

Antibody Drug Conjugates and the Link to Fighting Cancer

Antibody drug conjugates (ADCs) are a big deal in oncology. And rightly so: they combine highly specific targeting with a potent cytotoxic payload to deliver tremendous results. Since the approval of the first ADC in 2000, Mylotarg® for adults with acute myeloid leukemia (AML), ADCs have continued to make waves – something we can honestly say has revolutionised the way we approach oncology. Right now there are 15 approved ADC drugs for cancer indications, with reported hundreds more ADC candidates in pipelines or clinical trials,¹ testament to the extensive interest and promise of this therapeutic approach.

But we have to ask, why have they been so successful where small molecules haven't? What drives their success and potentially limits their efficacy? And what challenges still have to be overcome?

A Quick ADC Track Record

Despite their current success, ADCs' adoption was a little slow to start. Interestingly, the complexity of ADC design, the combination of a cytotoxic drug, an antibody, a linker, and of course the conjugation technology, was both the core of its success and the main reason it took so long for the field to initially take off. ADC developers had to wrestle with early issues such as the lack of stable conjugation methods, limited therapeutic profiles, and poorly designed linkers. However, advances in our understanding of selectivity, site-specific conjugation, and improved stability have led to this area of R&D thriving like it is.

Over the past two years, the ADC industry has witnessed significant milestones. A few of the most notable achievements include the approval of ADCs by the US Food and Drugs Administration (FDA), such as Elahere® (Mirvetuximab soravtansine) for ovarian cancer and Tivdak® (Tisotumab vedotin) for cervical cancer. We've also seen investment from big pharma into smaller biotech ADC companies that has brought not only a hefty dose of confidence but also a host of promising prospects to the field.

If we look at the R&D side of things, we've seen marked improvements in linker and conjugation chemistries that translate into clinical successes – you can see this in action when you take a look at Enhertu® (Trastuzumab deruxtecan), which is the first HER2-directed therapy for patients with HER2-low metastatic breast cancer to be approved in the EU.² Enhertu® was developed with a novel linker-payload combination (a tetrapeptide linker and an exatecan derivative as a payload), which makes it so effective in the treatment of many HER2-positive cancers.³

ADCs have a great track record, and with next-generation ADCs coming onto the scene, they look to be going from strength to strength. But what makes them so successful?

Of mAbs and Linkers

To get a better insight into why ADCs have been so successful we need to look at what they are and how they work. Central to this is their targeted approach to deliver potent cytotoxic drugs directly to cancer cells, and this is thanks to the inclusion of monoclonal antibodies (mAbs), which even on their own have transformed the way we approach cancer therapy.

But of course, while mAbs provide more targeted treatment compared to traditional chemotherapy, their efficacy when used in killing cancer cells alone is limited. This limitation birthed the concept of ADCs: "What if we link mAbs to cytotoxic drugs?"

And so an ADC combines the accuracy of a tumor-targeting mAb with a cytotoxic payload through an intricately designed chemical linker. While they may not be considered "magic bullets," (although some have called them "biological missiles"⁴) ADCs deliver highly specific and targeted chemotherapy while also aiming to minimise – though sadly not always eliminating – off-target effects.

Though it's easy to get caught up in mAb specificity and payload potency, it's this link between mAb and payload that plays a crucial role in how well the ADC functions. This is because that linker influences the ADC stability, payload release profiles, and,

ultimately, the therapeutic index (TI; the ratio between the toxic dose and the dose at which the drug becomes effective).

Ideally, a linker needs to avoid causing ADC aggregation and ensure a controlled release of the payload at the targeted sites while simultaneously minimising premature release in plasma. You can generally divide linkers into cleavable and non-cleavable. Cleavable linkers respond to external cues or enzymes, which enables the targeted release of the cytotoxic payload, whereas non-cleavable linkers provide plasma stability and controlled release through protease-mediated degradation. Both linker types offer unique advantages for optimising ADCs – but we'll come back to these in a bit. Let's clear something up first about ADCs and effective dosing.

They Work, But Not Like That

Contrary to one particular and widely quoted notion, the clinical data from human studies just don't support the idea that ADCs increase the therapeutic window by lowering the minimum effective dose (MED) or increasing the maximum tolerated dose (MTD) compared to small molecules.⁵ However, when administered at the MTD, ADCs do seem to show improved efficacy over small molecules in oncology trials.

While there's a distinct lack of direct trials comparing small molecules with ADCs, we can take a look at data from ADC trials and contrast them with trials involving analogous small molecules. Let's look at the 2021 DESTINY gastric cancer trial where the ADC Enhertu® showed an objective response rate (ORR) of 42%, while the ORR for the small molecule Irinotecan was 12.5%.⁵ ADC treatments frequently achieve ORRs above 40% in breast, gastric, urothelial, and lung cancer trials, whereas small molecules rarely exceed 25%. There's a degree of interpretation here, but these results would suggest that ADCs could offer greater beneficial outcomes for patients.

A recent discussion from Gerber et al. put forward a revised model where anti-tumour activities of ADCs, and by extension their TIs, aren't solely associated with changes in just their MTDs, but also in their MEDs.⁶ This ties

in with the way using their exposure-based TI calculation approach lets us more easily explain the improved anti-tumour activities of ADCs and highlight the success of new ADCs in the clinic.

If we want to optimise the TI ADCs, we have to explore dose optimization. Dosing regimens, including body weight capping, treatment duration, dosing frequency, and response-guided dosing are already being used to increase the therapeutic index of approved ADC treatments.⁷ Comparisons of the MTDs between approved ADCs and small molecule drugs with equivalent cytotoxic payloads indicate that ADCs do not increase the MTD. The reasons behind this are not fully understood, but factors such as payload-linker stability and deconjugation in plasma are believed to play a significant role.

So while ADCs do not improve the MED or significantly differ in MTD compared to small molecules, they do seem to show better efficacy in oncology trials. Dose optimisation and understanding the factors influencing ADC TI will help unlock their full potential in targeted cancer therapy.

The Link to Success

With all that in mind, let's return to linkers. Currently, most of the ADCs approved for

use or in clinical trials are produced by coupling a maleimide moiety on the linker payload to the antibody. The problem with this bioconjugation method is that it can lead to instability in the ADC, resulting in deconjugation via a retro-Michael reaction.⁸ Consequently, you can end up with a lot of variability in your drug-to-antibody ratio (DAR).

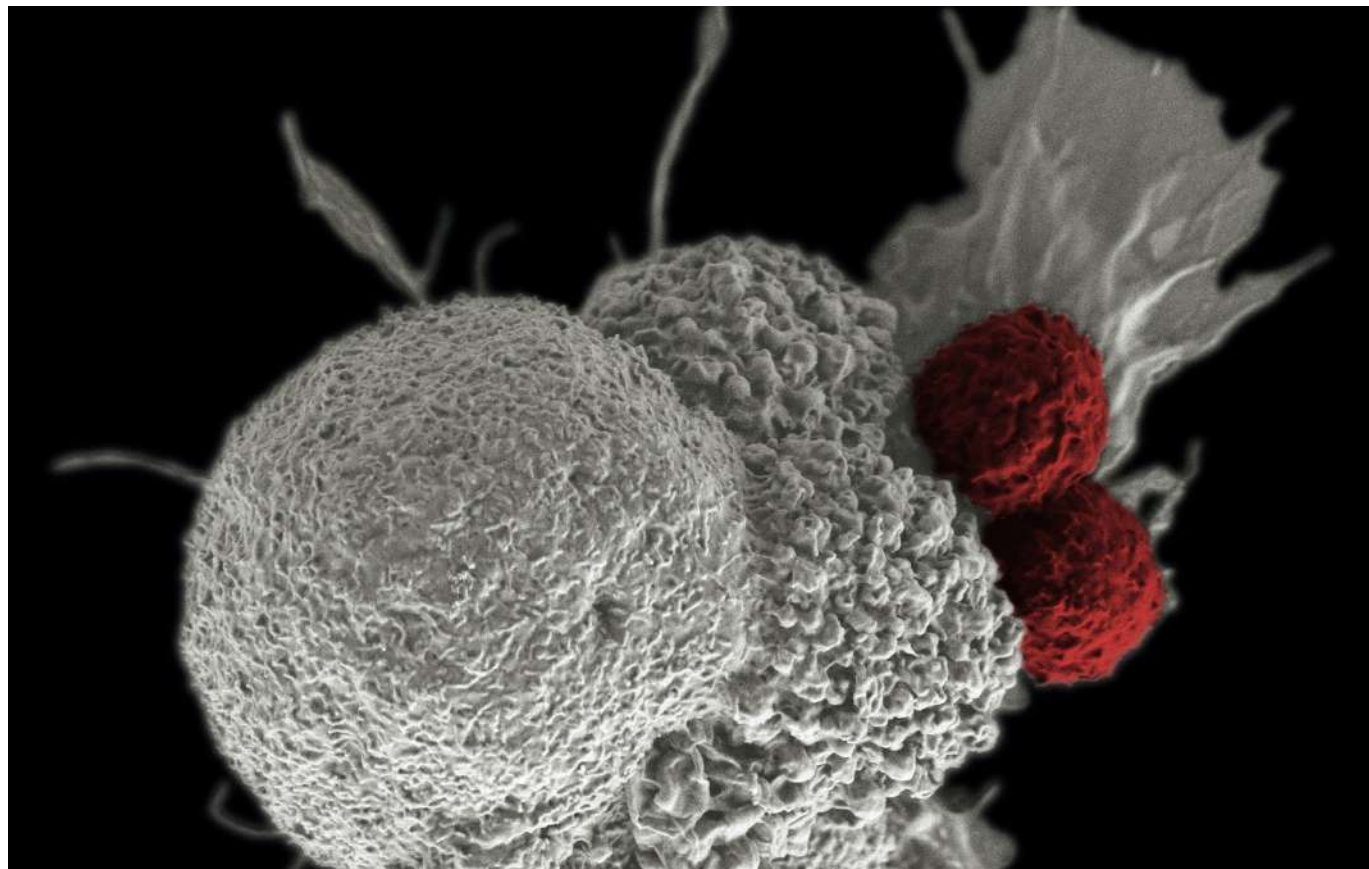
But like everything in biology, things are rarely simple. So, on one hand, deconjugation before engaging with target cancer cells can have a positive impact on reducing tumour cells by allowing the cytotoxic moiety to interact with cancer cells. On the other hand, this deconjugation can lead to a reduced concentration of the cytotoxic agent reaching the cancer cells, causing the unintended destruction of healthy cells. For example, deconjugation in plasma can result in albumin binding, leading to non-specific payload disposition and direct tumour albumin uptake.

As you can see, the linker is a major player in how successful your ADC is. And we're not suggesting that maleimide is a poor bioconjugation – not at all – it's just that there are better options. This is why we've seen a noticeable shift toward more site-selective conjugation technologies in pre-clinical and clinical-stage ADCs. These

advancements include ThioBridge™ cysteine re-bridging technology, peptide-directed lysine conjugations such as AJICAP™, and enzyme-directed approaches like microbial transglutaminase and GlycoConnect™ enzyme-mediated glycan remodelling technology.

If we take a deeper look at ThioBridge™, for example, we'll see how it overcomes some of the shortcomings of first-generation conjugation technologies. Unlike some of the first-generation conjugation technologies, ThioBridge™ delivers a more uniform profile compared to maleimide conjugation and enables site-specific conjugation to antibody interchain disulfides without requiring antibody engineering. Additionally, ThioBridge™ allows for stable attachment of the linker to the antibody through a 3-carbon bridge, resulting in optimised pharmacokinetic (PK) properties and architectural design flexibility. This conjugation technology lets scientists rapidly produce and profile ADCs in complex *in vivo* evaluations. Overall, ThioBridge™ gives people the power and control to generate homogeneous constructs with precise drug loading and predetermined, controlled sites of attachment.

It's these collective advancements in linker technology and bioconjugation that





will help deliver ADCs with improved safety, efficacy, and pharmacokinetic properties – which only bolsters their already incredible success in targeted cancer therapy.

Future Challenges and Opportunities

Despite rocketing to success, ADCs have faced challenges in their development – particularly in optimising the aspect of the monoclonal antibody, linker, and payload – but the ingenuity of scientists has so far overcome them.

But we're not done yet: there are more hurdles to clear and opportunities to seize. Bispecific antibody technology and dual-payload ADCs, for example, show tremendous potential in improving antibody internalisation and tumour specificity and overcoming the ever-present menace of drug resistance. Other groups are exploring alternative structures for ADCs, such as smaller molecular weight polypeptide fragments, which can overcome penetration efficiency issues and improve payload delivery. And then there's payload selection, something that's now expanded beyond cytotoxic drugs to include targeted and immune drugs, opening even more new possibilities for ADC therapies.

As long as there's room for improvement and challenges to address, the outlook for ADCs remains promising. A

better understanding of payload release mechanisms, exploration of unique linker strategies, and targeting strategies will continue to drive enhanced efficacy coupled with fewer off-target effects.

Decades of research and development have led to the success we now see with ADC therapies, and this benefits even more cancer patients. And as we strive forward, pushing the science and generating more and better drugs, the future of ADCs looks set to live up to the lofty claim of revolutionizing cancer therapy.

REFERENCES

1. *In Vivo* [Internet]. 2023 [cited 2023 Jul 11]. ADCs Coming Of Age: Deals, Targets And Catalysts. Available from: <https://invivo.pharmaintelligence.informa.com/IV147692/ADCs-Coming-Of-Age-Deals-Targets-And-Catalysts>
2. Enhertu approved in the EU as the first HER2-directed therapy for patients with HER2-low metastatic breast cancer [Internet]. 2023 [cited 2023 Jul 11]. Available from: <https://www.astrazeneca.com/media-centre/press-releases/2023/enhertu-approved-in-the-eu-as-the-first-her2-directed-therapy-for-patients-with-her2-low-metastatic-breast-cancer.html>
3. Shin SH, Park Y, Park SS, Ju EJ, Park J, Ko EJ, et al. An Elaborate New Linker System Significantly Enhances the Efficacy of an HER2-Antibody-Drug Conjugate against Refractory HER2-Positive Cancers. *Adv Sci*. 2021 Oct 18;8(23):2102414.
4. Fu Z, Li S, Han S, Shi C, Zhang Y. Antibody drug conjugate: the “biological missile” for targeted cancer therapy. *Signal Transduct Target Ther*. 2022 Mar 22;7(1):1–25.
5. Colombo R, Rich JR. The therapeutic window of antibody drug conjugates: A dogma in need of revision. *Cancer Cell*. 2022 Nov 14;40(11):1255–63.
6. Gerber HP, Gangwar S, Betts A. Therapeutic index improvement of antibody-drug conjugates. *mAbs*. 15(1):2230618.
7. Liao MZ, Lu D, Kågedal M, Miles D, Samineni D, Liu SN, et al. Model-Informed Therapeutic Dose Optimization Strategies for Antibody-Drug Conjugates in Oncology: What Can We Learn From US Food and Drug Administration-Approved Antibody-Drug Conjugates? *Clin Pharmacol Ther*. 2021 Nov;110(5):1216–30.
8. Alley SC, Benjamin DR, Jeffrey SC, Okeley NM, Meyer DL, Sanderson RJ, et al. Contribution of linker stability to the activities of anticancer immunoconjugates. *Bioconjug Chem*. 2008 Mar;19(3):759–65.



**Campbell
Bunce**

Campbell Bunce is Chief Scientific Officer (CSO) and Cambridge Site Head at Abzena. He leads a talented team of scientists across a diverse range of expertise and capabilities to support drug discovery, design, and developability; and cell line development. He ensures that Abzena's strong innovation focus and depth of scientific expertise is maintained through technological developments and works in partnership with clients to design and deliver solutions that support their program needs. Campbell has over 25 years of experience working in the biotech and diagnostics sectors. Before joining Abzena in 2015, he held multiple positions of increasing responsibility in Biotech including Head of Cellular Immunology at Cantab Pharmaceuticals, Director of Programs at Piramed Pharma, and R&D Director at Immune Targeting systems. Throughout his career he has applied innovative solutions for the design, manufacture and clinical evaluation of novel products including vaccines, biologics, and small molecules in multiple therapeutic areas. These include inflammation, cancer, infectious disease, and addiction. Campbell has a PhD in Immunology from the University of Manchester, an Executive MBA from Judge Business School, Cambridge University and has published a number of papers on cell-mediated immunity, immunotherapy and vaccines.