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Minireview

Physical and Chemical Stability of Antibody Drug Conjugates: Current Status

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ABSTRACT

Antibody drug conjugates (ADCs) are an emerging class of chemotherapeutic cancer treatment agents that combine the targeting specificity of antibodies with the efficient cell-killing potential of cytotoxic drugs. Unlike their protein and small-molecule therapeutic counterparts, the stability and degradation properties of ADCs are relatively unknown. Theoretically, ADC stability could be governed by properties and processes stemming from both the antibody and the linker-toxin chemistry. Recently, systematic studies of intrinsic ADC molecule stability have been presented in the primary literature. As there are burgeoning industrial and academic efforts aimed at developing optimized conjugation chemistries and antibody engineering approaches for next-generation ADCs, it is important to capture the current state of understanding of ADC stability. In this minireview, we discuss aspects of physical and chemical stability of ADCs gathered from a survey of the literature and illustrate how investigations into stability enable the development of more effective ADC molecules for the future.

Introduction

Antibody drug conjugates (ADCs) represent a new class of therapeutics in oncology. These hybrid molecules combine the site specificity of a monoclonal antibody (mAb) with the potent tumorigenic effects of cytotoxic compounds. Originally foreshadowed as Schofields magic bullets,[1] this vision is becoming a reality as 2 ADC products, Kadcyla and Adcetris, have recently received FDA approval, with over 38 different ADC molecules in clinical trials for solid and hematological malignancies as highlighted in a 2014 estimate.[2] Kadcyla consists of the monoclonal antibody Herceptin conjugated through Lysine side chains to the cell division maytansinoid inhibitor DM1. Lysine side chains are first converted to an activated form using the succinimidyl 4-(N-maleimidomethyl) cyclohexane-1-carboxylate (SMCC) linker. A free thiol installed on the terminus of the DM1 molecule then allows coupling to the maleimide-functionalized antibody. Adcetris comprises the human-mouse chimeric antibody cAC10 conjugated through cysteine residues to the tubulin polymerization inhibitor monomethyl auristatin E (MMAE). Partial disulfide reduction of the mAb is first carried out to provide a limited number of free thiols for subsequent conjugation. The ADC molecule offers a potentially more efficacious form of the mAb alone, while capitalizing on the tumor-killing activity of highly potent cytotoxic agents. Systemic administration of these potent anticancer drugs poses severe toxicity issues; therefore, the ADC platform provides a targeted delivery system to expand the therapeutic potential of these agents that may otherwise not be available to patients.

Although conceptually logical, ADCs are substantially more complex than either the mAb or small-molecule constituents because of the combined linker and toxin chemistry (linker payload) and the resultant heterogeneous distribution of modification sites. Characterization of mAbs themselves is not straightforward as there are a number of critical quality attributes (CQAs) that must be monitored to ensure final product acceptance. Included with this, characterization is a thorough understanding of the long-term stability of the drug molecule and drug product. Fortunately, the physical and chemical stability of therapeutic mAbs has been extensively studied, and degradation pathways are consistent with those generally observed for other proteins.[3-7] Characterization and stability studies are conducted throughout development of

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monoclonal antibodies to understand physicochemical liabilities and establish control strategies for ensuring appropriate product quality.

The degradation processes encountered by mAbs are directly relevant to ADCs, but additionally, the altered chemical and physical nature of the ADC compared to its mAb precursor introduces additional stability considerations that must be addressed during ADC drug development and manufacturing. Physical and chemical ADC, linker payload, and mAb instability could potentially impact the toxicity, immunogenicity, and efficacy of the molecule. Preparation of an ADC molecule involves a series of activation, conjugation, buffer exchange, or other handling steps to yield the ADC molecule from mAb and small-molecule starting materials. Although the impacts of some of these steps on stability of the finished ADC material are only beginning to emerge in the primary literature, the dense pipeline of next-generation ADCs in the development and clinical pipeline clearly warrants an assessment of the current state of information regarding ADC stability. This article provides a review of the current state of understanding in the primary literature pertaining to unique aspects of physical and chemical stability of ADCs and the measurement methods used. Regarding physical stability, the lessons learned can be applied across ADC formats with differing conjugation approaches. Chemical ADC stability investigations on the other hand have focused primarily on cysteine-based or maleimide-thiol conjugation, as specific degradation or reversibility pathways have been examined in detail. These studies have achieved the desired purpose of providing more stable and homogeneous ADC molecules. Conjugation approaches based on cysteines or engineered amino acids rather than lysine are also predominant in ADC stability investigations, as these technologies yield ADC molecules with lower drug loading and narrower distribution, attributes that have been established to provide a benefit to the therapeutic window.[8]

Physical Stability of Antibody Drug Conjugates

Instability Relating to Conjugation Processes

Study of the physical stability and degradation of ADCs primarily addresses mAb aggregation, and the increased propensity for aggregation brought about by processing steps or the physical impacts of the linker and drug modifications. Aggregation of therapeutic mAbs themselves has been extensively studied [7] and provides some of the necessary theoretical and conceptual framework for extension to ADCs. The impact of thiol modification or partial reduction in IgG1 antibodies on quaternary structure and binding properties has been established before the industrial pursuit of ADCs.[9] The first approved ADC, Mylotarg, consists of the calicheamicin derivative ozogamicin, conjugated to the anti-CD33 antibody gemtuzumab. This ADC is representative of the difficulties in linking hydrophobic small molecules to large hydrophilic proteins.[10] For Mylotarg, conjugation requires up to 20% dimethylformamide (DMF) to maintain solubility of the hydrophobic ozogamicin linker payload. The presence of DMF was demonstrated[10] to promote excessive (up to ca. 50%) aggregate formation as measured by size-exclusion chromatography (SEC). Conjugation additives such as glycerol, propylene glycol, and octanoic acid were shown to attenuate the aggregation problem and permit lower DMF concentrations during conjugation.

Focused physical stability assessment of ADCs was perhaps first described in comparisons of Trastuzumab (Tmab), lysine-activated Tmab T-MCC (activated using SMCC), and the fully conjugated ADC molecule T-DM1.[11] In a series of extended stressed-stability experiments analyzed by differential scanning calorimetry (DSC), SEC, liquid chromatography–mass spectrometry (LC-MS), and electrophoresis, it was clearly determined that T-MCC and T-DM1 were more prone to aggregation than Tmab, with the order of aggregation tendency being T-MCC>T-DM1>Tmab. In DSC experiments, reversible thermal transitions were observed for T-MCC, T-DM1, and Tmab; however, T-MCC also demonstrated a nonreversible component indicative of aggregation. The aggregation trend was also demonstrated in SEC analysis following 7-day storage at 40°C, where up to 32% abundance of high molecular weight species (HMWS) were observed in T-MCC. Covalent aggregation as a major contributor to these HMWS in T-MCC was confirmed by mass spectrometry and electrophoresis. Suppression of covalent aggregation was observed in the presence of added free amino acids (Cys, Tyr, Ser), lending support to the hypothesis that free exposed maleimido groups in T-MCC enable cross-linking. These findings illustrate the importance of controlling side reactions while working with maleimides or other active chemistries.

Thermal stability using DSC was studied by Acchione et al. [12] for a series of lysine-linked, thiol-linked, and carbohydrate-linked model IgG1-biotin conjugates. A primary observation from this work was that thiol coupling had a somewhat greater destabilizing effect on the antibody than lysine coupling. It was also observed that partial antibody reduction using TCEP had very minimal effect on overall thermal stability, suggesting that the observed loss of stability is not directly due to partial mAb reduction.

Temperature-Induced or Photo-Induced Instability

The typical stability testing strategy used across small-molecule and biomolecule therapeutics is to use elevated temperature exposure to accelerate the appearance of potential instability issues. Adem et al.[13] used temperature and ionic strength as stress conditions to examine the effects of increasing drug load of thiol-linked MMAE with up to 8 conjugated drugs per antibody. Antibody conjugates with different drug-to-antibody ratios (DARs) were isolated by preparative hydrophobic interaction liquid chromatography (HIC), allowing examination of effects relating to progressively increasing hydrophobicity of the MMAE. The formation of HMWS increased for higher DAR components, with the effect more pronounced in higher ionic strength conditions. The connection between increased hydrophobicity with higher DAR and lowered *in vivo* performance has also been established. Structural destabilization of higher DAR species presumably contributes to accelerated *in vivo* clearance. A recent examination of the impact of hydrophobicity by Lyon et al.[14] demonstrated that more hydrophilic auristatin ADCs obtained through hydrophilic linker design or attachment of polyethylene glycol (PEG) chains (“PEGylation”) could improve efficacy.

Temperature-induced aggregation as a function of increasing DAR was also examined by Beckley et al.[15] using a number of spectroscopic and separation tools. Cysteine-conjugated ADCs with varying DAR values were incubated at 40°C after which individual SEC fractions were collected and analyzed by DSC, far-UV circular dichroism spectroscopy, electrophoresis, and reverse-phase high-performance liquid chromatography (RP-HPLC). Taken together, it was shown that DAR 6 and DAR 8 species were far more prone to formation of aggregates under stressed conditions and that the HMWS observed in SEC analysis of stressed samples consists predominantly of DAR 6 and DAR 8 species. More specifically, DSC evidence suggests that partial unfolding of the C_H2/hinge region caused by conjugation is responsible for aggregate formation.

The most recent examination of physical instability by Guo et al.[16] features higher order structural analysis using advanced spectroscopic tools combined with calorimetry and molecular modeling. With these techniques, the irreversible destabilization of