

Mass Spectrometry & Regulatory Expectations for Multispecific Products

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**Symposium on the Practical Applications
of Mass Spectrometry in the Biotechnology Industry**

September 15-18, 2026

Rockville, MD

Everyone deserves
confidence in their *next* dose
of medicine.
Pharmaceutical quality
assures the
availability,
safety,
and efficacy
of *every* dose.

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IN A NUTSHELL:

Multispecific protein therapeutics may look like the mAbs they are derived from, but they aren't.

- They have more product-related impurities.
- Those impurities have greater unintended biological impact.
- They are harder to resolve (and therefore control) with conventional mAb QC methods.

Mass spectrometry is the foundation of the control strategy.

TYPES OF MULTISPECIFICS

- **Bispecific IgGs**
- **Multispecific antibody fragments (joined with linker(s))**
- **IgGs with tandem binding segments added**
- **Multispecific fusion proteins**
- **Multispecific antibody conjugates**

FDA APPROVED BISPECIFICS

Blincyto (Blinatumomab)	Amgen	α -CD3, α -CD19	2014
Hemlimbra (Emicizumab)	Genentech/Roche	α -FIXa, α -FX	2017
Rybrevant (Amivantamab)	Janssen Biotech	α -EGFR, α -MET	2021
Kimmtrak (Tebentafusp-tebn)	Immunocore	α -CD3, α -gp100/MHC	2022
Vabysmo (Faricimab-svoa)	Genentech/Roche	α -VEGF-A, α -Ang-2	2022
Tecvayli (Teclistamab-cqyv)	Janssen Biotech	α -CD3, α -BCMA	2022
Lunsumio (Mosunetuzumab-axgb)	Genentech	α -CD3, α -CD20	2022
Epkinly (Epcoritamab-bysp)	Genmab/ Abbvie	α -CD3, α -CD20	2023
Columvi (Glofitamab-gxbm)	Genentech	α -CD3, α -CD20	2023
Talvey (Talquetamab-tgvs)	Janssen Biotech	α -CD3, α -GPC5D	2023
Elrexio (Elranatamab-bcmm)	Pfizer	α -CD3, α -BCMA	2023
Imdellra (Tarlataamab-dlle)	Amgen	α -CD3, α -DLL3	2024
Ziihera (Zanidatamab-hrii)	Zymeworks/Jazz	Distinct HER2 epitopes	2024
Bizengri (Zenocutuzumab-zbco)	Merus	α -HER2, α -HER3	2024
Lynozyfic (Linvoseltamab-gcpt)	Regeneron	α -CD3, α -BCMA	2025

Bispecific Antibody Development Programs

Guidance for Industry

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research (CDER)
Center for Biologics Evaluation and Research (CBER)

May 2021
Pharmaceutical Quality/CMC

Quality attributes that may affect pharmacology should be studied, including antigen specificity; affinity and on- and off-rates; avidity (for bispecific antibodies 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.....**

The relative amounts of homodimers should be assessed. This evaluation is **particularly important for effector cell engaging constructs where homodimers of the anti-CD3 or anti-Fc engaging arm may lead to cytokine release.** Also, novel structures could potentially lead to increased immunogenicity.

MULTISPECIFICS ARE DIFFERENT: The Analytical Challenge



Multispecific protein therapeutics employ mechanisms through multiple functional modules such as T-cell redirection, receptor co-blockade, and tumor targeting and lysis.



Bispecific/multispecific antibodies often require correct pairing of distinct heavy and/or light chains, introducing multiple failure modes: homodimers, half-antibodies, light chain mispairing, and scrambled chain variants.



The wide array of formats continues to grow; each comes with its own impurity profile and analytical requirements.

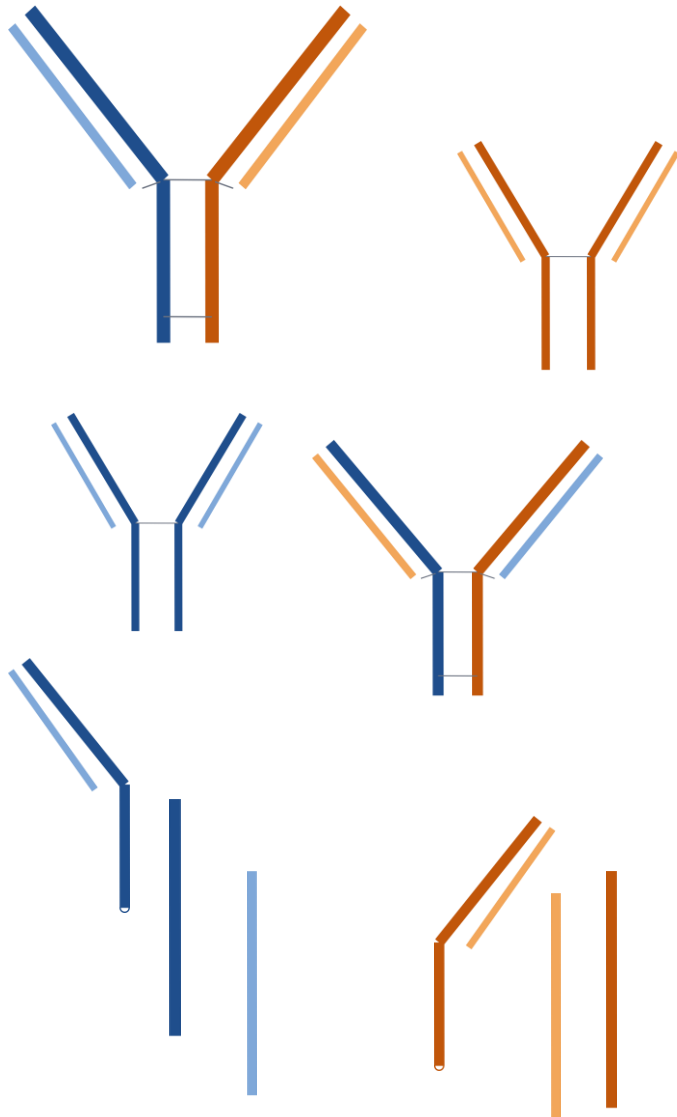


Standard platform mAb assays (SEC, CE-SDS, icIEF) often cannot resolve structurally similar misassembled variants



The analytical approach should be up to the complexity of the format.

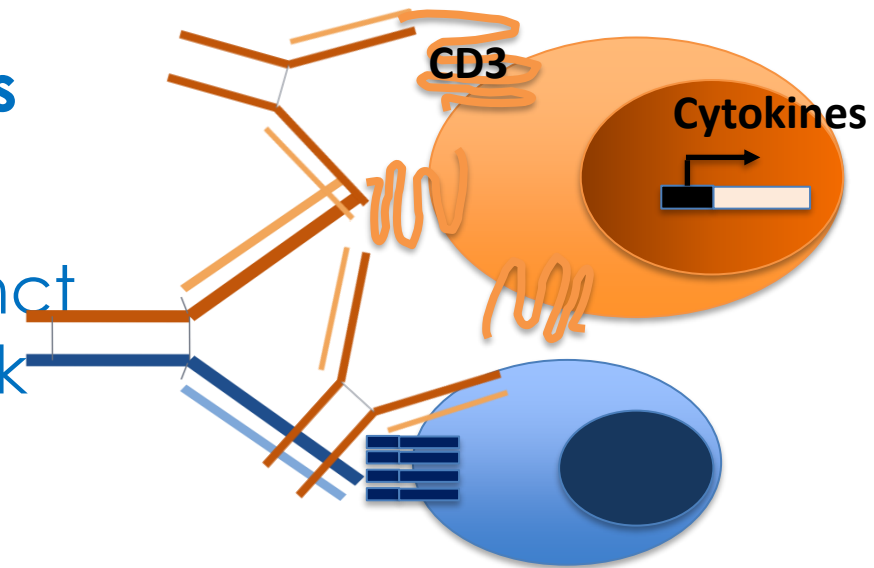
MULTISPECIFICS: Multiple failure modes



- **Product-related impurities include:**
 - homodimers
 - half-antibodies/unpaired chains
 - light chain mispairing (LC with wrong HC)
 - disulfide scrambled and/or incorrect assembly variants, and
 - truncation and fragmentation variants of fusion domains
- These are in addition to aggregates, which have more functional impact than with mAbs.
- **Should identify & control these impurities early.**

MULTISPECIFICS: Biological impact of impurities

- **Homodimers are often high-risk with regard to clinical relevance, particularly due to the prevalence of CD3 bispecifics/multispecifics**
- **Other impurities:**
 - **Aggregates can contain multiple distinct product-related impurities of distinct risk profiles. Can be hyperpotent.**
 - **Truncations often impact potency**
- **ICH Q6B: Specification is product-specific, based on impact to safety/efficacy**

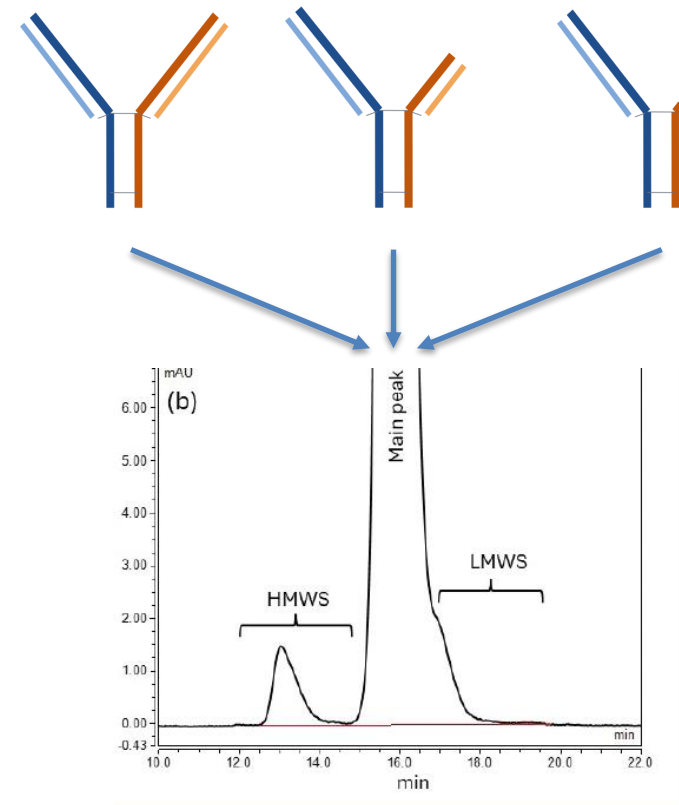


PRODUCT AND PROCESS

Understanding and Control

Most of these impurities will be CQA based on impact on safety and/or efficacy (ICH Q8).

- **Structural identification**
- **Biological risk stratification**
- **Quantitative measurement**
 - **Process understanding, control**
 - **Product quality control**
 - **Standard QC methods often lack required resolution**



Mass spectrometry is essential for this assessment throughout the product lifecycle.

EXAMPLES: MASS SPEC USE FOR MULTISPECIFICS



Mass Spectrometry is invaluable for identification of the many unique product-related impurities that arise from multispecifics and are crucial to qualify their analytical control.

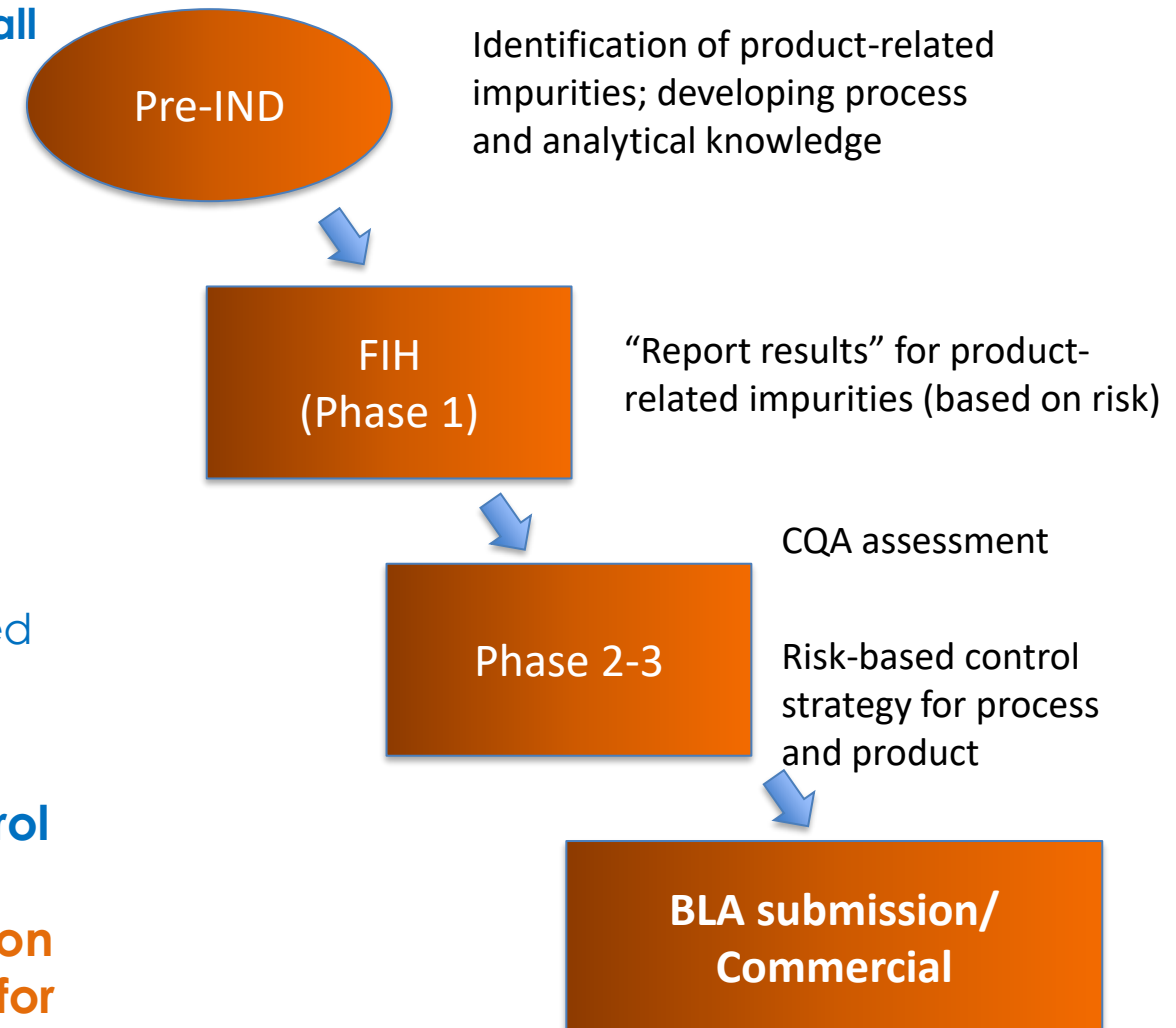
- **Intact mass analysis (ESI-MS or MALDI-TOF MS):** MW confirmation; can profile glycoforms, detect misassembled variants, but all based on size. First-line identity screen.
- **Native MS:** resolves non-covalent assemblies (heterodimer vs. homodimers) under near-physiological conditions; because keeps binding partners intact, can provide stoichiometry and half-antibody detection.
- **Reduced/non-reduced LC-MS:** identifies individual chain masses; detects LC mispairing directly, peptide mapping.
- **Peptide mapping (LC-MS/MS):** 100% sequence coverage, PTM localization, chain identity confirmation, clipping detection.
- **Multi-Attribute Method (MAM):** targeted peptide MS for routine monitoring - bridges characterization and QC release testing.
- **Subunit analysis (IdeS-MS):** has been used for more targeted evidence of correct assembly of subunits (Fab, Fc, Fd or Fd') with simpler spectra than full intact MS.

CHARACTERIZATION EXPECTATIONS

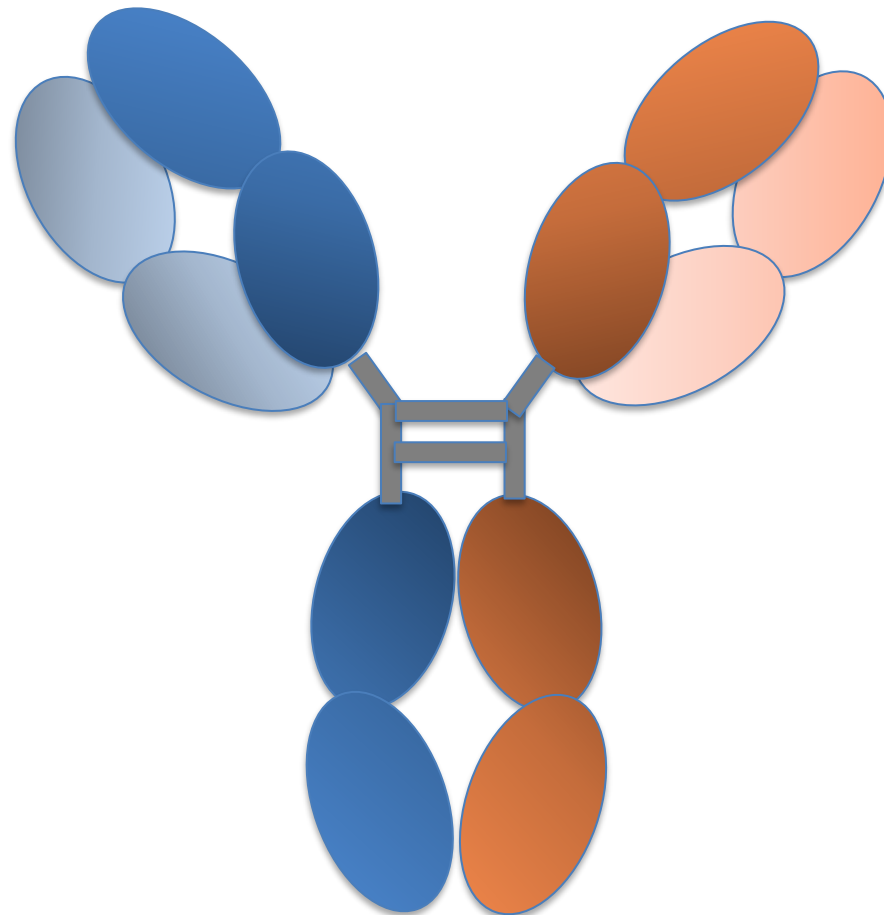
- **Direct analytical evidence of correct chain pairing, overall architecture, and understanding of impurities**
 - Typical mAb release assays may work, but will at a minimum ultimately require qualification by MS
- **Analytical methods for control-** “Report results” may be acceptable at early IND based on risk.
- **Potential impact of each PRI on safety, efficacy, and immunogenicity** must be formally evaluated.
- **Process linkage:** analytical thread from manufacturing controls to the final product specification.
- **Product knowledge:** Understanding which product-related impurities change on stability also crucial for licensure.

Validated assays must distinguish, measure, and control the product-related impurities through justified limits.

Mass spectrometry is generally required for qualification of these assays for intended purpose and sometimes, for QC itself.



CASE STUDIES



FDA: “Figure out what product-related impurities you have, what they do, and control them accordingly.”

“Bispecific-1 is produced using the proprietary super-duper-pairing sequence engineering to promote correct assembly. However, mispairing can still occur, resulting in homodimeric species (super-super or duper-duper), mispaired species (e.g. LC-super/HC-duper) or half-antibody species that lack bispecific activity. We note that the current specification includes analytical methods intended to monitor and control product-related impurities based on:

- Size (SEC-HPLC, reduced and non-reduced CE-SDS)
- Charge (icIEF)
- Hydrophobicity (RP-HPLC or HIC)

However, these methods may not be sufficiently sensitive or specific to detect and quantify mispaired bispecific species, scrambled species, or fragments. As development proceeds, support the adequacy of your analytical control strategy to assess bispecific antibody integrity and correct pairing.

Given the potential immunostimulatory impact of homodimers or aggregates, provide biological characterization data to support your proposed risk-based control strategy for product-related impurities.”

Case Study 1 – incomplete impurity profile



Pre-IND meeting packages and IND FIH submissions

- Have already done some preliminary work with mass-based MS methods to suggest that the current QC assays being used for release/ stability adequately resolve homodimers, mispaired impurities, unpaired chains, etc.
- Have considered CD3 homodimer (or immunostimulatory impurity) control
 - Appropriate biological characterization
 - Method to discriminate
 - Still working on numerical limits

GAPS

- **Only provided intact mass-based MS method data** for characterization
 - Other impurities?
 - Isobaric mispaired species? PTM? Disulfides scrambled?
- **Have had instances where product-related impurities have later been found to be hidden rather than absent.**

At this stage, appears to be fine. With final process & analytics? (Sent IR)



Case Study 2-



biological characterization and impurity identification

- There are CD3 homodimers; QC method
- Reporter gene assay requires simultaneous bispecific binding
 - Spike in CD3 homodimers and see inhibition of bioassay
 - Does this predict impact on cytokine release?

What other impurities?

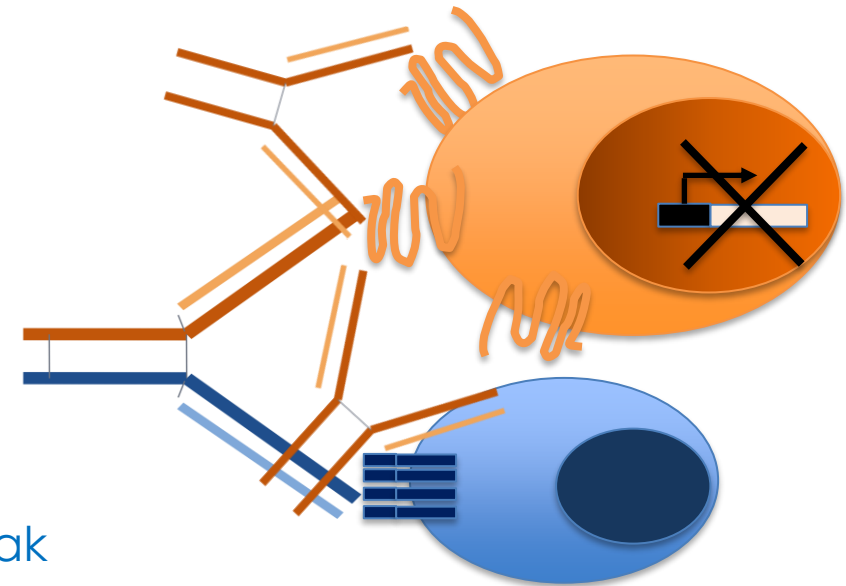
- 7-10% aggregates (HMW) by SEC

Aggregates are also higher risk with bispecifics than mAbs

- Can be high-avidity agonists
- Can be more immunogenic
- Could be multiple distinct product-related impurities in HMW peak

Mass spectrometry will be the best- and maybe only- way to identify the species contributing to the HMW. Once identified, will need:

- analytical method(s) to measure them, which allows
- an understanding of their formation and control during manufacturing



Case Study 3

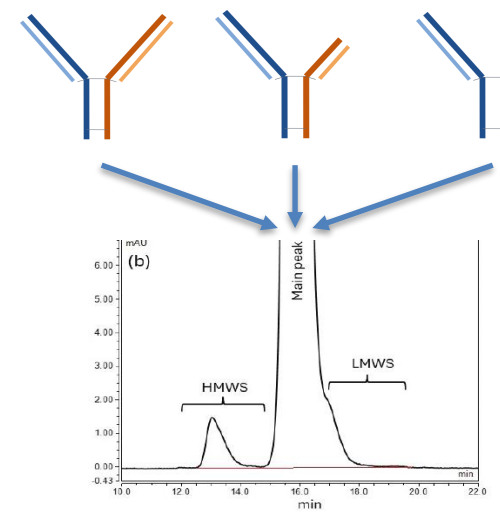
Tale of two BLAs

SEC- multiple species in the main peak (unresolved)

Reminder- While mass spec can resolve isobaric variants,

- SEC can't
- Reduced and non-reduced CE-SDS can't
- icIEF generally ambiguous....

So what's the control strategy going to be?



BLA 1: Unable to resolve fragmented impurity from intended product. Tried to justify based on process consistency, risk.

Unable to find the control strategy acceptable as it stood.

BLA 2: During development, used mass spec to characterize multiple methods. None were found to adequate to resolve and quantify the product-related impurities

Incorporated a mass spec method for QC control.

Tips to remember:

Multispecifics have

- more product-related impurities
- higher biological risk to these impurities
- difficult resolution of these impurities

Yet resolution is imperative.

Mass spectrometry is essential for the creation and qualification of a robust control strategy through multispecific product lifecycle.

Thank you!

- Marjorie Shapiro (retired)
- Kristen Nickens (Amgen)
- Leslie Rivera Rosado
- Brian Roelofs
- Samuel Mindaye
- Kelley Burrridge
- Frances Namuswe

