Dual-payload antibody-drug conjugates in triple-negative breast cancer: Preclinical advances and future perspectives (Review)

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Abstract. Triple-negative breast cancer (TNBC), a highly aggressive and challenging subtype of breast cancer, is distinguished by the absence of certain receptors, which affects the utilization of current treatment options. While some TNBCs could initially respond to chemotherapy, this approach is associated with high rates of recurrence and resistance mechanisms. In an effort to increase the selectivity and therapeutic efficacy of chemotherapy, antibody-drug conjugates (ADCs) have emerged to serve these purposes. However, tumor heterogeneity remains the major issue restricting their therapeutic effectiveness in TNBC, along with the emerging mechanisms of resistance associated with single-payload ADCs. The search for novel strategies is urgent. Dual-payload ADCs have been developed by incorporating two distinct payloads onto the same antibody to target a novel antigen overexpressed on TNBC, while also utilizing recent advances in the multi-target approach (bispecific antibodies) and chemoimmunotherapy to combat tumor heterogeneity, relapse and multiple mechanisms of resistance associated with TNBC. The present review summarizes the currently available preclinical dual-payload ADCs that target TROP2, CD276, EGFR and cMET, and future directions in an aim to provide a reference for researchers and facilitate further development. Notably, the most promising approach is TROP2-directed dual-payload ADCs, which could open new avenues in oncology.

Contents

1. Introduction
2. Key design requirements of dual-payload ADCs
3. Mechanisms of action of dual-payload ADCs
4. Dual-payload ADCs in TNBC: Preclinical drugs and advancements
5. Therapeutic rationale and challenges of dual-payload ADCs in TNBC
6. Conclusion and future perspectives

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1. Introduction

Triple-negative breast cancer (TNBC) is an invasive subtype of breast cancer, characterized by <1% expression of estrogen receptor and progesterone receptor, as determined using immunohistochemistry (IHC). Notably, TNBC is also characterized by absent or reduced levels of human epidermal growth factor receptor-2 expression by IHC, with negative results by *in situ* hybridization testing. It is associated with high rates of recurrence and metastasis with a short survival time, is more predominant in premenopausal women <40 years of age, and accounts for ~15-20% of all breast cancer cases (1-3). Moreover, it contributes to 40% of breast cancer mortality rates (4).

The treatment of TNBC represents a formidable challenge in oncology due to various clinical and biological reasons. TNBC is characterized by extensive intertumoral and intratumoral molecular heterogeneity; accordingly, TNBC can be classified into different subtypes based on the Lehmann classification as follows: Basal-like 1 (BL-1), basal-like 2 (BL-2), a mesenchymal subtype (M), an immunomodulatory subtype (IM), a mesenchymal stem-like subtype (MSL) and a luminal androgen receptor subtype (LAR) (5).

Subsequently, this classification system was refined into four tumor-specific molecular subtypes: BL1, BL2, M and LAR, after taking into consideration the contribution of transcripts from normal stromal and immune cells in the tumor environment (6). Although the heterogeneous nature of TNBC is associated with drug resistance and a poor prognosis, this genetic variation supports the need for individualized treatment rather than uniform treatment strategies, and can be utilized to identify therapeutic targets and enhance treatment response (7,8).

The management of TNBC primarily relies on a multimodal approach that depends on the stage of the disease, involving surgery and radiotherapy, with chemotherapy

remaining the standard treatment in both systemic neoadjuvant and adjuvant therapy, particularly for patients with early-stage disease (9). Developing resistance and off-target toxicity associated with chemotherapy highlight the requirement for a novel therapeutic strategy to counter the aggressiveness of TNBC (10).

Single- and dual-payload antibody-drug conjugates (ADCs) represent a promising therapeutic approach for the treatment of TNBC by selectively delivering cytotoxic payloads to target cells. Single-payload ADCs consist of a monoclonal antibody and a cytotoxic payload connected by a linker, in comparison to dual-payload ADCs, which consist of two distinct payloads conjugated to a monoclonal antibody (11). This field of anticancer drugs has grown rapidly over the past few decades; as of April, 2025, the US Food and Drug Administration (FDA) has approved 15 ADCs for the treatment of a wide range of solid tumors and hematological malignancies, of which four are approved for the treatment of breast cancer: trastuzumab emtansine, trastuzumab deruxtecan, sacituzumab govitecan (SG) and datopotamab deruxtecan (12). However, the clinical efficacy of ADCs is often limited by issues associated with tumor heterogeneity, relapse and intrinsic or acquired resistance (13).

To address the limitations of single-payload ADCs, particularly resistance mechanisms and tumor heterogeneity in TNBC, research efforts have increasingly focused on developing dual-payload ADCs as a promising drug class by taking advantage of targeting pivotal antigens associated with tumor proliferation, invasion and immune modulation, including trophoblast cell-surface antigen 2 (TROP2) and CD276 (also known as B7-H3), and applying the dual-target approach to present agents with potentially greater therapeutic efficacy than single-payload ADCs. The present review highlights the design, mechanism, recent advances, current and future perspectives of dual-payload ADCs for the treatment of TNBC. With a comprehensive understanding of these aspects, researchers could collaboratively contribute to the next generations of ADCs and to improved therapeutic outcomes for patients with TNBC.

2. Key design requirements of dual-payload ADCs

Dual-payload ADCs are designed using a humanized or chimeric IgG monoclonal antibody (mAb), which targets tumor-specific antigens, while simultaneously carrying two or more distinct payloads through cleavable or non-cleavable linkers. Beyond the design principles of conventional ADCs, dual-payload ADCs involve additional design considerations that need to be carefully considered (14). The ideal dual-payload ADC design should promote tumor cell death, induce apoptosis, exhibit a high target binding specificity and affinity, a good internalization rate, low immunogenicity, potent cytotoxic payloads and stable linkers (15) (Fig. 1).

mAbs. Humanized or fully human mAbs are the preferred delivery system to ensure high target-cell specificity and a long circulating half-life in the systemic circulation. The antibody component of the ADC recognizes and binds to cell-surface antigens that are highly expressed in tumor cells (16,17). Healthy cells lack the expression of this antigen or express only minimal levels of such an antigen. Moreover, the targeted antigen should be a cell surface antigen so that circulating mAbs can recognize and bind to it easily (18). Several novel dual-payload ADCs have entered preclinical evaluation, targeting antigens, such as TROP2, B7-H3 and epidermal growth factor receptor (EGFR), which are overexpressed in TNBC (19,20).

IgG1 antibodies are the preferred subclass due to their high stability in the bloodstream and robust activation of innate immune cells, such as natural killer (NK) cells and macrophages, through interactions with Fcγ receptors (21). Moreover, human IgG1 exhibits low immunogenicity, which minimizes the risk of hypersensitivity reactions and the formation of anti-drug antibodies (22). Bispecific antibodies (bsAbs) can also be used to bind either two distinct antigens or two non-overlapping epitopes on the same antigen (a subgroup termed 'biparatopic') (23,24). Bispecific antibodies help overcome drug resistance, further enhancing the therapeutic potential of dual-payload ADCs (21).

Payload. Cytotoxic agents are the core payloads in ADCs (25). In addition to payload criteria in a single ADC, a dual-payload ADC requires the conjugation of potent and/or synergistic payload combinations (e.g., dual anti-tubulin inhibitors, TOPI inhibitors and immunotherapy) to reduce the therapeutically effective dose required to kill tumor cells. Dual-payloads could have distinct mechanisms of action, biological activity and pharmacokinetic (PK) properties. Therefore, they have disparate effective dose ranges and toxicity profiles; consequently, it is essential to determine the potential toxicity of payload combinations and the overlapping toxicities of the two payloads (26).

Linker. The linker is the spacer component that chemically binds the cytotoxic payload to the antibody (27). The fundamental requirement of a linker is to ensure the chemical stability of the ADC within the bloodstream. Linker-drug stability plays a critical role in determining PK properties, selectivity, therapeutic index and the overall success of the ADC (28). Therefore, it is critical to optimize linker stability, hydrophilicity, steric hindrance and linker length, which can affect the drug antibody ratio DAR of ADCs and PK properties (29-31).

ADC linkers can be divided into two types according to the cleavage method: Cleavable and non-cleavable. Cleavable linkers are often less stable in the blood, resulting in various off-target effects. By contrast, non-cleavable linkers require internalization by the target cell and release a linker-payload. This latter mechanism does not produce efficient bystander killing and thus results in lower toxicity profiles (32,33).

Dual-payload ADCs may employ either a linear linker or a branched linker. Both the multifunctional adapter and multifunctional linker payload methods provide adaptable paths for dual-payload ADCs; each approach can contribute to optimizing drug efficacy and targeting precision through strategic adjustments in DAR and payload distribution. Moreover, the option of a non-cleavable linker instead of a cleavable one is a decision for a particular strategy in which the stability of the complex is more critical than the rapid release of the active fragment (34).

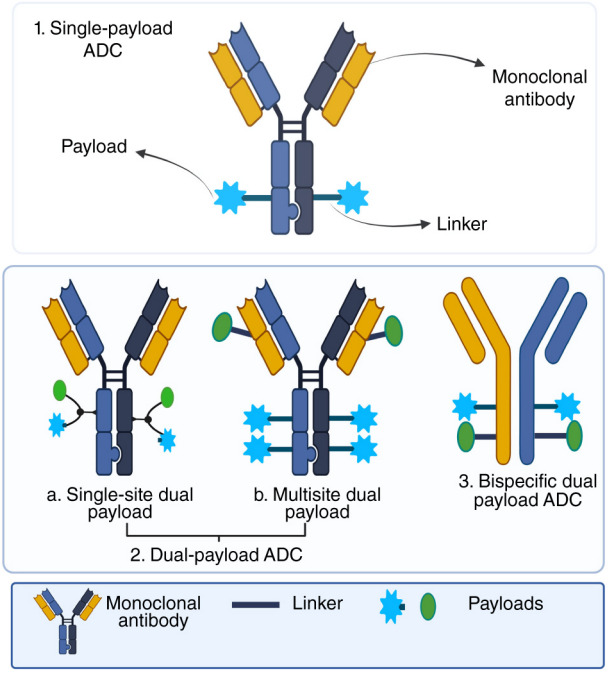

Figure 1. Schematic diagram illustrating the fundamental design components of a dual-payload ADC. (1.) ADCs consist of a mAb that targets a tumor-specific antigen and connects to only one cytotoxic payload via a cleavable or non-cleavable linker. (2.) Dual-payload ADCs: mAb carries two distinct payloads through a linker. There are two distinct conjugation strategies for dual-payload ADCs: (a.) Single-site dual-payload ADCs utilize one reaction site to conjugate a multifunctional linker; (b.) multisite dual-payload ADCs use two different conjugation sites on the mAb. (3.) Bispecific dual-payload ADCs: These conjugate a bispecific mAb that can bind two different antigens or epitopes, while carrying more than one payload simultaneously. The figure was created using BioRender. ADCs, antibody-drug conjugates; mAb, monoclonal antibody.

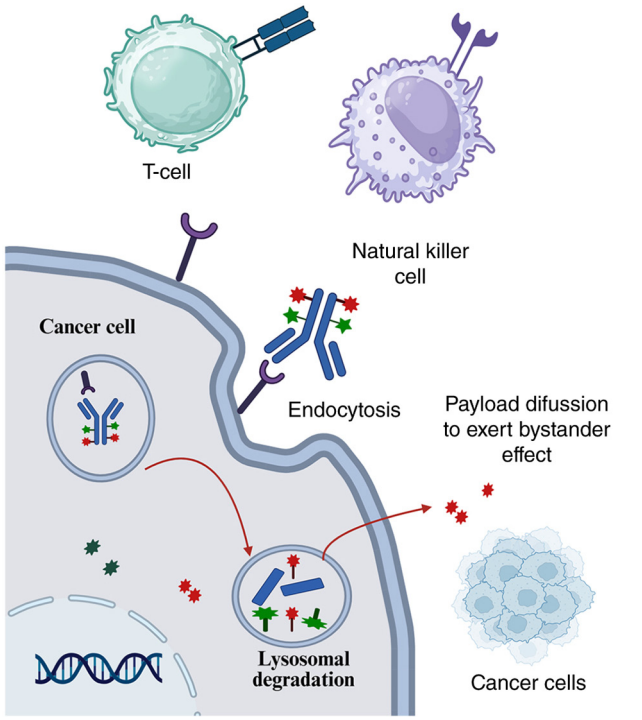

Figure 2. Mechanisms of action of dual-payload ADCs. Direct cytotoxicity: Following receptor-mediated endocytosis, the ADC is trafficked to the lysosome, where lysosomal proteases may cleave the linker or acid-mediated hydrolysis may occur, thereby releasing the payloads to exert their cytotoxic effects. In the event that the payload is sufficiently membrane-permeable, it may diffuse into neighboring cells and induce a bystander killing effect. In addition to direct cytotoxicity, immune-mediated mechanisms may contribute to the antitumor activity of the dual-payload ADCs. This has been investigated with CD276-directed dual-chemoimmunotherapy ADCs, in which CD276 targeting combined with an immunomodulatory payload, such as IMQ was associated with enhanced immune-cell infiltration and activation of the tumor microenvironment. The figure was created using BioRender. ADCs, antibody-drug conjugates.

DAR. DAR is defined as the average number of payloads conjugated to a single monoclonal antibody within an ADC. It is particularly challenging to adjust the total DAR and the ratio of the two payloads, which is critical for optimizing the therapeutic efficacy and safety profiles of different linker payload combinations. DAR measurement is pivotal in predicting essential properties, such as hydrophobicity, drug clearance, PK and biodistribution (35,36). A low DAR may reduce the antitumor efficacy, whereas an excessively high DAR increases hydrophobicity, promoting aggregation, reducing molecular stability and antigen binding affinity, leading to diminished biological activity. Although ADCs with a high DAR are generally more potent, they have been shown to exhibit more rapid clearance or efflux through multidrug resistance transporters, thereby reducing the therapeutic index (37).

3. Mechanisms of action of dual-payload ADCs

The mechanisms of action of dual-payload ADCs vary according to the targeted antigen, the dual-cytotoxic payloads, the monoclonal antibody and the linker connecting these components (38) (Fig. 2).

Upon binding to a specific cell-surface receptor antigen, the antibody-antigen complex is internalized via endocytosis, forming an endosome, which later fuses with a lysosome, where it is exposed to lysosomal proteases and degraded. The linker is cleaved in response to the acidic environment or the lysosomal proteases, thereby releasing the cytotoxic payloads into the cytoplasm (39).

Generally, chemotherapeutic agents exert their antitumor effects through three distinct mechanisms of action: Some drugs exhibit anti-tubulin activity or serve as DNA-damaging agents, whereas others function as topoisomerase inhibitors; the outcome is the apoptosis or necrosis of cancer cells (40,41).

In addition to direct cytotoxicity, dual-payload ADCs exert other mechanisms of action as immunomodulators, in which the antibody moiety is not only a vehicle for cytotoxic payloads or responsible for antigen recognition and targeting; this monoclonal antibody also promotes the activation of natural killer cells and T-cells in the tumor microenvironment (TME), as demonstrated by CD276 mAb-monomethyl auristatin F (MMAF)/IMQ (42,43).

Loo *et al* (44) found that an anti-CD276 mAb induced potent antibody-dependent cellular cytotoxicity (ADCC) in several tumor cell types. These findings suggest that CD276 mAb-MMAF/IMQ may also induce ADCC. In the study by Zhou *et al* (45), which investigated the anticancer mechanisms of this dual-payload ADC, the marked infiltration of activated cytotoxic CD8 T-cells, increased levels of CD45, the activation of natural killer cells and macrophage-mediated phagocytosis

were observed. Additionally, a certain level of cell death was observed in single-payload ADCs (mAb-MMAF and mAb-IMQ), whereas more extensive cell death was observed in the CD276 mAb-MMAF/IMQ group. These findings suggest that the dual-payload ADCs may enhance antitumor immune responses more potently compared with single-payload ADCs. However, further evidence is required to establish whether this effect is mediated by enhanced ADCC (45).

Apart from the selective drug delivery to tumor cells, some dual-payload ADCs have been found to have a bystander killing effect [such as in anti-TROP2 monomethyl auristatin E (MMAE)/F 4 + 2 ADC and 412a-MMAF + SN38), whereby cytotoxic drugs are diffused passively into neighboring cells, which can induce the death of cancer cells that exhibit a low receptor expression or none at all (46,47). This is crucial in heterogeneous tumors to enhance therapeutic efficacy by allowing cytotoxic payloads to eliminate antigen-negative tumor cells (48).

The bystander killing effect depends largely on factors, such as the extent of internalization after binding to a specific receptor antigen, the hydrophobicity of the cytotoxic payload and the type of linker, whether cleavable or non-cleavable. In the case of certain forms of cleavable linker, the drug could be released within the extracellular space before internalization, presenting an efficient bystander killing effect in the TME (49).

4. Dual-payload ADCs in TNBC: Preclinical drugs and advancements

Beyond the conventional single-payload ADCs, dual-payload ADCs combine the targeting specificity of monoclonal antibodies with the potent cytotoxic activity of two cytotoxic payloads to selectively eradicate cancer cells, including TNBC. Due to the heterogeneous nature of this disease, constructing dual-payload ADCs to target novel antigens that are overexpressed in TNBC, such as TROP2, is pivotal, as its overexpression is associated with tumor aggressiveness and a poor prognosis.

To enhance therapeutic strategies for TNBC with dual-payload ADCs, immunotherapy and bispecific antibodies have been employed in their design, targeting the overexpression of the immune checkpoint transmembrane, CD276, and the Toll-like 7/8 receptor (TLR7/8), which could overcome the limitations of immunotherapy in TNBC. Additionally, incorporating bsAbs further enhances their selectivity and efficacy.

TROP2-directed dual-cytotoxic payload ADCs. The TROP2 receptor is a type I surface glycoprotein receptor (50). It is consistently overexpressed in a variety of malignant tumors, including in >80% of patients with advanced-stage TNBC; TROP2 activation is also associated with several signaling pathways, depending on the tumor type, that affect cell proliferation and metastasis (51).

Stimulation of the TROP2 receptor leads to the increased expression of proto-oncogenes and anti-apoptotic proteins, which are responsible for the promotion of cell proliferation and the inhibition of the apoptosis of cancer cells (52). Moreover, the overexpression of TROP2 is associated with the increased expression of VEGF, a key mediator of tumor angiogenesis. Additionally, the activation of the TROP2 receptor stimulates the expression of genes that are responsible for preventing immune cell infiltration in the TME (53). TROP2 is also associated with a poor prognosis. Further investigations have also demonstrated that TROP2 gene deletion causes the suppression of TNBC growth *in vivo* and *in vitro*; therefore, TROP2 becomes a novel, attractive target for drug delivery (54).

TROP2 is the target of SG, a single-payload ADC. In April 2020, SG received accelerated approval from the FDA for patients with metastatic TNBC who have received at least two prior therapies for metastatic disease. In April 2021, SG received regular (full) FDA approval for use in patients with unresectable locally advanced or metastatic TNBC who had received two or more prior systemic therapies. This approval was supported by findings from the phase III ASCENT trial (55,56). TNBC has a high relapse rate, which may partly be due to multidrug resistance caused by SN38 (57). TROP2 is also expressed in normal tissues, such as the skin, leading to concerns about on-target, off-tumor toxicity. This encourages researchers to develop strategies that reduce multidrug resistance, mitigate toxicity and improve antitumor efficacy.

One of the strategies involved the discovery of a novel fully human mAb with reduced affinity to TROP2 and was engineered into homogenous dual-payload ADCs to successfully obtain anti-TROP2 MMAE/F and DuoDM/MMAE dual ADCs (58) (Table I). MMAE/F ADC is a dual-warhead ADC with MMAE and MMAF, functioning as anti-tubulin agents (59), while anti-TROP2 DuoDM/MMAE with duocarmycin DM functions as a DNA-damaging cytotoxic payload (60).

The combination of two payloads to target-resistant tumors, as in TNBC, can enhance antitumor activity by overcoming therapeutic resistance. Additionally, MMAE exhibits a bystander killing effect; to maximize the advantages of such an effect, one payload agent should be a small, lipophilic molecule (MMAE) to enhance penetration to the adjacent tumor cells, and the second one should be a larger and less hydrophobic agent (MMAF) to enhance retention within target antigen-expressing cells (61).

In 2026, Yamaguchi *et al* (58) demonstrated that anti-TROP2 MMAE/F with optimized antibody binding affinity exhibited significant antitumor activity in an immunocompromised orthotopic breast cancer model expressing TROP2 at a dose of 3 mg/kg, which was comparable to the efficacy of the sacituzumab-based variant. *In vitro*, MMAE/MMAF dual ADC did not demonstrate significant antitumor activity; this was probably due to the expression of multidrug resistance protein. By contrast, anti-TROP2 DuoDM/MMAE and SG surrogate led to the dose-dependent inhibition of tumor cell growth. *In vivo*, the antitumor activity of anti-TROP2 DuoDM/MMAE was demonstrated at an early phase of treatment with a dose of 3 mg/kg. However, tumor regrowth was observed 2 weeks following treatment, compared with the MMAE/MMAF dual-payload ADC. All animals remained tumor-free for >80 days (58).

As regards the toxicity profile according to the study by Yamaguchi *et al* (58), no significant acute toxicity was observed with the administration of ADCs; this may be due to the fact that the breast cancer models used in that experiment did not express the TROP2 as the same characterization that mimic human tissue; therefore, further investigations are required to

Table I. Summary of pre-clinical dual-payload ADCs.

Dual-payload ADCs	Target	Payloads	Conjugation method	DAR	Tested doses (mg/kg)	(Refs.)
mAb-MMAE/MMAF	TROP2	Anti-tubulin agents	MTGase conjugation	4+2	3	(58)
mAb-DuoDM/MMAE	TROP2	DNA damaging + anti-tubulin agents	MTGase conjugation	4+2	3	(58)
mAb-MMAF/IMQ	CD276	Anti-tubulin agent + TLR7/8 agonist	Site specific conjugation at lysin and cysteine residues	3 to 4+7 to 14 4+4.03	8, 16 and 24	(45,70)
mAb-DM1/IMQ	CD276	Anti-tubulin agent + TLR7/8 agonist	Site specific conjugation at lysin and cysteine residues	3.55+3.33	16	(70)
mAb-DXd/IMQ	CD276	TOP1 inhibitor + TLR7/8 agonist	Site specific conjugation at lysin and cysteine residues	8+2.9	16	(70)
412a-SN38+MMAF	EGFR x cMET	TOP1 inhibitor + anti-tubulin agent	Maleimide conjugation	Closer to 2	1, 3 and 9	(72)

mAb, monoclonal antibody; MMAE, monomethyl auristatin E; MMAF, monomethyl auristatin F; DuoDM, duocarmycin-based DNA-alkylating payload; IMQ, imiquimod; DM1, mertansine derivative 1; DXd, deruxtecan; TROP2, trophoblast cell surface antigen 2; EGFR, epidermal growth factor receptor; cMET, mesenchymal-epithelial transition factor; TLR7/8, Toll-like receptor 7/8; TOP1, topoisomerase I; DAR, drug antibody ratio; MTGase, microbial transglutaminase.

address the toxicity of anti-TROP2 dual-payload ADCs. This is particularly critical as SG exhibits a distinct toxicity profile arising either from the expression of TROP2 in normal tissues, such as keratinocytes and oral mucosa, manifesting as localized effects (mucositis and skin reactions), or from the toxicity associated with the SN38 payload, such as neutropenia, febrile neutropenia, anemia and leukopenia, among patients treated with SG (62,63).

CD276 (B7-H3)-directed dual-chemoimmunotherapy ADCs. Recent evidence indicates that TNBC has a high tumor mutation burden and the overexpression of immune checkpoints, such as B7-H3, which is expressed in >60% of all patients with TNBC. It is associated with poor patient survival and TNBC progression. Moreover, CD276 is involved in tumor growth, metastasis and treatment resistance. Therefore, CD276 has emerged as a potential target for anticancer therapy (64).

Several clinical and preclinical trials have demonstrated the efficacy of the combination of immune checkpoint and ADCs, including MORPHEUS-panBC and BEGONIA. However, several challenges are involved. These include overlapping toxicity between ADC and immunotherapy, as well as multiple resistance mechanisms, such as target antigen downregulation, resistance to the cytotoxic payload, impaired immune infiltration, or the upregulation of alternative immune checkpoints. These challenges are in addition to the limitations of single ADCs (65,66).

To address these challenges, Zhou *et al* (45) developed a chemoimmunotherapy conjugate by incorporating MMAF and TLR7/8 agonist (IMQ), on cysteine and lysine residues of anti-CD276 mAb. Preclinical data demonstrated that mAb-MMAF/IMQ exerted a synergistic effect *in vitro*. *In vivo*, a significant reduction in tumor burden was observed in immunocompromised and immunocompetent mouse models at 16 and 24 mg/kg. Additionally, mAb-MMAF/IMQ prevented tumor recurrence in both the patient-derived xenograft (PDX) model and the immunocompromised mouse model (45).

MMAF induces direct tumor cell death by blocking microtubule polymerization (67). IMQ inhibits cell proliferation via the MyD88/IRF7 signaling pathway through the activation of TLR7/8, which results in the secretion of several tumor cytokines (68). Moreover, the inhibition of the CD276 immune checkpoint by the mAb results in the activation of T-cells and natural killer cells in the TME (69). Thus, dual payload ADCs exert a synergistic antitumor effect and activate the immune system in the TME.

Building upon these findings, a subsequent preclinical trial developed a novel co-conjugation technology to conjugate highly potent payloads (MMAF, DM1 or DXd), which are microtubulin inhibitors or TOPI inhibitors, and IMQ to mAb-CD276 on cysteine and lysine. The optimized protocol achieved a high mAb conjugation efficacy, thus allowing the flexible conjugation of potent payloads, ensuring safety and optimizing therapeutic efficacy. Most notably, mAb-DXd/IMQ exhibited a significant potency compared to mAb-MMAF/IMQ and mAb-DM1/IMQ, *in vivo* at 16 mg/kg. This superior activity is likely attributable to its favorable biodistribution profile in TNBC; DXd causes DNA damage, which induces cell apoptosis regardless of the mitotic state. Moreover, it has a bystander killing effect that results in a significant tumor burden reduction (70).

Collectively, data confirmed that all three anti-CD276mAb ADCs are more effective in eradicating TNBC cells than single-payload ADCs, and address the off-target-induced

immune toxicity of free TLR agonists by integrating complementary anticancer mechanisms and an optimized conjugation protocol (71). This strategy has greater potential to bypass drug resistance and overcome tumor relapse, with a manageable systemic toxicity profile of anti-CD276 at all tested doses. Furthermore, neither the *in vitro* nor the *in vivo* models address advanced-stage metastatic disease or the complex mechanisms of therapeutic resistance. Therefore, the development of heterogeneous, spontaneous metastatic humanized mouse models in the future will be crucial for preclinical evaluation. Targeting a single antigen, CD276, may induce resistance to anti-CD276mAb ADCs in patients who do not express this antigen. Additionally, the maximum tolerated dose was not reached; therefore, further toxicologic studies are warranted (45,70).

Dual-payload bispecific ADCs. 412a-MMAF + SN38 is an innovative dual-payload ADC, composed of a bispecific EGFR x mesenchymal epithelial transition factor (cMET) antibody conjugated to two cytotoxic payloads (MMAF and SN38) utilizing a novel tri-functional linker, which has been designed to overcome mechanisms of resistance reported with single-payload ADCs in both dividing and non-dividing tumor cells.

The 412a-MMAF + SN38 ADC was generated using maleimide-based conjugation, resulting in an average DAR of ~2. As discussed above, ADCs with higher DARs are generally associated with increased cytotoxic potency; however, excessive drug loading may adversely affect pharmacokinetics, including a negative impact on therapeutic index due to faster clearance rate. Suggesting that dual-payload ADCs with a dual-target strategy may enhance antitumor activity without requiring a higher overall DAR (72,73).

BsAbs are engineered molecules constructed to target two distinct antigens or epitopes. Generally, bsAbs are generated by the incorporation of two different light chains and two different heavy chains (Fig. 1); the two binding variable domains allow the targeting of two antigens or epitopes to enhance therapeutic efficacy and reduce on-target, off-tumor toxicity (74). This field has evolved considerably; by early 2026, 15 bsAbs had received regulatory and/or conditional approval for hematological malignancies and solid tumors (75).

The distinctive structure of bsAbs and different design strategies permit a range of mechanisms of action, involving redirecting immune cells to the TME or blocking driver signaling pathways. bsAb in 412a-MMAF + SN38 targets simultaneously members of receptor belong to the tyrosine kinase family, including the EGFR and cMET receptor; blocking these receptors obstructs ligand-induced phosphorylation, hence affecting tumor proliferation, invasion and metastasis (76).

Apart from the direct cytotoxic effect of SN38 that functions as a potent topoisomerase I inhibitor, MMAF as an anti-tubulin agent and the bystander killing effect of SN38, amivantamab, a bispecific antibody targeting EGFR and MET, has been approved for EGFR-mutant non-small cell lung cancer. It has been reported to trigger an antitumor immune response through ADCC, antibody-dependent cellular phagocytosis activity and trogocytosis, involving the recruitment and activation of natural killer cells and macrophages through its Fc region. Moreover, Yoshichika *et al* (77) provided the first direct evidence from human clinical specimens of an immunostimulatory effect associated with amivantamab treatment. These findings may provide a rationale for investigating whether similar immune-mediated mechanisms contribute to the antitumor activity of 412a-MMAF + SN38 (72,77).

TNBC is a highly heterogeneous subtype of breast cancer, which is characterized by substantial variability in drug sensitivity and antigen expression; the engineering of dual-payload ADCs with dual-targeting bispecific antibodies could address intra- and inter-tumor heterogeneity. Furthermore, a bispecific antibody may reduce resistance caused by target antigen downregulation, and this strategy enhances the internalization of cytotoxic payloads into the tumor cells.

Recently, in the investigation of the novel 412a-MMAF + SN38 by Wilski *et al* (72) on multiple cancer cell lines, including TNBC, this drug was more effective in inhibiting tumor cell proliferation than single-payload ADCs. In the *in vivo* BxPC-3 human pancreatic cancer xenograft model, 412a-MMAF + SN38 more efficiently inhibited tumor growth compared to 412a-MMAF at a dose of 1 mg/kg. However, a surprising finding 412a-SN38 was not effective at any dose (Table I), this may be due to SN38 being a substrate of drug efflux pumps (72,78). Additionally, another mechanism of resistance associated with single-payload ADCs, chemotherapy and radiation is the development of dormant and non-dividing tumor cell populations; these cells are considered to contribute to tumor recurrence. 412a-MMAF + SN38 has been found to have a greater inhibitory activity when compared to single-payload ADCs in non-dividing (72).

5. Therapeutic rationale and challenges of dual-payload ADCs in TNBC

Dual-payload ADCs have been developed to address the limitations of single-payload ADCs, particularly in heterogenous tumors, such as TNBC. Incorporating two distinct payloads on a single monoclonal antibody could help overcome resistance mechanisms associated with the overexpression of drug efflux transporters, such as breast cancer resistance proteins, Multidrug resistance-associated proteins and P-glycoprotein (78). Furthermore, cancer cells could develop resistance to certain drug classes such as topoisomerase inhibitors. Therefore, delivering payloads with different mechanisms of action may help overcome resistance to individual agents (34).

Antigen heterogeneity is a hallmark of TNBC and represents a substantial obstacle that limits single-payload ADC efficacy. It may arise from antigen mutations, downregulation, or heterogenous antigen expression within tumors. This heterogeneity drives the production of subpopulations of cancer cells that arise within a single tumor site or patient, ultimately leading to tumor resistance (79).

The selection of a payload with a bystander killing effect, such as SN38 could overcome this limitation. However, the therapeutic effectiveness of SG may be diminished by several resistance mechanisms, including alteration in target antigen expression (78). Therefore, combining dual-targeting and dual-payload strategies has emerged as a potential approach to address tumor heterogeneity. For example, the

dual-payload ADC 412a-MMAF + SN38 has demonstrated antitumor activity in preclinical studies (72). Moreover, anti-CD276 mAb conjugated to an immunomodulator (IMQ) and a potent cytotoxic payload has a bystander killing effect, such as DXd, which may help address antigen heterogeneity and payload resistance by delivering two agents with distinct mechanisms of action. In addition, the immunomodulatory component may overcome the immunosuppressive TME characteristic of immunologically cold tumors, including TNBC (70).

Compared to combinations of two single-payload ADCs and combinations of single-payload ADC with immunotherapy or chemotherapy, the key innovation of dual-payload ADCs lies in their capability to deliver both payloads to the same cancer cell directly per single internalization of antibody. This is particularly critical in the case of heterogenous tumors, where the availability of antigen-binding sites may be limited (34). However, the design of dual-payload ADCs is complex and requires additional development steps compared to single-payload ADCs. This complexity is influenced by optimization of individual DAR of each payload and total DAR, the conjugation strategy and post-protein synthesis steps. Several conjugation approaches are available, and the selection of an appropriate method depends largely on process complexity, linker-payload characteristics, and antibody production platforms (34).

Furthermore, the conjugation strategy should provide flexibility in adjusting the DAR of each payload, allowing the physicochemical properties, efficacy, and toxicity profiles of ADCs to be optimized according to the disease target and payload combination. Yamaguchi *et al* (58) prepared anti-TROP2 MMAE/MMAF and anti-TROP2 DuoDM/MMAE using microbial transglutaminase and branched spacers to obtain homogenous dual-payload ADCs with DAR 4+2 and acceptable physicochemical properties. This DAR configuration exhibited favorable properties in preclinical studies, including a high monomeric fraction and antitumor activity in different breast cancer models (58). Additionally, a dual-payload ADC strategy may provide improved antitumor activity at a lower overall DAR, as demonstrated by 412a-MMAF + SN38 compared with single-payload ADCs having higher DAR (72).

The selection of payloads with different hydrophobicity profiles is critical for minimizing aggregation and potentially enhancing the bystander killing effect. For example, MMAE is more lipophilic than MMAF; therefore, combining these payloads results in dual-payload ADCs with intermediate hydrophobicity. Thus, the complementary properties of these two drugs may enhance cytotoxic activity against cancer cells when incorporated into a dual-payload ADC (51). Furthermore, the dual-payload ADC platform has the potential to reduce off-target toxicity by optimizing the dose of a highly potent payload and combining it with a less potent payload that may have more favorable PK properties. However, integrating two payloads with different toxicity profiles, PK properties, and potencies increases the complexity of ADC design (26).

Overall, dual-payload ADCs directed against different tumor-associated antigens have demonstrated encouraging antitumor activity in preclinical studies. However, several limitations associated with these preclinical findings remain regarding their safety and clinical applicability; neither the study by Yamaguchi *et al* (58) nor the study by Wilski *et al* (72) established the toxicity profiles of the TROP2-directed dual-payload ADCs and dual-payload bispecific ADC, respectively. Furthermore, CD276-directed dual-chemoimmunotherapy ADCs require additional toxicity studies, including determination of the maximum tolerated dose. Therefore, further studies are required to establish their safety and efficacy before their clinical potential can be determined (45,58,70,72).

6. Conclusion and future perspectives

Despite the promising preclinical results of dual-payload ADCs in TNBC treatment, the determination of their optimal design remains a time-consuming and costly process. Therefore, the integration of artificial intelligence and machine learning is accelerating the development of single-payload ADCs by optimizing linker-payload design, predicting PK properties, reducing on-target/off-tumor toxicity and predicting cytotoxic potency. These advances may represent a promising approach for the rational design and optimization of next generations of dual-payload ADCs (80,81).

Combination strategies integrating bispecific antibodies and dual-payload ADCs represent a promising approach to overcome the limitations of immune checkpoint inhibitor monotherapy, resistance, tumor heterogeneity and to enhance antitumor activity in TNBC through complementary mechanisms. The combination of KH815 with PD-L1/VEGF and B7-H3 mAb-DXd/IMQ with PD-L1/4-1BB demonstrated a significant enhancement of tumor growth inhibition compared to monotherapy in preclinical models. Moreover, the former induces long-term immunological memory response *in vivo* (82,83).

Triple-payload ADCs may represent a future direction for cancer therapy, particularly for aggressive types of cancer, such as TNBC. Triple-payload ADCs allow multiple cytotoxic payloads to be delivered to the tumor cells, enhancing the eradication of heterogeneous and resistant tumors. A triple-payload incorporating MMAE and two distinct topoisomerase-1 inhibitors conjugated to a novel Nectin-4 targeting antibody has been engineered and demonstrated potent anti-tumor activity in a low Nectin-4-expressing TNBC PDX model comparing to single payload ADCs (84).

Nanoantibody technology represents a potential advance for ADC design to overcome the large molecular weight of ADCs (>150 kDa) that affects payload penetration into solid tumors. Furthermore, engineering bsAbs as a conditionally activated prodrug in TME may be an avenue to mitigate off-target toxicity. Although there are currently limited data for such strategies in dual-payload ADC design. However, both may present an opportunity for further optimization of dual-warhead ADC development (85,86).

In addition to CD276, EGFR and cMET, which are attractive targets for TNBC drug development, TROP2 was the most extensively investigated target, owing to the clinical success of SG for TNBC and its overexpression in TNBC and other solid tumors, such as gastric, colorectal, pancreatic, squamous cell carcinoma of the oral cavity, non-small cell lung cancer and prostate cancer.

Building on these findings, several TROP2-directed dual-payload ADCs were developed, with preclinical antitumor activity demonstrated in various cancer models. Moreover, two Trop2-directed dual-payload ADCs, KH815 and ASP2998, have advanced to clinical trials for solid tumors (52,87,88). However, the available published evidence for KH815 and ASP2998 does not clearly establish whether patients with TNBC have been enrolled in these trials. Nevertheless, the clinical development of these agents, particularly KH815, represents progress toward the clinical evaluation of TROP2-directed dual-payload ADCs, particularly given the reported preclinical antitumor activity of KH815 in TNBC models (89).

In conclusion, to date, none of thes preclinical dual-payload ADCs discussed in the present review has been specifically evaluated in clinical trials for TNBC. Furthermore, preclinical findings may not necessarily translate into clinical benefits. Therefore, further preclinical and clinical studies are required to optimize ADC design, establish therapeutic efficacy compared with single-payload ADCs, understand the potential synergistic mechanisms of action, determine the therapeutic index and evaluate safety profiles. These efforts will be essential for maximizing the therapeutic potential of dual-payloads in TNBC. Furthermore, the progression of the anti-TROP2 dual-payload ADCs, KH815 and ASP2998, into clinical evaluation for solid tumors highlights progress towards the clinical transition of this therapeutic strategy. Overall, dual-payload ADCs represent an evolving approach in oncology, and continued research may help to establish their role in future cancer treatment strategies.

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FAA and MNA were involved in the conceptualization of the review and the approach to literature identification and synthesis. RJS, EGY, MNA and FAA were involved in the preparation of the figures and in the writing of the original draft of the manuscript. All authors edited and revised the manuscript. All authors have read and agreed to the final version of the manuscript. Data authentication is not applicable.

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Competing interests

The authors declare that they have no competing interests.

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