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Bioanalysis of antibody–drug conjugates

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Keywords: antibody–drug conjugate • bioanalytical strategy • drug–antibody ratio

Antibody–drug conjugate (ADC) biotherapeutic modality is a quickly advancing class of compounds generally developed for the treatment of various oncological conditions. ADCs are developed with the idea to combine high specificity of a cell surface targeting antibody component with the high cytotoxicity of a small molecule drug. Although only two therapeutics are currently approved in the USA (brentuximab vedotin/Adcetris, Seattle Genetics/Millennium Pharmaceuticals and adotrastuzumab emtansine/Kadcyla/T-DM1, Roche/Genentech), a significant and growing pipeline of ADCs exists across the industry. Small and large pharma as well as various biotech companies are actively working on identification of new cell surface targets, developing new highly potent small molecular weight toxins and ‘smart’ cleavable and noncleavable linkers. Optimization of the ADC stability *in vivo* while aiming for a cell specific intracellular release of the cytotoxic agent continues to be the main focus of research. Although ADCs are highly diverse in the nature of components used, they frequently share several common characteristics, including various degree of heterogeneity of the therapeutic substance and continuous dynamic change of the composition of ADC while in circulation. The location of the conjugation site and the number of small molecule drug moieties attached to the antibody are often the two major sources of ADC heterogeneity. The *in vivo* changes take place mainly due to spontaneous deconjugation of the small molecule drug and/or differential clearance rates of ADC components with different drug–antibody ratio (DAR) value.

These unique features of ADCs lead to particular and distinct bioanalytical challenges. Complexity of the ADC composition requires use of both ligand-binding assay (LBA) and LC–MS platforms. In addition, hybrid LC–MS platforms have been actively developed and applied in support of ADC bioanalysis [1]. A number of analytes have been applied to describe ADC exposure in nonclinical and clinical investigations. These generally include total antibody, conjugated antibody, antibody conjugated drug and unconjugated drug [2]. *Bioanalysis* has previously published a dedicated special issue focusing on ADCs and presenting original research, review papers and a position paper on bioanalysis of ADCs submitted by a working group from the American Association of Pharmaceutical Scientists [3]. As the investigations continue to advance, we are learning that the very nature of the data requested from a bioanalytical scientist differ depending on the phase of compound development. A stage specific BA support is now recognized as an essential element of bioanalytical strategy of ADCs. Early discovery phase warrants an investigation of *in vivo* stability of an ADC candidate by following average DAR value or presence and integrity of the drug moiety at a specific site on the antibody component. Understanding of metabolic profile of the small molecule drug component, whether released from the antibody moiety or still attached, is critical to enable selection of most appropriate ADC candidate. Other development phase specific analytes may include intracellular levels of the small molecule drug, conjugated antibody and antibody conjugated drug concen-

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trations in targeted tissue. During clinical development phase, it is critical to establish a correlative relationship between one or several components of the ADC analyte panel and various safety and efficacy signals.

The current issue of *Bioanalysis* presents the next installment in review of current data and opinions on BA of ADCs. In this issue the reader will find discussions of analytical approaches and platforms applied for ADC bioanalysis, immunogenicity potential of ADCs, ADC-related PK–PD modeling, literature reviews related to other topics of Bioanalysis of ADCs. *In vivo* stability and various forms of metabolic and catabolic processing of an ADC are recognized as important influencing factors for the therapeutic PK, efficacy and safety outcome. A detailed review of the bioanalytical approaches focusing on characterization of catabolism of ADCs is presented on example of Kadcyla® 33 (T-DM1) and a THIOMAB™ antibody drug conjugate [4]. Challenges in ADC discovery from a perspective of drug design and analytical characterization are presented Rao *et al.* [5]. In a related review manuscript various protocols applied for analysis of the *in vivo* biotransformation of ADCs are presented and substantiated by a survey of various types of biotransformation events reported in the literature [6]. Kumar *et al.* present a stage relevant approach to LBA support for ADC bioanalysis specifically focusing on the impact of the DAR parameter on LBA-based measurement of various ADC analytes [7]. As any other member of the biotherapeutic modality, ADCs present a risk of immune response. Molecular structure factors specific to ADCs that are capable of influencing the outcome of immune response induction are pre-

sented [8]. As a directly related topic, publication by Jani *et al.* [9] presents recommendations for development and validation of confirmatory antidrug antibody testing, a critical component of a tiered-based approach during immunogenicity evaluation for a biotherapeutic compound. Various bioanalytical protocols commonly applied for evaluation of ADC exposure allow for precise quantitation of various relevant analytes and moieties. A case study of an ADC with a microtubule polymerization inhibitor payload and cathepsin B cleavable linker mode of action is presented [10]. A review of application of PK–PD modeling and simulation for quantitative integration of the bioanalytical data is presented [11].

We continue to appreciate that due to a complexity of ADCs, it is highly challenging to recommend a single universal analyte that will allow for a robust exposure–response correlation. Each unique ADC structure may require a specific combination of analytes and a fit for purpose approach in navigating complexities of ADC exposure evaluation. Advanced requirements for bioanalysis of ADCs have challenged existing analytical platforms and will continue to lead us to new and exciting technologies and ideas.

Financial & competing interests disclosure

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