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Critical Quality Attribute D0 Impact in ADC Modality

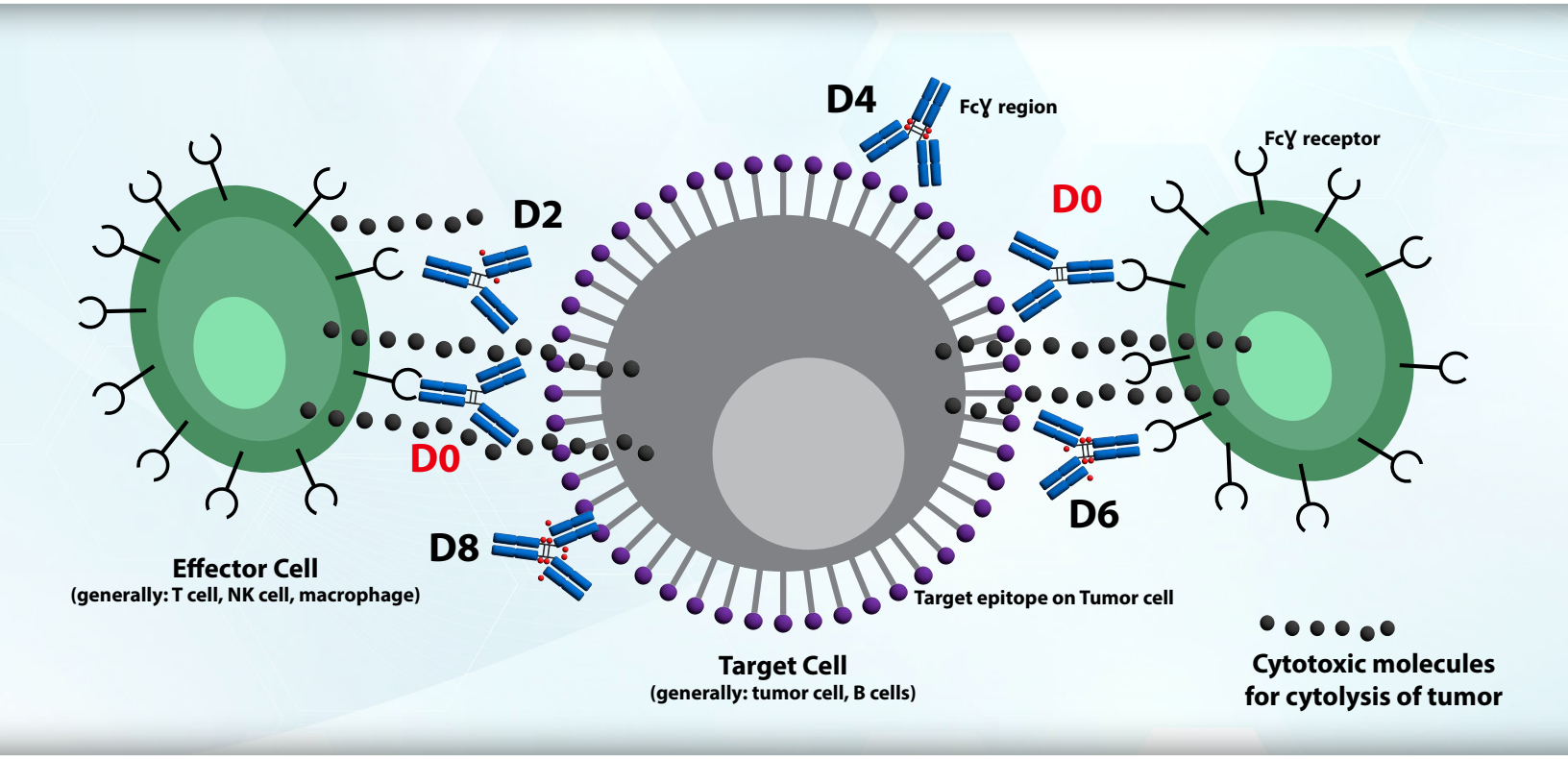

Figure: Illustration of D0 competing with ADC (D2 to D8) at similar binding sites of target cell to affect ADC action of Antibody Dependent Cell mediated Cytotoxicity (ADCC). (Image adapted from Axion Biosystems)

Antibody drug conjugates (ADC) are typically composed of an antibody chemically attached with small molecule drugs as payload through linkers[1]. **D0** refers to an antibody conjugated with no drug (0 payload or naked antibody[4])

This white paper discusses the CQA D0 impact in ADC modality, including:

- What is D0 in ADC?
- The relevance of D0
- D0 impact on ADC potency
- Differentiating D0 in ADC
- How to characterize DAR in ADCs

Read more

What is D0 in ADC?

Antibody drug conjugates (ADC) are typically composed of an antibody chemically attached with small molecule drugs as payload through linkers[1]. The drugs attach to the antibody in different proportions, which defines the drug-to-antibody ratio (DAR or abbreviated further as D). Different ratios indicate different degree of drug(s) appended to the antibody, affecting ADC potency and cytotoxicity to different extents, and are a critical quality attribute of ADCs[2,3].

D0 refers to an antibody conjugated with no drug (0 payload or naked antibody[4]), D1 conjugated with 1 drug, D2 conjugated with 2 drugs and so on. So far, the maximum number of drugs conjugated to an antibody is limited to 8 (D8)[2] with D4 being the most common[5].

Why does D0 still exist after drugs are conjugated to an antibody? Why is it relevant?

Drug conjugation is a chemical process often yielding a distribution, with existence of minute levels of unconjugated (D0) species. ADC stability during purification process and storage may further generate D0. Further, ADC in serum or plasma, as observed in animal or human PK time-course studies, the cleavable linkers of ADCs may get hydrolyzed at an appreciable rate, leading to appreciable D0 and payload. In such instances, the naked antibody (due to the premature release of payload) loses targeted cell killing (thus reducing efficacy), and the released payload in extra-tumoral compartments[6] can cause off-targeted cytotoxicity (safety concern).

How can D0 impact ADC potency?

Both D0 and ADC recognize the same epitope on the tumor / antigen surface, thus competing for the target binding affecting ADC potency, as illustrated in Figure. Additionally, effector function in ADC (with multiple payload) could be diminished, possibly due to masking Fc moiety of ADC by conjugated payload[1]. Overall, in case of ADC, the effector cell mediated cytotoxicity of target molecule maybe decreased due to (a) competition with D0 for the same epitope, (b) masking of Fc moiety by conjugated payload.

How can we differentiate D0 in ADC?

Binding studies, using ELISA and / or SPR are generally used to characterize the affinity and specificity of a therapeutic antibody to a specific purified antigen. The Fab portion (CDR) of a therapeutic antibody and its ADC counterpart remain the same, and therefore, there may not be significant difference in the performance of these molecules in binding to the desired antigen. Hence, such binding assays may not be sufficient for differentiating D0 in ADC.

These assays can still be used for product consistency in lot release due to their robustness and fast turnaround time. On the other hand, effector function assay can be achieved to differentiate D0 (without payload, not masking Fc moiety) from ADC (with multiple payloads, likely masking Fc moiety), as well as for cell-based assay with direct drug-induced cytotoxicity. These effector function studies, carried out mainly by Antibody Dependent Cell mediated Cytotoxicity (ADCC) or Antibody Dependent Cell mediated Phagocytosis (ADCP) or Complement Dependent Toxicity (CDC) assays form the backbone of potency experiments (Figure) and are likely to be performed early in development stages for evaluating efficacy and safety concerns.

How can we characterize DAR (e.g. D0) in ADCs?

Hydrophobic Interaction Chromatography (HIC), capable of separating the D0, D1, D2, etc. along with SEC-MS and UV spectroscopy, are often used for DAR distribution, identification, and quantitation, accompanied by Reverse Phase High Performance Liquid Chromatography (RP-HPLC) which provides DAR distribution in light-and-heavy-chain separately. Fractions collected from RP-HPLC or HIC can be analyzed by Liquid Chromatography-Mass Spectroscopy (LC-MS) for peak identity[7]. These techniques can be built concurrently to build a robust analytic technique for characterization and release testing of an ADC. A variety of immunological assays can also be employed to evaluate the ADC's capacity to engage the immune system to trigger effector functions. This approach, if incorporated early-on in drug development, can avoid surprises later[8].

Bio-analytical techniques used for assessment of potency are listed in the Table below.

Category	Binding assays	Cell-based assays
Type	Solid phase methods	Functional assays, for therapeutic action
Stage	IND /Phase I submission	Phase II / Phase III submissions

Techniques	• Direct /indirect ELISA
	• Competitive ELISA (AlphaLISA)
	• SPR/OCTET studies for biophysical binding between antibody and receptor.
		• Direct drug-induced cytotoxicity
		• Immunogenic cell death
		• Effector function:
		FcɣR- signaling by ADCC, ADCP, CDC assays, via
		  • Luminescence-based techniques
		  • Multicolor Flow cytometry-based cell killing assays

Table: Bio-Analytical techniques for potency or cytotoxicity assessment of ADCs

Capabilities at Crystal Bio

Crystal Bio is a leading Contract Research Organization (CRO) specializing in comprehensive analytical services for biotherapeutics. Our expertise covers a wide range of modalities, including Antibody Drug Conjugates (ADCs); mRNA-LNP therapeutics with our strategic partner CATUG; Monoclonal Antibodies; and Fusion Proteins- with a robust bio-analytical toolkit 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 accompanied by high-resolution LC-MS, SEC-MS, HIC, UV-VIS spectroscopy, RP-HPLC, IPRP-LC, LSD, LC-CAD, CE, cIEF, qPCR, ELISA, endotoxin, sterility, and bioburden 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.

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