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Challenges and Strategies to Enhance Drug Efficacy of the Approved Drugs for Chemoresistant Triple-Negative Breast Cancer: A Narrative Review

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Abstract

Triple-negative breast cancer (TNBC) has been shown to have a high relapse rate, high incidence of distant metastases, poor overall survival and chemoresistance. Resistance can arise from various factors, including the induction of cancer stem cells (CSCs), activity of ATP-binding cassette (ABC) transporters, hypoxic conditions and evasion of apoptosis. It is necessary to develop biomarker assays to predict chemoresistance (CR). Identification of CR-associated biomarkers will greatly enhance the therapeutic efficacy of drugs, in addition to improving the quality of life of patients. Recently, therapies tailored to specific biomarkers have been introduced for subsets of TNBC, and are now available in clinical practice. These include olaparib and talazoparib for carriers of BRCA1/2 germline mutations; and antibody-drug conjugate therapy targeting trophoblast cell surface antigen 2 (Trop2) for heavily pretreated metastatic TNBC (mTNBC). Additionally, therapies targeting various pathological molecular pathways are currently under investigation. Emerging biomarker-associated therapies, in combination with conventional therapeutic approaches, may overcome/hinder chemoresistance. This review address CR in TNBC while integrating insights into newly approved targeted therapies, biomarker-based patient selection and innovative strategies to overcome resistance mechanisms. This review uniquely combines scientific advances in biomarker-based therapies, clinical translation challenges and practical strategies, making it a valuable resource for guiding precision medicine in TNBC.

Keywords: Triple negative breast cancer, Chemoresistance, PARP, ADC

Introduction

Triple-negative breast cancer (TNBC) accounts for 15 - 20 % of all breast cancer cases and is considered a physiologically aggressive type of cancer. Women under 40 years of age and African American, Latina or BRCA mutation carriers are more likely to be diagnosed with this type of cancer [1]. It is characterized by the absence of the estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth receptor 2 (HER2); thus, it does not benefit from hormonal or trastuzumab-based therapy. Patients suffering from TNBC have a shorter surviving period compared to other variants of breast cancer, and 40 % of them dies within their initial 5 years of diagnosis [2]. It is a highly invasive disease and approx. The 46 % of patients will get metastatic tumours. The median survival time in TNBC patients is only 13.3 months. The brain and visceral organs are frequently affected by metastases, leading to recurrence in approximately 25 % of cases [3].

Patients are insensitive to both molecular-targeted therapy and endocrine therapy because of their distinctive molecular profiles. Therefore, chemotherapy is the preferred treatment for patients with TNBC in both the preliminary and advanced stages, because these patients do not respond to endocrine therapy or HER2 targeted therapy. The main drawbacks of chemotherapy include high recurrence, relapse, and poor overall or disease-free survival. There are 2 types of chemoresistance: Intrinsic and acquired chemoresistance. Patients with intrinsic resistance are

chemoresistant before the initiation of chemotherapy. In cases of acquired resistance, it develops during chemotherapy. A single factor was not responsible for causing either type of resistance.

According to the literature, approximately 40 - 70 % of TNBC patients show chemosensitive behaviour [clinical complete (disappearance of tumour) or partial response (decrease in tumour size of ≥ 50 %)] [4]. However, approximately 30 - 50 % of TNBC patients have residual disease burden II/III after neoadjuvant chemotherapy and at high risk of relapse (stable or progressive disease, RCB II/III; chemoresistant), with significantly worse survival, particularly in the first 3 years [5]. It depicts that annually approximately 53,000 - 89,000 TNBC patients (30 - 55 % of TNBC) will have residual cancer burden (RCB) II/III after neoadjuvant chemotherapy and reduced overall survival or disease-free survival. The prognosis can be improved by early diagnosis and appropriate therapy [6].

It is critical to identify effective therapeutic targets to overcome drug resistance and to improve diagnosis of TNBC. Approximately 18 drugs have been approved for the treatment of HER2-positive breast cancer by the Food and Drug Administration (FDA). However, the development of novel small-molecule drugs that specifically target chemoresistance in TNBC remains challenging. This article provides a comprehensive review of the current status of recommended drugs targeting chemoresistance, challenges associated with specific drugs, choice of patient selection and considerations to overcome these challenges.

Materials and methods

This review article explores the advancements that have been made in therapeutic biomarkers that are used in TNBC to assess the chemoresistance associated with approved drugs. We included studies assessing the efficacy of FDA approved drugs for chemoresistant TNBC. We performed an extensive search using 3 online databases: PubMed, Embase and Web of Science. The search was carried out from the beginning of the databases up until October 31st, 2024. To identify ongoing clinical trials for TNBC treatments, we also conducted a systematic search on ClinicalTrials.gov. We used a combination of the following keywords: Cancer, chemo-resistance, Triple-Negative Breast Cancer (TNBC), TGF-β, BRCA1/2, PARP inhibitors, small molecule, drugs, targeted therapies and biomarker-associated therapies for TNBC, clinical trials. In this study, inclusion criteria required participants were pathologically confirmed metastatic or locally advanced TNBC. The studies unrelated to TNBC, lacking clinical or preclinical evidence or gives inadequate mechanistic insights and case reports were excluded.

Discussion

Recently, therapies tailored to specific biomarkers have been developed for subsets of triple-negative breast cancer (TNBC) and now available in clinical practice. These include olaparib and talazoparib for carriers of BRCA1/2 germline mutations, anti-trophoblast cell surface antigen 2 (Trop2) antibody-drug conjugate therapy and Galunisertib targeting TGF-β.

Poly (ADP-ribose) polymerases (PARPs) and its inhibitors (PARPi)

Poly (ADP-ribose) polymerases (PARPs) facilitate the transfer of ADP-ribose via ADP ribosylation to target proteins with arginine, glutamate, aspartate, cysteine, lysine and serine acceptor sites [7]. It plays a crucial role in base excision repair (BER) in response to single-stranded DNA breaks (SSBs). Due to its function in DNA repair, inhibition of PARP leads to genomic instability and accumulation of damaged cells in a state of cell cycle arrest. Previous studies suggest that both the inhibition and hyperactivation of PARP can induce DNA damage and lead to apoptosis [8].

In humans, there are 17 proteins belonging to the PARP family. PARP inhibitors entrap PARP at the site of DNA damage and induce double-strand breaks. These breaks are not repaired in BRCA-positive tumors because of defects in the homologous recombination (HR) repair (HRR) pathway [9]. This justifies the use of PARP inhibitors in the management of BRCA1/2 BC patients. In addition to BRCA, other DNA repair proteins play an essential role in regulating the activity of PARP inhibitors. For example, deleterious variants in non-BRCA HRR genes, such as *DSS1*, *RPA1*, *CHK1*, *ARID1A*, *ATM*, *PALB2* and *RAD51*, lead to functional HRR deficiency and sensitivity to PARP inhibition [10]. HRR activity has also been shown to be reduced via the involvement of Krebs cycle genes, such as *IDH1* or *FH*, leading to oncometabolite production, such as

hydroxybutyrate and fumarate [11,12]. Additionally, mutations in BRCA associate protein (BAP1) prevent PARP-dependent recruitment of the polycomb deubiquitylase complex (PR-DUB) to sites of DNA damage and inhibit the DNA repair pathway [13]. Many studies have assessed the possible function of PARPs in advanced TNBC that resembles BRCA and has an HR deficit status [14]. These results suggest that any mutation in the HR genes may enhance the inhibitory activity of PARP inhibitors.

Currently, 6 small-molecule inhibitors of PARP got approval for clinical use: i) olaparib, ii) rucaparib, iii) niraparib, iv) talazoparib, v) veliparib and vi) pamiparib. All 6 inhibitors differed in their chemical structure, off-rate from the PARP site and preclinical potency. PARP trapping also requires a slow off rate and thus allows the establishment of PARPi at the PARP site for an extended duration or low dose requirement of the inhibitors [10]. It has been shown that Talazoparib and veliparib demonstrate highest and lowest PARP trapping ability, respectively [10]. In addition, the antitumor activity of pamiparib has been shown to be 10-fold higher than olaparib in a BRCA1-mutant breast cancer xenografts [15]. However, only 2 PARPi (olaparib and talazoparib) have received FDA approval for metastatic BRCA1/2-mutant breast cancer. Veliparib is currently being investigated as a combinatorial therapy for TNBC [10,16].

The use of PARPi in combination with chemo and immunotherapies has been demonstrated in **Table 1** [16-20]. To identify ongoing clinical trials for TNBC treatments, we conducted a systematic search on ClinicalTrials.gov. Current clinical trials are exploring the use of PARP inhibitors (PARPi) in combination with novel agents, including olaparib alone or in conjunction with AZD1775, a WEE1 checkpoint inhibitor and AZD6738, an ataxia telangiectasia and Rad3-related protein (ATR) inhibitor, as part of the VIOLETTE trial [21], ZEN003694 (a bromodomain inhibitor) with talazoparib in patients with TNBC without BRCA 1/2 germline mutations [22].

Table 1 List of clinical trials of PARPi with other chemo and immunotherapeutic agents.

Clinical trial number	Phase	Type of TNBC	Treatment plan	Prior therapy	Outcome of the study
PARP inhibitors with chemotherapeutic agents [16-20]					
NCT05128734	II	Metastatic or locally advanced triple-negative breast cancer (TNBC) having methylated DNA	TMZ arm: Temozolomide (TMZ): 50 mg/m^2 daily in cycles of 21 days TMZ + Olaparib arm: TMZ 75 mg/m^2 day 1 to day 7 plus Olaparib 200 mg on, day 1 to day 7 in cycles of 21 days	Information not provided	Study has not started yet.
BROCADE NCT01506609	II	TNBC with BRCA 1/2 germline mutation	VT arm: (veliparib + TMZ) VCP arm: (veliparib + carboplatin + paclitaxel), PCP arm: (placebo +carboplatin+paclitaxel)	Information not provided	VCP vs PCP: Median PFS: 14.1 and 12.3 months, interim median OS: 28.3 and 25.9 months, ORR: 77.8 and 61.3 % VT vs PCP, median PFS: 7.4 months, i OS 19.1 months, ORR: 28.6 %.

Clinical trial number	Phase	Type of TNBC	Treatment plan	Prior therapy	Outcome of the study
NCT01091454 (n = 46)	II	Information not available	Cisplatin at day 1 followed by brostallicin on day 2. This cycle was repeated for 21 days.	Information not provided	Confirmed (8CR and 1 PR) responses in 9 patients. DOR: 2.6 - 14.5 months. 3-months PFS: 51 %; 6-months PFS: 28 %. The pERK expression was negatively associated with 3-months PFS, but not with 6-months PFS. Cytoplasmic pERK expression was negatively associated with tumor response (p = 0.03).
NCT02158507 (n = 17)	NA	Metastatic or locally advance unresectable TNBC having no germline mutation	Lapatinib (1,250 mg/day) and veliparib (200 mg/12 h) for 28 days.	Anthracycline -based and taxane chemotherapy.	PR (partial response): 4 patients stable disease: 2 patients. No dose-limiting toxicities.
GeparOLA [NCT02789332] (n = 77)	II	Germline BRCA1/2 and tBRCA1/2 (43 patients having confirmed g/tBRCA1/2 mutation status)	PO arm: Paclitaxel 80 mg/m^2 and 100 mg olaparib for 12 weeks followed by epirubicin and cyclophosphamide PCb arm: Paclitaxel 80 mg/m^2 with carboplatin for 12 weeks followed by epirubicin and cyclophosphamide	Neoadjuvant therapy	pCR: PO arm: 55.1 % PCb arm: 48.6 %.
PETREMAC (n = 32)	II	TNBC patients were screened for the following biomarkers: *BLM*, *MSH6*, *BRCA1 MLH1*, *BRCA2 XPC*, *CHEK1 XPA*, *ERCC4 CHEK2*, *MRE11 BAP1*, *NBN MUTYH*, *PALB2 PARP10*, *SETD2 ERCC2*, *FANCA*, *FANCC*, *FANCD2*, *FANCE*, *FANCF*, *FANCG*, *ATRX*, *C11orf30_EMSY*, *MEN1*, *PTEN*, *BRIP1*, *CDK12* and *ATR*	Olaparib for up to 10 weeks before chemotherapy	Patients with primary TNBC > 2 cm	Complete or partial response = 18 patients (56.3 %). It was also observed that 16 of 18 responders had homologous recombination (HR) mutations and/or BRCA1 methylation. The HR and BRCA1 methylation rates were lower in non-responders (4/14 patients)

Clinical trial number	Phase	Type of TNBC	Treatment plan	Prior therapy	Outcome of the study
PARP inhibitors with immunotherapeutic agents [23-26]					
MEDIOLA NCT02734004	I/II	Germline BRCA1/2 mutated	Olaparib 300 mg on week 1 day 1 and MEDI4736 (1.5 g) every 4 weeks starting on week 5 day 1	Patients previously treated with cytotoxic chemotherapy (like anthracyclines and taxane) but no more than 2 cycles.	Median PFS: 4 - 9 months; median OS: 20.5 months. PMID: 32771088
NCT03801369	II	*BRCA*-wildtype mTNBC	olaparib on days 1 - 28 of each cycle and durvalumab iv over 1 h on day 1 of each cycle.	Information not provided	Median PFS: of 5.5 months. Identified over 30 biomarkers, with the optimal predictive value arising from biopsies.
TOPACIO/Key note-162 NCT02657889	II	BRCA mutation	TNBC patients received RP2D of Niraparib 200 mg once daily and Pembrolizumab 200 mg IV on Day 1 of each 21-day cycle.	Information not provided	ORR/DCR (disease control rate): 45 and 73 %, respectively.
IMpassion 130 NCT02425891	III	Information not provided	Experimental arm: Atezolizumab at a fixed dose of 840 mg iv on Days 1 and 15 of each 28-day cycle + Nab-Paclitaxel 100 mg/m^2 via iv on Days 1, 8 and 15 of each 28-day cycle. Placebo arm: In place of atezolizumab placebo is used remaining is same as experimental arm Placebo + Nab-Paclitaxel	Untreated patients were recruited	Median OS: experimental arm: 21·3 months, placebo arm: 18·7 months.

Challenges of PARPi in TNBCs

PARPi resistance

Resistance to PARPi is a major obstacle in the treatment of TNBC. There are several mechanisms that can lead to PARPi resistance, such as i) loss of non-homologous end joining, ii) loss of single-strand annealing (SSA) and alternative end joining (TMEJ), iii) reactivation of mutant BRCA1/2 due to genomic instability, iv) Increased RAD51 levels in BRCA2-deficient cells, v) restoration of ADP-ribosylation, vi) protection of replication fork either by preventing fork reversal or by inhibiting recruitment of nucleases, vii) reversal mutations and viii) potential reduction in intracellular concentrations of PARP inhibitors (PARPi) may occur due to enhanced drug efflux mechanisms [27].

Fork reversal aids in removing or bypassing replication blocks, allowing DNA replication to continue. Fork remodelers like SMARCAL1 are involved in this process. SMARCAL1 loss leads to PARP resistance in BRCA1/2 deficient cells by stabilizing the replication fork and reducing DNA

breaks [28]. FANCD2 suppresses MRE11-mediated fork degradation and its overexpression contributes to PARPi resistance [29]. Fork remodelers such as ZRANB3 and HLTF also influence PARPi resistance by safeguarding replication forks [28]. Reversion mutations in BRCA1/2-mutated cancers, and other HRR genes like RAD51C and PALB2, restore BRCA functionality and drive PARPi resistance *[30]*. Off-target effects and maintaining steady-state trough concentrations above the IC90 level further impact PARPi efficacy. High doses of olaparib showed better clinical outcomes, and cleaved PARP is considered a marker of PARPi sensitivity, as demonstrated in tumor mice treated with talazoparib and temozolomide, showing better responses with high cleaved PARP levels [31,32].

Critical health diseases

PARP inhibitor therapy may lead to severe complications such as myelodysplastic syndrome and acute myeloid leukemia (MDS/AML), particularly in individuals with a germline BRCA mutation. Additionally, biallelic mutations in BRCA2 are associated with Fanconi anemia, a genetic condition marked by congenital abnormalities and a significantly elevated risk of developing cancers, including AML. Therefore, olaparib carries a warning about the potential risk of developing MDS/AML. It was shown that MDS/AML developed in 2 % of BRCA1/2 mutated breast and ovarian cancer patients receiving olaparib therapy. The disease is fatal in most patients. These patients received therapy for at least 6 months and a maximum of 2 years. They also receive chemotherapy with platinum and/or other DNA-damaging agents [31]. The exact mechanism of disease toxicity in these patients remains unclear. Therefore, it would be beneficial to sequence patient DNA for genes associated with PARP mechanisms, specifically HR genes, both before and after chemotherapy initiation. This will help to uncover the mechanisms associated with disease toxicity in olaparib-receiving patients. Hematological toxicity presents a significant challenge in PARP inhibitor (PARPi) therapy, making it essential to closely monitor complete blood counts and, potentially, peripheral blood mononuclear cells (PBMCs) for the presence of micronuclei. Another rare side effect of PARP inhibitor therapy is pneumonitis, which has been documented in less than 1 % of women. Routine monitoring includes a complete blood count, renal and hepatic function tests and a pregnancy test, as conception is not permitted during treatment and for 6 months after therapy concludes [33].

Strategies to enhance efficacy of PARP inhibitors

It is essential to consider several factors before conducting clinical trials on PARP inhibitors, either in combination with chemotherapy or immunotherapy, such as i) Determination of chemoresistance for the study drug before starting chemotherapy. This is essential because not all patients are chemoresistant. Chemoresistance can be predicted by conducting biomarker analyses such as the Oncotype Dx test. ii) Analysis of HR mutations beyond BRCA1/2 and iii) Analysis of PD-1/PD-L1 expression in patients. iv) Inclusion of all types of samples, such as BRCA1/2 mutated, BRCA wild-type and BRCA1 mutated TNBC. v) Involvement of FDA-approved chemoresistant drugs such as crizotinib (a multitarget kinase inhibitor). There are several other studies which are ongoing on PARPi (Rucaparib, olaparib, Niraparib and Talazoparib) and anti-PD-1 therapies (Atezolizumab, Avelumab and Pembrolizumab) in TNBC patients such as NCT03101280, NCT03544125, NCT02657889, NCT02849496, NCT03330405 and NCT03801369). Notably, few of these trials are evaluating the effect of same PARPi (olaparib) and anti-PD-1 (Durvalumab) on TNBC patients. It would be more beneficial if these trials could be conducted in collaboration with each other to obtain faster results and the inclusion of a greater number of diverse samples.

Anti-trop2 antibody drug conjugate therapy

TACSTD2 gene encode the trophoblast cell surface antigen-2 (Trop-2), which is located on chromosome 1p32. It was originally identified in human placental trophoblasts. It is a transmembrane glycoprotein composed of 323 amino acids with a molecular weight of 36 KDa. It acts as a calcium transducer, and the release of calcium plays an indispensable role in cell cycle signaling pathways participating in many mechanisms, such as proliferation, migration, metastasis, apoptosis and invasion. It can also regulate cell cycle via the degradation of intracellular domain by

tumor necrosis factor alpha converting enzyme (TACE), γ-secretase and presenilin 1 (PS-1) and presenilin 2 (PS-2). It also activates AP1 via the phosphorylation of ERK1/2, which ultimately enhances angiogenesis, apoptosis, invasion and epithelial-to-mesenchymal transition (EMT). Therefore, this molecule is considered important for therapeutic targeting in cancer patients [34]. The molecule has many serine and tyrosine residues in its cytoplasmic tail, and mutations in these residues have been shown to inhibit the activity of trop2 [34]. However, the correlation between these mutations and tumorigenesis is unclear and requires further research. The expression of trop2 is high in many tumors, including TNBC. A possible reason for its high expression in tumor cells may be its presence on the surface of stem/progenitor cells and the involvement of transcriptional factors modulating their expression [35]. increased TROP-2 expression has been linked to poor survival outcomes in patients with breast cancer. The high expression of TROP-2 in cancer cells and its involvement in various tumor-promoting mechanisms have prompted further exploration of TROP-2 as a potential therapeutic target. Consequently, a variety of drugs have been rapidly developed, including monoclonal antibodies (mAbs), bispecific antibodies, virus-like particles (VLPs) and antibody-drug conjugates (ADCs). Antibody-drug conjugates (ADCs) consist of 3 main components: A monoclonal antibody (mAb) that targets a specific tumor antigen, a cytotoxic payload and a chemical linker that connects the 2 components. ADC binds to antibody-specific receptors on tumor cells, followed by its internalization via endocytosis or pinocytosis. This internalization leads to endosome formation. Acidic proteolytic or redox conditions in lysosomes or endosomes result in the release of the payload from the antibody. The payload then diffuses into the cytoplasm, leading to cell death. The primary factors to consider in the design of an antibody-drug conjugate (ADC) include the selection of a tumor-specific antigen, potency of cytotoxic payloads and choice of ADC linkers. Linkers are of 2 types i) cleavable linkers and ii) non-cleavable linkers. These linkers differ in their payload-release mechanisms. Non-cleavable linkers release payloads via degradation of the antibody backbone, whereas cleavable linkers release payloads via the action of enzymes or low pH [36]. Any instability or accumulation of linkers at off-target sites leads to toxicity. Thus, there are 2 major challenges in designing linkers: i) reduction of systemic toxicity, and ii) efficient release of payloads. Only a small amount of ADC (0.1 %) actually reaches the tumor tissue; thus, the potency of payloads must be high (nm-pm range) [37]. Therefore, optimal combinations of the linker and payload are required to address these 2 obstacles. Currently, multiple trop2 targeted ADCs are undergoing clinical investigation.

Sacituzumab govitecan (SG/IMMU-132)

It is a novel ADC composed of 3 major parts: i) humanized IgG1 mAb hRS7, ii) SN38 (topoisomerase I inhibitor) and iii) cleavable and pH-sensitive linker (CL2A). SN38 is an active metabolite of irinotecan that has been used to treat various types of cancers. There are 2 major reasons for using SN38 instead of irinotecan: i) The potency of SN38 is 2 - 3 times higher than that of irinotecan. ii) the cleavable linker of SG allows for both extracellular and intracellular release of SN-38 thus leading to “bystander effect.” This bystander effect is desirable, specifically in the case of heterogeneous tumors such as TNBCs. iii) High drug-to-antibody ratio (DAR) without compromising antibody binding or pharmacokinetic properties. iv) Reduced toxicity compared with other topoisomerase inhibitors [37]. A possible reason for the low toxicity could be the lower rate of glucuronidation of SN-38 molecules bound to the antibody. Glucuronidation of SN-38 by UGT1A1 leads to hematological toxicity [38]. The release of SN38 payload leads to DNA damage and cell death. Currently, a polymeric micelle formulation of SN-38, NK012, has been used in clinical trials for the treatment of mTNBC (NCT00951054). The details of clinical trials of SG have been demonstrated in **Table 2**.

SKB264: An innovative ADC

The payload of SKB264 consists of a topoisomerase I inhibitor, KL610023. It is a derivative of the belotecan. The remaining components were similar to those of the SG. SKB264 is a patented product from Kelun Biotech. Initial clinical results showed that SKB264 with a dose regimen of Q2W leads to a partial response in 40 % of advanced TNBC (PR: 2/5 TNBC) with manageable side effects in solid tumors. All TEAEs were clinically manageable and included nausea, alopecia, decreased neutrophil and white blood cell

counts and anemia [39]. No deaths were due to TEAE. Kelun-Biotech is also planning a Phase II trial of SKB264 with or without anti-PD-L1 monoclonal antibody as a first-line treatment for advanced TNBC.

Datopotamab deruxtecan (Dato-DXd)

Dato-DXd is composed of 3 components: i) humanized anti-TROP-2 IgG1 MAb, ii) topoisomerase I inhibitor payload (DXd; exatecan derivative) and iii) a tetrapeptide-based enzymatic cleavable linker (**Figure 1**). With a DAR of ~4:1, the linker is cleaved only in the presence of lysosomal proteases, and thus is highly stable in circulation. The payloads of Dato-DXd and SG differs in 3 major aspects i) more potency of DXd than SN-38 ii) longer half-life of DXd, and therefore, Q3W regimen is followed in case of DXd which is preferable over day 1 and day 8 Q3W schedule. iii) Improve LD50/ED50 ratio or therapeutic index. iv) less off-target effects; the payload inhibits the growth of tumor cells having high trop2 expression [37]. The details of clinical trials of Dato-DXd have been demonstrated in **Table 2**.

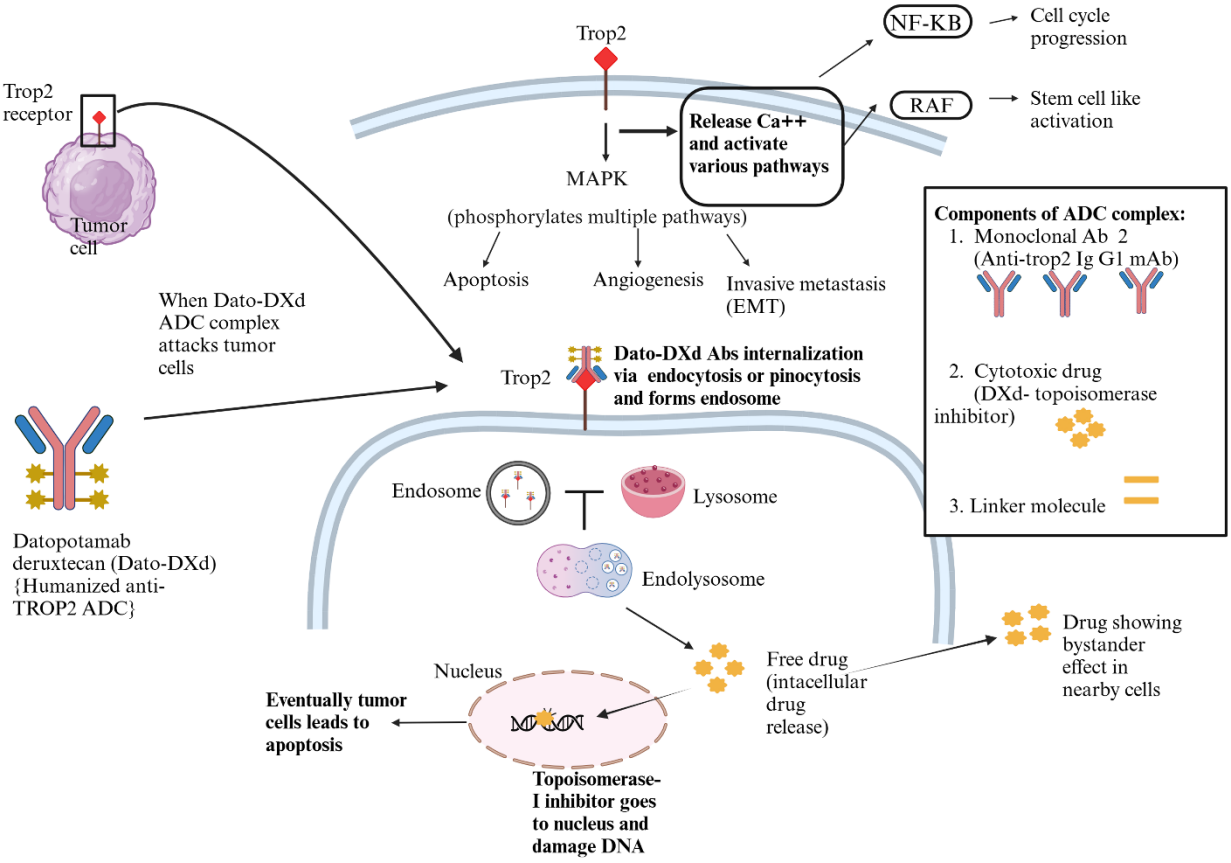

Figure 1 Mechanism of Datopotamab deruxtecan (Dato-DXd).

Trop2 is involved in the MAPK pathway. This pathway regulates apoptosis, angiogenesis, proliferation and epithelial-mesenchymal transition (EMT). It is also involved in the activation of various pathways, such as RAF and NF-κB, via the release of calcium ions from the sarcoplasmic reticulum. Owing to the high expression of Trop2 in tumor cells, it is used as a therapeutic target in cancer. The Dato-DXd ADC complex binds to trop2 in tumor cells, followed by its internalization via endocytosis or pinocytosis. This internalization leads to the formation of endosomes. Acidic conditions in lysosomes or endosomes result in the release of payload from the antibody. The payload then diffuses into the cytoplasm, leading to tumor cell apoptosis. It also shows a bystander effect, where the cytotoxic drug migrates to neighbouring cells and causes apoptosis.

Table 2 List of clinical trials of ADCs with other chemo and immunotherapeutic agents.

Clinical trial Number	Phase of study	Treatment	Prior treatment	Outcome
ADC (Antibody-Drug Conjugate) clinical trials [40-46]				
NCT01631552 (n = 4)	I/II	SG (Sacituzumab Govitecan): 8 to 18 mg/kg on days 1 and 8 of a 21-day cycle	Paclitaxel/carboplatin, capecitabine, cisplatin and carboplatin/gemcitabine	Dose of 8 - 10 mg/kg were better tolerated with minimal toxicity. The duration of response was lowest in patients receiving combinatorial therapy of Carboplatin/Gemcitabine (1.2 months). However, the other 3 patients had partial response (PR) with duration of response (DoR) of 10.4 (Paclitaxel/Carboplatin/SG), 6.9 (Capecitabine/SG) and 3.1 months (Cisplatin/SG).
ASCENT NCT02574455	III	SG arm: SG on Days 1 and 8 of 21-day cycle up to 29.6 months TPC (treatment of physician's choice) arm: Eribulin, vinorelbeine, gemcitabine or capecitabine as a single agent regimen	Taxane	(SG arm vs TPC arm) Median PFS: 5.6 vs 1.7 months Median OS: 12.1 vs 6.7 months Duration of response (DoR): 6.3 months vs. 3.6 months. However, there were 4 design features in this trial that favored the experimental arm or penalized the control arm: i) the trial was not completely blinded and thus overstated the effect of the experimental arm; ii) PFS is debatable in metastatic TNBC and can lead to biases. Both early stopping rules and informative censoring can bias the interpretation of PFS results; iii) the control arm was not a complete clinician choice; iv) dose reduction and supportive care recommendations for SG drug-treated patients favored the experimental arm. Therefore, it was not clear whether the outcome of the study would translate into the real world.
NCT05113966	II	Trilaciclib (240-mg/m^2) followed by 10 mg/kg of SG on days 1 and 8 of each 21-cycle.	≥ 2 prior systemic therapies [≥ 1 in the metastatic setting]	ORR: 23.3 % CBR: 46.7 % Median PFS: 4.1 months Median DoR: 9.1 months
TROPICON-PAN Tumour 01 NCT03401385 (n = 44)	I	Dato-DXd was given to the patients on day 1 of every 21 days cycle	Topo I exposed, sacituzumab govitecan, trastuzumab, deruxtecan and patritumab deruxtecan	ORR: 31.8 % Median DOR: 16.8 months DCR:79.5 % CBR:38.6 %

Clinical trial Number	Phase of study	Treatment	Prior treatment	Outcome
				Median PFS: 4.4 months Median OS: 13.5 months
NCT04039230 (n = 26)	II	10mg/kg of SG on days 1 and 8 + talazoparib 1mg on days 15 - 21.	Information not provided	Median OS: 18.0 months Median PFS: 6.2 months ORR: 30.1 % CBR: 53.8 %
Ongoing trials				
NCT03971409	II	Arm A: Binimetinib twice daily 15 days lead till 1 - 28 days + avelumab by iv over 60 min on day 1 and 15 + liposomal doxorubicin iv over 60 min on day 1. Arm B: 15day lead patients receive SG given on day 15 followed by day 8 and 15 of cycle 1; day 1, 8 and 21 of cycle 2; 1, 15 and 21 of cycle 3; day 8 and 15 of cycle 4 for 21 days cycle + 10 mg/kg avelumab for 60 min on day 1 and 15 of every 28 days cycle. Arm C: 15 days lead in of liposomal doxorubicin, followed by liposomal doxorubicin on day 1 + avelumab dosage same as upper	Should not have taken any prior treatment with SG and no more than 2 lines of chemotherapy	
NCT06328387	I/II	SG arm: SG given by iv 10 mg/kg on 1 and 8 day of 21 day cycle HCQ + SG arm: Hydroxychloroquine (HCQ) + SG through iv, 10 mg/kg on 1 and 8 day of 21 day cycles. T-DXd arm: Trastuzumab Deruxtecan (T-DXd) through iv, 5.4 mg/kg every 3 weeks. HCQ + T-DXd arm: HCQ + T-DXd as above-mentioned dose.	No more than 3 chemotherapy schemes	
NCT05520723	II	SG 10 mg/kg, iv on day1 and 8 of every 21 day cycle + Loperamide 2 mg	Patients must have treated with taxane but didn't have taken prior treatment with topoisomerase 1 inhibitors.	

Clinical trial Number	Phase of study	Treatment	Prior treatment	Outcome
		orally twice daily and 4 mg once a day for 3 consecutive days after taking SG during first 2 cycles + G-CSF (granulocyte colony-stimulating factor) 30 mu subcutaneously for 2 consecutive days, 48 days after taking SG.		

Challenges of ADCs

The variability in trop-2 expression

Given the heterogeneous nature of triple-negative breast cancer (TNBC), the expression pattern of Trop-2 can differ across various tumor stages, and even within distinct regions of the same tumor. Previous studies have emphasized the variability in Trop-2 expression levels across various stages of the disease and subtypes of TNBC. Expression of TROP-2 antibody was scored semi-quantitatively using the H-score. The study showed differential H-scores in TNBC patients (High H score: 97/589; medium H-score: 149/589; and low H-score: 343/589). TROP-2 expression was significantly associated with lymph node involvement, low continuous BMI and histological subtype. No significant correlations were observed between TROP-2 expression and tumor size, grade, stromal tumor-infiltrating lymphocytes or standardized mitotic index [47]. Therefore, Trop-2 poses a significant challenge for therapeutic antibody-drug conjugate (ADC) strategies.

The stability and resistance to ADCs

Any instability in ADCs can result in toxicity, which is closely related to the type of linker used. Studies have indicated that drug instability can lead to premature dissociation, resulting in adverse effects. Other mechanisms of resistance include i) altered target antigen expression, ii) defective internalization pathways, iii) lysosomal dysfunction and iv) off-target effects of payloads [48]. A recent study emphasized the generation of tumor neoantigens in response to low-dose radiation as a promising approach to overcome resistance to ADCs [49].

Lack of predictive biomarkers of efficacy

A clinical study has demonstrated a direct correlation between trop-2 expression and the clinical benefits of ADC. However, high expression does not significantly correlate with improved outcomes, possibly because of the bystander effect [50]. Therefore, other biomarkers associated with trop-2 expression may also serve as important predictors of treatment outcomes.

Strategies to enhance drug efficacy of ADCs

Most studies enrolled heavily pretreated patients with different drugs. It is advisable to include patients treated with the same drugs to evaluate the efficacy of SG. It is likely that the efficacy of SG may be different in patients treated with different types of drugs owing to their different mechanisms of action. Moreover, the genetic profiling of patients specifically for cell cycle or DNA repair genes will also be helpful in evaluating the effect of SG in TNBC patients. Using a cancer-specific antibody having high affinity for trop2, such as Hu2G10, ADAM10 and 2EF mAb, will further advance the research. The 2EF mAb prevents the formation of trop2 dimers and polymers; thus, the reachability of the antibody to the target site is high. Multi-target combination therapies, including the combination of ADCs or bi-specific antibodies, should also be explored for mTNBC. For example, in mCRC, the expression levels of Trop-2 and Nectin-4 suggest target-driven development of anti-Trop2 and anti-Nectin-4 ADCs [50]. Currently, there is no approved prognostic assay to determine the chemoresistance of tumors. Therefore, prognostic biomarker tests for commonly used drugs in TNBC will further help clinicians to predesign treatment schedules, reduce unnecessary exposure to drugs, and improve the quality of life of cancer patients.

Galunisertib

Galunisertib functions as an inhibitor of the tyrosine kinase transforming growth factor beta (TGFβ) type-1 receptor (TGFβR1) and exhibits potential antineoplastic properties. It has not got approval by FDA, however, is currently an investigational drug. Upon administration, it selectively binds to the kinase domain of TGFβR1, thereby blocking the activation of TGFβ-mediated signaling pathways (**Figure 2**). EMT is a key mechanism for tumor metastasis and invasion. TGF-β1 plays a key role in regulating epithelial-to-mesenchymal transition. The expression of TGF-β1 has been found to be significantly higher than other TNBC tissues and reduce the OS and PFS of TNBC patients [51]. Inhibiting the activation of the TGF-β1 pathway can block epithelial-mesenchymal transition (EMT), thereby hindering the progression of triple-negative breast cancer (TNBC). Galunisertib prevents the activation of the TGF-β canonical pathway by downregulating the phosphorylation of SMAD2, which is associated with the activation of pathogenic and inflammatory signals [52]. This molecule has the potential to inhibit the proliferation of tumor cells that overexpress TGF-β.

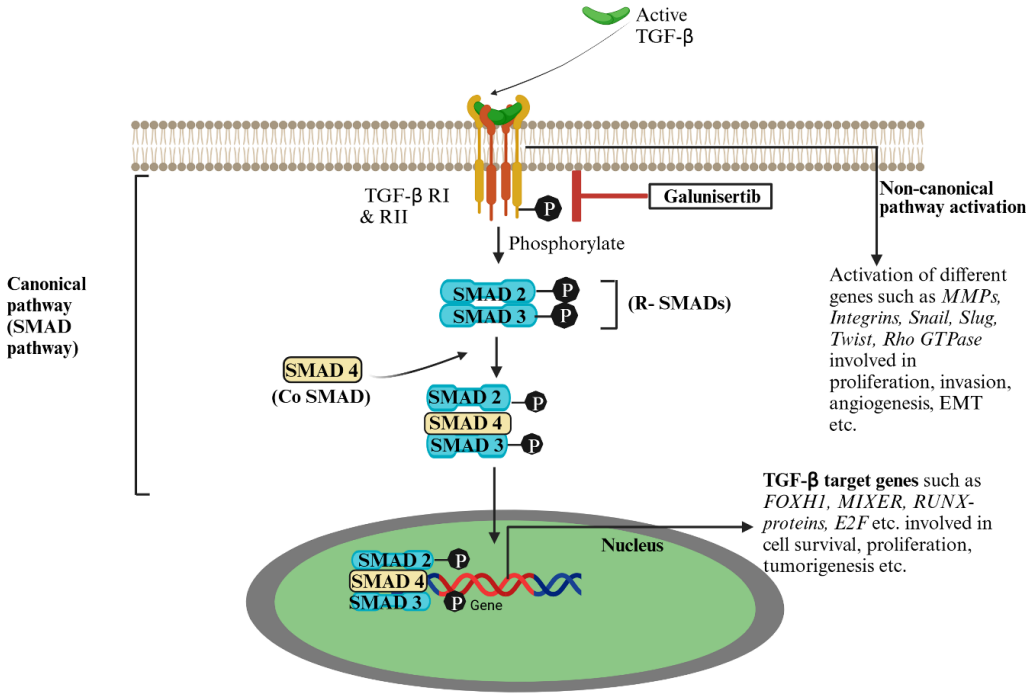

Figure 2 Mechanism of Galunisertib demonstrating inhibition of TGF-β signaling pathway.

TGF-β can induce TGF-β signaling via canonical or non-canonical pathways. Galunisertib has an inhibitory action against the TGFβ receptor internal domain that specifically downregulates the phosphorylation of SMAD. It eventually inhibits the canonical pathway by inhibiting the expression of genes, such as *FOXH1*, *MIXER* and *RUNX*.

Basal-like breast cancer exhibits a positive response to TGF-β inhibition, which has been shown to inhibit metastasis in animal models [52]. Furthermore, paclitaxel combined with galunisertib has additive antitumor activity in TNBC cells [52,53] . A Phase Ib Trial of Galunisertib (LY2157299) and paclitaxel was completed in patients with metastatic TNBC. Patients participating in this trial received both drugs, and the correct dose was estimated for future trials in mTNBC (NCT02672475). However, these results have not yet been reported.

In addition to Galunisertib, Fresolimumab (TGF-β antibody) has also been evaluated in clinical trials; however, the results were not as expected in breast cancer patients (NCT01401062). A possible reason for this failure could be the non-accessibility of TGF-β to its antibody.

Challenges

TGF-β-based TNBC therapy has several challenges: i) selectivity/specificity issues of TGF-βR inhibitors and ii) non-accessibility of TGF-β to mAbs. iii) Dual behavior of the molecule. Therefore, inhibitors are required to suppress the tumorigenic pathway while promoting the tumor-suppressive pathway. iv) Drug resistance related to TGF-β signaling has been reported to be associated with the loss of MED12 in tumors. Several drugs are currently being evaluated in clinical trials.

Conclusions and recommendations

Triple-negative breast cancer (TNBC) has the worst prognosis, with no significant improvements in progression-free survival (PFS) and overall survival (OS). Its heterogeneous nature involves multiple molecular mechanisms contributing to chemoresistance, which is the primary drawback of chemotherapy. Chemoresistant (CR) patients do not benefit from treatment, leading to decreased quality of life. To optimize cancer treatment, it is crucial to prioritize patient selection based on biomarkers, ensuring that therapies are personalized for maximum effectiveness and minimal side effects. Developing biomarker tests to identify chemoresistant tumors before or during early chemotherapy cycles is essential. These tests should be tailored for specific drug classes and based on their impact on survival rather than differential expression. Additionally, drug efficacy varies with tumor stage and nodal status, necessitating clinical trial data analysis based on TNBC characteristics. These strategies could inform therapeutic approaches for both early and advanced TNBC. Combinatorial therapies that integrate multiple approaches can effectively address challenges such as tumor heterogeneity and can also enhance outcomes of targeted therapy. Furthermore, utilizing FDA-approved chemoresistant drugs can offer a promising strategy to overcome resistance and improve patient responses and quality of life. These recommendations collectively provide a robust framework for advancing precision oncology.

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