

Opportunities and Challenges in the Development of Antibody-Drug Conjugate for Triple-Negative Breast Cancer: The Diverse Choices and Changing Needs

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Abstract

Triple-negative breast cancer (TNBC) is a highly heterogeneous breast cancer subtype, which is also characterized by the aggressive phenotype, high recurrence rate, and poor prognosis. Antibody-drug conjugate (ADC) is a monoclonal antibody with a cytotoxic payload connected by a linker. ADC is gaining more and more attention as a targeted anti-cancer agent. Clinical studies of emerging ADC drugs such as sacituzumab govitecan and trastuzumab deruxtecan in patients with metastatic breast cancer (including TNBC) are progressing rapidly. In view of its excellent clinical efficacy and good tolerability, Sacituzumab govitecan gained accelerated approval by the FDA for the treatment of advanced metastatic TNBC in 2020. This review discusses the treatment status and challenges in TNBC, with an emphasis on the current status of ADC development and clinical trials in TNBC and metastatic breast cancer. We also summarize the clinical experience and future exploration directions of ADC development for TNBC patients.

Keywords: Breast cancer; Triple-negative breast cancer; Antibody-drug conjugate; Clinical trials

Introduction to Triple Negative Breast Cancer (TNBC): Current Landscape of Classification, Heterogeneity, Treatment and Challenges

Breast cancer is one of the most common malignant tumors in

females, which has become a significant risk factor endangering the health of women [1]. In 2020 there were 2,261,000 new cases of breast cancer and 685,000 related deaths worldwide, according to the global cancer statistical report [2]. Breast cancer originates from the carcinogenic transformation of the intraductal epithelium, displaying a high degree of heterogeneity within the tumor and between the tumors of different patients. Heterogeneity refers to the diverse and varied nature of cancer cells, both within a single tumor and between different tumors of the same cancer type [3, 4]. Intratumor heterogeneity is manifested as the variations in genetic composition, phenotypic/functional features and drug sensitivity among cancer cells within a single tumor mass, which arises from the accumulation of genetic and epigenetic alterations in different subpopulations of cancer cells. Intertumor heterogeneity is characterized as the differences observed among the same type of tumors from different individuals. Tumors from different patients with the same cancer type can exhibit distinct genetic alterations, gene expression profiles, histological features, and clinical behaviors, including responses to treatment. The degree of heterogeneity is related to the risk of disease progression and treatment resistance and has become the basis for the classification of breast cancer [3, 4]. For example, the classification of breast cancers published at the 2013 St. Gallen International Breast Cancer Conference was based on molecular subtypes: luminal A (estrogen (ER)/progesterone (PR)+, human epidermal growth factor receptor 2 (HER2)-, Ki-67+ and low tumor (LT) grade; 20%), luminal B (ER/PR+ and LT; 20% , HER2-, Ki-67+ \geq 20%); Her2 + B2 (ER/PR+, HER2 overexpression); HER2 overexpression (ER-, PR-, HER2 overexpression); TNBC (ER-, PR-, HER2-) and other special subtypes [5]. TNBC is a highly malignant subtype of breast cancer with poor prognosis, accounting for 15-20% of breast cancer cases [6, 7]. TNBC is defined as a type of breast cancer that is negative for ER, PR and HER2 [7, 8]. Gene expression profiling analysis classifies TNBC as a subtype of basal-like breast cancer (BLBC), since there is approximately 56% overlap between TNBC and BLBC gene profiles, whereas the overlap between non-TNBC and BLBC was only 11.5% [9]. About 15% of TNBC cases carry mutations in *BRCA1* and *BRCA2* genes, the inheritable breast cancer susceptibility genes [10]. Meanwhile, TNBC also harbors a unique spectrum of genetic

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mutations, and recurrent mutations (such as *TP53* and *PI3K-CA* mutations) can drive tumor progression concurrently with rare mutations in TNBC [11]. The diversified genetic mutation landscape of TNBC hints the complicated etiology and disease progression. Compared with other breast cancer subtypes, TNBC usually occurs in young women and has a mortality rate up to 40% within 5 years of diagnosis [6]. Moreover, TNBC is highly invasive, with distant metastasis occurring in about 46% of cases. The median survival time after metastasis is only 13.3 months and the cancer recurrence rate is up to 25% [12]. Once the recurrence is diagnosed, the mortality rate of TNBC patients is as high as 75% within 3 months of cancer recurrence [13]. Therefore, TNBC is the most aggressive and threatening subtype of breast cancer, which is difficult to manage in clinical settings.

There is significant heterogeneity within TNBC tumor tissue. The study of early tumor heterogeneity focuses on the genomic, transcriptomic and proteomic features of TNBC cells [14]. In the past decade, accumulating evidence has highlighted the significance of heterogeneity in the tumor microenvironment. Especially after the advent of immunotherapy, the implication of both the intrinsic and extrinsic features of TNBC tumors in judging the prognosis and treatment response has been gradually realized [15, 16]. Furthermore, advances in single-cell analysis techniques have provided an unprecedented delineation of the heterogeneity of TNBC tumor cells and the tumor microenvironment [17, 18]. The early classification system TNBC-Type is a subtype categorization of six features at the level of gene expression (basal-like (BL)1, BL2): keratin 5/6, epidermal growth factor receptor (EGFR); mesenchymal (M): vimentin, fibronectin; mesenchymal stem-like (MSL): CD44, ALDH1A1; immunomodulatory (IM): programmed cell death ligand 1 (PD-L1), signal transducer and activator of transcription 1 (STAT1); androgen receptor (AR): androgen receptor; luminal androgen receptor (LAR): androgen receptor, Forkhead box protein A1 (FOXA1)), which reflects the heterogeneity of TNBC tumor cells [19]. Subsequent research proposed two TNBC subtypes based on the tumor microenvironment: the immunomodulatory subtype and the mesenchymal stem cell-like subtype, which is based on whether there are large numbers of tumor-infiltrating lymphocytes or stromal cells in the tumor tissue [20]. Along with this rationale, the revised TNBCType-4 classification system defines four subtypes: BL 1 and 2 subtypes, LAR subtypes, and M subtypes. Thus, the evolution of TNBC subtype classification is a typical example of how the intrinsic and extrinsic features of a tumor are taken into account as complementary information. A subsequent large cohort study verified that each TNBCType-4 category (including BL, LAR, and M subtypes) was characterized by specific features in the tumor microenvironment, including immune composition, angiogenesis, matrix composition and metabolic processes [21]. These findings also suggest the necessity of tailored therapy for each TNBC subtype to optimize treatment outcomes.

Intriguingly, intratumoral features and genetic mutations impinge on the tumor microenvironment. For example, the level of immune cell infiltration correlates with the genomic profile of TNBC. The low degree of clonal heterogeneity, somatic copy number changes, somatic mutations, and neoantigen burden in TNBC tumor samples is associated with higher

levels of immune cell infiltration [22]. The most common *TP53* gene mutation in TNBC contributes to the suppression of innate immune signals and promotes immune evasion [23, 24]. In addition, defects in DNA damage response in TNBC cells can also regulate intratumoral immunity through the cGAS-STING signaling pathway [25]. Notably, inherited *BRCA1* and *BRCA2* mutations also differentially regulate the immune microenvironment. Tumors with *BRCA2* deficiency are characterized by more abundant adaptive and innate immune-related gene expression [26]. An increasing number of studies focus on the interplay of the mutation profiles of cancer cells and the tumor microenvironment in TNBC, with the hope to deepen the understanding of the impact of intrinsic and extrinsic interaction on tumor evolution and treatment response.

Because of the specific molecular profile, TNBC is not sensitive to endocrine therapy or some molecular-targeted therapies, such as anti-angiogenic therapies and PI3K/AKT/mTOR inhibitors [27]. Cytotoxic systemic chemotherapy remains as the mainstream treatment for early and advanced TNBC [14]. In addition to conventional chemotherapeutics such as taxanes, anthracyclines, and cyclophosphamide, platinum-based regimens are also implemented as adjuvant chemotherapy for TNBC. Platinum-based reagents lead to apoptosis by inducing DNA damage, and clinical results have shown that platinum monotherapy is particularly effective for *BRCA1/2* mutation carriers. This may be explained by the fact that *BRCA1/2* mutations cause genomic instability and defective DNA repair, rendering tumor cells particularly sensitive to platinum-induced DNA damages [28-30]. The addition of platinum to neoadjuvant therapy can increase the complete response rate by 10-15%, although drug administration had to be discontinued due to hematologic toxic effect [29]. Targeted therapies focusing on key signaling pathways in TNBC, including EGFR, PI3K/AKT/mTOR signaling pathway, and poly ADP-ribose polymerase (PARP) inhibitors, have been approved or are currently in clinical trials [31]. Recently, immunotherapies such as immune checkpoint inhibitors (ICIs) are also being trialed for the treatment of TNBC. These drugs work by blocking immunosuppressive receptors and improving the cytotoxicity and proliferation of tumor-infiltrating lymphocyte [32, 33]. However, systemic toxicity limits the dosage of drug administration in chemotherapy and targeted therapy. Postoperative adjuvant chemotherapy cannot eradicate the tumor in most patients, and long-term targeted therapy inevitably leads to the development of drug resistance. The residual metastatic lesions after therapy eventually lead to tumor recurrence with cancer cells exhibiting increasingly aggressive characteristics [14, 34]. Antibody-drug conjugate (ADC) is a rapidly developing class of anticancer agents, which can reduce systemic toxicity through targeted delivery to cancer cells [35]. This review mainly focuses on the current landscape and challenges of ADC drug development for TNBC.

ADC: The Concept, History and Development

ADCs are complex targeted drugs consisting of cytotoxic drugs and antibody scaffolds. ADCs remain stable under normal

physiological conditions such as blood circulation, and upon binding to tumor cell surface antigens by the antibody, ADC-carried cargos (anti-neoplastic drugs) are released to induce tumor cell death [35]. The idea of using antibodies as targeted therapies for cancer dates back to the pioneering work of Paul Ehrlich in the early 20th century, when he proposed the need to develop drugs that could selectively target diseased cells without harming healthy tissue [36]. In the 1960s, researchers began exploring the application of antibodies to deliver cytotoxic drugs to cancer cells, including the use of antibodies to deliver radionuclide to tumors. However, due to the lack of suitable antibody targets, the immaturity of antibody engineering technology and the difficulty of drug-antibody conjugation, the development of ADC did not see much progress [36]. In 1975, the advent of monoclonal antibody technology paved the way for ADC development [37]. Gemtuzumab ozogamicin was the first Food and Drug Administration (FDA)-approved ADC in 2000 for the treatment of acute myeloid leukemia but was later withdrawn for safety reasons [38]. Despite the challenges, several ADCs have been approved for the treatment of various types of cancers [37].

The three key elements in developing an ADC drug are: the antibodies against specific tumor antigens, cytotoxic drugs (also called payloads or warheads), and the linkers that connect payloads to antibodies [37]. Linkers are biochemical compounds that attach the payloads to antibodies, which can be divided into cleavable and non-cleavable types according to their chemical property [39]. Non-cleaved linkers consist of stable bonds that are resistant to proteolytic degradation and therefore only undergo cleavage after lysosomal internalization and antibody degradation. Although these linkers are more stable than cleavable linkers, the drug internalization may be impaired due to the low membrane permeability. Instead, the breakdown of cleavable linkers depends on external factors such as pH (acid unstable linker), the presence of specific proteases (protease-cleavable linker) or glutathione reduction (disulfide bond linker) [39-41]. Currently, most ADC drugs are based on the internalizing mechanism, whereby ADC is internalized by endocytosis upon the binding to cancer cell surface antigen and degraded in lysosomes to release cytotoxic drug [37]. In addition to this canonical process, ADCs can also induce tumor cell death through “bystander effect”, an effect that occurs when cytotoxic payloads spread across the cell membrane to neighboring cells and induce apoptosis [42]. The key to the design of an internalizing ADC is that the relatively strong linker between the antibody and the payload, which remains stable under physiological conditions of plasma circulation but is rapidly lysed after endocytosis by tumor cells. This design selectively delivers payloads into tumors and at the same time limits adverse side effects due to off-target toxicity [43]. Non-internalizing ADCs do not require lysosomes to release payloads, and the linkers can be degraded in the tumor microenvironment to release the payload and exert cytotoxic effects [44]. Besides, non-internalizing ADCs usually do not require the overexpression of cancer cell surface antigens and can take advantage of several physiological features of the tumor microenvironment (such as pH and hypoxia) for targeted delivery [44]. Thus, non-internalizing ADCs are good candi-

dates to target cancers with dense tumor stroma, such as TNBC and pancreatic ductal adenocarcinoma (PDAC) [45].

The antibodies used for ADC development are humanized antibodies, with significantly attenuated immunogenicity compared to murine and chimeric monoclonal antibody [46]. Most antibodies are based on immunoglobulin (Ig)G1 isotype which are easier to produce than IgG2 and IgG4 [47]. IgG1-type antibodies retain higher immunogenic functions, supporting antibody-dependent cell-mediated cytotoxic (ADCC) and complement-dependent cytotoxic (CDC) responses [46]. But some ADCs contain specifically designed antibodies to minimize the immunogenicity (such as FC mutants of IgG1 isoforms) and avoid undesirable toxicity [46]. The cytotoxic drugs used for most ADC development fall into two major categories: tubulin inhibitors (such as Maytansine alkaloids) and DNA-damaging agents (such as calicheamicin) [41]. These drugs are characterized by an IC_{50} (the concentration that inhibits cell viability by 50%) in the nanomolar and picomolar range, which are highly toxic and has adverse toxic side effects when administered systemically [48]. The incorporation of these drugs into ADCs minimizes the toxicity in the bloodstream and avoids undesirable effects in other organs. Conversely, for less toxic drugs, ADC-released payload is not able to reach the IC_{50} and exert significant toxicity in tumor cells. Thus, this type of drugs has not been successfully developed as effective ADCs [46]. Indeed, the poor performance of the first batch of developed ADCs can be attributed to the low therapeutic index of the payload (e.g., anthracycline) and low antigen abundance on the surface of cancer cells [49].

Current Clinical Progress of ADC Development in TNBC

Clinical studies are under rapid progress on multiple ADCs targeting different antigens expressed in TNBC. These ADCs are usually constructed using cleavable linkers, and some ADCs targeting HER2 and trophoblast cell surface antigen 2 (TROP-2) have entered clinical phase III trials (Table 1).

ADCs targeting HER2 and TROP2 in phase III clinical trials

ADCs targeting HER2

HER2 is a member of the EGFR family and is overexpressed in 15-20% of breast cancer cases. Approximately 40-50% of HER2-negative breast cancer cases express HER2 at low levels (defined as immunohistochemistry (IHC) 1+ or IHC 2+/Ish-type breast cancer) [50]. In this class of TNBC patients, HER2 inhibitors did not improve clinical outcomes, as shown by the results of the NSABP B-47 clinical trial [51]. The new generation of anti-HER2 ADCs has a higher drug to antibody ratio (DAR), a cleavable linker, and a permeable payload that can induce bystander cytotoxicity. These features enable anti-HER2 ADCs to exert anticancer effect in the tumor environ-

Table 1. ADCs for Breast Cancer and TNBC Under Clinical Trials

ADC	Target	Antibody	Payload	Linker	DAR	Clinical trial identifier	Phase	Patient cohort	Patients number	Main results of the clinical trial
Trastuzumab deruxtecan (DS-8201a)	HER2	Trastuzumab	Deruxtecan (topoisomerase I inhibitor)	Proteolytically cleavable	7 - 8	NCT02564900	I	Pre-treated metastatic breast, gastric, gastroesophageal cancers	278	Controllable safety, and satisfactory objective response rate
Trastuzumab duocarmazine (SYD985)	HER2	Trastuzumab	Duocarmycin (DNA alkylating agent)	Proteolytically cleavable	7 - 8	NCT02277717	I	Locally advanced or metastatic BC	185	Both HER2-positive and negative patients showed objective response; controllable safety
Sacituzumab govitecan (IMMU-132)	TROP-2	Sacituzumab	SN-38 (topoisomerase I inhibitor)	Cleavable, pH sensitive	7.6	NCT01631552	I/II	HER2-positive advanced or metastatic BC	436	PFS was significantly improved compared to conventional chemotherapy
Datopotamab deruxtecan (DS-1062)	TROP-2	Datopotamab	Deruxtecan (topoisomerase I inhibitor)	Proteolytically cleavable	4	NCT02574455	III	Recurrent metastatic TNBC	468	Objective response and clinical efficacy; bone marrow toxicity
						NCT03401385	I	Advanced or metastatic TNBC	44	Compared to chemotherapy, PFS and OS were significantly improved; bone marrow toxicity and diarrhea
						NCT05374512	III	Locally recurrent, inoperable or metastasis TNBC	600	Preliminary results showed good objective response rate and controllable safety

Table 1. ADCs for Breast Cancer and TNBC Under Clinical Trials - (Continued)

ADC	Target	Antibody	Payload	Linker	DAR	Clinical trial identifier	Phase	Patient cohort	Patients number	Main results of the clinical trial
Patritumab deruxtecan (U3-1402)	HER3	Patritumab	Deruxtecan (topoisomerase I inhibitor)	Proteolytically cleavable	7-8	NCT02980341	I/II	HER3-positive metastatic BC	180	Objective response in HR+/HER2-, HER2+ and TNBC patients
Ladiratumumab vedotin (SGN-LIV1a)	Estrogen-regulated LIV-1 protein (LIV-1)	Ladiratumumab	Monomethyl auristatin E (microtubule inhibitor)	Proteolytically cleavable	4	NCT01969643	I	Locally advanced or metastatic BC	89	Interim results showed good tolerability and significant antitumor activity
Enfortumab vedotin-efv	Nectin cell adhesion molecule 4 (nectin-4)	Enfortumab	Monomethyl auristatin E (microtubule inhibitor)	Proteolytically cleavable	3.8	NCT04225117 (EV-202)	II	HR+/HER2- BC, and TNBC	240	The combination with pembrolizumab (PD-1 monoclonal antibody) showed antitumor efficacy and safety
Mirvetuximab soravtansine (IMGN853)	FR α	Mirvetuximab	Ravtansine (DM4) (microtubule inhibitor)	Cleavable	2	NCT03106077	II	FR α -positive, advanced TNBC	96	Terminated due to low frequency of FR α positivity and the lack of response
Farletuzumab ecteribulin (MORAb-202)	FR α	Farletuzumab	Eribulin (microtubule inhibitor)	Proteolytically cleavable	4	NCT04300556	I/II	FR α -positive, advanced solid tumors (including TNBC)	196	NA
AVID100	EGFR	MAB100	Mertansine (DM1) (microtubule inhibitor)	Proteolytically cleavable	NA	NCT03094169	I/II	Advanced epithelial tumors (including TNBC)	90	NA
Ozurifamab vedotin (CAB-ROR2-ADC)	ROR2	Ozurifamab	Monomethyl auristatin E (microtubule inhibitor)	Proteolytically cleavable	NA	NCT03504488	I/II	TNBC, non-small-cell lung carcinoma	120	NA
Anti-CA6-DM4 immun-conjugate (SAR566658)	Tumor-associated Sialoglycoprotein CA6	DS6	Ravtansine (DM4) (microtubule inhibitor)	Cleavable, glutathione dependent	1	NCT02984683	II	CA6- positive, metastatic TNBC	23	Discontinued due to limited efficacy

ADC: antibody-drug conjugate; TNBC: triple-negative breast cancer; DAR: drug to antibody ratio; HER: human epidermal growth factor; TROP2: trophoblast surface antigen 2; FR α : folate receptor α ; EGFR: epidermal growth factor receptor; ROR2: receptor tyrosine kinase-like orphan receptor 2; BC: breast cancer; NA: not available; PFS: progression-free survival; OS: overall survival; HR: hormone receptor.

ment where HER2 is poorly expressed.

Trastuzumab deruxtecan (T-Dxd, also called DS-8201A) contains an anti-HER2 human monoclonal IgG1, which is conjugated to the payload Dxd by an enzymatically cleavable peptide linker. Dxd, a derivative of exatecan mesylate, is a synthetic camptothecin analogue and a potent topoisomerase I inhibitor. The DAR of T-Dxd is 7 to 8, which indicates that each antibody is loaded with multiple drug molecules. T-Dxd showed therapeutic efficacy against HER2-low-expressing breast and gastric cancers in a mouse xenograft model, whereas another anti-HER2 ADC (T-DM1), which is composed of a monoclonal antibody trastuzumab and DM1 (emtansine), had no significant effects [52]. The first report of clinical efficacy of T-Dxd in HER2-negative metastatic breast patients, including TNBC, was based on an open-label phase I trial (NCT02564900) [53]. This study recruited patients with metastatic breast, gastric, and gastroesophageal cancer who had undergone previous treatment. A total of 54 patients with HER2-negative metastatic breast cancer were recruited. The antitumor activity of T-Dxd was observed in this cohort of heavily treated patients: the objective response rate (ORR) confirmed by independent central review was 20/54 (37.0%; 95% confidence interval (CI): 24.3% to 51.3%); median duration of response (MDOR) was 10.4 months (95% CI: 8.8 months until not evaluable). Interestingly, there was a significant difference in ORR according to hormone receptor (HR) status, with an ORR of 40.4% in HR-positive patients and 14.3% in patients with TNBC [54]. The difference in response rate may reflect underlying biological differences between HER2-low expression/HR-positive cases and TNBC cases. In terms of safety profile, the most common grade ≥ 3 treatment emergent adverse events (TEAEs) with an incidence $\geq 5\%$ were hypokalemia, thrombocytopenia, loss of appetite, febrile neutrophil reduction and diarrhea. Based on the results of phase I study, a multicenter, randomized, and open-label phase III study (DESTINY-Breast 04, NCT03734029) has been initiated with the plan to recruit 540 patients with advanced/metastatic HER2-low-expression breast cancer, including TNBC. The enrolled patients will receive either T-Dxd treatment or one chemotherapeutic of doctor's choice (capecitabine, eribulin, gemcitabine, paclitaxel, or albumin-binding paclitaxel) in 2:1 ratio [55]. The recently disclosed data showed that of the 557 patients who underwent randomization, 494 (88.7%) were HR-positive, and 63 (11.3%) were HR-negative. In the HR-positive cohort, the median progression-free survival (mPFS) was 10.1 months in the T-Dxd treatment group and 5.4 months in the doctor's choice chemotherapy group (hazard ratio for disease progression or death: 0.51; $P < 0.001$). The median overall survival (mOS) was 23.9 months and 17.5 months, respectively (hazard ratio for death: 0.64; $P = 0.003$). In all patients, the mPFS was 9.9 months in the T-Dxd group and 5.1 months the doctor's choice chemotherapy group (hazard ratio for disease progression or death: 0.50; $P < 0.001$), and the mOS was 23.4 and 16.8 months, respectively (hazard ratio for death: 0.64; $P = 0.001$). Grade 3 or higher-grade adverse events occurred in 52.6% of patients treated with T-Dxd, compared with 67.4% of those who received physician's choice chemotherapy [56]. This phase III trial demonstrated the superior therapeutic potential and safety of T-Dxd over conventional chemotherapy.

Trastuzumab duocarmazine (SYD985), an anti-HER2 ADC that carries duocarmycin (a DNA alkylating agent), is linked by a proteolytically cleavable linker with an average DAR of 7 - 8; Preclinical evaluation of SYD985 in HER2-positive tumor cells showed promising anticancer effect [57]. Subsequent preclinical study reported similar antitumor activities of SYD985 and T-DM1 in HER2-overexpressing breast cancer cells; however, in HER2-low-expressing cells, SYD985 had three to 50-fold higher tumoricidal effect and stronger bystander-killing activity [58]. Another study also confirmed the antitumor effects of SYD985 in HER2-low-expressing and T-DM1 resistant cancer cells in animal model [59]. The first human study of this ADC was performed in patients with metastatic cancers expressing HER2 (NCT02277717, phase I). In a cohort of 15 patients with low-HER2-expressing metastatic TNBC, SYD985 showed antitumor activity: in this cohort of patients with previous treatment, the ORR to SYD985 reached 40% (6/15) (median of previous treatment: 4). Of all 146 patients in the dose expansion cohort, 19% of cases discontinued drug administration because of TEAE, the most common being ocular toxicity (10%) [60]. Thus, SYD985 showed promising clinical response in heavily pretreated metastatic patients, including HER2-low-expressing breast cancer, with a manageable safety profile. A multicenter, open-label, and randomized phase III trial has been initiated to compare the efficacy and safety of SYD985 to physician-selected chemotherapy in patients with HER2-positive, unresectable, advanced/metastatic breast cancer (NCT03262935, TULIP Trial, phase III). The newly disclosed results showed that SYD985 significantly improved PFS in patients with HER2-positive, locally unresectable, advanced/metastatic breast cancer when compared with physician-selected chemotherapy, and the primary end point was reached. At present, there are no clinical studies focusing on the efficacy and safety of SYD985 in TNBC patients.

ADCs targeting TROP-2

TROP-2, also known as EGP-1, was initially described as a surface marker expressed in trophoblast cells, and subsequent studies characterized TROP-2 as a glycoprotein that is overexpressed in different epithelial tumors, including all types of breast cancer [61]. Immunohistochemical evaluation of TROP-2 suggested that approximately 80-93.4% of TNBC cases are TROP-2-positive [62, 63]. Therefore, TROP-2 is an important molecular target for ADC development in TNBC.

Sacituzumab govitecan (IMMU-132) is a TROP-2 antibody conjugated with a potent DNA-damaging agent SN-38 by a pH-sensitive cleavable linker. SN-38, an active metabolite of irinotecan, inhibits topoisomerase-I and causes DNA double-strand breaks. Although SN-38 was only moderately toxic (the IC_{50} was in the nanomolar range), the average DAR in IMMU-132 can reach 7.6, which facilitates more drug delivery to tumor [64]. *In vitro* study and pharmacokinetics analysis showed that about 50% of SN-38 is released from the antibody daily, and about 90% of the payload is released within 3 days [65]. IMMU-132 led to rapid tumor regression in a TNBC mouse model, showing a stronger antitumor effect than irinotecan [66]. The first human trial of IMMU-132 (NCT01631552,

phase I) enrolled a total of 25 previously treated patients with metastatic solid tumors, including four cases of TNBC [67]. Two patients showed objective responses, one of which was a TNBC subject. A subsequent phase II study evaluated the efficacy of IMMU-132 in metastatic TNBC patients who had undergone prior treatments (NCT01631552, phase II) [68]. In 69 enrolled patients, IMMU-132 demonstrated significant antitumor activity with an ORR of 30% (partial response = 19; complete response = 2), and the mDOR = 8.9 months (95% CI: 6.1 to 11.3). Notably, treatment response occurred earlier after drug administration (a median onset of 1.9 months after treatment initiation). The mPFS was 6.0 months (95% CI: 5.0 to 7.3) and the mOS was 16.6 months (95% CI: 11.1 to 20.6). The efficacy and safety of IMMU-132 in 108 patients with metastatic TNBC have been reported for this phase II clinical trial, confirming the potent anticancer activity of IMMU-132: the ORR was 33.3% (95% CI: 24.6% to 43.1%; including three complete responses and 33 partial responses); the mDOR was 7.7 months (95% CI: 4.9 to 10.8 months); the mPFS was 5.5 months (95% CI: 4.1 to 6.3) and the mOS was 13.0 months (95% CI: 11.2 to 13.7). Commonly observed side effects included nausea (67%), diarrhea (62%), fatigue (55%), neutrophil (64%), and anemia (50%) [69]. Based on the optimistic phase II trial results, Sacituzumab govitecan (IMMU-132) received accelerated FDA approval for metastatic TNBC patients who have received at least two prior therapies [70]. Recently, the confirmatory phase III trial results further validated the efficacy of IMMU-132 in patients with metastatic TNBC. A total of 468 TNBC patients who had previously received at least two treatments were randomized in a 1:1 ratio to receive IMMU-132 therapy or physician-selected chemotherapy (NCT02574455, stage III) [71]. After 17.7 months of follow-up, the mPFS was 5.6 months (95% CI: 4.3 to 6.3 months) and 1.7 months (95% CI: 1.5 to 2.6 months) in patients receiving IMMU-132 and chemotherapy, respectively; the mOS was 12.1 months (95% CI: 10.7 to 14.0) and 6.7 months (95% CI: 5.8 to 7.7), respectively. In IMMU-132 treatment group, bone marrow toxicity and diarrhea were the main adverse events. Thus, this clinical trial further demonstrated the superiority of anti-TROP2 ADCs over conventional chemotherapeutic agents for advanced TNBC patients with previous treatment. Additionally, IMMU-132 will be the first ADC to be evaluated in adjuvant therapy for primary TNBC with a high risk of recurrence (elevated levels of proliferation markers (Ki-67), higher tumor grade and presence of lymphovascular invasion). A phase III trial called SASCIA (NCT04595565) was planned to enroll 1,200 patients with HER2-negative breast cancer (including TNBC), and the inclusion criteria is the presence of residual tumor and high risk of cancer recurrence after neoadjuvant chemotherapy.

Datopotamab deruxtecan (Dato-Dxd) (DS-1062) is an ADC produced by a TROP-2 targeting monoclonal antibody (Datopotamab) attached to Dxd (a topoisomerase inhibitor) via a cleavable linker. Tropionpantumor01 (NCT03401385) was the first phase I clinical trial to evaluate the efficacy of monotherapy for this ADC, which recruited patients with advanced or metastatic TNBC who had previously been treated. The published preliminary results for the 24-patient TNBC cohort showed an ORR of 43% for Dato-Dxd monotherapy in

the pretreated patient population (median = 4 prior treatment, range 1 to 9). The only treatment-related adverse event with a frequency of more than 10% and above grade 3 was stomatitis (13%). Thus, Dato-Dxd has potential anticancer efficacy in patients with advanced or metastatic TNBC [72]. Notably, a phase III clinical trial (NCT05374512), named TROPION-Breast02, has been initiated in patients with locally recurrent inoperable or metastatic TNBC, and the enrolled patients are not suitable for PD-1/PD-L1 inhibitor therapy [73]. It is surmised that Dato-Dxd will be the second anti-TROP-2 ADC that could be applied in the clinical treatment of TNBC after IMMU-132.

HER2 and TROP-2 are the leading targets in ADC development for TNBC, and the clinical evaluation progresses rapidly. Sacituzumab govitecan (IMMU-132) received accelerated FDA approval for the treatment of patients with recurrent and metastatic TNBC after the promising results in phase II trial [70]. The phase III trial results also demonstrated the superior efficacy of trastuzumab deruxtecan over conventional chemotherapy. Sacituzumab govitecan and trastuzumab deruxtecan also showed good safety profiles compared with conventional drugs. For example, 52.6% of patients treated with trastuzumab deruxtecan had an adverse event of grade 3 or above, compared with 67.4% of patients treated with conventional chemotherapy [56]. Therefore, sacituzumab govitecan and trastuzumab deruxtecan represent the successful examples of ADC development for advanced and metastatic TNBC. In addition, the evaluation of datopotamab deruxtecan in patients with metastatic TNBC has entered phase III clinical trial. The rapid progress of anti-HER2 and anti-TROP-2 ADC clinical development has intensified the competition of pharmaceutical companies in ADC development for other targets.

Clinical progress of ADCs for other targets

ADC targeting HER3

HER3, a member of HER transmembrane receptor family, is highly expressed in different tumor types. Unlike other HER family members, HER3 lacks intrinsic kinase activity and functions to activate intracellular oncogenic signaling cascades by forming heterodimers with other receptor tyrosine kinase such as HER2 [74, 75]. HER3 upregulation and the downstream Akt signaling are important mediators of BL TNBC cell survival [76]. Patritumab deruxtecan (U3-1402) is an anti-HER3 ADC that is coupled to the topoisomerase I inhibitor (Dxd) via a peptide linker, with a DAR up to 7 - 8. U3-1402 exhibits dose-dependent and HER3-dependent antitumor activity in rat and monkey model, with no severe toxicity or side effects being observed [77]. An ongoing phase I/II trial is evaluating the safety, tolerability, and efficacy of U3-1402 in patients with HER3-overexpressing metastatic breast cancer (NCT02980341). This is the first-in-human study of patients with HER3-expressing metastatic breast cancer, and three cohorts are included: HR+/HER2-negative, high HER3 expression (64 cases), HR+/HER2-negative, low HER3 expression (21 cases) and TNBC with high HER3 expression (31

cases). In the cohort of previously treated TNBC patients, the ORR was 16.1% (95% CI: 5.5% to 33.7%) and the mPFS was 5.5 months (95% CI: 3.9 months to unevaluable). A total of six cases with treatment-related interstitial lung disease were observed in the entire patient population (two in the TNBC cohort, grade 1 and 2, respectively) [78]. These preliminary results suggest that U3-1402 has potential antitumor activity and controllable toxicity in metastatic TNBC patients with HER3-overexpression.

ADC targeting LIV-1

LIV-1 is a breast cancer-associated zinc transporter which was originally characterized as an estrogen-regulated gene in breast cancer cells. Studies in breast and prostate tumor models demonstrated that LIV-1 mediates epithelial-mesenchymal transition to promote the motility and metastasis of tumor cells. Thus, LIV-1 has been considered as a potential therapeutic target for breast cancer [79, 80]. Ladiratumumab vedotin (SGN-LIV1A) is an anti-LIV-1 ADC that consists of a humanized anti-LIV-1 monoclonal antibody (ladiratumumab) linked to a microtubule-disrupting agent monomethyl-auristatin E (MMAE) by a cleavable dipeptide linker, with a DAR at approximately 4. This ADC possesses tumoricidal activity against cancer cell lines expressing LIV-1, and *in vivo* evaluation demonstrated tumor regression in a mouse model of ER-positive breast and cervical cancer upon SGN-LIV1A treatment [81]. An ongoing phase I trial aims to evaluate the efficacy and safety of SGN-LIV1A in patients with advanced or metastatic breast cancer (NCT01969643). Upon the completion of dose escalation, the cohort was expanded to further assess the safety and antitumor activity of monotherapy in TNBC patients. In the combined dose-escalation and expansion cohort of 44 TNBC cases, the ORR was 32% and the mPFS was 11.3 weeks (95% CI: 6.1 to 17.1 weeks). In the entire cohort, the main grade 3 and 4 adverse events were neutrophil reduction (25%) and anemia (15%) [82]. Interim results from this phase I study showed promising antitumor activity and tolerance of SGN-LIV1A in TNBC patients. An expanded cohort registry for TNBC patients is currently underway. In another ongoing phase IB study (NCT03310957), the combination of GN-LIV1A with pembrolizumab (an anti-PD-1 monoclonal antibody) began to show good safety profile and treatment response in TNBC patients [83].

ADC targeting nectin-4

Nectin-4, a type 1 transmembrane protein, is the fourth member of the related immunoglobulin-like adhesion molecule family and participates in cell adhesion through its extracellular domain [84]. The oncogenic properties of nectin-4 promote tumor growth and proliferation in malignancies of different epithelial origins, including breast cancer [85]. In a study assessing nectin-4 protein expression in different primary tumor samples, 69% of 2,394 samples were found to express nectin-4, including 53% of the breast cancer samples [86]. In

the mRNA expression analysis of 5,673 invasive breast cancer samples, high expression levels of nectin-4 were associated with a dismal prognosis of TNBC patients [87]. Enfortumab vedotin-ejfv, an anti-nectin-4 ADC, consists of a humanized IgG1 antibody and the microtubule inhibitor MMAE (DAR: about 3.8). Preclinical experiments have shown that enfortumab vedotin has strong antitumor activity against HR-positive breast cancer and TNBC, leading to complete tumor regression in animal models [86]. An ongoing phase II clinical study of monotherapy (NCT04225117) will evaluate the efficacy of enfortumab vedotin in patients with metastatic solid tumors [88]. Patients with metastatic TNBC are included in six patient cohorts. Although nectin-4 expression is not included in the inclusion criteria, this study will review the expression of nectin-4 retrospectively. It is expected to reveal whether the antitumor activity of enfortumab vedotin reported in preclinical models of TNBC can translate into clinical efficacy in patients.

ADCs targeting folate receptor α (FR α)

FR α is overexpressed in several tumor types of epithelial origin [89]. In the immunohistochemical analysis of 71 primary breast cancer samples, FR α expression was found to be associated with TNBC, and this protein was expressed in 86% of metastatic TNBC specimens (n = 61) [90]. FR α was also demonstrated to be highly expressed in TNBC by other studies, and high FR α expression is a dismal prognostic factor in breast cancer patients [91, 92]. Mirvetuximab soravtansine (IMGN853) is an ADC consisting of a humanized monoclonal antibody targeting FR α and the microtubule inhibitor maytansine DM4, with a DAR of 2. This ADC demonstrated antitumor activity against FR α -expressing cancer cell lines in the xenograft models [93]. *In vitro* experiments have shown the long-term bystander killing effect of IMGN853, allowing its toxicity to spread to neighboring cells (regardless of FR α expression). Patients with advanced TNBC were recruited in a single-arm phase II trial (NCT03106077) to evaluate this ADC drug [94]. The assessment of FR α expression was performed in 80 of the 96 enrolled patients, of whom eight were confirmed as FR α -positive samples. However, in two FR α -positive patients who received IMGN853 treatment, no response was recorded. This clinical study was terminated early due to low frequency of FR α positivity and the lack of drug response. Another anti-FR α ADC drug MORAb-202, is a humanized monoclonal antibody farletuzumab linked to a synthetic analog of macrocyclic polyether chondroitin hydrochloride B (a microtubule inhibitor), with a DRA at 4 [95]. MORAb-202 has shown robust tumoricidal activity against FR α -expressing cancer cell lines in animal models, including lung and gastric cancers. This ADC exerted potent bystander killing activity and also displayed durable antitumor activity in a TNBC xenograft model [96]. Morab-202 has entered phase I/II clinical trial (NCT04300556), and the newly published phase I results showed that 22 patients with advanced solid tumors were enrolled on the basis of confirmed FR α expression (defined as $\geq 5\%$ of any cells being positively stained for FR α in immunohistochemical analysis). The most common hematologic adverse events during treatment were leukopenia

and neutrophil reduction (45% respectively, both at grade 1 or 2). One patient showed a complete response (5%), and nine patients had a partial response (41%, with one TNBC patient), with a total ORR of 46%. Based on these results, this study is expanding to recruit a cohort of TNBC patients [97].

ADC targeting EGFR

EGFR is highly expressed in a variety of epithelial tumors, including lung cancer, breast cancer, head and neck cancer, which is also a hot target for cancer therapy development [98]. EGFR is highly overexpressed in about 20% of TNBC patients. However, due to EGFR expression in normal skin cells, targeted and non-targeted toxicity has been a major concern. AVID100 is an ADC that targets both wild-type and mutant *EGFR*, and its payload is mertansine (DM1, a microtubule inhibitor). The antibody component in AVID100 (MAB100) also acts as an antagonist in the EGFR signaling pathway by competing for the ligand binding of EGFR. Although AVID100 has tumoricidal activity in breast, head and neck, and lung cancer cell lines, severe cytotoxicity was detected in keratinocyte [99]. In a phase I dose-escalation clinical study, AVID100 assessment was performed in 24 patients with advanced or metastatic epithelial malignancies (colorectal, ovarian, cervical). The most common adverse reactions were rash, nausea, and fatigue, but the drug was generally tolerated. Durable responses were observed in three of the patients without EGFR expression screening [100]. A multicenter, dose-escalating phase IIa trial is currently recruiting patients with advanced EGFR-overexpressing epithelial malignancies, including TNBC (NCT03094169), with the objective to assess the efficacy, safety, and tolerability of AVID100 in patients with EGFR-overexpressing solid tumors.

ADC targeting receptor tyrosine kinase-like orphan receptor 2 (ROR2)

ROR2 belongs to the cell surface receptor ROR subfamily and is a tumor-associated fetal protein that acts as an atypical WNT 5A receptor. Its expression level is correlated with clinical outcomes in breast cancer [101]. Ozuriftamab vedotin (CAB-ROR2-ADC) is an ADC consisting of the payload MMAE (microtubule inhibitor) and anti-ROR2 conditional active biological (CAB) antibody. CAB antibodies are produced using the specialized protein technology of BioAtla company, which are inactive in the microenvironment of normal tissues and become activated by glycolytic metabolites of cancer cells. After drug administration, ozuriftamab vedotin is preferentially activated in the tumor microenvironment. *In vitro* and *in vivo* experiments demonstrated the ability of ozuriftamab vedotin to suppress tumor growth of ROR2-expressing cell lines [102]. A multicenter, open-label, phase I/II study is currently recruiting patients with advanced solid tumors such as TNBC, non-small-cell lung carcinoma, and soft-tissue sarcoma to evaluate the safety and efficacy of CAB-ROR2-ADC (NCT03504488).

ADC targeting CA6

Anti-CA6-DM4 immunoconjugate (SAR566658) is an ADC composed of a humanized DS6 antibody against tumor-associated sialoglycoprotein CA6 and a microtubule inhibitor DM4. DS6 is able to recognize the Muc-1 tandem repeat domain in sialoglycoprotein CA6, which is expressed in multiple tumors including breast cancer [103]. The antitumor activity of SAR566658 against CA6-positive tumor derived from breast patients has been proved in the animal model [104]. The safety and maximum tolerated dose of SAR56658 was assessed in the completed phase I study (NCT01156870), which included 114 patients with advanced solid tumors with CA6 expression. At the dose of 190 and 90 mg/m² on days 1 and 8, tumor regression was observed in approximately 60% of patients, and 35% of patients showed tumor regression at the dose of 150 and 120 mg/m² every 2 weeks. Of note, three breast cancer patients showed partial response [105]. However, a clinical phase II trial on the evaluation of SAR566658 in CA6-positive metastatic TNBC was terminated due to limited efficacy [106].

Summary of Experience in Developing ADC for TNBC Patients

Although multiple ADCs have demonstrated therapeutic potential in advanced solid tumors and TNBC, the development of certain ADCs is discontinued due to the lack of efficacy and/or toxicity. For example, a clinical phase II trial of an anti-FR α ADC (mirvetuximab soravtansine) in patients with advanced TNBC was terminated due to the low FR α positive rate and the lack of drug response [94]. In addition, DAR may also be a key factor in dictating the *in vivo* pharmacodynamic effect. For example, SAR566658 (anti-CA6 ADC) has limited efficacy in patients with CA6-positive metastatic TNBC [106]. This is possibly due to the low DAR (DAR = 1), which restricts the capacity of drug delivery to reach an effective dose at tumor site. Another example of development failure is glembatumumab vedotin (GV). GV consists of an MMAE microtubule inhibitor and an antibody against the transmembrane protein GPNMB, which is expressed in approximately 40% of TNBC. Although early phase I/II results were encouraging, in an accelerated phase II trial that enrolled 327 patients with gpNMB-positive TNBC (NCT01997333), GV administration failed to achieve improvement in the primary end point, when compared to capecitabine chemotherapy control group (mPFS was 2.9 and 2.8 months, respectively). Further clinical evaluation of GV in TNBC was discontinued due to the lack of treatment superiority [107]. Another discontinued ADC for TNBC is PF-06664178, consisting of an anti-TROP-2 antibody and the payload Aur0101 (a microtubule inhibitor). This ADC showed severe toxicity in a phase I study of patients with advanced/metastatic solid tumors [108]. ADCT-502 is an ADC consisting of an anti-HER2 antibody linked to a pyrrole benzodiazepine dimer (PBD) cytotoxin, which was terminated after the phase I study of patients with advanced solid tumors due to a narrow therapeutic index [109]. Of note, a HER2-targeting ADC (XMT-1522), although showing good efficacy and toler-

ability in early study, was discontinued due to a competitive research and development (R&D) environment for HER2-targeted therapies. These examples highlight the challenge of translating ADCs into clinically safe and efficacious therapy in TNBC. The successful development of ADC not only requires the selection of appropriate target and payload, it is also necessary to ensure the stability of ADC in order to reduce the toxicity during delivery. Other than that, the fierce competition in the R&D market mandates the consideration of the formulation of novel ADCs with unique target and profile.

Sacituzumab govitecan (Immune-132), an anti-TROP-2 ADC, granted accelerated FDA approval for use in patients with recurrent and metastatic TNBC based on the superior efficacy and tolerability [70]. Multiple factors need to be taken into account for the successful clinical development of ADCs. ADC is a composite structure of advanced biomedical chemistry in which each individual component (monoclonal antibody, linker, and cytotoxic payload) impinges on its antitumor activity. Optimal configuration of each component is critical to achieve maximum antitumor activity and safety [110]. A new approach is to use bispecific monoclonal antibody to target two different epitopes. Preclinical studies have shown that ADCs based on bispecific HER2-targeting antibodies have antitumor activity against breast cancer cells with low HER2 expression [111]. This new type of ADC is currently in clinical development, such as ZW49 (a HER2-targeted biparatopic ADC targeting two epitopes located in the subdomain II and IV of HER2 extracellular domain) [112]. By recognizing more than one epitope on the same antigen, bispecific ADCs may enhance tumoricidal activity by improving cellular internalization, trafficking, or extracellular killing effects. Further advances in linker chemistry also provide a variety of technical platforms to optimize ADC stability during delivery [39]. Besides, target patient selection and stratification in the clinical trial is conducive to reveal the anticancer response in the most promising treatment population. Exploratory trials of ADCs in patients with solid tumors have adopted different approaches of patient selection, including strategies that do not implement antigen screening, screening strategies based on antigen expression, and retrospective evaluation of antigen expression in collected tumor samples [113]. All the approaches could possibly report meaningful antitumor activity against targets that are universally expressed in tumors, such is the case of TROP-2-targeted sacituzumab govitecan trial [68, 69]. However, for low-expression antigens or antigens showing intratumoral heterogeneity, proper patient screening strategies can be implemented to avoid dilution of treatment response in an unsuitable patient cohort. For antigens with unclear expression patterns, patient tumor samples should be rigorously collected in phase I trials to characterize antigen expression patterns and guide future clinical development activities.

Currently, the combination of ADC and other kinds of drugs is also being evaluated to achieve synergistic antitumor activity. For instance, PD-(L)1 ICI and Dato-Dxd are being evaluated in a phase IB/II clinical trial of TNBC patients (BEGONIA) [114]. The study is based on the rationale that cytotoxic payload of ADCs can induce immunogenic cell death to activate effector T cells, and the antitumor immunity can be further augmented by ICIs [115]. There is preclinical evidence

that ADCs carrying topoisomerase I inhibitors show potential synergistic antitumor effects with CTLA-4 or PD-1 inhibitor [116, 117]. The understanding of ADC resistance mechanisms also shed light on novel opportunities for therapeutic combinations. Previous studies reported that acquired resistance to ADCs is attributable to the increased expression of ATP-binding cassette transporters, which promotes the efflux of ADCs to extracellular environment [118, 119]. A preclinical study demonstrated that ABCG2 inhibitor treatment re-sensitizes resistant TNBC cells to sacituzumab govitecan [120]. This study serves as an example of targeting resistance mechanism to achieve better anticancer effect. Nevertheless, the clinical development of such drug combination may require rigorous patient screening. In addition, ADCs in combination with other targeted therapeutic agents, such as the combination of sacituzumab govitecan with PARP inhibitor, demonstrated synergistic antitumor effects in preclinical studies [121]. To validate the feasibility of such combination therapy, a phase I/II trial (NCT04039230) has been initiated to evaluate the dose-limiting toxicities of sacituzumab govitecan and talazoparib in patients with metastatic TNBC.

Conclusions and Future Perspectives

Current clinical results reveal promising therapeutic efficacy of sacituzumab govitecan and trastuzumab deruxtecan in patients with advanced metastatic TNBC, with a significantly higher rate of response over conventional chemotherapy. In a recently published phase III clinical trial progress, trastuzumab deruxtecan has also shown superior therapeutic potential and safety over conventional chemotherapy in patients with HER2-low-expression metastatic breast cancer. Although these ADCs are generally well tolerated, the potential adverse effects associated with the cytotoxic payload require continuous monitoring during treatment. Overall, sacituzumab govitecan (anti-TROP-2 ADC), trastuzumab deruxtecan (anti-HER2 ADC), and ladiratumumab vedotin (anti-LIV-1 ADC) represent the clinical development cases of ADCs targeting different surface antigens in TNBC. With the further evaluation of ongoing clinical trials, they are likely to be implemented as the first-line treatment for advanced TNBC. The accelerated approval of sacituzumab govitecan for the treatment of advanced TNBC after phase II clinical trial has also spurred ADC development for advanced metastatic solid tumors.

Given the broad therapeutic window of ADCs, the combination with other agents is a promising strategy to achieve antitumor synergy as well as to target intratumoral heterogeneity. ADCs in combination with other classes of agents, including PARP inhibitors and ICIs, are currently under clinical evaluation in TNBC patients. In addition to exploring ADCs in combination with drugs for treating advanced TNBC, future clinical development could also focus on the implementation of ADCs in treating early TNBC for the purpose of reducing cancer recurrence and metastasis. Whether the use of ADC as neoadjuvant therapy can maximize pathological complete remission remains to be evaluated in clinical trials. Furthermore, bispecific ADCs which target two different antigens may en-

hance antitumor activity by improving cellular internalization, trafficking, or extracellular killing effects [122, 123]. If these attempts to improve the efficacy of ADC are successfully translated into clinical practice, ADC may replace traditional chemotherapy as the first-line treatment for TNBC.

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Conflict of Interest

The authors declare that they have no conflict of interest to report regarding the present study.

Author Contributions

The authors confirm contribution to the paper as follows: study conception and design: Qi Tang, De Dian Chen; data collection: Ze Ying Li, Chun Xiao Ma; analysis and interpretation of results: Shao Qiang Zhou; draft manuscript preparation: Qi Tang, Hui Li, Xin Tong Zhao. All authors reviewed the results and approved the final version of the manuscript.

Data Availability

The data generated in this study are available upon request to the corresponding author.

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