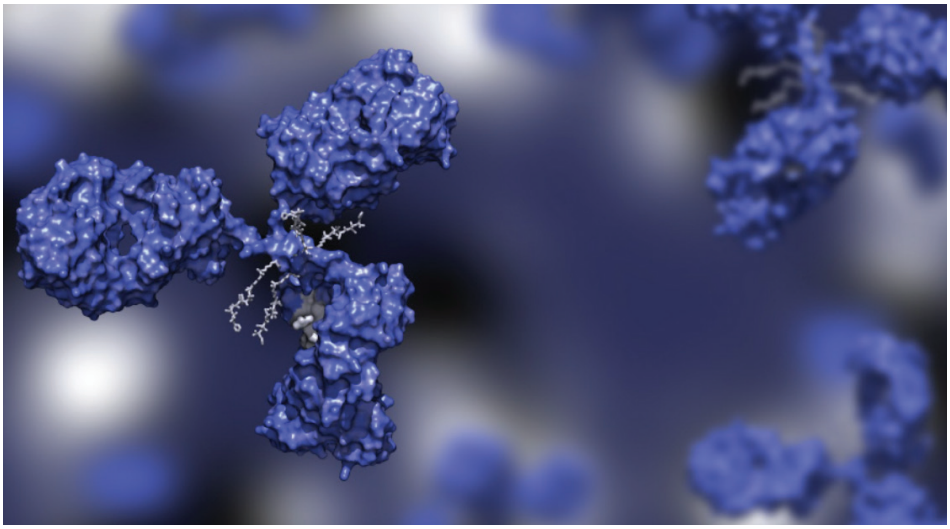
c&en | WHITE PAPERS

ANTIBODY-TARGETED PRECISION MEDICINES: STRATEGIES FOR OVERCOMING DEVELOPMENT CHALLENGES



Credit: Shutterstock

Antibody-targeted therapies encompass a broad range of biologics that have emerged as a flexible and powerful modality over the last twenty years. These therapies use antibodies to target cells with therapeutic drugs while limiting damage to healthy cells.

Antibody-drug conjugates (ADCs) are the most studied modality of antibody-targeted therapies. In 2000, gemtuzumab ozogamicin—brand name Mylotarg—became the first ADC to be approved by the US Food and Drug Administration.[1,2] As of April 2024, 15 ADCs have been approved globally for cancer.[2] At the end of 2024, over 200 companies were developing ADCs,[3] and the global market for them is projected to reach $25 billion by 2033.[4]

Since the development of ADCs, other modalities have emerged that could broaden the impact of antibody-based therapies by expanding beyond

cancer applications and by providing different mechanisms of action besides cytotoxicity. These modalities include antibody-oligonucleotide conjugates (AOCs), which deliver nucleic acids, and degrader-antibody conjugates (DACs), which target proteins for degradation.[5,6] The key advantage of antibody-targeted therapies is that they provide three points of control due to the antibody, linker, and therapeutic payload components. "The antibody gives you more selective delivery, and the payload gives you a more selective mechanism of action," says David Madge, vice president of discovery services for WuXi AppTec.

Antibody-targeted therapy development is a complex, multistep process. It's both aided and complicated by the expanding array of technologies and strategic choices at each step of discovery and development. This white paper offers an overview of the development of ADCs, DACs, and AOCs and highlights unique challenges facing researchers working on these medicines.

The specific combination of mAb, linker, and payload is dependent on the target and the mechanism of action.

Types of antibody-targeted therapies

Antibody-targeted therapies have three main structural elements:

- A monoclonal antibody (**mAb**) used to deliver therapy to targeted cells
- A **linker** molecule that connects the payload to the antibody
- A **payload**, such as a toxin, small interfering RNA, or degrader, that has a therapeutic effect when released within the target cell

ANTIBODY-DRUG CONJUGATES

Scientists developed ADCs to ensure that highly potent cytotoxic drugs targeting cancer cells were not affecting other cell types. Attaching the drug to a mAb allows the therapy to selectively target cancer cells (figure 1). Once internalized, the cytotoxic drug can be released inside the cell to cause cell death. Cytotoxic molecules such as microtubule inhibitors (auristatins and maytansinoids) currently dominate the ADC market.[7] But cytotoxic drugs alone have poor selectivity, and antibody-drug conjugates are typically more toxic than AOCs and DACs, making off-target toxicity a challenge even when ADCs are applied using a highly selective delivery mechanism.

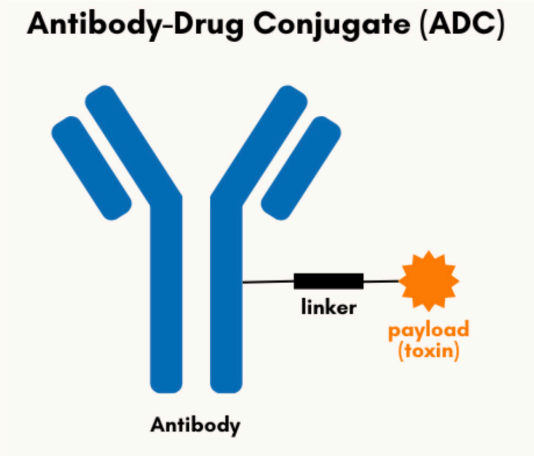


Figure 1: Antibody-drug conjugate structure

ANTIBODY-OLIGONUCLEOTIDE CONJUGATES

AOCs consist of a small interfering RNA (siRNA) molecule or an antisense oligonucleotide (ASO) linked to a mAb that guides internalization and release into the target cell (figure 2). These molecules are designed to bind target RNA, which can lead to messenger RNA (mRNA) degradation, exon inclusion or exclusion,

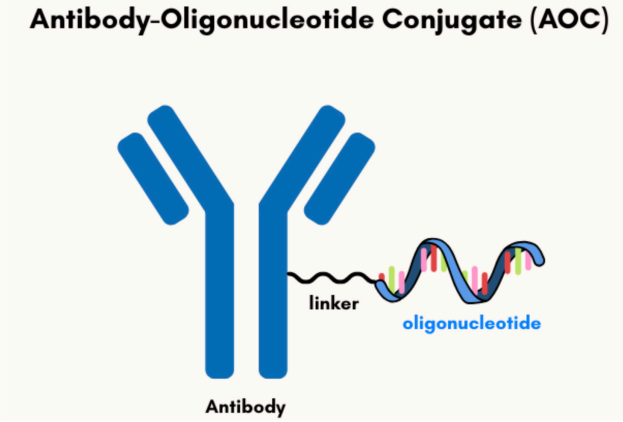


Figure 2: Antibody-oligonucleotide conjugate structure

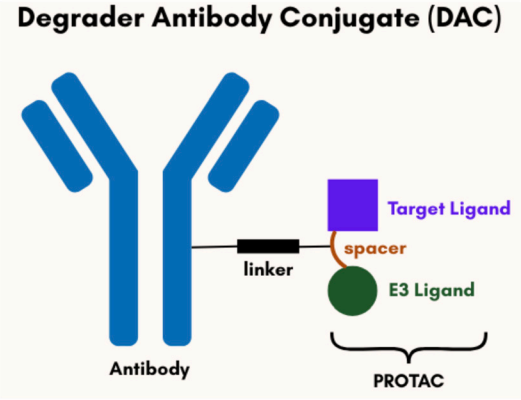


Figure 3: Degrader-antibody conjugate structure

changes to gene expression, or pre-mRNA splicing. Because the oligo sequence can be highly customized, both the mAb and payload components of the AOCs add specificity. Unconjugated siRNAs, ASOs, and phosphorodiamidate morpholino oligomers (PMOs) are already approved to treat a variety of diseases, such as cardiovascular, neuromuscular, and central nervous system diseases.[8]

AOCs are unique because they have therapeutic as well as diagnostic applications and can be used for imaging and detection during diagnosis. Madge noted that diagnostics is an area that has received increased attention in recent years, since the same mAb can be used to image the diseased cells and deliver the therapeutic agent selectively.

DEGRADER-ANTIBODY CONJUGATES

DACs consist of a mAb used to deliver a bifunctional molecule—a molecule that can bind to two proteins simultaneously—to a specific cell based on mAb binding (figure 3). While bifunctional molecules can serve many functions, in the case of DACs they're used to target proteins for degradation. These molecules consist of one ligand that binds the protein targeted for degradation and another that binds a component of the cell's degradation machinery.

Many types of degraders can be used in DACs, including

- PROTACs (proteolysis-targeting chimeras), which bring a target protein into proximity with an E3 ubiquitin ligase. E3 ligases can then attach ubiquitin molecules to the target protein, thus marking it for degradation (figure 4).[9]
- LYTACs (lysosome-targeting chimeras), which bring targeted surface proteins to a lysosome-targeting receptor, where they can then be internalized and degraded.[10]
- AUTACs (autophagy-targeting chimeras), which tag target proteins for degradation by binding them to a guanine derivative tag to recruit the autophagy machinery.[11]

DACs have diverse therapeutic applications, including cancer, central nervous system diseases, rare diseases, and inflammatory disorders.

DEVELOPMENT CHALLENGES FOR ANTIBODY-TARGETED THERAPIES

Although each has its own unique challenges, the development of ADCs, AOCs, and DACs shares a common workflow. Creating an antibody-targeted therapy includes designing the individual components of the therapeutic molecule, linking them together, then characterizing and optimizing the molecule and its biological effect. The choices made during each step of the workflow can impact

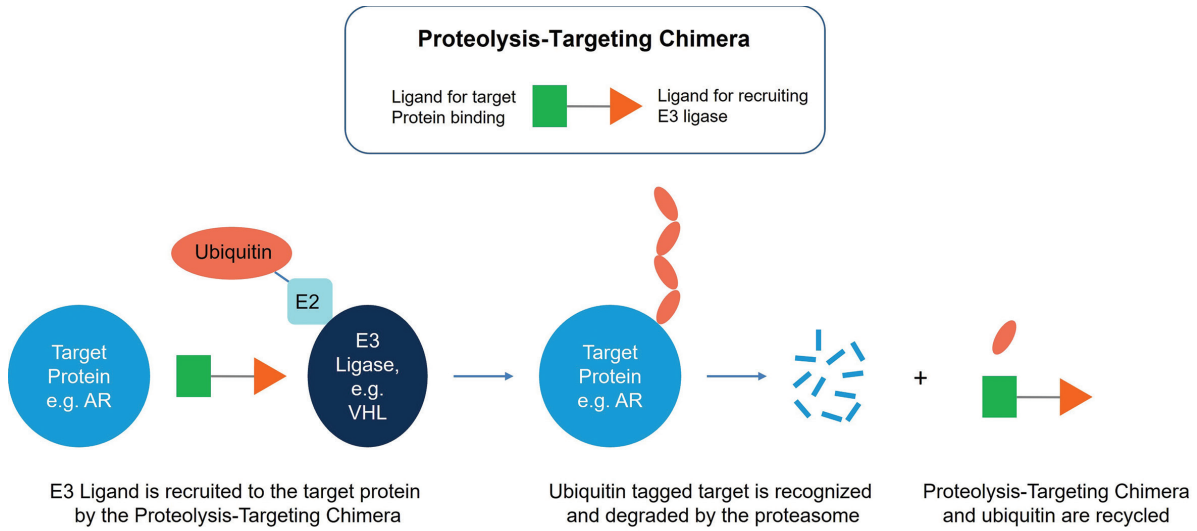


Figure 4: Proteolysis-targeting chimera mechanism of action

the molecule's off-target effects, payload release, internalization, stability, and drug resistance.

Identifying and engineering the mAb

The ideal mAb used in antibody-targeted therapies will bind targeted cells with high affinity but will not bind nontarget cells. The mAb should have a long half-life in blood and support efficient cellular uptake by the target cell. Once identified, a mAb used in antibody-targeted therapies can be further engineered to optimize these characteristics.

Off-target effects—or when the payload acts on nontarget cells—can be addressed by using a mAb with high specificity to the target cell. Off-target effects are a problem when the payload releases its cytotoxic effect on normal cells, as is often observed with ADCs. While these precision medicines are highly selective, off-target activity cannot be eliminated. Monoclonal antibodies occasionally bind nontarget cells or release the payload in circulation before it reaches the intended site, or the payload can permeate neighboring, nontarget cells once released.

STRATEGY TIP:

It's possible to use antibodies that have already been validated or pre-existing payloads to generate antibody-targeted therapies. This can help extend the life cycle of an existing drug.

Designing the payload

The payload of choice depends on the desired therapeutic effect. For ADCs targeting cancer cells, this payload would be cytotoxins. For AOCs, the payload would be nuclease-resistant oligonucleotides. For DACs, the payload would be a molecule that binds a target protein and a degradation-related protein to promote the breakdown of the intended protein.

These payloads all work by different mechanisms, and they each present unique challenges. ADCs use cytotoxic and nonspecific payloads, meaning they have the potential for off-target toxicity. This problem can be addressed with linker design and the drug-to-antibody ratio (DAR). There has been a lot of interest in

improving safety profiles by combining the selectivity of the delivery technology with that of a payload to further target specific cells or proteins.

In contrast to ADCs, the nucleic acid payloads for AOCs are more specific because they are based on oligonucleotide sequences designed to match cellular machinery through highly specific mRNA sequences. On their own, however, AOCs lack tissue selectivity and have low membrane permeability. Because of their large size, oligonucleotides are harder to characterize and purify, which introduces some chemistry challenges.

Nucleic acid payloads such as siRNA can also have a short half-life in circulation (6 min–1 h) and are subjected to degradation from nucleases.[12] This challenge can be addressed using chemical modification—such as changes to the nucleic acid backbone, sugar, or base—to increase nuclease resistance.[13] In fact, recent advances have resulted in therapies with required dosing as little as once-per-year.[14]

Choosing a linker

There are two general types of linkers. **Cleavable linkers** are those that can release the payload via various cellular mechanisms, including those that are upregulated in the target cell. For example, lysosomal protease–sensitive linkers are cleaved by enzymes such as cathepsin B, which is overexpressed in cancer cells.[15] Cleavable linkers must be stable in circulation to avoid releasing the payload too early. Madge offered another example: acid-sensitive linkers can give more selective cleavage in cell types with low pH, such as those in an inflammatory state.

In contrast, **noncleavable linkers** release the payload only when the antibody degrades. This degradation often occurs after internalization into the lysosome and has a lower risk for off-target effects. Examples of noncleavable linkers include maleimidocaproyl and maleimidomethyl cyclohexane-1-carboxylate.[16]

Steric hindrance from bulky linkers or blocked cleavage sites can impair payload release, so there is a trade-off between stability of the therapy and release. The choice between cleavable and noncleavable linkers can help address off-target toxicity and drug resistance. If the payload is released too early, such as in circulation, off-target effects may result. This is a greater concern with cleavable linkers, while noncleavable linkers can help reduce premature release.

However, cleavable linkers can help address drug resistance via a bystander effect, in which the payload can kill cells neighboring the target cell.[17] This is important because not every tumor cell will express the antigen targeted by the mAb. The bystander effect helps clear all tumor cells regardless of antigen expression and reduces the risk of drug resistance by preventing the regrowth of antigen-negative tumor cells.

Conjugation and characterization of antibody-conjugated therapy

Once all the components of the antibody-targeted therapy have been designed,

the next step is to attach the payload to the antibody. This can be done using non-site-specific or site-specific methods.

Non-site-specific methods, which were used in early ADC drug development, attach the payload to amino acids nonspecifically throughout the antibody. These methods are simpler and provide higher yields, but the resulting therapy is a heterogeneous mixture and can have poor uniformity and low stability, which negatively affects efficacy.

On the other hand, **site-specific conjugation** attaches the payload to predetermined sites on the antibody using genetic engineering, enzyme-catalyzed reactions, or selective chemical conjugation on specific functional groups. These methods have the advantages that the entire therapy is essentially identical and has a defined DAR. However, synthesizing antibody-targeted therapies using site-specific conjugation is more difficult, tends to have lower yields, and has a limited number of conjugation sites per molecule.

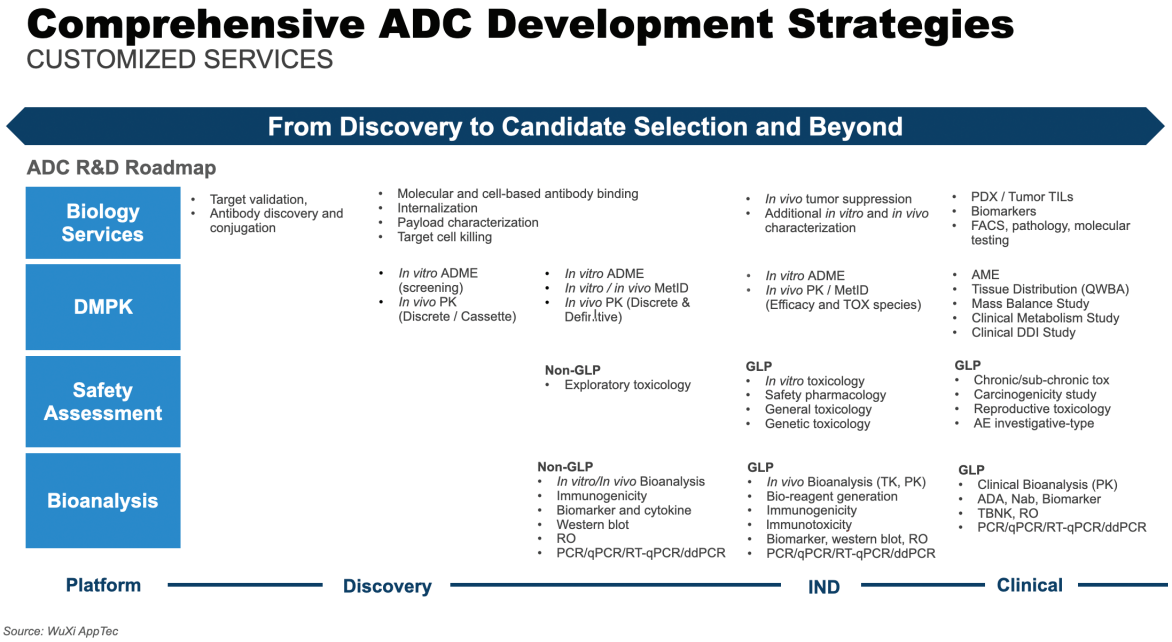
In characterizing ADCs, the DAR distribution and the average DAR can be measured using immunocapture and mass spectrometry. Most ADCs have a DAR between two and four.[18] For AOCs, the oligonucleotide-antibody ratio is usually less than four because the payload is typically extremely potent. Because degraders are less potent, they need to be delivered with a higher degrader-antibody ratio, according to Madge. Size-exclusion chromatography can be used to detect unconjugated antibodies, free payloads, and aggregates. Conjugation sites can be identified using peptide mapping with liquid chromatography–tandem mass spectrometry (LC-MS/MS).

Conjugation is more challenging with AOCs than with ADCs, because the former are larger molecules and are harder to purify and characterize. Because DACs require a high ratio of degrader to antibody, there can be issues with size and aggregation as these molecules become larger. Optimizing the payload to antibody ratio can help address the balance between efficacy and off-target effects. For example, an ADC with a high DAR could lead to increased off-target toxicity, but one with a low DAR could lead to low efficacy.

In vitro functional and stability studies

Once the antibody-targeted therapy has been designed, the next step is to see how it performs in vitro using a variety of assays depending on which function or characteristic is being studied. As a contract research, development, and manufacturing organization (CRDMO), WuXi AppTec offers a wide range of optimized assays to support in vitro evaluation of antibody-targeted therapies (figure 5).[19]

To **assess payload function**, assays such as fluorescence microscopy with labeled antibodies or oligonucleotides can be used to determine uptake of the therapy and trafficking. For ADCs, it's critical to determine how



Source: WuXi AppTec

Figure 5: Comprehensive antibody-drug conjugate research strategies at WuXi AppTec

the treatment affects cell viability and growth. This can be measured using cell proliferation assays, cytotoxicity assays to measure the killing effect on cancer cells, and cell cycle assays. Because bystander killing activity can be helpful for ADCs to target neighboring tumor cells, bystander killing assays can measure lysis of antigen-negative cells using fluorescence-activated cell sorting (FACS).

For AOCs, quantitative polymerase chain reaction (qPCR) or reporter assays can quantify the effect of the introduced oligonucleotide on target gene expression, and Western blots can be used to measure protein levels of the targeted gene. Several assays are used to gain insight into the effect of DACs on protein degradation, including:

- Western blots and enzyme-linked immunosorbent assays (ELISAs), to measure changes in protein levels
- Ubiquitination assays, to assess protein degradation levels
- FACS, to measure degrader-antibody conjugate internalization
- Cell viability assays, to determine the effects of protein degradation

To **assess stability**, in vitro studies can be performed by incubating the molecule complex in serum, then performing ELISAs and LC-MS/MS analysis to quantify the levels of the antibody-targeted therapy.

In vivo efficacy and pharmacokinetics-pharmacodynamics evaluation (PK/PD)
The last step of the antibody-targeted precision medicine development workflow is to investigate whether the payload performs the intended function in the correct cells and what happens to the therapy once inside the body. For instance, understanding the drug efficacy of ADCs against tumor cells could

involve using cell line-derived xenograft (CDX) tumor models or patient-derived xenograft (PDX) tumor models to assess endpoints like survival or tumor regression. WuXi AppTec provides a comprehensive panel of CDX and PDX models to cover multiple ADC targets to facilitate such studies.[20] Fluorescence microscopy can be used to monitor payload internalization, cellular uptake, and trafficking.

In addition, drug metabolism and pharmacokinetic (DMPK) studies are essential in the early stages of discovery to help predict how the therapy will behave in later stages of development. Understanding the fate of the payload once it enters the body using biotransformation studies can reveal how the payload is metabolized. These studies can be done by monitoring the blood, liver, and tumor cells (for ADCs) over time to observe the breakdown products of the therapy and how they behave. Tissue distribution studies can help scientists understand the biodistribution of the drug and assess the potential for any associated toxicity.

CONCLUSIONS

Modalities such as ADCs, AOCs, and DACs are expanding the toolbox of targeted therapies. Developing antibody-targeted drugs involves a complex workflow with many opportunities for optimization to address challenges such as off-target effects, payload release, and stability.

Navigating antibody development requires a trusted and experienced partner. WuXi AppTec is a world leader in providing end-to-end contract research, development, and manufacturing services to support all stages of antibody-targeted therapy research and development. WuXi AppTec offers state-of-the-art services for target identification, hit finding, customized linker synthesis, advanced conjugation and analytical techniques, in vitro functional and biophysical assays, and in vivo tumor models for evaluating PK/PD efficacy.

REFERENCES:

1. Virginia Metrangolo and Lars H. Engelholm, "Antibody-Drug Conjugates: The Dynamic Evolution from Conventional to Next-Generation Constructs," *Cancers* 16, no 2 (Jan. 2024): 447, https://doi.org/10.3390/cancers16020447.
2. Ruili Wang et al., "Antibody-Drug Conjugates (ADCs): Current and Future Biopharmaceuticals, *J. Hematol. Oncol*. 18, no. 51 (April 2025), https://doi.org/10.1186/s13045-025-01704-3.
3. "200+ Pharma Companies Vie for the Top Spot in the Expanding Antibody-Drug Conjugate Oncology Market," PR Newswire, Oct. 17, 2024, https://www.prnewswire.com/news-releases/200-pharma-companies-vie-for-the-top-spot-in-the-expanding-antibody-drug-conjugate-oncology-market--delveinsight-302278759.html.
4. Antibody Drug Conjugates Market Size is Expected to Reach USD 25.38 Billion by 2033, Growing at a CAGR of 8.84%: Straits Research," GlobalNewswire, Jan. 22, 2025, https://www.globenewswire.com/news-release/2025/01/22/3013689/0/en/Antibody-Drug-Conjugates-Market-Size-is-Expected-to-Reach-USD-25-38-Billion-by-2033-Growing-at-a-CAGR-of-8-84-Straits-Research.html.
5. Jinlan Jiao et al., "Overcoming Limitations and Advancing the Therapeutic Potential of Antibody-Oligonucleotide Conjugates (AOCs): Current Status and Future Perspectives," *Pharmacol. Res*. 209 (Nov. 2024): 107469, https://doi.org/10.1016/j.phrs.2024.107469.
6. Ki Bum Hong and Hongchan An, "Degrader-Antibody Conjugates: Emerging New Modality," *J. Med. Chem*. 66, no. 1 (Jan. 12, 2023): 140–48, https://doi.org/10.1021/acs.jmedchem.2c01791.
7. Dan-Yun Ruan, Hao-Xiang Wu, and Qi Meng, "Development of Antibody-Drug Conjugates in Cancer: Overview and Prospects," *Cancer Commun*. 44, no. 1 (Jan. 2024): 3–22. https://doi.org/10.1002/cac2.12517.
8. Barbora Malecova et al., "Targeted Tissue Delivery of RNA Therapeutics Using Antibody-Oligonucleotide Conjugates (AOCs), *Nucleic Acids Res*. 51, no. 12 (July 7, 2023): 5901–10, https://doi.org/10.1093/nar/gkad415.
9. Kyli Berkley et al., "Journey of PROTAC: From Bench to Clinical Trial and Beyond," *Biochemistry* 64, no. 3 (Feb. 4, 2025): 563–80, https://doi.org/10.1021/acs.biochem.4c00577.
10. Bhavana Ramadas, Pritam Kumar Pain, and Debasish Manna, "LYTACs: An Emerging Tool for the Degradation of Non-Cytosolic Proteins," *ChemMedChem* 16, no. 19 (Oct. 6, 2021): 2951–53, https://doi.org/10.1002/cmdc.202100393.
11. Daiki Takahashi et al., "Second-Generation AUTACs for Targeted Autophagic Degradation," *J. Med. Chem*. 66, no. 17 (Sept. 14, 2023): 12342–72, https://doi.org/10.1021/acs.jmedchem.3c00861.
12. Syed Saqib Ali Zaidi et al., "Engineering siRNA Therapeutics: Challenges and Strategies," *J. Nanobiotechnol*. 21, no. 1, 381 (2023), https://doi.org/10.1186/s12951-023-02147-z.DOI: 10.1186/s12951-023-02147-z
13. Priyanka Mangla, Quentin Vicentini, and Annabelle Biscans, "Therapeutic Oligonucleotides: An Outlook on Chemical Strategies to Improve

Endosomal Trafficking," *Cells* 12, no. 18 (Sept. 11, 2023): 2253, https://doi.org/10.3390/cells12182253.

14. Bowen Xiao et al., "Development, Opportunities, and Challenges of siRNA Nucleic Acid Drugs," *Mol. Ther.—Nucleic Acids* 36, no. 1 (March 11, 2025): 102437, https://doi.org/10.1016/j.omtn.2024.102437.
15. Vera S. Eragova et al., "Smart Delivery Systems Responsive to Cathepsin B Activity for Cancer Treatment," *Pharmaceutics* 15, no. 7 (June 29, 2023): 1848, https://doi.org/10.3390/pharmaceutics15071848.
16. Nareshkumar Jain et al., "Current ADC Linker Chemistry," *Pharm. Res*. 32, no. 11 (March 11, 2025): 3526–40, https://doi.org/10.1007/s11095-015-1657-7.
17. Janet M. Sasso et al., "The Evolving Landscape of Antibody-Drug Conjugates: In Depth Analysis of Recent Research Progress," *Bioconjugate Chem*. 34, no. 11 (Nov. 15, 2023): 1951–2000, https://doi.org/10.1021/acs.bioconjchem.3c00374.
18. Mahmood et al., "Clinical Pharmacology of Antibody-Drug Conjugates," *Antibodies*. 10, no. 2 (May 21, 2021): 20, https://doi.org/10.3390/antib10020020.
19. "Antibody Drug Conjugate—Molecule Synthesis," WuXi AppTec, https://wuxibiology.com/solutions/therapeutic-modalities/adc-molecules/.
20. Advanced Tumor models for Oncology and Immunotherapy Research," Wuxi Apptec, https://wuxibiology.com/biology-services/oncology-immunology/tumor-models/.

AMERICAN CHEMICAL SOCIETY
1155 Sixteenth Street, NW,
Washington, DC 20036