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KBI BIOPHARMA

Regulatory-Ready Characterization: From IND to BLA

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Global CDMO Providing Innovative Biologic Solutions

KBI Biopharma drives breakthroughs in biopharmaceutical development and manufacturing that positively impact the lives of patients worldwide.

30+
years of experience

1400+
client partners

300+
drug candidates

Mammalian
Delivering Innovative CLD to Vial Solutions

Analytical
Solving Analytical Challenges at Every Stage

Microbial
Elevating Microbial Expression and Manufacturing Efficiencies

Product Characterization Overview

- Development Stage Expectations:
  - Investigational New Drug (IND)
  - Investigational New Drug Amendment (INDa)
  - Biologics License Application (BLA)
- ADC case study
- Best practices and potential risks

Why Product Characterization Matters

3.2.S.3.1 Elucidation of Structure | Manufacturing Controls (CMC) | Common Technical Document (CTD)

Ultimate goal: Patient safety

Data Collection

- Primary & Higher Order Structure
- Biological Activity
- Variant Characterization
- Degradation Pathways

Enables

QTPP
Quality Target Product Profile

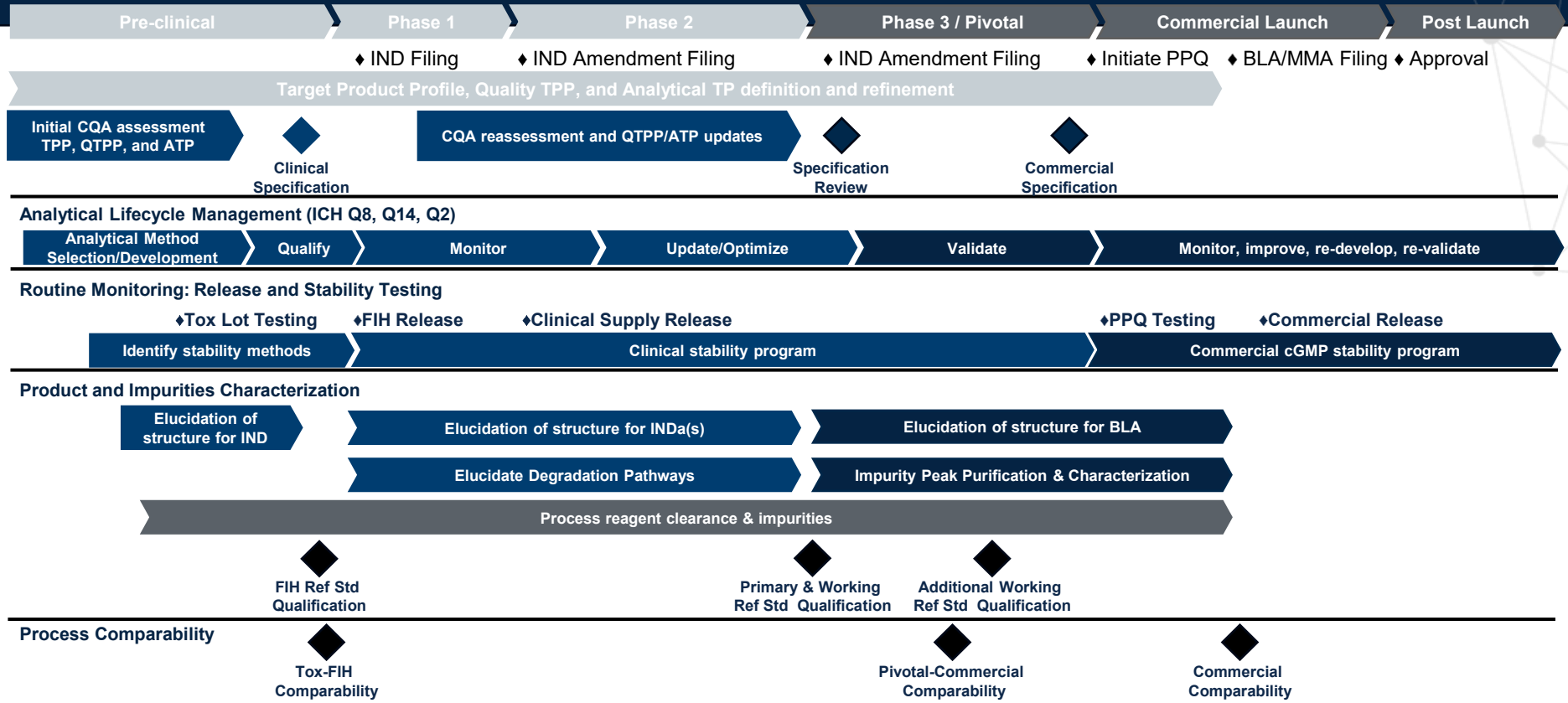
CQAs
Critical Quality Attributes

Control
Product and Process

Quality
Quality by Design

Biotherapeutic Development Phases

Biotherapeutic Development Phases



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Krishnan Sampathkumar, Brent S. Kendrick, John P. Gabrielson, Da Ren, Analytical control strategy for biologics. Part II: Roadmap for development and implementation, Journal of Pharmaceutical Sciences, Volume 114, Issue 8, 2025, 103834,

From IND to BLA: Increasing Analytical Expectations

IND EoS

- **Purpose:** Initial Characterization Assessment for First in Human (FIH) trials
- **Material:** Tox/Clinical Lot
- **Methods:** Platform
- **Duration:** 6 weeks

INDa EoS

- **Purpose:** Deeper Product Understanding in Phase II/III
- **Material:** Phase I/II DS and DP
- **Methods:** Optimized for product
- **Duration:** 3 – 5 months

BLA EoS

- **Purpose:** Full Package for Commercial Approval
- **Material:** Commercial Scale/PPQ Lot
- **Methods:** Qualified, product specific
- **Duration:** 9 - 12 months

Increasing Complexity

IND: Early-Stage Structural Characterization

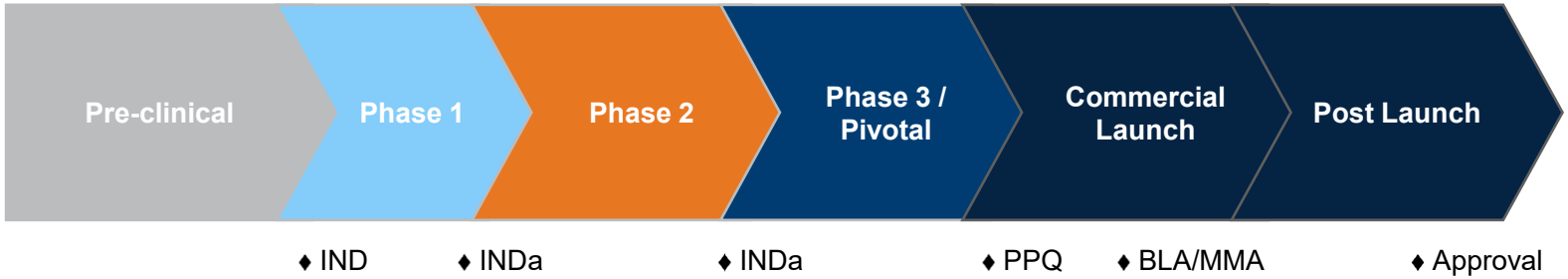
- Fast characterization focused on patient safety
- Approval required for Phase 1 (FIH) trials

Category	Method	Purpose
Primary structure	Intact mass	Mass measurements
	Reduced peptide mapping	Post Translational Modifications (PTMs)
Higher order structure	FTIR/Far UV CD spectroscopy	Secondary structure (i.e. alpha helices, beta sheets)
	Near UV CD spectroscopy	Tertiary structure
Biological Activity	Binding or Potency method	Structure function

Methods presented are based on mAb therapeutics. More complex biologics may utilize additional characterization techniques.

INDa: When Characterization Becomes Critical

INDa: Required for significant process changes



Common Triggers:

- Scale up (i.e., lab → pilot → GMP)
- New manufacturing site or CDMO
- Raw material changes (e.g., cell culture media)

- **Requirements:**
  - Comparability data using product-specific methods
  - Multiple lots to demonstrate no new safety risks

INDa: When Characterization Becomes Critical

Primary Structure

- Intact mass
- Peptide mapping
- Disulfide mapping
- Glycan profiling

Size Variants

- SEC
- SEC-MALS
- CE-SDS

Biological Activity

- FcRn binding
- Potency assay

Higher-Order Structure

- FTIR/Far UV CD spectroscopy
- Near UV spectroscopy
- DSC

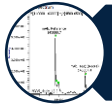
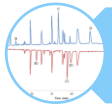
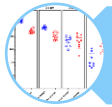
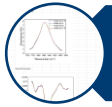
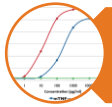
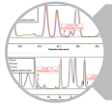
Charge Variants

- IEX
- cIEF

Particle Assessment

- MFI (Drug Product)

BLA: Complete Characterization Package

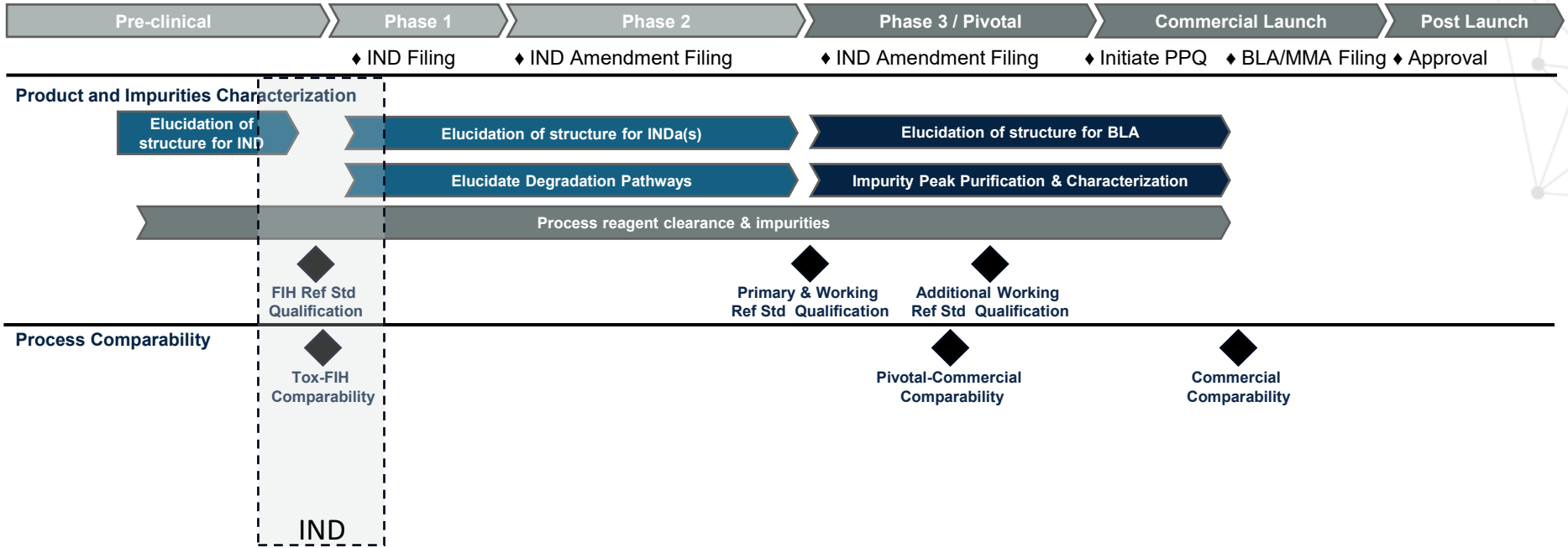
Characterization Activity	Rationale
 **Primary Structure** (Amino Acid Sequence)	Confirm fidelity of DNA transcription with 100% sequence confirmation on amino acid level
 **Primary Structure, Disulfide & Glycan Variants** (Post Translational Modifications)	Elucidate product attributes to enable QTPP, and analytical control strategy (> 0.1% abundance)
 **Product related substances & impurities** (Size, charge and hydrophobic variant peaks)	
 **Secondary/Tertiary Structure & Thermal Stability**	Ensure protein is properly folded with appropriate conformational stability
 **Biological Characterization**	Elucidate biological mechanism of action(s)
 **Degradation Pathways**	Elucidate product susceptibility to degradation under multiple stress conditions

Characterization Requirements: Side-by-Side View

Increasing Complexity

Feature	IND	INDa	BLA
Primary Structure	• Intact mass • Reduced peptide map	• Intact mass • Reduced peptide map • Disulfide map • Glycan map(s)	• Intact mass • Reduced peptide map(s) • Disulfide map • Glycan map(s)
High Order Structure	• FTIR/Far UV CD • Near UV CD	• FTIR/Far UV CD • Near UV CD • DSC	• FTIR/Far UV CD • Near UV CD • DSC
Impurity Characterization	Not required	Charge characterization of peak groups	Charge & size characterization of individual peaks
Biological Activity	Binding or potency assay	Binding & potency assays	Binding & potency assays
Degradation Pathways	Limited for early understanding of molecule stability	Limited to support other studies: comparability, formulation dev, method qualifications	Full degradation of key stress conditions: heat, pH, oxidation, light, agitation
Method Qualification	Not required	Limited, focus on method optimization for product	Qualification for characterization methods

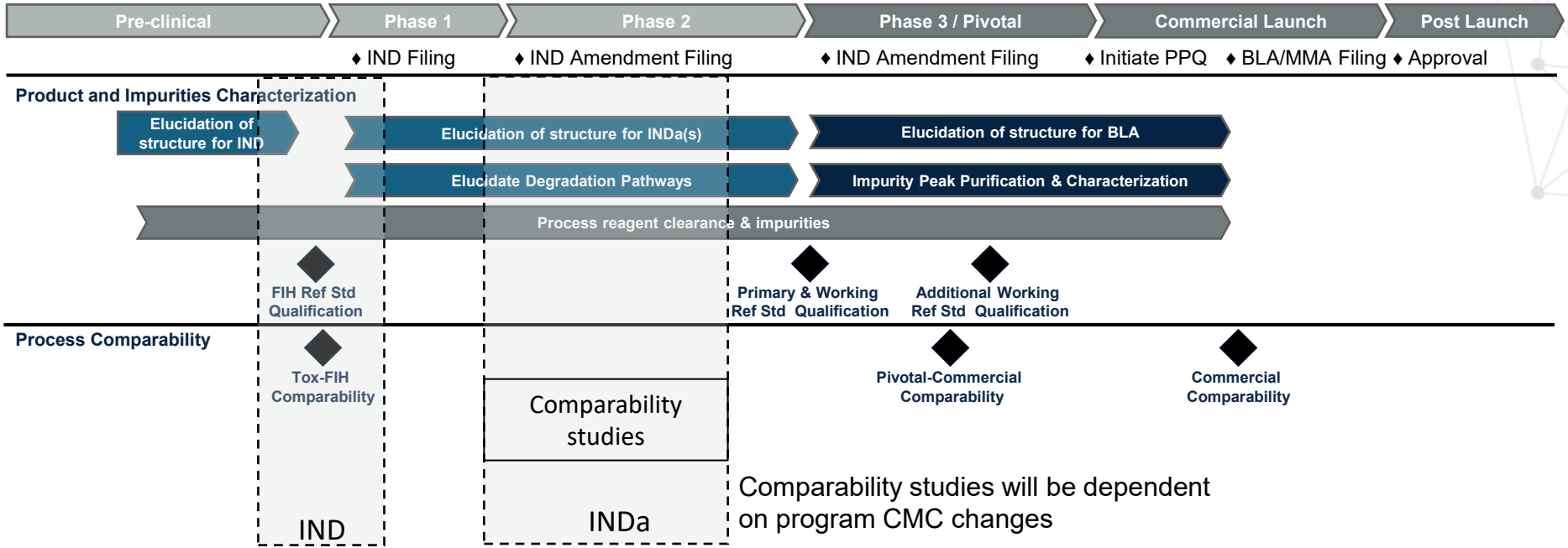
Project Timelines: How to be Efficient



Couple RS Characterization and/or Comparability with Product Characterization

Krishnan Sampathkumar, Brent S. Kendrick, John P. Gabrielson, Da Ren, Analytical control strategy for biologics. Part II: Roadmap for development and implementation, Journal of Pharmaceutical Sciences, Volume 114, Issue 8, 2025, 103834,

Project Timelines: How to be Efficient



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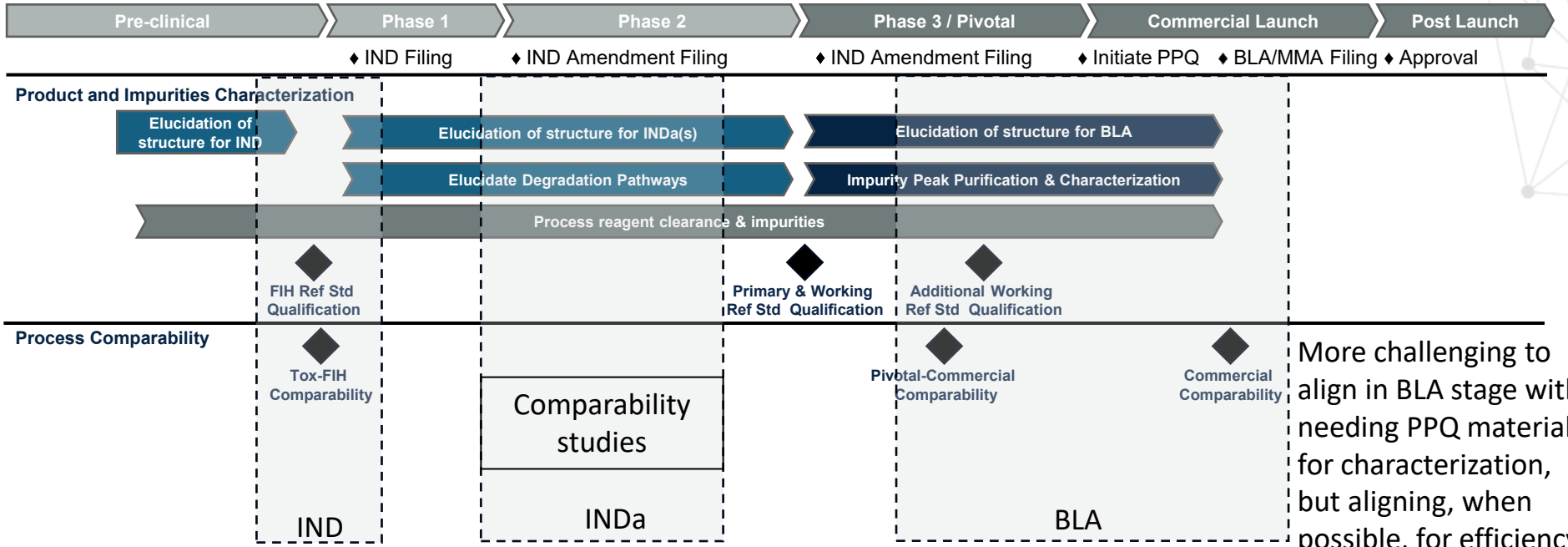

More challenging to align in BLA stage with needing PPQ material for characterization, but aligning, when possible, for efficiency

Couple RS Characterization and/or Comparability with Product Characterization

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Case Study:
ADC Characterization

Analytical

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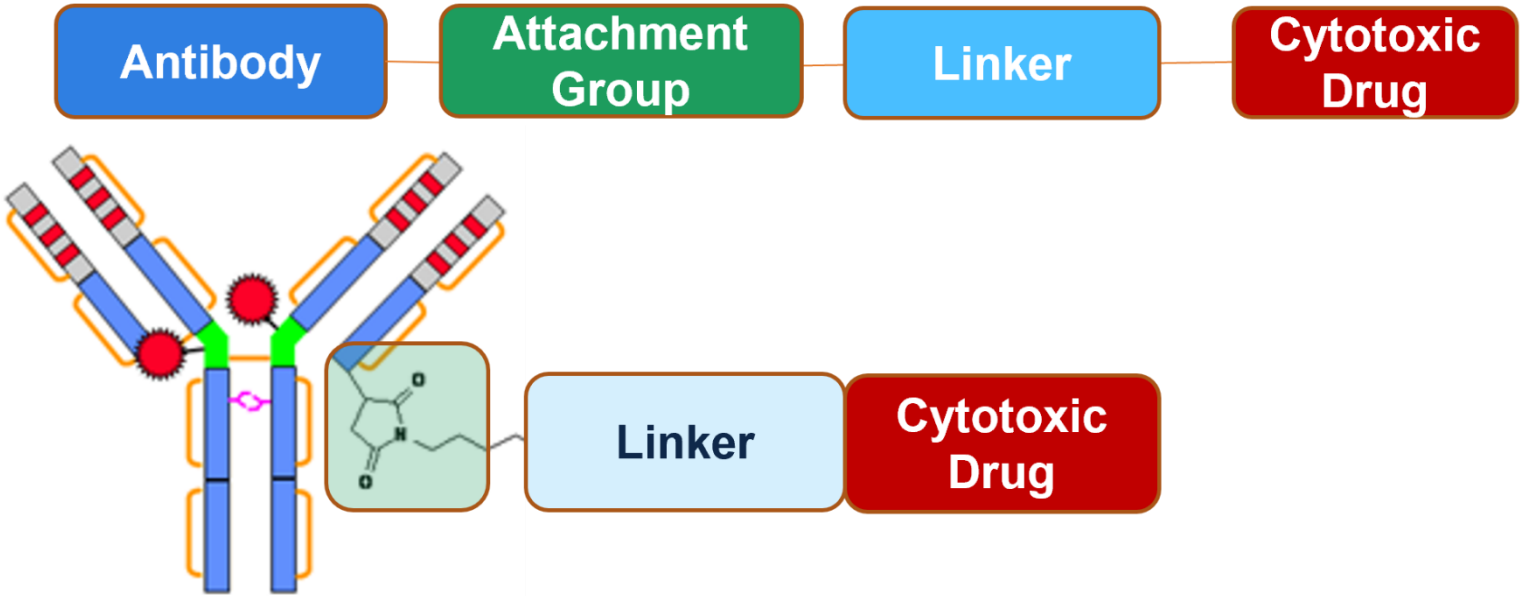
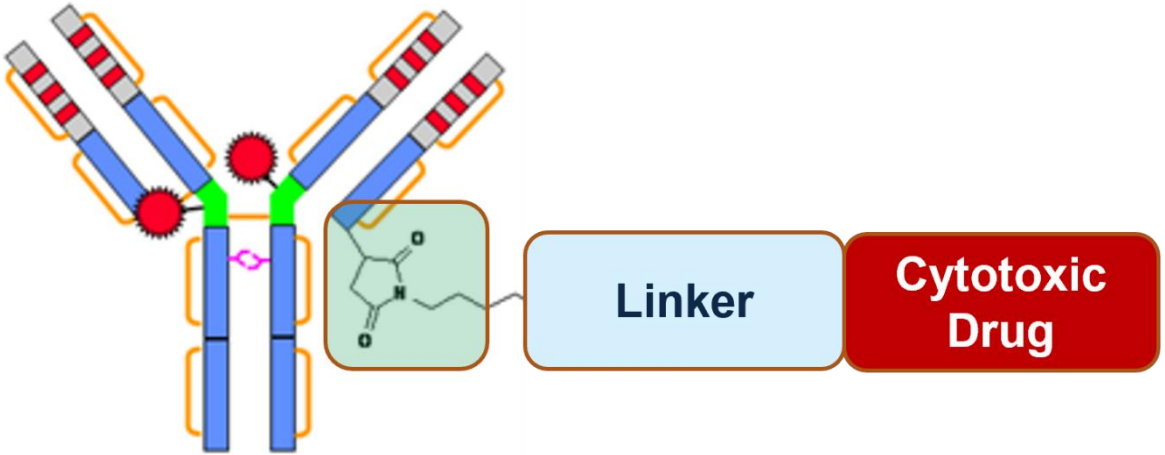
ADC Structure Includes Multiple Components for Characterization

CQAs for antibody, drug-linker, & combined conjugated product

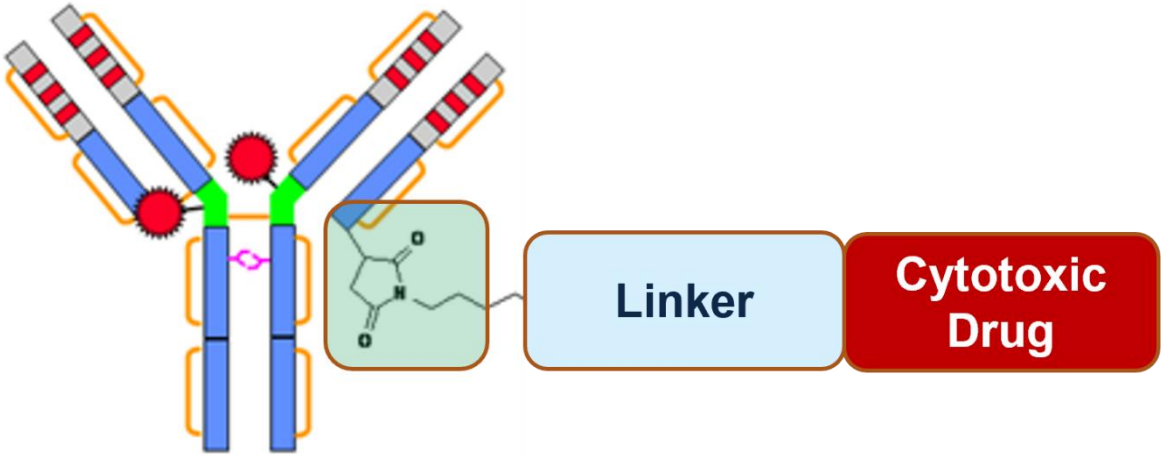


Commonly Known ADC CQAs



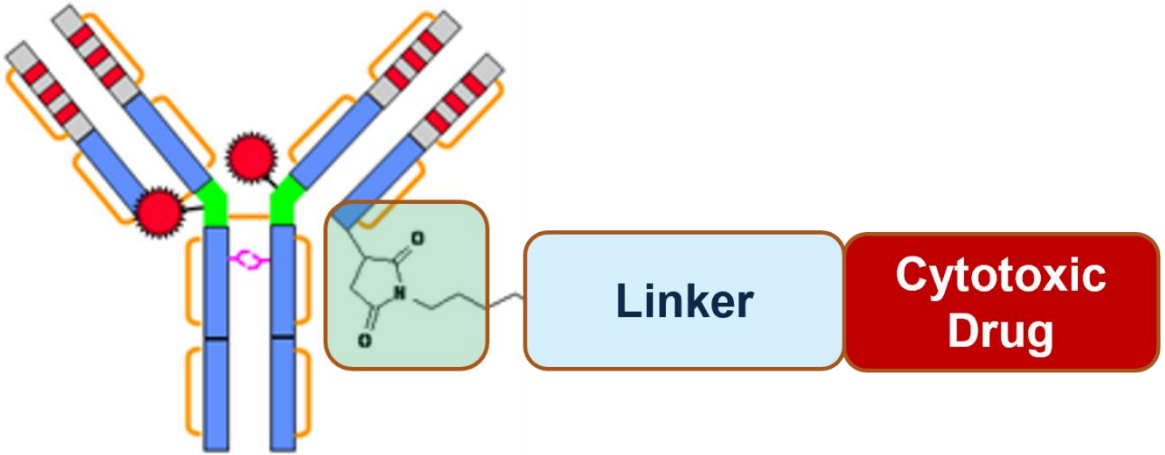
- Drug-to-Antibody Ration (DAR)

Common ADC CQAs



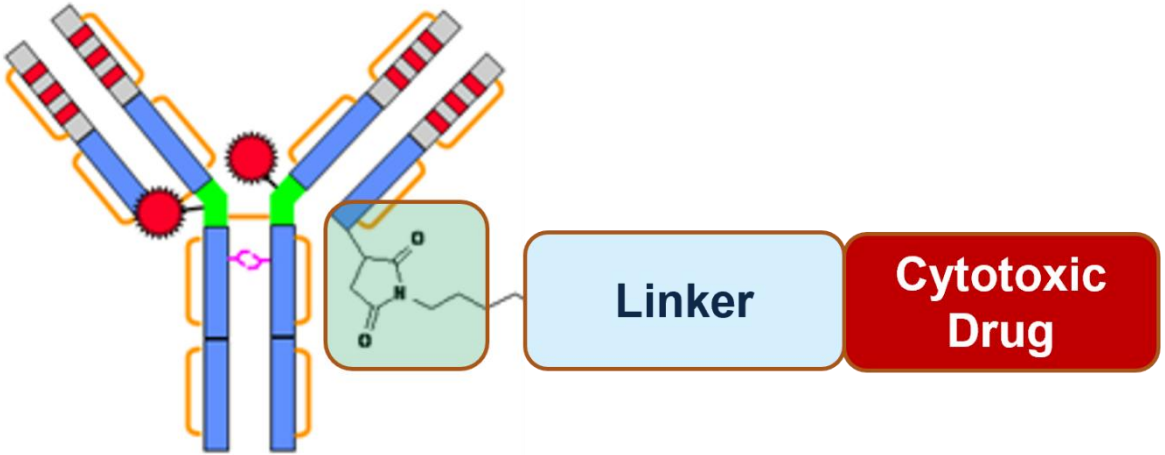
- Drug-to-Antibody Ration (DAR)
- Conjugation Site

Common ADC CQAs



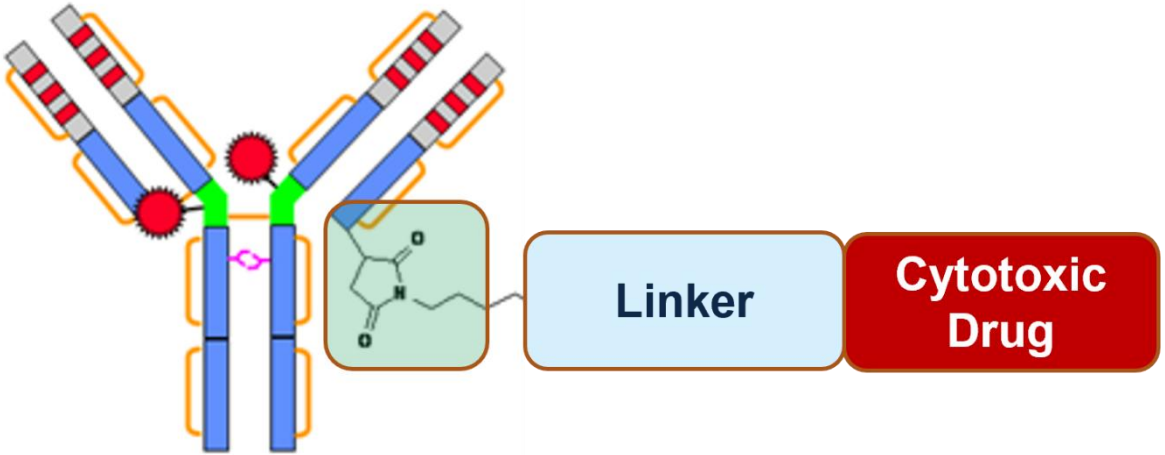
- Drug-to-Antibody Ration (DAR)
- Conjugation Site
- Free Drug

Common ADC CQAs



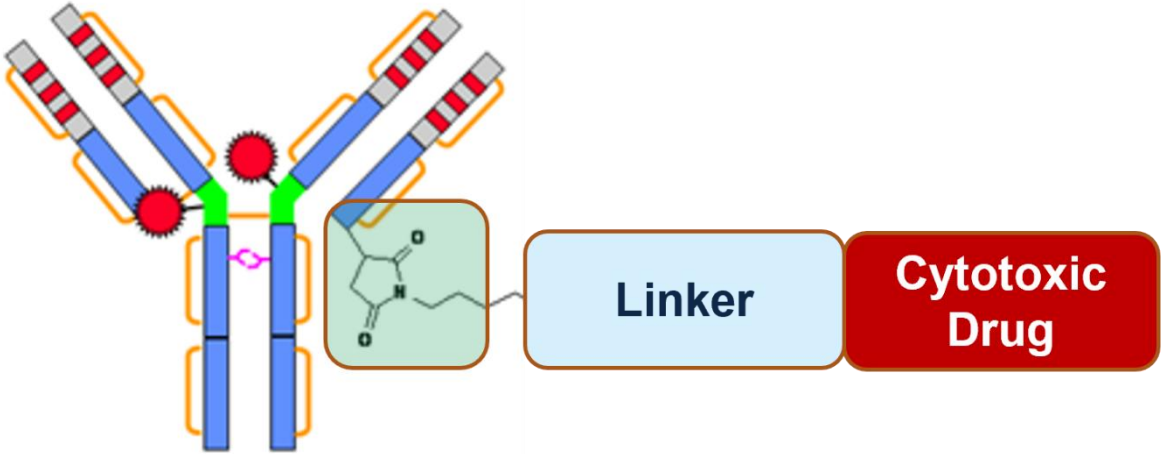
- Drug-to-Antibody Ration (DAR)
- Conjugation Site
- Free Drug
- Linker Stability

Common ADC CQAs



- Drug-to-Antibody Ration (DAR)
- Conjugation Site
- Free Drug
- Linker Stability
- Aggregation/Size Variants

Common ADC CQAs



- Drug-to-Antibody Ration (DAR)
- Conjugation Site
- Free Drug
- Linker Stability
- Aggregation/Size Variants
- Potency

IND: Fast Assessment for FIH Safety

DAR

- HIC

Free Drug

- Intact mass

Size Variants

- SEC
- CE-SDS

Conjugation Site

- Peptide mapping

Linker Stability

- HIC – changes in DAR levels

Potency

- Binding assay
- Cytotoxicity assay

Testing completed on using platform methods

INDa: Demonstrate Comparability For Site Change

DAR

- HIC
- Intact mass

Free Drug

- Intact mass
- RP-HPLC

Size Variants

- SEC
- CE-SDS
- SEC-MALS

Conjugation Site

- Peptide mapping

Linker Stability

- HIC – changes in DAR levels

Potency

- Binding assay
- Cytotoxicity assay
- Cell based bioassay

Testing completed on using product specific methods

BLA: Complete Characterization Package for Commercial Approval

DAR

- HIC
- Intact mass
- Fractionation of hydrophobic variants

Free Drug

- Intact mass
- RP-HPLC

Size Variants

- SEC
- CE-SDS
- SEC-MALS
- Fractionation of size variants
- SV-AUC

Conjugation Site

- Peptide mapping
- Intact mass

Linker Stability

- HIC – changes in DAR levels
- Degradation studies

Potency

- Binding assay
- Cytotoxicity assay
- Cell based bioassay

Testing completed on qualified characterization methods

Avoidable Mistakes That Delay Filings

Inadequate Qualification of Characterization Methods

- Not qualifying LC-MS and HOS methods
- Lack of method performance understanding at late-stage

Incomplete Characterization Studies

- Assessing only size OR charge variants (not both)
- Skipping hydrophobic variants when detected

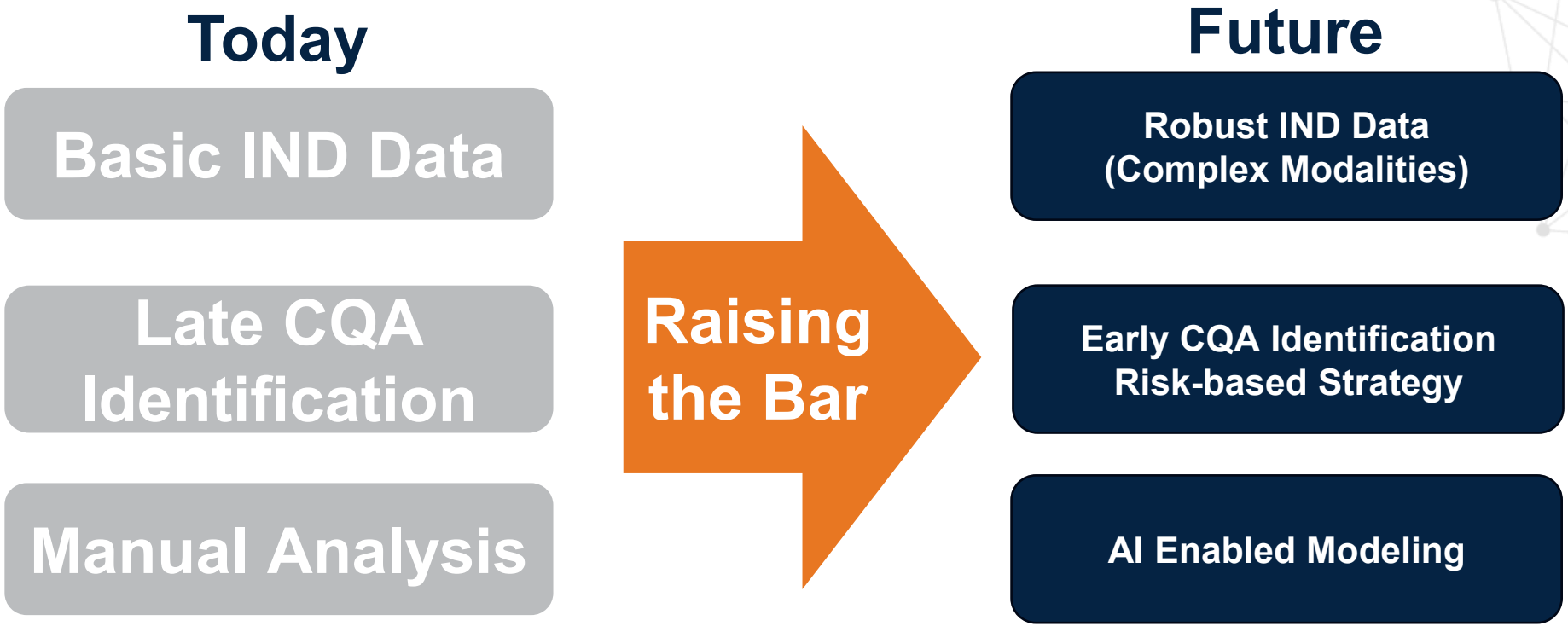
Delayed Characterization Timelines

- Starting characterization too late in development
- Discovering critical issues at BLA stage

Insufficient Comparability Data

- Incomplete methods to demonstrate impact on CQAs
- Not enough lots for stage of comparability

Regulatory Expectations - Where the Bar Is Heading



Bringing It All Together

- Product characterization is progressive – start light, end deep
- IND: Fast with a focus on patient safety
- INDa: Comparability studies with greater product understanding
- BLA: Complete package
- Regulatory success = scientific rigor + staged planning

Virtual Poster Presentation

65

LC-MS Characterization of an Unexpected C-Terminal HC Extension in a Therapeutic mAb

Topic: WCBP Virtual Poster

LC-MS characterization of an unexpected C-terminal HC extension in a Therapeutic mAb

Ben Zigterman

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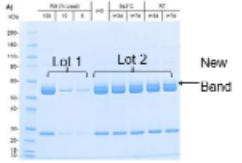
Introduction

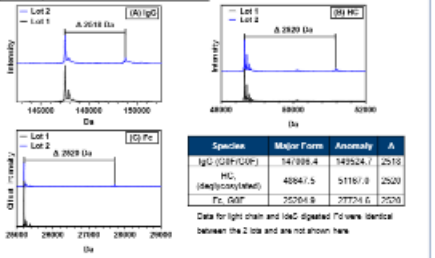
To ensure safety and efficacy therapeutic monoclonal antibodies (mAbs) require extensive molecular characterization, including identification and characterization of size variants. SDS-PAGE of an IgG1 drug candidate revealed an unexpected HMW band under reducing conditions.



Orthogonal IEF and SEC did not observe this anomaly, suggesting it comprised a HC size variant. Accordingly, LC-MS was conducted to distinguish among likely modifications (e.g. contaminants, glycovariants, and frameshift mutants)

Results

Mass Analysis Reveals Species +2448 Da and +2520 Da Greater than Expected, with Shift Localized to Fc



Species	Major Form	Anomaly	Δ
IgG (G0F/G0F)	147008.4	149524.7	2516
HC (deglycosylated)	48847.5	51167.9	2520
Fc, G0F	25264.9	27724.6	2520

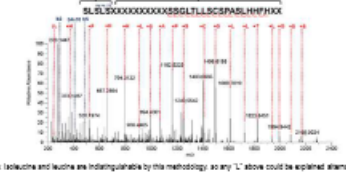
Data for light chain and IdeS digested Fd were identical between the 2 lots and are not shown here.

Deconvoluted masses of intact IgG (A), diglycosylated heavy chain (B), and IdeS digested Fc (C) shown above for each lot, showing the presence of mass variant exclusively in lot 2 and localizing the variant to the Fc.

The variant Fc resolved chromatographically, and the spectra of the variant peak is shown in panel (D).

Results Continued

Denovo Sequencing of C-terminal Extensions



Note: Isoleucine and leucine are indistinguishable by this methodology so any 'L' above could be explained alternatively be as 'I'

MS² data were sequenced de novo then compared the theoretical sequence from the expression vector, revealing that unknown species comprised C-terminal extensions caused by alternative splicing of the vector sequence.

Alternative Splicing Yields C-Terminal Extensions

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Science Driven. Customer Focused.

A Global CDMO Providing Innovative Biologic Solutions

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