Potent Promise

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Research into the use of antibody-drug conjugates as a means of targeted oncology treatment is off to a successful start, with both Adcetris and Kadcyla gaining approval. This should lead to further opportunities in developing this approach to target even more patients

For three decades, drug developers have attempted to combine the specificity of a monoclonal antibody for tumour cells with the potency of drugs that kill rapidly dividing cancer cells (see Figure 1). Within the past three years, two antibody-drug conjugates (ADCs) – Adcetris® (brentuximab vedotin) and Kadcyla® (ado-trastuzumab emtansine) – have been approved, validating the approach. These forerunners have highlighted the tremendous potential of ADCs, as well as opportunities to further improve this promising therapeutic approach.

Keywords

Antibody-drug conjugates
DNA damaging agents
Tubulin binder
Drug-to-antibody ratio
Potent drug payloads

First Steps

The early ADCs employed cytotoxic chemotherapies that were approved for systemic administration for the treatment of cancer, such as doxorubicin and methotrexate. While antibody conjugation of these drugs allowed them to be tumour-targeted, they were not sufficiently potent to provide clinical benefit, and development was discontinued.

Wyeth attempted to address this issue by conjugating highly potent DNA-damaging agents called calicheamicins. In 2000, the drug Mylotarg® (gemtuzumab ozogamicin) was granted accelerated approval by the FDA for the treatment of acute myelogenous leukaemia in elderly patients. Although Mylotarg was able to demonstrate clinical responses, the drug payload was relatively quickly released from the antibody in circulation, leading to a narrow therapeutic index. As a result, the product was voluntarily withdrawn from the market in 2010, after confirmatory trials revealed no significant clinical benefit, with an excess number of deaths in Mylotarg-treated patients compared to chemotherapy control.

Reaching the Market

The early experiences with ADCs highlighted the need for highly potent drug payloads, combined with stable linkers, to achieve suitable therapeutic index. Two companies – Seattle Genetics and ImmunoGen – have emerged with ADC platforms that have addressed these limitations, and they have led to the development of currently approved products – Adcetris and Kadcyla –as well as to more than 30 ADCs in clinical development.

Adcetris, from Seattle Genetics, uses a payload from the auristatin class. These are antimitotic agents that bind to tubulin and block microtubule polymerisation, leading to cell death. The auristatins are mechanistically similar to the vinca alkaloid class of chemotherapies, but are several hundred-fold more potent. In the case of Adcetris, auristatin is linked to cysteine residues on an anti-CD30 antibody by an enzyme-cleavable linker that is stable in plasma, but is cleaved to release the drug upon internalisation into a cell. Adcetris is approved in two types of lymphoma where the tumour cells express high levels of CD30.

Genentech licensed technology from ImmunoGen to develop Kadcyla. As with Adcetris, the drug payload here is a tubulin binder, of the maytansinoid class. The maytansinoids are several hundred-fold more potent as anti-cancer drugs, compared to chemotherapies approved for systemic administration. In Kadcyla, maytansine is conjugated to lysine residues on the anti-HER2 antibody, trastuzumab, using a non-cleavable linker. This linker only releases the drug when the ADC is internalised into a cell and the antibody is subsequently degraded. Kadcyla is approved for patients with advanced breast cancer carrying amplification of the HER2 gene (HER2-positive breast cancer).

New Opportunities

Adcetris and Kadcyla represent significant advances in the ability to target highly potent chemotherapies specifically to

cancer cells. However, they also highlight opportunities to further improve ADCs to achieve greater clinical benefit.

On average, there are three to four chemotherapy drugs per antibody in these two agents, which exist as heterogeneous mixtures of antibodies with a distribution of drug loads from zero to eight, and above. While higher drug loads would be expected to result in even more efficient drug delivery to tumours, it has been shown that an increase in drug load above four drugs per antibody leads to poor ADC exposure *in vivo*, decreased anti-tumour efficacy and increased toxicity (1).

Adcetris and Kadcyla have achieved success in patient populations with very high levels of target antigen expression. However, among the ADCs currently in clinical development, there have been variable levels of clinical activity reported, especially for solid tumour targets. One of the most advanced solid tumour ADCs – an anti-CD56 maytansinoid from ImmunoGen – recently reported negative results in a trial with small-cell lung cancer. One hypothesis for the mixed results is that even with highly potent payloads, ADCs with three to four drugs per antibody are not sufficiently potent to drive clinical benefit for antigens with lower tumour expression.

Both ADCs use natural product-derived drug payloads from the tubulin binder class, which interfere with microtubule formation. This mechanism has the benefit of preferentially killing rapidly proliferating cells, such as those in the tumour, while having little to no effect on non-dividing cells. However, the anti-tubulin class of agents could also limit the clinical benefit of anti-tubulin-derived ADCs, due to either acquired resistance or intrinsic resistance. For example, the tubulin binder class is largely inactive in colorectal cancer, potentially restricting the utility of tubulin-binder ADCs for colorectal cancer targets.

Diverse technologies and approaches are currently in development to address these opportunities to further improve ADCs.

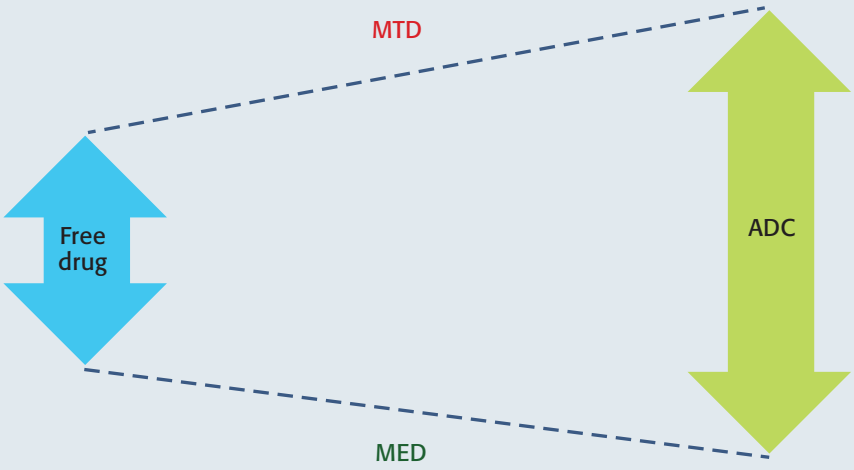

Figure 1: The goal of an ADC is to improve the therapeutic index by delivering the drug specifically to the tumour while sparing normal tissues, resulting in a potential increase in maximum tolerated dose (MTD) and a decrease in the minimally efficacious dose (MED)

Site-Specific Technologies

As previously discussed, while Kadcyla and Adcetris are the products of different conjugation technologies, they have a similar average drug-to-antibody (DAR) ratio and, in reality, represent mixtures of species with a range of zero to more than eight drugs per antibody. Higher drug loaded species are more rapidly cleared, and are hypothesised to contribute to ADC toxicity, while not improving anti-tumour efficacy.

To remove the undesirable higher DAR species and produce a more homogeneous drug product, various antibody technologies are being developed to conjugate the drug payload to an antibody in a more defined, site-specific manner. Genentech has developed a site-specific conjugation technology, THIOMAB, which introduces cysteine residues in the constant regions of an antibody. Using the same linker and auristatin payload found in brentuximab vedotin, the company has optimised the conjugation conditions for the engineered antibody to produce a THIOMAB-drug conjugate (TDC) (2). In the TDC, the drug payload is conjugated to the engineered cysteines, without the disruption of interchain disulfide bonds which occurs with the typical cysteine conjugation approach.

The site-specific TDC displays a narrow distribution of drug conjugate species, with an average DAR of 1.8. When the TDC was compared with a standard ADC (3.1 DAR), generated by inter-chain cysteine conjugation, the TDC showed similar anti-tumour efficacy, but when the toxicities of both conjugates were compared in rats and monkeys, the more homogeneous TDC was better tolerated than the more heterogeneous ADC. On balance, it was found that TDC improved the therapeutic index by approximately two-fold compared with the ADC. It was also demonstrated that

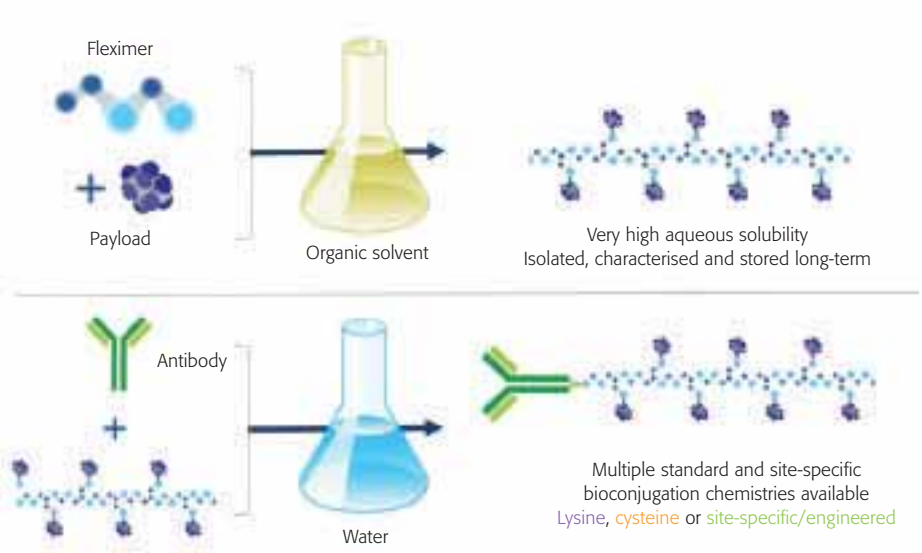

Figure 2: A Fleximer® ADC is prepared in a two-step process. First, the payload of interest is conjugated to the Fleximer polymer in an organic solvent optimally suited to the payload class. The resulting highly water-soluble Fleximer-drug conjugate can then be readily conjugated to the antibody via a variety of conjugation approaches including cysteine, lysine or site-specific/engineered

the variation of the specific site for conjugation could impact the stability and characteristics of the resultant ADC – not only the number, but the location of sites could influence the performance of the conjugate.

Taking it Further

While Genentech has not publically disclosed clinical data with a TDC, Seattle Genetics recently announced that SGN-CD33A – a site-specific anti-CD33 ADC designed using similarly engineered cysteine methods and conjugated with a pyrrolobenzodiazepine dimer payload – is currently being evaluated in a Phase 1 trial for the treatment of acute myeloid leukaemia (AML).

In addition to the site-specific cysteine incorporation approach, companies such as Ambrx, Sutro and Redwood Biosciences are using non-natural amino acid (nnAA) technology with orthogonal conjugation chemistries to facilitate site-specific ADC approaches. While the details of the specific approaches differ, the respective technologies provide alternatives to producing antibodies which incorporate nnAAs into the antibody, at specific locations in the protein sequence. The nnAAs so far incorporated have reactive groups not found in natural amino acids, and can be used to conjugate the drug payload in a chemospecific manner.

By generating a number of antibodies with specific conjugation sites at different positions along the protein sequence, more homogenous ADCs can be prepared, and the influence of the conjugation site on parameters such as stability, efficacy and tolerability can be evaluated. Furthermore, the nnAAs provide the opportunity to explore alternative bioconjugation chemistries and linkers, beyond those typically used for cysteine and lysine bioconjugation.

New Drug Payloads

One area of increasing interest in the ADC field is the identification and validation of drugs beyond auristatins and maytansines that can be effectively incorporated into ADCs. Because of the typical limited capacity of three to four drugs per antibody when directly conjugated, there has been a focus on identifying more potent drugs, as well as drugs that provide alternative mechanisms of action – including different resistance profiles – in comparison to the anti-tubulin agents widely used to date.

Pyrrolobenzodiazepines

One such class of drugs concerns the DNA-damaging agents represented by Spirogen's (recently acquired by MedImmune) pyrrolobenzodiazepines (PBDs), which are highly cytotoxic minor groove DNA cross-linking agents derived from the anthramycin class of natural products. *In vitro*, these drugs typically demonstrate

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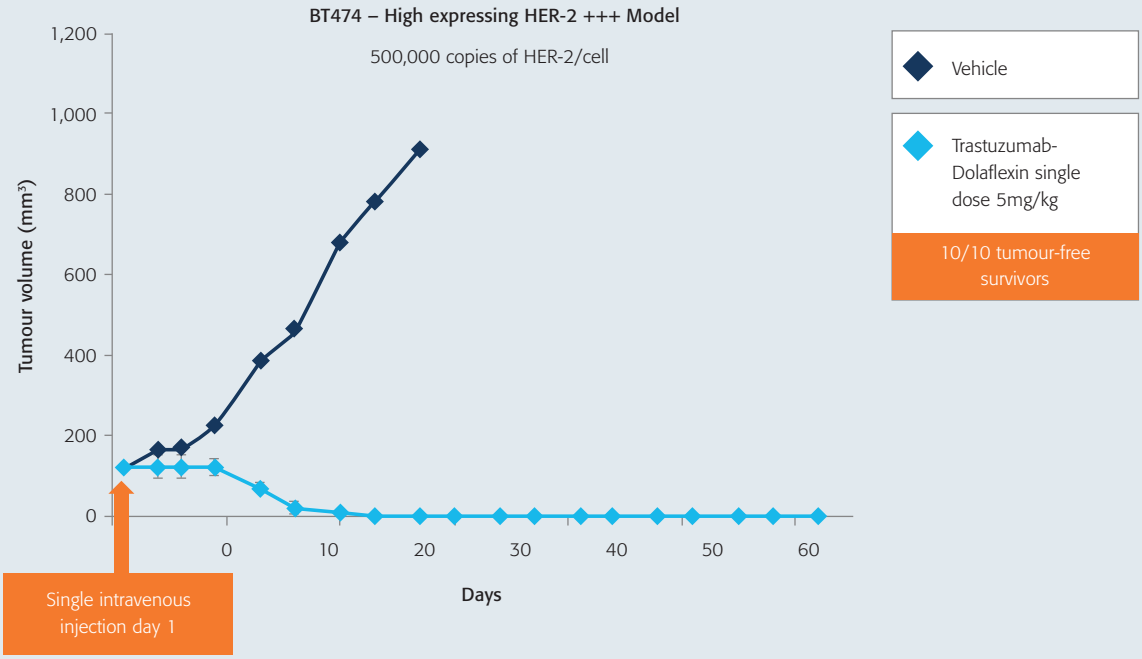

Figure 3: A single dose of trastuzumab-Dolaflexin in a mouse model of HER2+ breast cancer results in complete and durable tumour regressions

IC50 values in the low to mid picomolar range in a variety of cell types, and unlike the anti-tubulin agents, they can induce cell death in both dividing and non-dividing cells.

The most advanced ADC incorporating a PBD is that of Seattle Genetics, which partnered with Spirogen in the development of this payload class (3). Seattle Genetics explored the creation of ADCs targeted to the myeloid differentiation antigen CD33 for the treatment of AML. As reported by the American Association for Cancer Research in 2013, initial efforts to conjugate three to four PBDs per antibody via reduced interchain disulfides led to problems with aggregation, due to the highly hydrophobic nature of the PBD payload. However, by utilising a site-specific, engineered antibody approach and conjugating two PBDs per antibody, the issue of aggregation could be addressed. The resulting ADC showed high potency and tolerability in preclinical models, including multi-drug resistant models, and was advanced into clinical trials towards the end of 2013.

Duocarmycins

A second class of DNA-damaging agents includes duocarmycins, which are analogs of the natural product, CC-1065. Like the PBDs, these molecules also irreversibly bind to DNA in the minor groove, resulting in highly potent cell killing of both dividing and non-dividing cells. A Phase 1 clinical trial of the duocarmycin ADC MDX-1203, targeted to the CD70 antigen, was completed in November 2012 by Medarex/Bristol-Myers Squibb in patients with advanced/recurrent renal cell carcinoma or relapsed/refractory non-Hodgkin's lymphoma, although results have not been reported to date.

Synthon has also reported preclinical results with a HER-2 targeted duocarmycin ADC, which showed efficacy in a number of HER-2 positive models, as well as safety data in cynomolgus monkeys. Based on these results, it plans to initiate clinical trials with a trastuzumab biosimilar – a duocarmycin ADC – in the latter half of 2014.

As duocarmycins are highly hydrophobic molecules with a propensity to cause protein aggregation, companies such as Nerviano Medical Sciences have sought to identify more aqueous soluble analogs better suited for incorporation into ADCs. By including undisclosed solubilising moieties into their derivatives, they have demonstrated the preparation of ADCs with trastuzumab, which lacked aggregation, and demonstrated efficacy in HER-2 positive models.

Amanitin

Another highly potent payload class derived from a natural product is the RNA polymerase inhibitor, amanitin, which is also being investigated for incorporation into ADCs.

> An alternative approach to ADCs, designed to address and overcome several of the limitations inherent in directly linking a payload to an antibody, is currently in development

This bicyclic octapeptide, which is isolated from the extremely toxic Green Deathcap mushroom, interferes with the cellular transcription machinery, resulting in cell death of both dividing and quiescent cells at very low concentrations. Unlike the majority of payloads utilised for ADCs, amanitin is not highly hydrophobic. Heidelberg Pharma has reported the incorporation of amanitin into ADCs targeting the prostate-specific membrane antigen, and demonstrated efficacy both *in vitro* and *in vivo*.

Alternative Approaches

Mersana Therapeutics is developing an alternative approach to ADCs, designed to address and overcome several of the limitations inherent in directly linking a payload to an antibody. The approach relies on Fleximer® – a highly water-soluble polymer derived from naturally occurring carbohydrates. The specific properties of Fleximer allow for a wide variety of drug payload classes to be readily conjugated to the polymer with high drug loading, while maintaining excellent physicochemical properties. These resulting Fleximer-drug conjugates, which can be fully isolated, characterised and stored for prolonged periods, can be efficiently conjugated to antibodies under aqueous conditions via lysine, cysteine or site-specific bioconjugation methods (see Figure 2, page 18).

Mersana has also disclosed the development of an auristatin-Fleximer drug conjugation platform, termed Dolaflexin™, and demonstrated its advantages in the context of a trastuzumab-Dolaflexin ADC. By utilising a cysteine bioconjugation approach, a trastuzumab-Dolaflexin ADC with a DAR of 20 was prepared, and shown to maintain excellent physicochemical properties with no aggregation. When evaluated *in vivo*, a single dose of the trastuzumab-Dolaflexin ADC resulted in complete and sustained tumour regressions (see Figure 3, page 19). Furthermore, in contrast to the findings with conventional ADCs, the trastuzumab-Dolaflexin ADC maintained excellent pharmacokinetic properties and tolerability, despite the significantly higher drug load.

Summary

Kadcyla and Adcetris have established the viability of ADCs as a therapeutic modality for the treatment of cancer. Building on this foundation, next-generation technologies hold the promise of realising ADCs that are even more efficacious, better tolerated, and relevant for a broader population of patients.

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