Bispecific Antibody PK and ADA Bioanalysis: An experienced approach

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Introduction:

Recent regulatory approval of multiple bispecific antibody (BsAb) constructs has intensified efforts toward development of these complicated biologics. As each molecule contains multiple functional domains that interact with corresponding disease-related targets, BsAbs represent an expansive, diverse family of molecules offering potential benefits beyond those of traditional monoclonal antibodies (mAb). With a 2016 – 2021 compound annual growth rate (CAGR) of 100%, the global market opportunity for bispecific antibodies has been assessed at greater than US$30 billion.[1]

Applications for BsAbs range from cancer immunotherapy to treatments for hemophilia, ophthalmic disorders, Alzheimer's disease, and diabetes, as well as molecular diagnostics. More than 180 BsAbs are currently in preclinical development.[2] Seven have been approved by FDA, EMA, or NMPA (China), with four of these approvals in 2022 alone: Vabysmo® for wet, age-related macular degeneration and diabetic macular edema; Lunsumio® for management of follicular lymphoma; Tecvayli™ for relapsed and refractory multiple myeloma; and Cadonilimab®, for advanced cervical cancer.

BsAbs' complex mechanisms of action (MOA) present numerous challenges for developers, particularly in the realm of bioanalysis. For one, in designing a PK study to assess safety and efficacy, selecting which drug status to measure (free, partial bound, or full bound) can be problematic. Additionally, in immunogenicity evaluations, extra work is needed to determine which domain(s) are triggering immunogenicity. Here, we present a purposeful approach to these aspects of bioanalysis for BsAbs.

BsAbs' wide variety of forms and novel MOAs affect bioanalysis

In recent decades, strides in genetic engineering and pharmaceutical techniques have enabled the development of BsAbs in numerous formats. Many technical advances surrounding developability — such as stability, solubility, and the achievement of target product profiles — are in evidence.[3]

Structures include larger, IgG-like molecules with an Fc domain and non-IgG-like fragment-based molecules without an Fc domain. The IgG-like antibodies are designed with heterodimeric Fab (antigen binding) regions to bind different targets. The non-IgG-like Fc fragments trigger antibody-dependent cell-mediated cytotoxicity (ADCC) and phagocytosis as well as complement-dependent cytotoxicity (CDC).[4] BsAbs typically function via three general mechanisms of action (Figure 1):

1. The majority connect immune cells (e.g., T cells, NK cells) with tumor cells to potentiate cytotoxicity. Examples: BiTE, bispecific T cell engager (blinatumomab); DART, Dual affinity retargeting; TandAbs, tandem diabodies; Triomab (catumaxomab).
2. Some block the interaction of cell receptors with their cognate ligands to block tumor receptor signaling pathways. Examples: 2-in1-IgG/Tv-IgGs, tetravalent IgGs; CrossMabs; DAFs, dual-action Fab; DVD-Igs, dual variable domain immunoglobulins.
3. In one unique application, the BsAb acts as a catalyst by simultaneously cross-linking Factor IXa and Factor X in the clotting cascade to activate coagulation in the absence of Factor VIII. Knob-into-hole common light chain IgG.

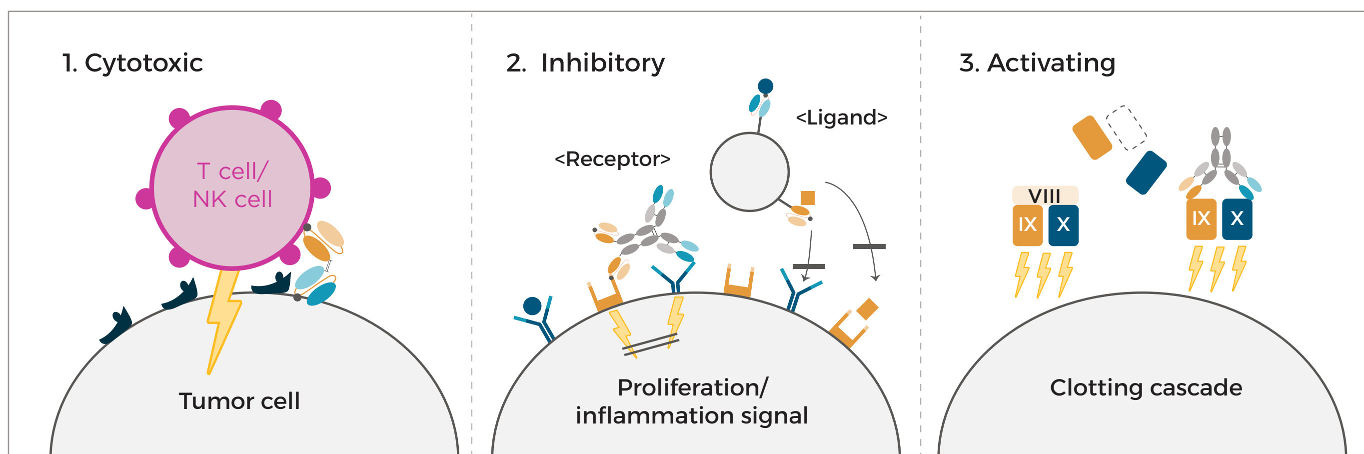

Figure 1. Popular mechanisms of action for bispecific antibodies.[5] Common MOAs aid the immune system in targeting undesirable cells or inhibit undesirable cell signaling.

By acting simultaneously to adhere to tumor cells, target CD3 on T cells, and recruit macrophages and NK cells, some BsAbs stimulate both the innate and adaptive immune systems.[6] Others may interfere with tumor functioning or minimize drug resistance by simultaneously blocking two tumor cell signaling pathways.[7]

While these options provide a wide range of opportunities for therapeutic intervention, the path from bench to bedside can be rocky. Even once antibody engineering and production challenges are addressed, optimal dosing must be determined and potential adverse systemic or off-target effects, such as cytokine release syndrome, must be recognized and mitigated. The drug pharmacodynamics and biodistribution are determined by all parts of the BsAb construct and their combination,[8] creating layers of complexity unseen in monoclonal antibody bioanalysis.

Bioanalytical considerations of BsAbs — PK

BsAbs contain multiple functional domains for interacting with their targets, once administered, they may exist in multiple binding states within biological matrices. These drugs may be present in unbound, partially bound or fully bound forms. Thus, appropriate bioanalytical assay(s) should be developed to measure the form(s) that directly impact pharmacokinetic and pharmacodynamic results.

Here, we propose three assay formats for measuring the different forms of bispecific antibodies. However, dedicated assays are usually not required for measuring BsAb in each state. Rather, bioanalysis teams and pharmacologists should collaborate to explore which drug status should be investigated in alignment with the study design.

Recommended strategies are as follows (Figure 2):

1. For studies in which unbound BsAb must be measured, two anti-ids (or targets) that bind separately with each BsAb domain should be used as capture and detection reagents. Only constructs with both binding sites free will be detected as they must first bind to the capture reagent and then bind the detection reagent.

2. When the partially bound form must be measured, the assay combines an anti-ID (or target) associated with the binding domain in question with a generic BsAb Fc binding reagent. Only BsAbs with the relevant target binding site free will be measured. In this case, the binding site NOT under consideration may be either free or bound. Unbound states for the various binding sites are considered via separate assays.

3. In an assay quantitating total BsAb, including free, bound and partially bound states, the capture and detection reagents are designed to avoid binding in the complementarity-determining regions (CDR). This ensures the assay will be unaffected by targets or the presence of anti-drug antibodies (ADA). Any binding by either of these will occur at variable regions and not interfere with this assay.

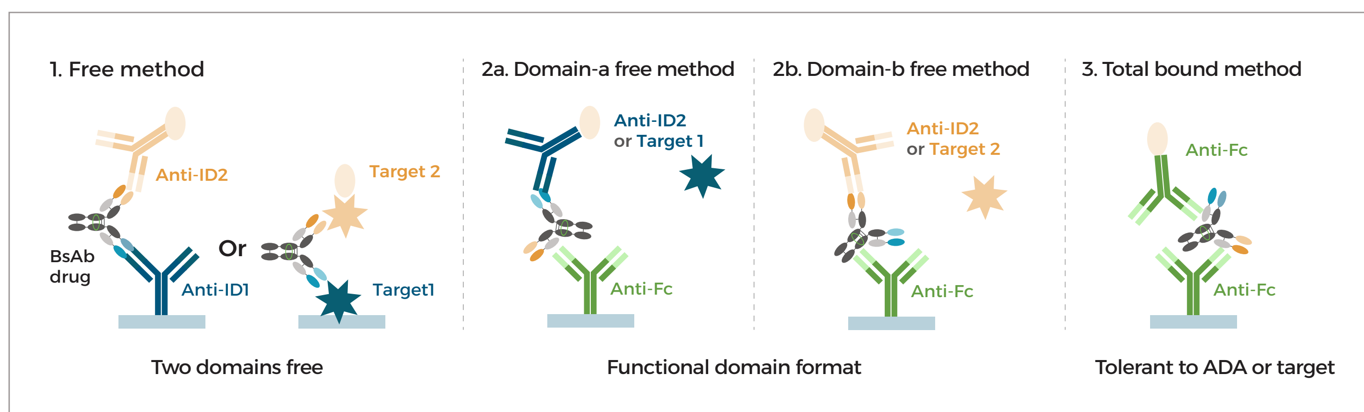

Figure 2. PK assay designs for measuring various forms of bispecific antibody. Specific strategies should be selected to complement the study design.

Evaluation of PK for BsAb is different from that of mAb. Typically, BsAb drugs exhibit more complex PK behaviors than their monoclonal counterparts. Developers must be alert for high inter-individual variability. This may arise from differences in patients and disease status, the target properties, or the effects of ADA.[9] Low distribution in the target area, whether plasma, solid tissue, brain or cell, is another common issue.

Bioanalytical considerations of BsAbs — ADA

Immunogenicity continues to be a significant challenge for BsAbs and other novel, multispecific antibody therapies. Implementation of a drug-specific immunogenicity risk assessment is crucial during early development to direct ADA monitoring and risk management efforts. ADA can impact drug circulation, alter half-life, neutralize the drug, or even trigger serious immune effects. All these activities influence the BsAb's PK/PD profile, drug efficacy and safety.

The immunogenicity assessment of BsAb is similar to that of monoclonal antibodies. However, the multiple BsAb binding domains that interact with various receptors to mediate clinical efficacy demand special consideration. It is common to develop assays to measure the immune response to each binding domain of the BsAb. Of course, sponsors are encouraged to discuss their individual biologics with FDA. In the meantime, we propose a three-tier ADA analysis strategy for BsAbs (Figure 3):

1. **Screening:** One sensitivity screening assay is developed to test all BsAb ADA samples. In a general bridge assay, any ADA present could bind to either arm of the capture BsAb and bind to either arm of a BsAb detection agent. A positive result tells us that ADA activity exists, but we don't yet know specifically which arm or arms are involved. To err on the side of caution, the screening cut point is selected to allow a 5% false positive rate for the presence of ADA. Samples that test negative are reported as ADA negative; samples with positive results are tested further by a confirmatory assay.

2. **Confirmatory testing:** To confirm whether a domain that screened positive does, indeed, trigger immunogenicity, a confirmatory assay is required. This test will also indicate which BsAb binding site or sites are involved. To confirm ADA binding with Domain A, for example, an excess of Domain A fragments would first be added to the assay to saturate any ADA domain A-type binding sites. The bridge assay is then performed. If the ADA is fully bound, it will not be detected by the capture and detection reagents, so the assay signal drops. When the percentage of signal drop is greater than the confirmatory cut point (% inhibition), the sample is reported to be positive for Domain A. A similar assay is then repeated for Domain B.

In general, to verify the immunogenicity triggers of BsAb, three confirmatory cut points (CCP) are developed: domain A CCP, domain B CCP, and BsAb CCP. By comparing the sample results with multiple CCPs, we conclude whether each sample is ADA positive and learn which corresponding domain is triggering the immune response.

3. **Titer testing:** A titer assay is then applied to semi-quantitate any samples confirmed as positive for ADA. This time, the titer cut point is calculated using a 1% false positive rate, for greater precision. ADA samples are often under two-fold serial dilution prior to the analysis.

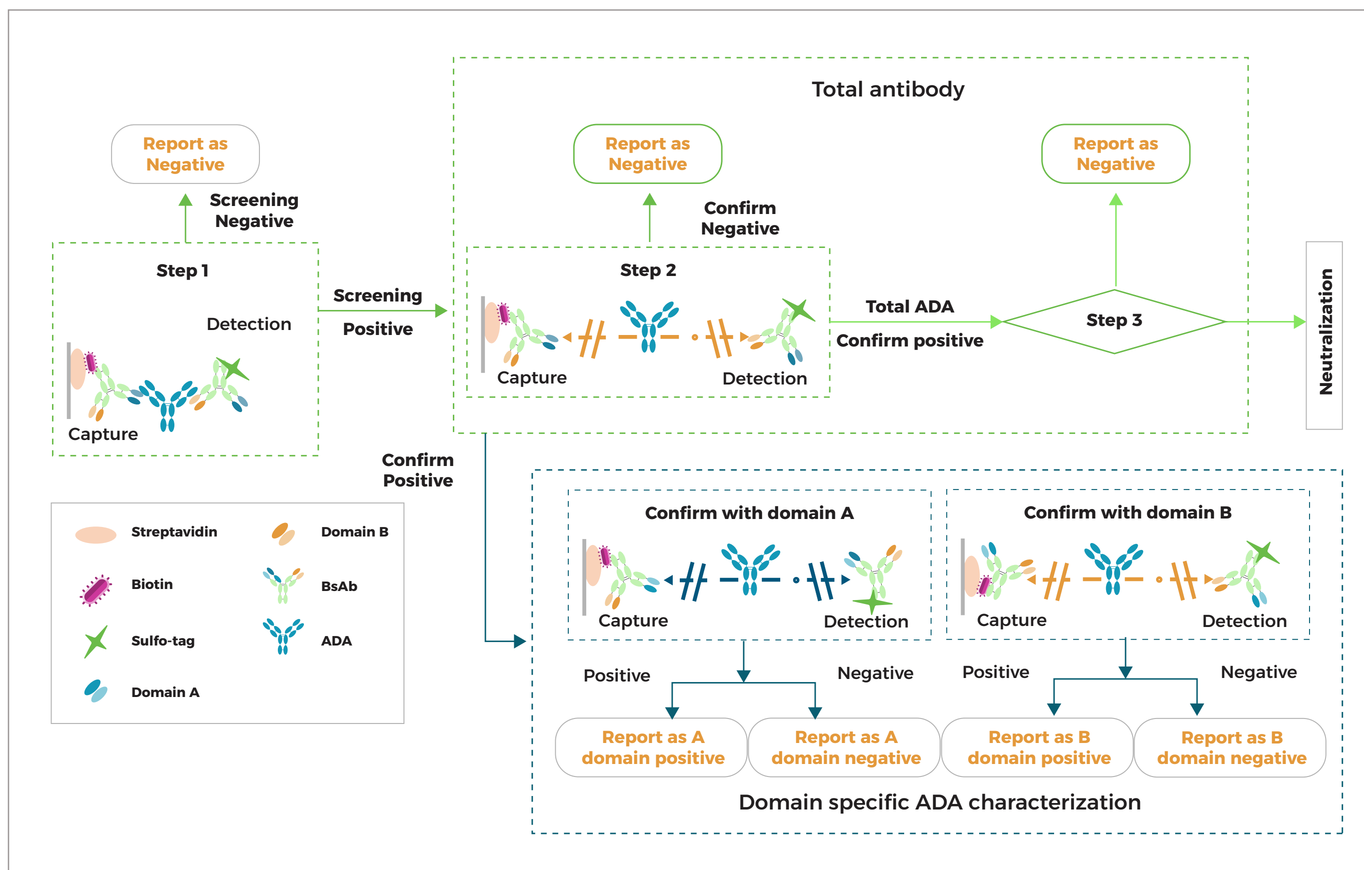

Figure 3. Immunogenicity assessment of bispecific antibody. In a series of steps, samples are tested for ADA activity; activity is confirmed and ADA specificity is uncovered; and ADA titers are semi-quantitatively determined.

Immunogenicity assessments for BsAb therapies have improved over the years but still require a great deal of forethought and expertise. Fundamentally, effective ADA screening assays borrow the analytical approach of single domain biologics, but then add domain specificity. Discovering and investigating potential immunogenicity of BsAbs is crucial for patient safety and the success of biologics.

Bioanalysis for BsAb PK and ADA evaluation demands an experienced team

Developers working on BsAb products can benefit from expert assistance to provide insights into the design of PK studies and data analysis for all stages of drug development, discovery through preclinical and clinical studies.

To help negotiate these constructs' complexity, a good bioanalysis team must first be able to understand the MOA. They must then be able to design a PK assay to measure the correct drug status, working closely with the reagent team to develop suitable anti-ID to use in the assays. In addition, the bioanalysis team must be adept at designing appropriate ADA assays to cover domain specificity assessment, while working with the reagent team to generate ADA positive controls and single domain fragments.

Given the versatile MOAs of BsAbs, success hinges on assay quality, which requires significant scientific and analytical experience. Before you embark on your next BsAbs journey, get in touch with our team of experts.

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