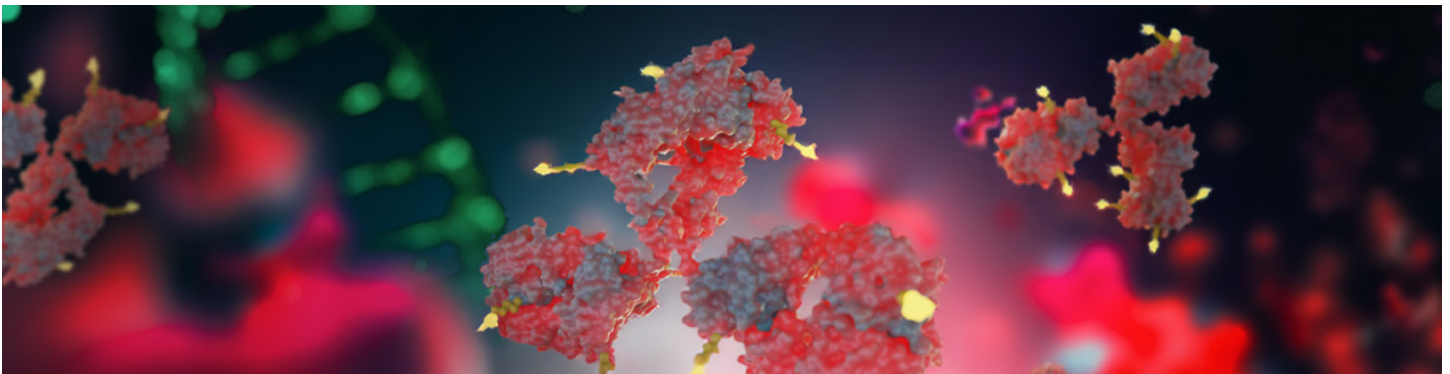


**Smarter ADCs
start with genetic
analysis insight**

Contents

Introduction	2
ADC Fundamentals	3
ADCs compared to other common therapeutic mAb modalities	3
Engineering and optimization of mAbs for ADC function	3
ADC structural components for design consideration	4
Summary	5
ADC research and development	6
Target antigen identification and characterization	6
Antibody discovery	7
mAb and payload conjugation	10
Summary	11
Factors for downstream product development	11
Process development (PD) and analytical development (AD)	11
Quality control (QC)	11
Clinical research and longitudinal monitoring	12
Conclusion	12



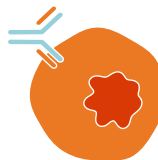
Introduction

Ongoing advancements in therapeutic monoclonal antibody (mAb) development are expanding the field beyond early IgG mAbs to include new modalities such as bispecific mAbs, antibody–drug conjugates (ADCs), and other engineered formats, with the aim of treating a broader range of diseases.^{1,2,3} ADCs combine the targeting precision of mAbs with the potency of cytotoxic payload to permeate directly inside target cells while minimizing off-target effects. Their growing importance is reflected in the broader antibody therapeutics landscape, with at least 212 approved antibody therapies worldwide as of August 2025, including 13 commercially available ADCs as of December 2024.^{1,3,4}

ADCs share several fundamental properties with other types of therapeutic mAbs but require additional design elements to help optimize conjugation and internalization properties. This eBook explores how genetic solutions can support R&D of ADCs across the full discovery pathway, including mAb design, conjugation site design, binding to the target cell, internalization, and bystander effects. Genetic solutions can play a valuable role in developing well-characterized ADCs and helping generate analytical insights that can inform ongoing ADC innovation.

Conventional IgG mAb

Binds a specific antigen on target or immune cells to initiate an immune response or modulate a pathway.



Bispecific mAb

Binds two different types of cells or pathway effectors simultaneously.



ADC

Binds the target cell and is internalized to deliver a cytotoxic drug directly inside the cell.



Cell death

Figure 1. Comparison of target cell elimination mechanisms by mAbs in common native IgG, bispecific, and ADC therapeutic modalities.

ADC fundamentals

ADCs compared to other common therapeutic mAb modalities

Unlike other therapeutic mAb modalities such as conventional IgG mAbs and bispecific mAbs that act outside the target cell, ADCs are internalized to kill cells from the inside (Figure 1).^{1,2,3,5}

- **Conventional IgG mAbs** are designed to destroy target cells externally. They bind a single antigen directly on the surface of the target cell or in the nearby environment to launch immune functions, block signaling, or neutralize a ligand or receptor.
- **Bispecific mAbs** also act extracellularly but can bind two different antigens simultaneously. They may be designed to bring immune cells closer to target cells; simultaneously block two signaling pathways; or bring multiple receptors together on the cell surface to trigger or disrupt signaling, activation, or inhibition pathways.
- **ADCs** are designed to kill target cells from the inside by combining a monoclonal antibody with a potent cytotoxic drug. The antibody binds to a target antigen on the target cell surface, triggering endocytosis and internalization of the ADC into the cell. Once inside, the payload is released and acts on critical intracellular processes, most commonly disrupting microtubule function or DNA integrity, to kill the cell.

Engineering and optimization of mAbs for ADC function

The three structural components of an ADC consist of a target-specific monoclonal antibody, a cytotoxic drug payload, and a chemical linker. The antibody serves as the delivery vehicle that binds target cells, while the payload acts as the weapon to destroy them. The linker ensures the ADC remains stable in circulation, releasing the drug only after internalization (Figure 2).^{6,7}

1. mAb design and optimization: ADC design begins with development and optimization of the mAb, the structural backbone of the ADC. Genetic solutions can be used to identify or analytically validate target antigens, engineer and confirm mAb variable region and conjugation site properties, accurately balance VH and VL gene ratios, and help ensure efficient antibody expression and proper HC/LC pairing.

2. mAb binding to the target antigen: Verifying that mAb binding activates the intended endocytic pathway and measuring associated molecular biomarkers can be important in correlating binding with internalization efficacy. A range of cell-based assays or imaging techniques are commonly used to assess antigen binding and internalization. However, genetic analyses may aid mAb optimization by associating mAb gene variants with high affinity binding, determining target antigen gene copy numbers to investigate binding variability, or evaluating immune-related gene

expression signatures to gain insights into unwanted immune response upon ADC target binding.

3. Internalization: Beyond high affinity antigen binding, maximizing ADC internalization requires optimization of bivalency, receptor clustering, and conjugation sites. Incorporating these factors into mAb gene design may help optimize ADC internalization with minimal immune response.

4. Cytotoxicity: Most currently approved ADCs induce cell death through DNA damage or microtubule disruption, although interest is growing in development of new mechanisms.⁴ Characterizing damage and resistance mechanisms through gene expression pathways, such as alterations in DNA repair, apoptosis, and cell-cycle regulation, may reveal insights into ADC-mediated cell death.

5. Bystander effects: When the released cytotoxic drug escapes into the surrounding microenvironment, it can kill neighboring cells even without mAb involvement. These “bystander effects” can be either beneficial if neighboring target cells are destroyed or harmful if healthy tissue is affected. Genetic regulation of pathways linked to these signals may present valuable analytical insights into ADC functionality.

ADC receptor target characterization

Before mAb development begins, the target antigen must be thoroughly characterized to confirm its biological relevance and suitability for antibody discovery. To maximize potential for strong ADC mAb action, the antigen should be overexpressed on target cells relative to healthy tissue, accessible for antibody binding, and capable of internalizing the antibody-antigen complex. Typically, clinical research samples and healthy tissue are analyzed using multi-omic analyses to confirm differential expression and internalization.

ADC structural components for design consideration

ADCs share several core features with other therapeutic mAbs, including target specificity, favorable binding properties, stability, and manufacturability. However, they also require additional design considerations to help enable site-specific drug conjugation, preservation of structural integrity after payload attachment, and efficient target-mediated internalization.

Optimizing heavy chain and light chain ratios

ADC binding, internalization, immunogenicity, and potency rely on balanced heavy chain (HC) and light chain (LC) pairing, variable region (VH, VL) design, and precise conjugation site selection to help produce a stable, high-affinity antibody that retains its payload in circulation and releases it upon internalization (Figure 3).^{4,6,7} Care must also be taken to minimize residue liabilities such as potential deamidation sites and charge variants that can reduce binding or stability, promote aggregation, or increase variability.

- Proper **HC-LC pairing** results in correct folding, prevents protein aggregation, and maintains antigen-binding integrity both before and after conjugation.
- The **HC-LC interface** acts as a scaffold to retain mAb folding, stability, and resilience even under the added strain of the conjugated payload.
- The **HC** contributes to immunogenicity and alterations in pharmacokinetics (PK). By fine-tuning Fc-mediated functions, the HC can be engineered to optimize half-life, maximize target cell exposure, and minimize immune-related toxicities.
- The **VH and VL** are engineered to maximize affinity and specificity for the target cell epitope. In ADCs, the variable regions carry the additional role of driving receptor-mediated internalization to enable efficient intracellular payload delivery with minimal off-target effects.

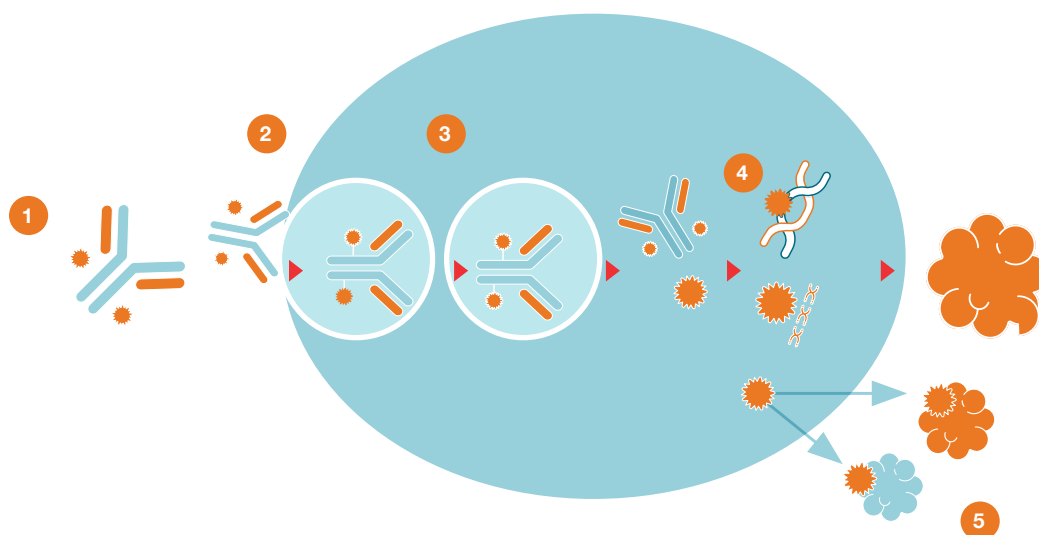


Figure 2. Genetic solutions enable exploration of mechanisms of action throughout the ADC pathway.

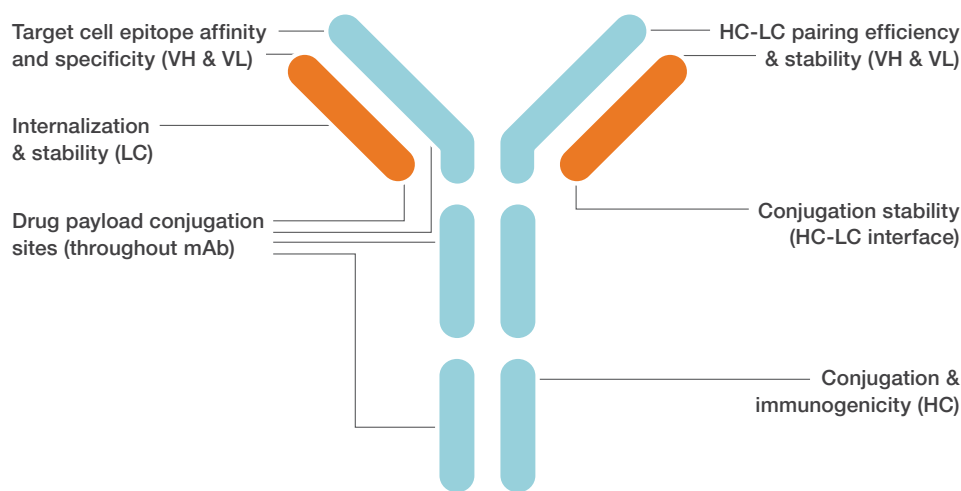


Figure 3. Key design elements for ADCs.

HC = heavy chain, LC = light chain,
VH = heavy chain variable region,
VL = light chain variable region.

Optimizing cysteine and lysine positions

Cysteine and lysine residues play dual roles in ADC design as both structural elements and payload conjugation sites (Figure 4).^{4,6,7} Careful management of both residue types is necessary for balancing structural integrity, conjugation consistency, and overall ADC quality (Figure 3).⁸

Native disulfide cysteines are integral for antibody folding, stability, and pharmacokinetics, and should be protected during conjugation to avoid uncontrolled payload attachment or product heterogeneity.

Lysine residues can offer a straightforward conjugation approach. However, because they are abundant and distributed across the antibody surface, consistent site selection can be difficult and potentially increase drug-antibody ratio (DAR) variability.

Conjugating at cysteine residues in the hinge region or interchain disulfide positions offers greater selectivity and improved homogeneity.

For maximum control, engineering cysteine motifs at specific locations in the mAb sequence enables precise, site-specific conjugation, supporting a consistent DAR and more predictable therapeutic profile.

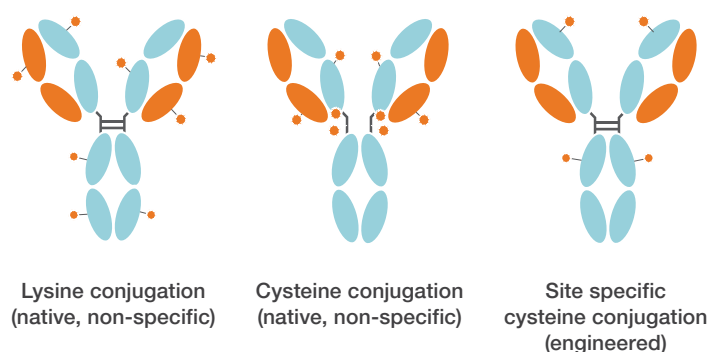


Figure 4. Lysine and cysteine conjugation site options.

Summary

ADC development requires co-optimization across multiple properties of the mAb including HC:LC pairing, target affinity and specificity, internalization efficacy, and cytotoxic payload delivery. Conjugation site distribution directly influences ADC homogeneity and performance. Understanding the genetic underpinnings of each component, including bystander effects and resistance mechanisms, supports more informed design decisions and stronger candidates entering preclinical development.

References

1. Chan AC, Martyn GD, and Carter PJ (2025) Fifty years of monoclonals: the past, present and future of antibody therapeutics. *Nat Rev Immunol* 25:745–765.
2. Kothari M, Wanjar A, Acharya S, et al. (2024) A comprehensive review of monoclonal antibodies in modern medicine: tracing the evolution of a revolutionary therapeutic approach. *Cureus* 16(6):e61983.
3. Liu JK. (2014) The history of monoclonal antibody development: progress, remaining challenges and future innovations. *Ann Med Surg (Lond)* 3(4):113–116.
4. Grairi M and Le Borgne M (2024) Antibody–drug conjugates: prospects for the next generation. *Drug Discov Today* 29(12):104241.
5. Lu RM, Chiang HL, Yuan JP, et al. (2025) Technological advancements in antibody-based therapeutics for treatment of diseases. *J Biomed Sci* 32(1):98.
6. Riccardi F, Dal Bo M, Macor P, et al. (2023) A comprehensive overview on antibody–drug conjugates: from the conceptualization to cancer therapy. *Front Pharmacol* 14:1274088.
7. Wu G, Yuan Z, Chen M, et al. (2026) Antibody–drug conjugates (ADCs): a review of structural design, technological evolution, and future perspectives. *Molecules* 31(7):1180.
8. Agarwal P and Bertozzi CR (2015) Site-specific antibody–drug conjugates: the nexus of bioorthogonal chemistry, protein engineering, and drug development. *Bioconjug Chem* 26(2):176–192.

ADC research and development

The mAb is the backbone that supports key functional ADC properties, and small sequence differences can lead to structural changes that potentially impact ADC performance. Antigen specificity and affinity, propensity to trigger unwanted immune response, DAR, internalization, and payload release must be co-optimized from the outset. Early integration of genetic technologies can supplement traditional protein analyses to help optimize mAb design.

Fundamental molecular objectives of ADC mAb development

ADC development shares several core molecular objectives with all therapeutic mAbs, including optimizing target epitope affinity, minimizing adverse immunogenicity and potentially destabilizing sequence risks, and maximizing structural stability. In addition, design strategies for ADCs should promote efficient internalization, optimize the DAR, and support efficient conjugation and release (Figure 5). Balancing these shared and complex objectives is important to creating a stable, potent, and manufacturable ADC therapeutic.¹

Humanization of ADCs minimizes potential immunogenicity

The cellular response triggered by mAb binding is a key distinction between extracellular-acting therapeutic mAbs and ADCs. Whereas some therapeutic mAbs are designed to initiate cell damage upon binding to the surface of the target cell, ADCs are designed to initiate endocytosis and internalization. To reduce immunogenicity risk, ADCs are commonly built from humanized or fully human mAbs, with Fc engineering used when needed to limit unwanted effector activity while preserving target specificity.

Fundamental molecular objectives

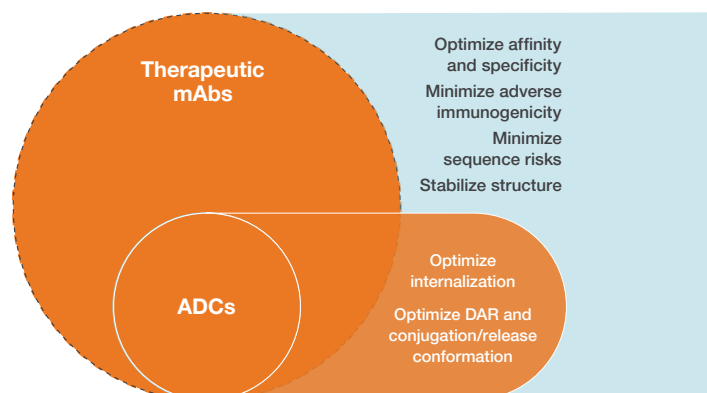


Figure 5. Fundamental molecular objectives of all therapeutic IgG mAbs and additional objectives specific to ADCs.

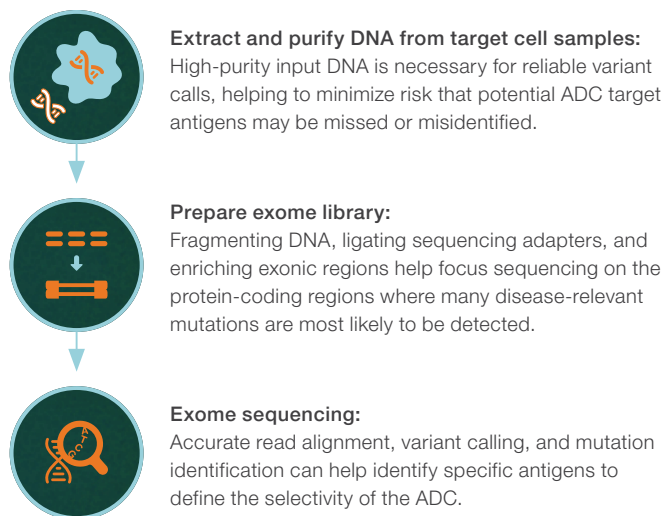


Figure 6. Steps in exome sequencing for ADC target antigen identification supported by genetic technologies.

Target antigen identification and characterization

The target characterization phase evaluates whether a cell-surface antigen is worth pursuing as an ADC target (see sidebar). When deployed early in the discovery phase before genetic diversity is established, genetic solutions can help maximize efficiency in downstream mAb screening and library engineering. Assessing whether there is high, selective expression on target cells and low expression on healthy cells can be done by measuring antigen gene expression with approaches such as qPCR, followed by confirmation that proteins are differentially expressed between normal and target cells. Disease-associated mutations and structural changes can be explored using Sanger sequencing and fragment analysis (Figure 6). Verifying target identity can be aided by exome sequencing, which may also help reveal other relevant low frequency variants. Assessing other functional properties, such as internalization efficiency and antigen shedding, is typically done using cell-based and protein-based methods such as flow cytometry, imaging, or binding assays.

Key properties of ADC target antigens

- High and selective expression on target cells and minimal on healthy tissue to reduce off-target toxicity
- Accessible for mAb binding on the target cell surface
- Internalizes efficiently after mAb binding to enable payload delivery
- Favorable trafficking to intracellular compartments that allow effective payload release.
- Low shedding into circulation, which could cause premature ADC binding.
- Homogeneous expression across the target cell population for reliable efficacy

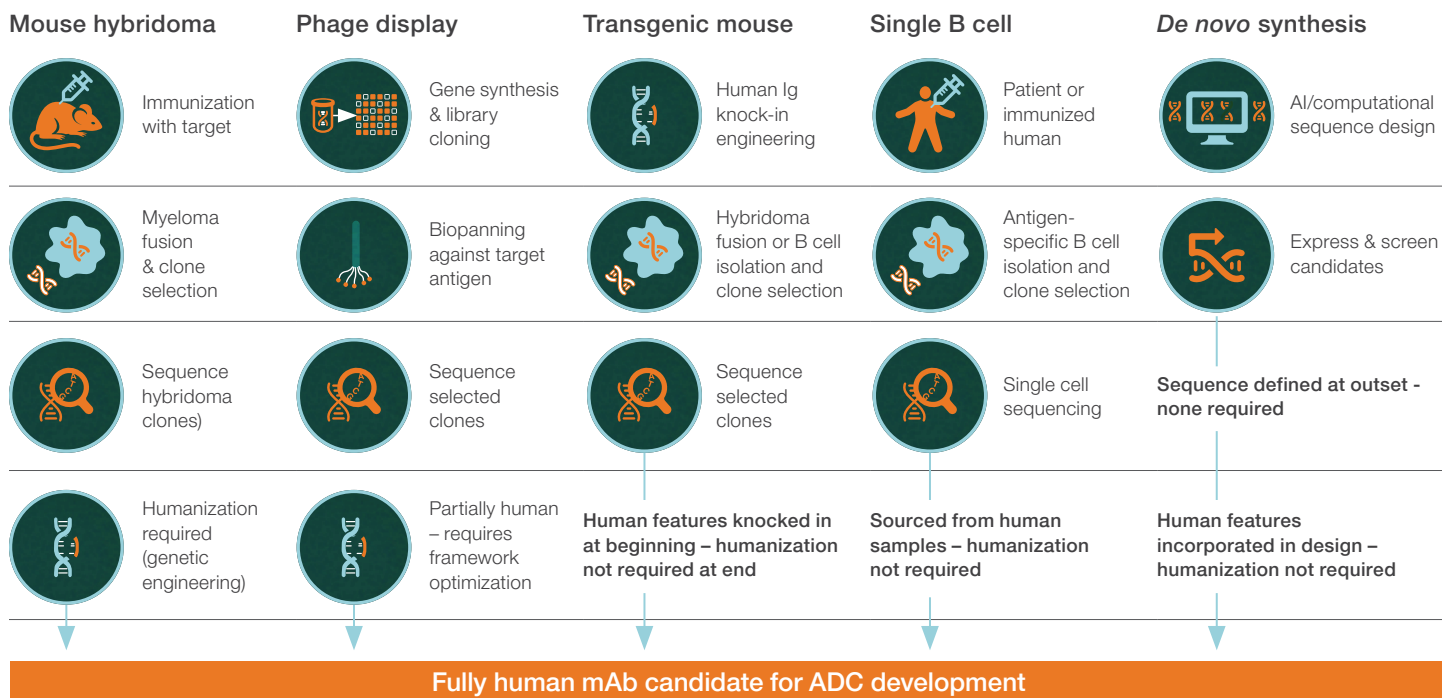


Figure 7. Comparison of methods used to generate diversity for mAb discovery.

Antibody discovery

Generate antibody diversity

Creating genetic diversity in the complementarity-determining region (CDR) maximizes the opportunity to identify mAb candidates with the binding properties needed for ADC development. There are several approaches to assembling large CDR collections to screen for optimal binding (Figure 7).² Hybridoma technology and phage display remain two of the most widely used approaches for antibody discovery, while additional strategies include isolating human mAbs from transgenic animals or single human B cells, as well as computational antibody design and de novo synthesis. De novo synthesis is gaining interest as protein analysis and computing technologies advance.² Genetic solutions including Sanger sequencing, fragment analysis, qPCR, and NGS help enable genetic engineering, screening, identification, and verification of key mAb sequence motifs in these methods.

Method 1: Selecting mAbs from hybridoma cells was one of the earliest methods to produce mAbs tailored to specific antigens of interest. It is highly reliable and efficient and is still commonly used to generate antibody diversity for therapeutic mAbs (Figure 8). Hybridomas preserve natural heavy/light chain pairing and can potentially yield strong target specificity and functional relevance early in selection. However, this method yields a narrower gene set than engineered libraries.^{2,3} For ADCs, mAbs derived from hybridomas also require humanization to avoid species-specific immune response.

Hybridoma-derived genes

Preserve natural VH–VL pairing in small sets of genes

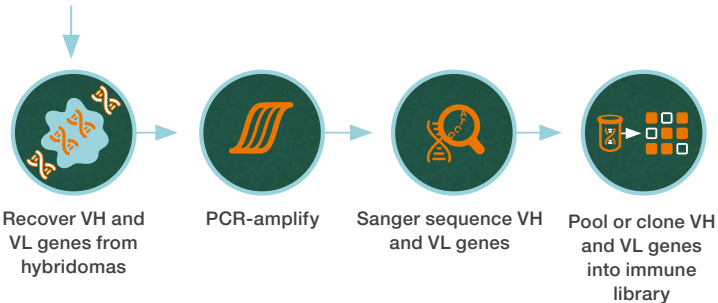


Figure 8. Steps in hybridoma-derived VH/VL gene library generation supported by genetic technologies.

- **Recover VH and VL genes from hybridomas:** Recovery and amplification of high-purity VH and VL DNA help reveal promising leads.
- **Sequence VH and VL genes:** Sanger sequencing captures the full diversity of the immune response at the sequence level.
- **Assemble VH and VL genes into an immune library:** A library of verified and pooled VH and VL genes provides a diverse collection of antigen-binding configurations to screen for potential ADC candidates.

Hybridoma technology

Hybridomas are created by fusing an antibody-producing B cell from an immunized mouse with an immortal myeloma cell. Each hybridoma cell line is characterized by the robust expression of a singular mAb, providing a source for ongoing production of identical antibodies. qPCR and DNA sequencing solutions can help verify clone identity.

Method 2: Phage display libraries can offer a vast genetic repertoire that can be used to rapidly screen for promising mAb candidates. Genetic engineering can enable greater design control than cell-based methods so multiple mAb characteristics can be optimized simultaneously (Figure 9). This multi-parameter method could result in a larger number of hits for ADC optimization. However, when libraries are developed using combinatorial genetic engineering, phage display iterations may introduce sequence liabilities or require additional work to confirm manufacturability.^{2,3}

Engineered genes

Create very large pools of randomized VL-HL pairing

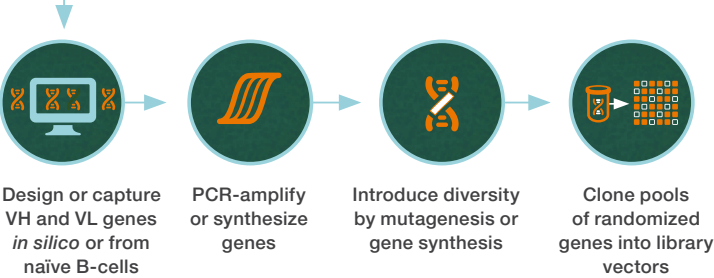


Figure 9. Steps in generating engineered VH/VL gene libraries supported by genetic technologies.

- **Design or capture VH and VL genes:** Library construction begins by designing VH and VL gene sequences in silico or capturing them directly from naïve B cells.
- **Amplify genes for the VH and VL library:** Amplification or synthesis of the VH and VL genes generates the raw material for library assembly.
- **Introduce diversity:** Diversity is introduced through intentional mutagenesis or gene synthesis, systematically randomizing key residues to greatly expand variation within the gene set.
- **Build randomized VH and VL gene library:** Libraries of randomized, cloned, and pooled VH and VL genes can produce very large and structurally varied antibody collections ready for screening against target-cell-specific antigens.

Select and optimize VH and VL genes

The VH and VL chains are cloned into phage display or mammalian expression systems with Fc or framework regions incorporated to tune function, stability, or conjugation compatibility.^{2,3} DNA sequencing can help verify structural integrity. Precise cloning, aided by DNA sequencing, supports high-yield production of full-length antibodies.

Phage display

Phage display was the first in vitro technique for antibody screening and is currently the most widely used method to establish antibody diversity. Phage display libraries for ADC development are constructed by cloning combinatorial libraries of VH and VL genes into phage vectors to express antibody fragments on the surface of bacteriophages.^{2,3}

Genetic engineering

Genetic engineering relies on recombinant DNA technology to combine DNA fragments from different sources to create a variety of genetic sequences not found in nature. The process involves isolating, cutting, and amplifying specific genes before inserting them into host organisms. Precise sequencing, fragment analysis, and PCR solutions are critical in helping ensure accurate and consistent constructs. This powerful methodology allows scientists to modify existing genes and mass-produce specific proteins.

Mammalian cell expression systems

Stable mAb producing cell lines are constructed by transfecting cells such as CHO or HEK293, with optimized antibody genes, allowing for complex post-translational modifications. In biopanning, these libraries display full-length mAbs on cell surfaces, enabling screening for the optimal ADC candidates.

Table 1: Comparison of hybridoma and phage display approaches to establishing genetic diversity.

Feature	Hybridoma (B-cell-derived)	Phage display (genetic engineering)
Source of diversity	In vivo immune response	In vitro designed libraries
Diversity size	Limited	Very large, highly customizable
VH–VL pairing	Natural, preserved	Often combinatorial or designed
Initial affinity	Typically, high (affinity-matured)	Variable; often lower initially
Epitope coverage	Biased by immunization	Broad, can target difficult epitopes
Immunogenicity risk	Higher (non-human origin, needs humanization)	Lower (use human frameworks)
Tunability	Limited control	Can design for ADC-specific properties
Screening effort	Lower (fewer, higher-quality hits)	Higher (more variants to triage)
Optimization required	Moderate (humanization, minor tweaks)	Higher (affinity maturation, stability optimization)
Speed to lead	Faster for good targets	Slower initial hit finding, faster iteration later
Design flexibility	Low	High (targeted mutations, CDR design)

Biopanning and genetic engineering are used iteratively to screen and enrich the antibody library for candidates with high-affinity target engagement and efficient internalization (Figure 10). Multiple rounds of biopanning selection combined with cell-based internalization assays help enable developers to simultaneously assess and refine both properties.

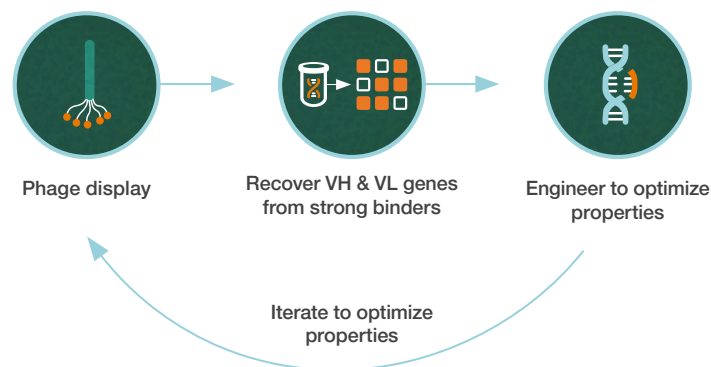


Figure 10. Steps in VH and VL gene selection supported by genetic technologies.

- **Display antibody fragments for screening:** Antibody genes from the VH and VL repertoire are cloned into phage display systems and expressed so each candidate antibody fragment is displayed on the surface of its host particle.
- **Recover VH and VL genes:** The library is exposed to the target antigen, then stringent washing enriches for high-affinity, specific binders. VH and VL genes from captured fragments are recovered by PCR, helping link functional properties to the molecular data needed to advance top candidates.
- **Engineer to optimize properties:** Recovered VH and VL genes may be revised using targeted mutagenesis or synthesis to help optimize properties before re-entering the display system for the next round of selection.
- **Narrow the pool of high-affinity candidates:** Recovered genes are repeatedly re-cloned, re-exposed to the target, and re-engineered, progressively narrowing the pool to the highest-affinity candidates.

Sequencing the VH and VL genes from lead clones serves four interconnected goals:

- Reveal motifs or residues in the antibody sequence that may create development, stability, or manufacturability risks
- Map cysteine and lysine residues for site-specific conjugation
- Support humanization to reduce immunogenicity risk in patients receiving the ADC.
- Verify that all intended modifications are accurately incorporated with no unintended mutations.

Sequence liabilities

Sequence liabilities in ADCs are amino acid motifs or residues in the antibody sequence that can create development, stability, immunogenicity, or manufacturability risks.⁴

These are some examples of sequence liabilities that can occur:

- **Deamidation:** Asparagine and glutamine residues in CDR regions may spontaneously deamidate, altering antibody charge and conformation, potentially reducing binding affinity and compromising ADC consistency.
- **Oxidation:** Methionine and tryptophan residues can be vulnerable to oxidation, which can destabilize the antibody structure, reduce target binding, and interfere with site-specific payload conjugation.
- **Aggregation-prone motifs:** Hydrophobic regions can drive antibody aggregation that can lead to reduced potency, higher immunogenicity risk, or manufacturability challenges that may be amplified by the presence of a conjugated cytotoxic payload.
- **Isomerization hotspots:** Residues such as aspartate can spontaneously rearrange over time and alter antibody structure, potentially reducing ADC stability, binding, or conjugation consistency.
- **Unpaired cysteines:** Unpaired cysteines can create reactive free thiols that lead to uncontrolled payload attachment, increased heterogeneity, and reduced ADC stability.
- **Protease-sensitive sites:** Some amino acid sequence motifs are susceptible to enzymatic cleavage, which can compromise antibody integrity, alter pharmacokinetics, or cause premature payload release.
- **Immunogenic sequences:** Some non-native or highly immunostimulatory regions may provoke anti-drug antibody responses, potentially reducing ADC efficacy.

Examples of ADCs that have been developed to minimize the impact of sequence liabilities include trastuzumab,⁵ brentuximab anti-CD30 antibody,⁶ and loncastuximab teserine.⁷

Humanization

Humanization is the process of modifying a non-human antibody, often using site-directed mutagenesis, to graft its antigen-binding CDRs onto a human antibody framework. This modification reduces immunogenicity while preserving ADC binding and conjugation.

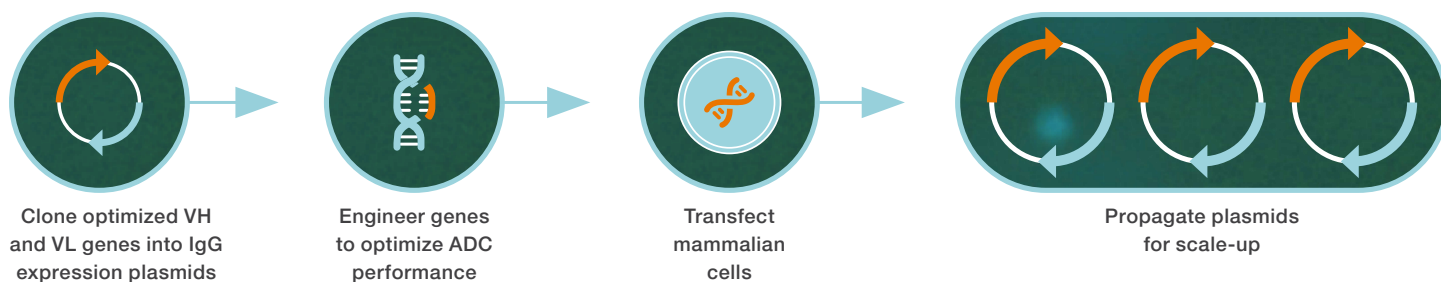


Figure 11. Steps in producing full-length IgG supported by genetic technologies.

Establish the mAb production system with full-length IgG in mammalian cell lines

Producing full-length IgG in mammalian cells (e.g. Chinese hamster ovary; CHO) marks the transition from gene-level optimization to functional protein, generating the intact antibody needed to evaluate binding, internalization, and conjugation properties (Figure 11).

- **Assemble VH and VL expression constructs:** Sequence-verified VH and VL genes are cloned into IgG expression plasmids to produce a structurally intact antibody ready for mammalian expression.
- **Engineer genes to optimize performance:** Additional genetic engineering may be needed to configure the expressed IgG for ADC development, including site-specific conjugation handles or Fc modifications to enhance stability or half-life.
- **Transfect expression plasmids for mAb expression:** Expression plasmids are transfected into mammalian cell lines, such as CHO or HEK293, for post-translational modification needed for proper folding, glycosylation, and assembly into functional IgG.
- **Propagate cells for scale-up:** Propagating successfully transfected cells helps generate sufficient IgG yields to support downstream functional assays, conjugation development, and early characterization of ADC candidates.

Well-characterized, stable cell lines are the foundation of reliable, high-quality mAb production. Clonal stability helps ensure each batch is genetically identical, minimizing variability in binding, internalization, and conjugation site availability, all of which can directly affect ADC performance. Single-cell subcloning may help establish genetically homogeneous populations, with sequencing used to verify VH and VL gene sequences across subclones. Profiling genetic variants can further support cell line identity tracking and help detect genetic changes that could compromise manufacturing consistency. Sequence-verified clones are cryopreserved into master cell banks to help establish a genetically defined, stable biological repository of the mAb DNA for future production.

mAb and payload conjugation

Payload selection

For ADCs, payload selection should not be conducted in isolation from mAb development. Endocytosis of the ADC constrains how much cytotoxic payload reaches the target cell. Therefore, ADC drugs are typically more potent than conventional chemotherapy drugs, driving selection toward highly cytotoxic agents that might otherwise be too dangerous systemically.⁸ Genomic and transcriptomic profiling can help identify the best target cells, mutations, or cytotoxic mechanisms for specific diseases, supporting smarter payload selection.

Conjugation strategy

Linker selection and conjugation are vital aspects of ADC development for consistent DAR, payload stability both before and during internalization, and payload release inside the cell.⁸ Given the high toxicity of ADC drugs, exceptional care must also be taken to minimize potential for free payload in the bloodstream. Traditional stochastic conjugation methods can result in heterogeneous mixtures with variable DAR, impacting efficacy and potency. Genetic engineering can be used to introduce non-canonical amino acids or engineered cysteines at precise locations for greater consistency.

Linker selection for ADCs also requires careful consideration.^{8,9}

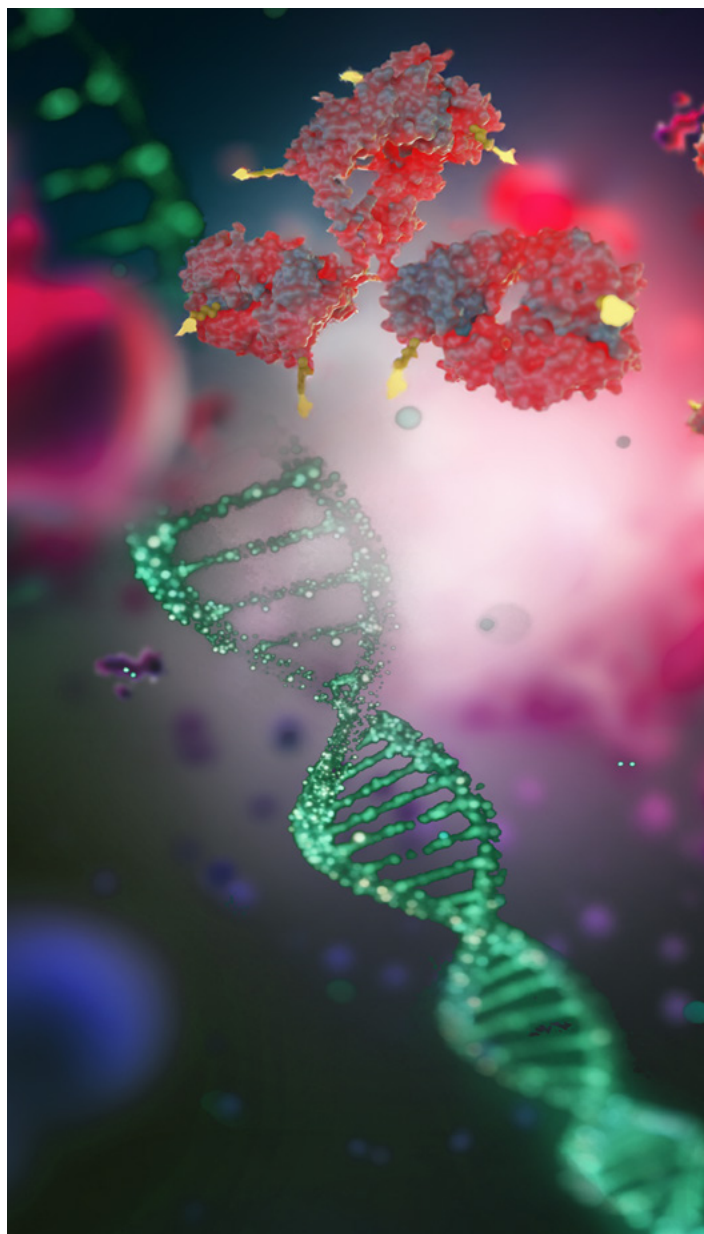
Two mechanisms are commonly used to release the payload once the ADC is inside the cell. Cleavable linkers exploit intracellular proteases or lysosomal conditions to detach the payload. Non-cleavable linkers rely on intracellular antibody degradation. Selection of the optimal linker is an iterative process. Transcriptomic and proteomic profiling can inform linker chemistry selection by helping identify disease-specific protease expression patterns or lysosomal conditions.

Summary

The primary objective of ADC R&D is to build a strong monoclonal antibody backbone, select the appropriate payload, conjugate the antibody with the drug, and test it for reactivity. Even small sequence changes can affect binding, internalization, stability, immunogenicity, and manufacturability. Genetic technologies can play a central role in helping shape monoclonal antibody design from the earliest stages. They can aid target identification, optimization of binding and internalization, and engineering of heavy and light chains for specificity, stability, and manufacturability.

References

1. Gairi M and Le Borgne M (2024) Antibody–drug conjugates: prospects for the next generation. *Drug Discov Today* 29(12):104241.
2. Han KH, Li YC, Parveen R, et al. (2025) Technologies for monoclonal antibody discovery and development. *Int J Mol Sci* 26(21):10470.
3. Liu JK (2014) The history of monoclonal antibody development: progress, remaining challenges and future innovations. *Ann Med Surg (Lond)* 3(4):113–116.
4. Raybould MJ, Marks C, Krawczyk K, et al. (2019) Five computational developability guidelines for therapeutic antibody profiling. *Proc Natl Acad Sci U S A* 116(10):4025–4030.
5. Matikonda SS, McLaughlin R, Shrestha P et al. (2022) Structure–activity relationships of antibody–drug conjugates: a systematic review of chemistry on the trastuzumab scaffold. *Bioconjug Chem* 33(7):1241–1253.
6. van de Donk NWCJ and Dhimolea E (2012) Brentuximab vedotin. *mAbs* 4(4):458–465.
7. Zammarchi F, Corbett S, Adams L, et al. (2018) ADCT-402, a PBD dimer–containing antibody drug conjugate targeting CD19-expressing malignancies. *Blood* 131(10):1094–1105.
8. Gu Y, Wang Z, and Wang Y (2024) Bispecific antibody drug conjugates: making 1+1>2. *Acta Pharm Sin B* 14(5):1965–1986.
9. Lei Y, Zheng M, Chen P et al. (2025) Linker design for the antibody drug conjugates: a comprehensive review. *ChemMedChem* 20(15):e202500262.



Factors for downstream product development

Process development (PD) and analytical development (AD)

During the PD and AD phases, process development teams optimize downstream manufacturing, leveraging analytical methods established during R&D to build efficient and reproducible processes that consistently meet quality attributes and chemistry, manufacturing, and controls (CMC) requirements. Understanding the mAb structural requirements and expression characteristics can help teams create procedures to maintain ADC stability, preserve conjugation site accessibility, and support consistent cell growth and mAb production. Analytical methods are developed in parallel to measure ADC properties such as binding activity, DAR, and conjugation site consistency. Genetic characterization of the cell line, including sequence confirmation and clonal stability data, can inform critical quality attributes and help define acceptable ranges for manufacturing parameters. These early molecular insights may help reduce risk of late-stage setbacks, simplify troubleshooting, and support CMC documentation and regulatory submissions.

Quality control (QC)

QC in ADC manufacturing relies heavily on the molecular and genetic groundwork established during R&D. Sequence-confirmed cell lines support a stable and traceable starting point, and ongoing genetic monitoring may help detect drift that could affect antibody expression or product quality. QC testing covers a broad range of attributes that may trace back to decisions made during antibody engineering including antibody identity, purity, potency, DAR distribution, and conjugation site consistency. Fragment analysis, sequencing, and expression profiling can support release and stability testing by helping confirm that the antibody component remains structurally intact and consistent over time. In addition to confirming ADC performance-related attributes, release testing should include sterility and mycoplasma analysis, to help ensure the product meets applicable release criteria. Sequencing microorganism contaminants may also be necessary for implementation of safeguards and ongoing process improvement. Maintaining tight control over these attributes is

vital because variability in the antibody portion of an ADC can potentially affect efficacy or toxicity. Together, the genetic and molecular work done during discovery and development can support a robust QC program, helping demonstrate that the product is consistent, potent, and well-understood.

Clinical research and longitudinal monitoring

Clinical research and longitudinal monitoring can generate rich datasets revealing how individual genetic profiles influence ADC response, resistance, and tolerability. During clinical research, genetic solutions can help enable tracking of mutations, expression levels, and genetic signatures that may reveal associations of molecular features with positive or adverse outcomes. Identified biomarkers may serve as surrogates for efficacy and progress monitoring. As genomic and clinical research data accumulate across programs and feed back into early R&D, they help create a growing knowledge base to support target selection, antibody engineering, and combination strategies for development of novel ADCs and the submission of the programs to the regulatory agencies.

Conclusion

The antibody therapeutics landscape is rapidly evolving, with ADCs emerging as an exciting modality to directly impact target cells while minimizing off-target effects. While ADCs share fundamental properties with other therapeutic mAbs, they demand additional engineering considerations for mAb affinity, immunogenicity, internalization, payload selection, and conjugation strategy.

Development of effective ADCs requires deep understanding of both the antibody and target antigen biology. Genetic solutions can yield valuable insights through the entire ADC R&D workflow from early discovery through engineering and manufacturing optimization, helping ensure candidates are well-characterized, and manufacturable. As clinical research data accumulates, these analytical technologies may play increasingly important roles in advancing more powerful ADC therapies in the future.

 Learn more at thermofisher.com/mab-development