Characterization of novel biological product modalities- A regulatory perspective

Session 2: Analytical Evolution – Understanding Novel Modalities

Leslie A. Rivera Rosado, Ph.D.
LCDR, U.S. Public Health Service Commissioned Corps
Lead Interdisciplinary Scientist
Division of Biotechnology Review and Research IV
Office of Biotechnology Products
Office of Pharmaceutical Quality
CDER, FDA

CASSS: CMC Strategy Forum North America ***July 2022***

Disclaimer

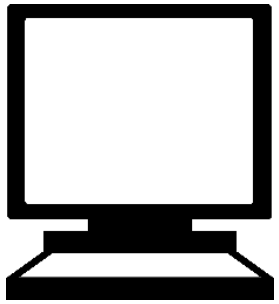
The views and opinions expressed should not be used in place of regulations, published FDA guidance, or discussions with the Agency

For official policy and guidance:

http://www.fda.gov

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.


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.

Background

- In general, novel modality products (e.g., bi- and multi-specifics, ADCs, fusion proteins) should be characterized and the manufacturing processes should be developed in accordance with standard monoclonal antibody and therapeutic biologics development practices.
  - Relevant quality attributes should be studied, including for example: antigen specificity; affinity and on- and off-rates; avidity (for BsAbs that target two molecules on the same cell); potency; product-related impurities such as aggregates, fragments, homodimers, and other mispaired species; stability; and half-life.

Reference: Bispecific Antibody Development Programs Guidance for Industry | FDA

Identity

- An identity test should uniquely identify the product.
- For bispecific, multi-specific, and multi-domain products, a single binding assay might not be sufficient to identify the product.
- The expectation is that the identity test(s) can unambiguously identify the product:
  - Multiple binding assays (e.g., one for each binding domain)
  - Peptide mapping
  - Bioactivity assay
  - A combination of assays (e.g., binding assay, bioactivity, purity methods)

Example

- A multi-domain product uses an ELISA assay using anti-[product] antibody as the main identity test.
- Comment to Sponsor:
  - Provide data to demonstrate the specificity of the anti-[product] antibody, i.e., whether the antibody is specific for [domain 1], [domain 2], or both domains together. Otherwise, develop an identity tests, such as peptide mapping, that will uniquely identify your product.

Antigen Specificity and binding affinity

- Demonstrate the ability of the product to recognize an antigen specifically as a unique molecular entity and distinguish it from another

- Antigen specificity and binding affinity information is usually provided in Module 3 as part of product characterization and Module 4 as part of the Pharmacology information.

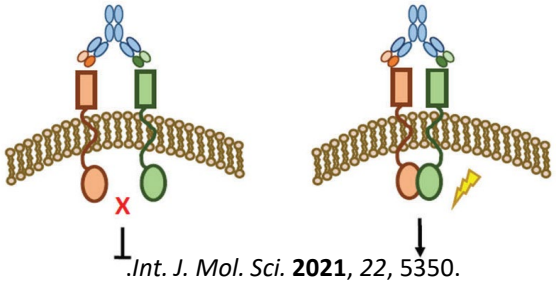
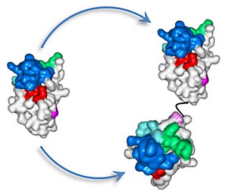
Example

- Bispecific antibody (BsAb) recognizing coagulation factors (F) IX/IXa and X/Xa
  - As EGF-like domains are expressed in other coagulation-related proteins, the binding selectivity and specificity to FVII, FIX, FX, FXII and protein C was tested by ELISA. The product did not bind FVII, FXII, or Protein C, demonstrating **binding specificity** for FIX/FIXa and FX/FXa.
  - The **binding affinity** for FIX/FIXa and FX/FXa was measured by SPR and found to be in the micromolar range

[Reference: Kitazawa et al. 2017]

Avidity

- Many novel designs incorporate multiple copies of one or more antigen binding domains, allowing for avidity binding of one or more targets
-

Examples	Modality	Demonstrate that the concurrent binding of the target antigens improve target selectivity:
	BsAbs targeting two molecules on the same cell  .*Int. J. Mol. Sci.* **2021**, *22*, 5350.	By demonstrating improved binding selectivity to a target population of cells expressing both antigens in a cell mixture containing 'non-target' cells expressing only 1 of the 2 antigens.
	For bivalent/ multi-valent  *Blood* (2011) 118 (3): 757–765.	By demonstrating increased, high avidity binding to the target compared to the monovalent counterpart

Biological activities and Potency

- Comprehensive biological characterization should be conducted using a panel of biological assays that assess the mechanism of action (MoA) in order to fully understand the relationship between the product's structure and its biological activity.
-

	Modality	Biological characterization
Examples	BsAb, BiTE, DARPin, nanobodies, multi-specific/multi-valent products, etc.	Target binding (for each binding module) Demonstrate concurrent binding to all targets (bispecificity) and intended biological effect Activation/inhibition of downstream signaling
	ADC	Binding assay Cell killing /cytotoxicity
	Products with an Fc domain (BsAb, ADC, Fc-fusion proteins, etc.)	Assessment of Fc effector functions (or lack thereof) • Binding to Fc gamma receptors • Binding to Fc neonatal receptor • Assessment of ADCC, CDC, ADCP, etc. as appropriate

Half life

For products with a Fc domain

(mAb and Fc-fusion proteins)

- Determination of binding to the neonatal Fc receptor (FcRn)

For products engineered to extend half-life

- by incorporating an anti-HSA domain
  - Determination of binding to albumin
- by fusing directly to albumin
  - Confirm albumin domain is present
- by PEGylation or albumination
  - Confirm presence, location, and stability of PEG moiety/ albumin

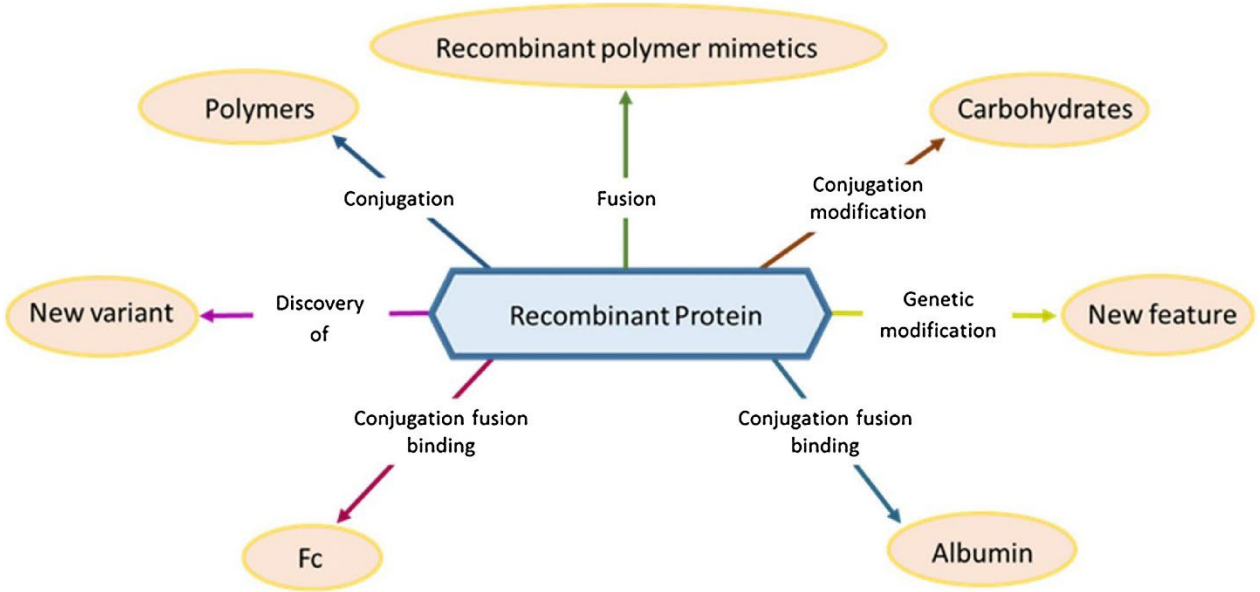


Fig. 1. Different strategies to extend the half-life of therapeutic proteins.

Reference: Strategies for the production of long-acting therapeutics and efficient drug delivery for cancer treatment – ScienceDirect

Product-related Impurities

- Product-related impurities expected for antibody products and other therapeutic proteins (e.g., aggregates, HMWS, LMWS, PTMs)
-

Examples	Modality	Impurities
	BsAb prepared by controlled reduction and oxidation of the parental monospecific antibodies	Residual parental antibodies
	BsAb produced by a single cell line (heavy chains engineered to preferentially heterodimerize)	Residual homo-dimers
	ADC PEGylated products	Unconjugated antibody/protein Modification-to-mAb ratio (e.g, DAR, # of PEG/mAb)

Process-related Impurities

- Process-related impurities expected for antibody products and other therapeutic proteins (e.g., resin leachates [protein A], HCP, DNA, inducers, antibiotics, manufacturing aids)
-

Examples	Modality	Impurities
	BsAb prepared by controlled reduction and oxidation of the parental monospecific mAb	Reducing agent
	ADC	Residual solvents Free drug Conjugatable impurities
	Products with unnatural amino acid(s) incorporated into the sequence for site specific modifications (e.g., PEGylation, albumination)	tRNA synthetase expressed in the production cell line to allow for product modification

Stability

- Stability indicating quality attributes expected for antibody products and other therapeutic proteins (e.g., size and charge variants, particles, potency)
- Examples

Modality	Stability
ADC	Assess the stability of the mAb-drug linkage • Unconjugated antibody • Drug-to-antibody ratio (DAR) • Free drug related impurities
PEGylated products	Assess the stability of the PEG linkage • Unconjugated antibody or protein • # PEG/mAb or protein • Free PEG

In closing

- All biologic products should be extensively characterized in order to fully understand the relationship between the product's structure, quality attributes, and biological activity.
- Known and potential mechanisms of action should be investigated/evaluated and understood.
- Special consideration should be given to the assessment and understanding of novel/potential degradation pathways, and potential impurities that could impact the product's safety and efficacy profile.

Acknowledgements

- Marjorie Shapiro
- Jin Wu
- Nailing Zhang
- Deborah Schmiel
- Eric Hales
- Zachary Kraus
- Gibbes Johnson

Thank you for your attention

Leslie.Rivera-Rosado@fda.hhs.gov