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Antibody-drug conjugates targeting TROP-2: Clinical development in metastatic breast cancer

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ABSTRACT

Antibody drug conjugates (ADCs) combine the potent cytotoxicity of chemotherapy with the antigen -specific targeted approach of antibodies into one single molecule. Trophoblast cell surface antigen 2 (TROP-2) is a transmembrane glycoprotein involved in calcium signal transduction and is expressed in multiple tumor types. TROP-2 expression is higher in HER2-negative breast tumors (HR+/HR-) and is associated with worse survival. Sacituzumab govitecan (SG) is a first-in-class TROP-2-directed ADC with an anti-TROP-2 antibody conjugated to SN-38, a topoisomerase inhibitor via a hydrolysable linker. This hydrolysable linker permits intracellular and extracellular release of the membrane permeable payload enabling the "bystander effect" contributing to the efficacy of this agent. There was significant improvement in progression free survival (PFS) and overall survival (OS) with SG versus chemotherapy in pretreated metastatic triple negative breast cancer (TNBC), resulting in regulatory approval. Common adverse events (AE) reported were neutropenia and diarrhea. SG also demonstrated clinical activity versus chemotherapy in a phase III trial of HR+/HER2-metastatic breast cancer (MBC) and is under evaluation in first-line metastatic and early stage TNBC as well. Datopotamab deruxtecan (Dato-DXd) is a TROP-2 ADC that differs from SG in that it has a cleavable tetrapeptide linker and a more potent topoisomerase inhibitor payload. This construct is highly stable in circulation with a longer half-life than SG, and undergoes cleavage in presence of intracellular lysosomal proteases. Dato-DXd demonstrated preliminary efficacy in unselected metastatic TNBC, with common AEs of low-grade nausea and stomatitis. Dato-DXd is being investigated in phase III studies in metastatic TNBC and HR+/HER2- MBC. These novel TROP-2 ADCs have the potential to deliver enhanced efficacy with reduced toxicity in MBC and possibly in early stage breast cancer (EBC).

1. Introduction to antibody drug conjugates

The identification of oncogenic drivers in various cancers paved the way for development of targeted therapies with monoclonal antibodies. These biological agents had the ability to precisely target tumors harboring the specific biomarker/alteration in contrast to the indiscriminate cell death caused by chemotherapy. In the past couple of decades, there have been major efforts to combine the specificity of monoclonal antibodies with potency of chemotherapy to generate antibody drug conjugates (ADCs). ADCs utilize target-specific antibodies as vehicles to deliver a potent cytotoxic to tumor cells while sparing healthy cells, thus limiting toxicity. This concept harks back to a theory proposed by Paul Ehrlich in the 1900s, where he envisioned a drug that could be delivered to the target cell via a "magic bullet" while sparing

Abbreviations: ADC, antibody drug conjugate; ADCC, antibody dependent cellular cytotoxicity; AE, adverse event; BC, breast cancer; CDC, complement dependent cytotoxicity; CI, confidence interval; DAR, drug to antibody ratio; Dato-DXd, Datopotamab deruxtecan; DFS, disease-free survival; DoR, duration of response; EBC, early stage breast cancer; FIH, first-in-human; HR, hazard ratio; HR+, hormone receptor positive; MAb, monoclonal antibody; MBC, metastatic breast cancer; NACT, neoadjuvant chemotherapy; ORR, overall response rate; OS, overall survival; pCR, pathological complete response; PFS, progression free survival; SG, Sacituzumab govitecan; T-DM1, Trastuzumab emtansine; T-DXd, Trastuzumab deruxtecan; TNBC, triple negative breast cancer; TPC, treatment of physician's choice; TROP-2, Trophoblast cell surface antigen-2.

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normal tissue [1]. The challenge in realizing this goal is underscored by the decades of research that finally culminated in the regulatory approval of the first ADC gemtuzumab ozogamicin (Mylotarg®) in 2000.

1.1. Components of an ADC

Antibody drug conjugates have 3 core components: a monoclonal antibody (MAb) targeting a specific tumor antigen, a cytotoxic payload, and a chemical linker connecting them. The basic mechanism involves binding of the antibody to the tumor antigen on the cell surface, followed by internalization of the ADC and lysosomal degradation. This results in release of the active cytotoxic agent into the cytoplasm which results in tumor cell death. Some ADCs also induce antibody dependent cellular cytotoxicity (ADCC) and complement dependent cytotoxicity (CDC) contributing to the antitumor efficacy of these agents [2].

The foremost consideration for design of the ADC is the selection of a tumor-specific antigen accessible to antibody binding on the tumor cell surface. The overexpression of the target antigen on tumor vs healthy cells is key to precise ADC delivery resulting in high specificity as well as efficacy. Targets that are oncogenic drivers are less likely to be down-regulated as a mechanism of drug resistance and may thus be a better choice as the antigen to be targeted by ADCs.

Payloads in ADCs need to have high potency (nano-picomolar range) to precipitate cell lysis once delivered into the tumor, since a very small fraction of the ADC (~0.1%) actually reaches the tumor tissue [3,4]. The potency of these payloads is often the source of the toxicity associated with ADCs and some of these toxicities are usually characteristic of the class of payload (ex: peripheral neuropathy is attributed to microtubule inhibitors like DM1, DM4, MMAE) [5]. Furthermore, payload drugs must be impervious to efflux proteins in order to remain in the target tumor cells.

The linker is attached to the MAb at a specific number of sites depending on the conjugation chemistry utilized. This linker has a dual role; one is to avoid the premature release of the payload which leads to unwanted systemic toxicity [6] and the second is to enable efficient release of payload once inside the tumor cell [7]. Cleavable linkers are designed to release the payload of the ADC under acidic conditions or in presence of proteolytic enzymes; examples include mirvetuximab soravtansine and sacituzumab govitecan. ADCs with cleavable linkers tend to have varying degrees of stability in circulation and may degrade over time in the plasma [6]. Non-cleavable linkers on the other hand, are in stable in plasma and release the payload only after intracellular lysosomal degradation of the ADC [6]. Trastuzumab emtansine (T-DM1) is the best-known example.

1.2. Mechanism of action of an ADC

ADCs essentially deliver a cytotoxic agent to tumors with high selectivity, minimizing systemic exposure and thereby toxicity, thus improving therapeutic index. Binding of the ADC to a receptor on the cancer cell results in a complex that is internalized via clathrin- or caveolae-mediated endocytosis or via pinocytosis [8,9]. This internalization leads to an inward budding resulting in endosome formation before fusing with lysosomes [10]. Once inside the lysosomes or endosomes, acidic, proteolytic or redox conditions result in release of the payload from the antibody. The payload then diffuses into the cytoplasm and acts on the target substrates precipitating cell death.

1.2.1. Bystander effect

Some payloads have high membrane permeability and can diffuse out into neighboring tumor cells that may or may not express the target antigen and promote their death as well. This phenomenon is known as "bystander effect". Bystander killing is influenced by extent of ADC internalization, presence of cleavable or non-cleavable linker, and the nature of the cytotoxic payload [11]. Recent studies indicate that internalization and intracellular linker cleavage may not be essential for ADC processing in all cases. After binding of the ADC to the target antigen, but before internalization, ADCs can be cleaved by extracellular enzymes (like Cathepsin B) released by the surrounding tumor cells and tumor associated macrophages, releasing the cytotoxic payload that penetrates surrounding "bystander" cells resulting in cell death [12].

1.2.2. Effect of payload

Payloads are usually small molecule agents that may be too toxic as stand-alone drugs but are potent enough to cause cell lysis at IC_{50} in the nano-picomolar range. Payloads utilized in current ADCs can be broadly categorized as agents that 1) disrupt microtubule polymerization ex: maytansoids like DM1 and DM4, auristatin based agents (MMAE, MMAF), 2) DNA damaging agents like calicheamicin or duocarmycin, or 3) topoisomerase I inhibitors (SN-38 and derivatives or DXd) [13].

The drug to antibody ratio (DAR) is the number of cytotoxic payloads loaded onto the antibody and is an important feature that may influence the efficacy of an ADC. A higher DAR does not necessarily imply greater efficacy but a low DAR may impact efficacy [14].

Of the dozens of ADCs in clinical development for cancer, three ADCs have FDA approval for treatment of metastatic breast cancer (MBC); sacituzumab govitecan - a Trophoblast agent-2 (TROP-2) targeting ADC and two HER2-directed ADCs - T-DM1 and trastuzumab deruxtecan (T-DXd).

1.3. TROP-2 protein

TROP-2 is a transmembrane glycoprotein with both extracellular and intracellular components that is involved in calcium signal transduction [15]. It was first discovered by Lipinski et al. who raised antibodies against the human choriocarcinoma cell line [16]. These initial experiments demonstrated TROP-2 expression in normal trophoblasts and allowed for trophoblast cell growth, migration, and proliferation [17]. TROP-2 has been implicated in several cell signaling pathways including intracellular calcium transduction, MAPK signaling pathway, RAF, NF-κB and Cyclin D/E among others [15,18].

While initial investigation focused on TROP-2 expression in normal tissue, subsequent analysis showed that TROP-2 is upregulated in cancer cells when compared to normal cell counterparts [19]. This increased expression has been seen in many different tumor types including breast cancer, colon cancer, non-small cell lung cancer (NSCLC), esophageal squamous cell cancer, thyroid cancer and hepatobiliary cancers, raising the possibility of TROP-2 as a tumor agnostic biomarker [17,18,20]. The reason for TROP-2 upregulation in cancer cells is unclear, however it is postulated that TROP-2 has critical regulatory effects on cell proliferation and invasion, meaning that overexpression would lead to selective tumor progression [21]. In fact, preclinical data suggests that TROP-2 overexpression stimulates tumor growth while TROP-2 knock-down inhibits tumor growth [19].

In breast cancer specifically, elevated TROP-2 expression has been associated with worse survival [22]. *TROP-2* gene expression has been detected in all breast cancer subtypes with higher levels noted in HR+/HER2-and triple negative breast cancer (TNBC) compared to HER2+ disease [23]. Genomic analyses of TNBC also identified *TROP-2* as an attractive candidate for targeted therapy [24] and led to further investigation of TROP-2 as a new therapeutic target.

1.4. Sacituzumab govitecan: TROP-2 ADC

Initial preclinical therapeutic development focused on the combination of anti-TROP-2 antibodies with several chemotherapeutic partners including doxorubicin and microtubule inhibitors [25,26]. Among the most promising of these was sacituzumab govitecan (SG), a novel ADC that combines the humanized RS7 (hRS7) anti–TROP-2 MAb to SN-38, an active metabolite of irinotecan, using a hydrolysable linker (Fig. 1). The anti-TROP-2 MAb allows SG to bind to the surface of cells expressing TROP-2 and is transported to intracellular lysosomes [27]

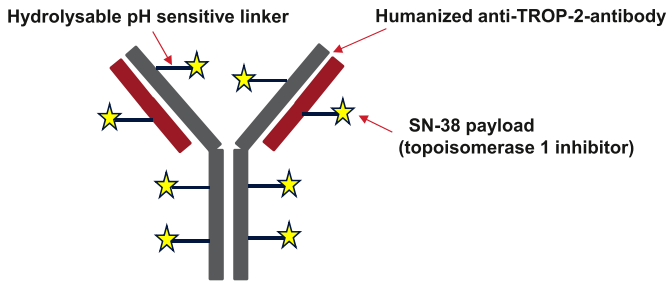

Fig. 1. Sacituzumab govitecan: example of an antibody drug conjugate.

and hydrolyzed releasing SN-38 into the cell. SN-38 inhibits activity of topoisomerase I, thereby disrupting cellular apoptosis and resulting in DNA damage [18,28].

There are several reasons why SG is uniquely suited as an ADC. Firstly, its active metabolite SN-38 is estimated to be 2–3 times more potent than irinotecan and is also membrane permeable, enabling it to exert a "bystander effect" [28,29]. Second, the hydrolysable linker of SG allows for extracellular release of SN-38 in addition to intracellular release, thereby creating another mechanism for "bystander effect". This potential for extracellular release may be particularly beneficial in tumors with heterogenous TROP-2 expression [18,28,29]. Third, SG offers a high DAR of 7.6:1. Prior ADCs, such as T-DM1, had DARs of 4:1 or less, however the unique antibody and linker design allows for SG to maintain a higher DAR without compromising antibody binding or pharmacokinetic properties [27,30]. Finally, SG showed reduced toxicity compared to other topoisomerase inhibitors, particularly with less severe diarrhea. This is postulated to be due to the lower rate of glucuronidation of SN-38 molecules bound to antibody, as opposed to SN-38 that is metabolized directly from irinotecan [31].

1.5. Sacituzumab govitecan in TNBC

Initial clinical trials focused on the use of SG in metastatic TNBC (Table 1). A first in-human phase I/IIb basket trial (NCT01631552) enrolled 25 patients with solid tumors, including 4 patients with TNBC [32]. Patients were enrolled at doses ranging from 8 to 18 mg/kg on Day 1 and 8 of a 21-day cycle. They were not pre-selected based on TROP-2 expression. Dose-limiting neutropenia was observed at the 12 mg/kg dose and thus a dose of 10 mg/kg was selected for further study. Of the four TNBC patients, three had partial response (PR) with duration of response (DoR) of 10.4, 6.9, and 3.1 months respectively.

This led to IMMU-132-01 (NCT01631552), a phase I/II multi-center basket trial which enrolled 108 patients with TNBC and a least 2 prior lines of chemo to receive 10 mg/kg of SG on Day 1 and 8 of a 21-day cycle [33]. Overall response rate (ORR) was 33.3% (36 patients) including 3 complete responses (CR), median DoR was 7.7 months (95% confidence interval [CI], 4.9 to 10.8), and clinical benefit rate (CBR) was 45.4%. Efficacy was not affected by prior exposure to taxanes and anthracyclines, suggesting a lack of cross-resistance to previous cytotoxic therapy. The most common AE was diarrhea (62%) with the majority being low grade and only 8% having grade 3 or higher. Other common AEs of any grade were fatigue, nausea, neutropenia and anemia. The most common grade 3 or greater AEs were neutropenia (26%) and anemia (11%). The rate of AEs leading to treatment discontinuation was low (3%). Overall, these results supported ongoing evaluation of SG in refractory metastatic TNBC as a potentially efficacious treatment with a favorable safety profile.

Building on IMMU-132-01, the ASCENT trial (NCT02574455) randomized 468 patients with TNBC with at least 2 prior lines and a taxane in a 1:1 fashion to receive SG vs single-agent chemotherapy with eribulin, vinorelbine, capecitabine, or gemcitabine [34]. Patients with stable brain metastases were allowed to participate in the trial but were excluded from final analyses. A median PFS of 5.6 months with SG and

Table 1
Published data with TROP-2 ADCs in breast cancer.

NCT Number/ Trial name (if applicable)	Trial Design/Patient Population	Clinical Trial Data	Reference
Sacituzumab govitecan			
NCT01631552	FIH trial evaluating multiple doses of IMMU-132 (Sacituzumab govitecan) in patients with advanced solid tumors including TNBC (N = 25)	DLT: neutropenia; MTD: 12 mg/kg Neutropenia was the most common G3 AE (n = 9) Two PRs per RECIST v1.1 (TNBC, colon cancer) SD: 64%; Survival: 15–20+ months in 25% of patients	32
	Phase II single arm expansion of Sacituzumab govitecan (10 mg/kg) in heavily pretreated TNBC (N = 69)	ORR: 30%, CBR: 46%. Median PFS: 6 months; and median OS: 16.6 months G3 AEs: neutropenia and other hematologic toxicities, diarrhea. Febrile neutropenia: 7%	68
	Ph I/II trial of Sacituzumab govitecan in TNBC treated with 2 prior lines of therapy including taxanes (N = 108)	ORR: 33.3%, CBR: 45.4%. Median PFS: 5.5 months; and median OS: 13 months. G3 AEs: neutropenia and other hematologic and gastrointestinal toxicities like diarrhea. Febrile neutropenia: 7%	33
NCT02574455 ASCENT	Phase III trial of Sacituzumab govitecan vs TPC in relapsed/refractory metastatic TNBC (N = 468)	Statistically significant improvement in median PFS and OS with SG compared to TPC. Myelosuppression and diarrhea were more frequent with SG	34
NCT04230109 neoSTAR	Phase II neoadjuvant trial with Sacituzumab govitecan administered q21 days × 4 cycles in patients with T1c-T4, node± TNBC (N = 50)	pCR (ypT0/isN0) was 30%. Most common AEs were nausea, fatigue and alopecia. G3/4 neutropenia and G3 diarrhea were also observed.	35
NCT Number/ Trial name (if applicable)	**Trial Design/ Patient Population**	**Clinical Trial Data**	**Reference**
NCT01631552	Ph I/II single arm basket trial of Sacituzumab govitecan including HR+/HER2- MBC treated with prior ET and chemotherapy (N = 54)	ORR 31.5%, median DoR: 8.7 months; median PFS and OS: 5.5 months and 12 months respectively. G3 neutropenia: 50%	43
NCT03901339 TROPICS-02	Phase III trial of Sacituzumab govitecan vs TPC in HR+/HER2- MBC (N = 468)	Statistically significant improvement in median PFS and OS with SG compared to TPC G3 neutropenia 51% on SG vs 38% on TPC, and G3 diarrhea 9% with SG vs 1% with TPC. Febrile neutropenia comparable on both arms. Unplanned subgroup analysis showed superior median PFS in patients with HER2-low	45, 46, 49

(*continued on next page*)

Table 1 (*continued*)

NCT Number/ Trial name (if applicable)	Trial Design/Patient Population	Clinical Trial Data	Reference
		and HER2 IHC 0 tumors (local testing) with SG compared to TPC	
Datopotamab deruxtecan			
NCT03401385 TROPION PanTumor01	FIH trial with Datopotamab deruxtecan in refractory metastatic TNBC (N = 44)	ORR 34% (ORR-52% in patients without prior topoisomerase 1 inhibitor-based ADC) G3 stomatitis in 9.1% and G3 nausea in 6.8% of patients	55
NCT03742102 BEGONIA	Phase Ib/II platform trial with Datopotamab deruxtecan + durvalumab in first-line metastatic TNBC (N = 29)	Confirmed ORR: 74%; median DoR: not yet reached. Responses seen regardless of PD-L1 expression. No DLTs reported; G3 stomatitis in 14%. No ILD/pneumonitis or neutropenic events reported	62

Abbreviations: ADC = antibody drug conjugate; AE = adverse events; CBR= Clinical benefit rate; DLT = dose limiting toxicities; DoR = Duration of response; FIH = First-in-human; G = grade; HR = hazard ratio; HR+ = hormone receptor positive; IHC = Immunohistochemistry; ILD = Interstitial lung disease; MBC = metastatic breast cancer; MTD = maximum tolerated dose; NACT = neoadjuvant chemotherapy; OS = overall survival; PFS = progression free survival; PR = partial response; pCR = pathologic complete response; TNBC = triple negative breast cancer; TPC = treatment of physician's choice chemotherapy.

1.7 months with chemotherapy (HR 0.41; 95% CI, 0.32 to 0.52; P < 0.001) and median OS of 12.1 months with SG vs 6.7 months with chemotherapy (HR 0.48; 95% CI, 0.38 to 0.59; P < 0.001), and DoR of 6.3 months with SG vs 3.6 months with chemotherapy (HR 0.39; 95% CI, 0.14 to 1.07) were reported. Efficacy analysis of the full population, including those patients with brain metastases, found a median PFS of 4.8 months with SG and 1.7 months with chemotherapy (HR 0.43; 95% CI, 0.35 to 0.54). Overall, the results of ASCENT demonstrated a statistically significant survival benefit with the use of SG over single-agent chemotherapy for patients with metastatic TNBC in the second line setting and beyond. The incidence of AEs, particularly neutropenia and diarrhea, was consistent with prior early phase studies of SG and were more common in the SG group compared to chemotherapy. However, despite the higher rate of toxicity with SG, only 5% of patients treated with SG had to discontinue treatment due to adverse AE, indicating that these AEs can be effectively medically managed.

NeoSTAR (NCT04230109) evaluated SG for TNBC in the neoadjuvant setting for localized disease [35]. After four cycles of SG, patients with biopsy proven residual disease had the option of continuing with further neoadjuvant therapy. Fifty patients were enrolled in the study with the majority (62%) being node-negative. Most patients had radiologic response after 4 cycles (62%) and 26 (52%) proceeded straight to surgery with a pCR rate of 30% (Table 1). The remaining 24 patients received additional neoadjuvant chemotherapy (anthracycline-based regimen vs carboplatin/taxane vs docetaxel/cyclophosphamide) with 6 of them achieving pCR. While this was an early phase trial with a small population size, these results suggest that ADCs could be used to select patients with good prognostic features that may be able to be spared further chemotherapy.

There are several ongoing trials for SG in TNBC. Among the early phase trials is Saci-IO (NCT04468061) a phase II trial randomizing patients with chemotherapy naïve metastatic TNBC to SG with or without pembrolizumab [36]. Morpheus TNBC (NCT03424005) is a phase Ib/II study that will enroll multiple parallel arms for patients with metastatic TNBC in the first- or second-line setting for those who are immunotherapy naive [37]. Other trials evaluating the combination of SG with immunotherapy include InCITe (NCT03971409), a phase II trial evaluating SG with avelumab vs avelumab with liposomal doxorubicin with or without binimetinib for metastatic TNBC and, and a phase I/II trial combining SG with cyclophosphamide and two novel agents, N-803, and PD-L1 t-haNK (NCT04927884) [38,39]. N-803 is a protein complex which functions as a super-agonist of IL15 and PD-L1 t-haNK is an engineered natural killer cell which expresses IL2, CD16 and a high affinity receptor for PD-L1. Finally, a phase I/II trial is combining SG with talazoparib in patients with metastatic TNBC (NCT04039230) [40]. There are two ongoing phase III trials of note: i) ASCENT-03 evaluating SG versus physician's choice of chemotherapy in patients with metastatic TNBC with PD-L1 negative tumors or PD-L1 positive and have already undergone treatment with immune checkpoint inhibitor (CPI) [41] and ii) ASCENT-04 evaluating pembrolizumab combined with either SG or physician's choice of chemotherapy in patients with metastatic TNBC and PD-L1 positive (combined positive score >10) tumors [42] (Table 2).

1.6. Sacituzumab govitecan in HR + breast cancer

The success of SG in TNBC prompted its evaluation in other breast cancer subtypes, particularly HR + disease. IMMU-132-01 was a phase I/II single-arm basket trial (NCT01631552) that enrolled 54 patients with metastatic HR+/HER2-breast cancer that had progressed on endocrine therapy (ET) and at least one line of chemotherapy [43]. At a median follow-up of 11.5 months, ORR was 31.5% with 17 partial responses. Median DoR was 8.7 months, median PFS was 5.5 months (95% CI 3.6–7.6), and median OS was 12 months (95% CI 9.0–18.2) (Table 1).

This led to the phase III trial TROPICS-02 (NCT03901339) which evaluated SG in metastatic HR+/HER2-disease [44] vs. TPC chemotherapy (eribulin, gemcitabine, capecitabine or vinorelbine). Eligible patients had HR+/HER2- MBC with 2–4 prior lines of chemotherapy for metastatic disease, and prior therapy including a taxane, CDK4/6 inhibitor and ET. Five hundred and forty-three patients were randomized 1:1 to receive SG or chemotherapy. At the first interim analysis (IA), SG was associated with a statistically significant improvement in PFS compared to TPC (5.5 vs 4.0 months, HR 0.66; 95% CI, 0.53–0.83; P = 0.0003) [45]. In a planned second IA with a median follow-up of 12.5 months, treatment with SG was associated with statistically significant improvement in OS (14.4 vs 11.2 months; HR 0.79; 95% CI, 0.65–0.96 P = 0.020) compared with TPC [46]. In addition to PFS and OS, ORR (21% vs 14%, HR 1.63; 95% CI 1.03–2.56, $P = 0.035$) and CBR (34% vs 22%; HR 1.80; 95% CI 1.23–2.63, $P = 0.003$) were significantly higher in patients receiving SG versus chemotherapy (Table 1). More patients in the SG group had grade 3 or greater AEs with 74% vs 60% in the chemotherapy group. The most common of these were neutropenia (51% vs 39%) and diarrhea (10% vs 1%). Interestingly, an analysis of patient reported outcomes revealed an association between improved quality of life with use of SG compared to chemotherapy as determined by responses to European Organization for Research and Treatment (EORTC) quality of life questionnaire-C30 [47]. Median time to deterioration in global health/quality of life was longer with SG compared to chemotherapy (4.3 vs 3 months, HR 0.75; 95% CI 0.61–0.92, p = 0.006). Due to interest in efficacy in patients with low HER2 expression (HER2 1+ IHC or 2+ IHC without ERBB2 overexpression by in situ hybridization) following the results of the DESTINY Breast04 trial [48], an unplanned sub-group analysis was conducted. SG appeared to have similar efficacy in both HER2 0 and HER2 low subgroups, with median PFS in HER2 low 6.4 vs 4.2 months (HR 0.58 (0.42–0.79) P < 0.001) for SG vs TPC, respectively [49]. Overall, these promising results point to an expanded role for SG in the treatment of pre-treated, endocrine resistant HR+/HER2- MBC.

Further studies to establish the role of SG in HR+/HER2-breast cancer (BC) are needed and are ongoing. SASCIA is a phase III trial for

Table 2
Phase III trials of TROP-2 ADCs.

	NCT04639986 EVER-132-002	NCT04595565 SASCIA	NCT05104866 TROPION-Breast01	NCT05374512 TROPION-Breast02	NCT05382299 ASCENT-03	NCT05382286 ASCENT-04
Stage	IV	Localized	IV	IV	IV	IV
Subtype	HR+/HER2-	HER2-	HR+/HER2-	TNBC	TNBC	TNBC
Characteristics	*i)* 2–4 prior lines chemotherapy *ii)* Prior taxane, prior hormone therapy *iii)* Asian population	*i)* Residual disease after NACT	*i)* 1–2 prior lines chemotherapy *ii)* Prior endocrine therapy	*i)* Not candidates for PD-1/PD-L1 inhibitors	*i)* No prior systemic therapy for LA/MBC ii) (PD-L1) negative at screening or PD-L1 positive if treated with anti-PD-(L)1 inhibitor in the (neo)adjuvant setting	*i)* No prior systemic therapy for LA/MBC *ii) PD-L1 + tumor*
Number of patients	Est 330	Est 1200	Est. 700	Est. 600	Est 540	Est 440
Intervention	Sacituzumab govitecan vs chemotherapy (eribulin, vinorelbine, capecitabine, gemcitabine)	Sacituzumab govitecan vs chemotherapy (capecitabine, carboplatin, cisplatin)	Datopotamab deruxtecan vs chemotherapy (eribulin, vinorelbine, capecitabine, gemcitabine)	Datopotamab deruxtecan vs (paclitaxel, nab-paclitaxel, capecitabine, carboplatin, or eribulin)	Sacituzumab govitecan vs chemotherapy (paclitaxel or nab-paclitaxel, gemcitabine & carboplatin)	Sacituzumab govitecan + pembrolizumab vs Pembrolizumab + chemotherapy (paclitaxel or nab-paclitaxel, gemcitabine & carboplatin)
Primary Endpoint	PFS	Invasive DFS	PFS & OS	PFS & OS	PFS	PFS

Abbreviations: TNBC = triple negative breast cancer; HR+ = hormone receptor positive; Est = estimated; DFS = disease free survival; OS = overall survival; PFS = progression free survival.

patients with localized HER2- BC (including TNBC and HR+) with residual disease after neoadjuvant chemotherapy [50]. Patients are randomized to receive 8 cycles of SG or physician's choice of therapy (capecitabine vs platinum-based chemotherapy vs observation) (Table 2). Saci-IO HR+ (NCT04448886) is a phase II trial evaluating the use of SG with or without pembrolizumab in patients with metastatic HR+/HER2- BC following progression on ET [51]. Finally, a phase II study (NCT04647916) is evaluating the efficacy of SG in patients with metastatic HER2- BC and brain metastases [52]. The primary outcome of this study is CNS response.

2. Datopotamab deruxtecan (Dato-DXd)

Dato-DXd is a TROP-2-directed ADC where a humanized anti-TROP-2 IgG1 MAb is attached to a topoisomerase I inhibitor payload via tetrapeptide-based cleavable linker. The DAR in this construct is ~4:1 [53]. It is highly stable in circulation due to the linker which is designed for cleavage only in the presence of lysosomal proteases. Although the payload (DXd) shares the same mechanism of action (MOA) as the payload (SN-38) in SG, there are some important differences between them. DXd is 10X more potent than SN-38. The longer half-life of Dato-DXd allows a Q3 week dosing schedule which is preferable over the D1 and D8 Q3 weeks schedule for SG. Dato-DXd has an improved therapeutic index with just 5% of the payload released after 21 days as opposed to 90% of payload released after 3 days with sacituzumab [54].

In preclinical studies, Dato-DXd inhibited growth in cell lines of multiple tumor types that expressed high levels of TROP-2, but not those with low TROP-2 [53]. All these cell lines were sensitive to DXd alone while datopotamab or an isotype control IgG ADC alone were not active thus confirming that expression of TROP-2 on the cell surface is essential for Dato-DXd activity. Studies in xenograft mouse models demonstrated that treatment of TROP-2-expressing tumors with Dato-DXd led to accumulation of DXd and DNA damage in tumor cells resulting in inhibition of tumor growth [53].

2.1. Clinical trials with Dato-DXd

The first-in-human (FIH) trial with Dato-DXd enrolled unselected patients with advanced solid tumors including TNBC and HR+/HER2- MBC. Forty-four patients with metastatic TNBC and a median of 3 prior regimens for advanced disease were treated with Dato-DXd [55]. Nearly a third had *de novo* MBC and over two-thirds had received 2 or more priors for MBC. Eleven percent of patients had brain metastases. Thirty percent of patients (n = 13) had received prior topoisomerase-I inhibitor-based ADC, SG (n = 10), and a DXd-based ADC (n = 3). An ORR of 34% was observed with confirmed responses in 32% of patients (ORR 52% and confirmed responses in 48% in SG/DXd naïve patients). Median DoR was not reached (Table 1). Grade 3 or greater AEs were seen in 45%, and the most common AEs were nausea (66%) and stomatitis (55%); majority of these were grade 1–2, but 9% grade 3 stomatitis was reported. The frequency of diarrhea and hematologic toxicity was low. There were no adjudicated cases of drug-related interstitial lung disease (ILD). Based on these encouraging data, a phase III trial evaluating Dato-DXd vs investigator choice of chemotherapy as first-line therapy for metastatic TNBC was initiated [56].

The FIH trial also enrolled patients with refractory HR+/HER2- MBC and although these data have not been publicly shared, a randomized phase III trial of Dato-DXd vs TPC in HR+/HER2- MBC previously treated with 1–2 lines of chemotherapy is underway [57] (Table 2).

Chemotherapy, including topoisomerase I inhibitors, can enhance the efficacy of CPIs providing an opportunity to combine immune CPI therapy with Dato-DXd [58]. In syngeneic mouse models, DXd bearing ADCs sensitized tumors to CPI, potentially through enhanced antitumor immunity caused by the delivered DXd payload [59,60]. This was demonstrated with T-DXd and there are ongoing preclinical studies with Dato-DXd [59]. BEGONIA is a phase Ib/II trial testing this concept in the clinical setting with the durvalumab + Dato DXd combination in first-line metastatic TNBC [61]. In the 27 patients evaluable for response, the confirmed ORR was 74% (18/27 PR), regardless of PD-L1 expression (evaluated by the SP263 assay) [62]. Most patients had durable responses and median DoR was not reached. Stomatitis and alopecia were the most frequent toxicities, mostly low-grade events, although grade 3 stomatitis was reported in 4 patients (14%) and ~14% of patients required dose reductions due to stomatitis. There were no cases of ILD/pneumonitis and no neutropenic events were reported [62]. The low rates of hematologic toxicities observed with Dato-DXd may enable combinations with PARP inhibitors. This doublet therapy is attractive since both agents target DNA damage and repair pathway [63]. TROPION Pantumor-03 is being planned (NCT05489211) in patients with advanced solid tumors (although not breast cancer) with

Dato-DXd plus AZD5305 (a potent inhibitor of PARP1 with significant PARP1 DNA trapping activity at doses that showed no PARP2 activity) included as one of the treatment arms.

2.2. Adverse events with TROP-2 ADCs

ADCs generally incorporate highly potent and toxic drugs to achieve maximum cytotoxic effects on the target tumor cells. Hence, the side effect profile of these ADCs may be characteristic of the cytotoxic payload. Some of the most common AEs reported on the sacituzumab arm in the pivotal ASCENT trial were neutropenia (63%), diarrhea (59%) and nausea (57%) [34]. Neutropenia was also the most frequent grade 3/4 AE (51%) and the incidence of grade 3 and 4 febrile neutropenia was 5% and 1% respectively. The incidence of grade 3 diarrhea was 10% with SG; no grade 4 events were noted. Both neutropenia and diarrhea are known side effects of SN-38 that is derived from irinotecan. These clinically relevant AEs were managed by utilizing standard supportive care measures. The reported incidence of ILD was very low with one patient having grade 3 pneumonitis following the last dose of SG therapy. It was resolved in 7 weeks without any sequelae [64]. Phase III TROPICS-02 study in HR+/HER2- MBC also reported similar rates of neutropenia (all grade 70%; Grade 3–51%) and diarrhea (all grade 57%; Grade 3–9%) [46].

A detailed biomarker analysis from the ASCENT trial demonstrated that the toxicity profile of sacituzumab was not influenced by the level of TROP-2 expression in the tumor [65]. In a retrospective exploratory analysis in the phase I/II basket trial with SG, the incidence of neutropenia, but not diarrhea, was shown to increase with increasing *UGT1A1*28* copy number [33]. A detailed exploratory safety analysis from the ASCENT trial also revealed that patients with *UGT1A1*28/*28* genotype (13% of patients on the SG arm) had slightly higher rates of neutropenia (59%) compared to the heterozygous (47%) and wild type (53%) variants but considerably higher rates of febrile neutropenia (18% vs 5% vs 3% respectively) [64]. However, rates of grade $\geq$3 treatment-related diarrhea, although more common in *UGT1A1*28/*28* genotype, were not that different between the genotypes (15% vs 9% vs 10% respectively). The authors noted that rates of treatment discontinuation for SG due to toxicities was low in patients with the *UGT1A1*28/*28* genotype (6%). One caveat from these exploratory analyses was the low frequency of the *UGT1A1*28/*28* genotype that precluded any firm conclusions on the differences in AEs. While mandatory prescreening for *UGT1A1* genotype is not required and *UGT1A1* status does not warrant upfront changes in treatment or management, individuals with *UGT1A1*28/*28* genotype should be closely monitored for neutropenia and diarrhea and dose reduced in the event of toxicity [64].

Dato-DXd treatment in heavily pretreated TNBC resulted in a low frequency of hematologic toxicities and diarrhea in the TROPION PanTumor01 trial [55]. The stable linker and the long half-life of Dato-DXd may result in less payload release into the plasma limiting myelosuppression [53]. The most common AEs reported in this trial were mainly low-grade nausea (~60%) and stomatitis (~45%), although grade 3 stomatitis was reported in ~10–12% of patients [55]. There were no cases of adjudicated drug-related ILD noted on this trial. A similar toxicity profile was observed in the BEGONIA trial in the Dato-DXd + durvalumab arm with all grade stomatitis in 69% of patients and all grade nausea and alopecia in 66% of patients. Grade 3 stomatitis was reported in 14% of patients and 21% had grade 2 nausea and alopecia [62]. Stomatitis led to dose reductions in 14% of patients necessitating prophylactic measures and implementation of toxicity management guidelines. No neutropenic events or cases of ILD/pneumonitis were reported.

2.3. Predictive biomarkers of TROP-2 directed ADCs

Currently there are no predictive biomarkers for treatment decisions regarding SG or Dato-DXd. In the ASCENT study, an exploratory biomarker analysis for patients undergoing SG treatment was performed using BRCA1/2 status as well as an H-score. The H-score is a score that utilizes an IHC stain for TROP-2 on tumor tissue and evaluates the intensity and percentage of cells that stain positive [34,67]. Two hundred and ninety patients in the study (60%) had evaluable specimens and patients were divided into those having high, medium and low H-scores. In patients treated with SG, those with high H-score had numerically higher OS compared to those with low score (14.2 vs 9.3 months) but this difference was not statistically significant. However, the small number of patients with low TROP-2 expression limited the analyses. Similarly, no difference in efficacy was noted for those with or without a BRCA mutation. An earlier phase I/II trial noted numerically higher PFS in those with moderate to high TROP-2 expression but again, this result was not statistically significant [68]. More investigation is needed to identify biomarkers predictive of benefit to TROP-2 ADCs.

3. Conclusion

ADCs have greatly expanded treatment options for patients with breast cancer (BC). They are able to deliver a potent cytotoxic that could not be given alone to the tumor through selective antibodies. Early efforts at implementing ADCs in MBC focused on HER2-directed agents and subsequent ADCs have expanded to target TROP-2 across multiple breast cancer subtypes. Sacituzumab govitecan, a TROP-2 ADC was initially approved for metastatic TNBC regardless of TROP-2 expression and most recently reported significant improvements in PFS and OS in hormonally driven BC as well. Data from trials with Dato-DXd in TNBC and HR + disease are likely to further impact treatment in this space in the near future as well.

Given the astoundingly poor performance of single agent chemotherapy in both TNBC and endocrine-resistant ER + MBC and the significant improvements with multiple ADCs compared to standard of care chemotherapy, it is anticipated that ADCs will continue to outperform chemotherapy in even earlier lines of breast cancer. Most ADCs also boast an attractive side effect profile compared to standard chemotherapy, and so far, are reasonable treatment choices across a wide spectrum of target protein expression.

Combinations are of interest with ADCs, however, it remains to be determined whether a) they add additional efficacy to a class of compounds that are already quite active, and b) if combinations add toxicity, making them undesirable. Ongoing trials are replacing conventional chemotherapeutic agents with SG as a partner for immunotherapy in the front-line setting for metastatic TNBC (ASCENT-04, Saci-IO TNBC, Morpheus TNBC) and in recurrent TNBC (InCITe) as well as refractory HR+/HER2- MBC (Saci-IO HR+). This strategy avoids use of drugs that patients may have been exposed to in the (neo)adjuvant setting. Moreover, SG does not cause the permanent toxicities like neuropathy and cardiac issues associated with taxanes and anthracyclines used to treat TNBC. Other combinations that can be envisaged include targeted therapies and a trial of SG + alpelisib in PIK3CA mutant HER2- MBC is already underway (NCT05143229) but may magnify GI toxicity [69].

In early stage disease TNBC, the modest pCR rates with 12 weeks of SG as neoadjuvant treatment suggest that the optimal duration of therapy needs to be evaluated and combination therapies must be explored to improve outcomes. Single agent SG is under evaluation in patients with residual disease after neoadjuvant chemotherapy for HER2- BC (SASCIA) and early safety analyses noted that patients treated with SG reported higher frequency and higher-grade AEs compared to those treated with capecitabine on the control arm [70]. This does underscore the need to adhere to guidelines for supportive care therapies, especially in the curative setting.

The success of targeting TROP-2 via ADCs in MBC and urothelial cancer and ongoing trials in NSCLC have established TROP-2 targeting as a valid and fruitful strategy. Future research needs to focus on optimizing the side effect profile and identification of predictive biomarkers

to tailor treatment.

Novel ADC agents are anticipated and are currently being explored in clinical trials. These include patritumab deruxtecan, a HER3-targeting ADC with the same payload as T-DXd and enfortumab vedotin with an antibody targeting Nectin-4 and MMAE as the payload. One very important consideration will be sequencing and mechanisms of resistance. It is unclear whether resistance will occur from loss of target expression from the cell surface, resistance to the payload, or alternative mechanisms. This could greatly impact our ability to use ADC after ADC and how we prioritize and sequence emerging ADC agents.

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