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Critical Quality Attribute (CQA) in Antibody Drug Conjugate (ADC) Modality

Part 2 of a 3-Part Series: Assessment of Critical Quality Attributes for Relevant Modalities

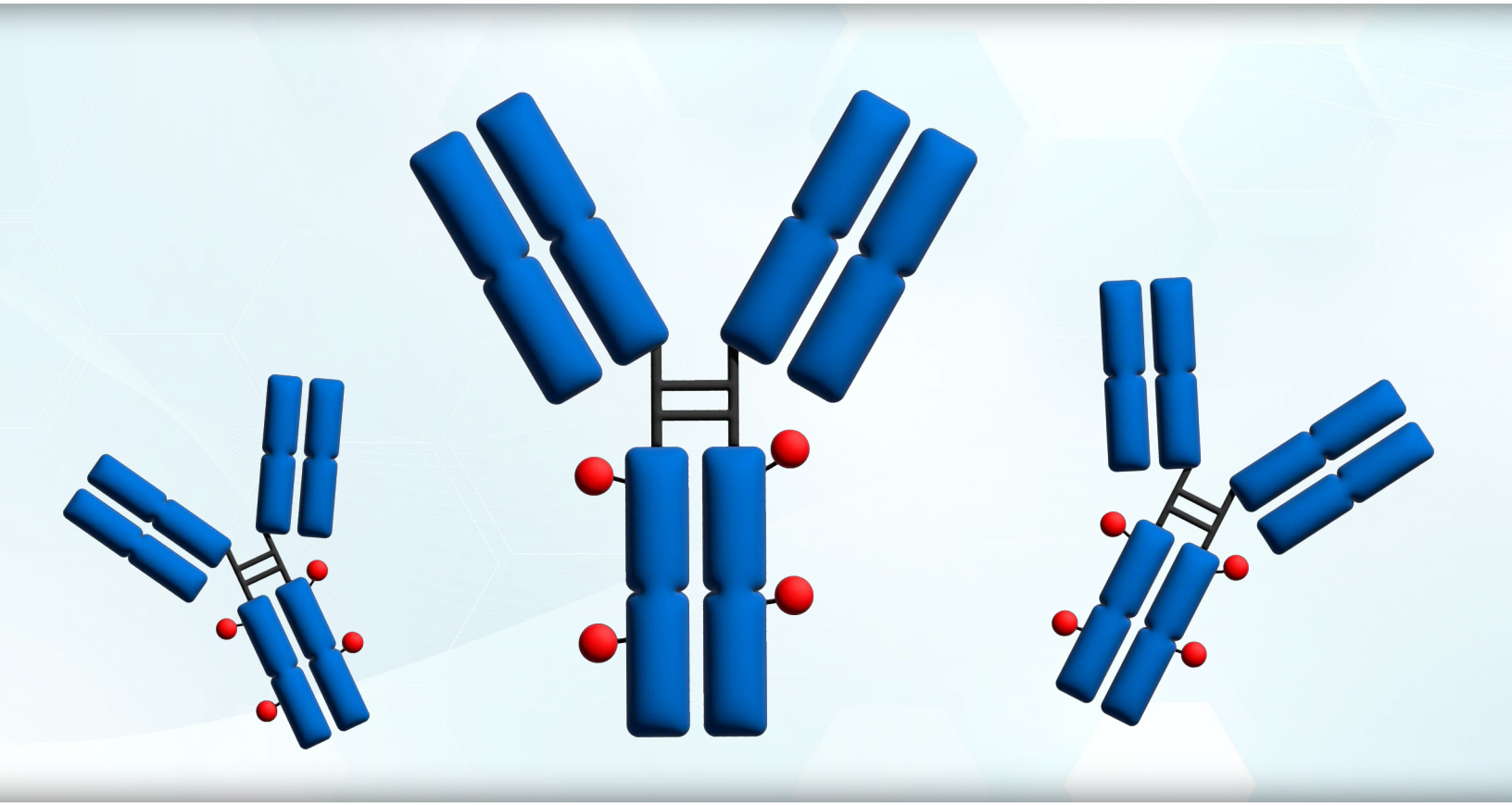

Figure 1: ADC structure (adopted from Signal Transduction and Targeted Therapy).[1]

Following the successful launch of **CQAs in LNP-mRNA Modality** (*July 8, 2024*), we continue the series with **CQA in ADC Modality**.

This white paper discusses:

- ADC components
- ADC-related CQA
- Impact on safety and efficacy

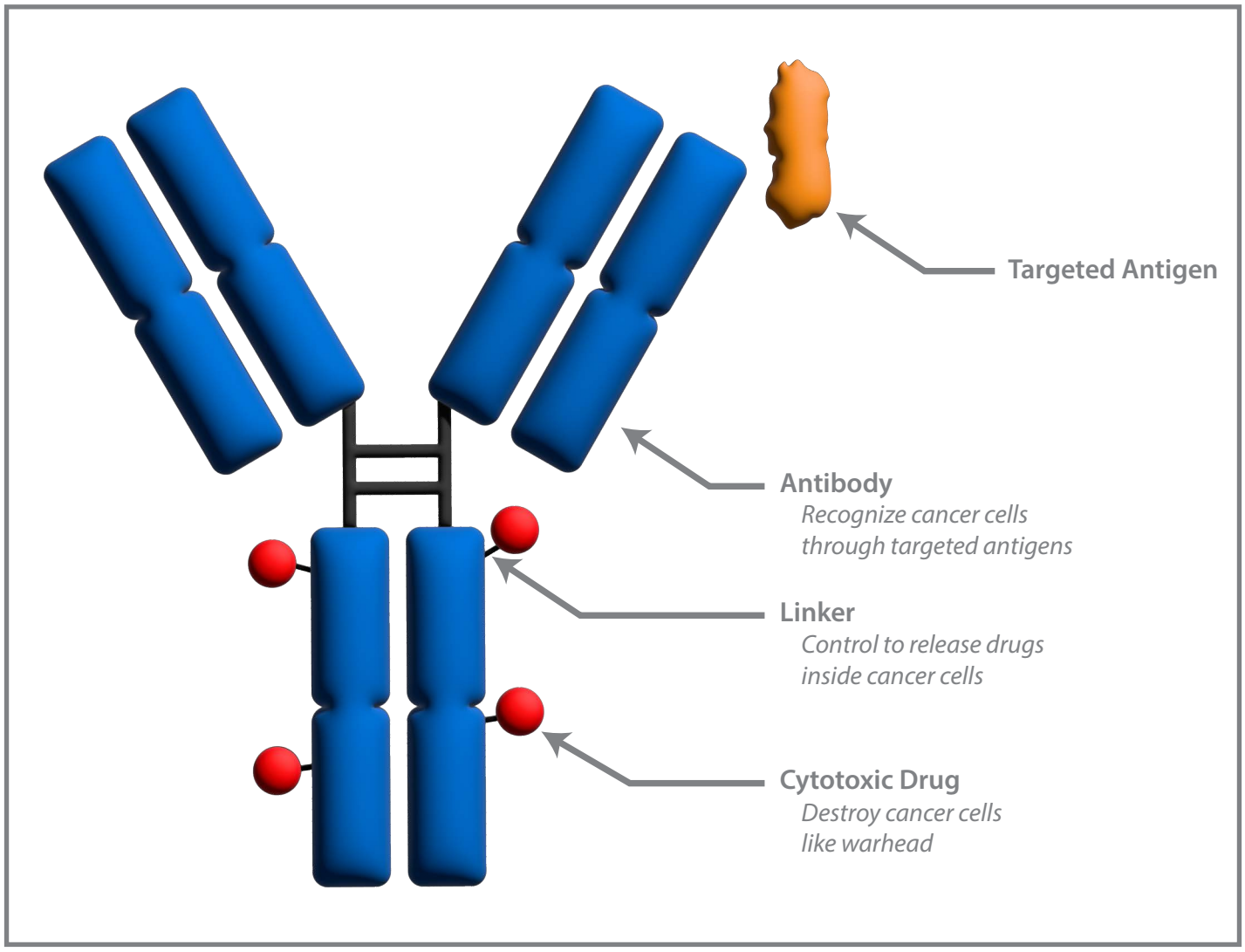

Figure 1: ADC structure (adopted from Signal Transduction and Targeted Therapy).[1]

ADC Components

Antibody-drug conjugates (ADCs) consist of three main components[2] :

1. Antibody: engineered to target specific antigen(s), mainly as mAb but can be bi-specific.
2. Linker: conjugated between a cytotoxic drug and an antibody at Cys or Lys residue, mainly as cleavable but can be non-cleavable.
3. Drug: synthetical small molecule functioning as cytotoxin (warhead or payload).

Heterogeneities of CQAs are anticipated in consideration of the 3 components in ADC.[3] The influences in potency, stability, and toxicity will be discussed with evaluation of risk assessment.

Category	CQA (ADC)	Regulatory requirement	Impact on Safety and Efficacy[3]
Drug to Antibody ratio (DAR) [4]	Yes	Yes	• High level of DAR affects the safety profile of the ADC. • Low level of DAR leads to a decrease in the efficiency of ADC.
Monoclonal antibody size variants (Aggregates)	Yes	Yes	• Size variants (aggregates, particles, fragments) can directly affect the efficacy and safety of antibody-drug conjugates.
Payload distribution	Yes	Yes	• Random distribution can cause un-controllable efficacy and safety.
Unconjugated Antibodies (naked antibody)	Yes	Yes	• Unconjugated antibody competes with ADC for binding antigen, reducing efficacy. • Unconjugated antibodies are susceptible to aggregation upon exposure to high temperatures, increasing the immunogenicity issue.
Unconjugated drug (D0)	Yes	Yes	• ADC without drug (no payload or D0), premature release of drug outside of targeted cancer region, can cause safety concern (off-target cytotoxicity). • In addition, D0 also competes with ADC for binding antigen, reducing efficacy.
Charge variants (acidic main and basic)	Yes	Yes	• Charge variants can potentially influence the stability and biological activity of these molecules.

Table 1: Summary of ADC-related CQA.[2]

ADC-Related CQA

Many of these quality attributes are expected to be crucial for ADCs; nonetheless, experimental validation is necessary to ascertain their criticality for a specific ADC.

As per regulatory ICH guidelines Q8 R(2)[6], a risk assessment for each quality attribute must be evaluated for potential impact on the patient. This involves employing a scoring system that considers the impact and uncertainty of each attribute, which have been provided previously.[5]

3-Part Series: Assessment of Critical Quality Attributes for Relevant Modalities

In this series, we will assess Critical Quality Attributes (CQAs) relevant to 3 modalities:

1. mRNA-LNP (Lipid Nano Particle) - *previous paper*
2. Antibody-Drug Conjugate (ADC) - *this paper*
3. Monoclonal Antibody (mAb) - *coming soon*

Capabilities at Crystal Bio

Crystal Bio is a leading Contract Research Organization (CRO) specializing in comprehensive analytical services for biotherapeutics. Our expertise covers many modalities, including Antibody Drug Conjugates, Monoclonal Antibodies, Fusion Proteins, and mRNA-LNP Therapeutics with our strategic partner CATUG. We provide all necessary analytical tools to measure the related CQAs, comprising High-resolution LC-MS, liquid-based chromatography (HIC, IEX, SEC, RPLC, CE, and cIEF), along with comprehensive bio-analytical tool comprising various binding and cell-based assays, effector function-based assays like surrogate cytotoxicity assays and direct ADCC, ADCP and CDC assays, anti-ADC-antibody assays, qPCR, ELISA, Endotoxin, Sterility, Bioburden, and Cell-based Bioassay, etc. Our capabilities also extend to method development and analytical characterization of biotherapeutics. This holistic approach ensures compliance with stringent regulatory requirements outlined in the CMC section, making us a valuable partner for pre-IND, Phase I, and subsequent submissions.

References:

1. Signal Transduction and Targeted Therapy. 2022, 7 (1): 93-117.
2. Challenges and new frontiers in analytical characterization of antibody-drug conjugates MABS 2018, VOL. 10, NO. 2, 222–243
3. Improving the Quality of Antibody Drug Conjugates by Orthogonal, Agilent Technologies, Inc, 2024 Printed in USA April 8, 2024, 5994-5089EN
4. Review of Antibody-Drug Conjugates, and methods in molecular Biology Series. mAb 6:1,30-33; January/February 2014; @ 2014 Landes Bioscience.
5. Assessment of CQA in mRNA-LNP modality published on LinkedIn on 8 July 2024.
6. International Council for Harmonization (2009). Guidance for Industry, Q8 (R2) pharmaceutical development.