ANTIBODY – DRUG CONJUGATES: CATALYSTS FOR CHEMISTRY

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Introduction
Antibody-drug conjugates (ADCs), also known as armed antibodies, are highly anticipated to be the source of next generation frontline oncolytic therapy. They marry the selective targeting properties of antibodies with the potency of cytotoxic small molecules. The antibody component targets and adheres to a selected antigenic cell-surface receptor, ideally only expressed on the target cancer cell. Once bound, the ADC is internalized through endocytosis, and the cytotoxic payload is then released in the lysosomal cellular compartment providing precise, selective delivery to cancerous cells. With the development and subsequent marketing approval of Mylotarg® (2000), Adcetris® (2011) and Kadcyla® (2013), there has been exponential growth in ADC research and development (Figure 1). Estimates place the global ADC market at $10 billion annually after 2024 with seven to 10 new commercial ADC launches projected in the next decade. No doubt, this explosive track will create opportunities for CROs and CMOs with the expertise necessary to work with these entities. The development of ADCs brings many challenges, however. Multiple disciplines across drug development must engage to successfully discover, develop, evaluate and eventually manufacture a therapeutically relevant ADC.

To illustrate, large macromolecular ADCs have a complex architecture whose assembly, manufacture and analysis presents challenges for organizations without significant experience in biological conjugation, optimization and the development of the important chemical linkers that are critical for effectively tethering the small molecule payload to the antibody. As such, ADCs are good candidates for outsourcing to experienced CROs, particularly the technically demanding processes of preparing the linker payload and developing an optimized conjugation process.

AMRI offers expertise for ADC discovery and development, particularly through its NY Integrated Discovery Hub. AMRI has a broad library of natural products, many of which are potent and biologically active and have great potential as ADC payloads. AMRI also offers expertise in antibody suitability testing and prioritization, general process research on ADCs, MS analysis and characterization of ADCs, the production of pyrogen-free, sterile ADC for in vivo, preclinical studies as well as pharmacokinetic and pharmacodynamic analysis.

AMRI’s expertise is not limited to discovery but also spans development. The company has conducted numerous projects involving the synthesis of linker-warhead conjugates under cGMP guidelines for the eventual manufacture of ADCs for clinical trials. Such expertise in both discovery and development is rare in the CRO industry. Notably, AMRI partnered with Seattle Genetics for the cGMP manufacturing of the proprietary drug linker section of Adcetris®. In this article, we discuss the complexities of developing linkers – chemical moieties which attach a drug payload to an antibody.
ADC Anatomy

Structurally, ADCs consist of an antibody, typically a humanized monoclonal (mAb) of the IgG class, a chemical linker of variable composition and a terminal payload, most frequently a cytotoxin (Figure 2). The payload and linker are miniscule in size compared to the 150 kDa mAb. Multiple linker-payload units are affixed to an antibody, typically up to eight. The ratio of linked drug to antibody (drug antibody ratio or DAR) is a critical factor to consider when designing an ADC. Early ADC research found that high DAR was associated with increased clearance and the potential for aggregation. Equally, increased toxicity may be a likely outcome of high DAR ADCs. Antibodies with sitespecific conjugation chemistries are being developed to carefully control DAR and improve ADC homogeneity. Modification strategies include introduction of cysteine residues (example HC-A114C, Genentech7), glutamine residues8, peptide tags9, unnatural amino acids10 and chemoenzymatic functionalization at the conserved heavy chain glycosylation sites at Asn29711.

The linker moiety is deceptively simple in concept, normally represented in graphics as a mere bridge connecting the payload to the antibody. In practice, the linker is one of the most important factors in the overall performance of the ADC. The linker-payload requires highly skilled scientists to design and construct, as multiple complex chemistry reactions are required to attach the payload to the linker and then the linker-payload to the antibody.

The linkers themselves are demanding to design and develop. Several classes of linkers are cleaved to release the active form of the payload once the ADC is inside the cell. In contrast to this deliberate intracellular frailty, high plasma stability in a linker is essential to avoid premature release of the payload and indiscriminate cell killing. Notably, antibodies can circulate in the bloodstream for several days. Therefore, linkers need to match this level of plasma stability. Currently, the range of payloads for ADCs is quite limited with tubulin inhibitors auristatins (MMAE and MMAF) and maytansinoids (DM1 and DM4) comprising 80% of the payloads in current clinical development.12 The paucity of diversity in both mechanism and payload identity is an opportunity for the future development of ADCs, but until new payloads are developed, varying linker chemistry can modulate the physicochemical properties of the ADC. Natural product samples have been a rich source of highly potent, cytotoxic compounds including antibiotics (e.g. penicillins) and anti-cancer (e.g. vinca alkaloid) drugs, but their production, isolation, structural elucidation and incorporation into a druglinker reagent for production of an ADC requires specialized resources (strain and sample libraries), experience, capabilities and expertise.

Linker Chemistry – Antibody Side

Conjugation chemistry to antibodies has historically relied on the sulfhydryl or amino groups found in the natural amino acids. Linker-payload reagents with a terminal antibody-side maleimide or activated ester are frequently encountered (Figure 3). Conjugation chemistry to these groups can be controlled but are random in that the product ADC consists of multiple species. Heterogenous ADCs are more difficult to manufacture due to batch variability, and biologically, individual ADC species offer different profiles of target activity, toxicity and clearance. More recently, site-specific conjugation strategies have opened up the options to include engineered thiols, non-natural amino acids, aldehydes and other groups in the antibody.13,14 These strategies provide greater control of DAR, simplify the manufacturing process, improve ADC homogeneity6,8 and can lead to improvements in efficacy and therapeutic index7.
Linker Chemistry – Payload Side
In the case of the auristatins and maytansinoids, amine or thiol groups, respectively, connect the payloads to the linker. A thiol-bearing spacer was introduced in maytansine to incorporate a release mechanism. New technologies that use other functional groups, such as hydroxyl, are sought to open up new payload options and linker chemistries.

Payload Release – Cleavable and Non-cleavable Linkers Innovation
Linkers are categorized as cleavable or non-cleavable. Non-cleavable linkers do not fragment. Instead, proteases digest the antibody protein backbone leaving the linkerpayload tagged with a terminal amino acid residue. Kadcyla® is an example of this type (Figure 4). Cleavable linkers fragment depending upon the environment within a cell. The three common cleavage mechanisms are enzymatic, disulfide and pH. Adcetris® uses a valine-citrulline para-aminobenzyl alcohol motif as part of the linker. Amide bond cleavage of the substrate by a protease results in release of the parent payload after 1,6 – elimination of the $p$-aminobenzyl carbamate moiety. Mylotarg® uses a two-step cleavage method using both pH and disulfide triggers. The acyl hydrazide within the linker is thought to first hydrolyze under the low pH environment of the lysosome (pH ~5). The disulfide is then reduced by glutathione, which is abundant in cancer cells.

ADC CROs
CROs vary in their competence to deliver quality ADC research. Experienced staff is crucial, as working with both payloads and linkers present challenges. Linkers, which are designed to release their payloads, are sensitive by design and subject to degradation. Poly functional payloads can possess complex stereochemistry or issues of regiochemistry requiring selective transformations and non-routine purifications. In addition, payloads are usually highly potent compounds and available in very limited quantities requiring expert handling during linker-payload reagent synthesis and conjugation reaction process research. Chemists with expertise in small scale, natural product chemistry and biologists with protein chemistry expertise are well suited for ADC work. CROs should have proper support facilities such as high potency suites with laminar flow hoods for safe handling of highly potent cytotoxins, protein expression and engineering laboratories for antibody engineering and production and high resolution mass spectrometry for comprehensive analysis of monoclonal antibodies and ADCs.

Conclusion
The growth track of ADC-based pharmaceuticals is unquestionably on the upswing. As the ADC space matures, novel antibodies, linkers and payloads will be developed to keep pace with the goal to deliver effective medicines with improved safety. Increased pressure to create homogenous ADCs will drive new conjugation approaches to control DAR. ADC discovery will catalyze the development of new chemistry and create opportunities for CROs and CMOs with the expertise required to work with these entities. With deep expertise in payload and linker chemistry, protein production, ADC process development and sterile, pyrogen-free manufacture of ADCs, along with state-of-theart facilities in its integrated drug discovery center, AMRI has the capabilities to meet the needs of our customers who are pursuing ADC drug discovery and development.
About AMRI
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