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Current methods for the synthesis of homogeneous antibody–drug conjugates

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ABSTRACT

Development of efficient and safe cancer therapy is one of the major challenges of the modern medicine. Over the last few years antibody–drug conjugates (ADCs) have become a powerful tool in cancer treatment with two of them, Adcetris® (brentuximab vedotin) and Kadcyla® (ado-trastuzumab emtansine), having recently been approved by the Food and Drug Administration (FDA). Essentially, an ADC is a bioconjugate that comprises a monoclonal antibody that specifically binds tumor surface antigen and a highly potent drug, which is attached to the antibody *via* either cleavable or stable linker. This approach ensures specificity and efficacy in fighting cancer cells, while healthy tissues remain largely unaffected.

Conventional ADCs, that employ cysteine or lysine residues as conjugation sites, are highly heterogeneous. This means that the species contain various populations of the ADCs with different drug-to-antibody ratios (DARs) and different drug load distributions. DAR and drug-load distribution are essential parameters of ADCs as they determine their stability and efficacy. Therefore, various drug-loaded forms of ADCs (usually from zero to eight conjugated molecules per antibody) may have distinct pharmacokinetics (PK) *in vivo* and may differ in clinical performance. Recently, a significant progress has been made in the field of site-specific conjugation which resulted in a number of strategies for synthesis of the homogeneous ADCs. This review describes newly-developed methods that ensure homogeneity of the ADCs including use of engineered reactive cysteine residues (THIOMAB), unnatural amino acids, aldehyde tags, enzymatic transglutaminase- and glycotransferase-based approaches and novel chemical methods. Furthermore, we briefly discuss the limitation of these methods emphasizing the need for further improvement in the ADC design and development.

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Contents

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1. Introduction

The idea behind targeted anticancer therapies originates from the 'magic bullet concept' which was introduced at the beginning of the 20th century by Paul Ehrlich, the father of modern immunology and chemotherapy. Ehrlich proposed that in order to reduce adverse effects of toxic molecules on healthy tissues drugs should be selectively delivered to disease-causing cells (Strebhardt and Ullrich, 2008). Realization of Ehrlich's vision became possible when production of monoclonal antibodies, that provide excellent specificity and high affinity of binding to antigens, was developed in the mid-70s (Kohler and Milstein, 1975). Monoclonal antibodies against tumor specific antigens can be labeled either with a particle emitting radioisotope (radioimmunotherapy, RIT) or with a highly potent drug resulting in antibody–drug conjugates (ADCs). Both strategies allow one to specifically destroy cancer cells. Nowadays, two radio-immunoconjugates, ^{131}I-tositumab (Bexxar®, GlaxoSmithKline) and ^{90}Y-ibritumomab tiuxetan (Zevalin®, Bayer Schering Pharma AG/Spectrum Pharmaceuticals) are approved for treatment of non-Hodgkin's lymphoma (Bodet-Milin, 2013; Chamarthy et al., 2011). Currently,^{177}Lu and ^{211}At radio-immunoconjugates targeting colon cancer are intensively investigated (Eriksson et al., 2012, 2014). Conjugation of cytotoxic payloads to monoclonal antibodies, that bind tumor cell surface antigens, enables to target and deliver drugs to cancer cells leaving normal cells largely unaffected. Importantly, this approach takes advantage of highly potent cytotoxic molecules that would be too toxic for use in conventional chemotherapy. Therefore, ADCs constitute a precise and powerful tool in fighting cancer. The research in the ADC field has been extremely intense in the past 10 years. This resulted in the approval of two ADC therapeutics, brentuximab vedotin (Adcetris®, Seattle Genetics) and ado-trastuzumab emtansine (Kadcyla®, Genentech) by the Food and Drug Administration (FDA) in 2011 and 2013, respectively. Furthermore, approximately 40 ADCs are currently undergoing clinical trials. Despite the tremendous progress in ADC technology, further improvement is necessary to ensure safety and efficacy of ADC-based products. One of the main challenges in ADC design is homogeneity of ADC molecules. Currently available ADCs are heterogeneous as they have zero to eight drug molecules per antibody. It has been reported that heterogeneity of ADC species can influence its pharmacokinetics (PK) and *in vivo* performance (Hamblett et al., 2004; Jackson et al., 2014; Junutula et al., 2008a; Strop et al., 2013). Therefore, biotechnology companies and academic units are intensely focused on establishing novel reliable methods for site-specific conjugation of cytotoxic agents to monoclonal antibodies (Table 1). The outcome of their effort has recently been summarized in a few excellent reviews. Agarwal and Bertozzi (2015) and Cal et al. (2014) in their articles discuss details of chemical aspects of site-specific conjugation methods. Behrens and Liu (2014) and Panowksi et al. (2014) give a general overview on well-defined ADC design and production. In our review we describe novel approaches towards homogeneous ADC, including those that are not discussed in above-mentioned reviews.

2. Conventional conjugation methods and their limitations

Essentially, an ADC contains three main components: a monoclonal antibody, a cytotoxic agent and a synthetic linker that is required to attach the drug to the antibody. Conventional conjugation methods employ surface-exposed lysine or interchain cysteine residues as attachment sites for linker-drug molecules. A human IgG comprises about 100 lysine residues. Mass spectrometry analysis of the huN901-DM1 antibody–drug conjugate revealed that potentially 40 of them can be modified with the DM1 cytotoxic drug (Wang et al., 2005). Lysine conjugation results in zero to eight drug molecules per antibody. This implicates that a tremendous number of over one million different ADC species can be generated using this unspecific approach (Wang et al., 2005).

Cysteine conjugation occurs after reduction of four interchain disulfide bonds, which leads to eight thiol groups that are available for linker-drug molecules. In this strategy, drugs are coupled to even number of cysteines (2, 4, 6 or 8) (Hamblett et al., 2004; Sun et al., 2005; Willner et al., 1993).

Table 1
Current site-specific conjugation methods.

Company/institution	Conjugation strategy	Antibody engineering	Chemistry (non-enzymatic reactions)	DAR
Genentech Seattle Genetics	Conventional lysine and cysteine conjugation Lewis Phillips et al. (2008) and Senter and Sievers (2012)	Not required	Thiol–melimide Primary amine-NHS-ester (coupling linker-drug to a native antibody)	3–4
Sutro Biopharma Ambrx	Incorporation of unnatural amino acids into antibodies Axup et al., 2012 and Zimmerman et al., 2014	Required	Click chemistry oxime ligation (coupling linker-drug to an incorporated unnatural amino acid)	2
National Cancer Institute	Incorporation of selenocysteine into antibodies Hofer et al. (2009)	Required	Selenol-maleimide Selenol-iodoacetamide (coupling linker-drug to an incorporated selenocysteine)	2
Rinat-Pfizer	*Streptoverticillium mobaraense* transglutaminase (mTG) Specifically recognizes and modifies genetically introduced glutamine tag (LLQGA) with a primary amine-containing linker-drug module Strop et al. (2013)	Required	–	1.8–2
Sanofi-Genzyme	Glycoengineering Site-specific introduction of sialic acid with the use of galactosyl- and sialytransferases Zhou et al. (2014a)	Not required	Oxime ligation (coupling linker-drug to a modified Fc glycans)	~1.6
Innate Pharma	Microbial transglutaminase (MTGase) Enzymatic conjugation of a primary amine-containing linker/linker-drug module to glutamine specifically recognized by MTGase Dennler et al. (2014)	Required	Thiol–maleimide Click chemistry (coupling drug to linker-antibody)	2
Redwood Bioscience	Formylglycine generating enzyme (FGE) Converts cysteine located in the CXPXR consensus sequence to formylglycine (FGly) Drake et al. (2014)	Required	Hydrazino-*iso*-Pictet-Spengler ligation (coupling linker-drug to FGly)	2
UCL Cancer Institute	Next generation maleimides (NGMs) Rebridge reduced interchain disulfide bonds of a native antibody Schumacher et al. (2014)	Not required	Reaction between thiols and leaving groups of the NGM linker-drug (coupling linker-drug to a native antibody)	1 2 3 4
PolyTherics	Bis-alkylating reagents Rebridge reduced interchain disulfide bonds of a native antibody Badescu et al. (2014)	Not required	Micheal addition and elimination reactions (coupling linker-drug to a native antibody)	2 4

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