Novel modalities may warrant novel product quality controls

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DISCLAIMER

The views presented today do not represent official FDA policy, but rather represent my opinion based on my experience as a reviewer of monoclonal antibodies and related products at the FDA.

Novel Modalities May Lead to Novel Product Quality Concerns

Generally, similar controls and characterization are required for bispecific, multispecific, Fc fusion proteins, and antibody-drug conjugates (ADC) drugs as for a conventional mAb drugs.

- These include assays for identity, appearance, purity and impurities, potency, quantity, secondary and tertiary structure, etc.

The unique/peculiar characteristics of mAb derivatives may indicate the need for additional testing/controls and characterization.

- For instance, ADC commonly have additional testing for drug to antibody ratio (DAR), free drug, and characterization of the location of drug conjugation sites.

Novel Modalities May Lead to Novel Product Quality Concerns

For bispecific, multispecific, and Fc fusion proteins additional testing, controls, and characterization may be recommended, although should be determined on an individual case basis or are specific to certain subtypes, in contrast to ADC for which DAR is generally expected.

The need for additional testing of bispecific, multispecific, and Fc fusion proteins will vary because of the great variety of engineered structures that can serve many functions with various mechanisms of action.

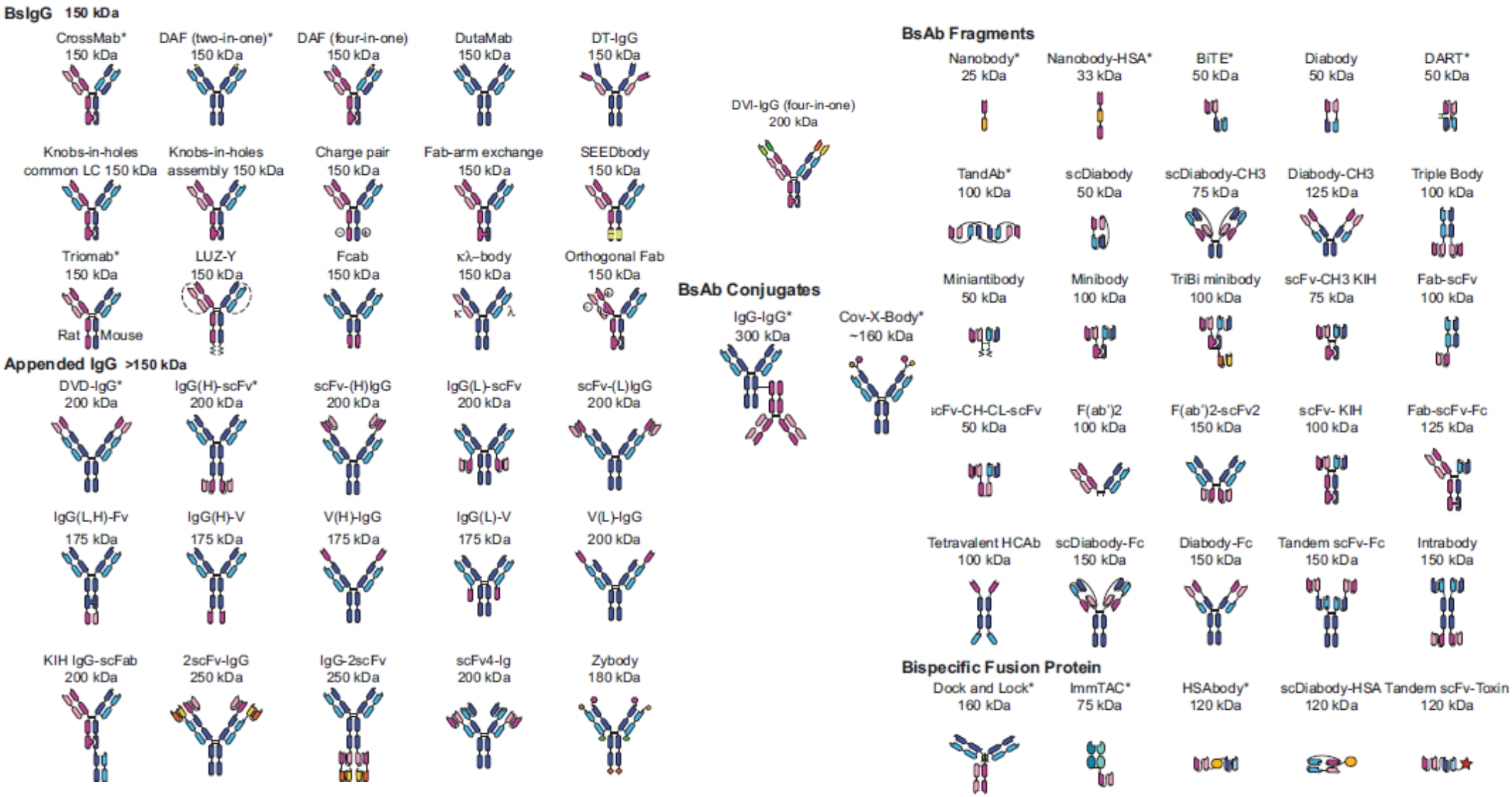
The additional testing may be a one-off study or incorporated into routine testing, such as in-process control, DS/DP release testing, and DS/DP stability testing.

Novel Modalities May Lead to Novel Product Quality Concerns

Additional testing, controls, and characterization may be indicated by these characteristics (with examples):

- novel assembly and/or stability issues from the particular structure and molecular design, e.g. engineered H chain heterodimers (i.e., knobs in holes, electrostatic steering, Fab-arm exchange, LUZ-Y mAbs, etc.),
- the intended mechanism of action (MoA), e.g. simultaneous binding of 2 or more different antigens, or
- dosing accuracy/safety issues, e.g., dilution for very low dose with risk of CRS.

A Variety of Novel Molecular Designs



Spiess et al. Molecular Immunology 2015

Stability of Fab Arm Heterodimers

Bispecifics with Engineered H chain Heterodimers

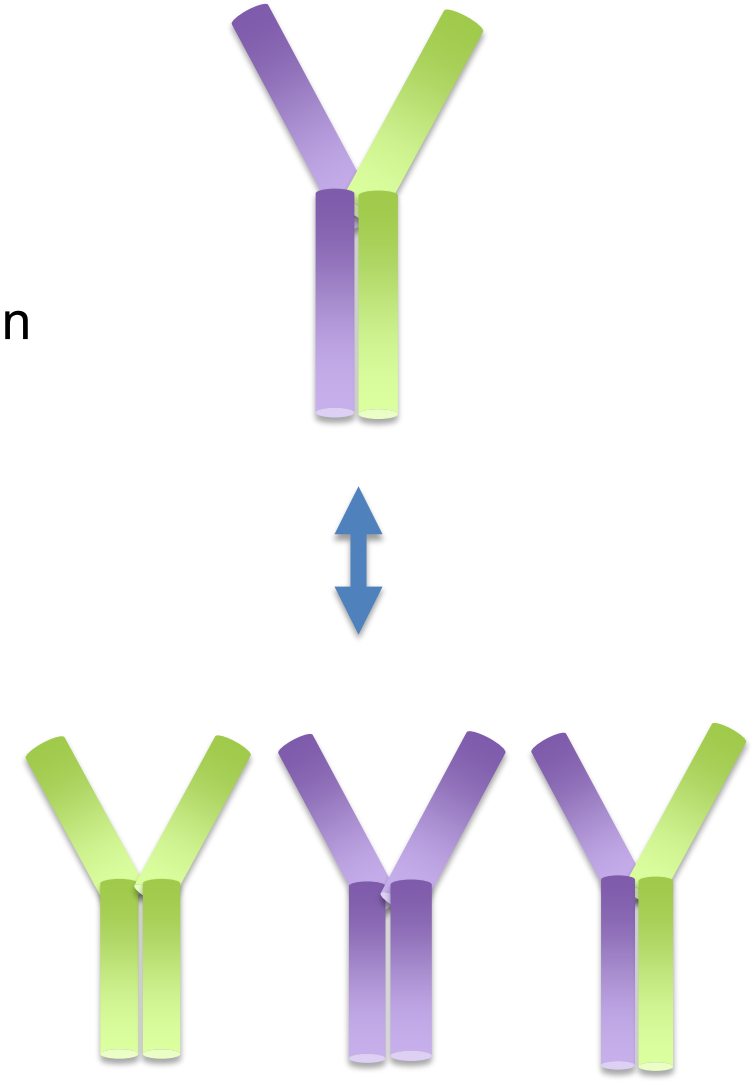
Antibody heterodimer pairing can be driven by protein engineering strategies such as knobs into holes, electrostatic steering, strand-exchange engineered domain (SEED), Fab-arm exchange, C terminal leucine zippers (LUZ-Y), CrossMab etc.

Sponsors should include an assay to quantitate the purity of the bispecific heterodimer at drug substance and drug product release in an IND application. Often the assay is not included in stability testing.

The stability of the heterodimers upon long term storage likely differ among the engineering strategies (knobs into holes, SEED, electrostatic engineering etc.) and the individual constructs.

Heterodimers engineered to strongly disfavor homodimer formation may be quite stable – this characteristic should be supported by data.

Compiling long term heterodimer stability data during clinical development, may support reducing the burden of future testing.



Bispecifics with Engineered H Chain and/or L Chain Heterodimers: Correct HL Chain Pairing

The κλ bodies (2H2L) are composed of a common H chain with a κ and λ L chain. The correct bispecific κλ body is purified away from the monospecific product impurities using two sequential affinity purification steps.

Engineering strategies in which heterodimers are assembled and enriched by purification during manufacture may be more susceptible to reassort upon storage.

Alternately, a common L chain can be used with electrostatically engineered H chain heterodimers such as emicizumab (IgG4, anti-factor IX, anti-factor X).

Various CrossMab strategies of exchanging H and L chain domains of the Fab fragments drives the correct association of H with L chains in conjunction with H chain heterodimers by knob-into-hole or electrostatic engineering. Depending on the CrossMab, undesired side products are formed and removed by purification.

Given the diversity of potential engineered structures, a purity assay should quantitate the intended bispecific heterodimer at DS and DP release (IND application) and stability.

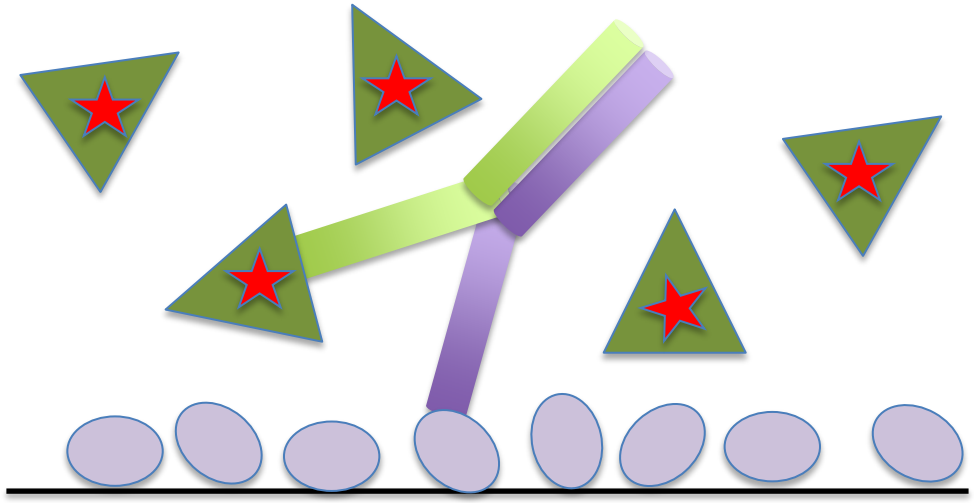
Confirmation of Function: Simultaneous Binding of Target Antigens

Intended MoA Involves Simultaneous Binding of Different Targets

If **simultaneous** binding of different targets is key to a bispecific's function, it should be confirmed early, and supporting data included in the IND application.

At IND submission not necessary by a cell based or potency bioassay, but some confirmation that 2 or more targets antigens can be bound by each bi-/multi-specific at the same time.

Complex multispecific Ab, Ig like or compact scFv/nanobodies, may encounter steric constraints when trying to simultaneously engage targets – distance separating binding domains and molecule flexibility.



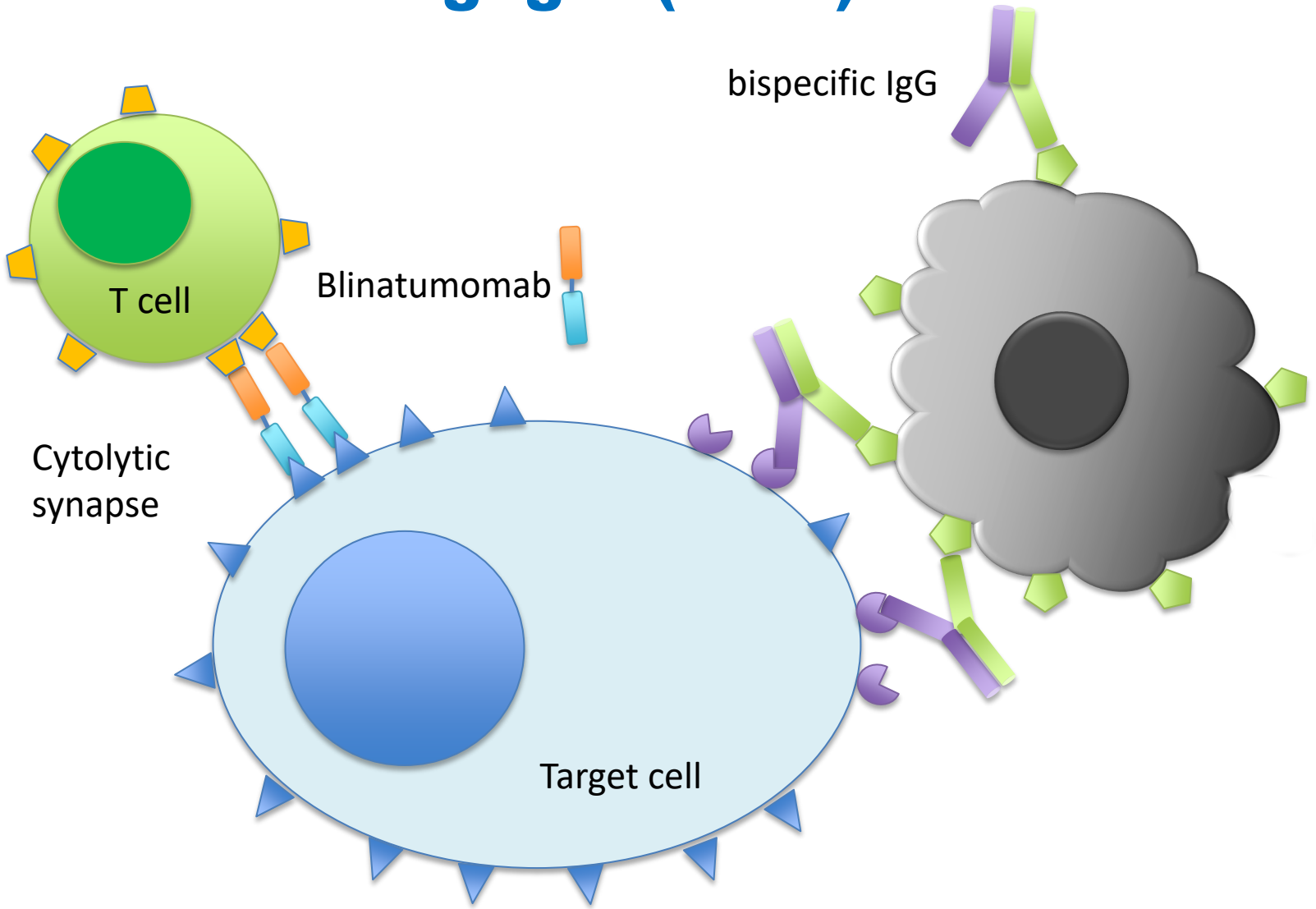
E.g. ELISA, FITR, SPR

Simultaneous Bispecific Binding to Targets

Data from binding assays used during initial development to screen candidate molecular formats may be sufficient – if the assay is designed to confirm simultaneous binding between one bispecific/multispecific molecule and each target antigen.

Safe and Accurate Administration of Low Dose, Highly Potent Bispecifics that Redirect Effector Cells

Effector Cell Targeting Bispecific Abs: Blinatumomab a T cell Engager (BiTE) & NK cell



Active cell lysis may trigger cytokine release syndrome (CRS) or tumor lysis syndrome.

Low Dose Accuracy and Safety Considerations for Very Potent Drugs

For highly potent drugs, such as a bispecific that directs cytotoxic effector cells (e.g. T cells, NK cells etc.) to target tumor cells, there may be an immediate safety issue at new IND submission, if:

- Low starting dose of an NME that has a risk of CRS or tumor lysis syndrome (or other potential toxicity), and
- The starting dose requires dilution of DP by the pharmacists to a low concentration for infusion

These characteristics will require a **thorough Compatibility study** prior to initiating a first in human (FIH) trial.

e.g. Blinatumomab

Licensed Low Dose Bispecific: Blinatumomab

Blinatumomab

- α-CD3, α-CD19 BiTE
- ~ 54 kDa, single chain
- ~2 hr serum half-life
- Treatment for B-cell precursor ALL
- Box warning for CRS & neurotoxicity
- Dosed at 9 µg/day for 1 week, then 28 µg/day in ≥45 kg patients (5 to 15 µg/m^2/day for <45 kg patients) continuous IV for 4 weeks
- Diluted in saline containing IV solution stabilizer

Properties of Proteins and Biologics

Low protein binding ≠ **No** protein binding

Proteins are generally less stabile in dilute solutions.

Therefore, the lower the dose & higher the risk, the greater the scrutiny.

Compatibility Studies For Low Dose/High Potency/High Risk Prior to FIH Study

Compatibility study should:

- **Test all the administration materials** (material composition and perhaps brand) of IV lines, in-line filters, IV bags &/or syringes that will be used in the clinic, the in manner that they will be used (i.e., priming of tubing, etc)

- Test the most dilute drug solution proposed in the trial *
  - a more sensitive assay may need to be qualified to measure drug concentration than the assay used for DS/DP release. This data should be available for review.
  - A bracketing approach is generally acceptable if justified.

- Test under **real use** or **worst case** conditions, regarding length of time – total administration set contact time (from preparation to end of infusion + extra time), temperature(s), and light from pharmacy preparation to end of administration

* If technically not feasible, confer with regulators.

Compatibility Studies For Low Dose/High Potency/High Risk Prior to FIH Study

Compatibility study should:

- Test at realistic timepoints, assay drug that would be delivered to patients
  - from needle tip, can you accurately administer the dose you propose?

- Provide the Pharmacy manual with the full instructions for drug preparation and administration

- Assays for drug concentration and activity, binding or potency to avoid a trial hold
  - stability indicating analytical assays, such as SEC for aggregates or charge isoforms may also be needed

Summary

The potential diversity of bispecific Abs, multispecific Abs, and fusion proteins, the variety of engineering strategies to create them, and the great functional diversity is not suited for easy categorization or generalized recommendations for additional product quality controls and characterization.

Some popular subtypes of formats and functions can be grouped to make specific recommendations regarding additional characterization, release, and stability testing.

The concerns for novel modalities at the initial IND application and/or early development would focus on patient safety, product stability, potential immunogenicity, and confirmation of functionality of novel structures.

Note that the recommendation for thorough compatibility studies for low dose, highly potent biologic drugs is not confined to bispecifics that redirect immune effector cell, but to potent biologics for which the DP is diluted in the pharmacy prior to administration.

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