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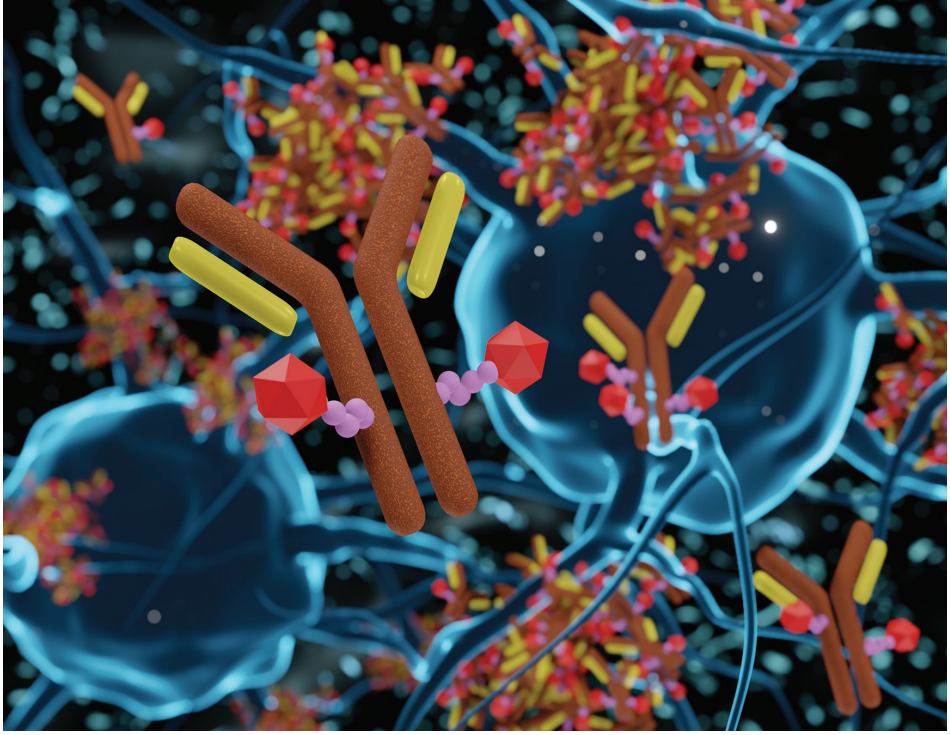
POWERING UP ANTIBODY-DRUG CONJUGATES WITH PROTACS

For decades, pharmaceutical interventions for cancer had been limited to small-molecule drugs that kill tumor cells or inhibit their ability to divide and proliferate.[1] Though these cell-killing compounds continue to be an essential tool in cancer treatment, a key drawback is their inability to directly target tumor cells; that leads to undesirable destruction of healthy cells and a trove of unpleasant side effects for patients.

The past 20 years have brought a new generation of cancer therapeutics designed to pinpoint tumor cells or cellular processes involved with tumor cell growth. From small-molecule kinase inhibitors to monoclonal antibodies, dozens of targeted therapies are now established in the clinic and are a standard of care in many cancer types.[1,2] One of the most rapidly growing areas of research and clinical development in the field of targeted therapies are antibody-drug conjugates (ADCs).[2]

ADCs consist of an antibody bound to a cytotoxic small molecule through a short molecular linker. These macromolecules use antibodies' unique ability to bind specific antigens to deliver potent small-molecule anticancer drugs directly to cancer cells. Though the drugs, or payloads, are typically more cytotoxic than traditional synthetic chemotherapeutics, "the amount of payload you need is significantly reduced because you're delivering it, in theory, exactly to the site of interest," says Santosh Kulkarni, head of medicinal chemistry at Syngene International. For this reason, ADCs are sometimes called biological missiles.[2]

The first ADC approved by the US Food and Drug Administration, in 2000, was for the treatment of acute myeloid leukemia. It consists of an anticancer molecular payload joined to an antibody that binds a receptor protein that is overexpressed in cells destined to become leukemia but not in healthy stem cells.[2,3] There are now 14 ADCs approved worldwide for treatment of blood cancers and solid tumors and over 100 in clinical trials.[2]



In this illustration, an antibody (brown and yellow) carries a drug (red) to cells in a brain tumor.

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One of the key issues for developers of ADCs is preparing them. In contrast to small-molecule drugs or monoclonal antibodies, ADCs are complex macromolecules and thus represent a significant synthetic hurdle. The success or failure of an ADC hinges on the ability of all three components—antibody, payload, and linker—to function in balance, says Paul Hogg, vice president of medicinal chemistry at ADC Therapeutics. Bringing these pieces together to manufacture ADCs for commercial use or clinical studies has also been a major challenge—one that is largely being met by developing complex supply chains and outsourcing the work to specialized contract development and manufacturing companies (CDMOs).[4,5]

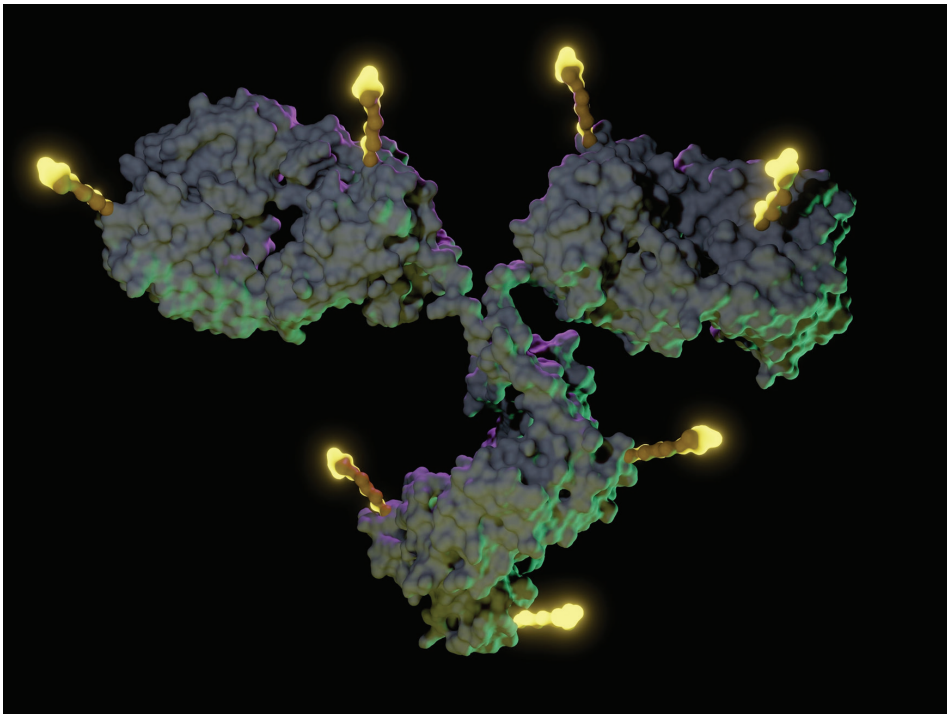
In addition, as ADCs prove themselves in the clinic, countless opportunities to innovate have emerged, such as varying payload types and expanding disease indications beyond cancer. Designing, optimizing, and manufacturing the next generation of ADCs require integrated teams with a deep understanding of chemistry, biology, and pharmacology, as well as facilities equipped to consistently deliver sensitive, potent biologics at scale.

NO WEAK LINKS

The key advances in ADC technology over the past 2 decades have been optimizing linkers and bioconjugation strategies to attach payloads, Hogg says. Linkers in early ADCs were designed to break down in the relatively acidic environment within cancer cells and tumors' microenvironments.[6] But these linkers were also found to hydrolyze while in circulation, which resulted in the payload being released before its delivery to the antibody's target and in off-target toxicity.[2]

These labile linkers limited researchers to using less potent payloads, which led to less effective therapeutics. But progress made in linker design has helped solve this problem. More recently developed ADCs use linkers that rely on changes in oxidation state or on cellular enzymes to trigger cleavage when the targeted cell takes up the ADC. Noncleavable linkers also exist; these show the greatest stability in circulation, yet rely on complete cellular degradation of the antibody to release the payload.[7]

To attach the linker-payload onto an antibody, early ADCs depended on random bioconjugation methods to form either disulfide or amide linkages at random cysteine or lysine residues.[2] The average number of payload molecules linked to the antibody, or the drug-to-antibody ratio (DAR), ranged from zero to eight and was inconsistent within a given ADC batch. Furthermore, ADCs with high DARs (six or eight) are very hydrophobic and tend to form aggregates. Inconsistent bioconjugation methods also impacted the pharmaceutical properties of these ADCs, causing faster clearance and limiting therapeutic efficacy.



In this illustration, an antibody carries several drug molecules (yellow) ready to deliver the therapeutics to the antibody's target, such as a tumor cell.

Credit: Love Employee/Getty Images

New bioconjugation methods provide opportunities for chemists to control and optimize the DAR. Installing nonnatural amino acids residues in an antibody provides unique reaction sites, such as alkynes and azides, for conjugating drugs via click reactions. Refined chemical conjugation methods target specific cysteine and lysine residues. For example, Seattle Genetics, which launched the ADC Adcetris in 2011, developed a conjugation technique to target disulfide linkages in an antibody's hinge region.[8]

Even with these advances, the linker and bioconjugation method used in any ADC program must be carefully selected to balance kinetics of payload delivery and release, potency of the payload, and the structural integrity of the antibody. "Optimizing this chemistry, although it looks very simple, can be very challenging," says Lakshindra Chetia, a lead investigator at Syngene.

Integrated research teams that blend synthetic, biological, and analytical skill sets can achieve quick progress in overcoming challenges to advancing ADC candidates to the clinic. For example, Chetia and Kulkarni recently collaborated with one of Syngene International's clients to develop ADCs carrying proprietary payloads. One team prepared a payload library consisting of the client's proposed compounds and compounds selected by Syngene researchers, while another developed custom bioassays to screen the compounds' activity.

After identifying hit compounds, the synthesis team prepared various linker-payload candidates, which it then handed off to the bioconjugation lab to synthesize the final ADC candidates. The entire project took less than a year to complete. "When you have the necessary capabilities as well as expertise brought together in one organization, you can quickly check ideas and set your research program on the right path," Kulkarni says.

PROTACS: PART OF THE NEXT GENERATION OF ADCS?

As linker and bioconjugation strategies for ADC preparation continue moving forward, researchers are looking beyond cytotoxic payloads. "We've got the background now to troubleshoot quickly, solve problems, and the technology to analyze the data," Hogg says. "It's all down to imagination now. As chemists and scientists in this area, it's up to us to be creative."

One ADC alternative that is capturing scientists' imagination: antibody linked-PROTACs.

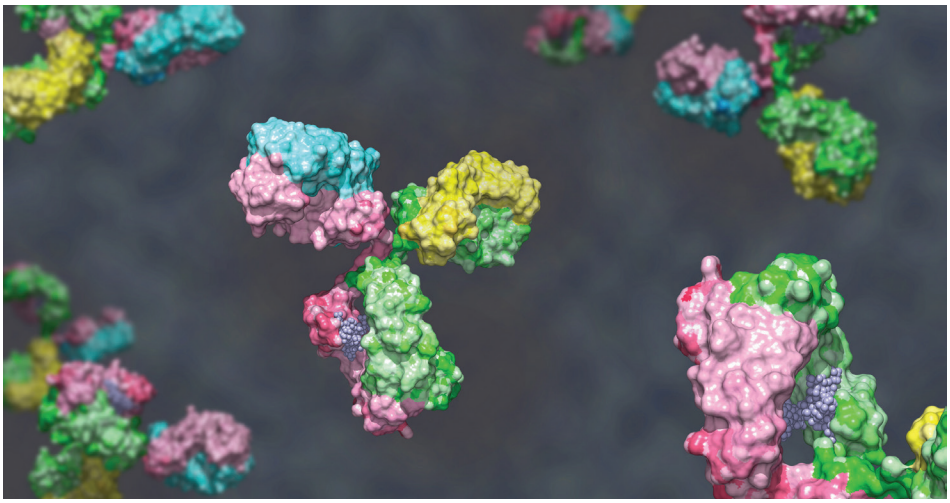
Proteolysis-targeting chimeras (PROTACs) are bifunctional small molecules that promote targeted degradation of a specific protein of interest (POI) by the proteosome.[9] They consist of a targeting ligand that binds to the POI; a stable linker; and a recruitment ligand that is designed to bind a ubiquitin E3 ligase complex. When the PROTAC, POI, and E3 ligase come together to form a three-part complex, the ligase machinery adds the protein ubiquitin to the nearby POI, marking it for cellular degradation. After this labeling, the PROTAC may go on to form another trimer complex to catalyze the destruction of another copy of the POI.

Although no PROTACs have yet been approved for clinical application, they are an active area of clinical research, with 12 candidates in clinical trials to treat diseases including breast and prostate cancer.[9] Specificity is a current obstacle with PROTACs. Most recruit a particular E3 ligase complex found in many cell types throughout the human body. So while a PROTAC may target a protein of interest for degradation, it currently does not work in a cell- or tissue-specific manner.

Since antibodies offer site specificity, however, the concept of PROTAC-antibody conjugates is clinically appealing. “Though this is a very new area of study, the properties of ADCs could marry very well with PROTACs,” Kulkarni says.

The first papers reporting PROTAC-antibody conjugates as a proof of concept were published in 2019 and 2020.[10–12] A pair of papers published in 2021 systematically explored the properties of PROTAC-antibody conjugates designed to deliver a PROTAC to a prostate cancer cell line.[13,14] The authors demonstrated selective delivery and specific protein degradation. But they encountered trouble with identifying a location to attach the linker and noticed that antibodies with high DARs experienced aggregation. Similar difficulties were overcome with traditional ADCs, which suggests that PROTAC-antibody conjugates could become a viable drug platform in time.

“If we could have tissue- and target-specific degrader activity from E3 ligases, we could make a superclean drug,” says David Langley, executive director of platform chemistry at Arvinas. Though PROTACs’ mechanism of action will still limit their application to diseases caused by gain of function proteins[9], PROTAC-antibody conjugates could have an impact in oncology, neurology, virology, agriculture, and many other areas, he adds. “There’s a lot of work to be done, but it’s an exciting road forward.”



In this illustration of an engineered antibody, a bispecific antibody contains two different heavy chains (green and pink) and two different light chains (blue and yellow), which creates two unique recognition sites at the antibody tips.

Credit: Huen Structure Bio/Shutterstock

PARTNERING FOR SUCCESS

The unique advantages that ADCs offer arise from the melding of powerful biological and chemical motifs. Likewise, developing and delivering new ADCs require integrated teams of experts working in facilities that can handle potent compounds and sensitive biological reagents. For the pharmaceutical companies, partnering with CDMOs that have this infrastructure can provide an accelerated route to targeted outcomes, as well as a research partner that can suggest new

routes of investigation. "Our expertise and experience help us to not just work on something that is new but to also invent structures and create new methods," Syngene's Kulkarni says. "That's especially needed for these new modalities, and it's something we've done quite often with ADCs and now with PROTACs."

BISPECIFIC ADCS: AN EMERGING TARGETING TOOL

Engineered antibodies are not limited to those that recognize a single antigen. In a bispecific antibody, the two available binding sites are different and thus can bind two distinct antigens.[15] Antigens can be present on two different targets, such as a tumor cell and an immune cell, or be present on the same target, such as two different receptor proteins on a tumor cell. There are several bispecific antibodies approved for therapeutic use in disease including hemophilia and various cancers.[16]

As bispecific antibodies find increased use in therapeutic applications, there is also growing interest in developing bispecific ADCs.[17] Compared with traditional ADCs, bispecific ADCs may potentially improve targeting specificity and internalization of the ADC into cells; that could in turn reduce toxicity and enhance overall efficacy. Also, because bispecific antibodies target two distinct antigens, the risk of developing resistance to the ADC therapy is lowered. There are currently several early-stage clinical trials underway using bispecific ADC therapies for cancer treatment.[18]

Like traditional ADCs, bispecific ADCs present challenges to achieving a stable, homogenous, and effective therapeutic. The bispecific antibody component introduces new and significant challenges.[19,20] Antibodies are made of two heavy and two light chains, which in normal antibodies are identical. But as these chains are broken, recombined, and engineered to form a bispecific antibody, mismatches are common.

Specific strategies have been developed to control this process. To minimize variability in the antibody structure and the DAR, expertise in synthetic biology and bioconjugation strategies is required when preparing bispecific ADCs. CDMOs like Syngene International can bring scientists together with know-how in these and other areas to streamline development of complex and novel therapeutics. Syngene has experience in preparing and screening ADCs as well as bispecific and trispecific antibodies.

REFERENCES:

1. Lei Zhong et al., "Small Molecules in Targeted Cancer Therapy: Advances, Challenges, and Future Perspectives," *Signal Transduction Targeted Ther.* 6, no. 201 (May 31, 2021): 201, https://doi.org/10.1038/s41392-021-00572-w.
2. Zhiwen Fu et al., "Antibody Drug Conjugate: the "Biological Missile" for Targeted Cancer Therapy," *Signal Transduction Targeted Ther.* 7, no. 93 (March 22, 2022): 93, https://doi.org/10.1038/s41392-022-00947-7.
3. Joseph E. Maakaron et al., "CD33-Targeted Therapies: Beating the Disease or Beaten to Death?," *J. Clin. Pharmacol.* 61, no. 1 (January 2021): 7–17, https://doi.org/10.1002/jcph.1730.
4. Rick Mullin, "New Day for Antibody-Drug Conjugates," *Chem. Eng. News* 100, no. 17 (May 16, 2022): 16–18, https://cen.acs.org/pharmaceuticals/biologics/New-day-antibody-drug-conjugates/100/i17.
5. Caylee Donaldson, CDMOs in the Preclinical ADC Space: A Benchmarking Study, Hanson Wade Intelligence, (Sept. 1, 2022), https://hansonwade-intelligence.com/cdmos-in-the-preclinical-adc-space-a-benchmarking-study/

6. Erez Persi et al., "Systems Analysis of Intracellular pH Vulnerabilities for Cancer Therapy," *Nat. Commun.* 9, no. 2997 (July 2018): 1–11, https://doi.org/10.1038/s41467-018-05261-x.
7. Kyoji Tsuchikama and Zhiqiang An, "Antibody-Drug Conjugates: Recent Advances in Conjugation and Linker Chemistries," *Protein Cell* 9, no. 1 (January 2018): 33–36, https://doi.org/10.1007/s13238-016-0323-0.
8. Ann M. Thayer, "Site Specific: Developers Aim to Create Well-Defined Drug Conjugates," *Chem. Eng. News* 92, no. 3 (Jan. 20, 2014), https://cen.acs.org/articles/92/i3/SITE-SPECIFIC.html.
9. Miklós Békés et al., "PROTAC Targeted Protein Degraders: The Past is Prologue," *Nat. Rev. Drug Discovery* 21 (March 2022): 181–200, https://doi.org/10.1038/s41573-021-00371-6.
10. Thomas H. Pillow et al., "Antibody Conjugation of Chimeric BET Degrader Enables In Vivo Activity," *ChemMedChem* 15, no. 1 (Jan. 7, 2020): 17–25, https://doi.org/10.1002/cmdc.201900497.
11. Peter S. Dragovich et al., "Antibody-Mediated Delivery of Chimeric Protein Degraders Which Target Estrogen Receptor Alpha (ERα)," *Bioorg. Med. Chem. Lett.* 30, no. 4 (February 2020): 126907, https://doi.org/10.1016/j.bmcl.2019.126907.
12. María Maneiro et al., "Antibody-PROTAC Conjugates Enable HER2-Dependent Targeted Protein Degradation of BRD4," *ACS Chem. Bio.* 15, no. 6 (June 19 2020): 1306–12, https://doi.org/10.1021/acschembio.0c00285.
13. Peter S. Dragovich et al., "Antibody-Mediated Delivery of Chimeric BRD4 Degraders. Part 1: Exploration of Antibody Linker, Payload Loading, and Payload Molecular Properties," *J. Med. Chem.* 64, no. 5 (February 2021): 2534–75, https://doi.org/10.1021/acs.jmedchem.0c01845.
14. Peter S. Dragovich et al., "Antibody-Mediated Delivery of Chimeric BRD4 Degraders. Part 2: Improvement of In Vitro Antiproliferation Activity and In Vivo Antitumor Activity," *J. Med. Chem.* 64, no. 5 (March 11, 2021): 2576–2607, https://doi.org/10.1021/acs.jmedchem.0c01846.
15. Jiabing Ma et al., "Antibodies: From Research to Clinical Application," *Front. Immunol.* 12 (May 2021): 1–19, https://doi.org/10.3389/fimmu.2021.626616.
16. "Therapeutic Monoclonal Antibodies Approved or in Regulator Review," The Antibody Society, accessed Feb. 12, 2023, https://www.antibodysociety.org/antibody-therapeutics-product-data/.
17. Antoine Maruani, "Bispecifics and Antibody-Drug Conjugates: A Positive Synergy," *Drug Discov. Today: Technol.* 30 (December 2018): 55–61, https://doi.org/10.1016/j.ddtec.2018.09.003.
18. Biopharma PEG, "Bispecific Antibody Drug Conjugates (ADCs): Emerging Trends," Aug. 11, 2022, https://www.biochempeg.com/article/290.html.
19. Ulrich Brinkmann , "The Making of Bispecific Antibodies," *MAbs* 9, no. 2 (Jan. 10 2017): 182–212, https://doi.org/10.1080/19420862.2016.1268307.
20. Qiong Wang et al., "Design and Production of Bispecific Antibodies," *Antibodies* 8, no. 3 (August 2019): 43–72, https://doi.org/10.3390/antib8030043.

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