can test separately
  - May be administered separately (same or different day)
- **Bispecifics**
  - **Single molecular entity with two specificities**
  - **May not be feasible/reasonable to test individual targets separately**
  - **Fixed dose is administered**

Rationale for the Development of Bispecifics

- There have been many great advances in the development and commercialization of therapeutic monoclonal antibodies across indications; however, there still remain unmet medical needs
- New strategies being developed to engage/recruit multiple targets with one therapeutic
- The benefit of proximity in engagement between target and recruited effector cell and potential synergistic effect
- Single dosage forms can be more convenient than co-administration
- Advances in biotechnology have improved the ease of manufacturing bispecific molecules

Increasing trend in bispecific Investigational New Drug (IND) application submissions to the FDA between 1994 to date



FDA Draft Guidance for Industry: Bispecific Antibody Development Programs

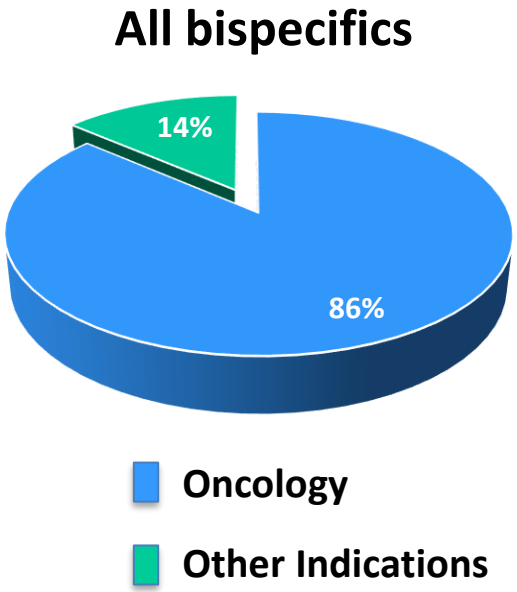
- April 2019, FDA published draft guidance regarding considerations for bispecific program development: clinical, non-clinical, CMC
- Two general categories of bispecifics (many formats):
  - Bridges two target cells (non-IgG-like): bridge immune effector cells with particular tumor-associated antigens to facilitate cell killing
  - Does not bridge target cells (IgG/IgG-like): targets two soluble cytokines or binds different epitopes of the same tumor or viral antigen
- General considerations:
  - Do both targets need to be engaged simultaneously?
  - What is the affinity and on/off rates of each arm for its target?
  - Is there a potential synergistic effect when binding both targets?

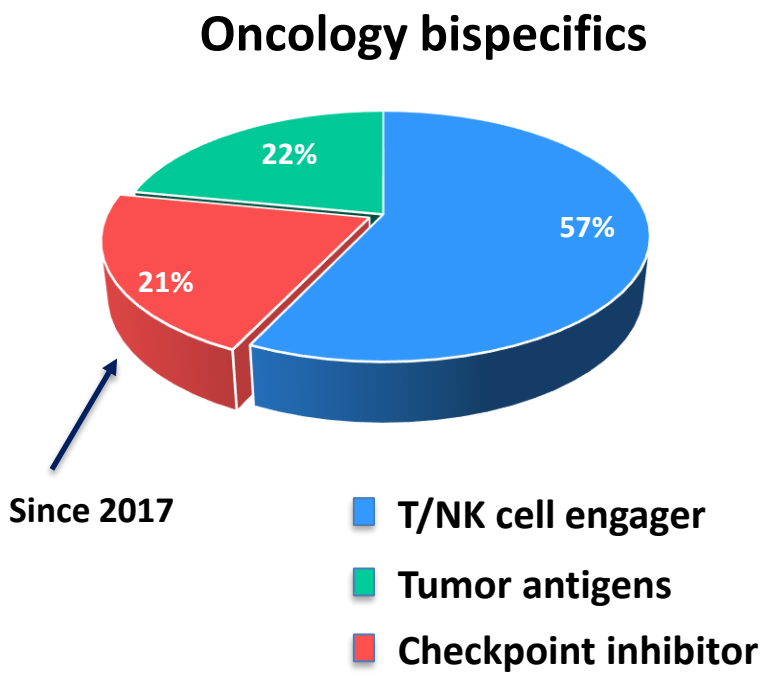
FDA Draft Guidance for Industry: Bispecific Antibody Development Programs

- CMC Quality considerations - Unique characterization and control considerations for the different bispecific formats:
  - Stability
  - Fragmentation/aggregation/immunogenicity (out of the scope of this presentation)
  - Antigen specificity
  - Affinity
  - On and off rates
  - Avidity (molecules with two targets on the same cell)
  - Potency (effector function?)

Well-developed bioassay(s) based on understanding of the mechanism(s) of action

Trends in bispecific targeted indications and mechanism(s) of action





Common targets include CD3, CD16A, CD137-mediated cytotoxicity or immune checkpoint-induced T cell activation and engagement with tumor cell killing

Less common formats include antagonists of receptors and downstream signaling, soluble ligands/cytokines, agonists, or bispecific antibody drug conjugates

Potency Assays Pose Common Challenges for Bispecific Development Programs

- Reflect the primary presumed mechanism(s) of action
  - Is simultaneous binding of both antigens a necessary aspect of the mechanism of action?
  - Can the potency assay capture simultaneous binding?
  - Cell-based assay?
  - Is there an Fc region and does it contribute to the mechanism of action?
- Sensitive to structural changes of the bispecific/is stability-indicating
- Assay(s) capable of being validated for use as commercial release and stability tests
- Representative potency assays should be in place prior to initiation of pivotal clinical studies

Types of potency assays typically seen in early stage bispecific development programs

- Non-cell based assays
  - Enzyme linked immunosorbent assay (ELISA)
  - Surface plasmon resonance (SPR)
  - Time resolved fluorescence resonance energy transfer (TR-FRET)
  - Reporter gene assays

- Cell based assays
  - Cytotoxicity assays
  - Apoptosis assay

Types of potency assays typically seen in early stage bispecific development programs

- Most bispecific products employ a single bioassay as part of release and stability testing
- Two bioassays are commonly employed for IgG/IgG-like bispecifics targeting either two soluble ligands/cytokines or antigens co-expressed on the same tumor cell
- CD3-based reporter gene assays are commonly employed for bispecifics targeting CD3 and an antigen overexpressed on a specific tumor cell
- Cytotoxicity and apoptosis assays are commonly employed for non-IgG format bispecifics

Examples of FDA-Sponsor communications regarding bispecific bioassay development at different phases

Example 1: First in human study

Sponsor: Are the proposed bispecific X drug substance and drug product release and stability specifications acceptable for the initiation of a phase 1 IND submission?

FDA: While the current dual antigen ELISA binding assay proposed to evaluate the potency of bispecific X as part of drug substance (DS) and drug product (DP) release and stability testing appears sufficient for initiation of phase 1 clinical studies, a potency assay (with scientifically justified acceptance criteria) that reflects the primary presumed in-vivo mechanism of action of bispecific X should be included in the DS and DP release/stability specifications prior to initiation of pivotal clinical studies. Sample retains from clinical lots should be appropriately stored to enable bridging of the potency assay(s) to ensure lot-to-lot consistency and aid in the interpretation of clinical study data.

Example 2: End of Phase 1

Sponsor: Does the Agency agree that the two independent ELISA binding assays and two independent ELISA-based blocking assays are appropriate for the drug substance and drug product release and stability testing for a phase 2 clinical study?

FDA: The proposed ELISA-based potency assays appear generally acceptable for initiation of a phase 2 clinical study; however, provide a scientific justification in the IND for how the testing of simultaneous binding to both antigens reflect the mechanism of action.

Sponsor: Does the Agency agree that the described ELISA-based potency assays are appropriate for drug substance and drug product release and stability testing as part of a BLA submission?

Example 2: End of Phase 1

FDA: It is premature to make a determination on the adequacy of the proposed test methods for the BLA stage because insufficient data (e.g., method validation) and information (e.g., justification that the assays are reflective of the mechanism of action) were provided. As a general matter, FDA recommends that a cell-based potency assays be developed for the control of bispecific antibodies that reflect the mechanism(s) of action prior to the initiation of pivotal clinical studies.

Sponsor: Due to the complexity of the product as a bispecific antibody, there is no guarantee that cell-based bioassays can be developed as a validated release test. We will continue to communicate with the FDA regarding the bioassay development strategy prior to the initiation of pivotal clinical studies and/or a BLA submission.

Example 3: End of Phase 1/End of Phase 2; Comparability

Sponsor: Does the Agency agree with the proposed comparability plan to support the drug substance manufacturing process scale up and site transfer and drug product manufacturing process site transfer for use in the proposed phase 2 clinical study?

- Comparability plan includes two TR-FRET binding assays to asses product potency
- One pre-change (phase 1 process) and one post change (phase 2 process) lot

FDA: The proposed strategy to evaluate the analytical comparability between phase 1 and phase 2 bispecific X material appears generally acceptable. However, for any future comparability studies, FDA recommends that a cell-based potency assay reflecting the primary presumed in-vivo mechanism of action be included.

Example 3: End of Phase 1/End of Phase 2; Comparability

Sponsor: Does the Agency agree with the proposed comparability plan to support the additional drug substance and drug product manufacturing process scale up and site transfer to support the commercial manufacturing process?

- Comparability plan includes two TR-FRET binding assays to asses product potency
- Three pre-change (phase 2 process) and three post change (commercial process) drug substance lots
- Two pre-change (phase 2 process) and two post-change (commercial process) drug product lots

FDA: For late stage manufacturing changes, the comparability exercise should be as comprehensive as one conducted for an approved product. FDA recommends that at least three lots of post-change drug substance and drug product material be included in the comparability studies. A cell-based potency assay reflecting the in-vivo mechanism of action should be included in the comparability study used to support comparability between late stage/commercial processes.

Example 3: End of Phase 1/End of Phase 2; Comparability

- In a subsequent communication regarding the adequacy of the proposed comparability exercise between the phase 2 process and commercial process, a new cell-based potency assay based on inhibition of target downstream signaling was proposed.

- Based on the information provided the general approach appeared acceptable.

Examples of bispecific cell-based bioassay (phase 1 IND submission)

Example 1: A bispecific designed to recruit CD16A+ cytotoxic effector cells for the lysis of XX target cells

- Molecule format –tetravalent bispecific CD16A/CDXX tandem diabody
  - Two in vitro assays developed based on simultaneous binding to both antigens
    - ELISA including both antigens
    - Cell-based cytotoxicity assay using primary NK cells
      - Significant variability observed in cell-based assay format
  - The sponsor proposed to use the ELISA as a surrogate for the cell-based cytotoxicity assay due to variability in cell-based assay
    - Early phase bridging data provided
- FDA recommend that alternate cell-based platforms be explored for the development of the cytotoxicity assay, such as use of an NK cell line instead of primary NK cells. Also, maintain both assays as part of characterization over the course of development.

Example 2: A bispecific designed to crosslink CD3+ T-cells with antigen expressed on the surface of cancer cells leading to tumor cell lysis

- Molecule format: homodimeric antibody with two IgG variable domains linked to a human IgG constant domain
- Two separate cell-based direct binding assays for each target included as part of characterization
  - Primary cells not used
  - A redirected T-cell cytotoxicity assay included at release
    - Assay includes target-expressing cancer cell line and a human cytotoxic T-cell derived effector cell lines
- Assays appropriate for current stage of development

General considerations for bioassay development – no exceptions for bispecific bioassays

- Assay(s) provides meaningful, interpretable and consistent results
- Assay(s) ensures patient safety
- Analytical information generated over the course of development using different assays can be bridged
- Appropriate reference standards/internal assay controls should be considered for inclusion in assay format
- Method performance capabilities including specificity, linearity, accuracy, precision, robustness, and stability should be determined

Retain samples

- Manufacturing changes and analytical method changes are common in bispecific antibody development programs

- Retain samples are critical to bridge different assay results generated over development

- Retain samples should be stored under appropriate conditions to ensure stability (e.g., -70°C)

- Retain samples used in comparative studies should include samples that represent, when possible, pivotal clinical trial material

Conclusion

- Bispecific bioassays are critical aspects to a well-designed development program
- Bispecific bioassay design may be dictated by bispecific antibody targets and format
- Challenges exists in bioassay development for bispecifics due to complex mechanisms of action
- Well-developed bispecific bioassays are expected to be incorporated into the release and stability programs prior to initiation of pivotal clinical studies
- Bispecific bioassays are expected to be validated by the time of licensure

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