Chemically Programmable Antibody-Drug Conjugates: Strategies from Targeting Programmability to Payload Activation

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Abstract. Antibody-drug conjugates (ADCs) are now highly effective anticancer drugs, but their molecular functions are relatively fixed at the time of production and cannot adapt to changes in the tumor microenvironment. Chemical programmability refers to the ability to modify a predefined antibody platform after scaffold construction by exchanging, activating, or reorganizing functional modules, providing modularity and control over various functional levels in ADC design through a versatile antibody platform that can be chemically altered in terms of targeting and effector functions. Hardware-level programmability refers to the engineering of universal antibody scaffolds and conjugation sites; software-level programmability refers to exchangeable targeting ligands, payloads and release mechanisms that define therapeutic functions. The five functional levels of chemically programmable strategies are: targeting programmability, assembly programmability, target-engagement programmability, conjugation programmability, and payload-activation programmability. We trace the conceptual origins to chemically programmed antibodies using catalytic antibody 38C2 and examine the core chemical toolbox, including inverse electron-demand Diels-Alder click chemistry, click-to-release technology, sulfur (VI) fluoride exchange chemistry, and non-canonical amino acid-based conjugation. These tools have been applied in four ways: in vivo click-driven assembly, small-molecule reprogramming, antibody fragment platforms and bispecific ADCs. Key challenges are discussed alongside future directions such as integrated multi-level programmable design.

Keywords: antibody-drug conjugates, chemical programmability, targeting programmability, click chemistry, bioorthogonal chemistry.

1. Introduction

Antibody-drug conjugates (ADCs) are constructed by linking a target-recognising monoclonal antibody to a toxic small-molecule agent via a chemical linker. Since the first ADC approval in 2000, the technology has been developing steadily, and newer agents such as trastuzumab deruxtecan (T-DXd) have added humanized antibodies, cleavable linkers, site-specific conjugation and topoisomerase I inhibitor payloads [1]. As of 2025, 15 ADCs have been approved for clinical use, with numerous additional candidates in clinical development [2].

ADC efficiency in solid tumors has not improved significantly to date. Several physical barriers have been established by the tumor microenvironment to restrict ADC penetration, and generally, tumor uptake typically amounting to less than 0.1% of the administered dose per gram of tissue [3]. Intratumoral heterogeneity results in different antigen expressions, and antigen loss, along with drug efflux pump upregulation and impaired trafficking, promotes acquired resistance [4]. The root cause of these limitations lies in the monolithic design of conventional ADCs: molecular functions—targeting specificity, payload conjugation site, drug-to-antibody ratio, and payload release kinetics—are largely predefined during manufacture, rendering them static properties incompatible with dynamic tumor evolution [5].

Next-generation ADC architectures, such as bispecific ADCs and probody-drug conjugates, aim to add regulation to targeting modules [6]. A 2024 review reported that 27 bispecific ADCs had entered clinical trials [7], and izalontamab brengitecan (BL-B01D1) had shown positive phase III results [8]. At present, these strategies are mainly antibody engineering and have not fully realized the prospects of chemical approaches.

Chemical programmability introduces a new model by adding modularity and controllability to all levels of function: targeting programmability (redirecting specificity to different antigens), assembly programmability (modular construction from components), target-engagement programmability (regulating binding avidity and spatial organisation), conjugation programmability (site-specific payload attachment with fixed stoichiometry), and payload-activation programmability (controlled release triggered by external or microenvironmental stimuli). The start of this idea is chemically-programmed antibodies (cpAbs), which have small-molecule ligands attached at specific locations on catalytic antibodies to add new binding functions [9-10]. Bioorthogonal chemistry and click chemistry have matured to some extent, and now they are being applied in vivo. This paper will systematically introduce the concept system, core chemical toolbox, implementation path, and related problems of chemically programmable strategies for ADCs at the five functional levels. The conceptual framework has been further developed through several comprehensive reviews that have shaped the field [11-14].

2. Conceptual Framework of Chemically Programmable ADCs

2.1. Origin: From "One Antibody-One Target" to Chemical Programming

Conventional monoclonal antibodies generally have a one-to-one correspondence between antibodies and antigens. Specificity of the target is realized via biological pathways and remains unchanged after the antibody is produced.

The foundation for chemically programmable antibodies (cpAbs) was laid in 1995, when Wagner and his colleagues reported the catalytic antibody aldolase 38C2; it has an abnormally nucleophilic lysine residue at its active site and was produced by reactive immunization with a 1,3-diketone hapten [10]. This lysine can form a stable covalent bond with small-molecule ligands that contain a 1,3-diketone or beta-lactam group. Based on this tool, Rader and his colleagues in 2003 first presented the concept of cpAb by demonstrating that small-molecule ligands could be covalently attached to 38C2 to provide new binding specificities [11]. The target specificity is no longer determined by the antigen-binding site of the antibody but by the conjugated small-molecule ligand; therefore, the antibody has changed from a targeting element to a functional carrier of the pharmacophore. In 2006, Guo and his group further developed this idea to propose an improved "proadapter" assembly strategy that extended the scope and in vivo application of the cpAb approach [9].

2.2. Functional Levels of Chemical Programmability

To organize the various chemical strategies reported in the literature systematically, this review puts forward a two-level framework for chemical programmability of ADCs. Programmability, as defined in this framework, requires that a predefined molecular platform can be reconfigured after scaffold establishment by exchanging, activating, or reorganizing functional modules without redesigning the entire antibody architecture. Therefore, a programmable system is not a fixed conjugate with all functional parameters set at the time of production. Chemical programmability has the following two kinds. Hardware-level programmability refers to the engineering of antibody scaffolds and conjugation sites, such as non-canonical amino acid incorporation and SuFEx-reactive handles, which provide defined, universal interfaces for subsequent functionalisation. The above changes are not therapeutic in nature themselves; rather, they offer a stable foundation for adding various functional modules. Software-level programmability refers to the exchangeable functional modules themselves—targeting ligands, payloads, and release mechanisms—whose identities determine therapeutic specificity and activity. Together, the two levels make up the entire chemical programmability system. The five functional levels in the system have been set up to cover all parts of chemical programmability.

Targeting programmability refers to the ability to redirect an ADC’s specificity to different antigens by exchanging the targeting ligand. Targeting specificity in traditional ADCs is permanently fixed by the complementarity-determining regions of antibodies. Chemical programming relocates

this specificity to small-molecule ligands, peptides, or aptamers that can be readily exchanged, enabling rapid adaptation to antigen drift or patient-specific target profiles [15,16].

Assembly programmability is a modular construction of ADC components—antibody scaffold, targeting ligand, linker and payload—through chemical reactions rather than covalent fixation during manufacture. Thus, the same antibody scaffold can be attached to various targeting ligands or payloads through different reaction paths for flexible and rapid construction of ADC variants [5].

Target-engagement programmability is the capacity to regulate binding avidity, valency and spatial organisation of targeting modules. Chemical modification can be used to control both the number and arrangement of targeting ligands on the antibody scaffold precisely, thereby tuning binding affinity and cellular-internalisation kinetics [17-18]. Affinity modulation can be used to improve tumor penetration; for example, intermediate-affinity antibodies distribute more evenly in the tumor than high-affinity ones due to a reduced binding-site barrier effect [19].

Conjugation programmability refers to the capability to control the site, stoichiometry and homogeneity of payload attachment in a way that allows the same scaffold to be modified with different payloads, DARs or conjugation patterns without altering the antibody. Conventional conjugation relies on native lysine or cysteine residues and yields heterogeneous products; chemically programmable conjugation, however, can achieve site-specific conjugation with defined drug-to-antibody ratios (DARs) through non-canonical amino acid incorporation, enzymatic modification, or bioorthogonal reactions [20,21].

Payload-activation programmability is the capacity to regulate the time, position and way of release of the payload. Through click-to-release linkers, enzyme-cleavable sequences, or photoresponsive groups, payload release can be triggered by external stimuli (e.g., tetrazine administration, light) or internal microenvironmental cues (e.g., tumor-specific proteases, acidic pH), achieving spatiotemporal control of cytotoxicity [22,23].

The five functional levels and the hardware/software division are, together, the conceptual system proposed in this paper for analysing and classifying chemically programmable ADC strategies. The five levels above are not mutually exclusive; rather, they can be integrated into a single ADC design to achieve multi-dimensional programmability. Together, they constitute the foundation of chemical programmability; that is to say, the antibody scaffold is a universal platform for chemical modification and various functionalisations.

2.3. Implications for ADC Design

A multi-level chemical programmability framework has been proposed for ADC design. First, it separates the traditional connection among targeting, payload delivery and payload release into independent functional modules for optimisation. Secondly, it supports fast iteration and customisation; a single antibody scaffold can be reconfigured for various antigens, different payloads or different release mechanisms by modifying only the chemically linked parts. Third, it offers a flexible solution for tumor heterogeneity and acquired resistance: when a tumor loses the target antigen or increases expression of drug efflux pumps, the ADC can be reprogrammed to target another antigen or deliver a different payload without modifying the whole molecule.

This framework also clarifies the relationship between chemical programmability and other next-generation ADC strategies. Bispecific ADCs, for instance, are created by protein engineering of antibody structures to achieve dual targeting [7], and probody-drug conjugates are another type of target-engagement programmability realized through reversible masking [24]. Chemically, it offers good modularity, is readily synthesizable, and new functions that cannot be obtained by recombinant DNA technology can be added [21].

3. Chemical Toolbox for Programmable ADCs

Chemically programmable ADCs use high-precision chemical reactions for site-specific conjugation, controlled release and in vivo assembly. The four main reaction classes and their different functions in the multi-level programming environment are introduced in this section.

3.1. Inverse Electron-Demand Diels-Alder (IEDDA) Reaction: Enabling Assembly and Targeting Programmability

The IEDDA reaction between a tetrazine and a strained alkene, generally trans-cyclooctene (TCO), has become the first choice for bioorthogonal conjugation. This catalyst-free cycloaddition has high selectivity and efficiency, with a second-order rate constant of more than 800 $M^{-1}s^{-1}$, and is therefore highly suitable for low-reactant-concentration environments in vivo.

The two functions of IEDDA in chemical programmability are as follows. First, it can realize assembly programmability by allowing modular conjugation of targeting ligands or payloads to the antibody scaffold in a site-specific manner. Second, it is targeting programmable; that is, by exchanging targeting modules, one can change the specificity of a TCO-modified antibody to various other targets without modifying the antibody itself.

IEDDA has been used for site-specific conjugation in ADC development, and Tomarchio and his colleagues recently reviewed the applications of tetrazine-trans-cyclooctene ligation in antibody conjugation and pretargeted strategies systematically [25]. In addition to ex vivo conjugation, IEDDA has been widely used in pretargeted imaging and radioimmunotherapy to administer a TCO-modified antibody first, followed by a rapidly and selectively ligating radiolabeled tetrazine probe in vivo. The same ideas are now being applied to ADC development, and IEDDA can achieve both site-specific payload installation and in vivo assembly [5].

3.2. Click-to-Release Technology: Enabling Payload-Activation Programmability

IEDDA is used to achieve stable conjugation, and then click-to-release chemistry is employed to release the payload. A TCO derivative is used as a cleavable linker in this design, and after tetrazine reaction, the TCO undergoes rearrangement to release the payload.

The function of click-to-release technology is payload-activation programmability, which can be triggered in space and time by an external chemical stimulus. This is not the same as a typical ADC linker that releases the payload through intracellular enzymes or pH changes and cannot be controlled externally.

Bishydroxy-trans-cyclooctene (C2TCO), a symmetrical TCO derivative, has also been used to demonstrate the advantages of the click-to-release method for ADC applications by achieving efficient payload release upon tetrazine treatment of a PD-L1-targeting ADC [26]. Clinical translation is underway: TGW101, developed by Tagworks Pharmaceuticals, is the first bioorthogonal click chemistry-activated ADC in human trials, and it uses a two-step mechanism for controlled payload release without requiring tumor cell internalization [5].

3.3. Sulfur(VI) Fluoride Exchange (SuFEx) Chemistry: Enabling Target-Engagement Programmability

SuFEx chemistry can link sulfur(VI) fluorides and nucleophiles efficiently. Sulfur(VI) fluorides have good stability in the presence of water and are unreactive with free amino acids at physiological temperatures; however, they can undergo facile nucleophilic displacement near the target and thus SuFEx is ideal for covalent modification of proteins.

SuFEx chemistry has a function of target-engagement programmability; it can permanently bind to a target antigen via proximity-enabled reactivity. Covalent binding is not restricted by dissociation kinetics and, thus, can achieve extended target occupancy to circumvent dissociation-limited efficacy.

A typical application is the development of covalent affibody-drug conjugates. Researchers introduced fluorosulfate-L-tyrosine at a specific site of a HER2-targeting affibody. After binding to

HER2, the FSY residue forms a covalent bond with a nearby histidine via a proximity-enabled SuFEx reaction. Covalent bonding will be formed between them to increase the concentration of the drug at the tumor site. In HER2-positive ovarian tumor models, the covalent affibody-drug conjugate nanoagent had a tumor inhibition rate of 90.03%, and the non-covalent one was only 64.25% [27].

Therefore, in this case, SuFEx chemistry programs the interaction between the therapeutic binder and its target through proximity-enabled covalent bond formation rather than controlling payload-antibody conjugation. SuFEx-mediated target engagement is not a typical conjugation method, and thus it is considered a different kind of chemical programmability at the target-engagement level.

3.4. Non-Canonical Amino Acid (ncAA)-Based Conjugation: Enabling Conjugation Programmability

While traditional conjugation relies on native lysine or cysteine residues yielding heterogeneous products, non-canonical amino acid (ncAA) incorporation enables introduction of uniquely reactive handles at precisely defined positions.

The function of ncAA-based conjugation is conjugation programmability; it can precisely control the site and stoichiometry of conjugation to obtain homogeneous ADCs with a fixed DAR and consistent pharmacokinetics [20,21].

The most widely used method introduces p-azido-phenylalanine through amber codon suppression for site-specific conjugation via strain-promoted azide-alkyne cycloaddition (SPAAC). This copper-free bioorthogonal reaction avoids metal-induced toxicity and is therefore compatible with cells. The Albubody platform employs this strategy for site-specific MMAE conjugation. The resulting conjugate retained HER2 binding affinity, showed HER2-specific cytotoxicity, and had prolonged systemic exposure and superior anti-tumor effects in HER2-positive xenograft models [28].

The concept origin of conjugation programmability is introduced in Section 2.1, and the history of the cpAb approach is also presented there.

Therefore, ncAA incorporation adds hardware-level programmability by providing genetically encoded, site-specific conjugation handles that are independent of native residues, and the identity of the conjugated module remains a software-level option.

4. Representative Strategies and Architectures

The following sections will present four representative strategies and architectures that demonstrate how chemical programmability has been applied in practice. Although these methods differ in their technical foundations and levels of development, together they demonstrate the extensive scope of chemical programmability for all kinds of molecules, including in vivo assembly of approved components (Pathway I), the earliest chemical reprogramming paradigm (Pathway II), fragment-based platforms designed to resolve the penetration-half-life trade-off (Pathway III), and bispecific constructs optimized by site-specific conjugation chemistry (Pathway IV). Each is a specific realisation of the five functional programmability levels mentioned in Section 2.2.

4.1. Pathway I: In Vivo Click-Driven in Situ Assembly

The most direct implementation of chemical programmability is in vivo assembly; antibody and ADC components are independently functionalised with complementary click handles and administered sequentially to assemble inside the body via bioorthogonal ligation. This pathway integrates assembly programmability (modular construction in vivo), targeting programmability (different targeting modules can be combined with the same antibody), and conjugation programmability (site-specific attachment of click handles).

Simó and his colleagues published a landmark study in Nature (2026) [5]. Therapeutic antibodies were conjugated to TCO, and ADCs were functionalised with tetrazine. After sequential administration, the two components were in vivo ligated to generate functional antibody-ADC click constructs. The approach demonstrated improved antitumor activity relative to standard ADC

monotherapy and was effective for treating tumors with low, ultra-low, negative or heterogeneous HER2 expression. In HER2-positive gastric cancer xenografts, the antibody-ADC click combination achieved a tumor growth inhibition (TGI) of 86.3%, and this was significantly higher than that of ADC monotherapy at 54.7%. Notably, the approach restored sensitivity in tumors that had acquired resistance to T-DXd, and the click-assembled construct achieved a TGI of 72.8% compared with 28.1% for the standard ADC. The approach does not require extensive antibody re-engineering, making it readily translatable using existing FDA-approved antibodies and ADCs. This strategy represents a promising pathway for rapidly generating dual-targeting ADCs from approved components.

4.2. Pathway II: Chemical Reprogramming of Full Antibodies with Small-Molecule Ligands

This historically earliest pathway involves chemically reprogramming full-length antibodies by conjugating small-molecule targeting ligands directly to the antibody scaffold. This pathway primarily embodies targeting programmability—the antibody's specificity is redirected by the conjugated small molecule—and conjugation programmability through site-specific attachment to the antibody's active-site lysine.

Sinha and his colleagues reported in 2008 cpADCs prepared with aldolase antibody 38C2 and small-molecule integrin α(v)β(3) antagonists. These constructs efficiently bound α(v)β(3)-expressing breast cancer cells and showed no binding to cells expressing only α(v)β(5), demonstrating that the small-molecule ligand conferred this specificity entirely [29]. This pathway enables targeting of antigens for which high-quality antibodies are difficult to generate, and rapid specificity switching can be achieved by changing the conjugated molecule; thus, both cost and development time are reduced compared to antibody engineering. Comprehensive reviews of the cpAb field have provided further details on the concept of chemical reprogramming [15-16].

4.3. Pathway III: Small-Size Antibody Fragment Platforms

While full-length IgG (~150 kDa) restricts tumor penetration, antibody fragments exhibit superior penetration but are rapidly cleared. Chemical programmability offers a way to achieve this via reversible modification, adding target-engagement programmability (masking and unmasking of binding activity) and conjugation programmability (site-specific attachment of payloads and PEG chains).

The Antibody Fragment-Cleavable PEGylated Drug Conjugate (AFCDC) platform uses a bifunctional non-canonical amino acid to perform site-specific dual conjugation, attaching both the payload and an MMP2-cleavable PEG chain. PEGylation prolongs the circulation time, and tumor proteases degrade PEG chains at the tumor site to enhance tissue penetration. The AFCDC platform had better intratumoral penetration and efficacy in HER2-positive tumor models, with a TGI of 89.2% compared to 72.5% for T-DXd at the same dose [3]. Besides scFv, this chemical strategy may also be applied to other fragment types such as nanobodies and minibodies to address the problem of a short half-life for fragments generally.

4.4. Pathway IV: Bispecific ADCs—Chemical Conjugation as an Enabling Layer

Bispecific ADCs (bsADCs) are the combination of biologically encoded targeting systems and chemically programmable payloads. Dual-antigen recognition is realized by antibody engineering, that is, the arrangement of two binding domains in a single construct; chemical conjugation adds programmability through payload selection, linker design and conjugation control. The two are different, so the target specificity of bsADCs cannot be achieved by chemical programming; instead, a strategy of chemical conjugation can be employed to control the attachment and release of payloads precisely to enhance therapeutic effects.

JSKN003 is a typical example of a biparatopic HER2-targeting ADC that builds upon the bispecific antibody scaffold KN026 and achieves dual epitope recognition through protein engineering. Thus, this targeting ability is the result of protein engineering programmability rather than chemical modification. The chemical contribution of JSKN003 is conjugation programmability;

site-specific glycan conjugation can be employed to precisely attach payloads and, thus, achieve superior structural homogeneity and reduced aggregation compared with traditional thiol-maleimide chemistry [7]. Chemical conjugation can enhance the therapeutic effects of a protein-engineered antibody architecture without changing its dual-targeting function, as shown in the above example. As of early 2024, more than 20 bispecific ADCs had entered clinical trials, and dual-payload designs and immune-stimulating antibody conjugates had also been applied [6,7].

5. Challenges and Perspectives

Although they are promising, many problems still need to be solved before chemically programmable ADCs can be widely used in clinical practice.

5.1. Chemical Stability and In Vivo Assembly Efficiency

Chemically programmable ADCs add more chemical complexity to traditional ADCs, and there are problems with the stability of the bioorthogonal handles and the efficiency of assembly and release.

Click-handle Stability in Circulation. Bioorthogonal handles for in vivo click chemistry, such as trans-cyclooctene (TCO) and tetrazine derivatives, need to be chemically stable and reactive in the bloodstream until they meet a suitable reaction partner. However, TCO is known to isomerise slowly from the reactive trans form to the less reactive cis form under physiological conditions, thus reducing the effective concentration of reactive handles available for in vivo ligation [5,13]. Tetrazine derivatives are also susceptible to degradation by nucleophilic attack of endogenous thiols or serum proteins, which may reduce their reactivity and thus lower the efficiency of the click reaction [5,25]. These stability problems are more pronounced when there is a long delay between the administration of the TCO-modified antibody and the tetrazine-modified ADC, as the reactive handles may degrade before assembly takes place. Strategies to address this problem include the development of stable TCO derivatives with a low-isomerisation rate and the design of tetrazine analogues with improved serum stability, both of which are still active research directions [13].

Incomplete Payload Release. Click-to-release strategies can realize spatio-temporally controlled payload release, but they are not inherently quantitative. The efficiency of payload release is influenced by several factors, such as the kinetics of the tetrazine-TCO reaction, the structural accessibility of the cleavable linker, and the stability of the intermediate species formed during the reaction [5,13]. An incomplete release will reduce the therapeutic effect, as only some of the ADC molecules reach the tumor, and insufficient quantities of the cytotoxic payload are delivered. An incomplete release may also lead to an adverse safety profile due to the accumulation of partially degraded or incompletely released conjugates in normal tissues and off-target effects. Recently, in the effort to enhance the release efficiency, researchers have optimized the electronic and steric properties of the tetrazine and TCO components to accelerate the reaction and ensure complete cleavage in a physiological environment [13].

PK/PD Complexity. Conventional ADCs are already complex in their pharmacokinetic and pharmacodynamic profiles; chemical programming introduces additional complexity through in vivo assembly kinetics and reaction efficiency. For in vivo click assembly, the TCO-tetrazine reaction should have both high speed and selectivity at nanomolar physiological concentrations. For a click-to-release strategy, the time and dosage of the trigger molecule need to be optimized to achieve controlled release without excessive systemic exposure [5]. Quantitative PK/PD modeling will need to be performed to predict the behavior of this multi-component system and determine a suitable dosing regimen [17-18].

5.2. Immunogenicity Risk

Non-natural chemical groups, such as TCO, tetrazine, SuFEx electrophiles and non-canonical amino acids, may be immunogenic and have not been studied clinically. The immune system may recognize the chemical modification as a foreign body and therefore destroy the drug, increase the

rate of its degradation, or cause an allergic reaction. However, the following ways can be used to manage the risk: these chemical handles are generally small and may be less immunogenic than protein-based modifications; site-specific conjugation reduces structural heterogeneity, which is itself a known cause of immunogenicity; and clinical experience with PEGylated proteins and other chemically modified biologics has provided a reference for managing immunogenicity through formulation and dosing strategies. Monitoring by ADA and T-cell epitope mapping are used in the immunogenicity evaluation of preclinical and clinical studies [6].

5.3. Multi-Component CMC, Manufacturing, and Regulatory Complexity

The many parts in chemically programmable ADCs pose considerable problems for chemistry, manufacturing and controls (CMC), as well as for regulatory approval.

Manufacturing and Quality Control. Multiple chemical steps need to be performed with high precision, such as antibody modification with click handles, synthesis and conjugation of the ADC component, and -in the case of in vivo assembly strategies- separate manufacturing of two or more components that each must meet high-quality standards. Key QC indicators are conjugation efficiency (the proportion of antibody molecules that have been successfully modified with the targeting ligand), site specificity (the spatial distribution of modifications), product homogeneity (the spread of DAR values), and stability (the integrity of chemical bonds during storage and in the body). Recently, new modular conjugation platforms have shown promise for large-scale production [28, 20].

Regulatory Complexity. The Regulatory Path for chemically programmable ADCs is less clear than that for traditional ADCs. A typical ADC is a single molecule that requires only one regulatory filing, but chemically programmable approaches, especially those involving in vivo assembly, may need to be evaluated for several components individually and together. Regulatory frameworks for ADCs are evolving. In March 2024, the FDA issued its first dedicated ADC guidance—Clinical Pharmacology Considerations for Antibody-Drug Conjugates—which addresses bioanalytical methods, dosing strategies, immunogenicity, and drug-drug interactions. The EMA has not yet issued an ADC-specific guideline, but the Oncology Working Party has provided guidance on the expectation of comparative evidence for ADCs in oncology submissions. The FDA's Office of Combination Products has established general frameworks, including Principles of Premarket Pathways for Combination Products. However, no particular guidance has been issued on bioorthogonally assembled or in vivo click-assembled ADC systems, and thus the required preclinical data package, toxicology study design and clinical development pathway remain unclear. Sponsors must address whether the individual components are evaluated separately or only in combination. What is the appropriate comparator for clinical trials? How are the pharmacokinetics of the assembled product different from those of its individual components? These considerations will require early and frequent engagement with regulatory authorities during the development process.

5.4. Future Directions

The multi-level chemical programmability framework opens several promising directions for future research. The next horizon is integrated multi-level programmable design; targeting programmability, assembly programmability, target-engagement control, conjugation precision and payload-activation triggerability will all be integrated into a single ADC platform for truly adaptive therapeutics.

Expanding the chemical toolbox with new bioorthogonal reactions that offer faster kinetics, better biocompatibility, and orthogonal selectivity will further enhance the scope and precision of chemical programmability. The development of mutually orthogonal click pairs can be used to sequentially connect multiple components or release different payloads in sequence from the same construct. Dual-payload delivery can address antigen heterogeneity and multiple resistance mechanisms simultaneously. Bystander effect: Released payload diffuses to kill neighbouring antigen-negative cells, and this can be modulated by chemical programming of payload lipophilicity and linker design [22,23]. Clinical translation of candidates such as TGW101 has provided early validation for the

development of chemically programmed ADCs [5,6]. Continuous investment in the fundamentals of chemistry and translational research is required to realize the full potential of this emerging paradigm.

6. Conclusion

Chemical programmability fundamentally redefines ADC design by introducing modularity and controllability across multiple functional layers that are traditionally fixed during manufacture. Five levels of function for chemically programmable strategies have been explored in this review: targeting programmability, assembly programmability, target-engagement programmability, conjugation programmability and payload-activation programmability.

cpAbs provided the conceptual basis and, using catalytic antibody 38C2, showed that a single antibody scaffold could be repurposed for multiple targets. These ideas have been extended by IEDDA, click-to-release, SuFEx and ncAA-based conjugation to achieve various degrees of programmability. Four implementation pathways have appeared: in vivo click-driven assembly, small-molecule reprogramming, fragment-based platforms, and bispecific ADCs, all of which are highly programmable.

Challenges remain in pharmacokinetic complexity, immunogenicity, and manufacturing. Future directions include integrated multi-level programmable design, expanded chemical toolboxes, and dual-payload delivery. Chemically programmable ADCs represent a fundamental shift from static, predefined therapeutics to dynamic, adaptable platforms, holding potential to overcome the inherent limitations of conventional ADCs and extend targeted therapy to broader malignancies.

References

[1] Narayan, P., Osgood, C. L., Singh, H., Chiu, H.-J., Ricks, T. K., Chiu Yuen Chow, E., Qiu, J., Song, P., Yu, J., Namuswe, F., Guiterrez-Lugo, M., Hou, S., Pierce, W. F., Goldberg, K. B., Tang, S., Amiri-Kordestani, L., Theoret, M. R., Pazdur, R., & Beaver, J. A. (2021). FDA Approval Summary: Fam-Trastuzumab Deruxtecan-Nxki for the Treatment of Unresectable or Metastatic HER2-Positive Breast Cancer. *Clinical Cancer Research*, *27*(16), 4478–4485. https://doi.org/10.1158/1078-0432.CCR-20-4557

[2] Wang, R., Hu, B., Pan, Z., Mo, C., Zhao, X., Liu, G., Hou, P., Cui, Q., Xu, Z., Wang, W., Yu, Z., Zhao, L., He, M., Wang, Y., Fu, C., Wei, M., & Yu, L. (2025). Antibody–Drug Conjugates (ADCs): current and future biopharmaceuticals. Journal of Hematology and Oncology, 18(1), Article 51. https://doi.org/10.1186/s13045-025-01704-3

[3] Wang, X., Hou, X., Tan, L., Chen, C., Chang, L., Han, B., Xing, Y., Zeng, Z., Zhou, Z., Lu, Q., Xie, L., Lu, Z., Zhao, K., Cao, B., Liu, T., & Wang, Y. (2026). Tumor Microenvironment-Responsive Antibody Fragment-Cleavable PEGylated Drug Conjugates (AFCDC) for Improved Tumor Penetration and Antitumor Efficacy. *Journal of Medicinal Chemistry*, *69*(9), 10169–10182. https://doi.org/10.1021/acs.jmedchem.5c03215

[4] Hao, Y., & Song, Z. (2025). Mechanisms of resistance to antibody-drug conjugates in cancer therapy: molecular basis and therapeutic strategies. *Cancer Drug Resistance*, *8*, Article 59. https://doi.org/10.20517/cdr.2025.148

[5] Simó, C., Vanover, A. C., D'Oliveira Albanus, R., Panikar, S. S., Shmuel, S., Benton, A., Giraldo-Guzman, J., Luna, J. M., Xu, Y., Berry, N. K., Keltee, N., Liu, J., Dehdashti, F., & Pereira, P. M. R. (2026). Modular in vivo antibody–ADC click to reverse drug resistance in tumours. *Nature (London)*. https://doi.org/10.1038/s41586-026-10789-w

[6] Tsuchikama, K., Anami, Y., Ha, S. Y. Y., & Yamazaki, C. M. (2024). Exploring the next generation of antibody–drug conjugates. *Nature Reviews. Clinical Oncology*, *21*(3), 203–223. https://doi.org/10.1038/s41571-023-00850-2

[7] Gu, Y., Wang, Z., & Wang, Y. (2024). Bispecific antibody drug conjugates: Making 1+1>2. *Acta Pharmaceutica Sinica. B*, *14*(5), 1965–1986. https://doi.org/10.1016/j.apsb.2024.01.009

[8] Yang, Y., Zhou, H., Tang, L., Qiu, S., Han, Y., Ji, D., Chen, X., Lei, F., Qu, S., Deng, B., Chen, L., Huang, J., Guo, Y., Liu, Z., Chen, D., Li, J., Shu, X., Qin, Y., Fu, Z., … Zhang, L. (2025). Izalontamab brengitecan, an EGFR and HER3 bispecific antibody–drug conjugate, versus chemotherapy in heavily pretreated recurrent or metastatic nasopharyngeal carcinoma: a multicentre, randomised, open-label, phase 3 study in China. *The Lancet (British Edition)*, *406*(10516), 2235–2243. https://doi.org/10.1016/S0140-6736(25)01954-3

[9] Guo, F., Das, S., Mueller, B. M., Barbas, C. F., Lerner, R. A., & Sinha, S. C. (2006). Breaking the One Antibody-One Target Axiom. *Proceedings of the National Academy of Sciences - PNAS*, *103*(29), 11009–11014. https://doi.org/10.1073/pnas.0603822103

[10] Wagner, J., Lerner, R. A., & Barbas, C. F. (1995). Efficient Aldolase Catalytic Antibodies That Use the Enamine Mechanism of Natural Enzymes. *Science (American Association for the Advancement of Science)*, *270*(5243), 1797–1800. https://doi.org/10.1126/science.270.5243.1797

[11] Rader, C., Sinha, S. C., Popkov, M., Lerner, R. A., & Barbas, C. F. (2003). Chemically Programmed Monoclonal Antibodies for Cancer Therapy: Adaptor Immunotherapy Based on a Covalent Antibody Catalyst. *Proceedings of the National Academy of Sciences - PNAS*, *100*(9), 5396–5400. https://doi.org/10.1073/pnas.0931308100

[12] Beck, A., Goetsch, L., Dumontet, C., & Corvaïa, N. (2017). Strategies and challenges for the next generation of antibody–drug conjugates. *Nature Reviews. Drug Discovery*, *16*(5), 315–337. https://doi.org/10.1038/nrd.2016.268

[13] Khongorzul, P., Ling, C. J., Khan, F. U., Ihsan, A. U., & Zhang, J. (2020). Antibody–Drug Conjugates: A Comprehensive Review. *Molecular Cancer Research*, *18*(1), 3–19. https://doi.org/10.1158/1541-7786.MCR-19-0582

[14] Joubert, N., Beck, A., Dumontet, C., & Denevault-Sabourin, C. (2020). Antibody–Drug Conjugates: The Last Decade. *Pharmaceuticals (Basel, Switzerland)*, *13*(9), Article 245. https://doi.org/10.3390/ph13090245

[15] Rader, C. (2014). Chemically programmed antibodies. *Trends in Biotechnology (Regular Ed.)*, *32*(4), 186–197. https://doi.org/10.1016/j.tibtech.2014.02.003

[16] Popkov, M., Rader, C., Gonzalez, B., Sinha, S. C., & Barbas, C. F. (2006). Small molecule drug activity in melanoma models may be dramatically enhanced with an antibody effector. *International Journal of Cancer*, *119*(5), 1194–1207. https://doi.org/10.1002/ijc.21924

[17] Cilliers, C., Menezes, B., Nessler, I., Linderman, J., & Thurber, G. M. (2018). Improved Tumor Penetration and Single-Cell Targeting of Antibody–Drug Conjugates Increases Anticancer Efficacy and Host Survival. *Cancer Research (Chicago, Ill.)*, *78*(3), 758–768. https://doi.org/10.1158/0008-5472.CAN-17-1638

[18] Tsumura, R., Manabe, S., Takashima, H., Koga, Y., Yasunaga, M., & Matsumura, Y. (2018). Influence of the dissociation rate constant on the intra-tumor distribution of antibody-drug conjugate against tissue factor. *Journal of Controlled Release*, *284*, 49–56. https://doi.org/10.1016/j.jconrel.2018.06.016

[19] Rudnick, S. I., Lou, J., Shaller, C. C., Tang, Y., Klein-Szanto, A. J. P., Weiner, L. M., Marks, J. D., & Adams, G. P. (2011). Influence of Affinity and Antigen Internalization on the Uptake and Penetration of Anti-HER2 Antibodies in Solid Tumors. *Cancer Research (Chicago, Ill.)*, *71*(6), 2250–2259. https://doi.org/10.1158/0008-5472.CAN-10-2277

[20] Walsh, S. J., Bargh, J. D., Dannheim, F. M., Hanby, A. R., Seki, H., Counsell, A. J., Ou, X., Fowler, E., Ashman, N., Takada, Y., Isidro-Llobet, A., Parker, J. S., Carroll, J. S., & Spring, D. R. (2021). Site-selective modification strategies in antibody-drug conjugates. *Chemical Society Reviews*, *50*(2), 1305–1353. https://doi.org/10.1039/d0cs00310g

[21] Aigbogun, O. P., Phenix, C. P., Krol, E. S., & Price, E. W. (2023). The Chemistry of Creating Chemically Programmed Antibodies (cPAbs): Site-Specific Bioconjugation of Small Molecules. *Molecular Pharmaceutics*, *20*(2), 853–874. https://doi.org/10.1021/acs.molpharmaceut.2c00821

[22] Li, F., Emmerton, K. K., Jonas, M., Zhang, X., Miyamoto, J. B., Setter, J. R., Nicholas, N. D., Okeley, N. M., Lyon, R. P., Benjamin, D. R., & Law, C.-L. (2016). Intracellular Released Payload Influences Potency and Bystander-Killing Effects of Antibody-Drug Conjugates in Preclinical Models. *Cancer Research (Chicago, Ill.)*, *76*(9), 2710–2719. https://doi.org/10.1158/0008-5472.CAN-15-1795

[23] Ogitani, Y., Aida, T., Hagihara, K., Yamaguchi, J., Ishii, C., Harada, N., Soma, M., Okamoto, H., Oitate, M., Arakawa, S., Hirai, T., Atsumi, R., Nakada, T., Hayakawa, I., Abe, Y., & Agatsuma, T. (2016). DS-8201a, A Novel HER2-Targeting ADC with a Novel DNA Topoisomerase I Inhibitor, Demonstrates a Promising Antitumor Efficacy with Differentiation from T-DM1. *Clinical Cancer Research*, *22*(20), 5097–5108. https://doi.org/10.1158/1078-0432.CCR-15-2822

[24] Desnoyers, L. R., Vasiljeva, O., Richardson, J. H., Yang, A., Menendez, E. E. M., Liang, T. W., Wong, C., Bessette, P. H., Kamath, K., Moore, S. J., Sagert, J. G., Hostetter, D. R., Han, F., Gee, J., Flandez, J., Markham, K., Nguyen, M., Krimm, M., Wong, K. R., … Lowman, H. B. (2013). Tumor-Specific Activation of an EGFR-Targeting Probody Enhances Therapeutic Index. *Science Translational Medicine*, *5*(207), Article 207ra144. https://doi.org/10.1126/scitranslmed.3006682

[25] Tomarchio, E. G., Turnaturi, R., Saccullo, E., Patamia, V., Floresta, G., Zagni, C., & Rescifina, A. (2024). Tetrazine–trans-cyclooctene ligation: Unveiling the chemistry and applications within the human body. *Bioorganic Chemistry*, *150*, Article 107573. https://doi.org/10.1016/j.bioorg.2024.107573

[26] Arun, V., Lee, M., Choi, H., Lee, S., Choi, J., Yoo, T. H., Kim, W., & Kim, E. (2025). Easy Access to Bioorthogonal Click-to-Release Reagent Bishydroxy-trans-cyclooctene (C2TCO) and Harnessing of Its Rapid Labeling and Dissecting Feature in Multicycle Imaging. *Bioconjugate Chemistry*, *36*(5), 930–935. https://doi.org/10.1021/acs.bioconjchem.4c00495

[27] Gao, W., Yang, X., Li, Q., Liu, Y., Huang, W., Xia, X., & Yan, D. (2025). Covalent Affibody-Molecular Glue Drug Conjugate Nanoagent for Proximity-Enabled Reactive Therapeutics. *Advanced Science*, *12*(10), Article 2412273. https://doi.org/10.1002/advs.202412273

[28] Kwon, N. H., Lee, J. H., Kim, Y., Hahn, Y. S., & Kwon, I. (2025). Albubody: an engineered scFv variant platform for site-specific drug conjugation and enhanced tumor efficacy. *Journal of Controlled Release*, *386*, Article 114165. https://doi.org/10.1016/j.jconrel.2025.114165

[29] Sinha, S., Huang, Z., & Felding-Habermann, B. (2008). Chemically programmed antibody-drug conjugates. *Cancer Research*, *68*(9_Supplement), 4060. https://aacrjournals.org/cancerres/article/68/9_Supplement/4060/548901