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Review article

Catabolism of antibody drug conjugates and characterization methods

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ABSTRACT

Antibody drug conjugates (ADCs) are large molecule therapeutics in which a cytotoxic payload is conjugated to a monoclonal antibody (mAb) via a linker. The molecules are designed to selectively bind to target-expressing cells, thus delivering therapeutic agents directly to the tumor. Chemical and enzymatic stability prior to reaching the target is an important factor for ADCs since it impacts their safety, efficacy, and pharmacokinetics (PK). One of the main reasons for off-target effects of ADCs is premature release of cytotoxic agents, either in the blood stream or at non-specific sites. Once an ADC is internalized by target-expressing cells, the cytotoxic payload and/or related catabolites are released through chemical or enzymatic cleavage within the cells. In some cases, the released payload and/or catabolites are effluxed into the systemic circulation and follow a small molecule disposition path. Since doses of ADCs are low, the concentration of cytotoxic payload and related catabolites/metabolites range from ng to µg levels in systemic circulation or tumors in clinical studies. Hence, it is challenging to identify these species without prior knowledge of the pathways of catabolism. The current review summarizes the mechanism of cleavage/catabolism of various types of linkers and available in vitro, in vivo, and bioanalytical methods for evaluation of catabolism of ADCs.

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Abbreviations defined at first use in document

A_{Ab}	active antibody
ADC	antibody drug conjugate
Baf A1	bafilomycin A1
C_{Ab}	conjugated antibody
CES1c	carboxylesterase 1C
CLF	crude lysosomal fraction
DAR	drug to antibody ratio
DDI	drug-drug interaction
HL	Hodgkin lymphoma
LBA	ligand binding assay
LC–MS	liquid chromatography and mass spectrometry
LSC	liquid scintillation counter
mAb	monoclonal antibody
mc-MMAF	maleimide-caproyl-monomethyl auristatin F
MMAD	monomethyl auristatin D
MMAE	monomethyl auristatin E
PABC	*p*-aminobenzyl carbamate
PBD	pyrrolobenzodiazepine
PK	pharmacokinetic(s)
T_{Ab}	total antibody
U_{Ab}	unconjugated antibody
val-cit	valine-citrulline

Abbreviations not defined in document

ACN
ALPAPIE
DTT
ELISA
FDA
HPLC
PD
pKa
US

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1. Introduction

The achievement of balance between potency and safety for cancer drug development is still challenging. Recently, there has been increased interest in identifying and optimizing the delivery strategies of cytotoxic molecules to minimize systemic toxicity.[1] Antibody drug conjugation is a rapidly evolving area of drug discovery for target drug delivery of small molecules selectively to tumors.[2] ADCs are large molecular weight compounds where a small molecule is linked to a mAb via chemical linkers. The conjugation of small molecules with a mAb is typically achieved via the amino group of lysines or the thiol residue of cysteines on the carrier antibody molecule through a chemical linker (cleavable, non-cleavable, reducing, or acid labile).[3] The antibody selectively binds to an antigen expressed on the surface of the tumor cell and the ADC is internalized and the antibody component is degraded through endosomal and/or lysosomal proteases or pH-dependent cleavage, followed by release of small molecule drug. The selective delivery of the cytotoxic agent minimizes systemic exposure and hence, increases efficacy and decreases drug-associated toxicity.[4] In addition to target drug delivery, ADCs also possess improved PK properties, including low volume of distribution and prolonged elimination. Currently, two ADCs (Adcetris® and Kadcyla®) are marketed and many are at various stages of clinical development.[5] To date, ADCs are generally developed in oncology therapeutic areas since they provide significant advantage in enhancing potency and minimizing toxicity associated with cytotoxic agents.

The withdrawal of gemtuzumab ozogamicin (Mylotarg®), the first approved ADC, from the market due to safety concerns associated with premature release of cytotoxic payload highlights that additional understanding of payload release mechanisms for this class of compounds is needed.[6] Drug-linkers are important determinants of ADC stability and many pharmaceutical companies are focused on developing improved linker technology that can enhance stability. Improving stability of the conjugated linker and payload in systemic circulation is a crucial and effective way of maximizing tumor exposure while minimizing release to non-target sites, thus improving the therapeutic index. The linkers are classified as cleavable and non-cleavable.[7] The cleavable linkers include peptide linkers (cleaved by proteolytic enzymes), hydrazones (cleaved at low pH), and hindered disulfides (reduced in the cell). ADCs with non-cleavable linkers are hydrolyzed at the site of conjugation leaving the linker-payload with an amino acid (s) residue where conjugation occurred.[7] Understanding the mechanism of payload release and other catabolic pathways is necessary to develop efficacious and safe ADCs. Since ADC discovery and development is an evolving area of research, there are only few robust methods available to study stability, the payload release mechanism, and catabolism in vitro and in vivo. This article provides an overview of linker cleavage pathways, in vitro and in vivo methods for assessment of release of payload-related species, and current bioanalytical methods utilized to determine small molecule-related catabolism of ADCs.

2. Catabolism/biotransformation of ADCs

ADCs are designed to be stable in circulation and retain specificity and affinity of the unconjugated antibody (U_{Ab}). In practice, ADCs tend to have a shorter half-life than the non-conjugated antibody, and often show instability in circulation.[8] The choice of linker used to conjugate the toxicophore and mAb can impact both linker stability and the payload release mechanism. Ultimately, the proper selection of antibody, linker, and payload will impact the ADC efficacy, safety, therapeutic index, exposure, and mechanism of catabolism. Upon internalization into cancer cells, the ADC may be subjected to hydrolysis due to the low pH environment, amide or ester cleavage by proteases, or disulfide reduction to release the payload.[9] The lysosomal proteolytic enzyme cathepsin B has been reported to be involved in payload release of several enzymatically cleavable ADCs. The low pH of the lysosome, at pH 4.6–5.0, can result in payload release via hydrolysis for certain linkers (e.g., hydrazone linkers) (Fig. 1). Non-cleavable ADC linkers are intended to exhibit improved stability in circulation but degrade upon internalization to the target cell. The metabolic products of non-cleavable linkers, such as those containing maleimide, are often payload-linkers attached to an amino acid, cysteine or lysine.[10] Accurate quantification of released products is a critical

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