Ultra-Stable Linker Strategies in Antibody–Drug Conjugates: Balancing Plasma Stability with Controlled Cytotoxic Payload Release

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ABSTRACT

Targeted drug delivery has emerged as a transformative approach in cancer therapy, allowing selective destruction of malignant cells while minimizing systemic toxicity. Antibody-drug conjugates (ADCs) and other targeted delivery systems rely heavily on chemical linkers that connect highly potent cytotoxic agents to targeting ligands. Among these, ultra-stable chemical linkers have gained significant attention due to their ability to remain intact during systemic circulation while ensuring precise intracellular release of therapeutic payloads. The physicochemical properties of linkers directly influence pharmacokinetics, therapeutic index, efficacy, and safety. Recent advances in linker chemistry have enabled the development of enzyme-responsive, pH-sensitive, reduction-sensitive, and bio orthogonal cleavage mechanisms, improving payload release specificity. This review discusses the chemistry, design principles, mechanisms of action, recent advances, clinical applications, challenges, and future perspectives of ultra-stable chemical linkers used for controlled cytotoxic payload delivery.

Keywords: Antibody-drug conjugates, Ultra-stable linkers, Cancer therapy, Drug delivery systems, Linker chemistry

INTRODUCTION

Cancer remains one of the leading causes of mortality worldwide despite continuous advancements in diagnosis and treatment. Conventional chemotherapy lacks selectivity, resulting in significant toxicity to healthy tissues. Targeted drug delivery systems, particularly antibody-drug conjugates (ADCs), have revolutionized oncology by selectively delivering potent cytotoxic agents to tumor cells. Antibody–drug conjugates (ADCs) represent a class of cytotoxic cancer therapies that combine the precision of monoclonal antibodies with the potent cell-killing activity of cytotoxic drugs. This allows them to selectively kill cancer cells, overcome resistance, reduce toxicity to healthy tissues, and expand treatment options for difficult subtypes.

An ADC consists of three essential components: ADC development play a significant role oncology toward increasingly demand and personalized targeted specific therapies [1].

Targeting antibody- Targeting antibodies have become a cornerstone of modern targeted cancer therapy due to their remarkable ability to selectively recognize and bind specific antigens that are overexpressed on the surface of tumor cells. Unlike conventional chemotherapeutic agents, which often lack specificity and cause widespread toxicity to normal tissues, monoclonal antibodies (mAbs) enable the precise delivery of therapeutic agents to malignant cells while minimizing off-target effects. This high degree of specificity has led to the development of antibody-based therapeutics, particularly antibody-drug conjugates (ADCs), which combine the selectivity of antibodies with the potent cytotoxic activity of small-molecule drugs.

Thus, aiming to combine the strong cytotoxicity of chemotherapy with the high specificity of targeted therapy, antibody drug conjugates (ADCs) are designed to selectively deliver cytotoxic payloads directly to target cancer cells [2].

In ADCs, the targeting antibody serves as the critical component responsible for recognizing tumor-associated antigens, facilitating selective accumulation of the conjugate at the tumor site. Upon binding to the target antigen, the ADC is internalized through receptor-mediated endocytosis, followed by intracellular processing that releases the cytotoxic payload. This targeted delivery strategy enhances the therapeutic index of highly potent cytotoxic agents that would otherwise be too toxic for systemic administration. Furthermore, the antibody itself may contribute to antitumor activity through mechanisms such as antibody-dependent cellular cytotoxicity (ADCC), complement-dependent cytotoxicity (CDC), and inhibition.

Cytotoxic payload- The cytotoxic payload is an effector molecule of ADCs. Various alkylating agents and topoisomerase inhibitors, tyrosine kinase inhibitors immune stimulants, protein synthesis inhibitors and proteolysis targeting agents.[3,4,5,6], Although a diverse range of cytotoxic agents has been investigated as payloads for antibody-drug conjugates (ADCs), only three major classes have been incorporated into FDA-approved therapies. These include microtubule inhibitors, such as auristatins (monomethyl auristatin E [MMAE]) and maytansinoids (DM1 and DM4); topoisomerase I inhibitors, including deruxtecan and SN-38 (the active metabolite of irinotecan); and DNA-alkylating agents, represented by duocarmazine. Numerous other cytotoxic payloads are currently under preclinical or clinical evaluation, while some remain limited to applications in hematological malignancies.

Chemical linker - The linker is a critical component of any ADC. The nature of the linker and spacer determines important features of ADCs, such as hydrophobic or lipophilic properties of payload, nontarget payload delivery, systemic toxicity, and most importantly, bystander killing[7]. The selection of an appropriate linker is a critical aspect of antibody-drug conjugate (ADC) design and is influenced by several factors, including the intended therapeutic application, the physicochemical properties and potency of the cytotoxic payload, and the desired pharmacokinetic profile of the conjugate. Linkers used in ADCs are broadly classified into two categories: cleavable and non-cleavable linkers. Cleavable linkers are engineered to release the payload in response to specific intracellular conditions and are further categorized into peptide linkers, which are degraded by lysosomal proteases (e.g., the valine–citrulline [Val–Cit] linker); disulfide linkers, which are cleaved in the reducing intracellular environment rich in glutathione; and hydrazone linkers, which undergo hydrolysis under the acidic conditions of endosomes and lysosomes. The choice of linker plays a crucial role in determining the stability, efficacy, and safety of the ADC by controlling the site and timing of payload release.

Structure and Mechanism of Action of Antibody-Drug Conjugates Adc

The idea behind ADCs is the optimal delivery of a highly potent payload to its target using a specific carrier. To Avoid Gastric acid and proteolysis enzyme degradation antibody drug conjugates administered by Intravenous route[8]. Ideally, the monoclonal antibody (mAb) component of an antibody–drug conjugate (ADC) should recognize and bind to antigens that are exclusively or highly selectively expressed on tumor cells, while exhibiting minimal or no expression on normal healthy tissues. Such tumor-specific antigen expression is a critical prerequisite for maximizing therapeutic efficacy and minimizing off-target toxicity [9]. Following specific binding to the target antigen, the antibody–drug conjugate (ADC)–antigen complex is internalized into the tumor cell through receptor-mediated endocytosis, facilitating intracellular delivery of the cytotoxic payload.[10]. After receptor-mediated endocytosis, the internalized ADC–antigen complex is transported from early to late endosomes, during which recycling-associated proteins are eliminated. Fusion of late endosomes with lysosomes generates an acidic, protease-rich environment containing enzymes such as cathepsin B and plasmin. This environment facilitates linker cleavage, leading to the efficient intracellular release of the cytotoxic payload into the cytoplasm, where it induces targeted tumor cell death.[11][12].

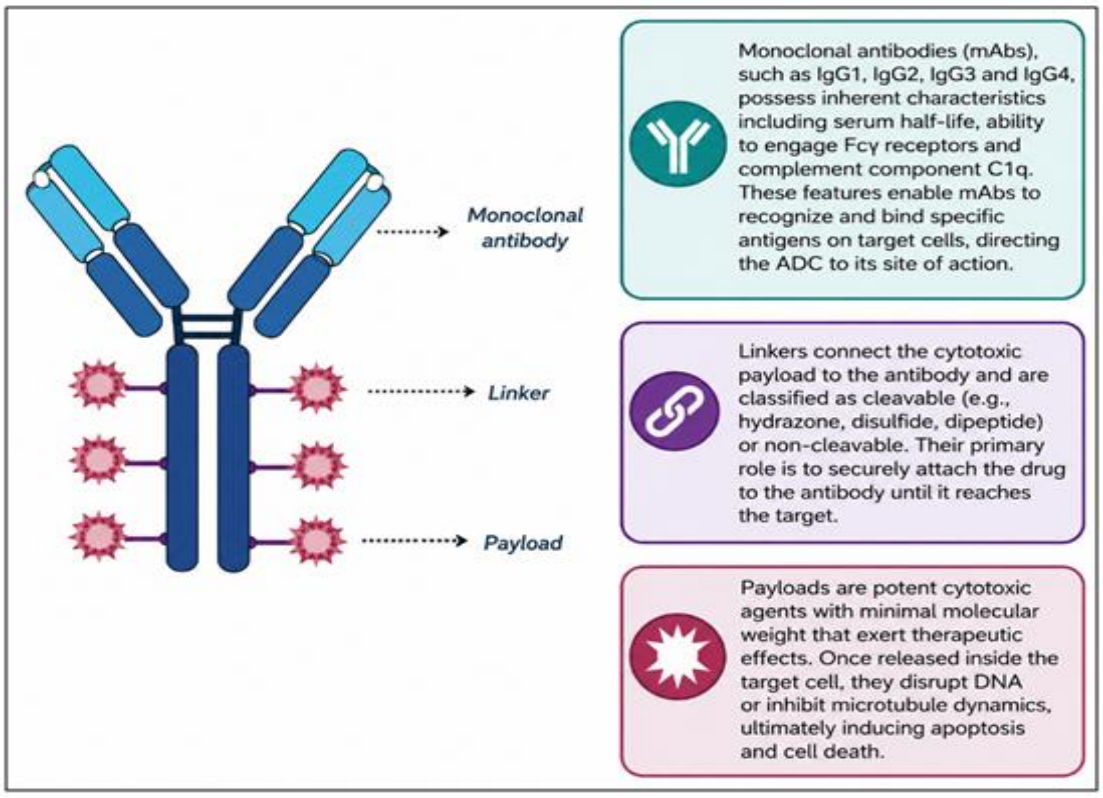


Fig. Schematic representation of the modular components of an Antibody-Drug Conjugate (ADC). ADC consists of a monoclonal antibody (blue), a linker (blue lines) and the cytotoxic drugs (gray/red).In this picture, the representative ADC on the left has a Drug-to-Antibody ratio equal to 4.

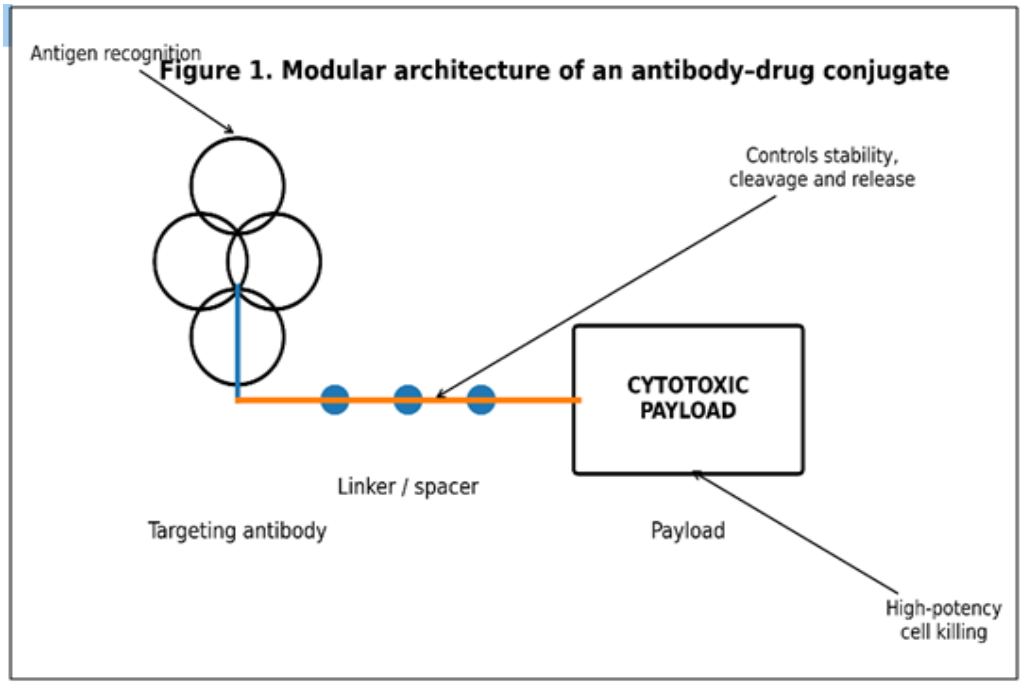


Fig .Modular architecture of an antibody–drug conjugate

2. Importance of Chemical Linkers

Chemical linkers determine.

- ✓ Drug release kinetics
- ✓ Therapeutic index
- ✓ Pharmacokinetics

- ✓ Drug-to-antibody ratio (DAR)
- ✓ Overall efficacy
- ✓ Safety profile
- ✓ Plasma stability

An ideal linker should possess:

- ✓ High stability in blood circulation
- ✓ Minimal premature degradation
- ✓ Efficient intracellular cleavage
- ✓ High reproducibility
- ✓ Low immunogenicity
- ✓ Simple synthesis
- ✓ Scalability

Classification of Chemical Linkers; Chemical linkers are broadly classified into:

A. Cleavable Linkers- Cleavable linkers constitute the predominant class of ADC linkers owing to their capacity to respond to distinct biochemical cues. They are specifically designed to be cleaved by changes in pH, elevated intracellular reducing conditions, or lysosomal proteases, thereby ensuring site-specific release of the cytotoxic payload after cellular internalization and enhancing the therapeutic selectivity of ADCs.[13]. There are different kinds of cleavable linkers used inADC design as mentioned below.

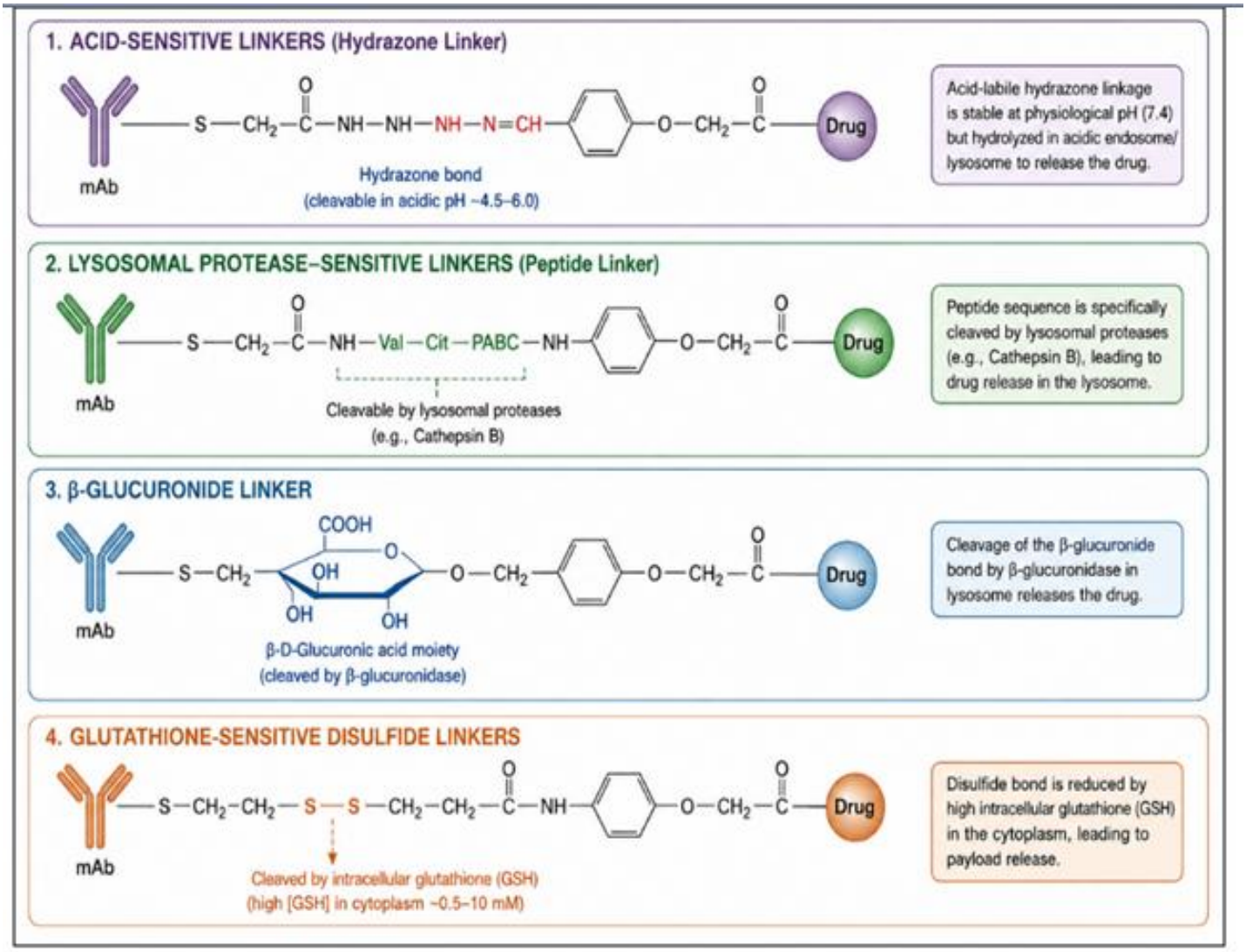
Types include

a) Acid-sensitive linkers- Acid-sensitive linkers exploit the pH differences between the bloodstream and intracellular vesicular compartments to achieve selective drug release. These linkers exhibit high stability at the physiological pH of systemic circulation but undergo hydrolytic cleavage after ADC internalization into tumor cells. In the acidic milieu of endosomes (pH 5.0–6.0) and lysosomes (pH 4.5–5.0), hydrazone-based or other acid-labile bonds are cleaved, facilitating efficient intracellular liberation of the cytotoxic payload and improving the therapeutic specificity of ADCs[14]. IMMU-110 consists of a humanized anti-CD74 monoclonal antibody conjugated to doxorubicin via an acid-sensitive hydrazone linker. The ADC exhibited promising preclinical efficacy against multiple myeloma and demonstrated acceptable tolerability in non-human primate studies, highlighting the therapeutic potential of acid-cleavable linker technology for targeted cancer treatment.[15].Examples, Hydrazone, Acetal Cleavage occurs in acidic lysosomes (pH 4.5–5.5).

b) Lysosomal protease–sensitive. Peptide-based, lysosomal protease-sensitive linkers constitute the most widely employed class of enzymatically cleavable linkers in ADC development. Their design exploits the elevated lysosomal protease activity, particularly that of cathepsin B, commonly observed in tumor cells. After antigen-specific binding and receptor-mediated internalization, the ADC is trafficked to lysosomes, where proteolytic cleavage of the peptide linker enables site-specific release of the cytotoxic drug. This enzyme-responsive strategy improves drug selectivity and minimizes premature payload release in systemic circulation.[15]. Peptide-based (lysosomal protease-sensitive) linkers are among the most widely employed linker platforms in ADCs because of their high plasma stability and efficient intracellular activation. These linkers remain resistant to cleavage under physiological pH and in the presence of serum protease inhibitors, ensuring minimal drug leakage during circulation. Following antigen-specific binding and receptor-mediated endocytosis, the ADC is trafficked to lysosomes, where elevated activity of proteases, particularly cathepsin B and related cathepsins, facilitates selective cleavage of the peptide linker. This enzymatic activation enables controlled intracellular release of the cytotoxic payload, thereby maximizing antitumor efficacy while limiting off-target toxicity[16].Examples-Valine-Citrulline, Valine-Alanine ,Gly-Phe-Leu-Gly.

c) Glucuronide Linker: Another protease-sensitive linker is the b-glucuronide linker that is recognized and hydrolyzed by b-glucuronidase for the drug release.[17]. This selective site of action allows for the cleavage of the glycosidic linkage of the b-glucuronidase–sensitive b-glucuronide linker, thereby enabling the selective release of cytotoxic payloads.

d) Glutathione-Sensitive Disulfide Linkers: Glutathione-sensitive disulfide linkers represent another important class of cleavable linkers widely employed in the design of antibody–drug conjugates (ADCs). These linkers exploit the substantial difference in glutathione (GSH) concentrations between extracellular and intracellular environments to achieve selective payload release. Glutathione, a low-molecular-weight thiol and a major intracellular reducing agent, is present at relatively high concentrations within the cytoplasm (approximately 0.5–10 mM), whereas its extracellular concentration in plasma is considerably lower (typically 2–20 µM). Following receptor-mediated internalization of the ADC into tumor cells, the elevated intracellular GSH levels reduce the disulfide bond, resulting in linker cleavage and controlled release of the cytotoxic payload. This redox-responsive mechanism enhances intracellular drug delivery while minimizing premature drug release during systemic circulation.[18]



B. Non-cleavable Linkers- Unlike cleavable linkers, non-cleavable linkers are characterized by highly stable covalent bonds that are not readily susceptible to proteolytic degradation, ensuring improved plasma stability and controlled intracellular payload release following antibody degradation [19]. The therapeutic activity of antibody–drug conjugates (ADCs) containing non-cleavable linkers relies on receptor-mediated internalization of the ADC–antigen complex, followed by lysosomal degradation of the monoclonal antibody component. This process generates an active drug–amino acid metabolite that is retained within the target cell, where it exerts its cytotoxic effect and induces tumor cell death. Owing to their high stability in systemic circulation, non-cleavable linkers minimize premature payload release, thereby reducing off-target toxicity and limiting damage to healthy tissues[20]. Example of Non-cleavable linkers are SMCC (Succinimidyl-4-(N-maleimidomethyl) cyclohexane-1-carboxylate).Payload released only after complete lysosomal degradation of antibody. The advantage of non-cleavable linkers that they have excellent plasma stability and reduced systemic toxicity.

What Makes a Linker "Ultra-Stable"?

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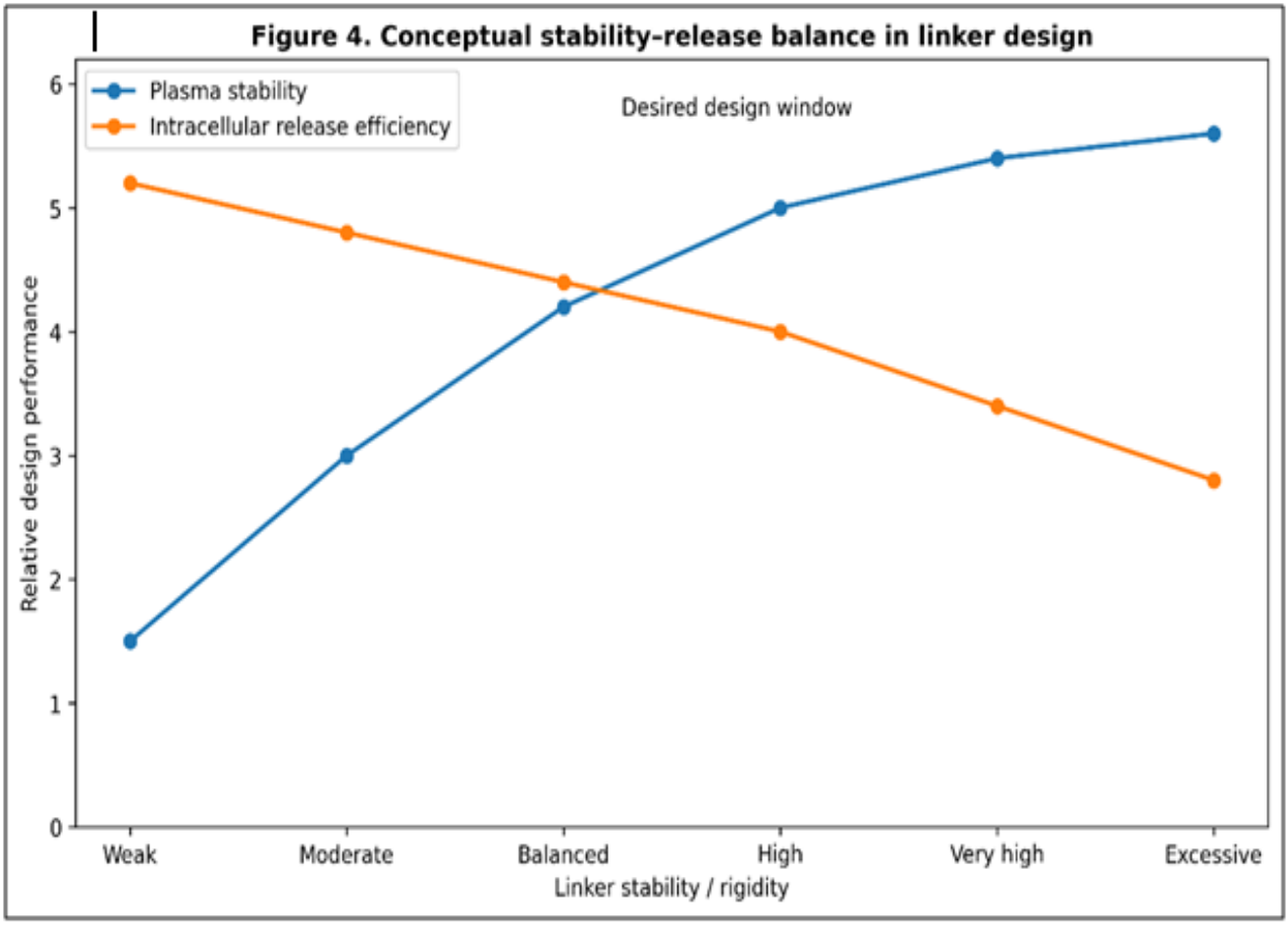
Ultra-stability is best defined operationally rather than as a single chemical class. An ultra-stable linker should resist hydrolysis, enzymatic degradation and unwanted chemical exchange during circulation while retaining a predictable pathway for intracellular payload liberation. This concept is therefore compatible with both non-cleavable linkers and highly stable cleavable systems. The ideal linker should simultaneously achieve: high plasma stability; low nonspecific release; predictable intracellular cleavage; acceptable synthesis and scale-up; low immunogenicity; appropriate hydrophilicity; compatibility with the selected payload; and reproducible conjugation. Importantly, excessive stability can be counterproductive if it prevents sufficient intracellular release. The design target is a controlled stability–release window rather than maximum inertness.



Conceptual stability–release balance. The optimum lies between premature systemic cleavage and excessive intracellular persistence; the curves are illustrative, not experimental measurements.

Recent Advances in Ultra-Stable Linker Chemistry

1. Self-immolative Linkers Examples- p-Aminobenzyl carbamate (PABC) it has Rapid payload release, with High efficiency, improved pharmacodynamics.

2. Bioorthogonal Linkers- This linker have High specificity & Minimal off-target reactions.

3. β-Glucuronide Linkers-Activated by β-glucuronidase enzymes overexpressed in tumors. It Improved tumor specificity Reduced toxicity.

4. Sulfatase-sensitive Linkers-Activated by lysosomal sulfatases. Use in Emerging ADCs.

5. Peptide-Based Ultra-Stable Linker-Val-Cit , Val-Ala it have high plasma stability and Efficient lysosomal cleavage.

Factors Affecting Linker Stability

Factor	Effect on ADC Behavior	Design Implication
Bond type	Controls chemical and enzymatic susceptibility	Select cleavage chemistry appropriate to the intended compartment
Steric environment	Can shield or expose labile bonds	Use steric effects to tune plasma stability and cleavage rate
Hydrophobicity	Influences aggregation, nonspecific uptake, and clearance	Balance payload/linker polarity with potency
Electronic effects	Modify hydrolysis and fragmentation rates	Tune substituents around the trigger and spacer
pH	Determines acid-triggered cleavage	Maximize physiological-to-endosomal selectivity
Protease activity	Controls peptide-linker processing	Match substrate sequence to intracellular enzyme activity
Redox state	Controls disulfide reduction	Consider tissue and tumor heterogeneity
DAR (Drug-to-Antibody Ratio)	Changes exposure, stability, and clearance	Optimize together with linker and conjugation site
Conjugation site	Can alter antibody structure and stability	Prefer controlled, reproducible attachment.
Tumor biology	Determines antigen density, internalization, and release.	Select linker strategy together with target biology.

Clinical Applications

Ultra-stable linker technology has significantly improved several FDA-approved ADCs.

ADC	Linker	Payload	Target
Trastuzumab emtansine (T-DM1)	Non-cleavable SMCC	DM1	HER2
Brentuximab vedotin	Val-Cit	MMAE	CD30
Enfortumab vedotin	Val-Cit	MMAE	Nectin-4
Polatuzumab vedotin	Val-Cit	MMAE	CD79b
Sacituzumab govitecan	Hydrolysable CL2A	SN-38	Trop-2
Trastuzumab deruxtecan (T-DXd)	Tetrapeptide linker	DXd	HER2

Recommended Research and Reporting Framework

For future studies, linker performance should be reported using a common set of parameters so that different platforms can be compared. A useful framework is: chemical stability → conjugation homogeneity → plasma stability → intracellular release → payload activity → pharmacokinetics → efficacy → toxicity. This sequence links molecular chemistry to clinical outcome and strengthens the translational value of linker research.

Development Stage	Recommended Readouts
Chemistry	Synthetic yield, purity, hydrolytic stability, trigger specificity
Conjugation	Site occupancy, DAR distribution, aggregation, antibody integrity
In vitro stability	Intact ADC, free payload, metabolite profile in plasma/serum
Cellular mechanism	Internalization, trafficking, cleavage, and intracellular payload concentration
Pharmacology	Potency, bystander activity, PK/PD relationship
Safety	Off-target release, organ toxicity markers, therapeutic index
Translation	Manufacturability, batch reproducibility, formulation stability, and regulatory characterization

CONCLUSION

Ultra-stable linker technology should be viewed as a central engineering principle in ADC development rather than as a single class of chemical structures.

The most successful linker systems achieve a controlled compromise: they remain sufficiently stable during systemic circulation to prevent premature payload leakage, yet become efficiently activated or processed after productive uptake by the target cell. This balance directly influences pharmacokinetics, payload exposure, bystander activity, efficacy and toxicity.

Current evidence indicates that linker performance depends on the complete ADC architecture, including antibody, antigen density and internalization, conjugation site, DAR, spacer properties and payload characteristics.

Cleavable peptide, acid-sensitive, redox-sensitive and enzyme-responsive linkers provide programmable intracellular release, whereas non-cleavable systems offer exceptional circulation stability through payload release after antibody degradation. Neither strategy is universally superior; the appropriate choice is determined by the biology of the target and the physicochemical requirements of the payload.

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