Regulatory Toxicology and Pharmacology 67 (2013) 382–391

Contents lists available at ScienceDirect

Regulatory Toxicology and Pharmacology

journal homepage: www.elsevier.com/locate/yrtph

Considerations for the nonclinical safety evaluation of antibody drug conjugates for oncology

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ARTICLE INFO

Article history:
Received 2 April 2013
Available online 5 September 2013

Keywords:
Biotherapeutics
Payload
Antibody drug conjugates
Cytotoxin
Linker
Safety assessment
Regulatory toxicology

ABSTRACT

Antibody drug conjugates (ADCs) include monoclonal antibodies that are linked to cytotoxic small molecules. A number of these agents are currently being developed as anti-cancer agents designed to improve the therapeutic index of the cytotoxin (i.e., cytotoxic small molecule or cytotoxic agent) by specifically delivering it to tumor cells. This paper presents primary considerations for the nonclinical safety evaluation of ADCs and includes strategies for the evaluation of the entire ADC or the various individual components (i.e., antibody, linker or the cytotoxin). Considerations are presented on how to design a nonclinical safety assessment program to identify the on- and off-target toxicities to enable first-in-human (FIH) studies. Specific discussions are also included that provide details as to the need and how to conduct the studies for evaluating ADCs in genetic toxicology, tissue cross-reactivity, safety pharmacology, carcinogenicity, developmental and reproductive toxicology, biotransformation, toxicokinetic monitoring, bioanalytical assays, immunogenicity testing, test article stability and the selection of the FIH dose. Given the complexity of these molecules and our evolving understanding of their properties, there is no single all-encompassing nonclinical strategy. Instead, each ADC should be evaluated on a case-by-case scientifically-based approach that is consistent with ICH and animal research guidelines.

1. Introduction

The number and types of biotherapeutics under development for the treatment of human diseases have greatly expanded over the past several decades. This diversity has been driven by scientific advancements as well as the need for innovative new treatments. One particularly exciting approach for oncology indications involves the use of antibodies to provide preferential cell targeting capabilities for the delivery of a cytotoxin to cancer cells. The objective underlying the development of these macromolecules, which have been termed antibody drug conjugates (ADCs) or immunoconjugates, is to decrease the off-target toxicities and, thereby, improve the therapeutic index of the toxin (Wu and Senter, 2005; Schrama et al., 2006; Ricart and Tolcher, 2007). A wide variety of macromolecules have been conjugated to naturally existing toxins, radioisotopes and small molecule cytotoxic

Abbreviations: ADC, antibody-drug conjugate; ADME, absorption, distribution, metabolism and elimination; EFD, embryofetal development; ELISA, enzyme-linked immunosorbent assay; FIH, first-in-human; GLP, Good Laboratory Practice; hERG, human ether-à-go-go related gene; HNSTD, highest non-severely toxic dose in non-rodent species; ICH, International Conference on Harmonisation; LBA, ligand binding assay; LC/MS/MS, liquid chromatography–tandem mass spectrometry; MS, mass spectrometry; MTD, maximum tolerated dose; PD, pharmacodynamics; PK, pharmacokinetics; STD_{10}, severely toxic dose in 10% of rodents; TCR, tissue cross-reactivity; TK, toxicokinetics; V_d, volume of distribution.

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http://dx.doi.org/10.1016/j.yrtph.2013.08.017

drugs. The delivery scaffolds of these macromolecules have ranged from targeted fusion proteins to monoclonal antibodies, each with the goal of conferring *in vivo* dispositional (i.e., absorption, distribution, metabolism, elimination (ADME)/pharmacokinetics [PK]) specificity to favorably improve the tolerability and/or efficacy of conjugates. The focus of this paper is on the compounds that use a monoclonal antibody platform to which a cytotoxic drug has been linked although the principles may apply to the development of a wider spectrum of conjugate drugs.

The theoretical mechanistic basis for the ADC approach includes its binding to the cellular target, which triggers internalization followed by the intracellular release of the cytotoxin (Fig. 1). While this approach may seem straight forward, the translation to clinical practice has proven to be more difficult. Initial attempts failed due to inappropriate linker systems or insufficiently potent cytotoxins, resulting in unfavorable therapeutic indices. Although some ADCs in development use standard chemotherapeutic agents, many current efforts are focused on the use of highly potent cytotoxic molecules, such as derivatives of calicheamicin, maytansine and auristatins (Senter, 2009; Ducry and Stump, 2010). A number of these molecules have demonstrated substantial *in vivo* antitumor activity; some selected examples of ADCs currently undergoing clinical development are presented in Table 1. Linker systems such as hindered disulfides, peptides and noncleavable thioethers have been designed to maximize the stability of the ADC in circulation while still providing a preferential release of the drug intracellularly (Table 1) (Wu and Senter, 2005; Ricart and Tolcher, 2007; Schrama et al., 2006). To translate the fundamental advantage of ADCs of targeting tumor-selective or tumor-specific antigens in clinical practice, it is essential that linkers be of sufficient stability to minimize systemic exposure to the cytotoxin and the attendant toxicities while still providing the intracellular release of the cytotoxic agent. The selection of the most appropriate linker should include the assessment of various dispositional (i.e., target cell delivery) and toxicologic characteristics during the discovery research phase. Optimizing the stability of the cytotoxin, linker and antibody with the resultant effects on toxicity and efficacy remain active areas of research as these characteristics may be disease, target, and/or cytotoxin-dependent.

Although ADCs are diverse in their structures, targets, mechanisms of action and toxicities, there is a need to provide a framework for the key principles in building a practical, scientifically-based strategy to guide evaluation of their nonclinical safety. After the failures with the first generation of ADCs, efforts were focused on engineering antibodies and linkers of ADCs that would improve PK and/or targeted disposition characteristics to improve the therapeutic index. The first of these new ADCs (i.e., brentuximab vedotin) received marketing approval in the United States in 2011 and the European Union in 2012 for the treatment of relapsed/refractory Hodgkin lymphoma and systemic anaplastic large cell lymphoma. In 2013, ado-trastuzumab emtansine was approved in the United States for treatment of HER2-positive (HER2+) metastatic breast cancer patients who previously received trastuzumab and/or a taxane and should have received prior therapy for metastatic disease or developed disease recurrence during or within six months of completing adjuvant therapy.

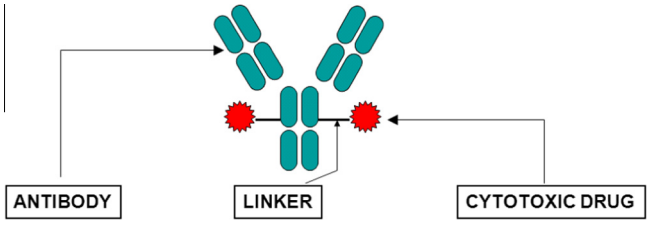

Fig. 1. Structure of a typical antibody-drug conjugate. Small molecule cytotoxic drugs are conjugated to antibodies by a linker molecule to create ADCs (antibody drug conjugate).

The chemical complexity of ADCs raise unique issues related to the development plan which includes details involved in the planning and conduct of the various nonclinical studies. Some of the important choices for toxicology studies include the selection of the types of studies to be conducted, the choice of animal species, evaluation of immunogenicity, analyte choices for bioanalysis plus the pharmacokinetics/toxicokinetics (PK/TK) and ADME characteristics that need to be defined. The primary objective of this publication is to provide guidance on important issues to consider when designing the nonclinical safety assessment programs for oncology-based ADCs.

2. Challenges and considerations for the design of nonclinical toxicologic program for ADCs

2.1. Selection of toxicologically-relevant animal models

The principles for the selection of animal models for the ADC safety assessment are fundamentally similar to those for other biotechnology-derived products. Since the ultimate goal of nonclinical toxicology studies is to predict potential safety risks in humans, animal species are selected with the expectation that they will respond to the ADC in a pharmacologically similar manner as humans. As ADCs consist of a monoclonal antibody, linker and cytotoxic components, the biological activity profiles of each should be considered when selecting the relevant and/or appropriate species (Fig. 2). For biotherapeutics, the International Conference on Harmonisation (ICH) S6(R1) (2012) Guideline, *Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals* (2012) defines an appropriate species as one in which the drug candidate is pharmacologically active due to the expression of a receptor or an epitope. This guidance indicates that safety should typically be evaluated in two relevant species (one rodent and one non-rodent). This requirement may be altered to only one relevant species if a second cannot be identified or if the biological activities are well understood. The use of a single relevant rodent species is also supported by the ICH S9 (2010) guideline which states that this alternative approach may be sufficient for conducting repeat-dose toxicology studies with genotoxic drugs that target rapidly dividing cells. The ICH S6(R1) (2012) guideline also supports the possible use of one species for subsequent repeat-dose toxicity studies provided the toxicity profile of the two species is comparable in the short-term studies.

The relevance of a particular animal species should be established by demonstrating that the affinity, potency and/or intended pharmacologic function of the ADC are comparable for the animal and human target. If the similarities are equivocal regarding binding, activity or the homology of the physiologic pathway, then all of the data should be collectively weighed to best ascertain the most appropriate and relevant animal species. If the ADC is internalized for activity, then homology of the physiologic pathway may be of limited importance. The determination of the relevance of an animal species can be complicated by differences in affinity and/or activity including differences in on/off rates of binding to the antigen (Fujimori et al., 1989; Adams et al., 2001). As with other biological products, a standard two mammalian (i.e., rodent and non-rodent) species paradigm for safety assessment may be complicated by species differences in target tissue binding and/or biological activity (ICH S6(R1), 2012). The ability to identify two species that are pharmacologically and toxicologically relevant may not always be possible, as many monoclonal antibodies will only bind to the target antigen in non-human primates. In these