Trop-2 as a Therapeutic Target in Breast Cancer

Subjects: Oncology

Contributor: Elizabeth Sakach, Ruth Sacks, Kevin Kalinsky

Trop-2 is an exciting, new target for the treatment of breast cancer. Trop-2 is found at high levels in multiple cancers such as prostate, pancreatic, urothelial, lung, and breast cancer. Among different breast cancer subtypes, Trop-2 is most highly expressed in triple negative breast cancer. Drugs that inhibit Trop-2 are now an important treatment option for patients with metastatic triple negative breast cancer, for whom few treatment options exist.

Keywords: Trop-2 ; breast cancer ; antibody drug conjugate

1. Introduction

Human trophoblastic cell surface antigen 2 (Trop-2) is a transmembrane calcium signal transducer highly expressed in multiple tumor types on the membrane surface of epithelial cells. Under physiologic conditions, Trop-2 plays a critical role in embryonic development, placental tissue formation, and stem cell proliferation. Trop-2 is expressed at low levels on the surface of many types of normal epithelial cells such the heart, liver, kidney, and lung. Expression on these normal epithelial cells is at a much lower level than in epithelial tumors, making Trop-2 an ideal therapeutic target [1][2][3]. When Trop-2 is overexpressed, it acts as an oncogene promoting tumor proliferation, growth, invasion, and metastasis in epithelial cancers such as breast, colon, prostate, pancreatic, urothelial, and lung [4][5][6][7][8][9][10][11]. High Trop-2 expression is associated with a poor prognosis in multiple cancer types, with worse overall survival and disease-free survival outcomes [12][13][14][15][16].

2. Trop-2 Expression in Breast Cancer

Trop-2 is overexpressed in all breast cancer subtypes, however it is most elevated in triple negative breast cancer (TNBC) as compared to estrogen receptor positive (ER+) or HER-2+ tumors [2][16]. Aslan et al. evaluated Trop-2 protein levels in breast cancer tumors by immunohistochemistry (IHC). High levels of Trop-2 expression were found in 50% of ER+ (n = 22), 74% of HER2+ (n = 35), and 93% of TNBC samples (n = 28) [16].

Vidula et al. 2017, studied the associations of Trop-2 expression with clinical characteristics and outcomes from microarray data from neoadjuvant I-SPY1, METABRIC, and TCGA patient databases. In all 3 datasets, Trop-2 had a wide range of expression in all breast cancer subtypes, particularly luminal A and TNBC. Presence of Trop-2 was associated with expression of genes central for cell epithelial transformation, adhesion, and proliferation. Trop-2 expression was inversely related to the expression of immune genes, potentially affecting tumor growth. These findings supported the ability of Trop-2 to be used as a therapeutic target across a variety of breast cancer subtypes [17].

2.1. Trop-2 as an Oncogene in Breast Cancer

Trop-2 is a critical element in TNBC tumor growth. Trop-2 gene deletion and gene silencing has been found to suppress TNBC cell growth in vitro and in vivo. Aslan et al. studied Trop-2 gene deletion via CRISPR/Cas9 technology in TNBC cells that endogenously express Trop-2. The loss of Trop-2 significantly suppressed TNBC cell growth in colony formation and proliferation assays. Using small hairpin RNA to create Trop-2 knockdown cells, downregulation of Trop-2 also significantly impaired the colony-forming ability and proliferation of TNBC cell lines. Downregulation of Trop-2 dramatically decreased the invasion ability of the TNBC cell lines in three-dimensional Matrigel drop invasion assays [16].

In preclinical models, Aslan et al. subcutaneously implanted Trop-2 depleted TNBC cells into mice. Tumor volumes were measured every three days. With the downregulation of Trop-2 in the implanted TNBC mouse tumors, there was a significant delay in tumor growth and decrease in tumor weight. The Trop-2 gene knockout models demonstrated the oncogenic potential of Trop-2 expression in TNBC [16].

Further studies by Aslan et al. suggest Trop-2 may lead to an oncogene-mediated metabolic reprogramming in TNBC by regulating a group of metabolic genes and oncogenes. The investigators evaluated changes in protein levels in TNBC tumors upon modulation of Trop-2. Trop-2 expression was found to be related to increased levels of a 5-gene metabolic signature (comprising of TALDO1, GPI, LDHA, SHMT2, and ADK). This 5-gene metabolic signature was associated with oncogenic metabolism and poorer overall survival in early stage breast cancers. Aslan et al. found this data was clinically correlated, as patients with the 5-gene metabolic signature had worse overall survival and disease-free survival in 12 different mRNA expression datasets of breast cancer patients [16].

As an oncogene, Trop-2 plays a role in several major signaling pathways involved in cell proliferation, but its precise role in these pathways is not completely understood. Trop-2 acts as a calcium signal transducer, leading to activation of various tumorigenic pathways including NF-KB, cyclin D1, and ERK [18][19][20][21][22]. Through calcium-mediated signal cascades, Trop-2 activates the ERK1/2-MAPK pathways, which modulate cell cycle progression and promote the evasion of apoptosis [22]. The Bcl-2 family is a crucial checkpoint in apoptosis, comprising anti-apoptotic proteins Bcl-2, Bcl-xl, and Mcl1 and pro-apoptotic proteins, Bax, Bak, and Bim [23]. The expression of Bcl-2 and Bax can be regulated by the MEK/ERK pathway [24][25].

Lin et al. proposed that a fragment antigen-binding fragment (Fab) against Trop-2 could inhibit the evasion of pro-apoptotic pathways and potentially induce apoptosis to optimize responses to anti-cancer therapeutics. The group employed a human Fab phage library to isolate a human Fab that recognized the extracellular domain of Trop-2 [26]. Specific binding of Trop-2 Fab to Trop-2 on the surface of breast cancer cells was confirmed by ELISA, flow cytometry, and fluorescent staining. Investigators performed an MTT assay, a colorimetric assay to assess metabolic activity, and showed that Trop-2 Fab was effective at inhibiting proliferation in the TNBC cell line MDA-MB-in a dose-dependent manner. Immunofluorescence staining and Western blot analysis showed Trop-2 Fab upregulated Bax expression, a pro-apoptotic protein, and downregulated Bcl-2 expression, an anti-apoptotic protein, suggesting a Trop-2 Fab could induce apoptosis in TNBC cells.

In vivo, the researchers confirmed an antitumor effect of the Trop-2 Fab in a breast cancer xenograft model. The tumor inhibition rate was 28% in the high dose Trop-2 Fab (30 mg/kg) treated group. Consistent with in vitro studies, IHC and Western blot analysis of excised TNBC tumors from mice showed significantly downregulated Bcl-2 expression and upregulated Bax expression compared with the control group in treated Trop-2 Fab mice. The group found that high concentrations of Trop-2 Fab allowed for significant tumor inhibition, confirming the hypothesis that Trop-2 could be a candidate for the therapeutic inhibition of TNBC [26].

2.2. Trop-2 as Determinant for Breast Cancer Survival

Trop-2 promotes migration of invasive breast cancer cells by inducing the epithelial-mesenchymal transition (EMT) which contributes to metastasis. Increased Trop-2 expression is associated with lymph node involvement and distant metastasis [27]. In a study evaluating Trop-2 expression in 127 patients with early stage TNBC, patients with high Trop-2 expression as determined by IHC, had higher rates of nodal involvement (53%) compared to patients with medium (23%) and low (21%) Trop-2 expression ($p = 0.03$). Lymphovascular invasion was also more frequent and found in 65% of patients with high Trop-2 versus 15-16% of patients with medium and low Trop-2 expression, respectively ($p \leq 0.001$) [28].

Trop-2 is synthesized in the endoplasmic reticulum, glycosylated in the Golgi apparatus, and sorted to the cell membrane. Trop-2 can then be activated by antibody-mediated cross-linking of cell-surface molecules or cleaved in the intramembrane. Some Trop-2 is also retained in intracellular compartments at different levels in various tumors. Ambrogi et al. found that the activation state of Trop-2 is a critical determinant of breast cancer tumor progression and could implicate Trop-2 as a novel prognostic indicator in breast cancer. Observations by this group found that Trop-2 localization and glycosylation are associated with worse overall survival, whereas intracellular retention is associated with less frequent disease relapse and therefore better overall survival [29]. By localizing Trop-2 expression via IHC in 702 breast tumor samples from patients, Ambrogi et al., found that localization of Trop-2 in the membrane is an unfavorable prognostic factor for overall survival (HR 1.63; $p = 0.04$), whereas intracellular Trop-2 had a favorable impact on prognosis and disease relapse (HR 0.48; $p = 0.003$) [29].

Lin et al. examined a panel of invasive ductal carcinoma (IDC) tissues ($n = 82$) and adjacent non-malignant tissue controls ($n = 70$) from patients undergoing surgery at a single institution [30]. None of the patients had received neoadjuvant chemotherapy or immunotherapy. The investigators evaluated Trop-2 expression by IHC and found significantly higher Trop-2 expression in IDC tissue compared to normal controls. High expression of Trop-2 was related to increased histologic grade ($p = 0.002$), P53 status ($p = 0.004$), cyclin D1 status ($p < 0.01$), lymph node metastasis ($p < 0.01$), distant metastasis $p = 0.004$) and more advanced TNM staging ($p < 0.01$). In contrast, no statistically significant association was

found between Trop-2 expression and age at diagnosis, tumor size, hormone receptor status, HER-2 status, or Ki-67. The strongest predictors of poor survival were high Trop-2 expression (p = 0.03), high cyclin D1 expression (p = 0.04), and lymph node metastasis (p = 0.06) [30]. Trop-2 is associated with high cyclin D1 expression as Trop-2 must bind to cyclin D1 to become an oncogene [31]. The binding of the two molecules affects the stability of cyclin D1 and increases cell proliferation and survival [1]. Silencing of this fusion protein has been shown to inhibit tumor growth [32].

3. Trop-2 as a Therapeutic Target: From Bench to Bedside

Precision medicine has opened the door to new possibilities in the world of targeted therapy development. Given the unmet need for innovative, new therapies in the treatment of TNBC, the emergence of Trop-2 is an exciting path forward as Trop-2 expression is reported in ~85% in TNBC tumors [16][33]. The first FDA (Food and Drug Administration) approved Trop-2 inhibitor for the treatment of TNBC is an antibody-drug conjugate (ADC). Over the past two decades, ADCs have moved to the forefront of cancer care and precision medicine. By targeting specific antigens on tumor cells, ADCs have become a leading therapeutic in Trop-2 inhibition [34].

4. Anti-Trop-2 Antibody Drug Conjugates: An Exciting Path Forward

ADCs are comprised of a monoclonal antibody, a cytotoxic agent (payload), and a linker that connects the monoclonal antibody to the cytotoxin. For Trop-2 directed ADCs: (1) the monoclonal antibody recognizes and binds to Trop-2 on the tumor cell; (2) the payload is internalized into the tumor cell; (3) the payload undergoes intracellular trafficking that carries it to the lysosomes; (4) following antibody catabolism and hydrolysis of the linker, the payload is released and induces apoptotic cell death [34][35][36]. Neighboring cancer cells (even with no target antigen present) are impacted by the bystander effect, which occurs when the cytotoxic payload is released from the target cell or within the extracellular space contributing to an augmented anti-tumor effect [37][38].

ADCs are a rapidly expanding class of agents with at least 160 drugs in the preclinical and clinical space [39][40]. Three ADCs are FDA approved for breast cancer treatment, but only one of these targets Trop-2. Extensive efforts are being made in ongoing trials to harness the effect of Trop-2 inhibition through ADC therapeutics.

4.1. Sacituzumab Govitecan: The First FDA Approved Anti-Trop-2 ADC

In April 2020, the FDA granted accelerated approval to sacituzumab govitecan-hziy (Trodelvy), a Trop-2 directed ADC, for patients with unresectable locally advanced and metastatic TNBC who received two or more prior lines of therapy for metastatic disease. Sacituzumab govitecan is composed of a humanized anti-Trop-2 immunoglobulin (IgG) antibody conjugated through a hydrolysable linker to SN-38, the cytotoxic agent or payload. SN-38 is a topoisomerase I inhibitor and active metabolite of irinotecan, which induces double-stranded breaks in DNA [41][42]. Irinotecan, the prodrug of SN-38, has limited delivery of the active metabolite, SN-38, due to the 100 to 1000-fold higher potency it carries in comparison to irinotecan. This high potency causes toxicity and poor tolerability with 1/3 of patients experiencing grade 3 or 4 diarrhea [43]. Sacituzumab govitecan can deliver higher levels of SN-38 with an improved tolerance profile [44].

4.1.1. Sacituzumab Govitecan in TNBC

The first-in-human study of sacituzumab govitecan originated as a basket trial which included 25 patients with 10 different epithelial cancers, including metastatic TNBC (n = 4), whose tumors had progressed on conventional treatments. Three of 25 patients had a >30% reduction in their tumor size, and one of these significant responders was a patient with metastatic TNBC [45]. A review of the initial data indicated improved response rates and clinical benefit in patients with TNBC given the starting dose of 10 mg/kg. Neutropenia was the only dose-limiting toxicity and 1 patient at this dose level experienced grade 3 diarrhea [46].

The efficacy of sacituzumab govitecan in TNBC was further evidenced in the multicenter, phase I/II, single-arm trial IMMU-132-01 that evaluated patients with advanced solid tumors, including 108 patients with metastatic TNBC who had received at least 2 therapies for metastatic disease [47]. This heavily pretreated population of metastatic TNBC demonstrated an overall response rate (ORR) of 33.3% (95% CI 24.6–43.1) and median response duration of 7.7 months (95% CI 4.9–10.8). The most common adverse events were nausea (67%), diarrhea (62%), fatigue (55%), and myelosuppression (74%) along with 9.3% of patients experiencing neutropenic fever [47][48]. Based on the findings from this trial, sacituzumab govitecan received accelerated approval by the FDA for the treatment of patients with metastatic TNBC.

In the randomized phase III ASCENT trial, the use of sacituzumab govitecan was compared against chemotherapy of physician's choice (eribulin, vinorelbine, capecitabine, or gemcitabine) in the treatment of 468 patients with relapsed or refractory metastatic TNBC. The median age of participants was 54 years, all patients had previous taxane exposure. The trial achieved its primary endpoint with a mPFS of 5.6 months with sacituzumab govitecan and 1.7 months with chemotherapy (HR for disease progression or death, 0.41; $p < 0.001$). The mOS was 12.1 months with sacituzumab govitecan and 6.7 months with chemotherapy (HR for death, 0.48; $p < 0.001$). The percentage of patients with an objective response was 35% with sacituzumab govitecan and 5% with chemotherapy (**Table 1**). Myelosuppression and diarrhea were more frequent with sacituzumab govitecan than with chemotherapy. Grade 3 or higher neutropenia was reported in 51% of patients treated with sacituzumab govitecan and 33% with chemotherapy, leukopenia in 10% and 5%, anemia in 8% and 5%, and diarrhea in 10% and <1% of patients, respectively [49].

Table 1. Outcomes in the ASCENT and TROPiCS-02 trials.

Clinical Trial	Clinical Outcomes	Full Population		Without Brain Metastases	
		Sacituzumab Govitecan	Chemotherapy	Sacituzumab Govitecan	Chemotherapy
Metastatic TNBC ASCENT Trial		*N* = 267	*N* = 262	*N* = 235	*N* = 233
	Median PFS, mo (95% CI)	4.8 (4.1–5.8)	1.7 (1.5–2.5)	5.6 (4.3–6.3)	1.7 (1.5–2.6)
	Median OS, mo (95% CI)	11.8 (10.5–13.8)	6.9 (5.9–7.7)	12.1 (10.7–14.0)	6.7 (5.8–7.7)
	Objective Response Rate no. of patients (%)	83 (31)	11 (4)	82 (35)	11 (5)
	Clinical Benefit Rate * no. of patients (%)	108 (40)	21 (8)	105 (45)	20 (9)
Metastatic HR+HER2- TROPiCS-02 Trial		*N* = 272	*N* = 271		
	Median PFS, mo (95% CI)	5.5 (4.2–7.0)	4.0 (3.1–4.4)		
	6-month PFS rate (95% CI)	46.1 (39.4–52.6)	30.3 (23.6–37.3)		
	9-month PFS rate (95% CI)	32.5 (25.9–39.2)	17.3 (11.5–23.2)		
	12-month PFS rate (95% CI)	21.3 (15.2–28.1)	7.1 (2.8–13.9)		
	Median OS, mo (95% CI)	14.4	11.2		
	Objective Response Rate no. of patients (%)	57 (21)	38 (14)		
	Median Duration of Response, mo (95% CI)	8.1 (6.7–9.1)	5.6 (3.8–7.9)		

An exploratory analysis was subsequently performed that assessed the potential clinical utility of Trop-2 expression. Patients with high, medium, and low Trop-2 expression that received sacituzumab govitecan demonstrated greater overall response rates (44%, 38%, 22%, respectively) compared to physician's choice chemotherapy (1%, 11%, 6%). The majority of tumors had high expression of Trop-2 (n = 85; 56%), with a small proportion of tumors with low Trop-2 expression (n = 27; 18%) [50]. Regardless of Trop-2 expression, however, all patients with metastatic TNBC benefited from sacituzumab govitecan in comparison to physician's choice chemotherapy. Trop-2 expression is not currently recommended to be checked as a biomarker to predict a benefit to sacituzumab govitecan.

4.1.2. Sacituzumab Govitecan in Hormone Receptor Positive Breast Cancer

Given Trop-2 expression is also observed in hormone receptor positive subtypes, sacituzumab govitecan was evaluated for the treatment of HR+ (hormone receptor positive), HER2- metastatic breast cancers. As patients with HR+/HER2- develop tumor progression on endocrine therapy and targeted agents, this subtype remains challenging to treat, as options are limited to sequential single agent chemotherapy, which has lower response rates and greater toxicity. The HR+/HER2- cohort in IMMU-132-01 showed promising results in 54 patients who had received at least 1 line of hormone-based therapy and at least 1 prior chemotherapy in the metastatic setting. Overall response rates were 31.5% (95% CI 19.5–45.6%) and median PFS was 5.5 months (95% CI 3.6–7.6) [51].

In the randomized phase III TROPiCS-02 study, the use of sacituzumab govitecan was further evaluated in an endocrine resistant HR+/HER2- population whose tumors had progressed on at least 2–4 lines in the metastatic setting, with a median of 3 prior chemotherapies. Sacituzumab govitecan demonstrated an improvement in ORR (57% vs. 38%) and PFS (5.5 vs. 4.0 months; HR 0.66; p = 0.0003) compared to physician's choice of chemotherapy (eribulin, vinorelbine, capecitabine, or gemcitabine) (**Table 1**) [52]. Overall survival data was presented at the European Society of Medical Oncology (ESMO) 2022 meeting with a median follow-up of 12.5 months, with 390 OS events. Sacituzumab govitecan improved mOS compared to physician's choice chemotherapy (14.4 vs. 11.2 months; HR 0.79, p = 0.020) [53].

4.2. Daptopotamab Deruxtecan

The safety and efficacy of a new Trop-2 ADC, daptopotamab deruxtecan (dato-Dxd), is being investigated in the phase I study TROPION-PanTUMOR01 among patients with advanced solid tumors, including relapsed/refractory TNBC following standard of care treatment [54]. Dato-Dxd is a topoisomerase I inhibitor (exatecan derivative) attached to a humanized immunoglobulin G (IgG1) monoclonal antibody via a cleavable peptide linker and cysteine conjugation using thioether bonds [55][56]. Patients with TNBC enrolled on TROPION-PanTUMOR01, had received a median of 3 prior therapies (range 1–10) with 91% of patients having received a prior taxane and 30% a prior topoisomerase I inhibitor-based ADC. The interim data from the cohort of 44 patients with advanced or metastatic TNBC demonstrated an overall response rate by blinded independent central review (BICR) of 34% at a median follow up of 7.6 months and a disease control rate of 77%. Patients who had not received prior treatment with a topoisomerase I inhibitor-based ADC had better overall response rates (52%) and disease control rates (81%). The most common adverse events were nausea (58%), stomatitis (53%), alopecia (35%), vomiting (35%), and fatigue (33%) [54]. There is an ongoing dose expansion cohort in patients with HR+/HER2- breast cancer and the data will be presented at the San Antonio Breast Cancer Symposium in 2023 (NCT03401385).

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