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Editorial

Site-Specific Bioconjugation in Antibody−Drug Conjugates: From Conjugation Chemistry to Precision Molecular Design

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1. INTRODUCTION

Antibody−drug conjugates (ADCs) have emerged as one of the most successful therapeutic modalities in oncology, combining the target selectivity of monoclonal antibodies with the extraordinary potency of small-molecule cytotoxic agents. The original "magic bullet" paradigm established proof of principle but also exposed a fundamental constraint: biological targeting alone does not guarantee therapeutic precision. Early ADCs relied on murine antibodies, labile linkers, and moderately potent chemotherapeutics such as methotrexate, vinblastine, and doxorubicin.[1−3] Subsequent advances in antibody humanization, linker stability, and payload potency transformed the field, and the clinical success of multiple approved agents—including trastuzumab emtansine, trastuzumab deruxtecan, sacituzumab govitecan, enfortumab vedotin, mirvetuximab soravtansine, and datopotamab deruxtecan—has demonstrated that no single molecular component alone determines therapeutic efficacy.[4−10] Most of current approved ADCs remain chemically heterogeneous, produced through stochastic modification of lysine or cysteine residues that generates a broad distribution of drug-to-antibody ratios (DARs).[11−17] This heterogeneity has not precluded regulatory approval, but it does mean that clinical efficacy and safety often reflect a population-averaged pharmacologic outcome rather than a predictable structure−activity relationship.

Site-specific bioconjugation technologies—including engineered cysteines, enzymatic ligation, glycan remodeling, and noncanonical amino acid incorporation—install payloads at predetermined locations within the antibody scaffold, converting conjugation from probabilistic chemistry into controlled molecular engineering. In this Editorial, we argue that site-specific bioconjugation should be viewed not merely as another conjugation technology, but as an enabling design principle reflecting the continuing evolution of medicinal chemistry toward precision molecular engineering. We first examine how and why this shift occurred and what molecular precision contributes, then summarize the major site-specific conjugation platforms that have emerged over the past two decades, and finally review representative clinical programs—including PF-06804103, STRO-001/STRO-002, pivekimab sunirine, and micvotabart pelidotin (MICVO/PYX-201)—to illustrate how precise molecular architecture is reshaping the design, evaluation, and future direction of ADC therapeutics.

2. EVOLUTION OF ADC DESIGN

The development of ADCs illustrates a broader transformation occurring throughout medicinal chemistry, in which drug discovery has progressively shifted from empirical optimization toward mechanism-based molecular engineering. First-generation ADCs established the feasibility of targeted drug delivery but also revealed challenges associated with linker stability, payload selection, target expression, and systemic toxicity. Subsequent innovations in antibody engineering, linker chemistry, and payload pharmacology dramatically improved therapeutic performance, ultimately leading to the multiple regulatory approvals noted above.[18,19]

Despite these advances, conjugation chemistry itself remained relatively unchanged for most approved products.[11,15,20−22] Stochastic lysine- and cysteine-based strategies proved sufficiently robust to support commercial manufacturing and clinical success, but because an IgG molecule contains roughly 90 lysine residues and four interchain disulfide bonds, partial conjugation generates a distribution of DAR species typically spanning 0−8—a heterogeneity that is not merely a manufacturing characteristic but one that directly shapes pharmacology. High-DAR species tend to aggregate, clear faster from plasma, and drive off-target toxicity, whereas low-DAR species retain favorable pharmacokinetics but insufficient potency; conventional ADCs therefore behave as ensembles of related molecules with varying biological properties rather than as a single, well-defined therapeutic entity.[12,13] As ADCs expanded beyond traditional cytotoxic mechanisms toward bystander killing, immune modulation, extracellular targeting, and tumor-microenvironment-directed therapies, it became increasingly important to distinguish biological effects driven by target biology and payload pharmacology from those attributable to this molecular variability—making molecular architecture itself an experimentally controllable variable rather than merely a consequence of manufacturing.

This conceptual shift—reframing ADC development from a formulation challenge into a molecular engineering problem—marks an important milestone. Conjugation chemistry is no longer viewed solely as the process of attaching a payload to an antibody, but as a means of controlling molecular structure in ways that directly influence biological function. Site-specific bioconjugation has emerged as the key enabling technology within this evolving framework—not to replace conventional conjugation, which continues to underpin many successful therapies, but

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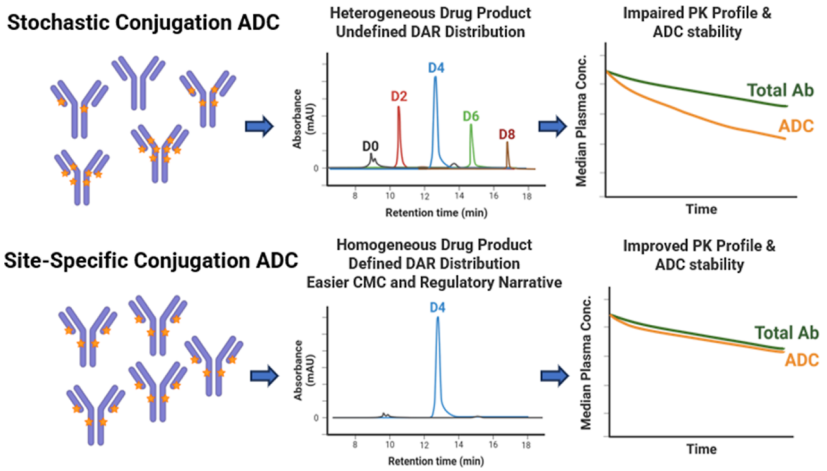

Figure 1. Comparison and impact between stochastic conjugation ADC and site-specific conjugation ADC.

to provide an experimental foundation that supports increasingly sophisticated medicinal chemistry and translational science.

3. WHY MOLECULAR PRECISION MATTERS

By fixing attachment site and stoichiometry, site-specific bioconjugation minimizes hydrophobic heterogeneity, stabilizes systemic exposure, and enables predictable intracellular payload delivery—allowing ADC behavior to be interpreted through defined structure−activity relationships rather than as an average over a heterogeneous mixture (Figure 1). Among the structural variables this controls, DAR remains one of the most influential: increasing DAR enhances payload delivery but can also increase hydrophobicity, aggregation, plasma clearance, and systemic toxicity, whereas excessively low DAR preserves favorable pharmacokinetics at the cost of antitumor activity. Successful ADC design therefore requires balancing payload potency against molecular stability and systemic exposure rather than maximizing any single parameter.

The site of attachment itself is equally important. Conjugation at different locations on the antibody can alter conformational stability, Fc receptor interactions, plasma disposition, intracellular trafficking, and susceptibility to proteolytic cleavage. Conventional stochastic conjugation averages these effects across heterogeneous molecular populations, obscuring mechanistic interpretation; site-specific conjugation reduces this variability and allows direct investigation of how molecular architecture governs pharmacological behavior.[23,24]

Molecular precision, however, should not be equated with superior efficacy. The continued clinical success of conventionally conjugated ADCs demonstrates that heterogeneous conjugation remains an effective, clinically validated strategy. Rather, the principal advantage of site-specific conjugation lies in the mechanistic clarity it provides—clarifying how molecular architecture shapes pharmacokinetics, biodistribution, payload release, efficacy, and safety—and in the experimental reproducibility and structure-activity relationships it enables to guide rational optimization. This advantage becomes increasingly relevant as ADCs move beyond conventional intracellular cytotoxic delivery toward extracellular matrix targeting, immune modulation, targeted protein degradation, and other emerging mechanisms that demand corresponding advances in molecular control.

4. SITE-SPECIFIC BIOCONJUGATION TECHNOLOGIES

Multiple strategies have been developed for site-specific conjugation, each balancing practicality, stability, and scalability differently. Although mechanistically distinct, they share a common design logic: incorporation of a unique reactive handle that permits selective modification while preserving antibody folding, antigen recognition, and Fc-mediated trafficking. No single platform has emerged as universally optimal; rather, the appropriate strategy depends on the antibody scaffold, the payload's physicochemical properties, and the intended mechanism of action.

Over the past two decades, several complementary strategies have emerged to achieve this objective. Engineered cysteine platforms, pioneered by THIOMAB[25] and subsequently expanded through symmetric DAR4 designs,[26−29] demonstrated that genetically encoded reactive thiols could produce homogeneous ADCs while preserving antibody structure and function. Alternative approaches—including noncanonical amino acid incorporation,[30−34] aldehyde-tag chemistry,[35−38] glycan-directed conjugation,[39,40] transglutaminase- and sortase-mediated ligation,[41−47] and affinity-guided lysine modification[48,49]—achieve the same goal through different chemical and enzymatic mechanisms (Table 1). Although these technologies differ in implementation, they share a common design philosophy: introducing a unique, selective conjugation site that minimizes molecular heterogeneity while maintaining manufacturability and biological activity.

Importantly, no single platform has emerged as universally superior. The optimal strategy depends on the antibody scaffold, payload physicochemical properties, desired DAR, mechanism of action, and manufacturing requirements. Collectively, these technologies illustrate the maturation of ADC development from empirical conjugation chemistry toward rational molecular engineering, where conjugation is viewed as an integral component of medicinal chemistry design rather than simply a manufacturing process. As ADC mechanisms continue to expand beyond intracellular cytotoxic delivery to include extracellular matrix targeting, immune modulation, and novel payload classes, molecular precision will become increasingly valuable for establishing reproducible structure−activity relationships and accelerating translational development.

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Table 1. Evolution of Site-Specific Bioconjugation Platforms

Platform	Conjugation Principle	Representative Company/Example
Engineered cysteine conjugation	Maleimide coupling to introduced thiols	Genentech (Thiomab);[25] Pfizer (kK183/K290)[27,28]
Transglutaminase-mediated conjugation	Enzymatic amide bond to endogenous Q295 or enzymatic coupling via short reactive peptide tag	Pfizer;[42] Araris[43,44]
Aldehyde Tag (SMARTag)	CxPxR motif insertion for FGE-generated formylglycine + aldehyde handle for HIPS conjugation	Redwood Bioscience/Catalent (TRPH-222)[35,36]
Noncanonical amino acid incorporation	Orthogonal ligation (i.e. oxime ligation) at ncAA	Ambrx (ARX788);[30] Sutro (STRO-001/STRO-002)[31−34]
Glycan-directed conjugation	Fc N297-glycan remodeling via copper-free "click" chemistry	Synaffix (GlycoConnect)[39,40]
Sortase-mediated conjugation	Transpeptidation between LPXTG-tagged antibody and glycine-modified payload	NBE Therapeutics[45−47]
Affinity-guided conjugation	Fc-affinity peptide directs native lysine (K248 & K288) modification by installing a thiol handle	Ajinomoto (AJICAP reagent)[48,49]
Emerging precision conjugation platforms		Dual payloads, programmable conjugation, multifunctional ADCs

5. LESSONS FROM CLINICAL TRANSLATION

Ultimately, the impact of any molecular engineering strategy is determined not by its chemical elegance but by its contribution to patient outcomes. The clinical case studies below illustrate how these site-specific conjugation principles translate into pharmacologic outcomes across distinct therapeutic settings.

PF-06804103 was one of the earliest clinical demonstrations of symmetric DAR4 cysteine engineering, applied to a HER2-directed ADC.[27,29] Although development of the molecule was ultimately discontinued as the HER2 therapeutic landscape evolved rather than because of limitations of the conjugation platform itself, the program established the clinical feasibility of producing chemically defined ADCs with predictable pharmacokinetic behavior and reproducible drug loading, and demonstrated that molecular precision could be translated from protein engineering into large-scale clinical development.

STRO-001 and STRO-002 were developed using genetically encoded noncanonical amino acids incorporated through cell-free protein synthesis, achieve exceptional control of conjugation site and stoichiometry.[31−34] Beyond their technological innovation, these programs demonstrate how chemically homogeneous ADCs facilitate systematic investigation of structure-activity relationships and accelerate rational optimization during drug discovery, displaying high serum stability and controlled pharmacokinetics in early clinical evaluation.

Pivekimab sunirine (IMGN632) is a CD123-directed ADC constructed with engineered cysteine sites, with conjugation via a cleavable linker−payload architecture generating an approximately DAR2 molecule.[50,51] Despite the hydrophobicity of the payload, a relatively uniform DAR2 species was obtained, and this ADC demonstrated prolonged systemic exposure in vivo. in clinical studies, the agent showed activity in acute myeloid leukemia and high response rates in blastic plasmacytoid dendritic cell neoplasm (BPDCN).[51,52] Importantly, these responses occurred in a disease characterized by high and relatively homogeneous CD123 expression, and the safety profile was consistent with target-mediated hematologic toxicity rather than nonspecific off-target exposure. These findings illustrate a central principle: structural homogeneity does not inherently broaden the therapeutic window but allows toxicity to track with antigen distribution. Site-specific conjugation improves interpretability but does not eliminate mechanism-based toxicities. Pivekimab sunirine-pvzy (Decnupaz) was approved by the U.S. FDA in May 2026 for adults with BPDCN.

Micvotabart pelidotin (MICVO/PYX-201) is an extracellular matrix-targeted ADC directed against the extradomain-B of fibronectin (EDB+FN). Unlike internalizing ADCs, this molecule was designed to release payload within the extracellular matrix of the tumor microenvironment following protease cleavage of the linker, enabling diffusion-mediated cytotoxicity. Defined cysteine conjugation (Figure 2) yields a DAR ≈4 molecule with reproducible pharmacokinetics. Preclinically, MICVO showed a therapeutic index of 4−16 at HNSTD ≥12 mg/kg, versus 2−4.9 at a lower HNSTD (≥5 mg/kg) for a conventionally conjugated counterpart.[28] Preclinical models also demonstrated activity across several tumor types,[53] consistent with a mechanism dependent on payload diffusion rather than cellular antigen internalization. MICVO is currently under clinical investigation both as monotherapy (NCT05720117) and in combination with pembrolizumab (NCT06795412) for the treatment of advanced solid tumors. Early clinical evaluation showed tolerability and preliminary antitumor activity, with systemic exposure consistent with a stable circulating ADC species.[54] The program demonstrates how site-specific conjugation supports reproducible pharmacokinetics in an extracellular matrix-targeted ADC, enabling mechanistic evaluation of payload release within the tumor microenvironment and illustrating the expanding biological applications of precision molecular design.

Collectively, these programs demonstrate that the principal contribution of site-specific bioconjugation lies not in establishing a universally superior ADC platform, but in expanding the experimental framework through which molecular design can be related to biological function. Whether the objective is improving therapeutic index, optimizing pharmacokinetics, investigating extracellular drug delivery, or establishing predictive exposure−response relationships, molecular precision strengthens translational understanding and supports increasingly rational therapeutic development.

6. FUTURE OPPORTUNITIES

The evolution of ADCs is increasingly shaped by biological mechanism rather than conjugation chemistry alone. The field's future progress will depend less on identifying a universally superior conjugation chemistry than on integrating molecular precision with advances in target biology, medicinal chemistry, protein engineering, and quantitative translational science.

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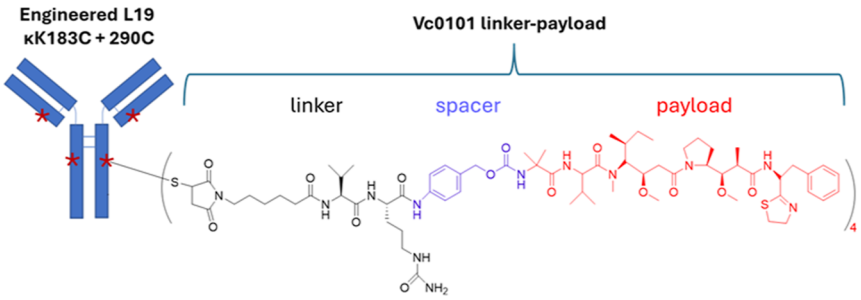

Figure 2. Structural representation of the DAR4 ADC MICVO. MICVO is composed of an anti-EDB+FN mAb derived from the L19 clone. The antibody was engineered with cysteines κK183C and K290C for site-specific conjugation. The auristatin Aur0101 payload was conjugated to the mAb via the PABC spacer and a mc-Val-Cit linker (vc0101 linker-payload).

Payload diversification. Although auristatins, maytansinoids, camptothecin derivatives, and DNA-damaging agents have demonstrated remarkable clinical success, future ADCs are likely to expand toward payloads whose activity depends on exposure kinetics rather than maximal potency—transcriptional inhibitors, protein degraders, immune agonists, radiotherapeutics, and nucleic-acid-based cargos. The use of dual payloads, attached in various ratios to a single targeting moiety with distinct mechanisms of cell killing, has also gained momentum. Such payloads require controlled release profiles, making predictable conjugation stoichiometry experimentally important for interpreting pharmacology.

Context-dependent linkers. Linkers are increasingly designed as programmable molecular switches that respond to local biochemical environments—protease activity, pH gradients, or oxidative stress—rather than serving solely as passive connectors. In these systems, therapeutic outcome depends on release location rather than circulation half-life alone, and uniform attachment sites help decouple linker behavior from distributional variability.

Scaffold and targeting architectures. Bispecific antibodies, biparatopic constructs, conditionally activated antibodies, antibody fragments, and other alternative scaffolds aim to improve tissue penetration and subcellular trafficking. As molecular formats diversify, defined conjugation becomes useful for maintaining comparable exposure across structurally different targeting platforms.

Combination pharmacology. ADCs are increasingly integrated with checkpoint inhibitors, DNA-damage response modulators, or targeted therapies, with the ADC functioning as a spatially restricted drug-delivery system rather than a standalone cytotoxic agent. Controlled drug loading facilitates prediction of overlapping toxicities and optimization of dosing schedules.

Resistance biology. Resistance mechanisms—antigen heterogeneity, impaired internalization, drug efflux, or microenvironmental protection—may be addressed through extracellular delivery strategies, dual payloads, immune-mediated mechanisms, or combination treatments. In such approaches, interpretability of exposure–response relationships becomes more valuable than maximal potency alone.

Predictive modeling and biomarker selection. Artificial intelligence, machine learning, quantitative systems pharmacology, structural modeling, and spatial pathology analyses increasingly rely on high-quality datasets that accurately relate molecular structure to biological behavior. By minimizing structural heterogeneity, site-specific conjugation strengthens the predictive value of these models and supports increasingly rational therapeutic optimization; emerging extracellular targeting strategies, exemplified by MICVO and related approaches, further illustrate how molecular precision can enable entirely new mechanisms of therapeutic intervention.

Importantly, the future of ADCs is unlikely to be defined by any single optimal conjugation platform. Conventional stochastic conjugation will remain a highly successful and clinically validated strategy for many applications, particularly where potency dominates performance, while site-specific constructs are most informative when distribution, release location, or immune interaction determine activity. Future progress will therefore arise not from replacing existing methodologies but from matching payload mechanism, target biology, and molecular architecture—expanding the molecular design toolbox available to medicinal chemists rather than seeking a single superior technology.

7. CONCLUDING PERSPECTIVE

The remarkable progress of antibody–drug conjugates over the past two decades[18,19,24,55] reflects the convergence of advances in medicinal chemistry, protein engineering, cancer biology, and translational science. ADCs have evolved from proof-of-concept targeted cytotoxic agents into sophisticated molecular therapeutics whose performance depends on the coordinated integration of multiple molecular components rather than any single technological innovation.

Within this broader context, site-specific bioconjugation represents an important milestone in the maturation of ADC design. Its significance extends well beyond generating homogeneous molecular products: by reducing structural variability and enabling precise control of molecular architecture, it provides a framework for understanding how antibody engineering, linker chemistry, payload pharmacology, and tumor biology collectively determine therapeutic performance—transforming conjugation chemistry from a manufacturing process into an enabling element of rational molecular engineering.

The future of ADC development will not be determined by identifying a single, universally superior conjugation chemistry. The extraordinary clinical success of conventionally conjugated ADCs clearly demonstrates that heterogeneous conjugation remains an effective and highly validated therapeutic strategy. Rather than replacing these established approaches, site-specific bioconjugation complements them by enabling more sophisticated mechanistic investigation and more robust structure–function relationships. Although most currently FDA-approved ADCs do not yet employ site-specific conjugation, the field is advancing rapidly, and next-generation site-specific

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ADCs are well positioned to provide clearer mechanistic insight into how conjugation, target biology, and payload design interact to shape clinical outcomes.

Perhaps its greatest contribution is conceptual: as ADCs evolve from empirically optimized cytotoxic agents into precision-engineered molecular therapeutics, conjugation chemistry becomes an integral component of biological design rather than simply a means of attaching a payload. Looking ahead, continued advances in protein engineering, linker innovation, payload diversification, computational biology, systems pharmacology, and spatial biology will further expand the capabilities of ADC therapeutics, and future success will depend on integrating these disciplines into coherent molecular designs that translate efficiently from laboratory discovery to clinical benefit. Ultimately, the future of ADCs will be defined not simply by greater molecular precision, but by greater biological understanding—and site-specific bioconjugation should be viewed not as an endpoint in itself, but as an enabling design principle that supports the rational development of the next generation of precision therapeutics.

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