

TROP2 as Patient-Tailoring but Not Prognostic Biomarker for Breast Cancer

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Purpose: Trophoblast cell surface antigen 2 (TROP2) has emerged as a promising target of antibody-drug conjugates (ADCs) for triple-negative breast cancer (TNBC), as well as other breast cancers (BCs). This study aims to investigate the biomarker value of TROP2 for patient-tailoring and prognostic for BC patients, including TNBC.

Methods: The levels of TROP2 expression in 404 Chinese BC tissues on tissue microarrays (TMAs) were quantified by immunohistochemistry and their correlations to the clinicopathological factors and the overall survival rate were analyzed. Also, BC cell lines and patient-derived organoids (PDOs) with different TROP2 expression levels were employed to investigate the correlation between TROP2 expression levels and the therapeutic responses to DS001, a TROP2-directed ADC molecule with stable linker and potent payload.

Results: TROP2 overexpression was identified in significantly more ($P = 0.046$) tumor tissues (41.08%, 99/241) than normal adjacent tissues (31.29%, 51/163) from Chinese BC patients, and in significantly more ($P = 0.024$) TNBC patients (59.38%, 19/32) than in other BC types (38.28%, 80/209). BC cell line with the lowest TROP2 expression level failed to respond to DS001 treatment. The levels of TROP2 expression were determined to be significantly correlated with the potencies of DS001 treatment, but not with the overall survival rates of the patients.

Conclusion: Our results demonstrated that TROP2 could serve as a patient-tailoring and predictive biomarker for ADC therapeutics but not as a general prognostic biomarker to predicate patient survival.

Keywords: TROP2, breast cancer, triple-negative breast cancer, antibody–drug conjugate, biomarker

Introduction

With more than 2.3 million estimated new cases in 2020, breast cancer (BC) is the most commonly diagnosed cancer (~24.5%) and the leading cause of cancer death (~15.5%) in women.¹ Clinically, BC can be divided into different subtypes by their histopathological appearance and expression status of the estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER2).² Triple negative breast cancer (TNBC) is characterized by the absence of any biomarkers mentioned above. Although TNBC represents only ~15% of all BC cases, it is highly aggressive and metastatic with poor prognosis,³ and accounts for ~25% of all BC-related deaths.⁴ Surgery, chemotherapy and radiotherapy had been the only treatment options for TNBC before the approval of programmed death-ligand 1 (PD-L1) antagonist atezolizumab in combination with nab-paclitaxel for PD-L1⁺ TNBC (approximately 40%).⁵ However, the development of active, specifically targeted therapies for TNBC had been impeded by the lack of biomarkers.

Recently, human trophoblast cell surface antigen 2 (TROP2), also termed epithelial glycoprotein 1 (EGP1), a 36 kDa transmembrane protein consisting of 323 amino acids and four N-linked glycosylation sites, has emerged as an important therapeutic target of antibody–drug conjugate (ADC) treatment for various solid tumors including TNBC and HER2⁺ BC.^{6,7} TROP2 was first described in 1981 as a protein highly expressed on the surface of trophoblast cells⁸ but later on revealed to play complicated roles in cancer cell growth, proliferation, migration, invasion and survival.⁹ Its over-expression was demonstrated in various tumor types and correlated with unfavorable prognosis and increased risk of metastasis.¹⁰ Unlike HER2, ER or PR, although TROP2 overexpression in BC was revealed previously,¹¹ whether it correlates with patient responsiveness to ADC treatments or with unfavorable prognosis has not been well established.

Sacituzumab govitecan-hziy (SG), the first TROP2-directed ADC that was approved by the FDA for treating unresectable locally advanced or metastatic TNBC,¹² does not require companion diagnostics (CDx) to measure the expression of TROP2 before treatment. In the Phase III confirmatory clinical study ASCENT (NCT02574455), all TNBC patient subgroups with variable TROP2-expression levels could benefit from SG, but in different degrees (PFS: TROP2-high 6.9 vs TROP2-medium 5.6 vs TROP2-low 2.7 months, OS: TROP2-high 14.2 vs TROP2-medium 14.9 vs TROP2-low 9.3 months, ORR: TROP2-high 44% vs TROP2-medium 38% vs TROP2-low 22%).⁶ Partnering with Immunomedics, Everest Medicine is seeking Chinese market entry for SG. Although target-based tailoring might not be necessary for SG, which adopted an unconventional molecule design with unstable linker CL2A and weak payload SN-38, it could benefit ADC molecules with more stable linkers and more potent payloads.

DS001 is a TROP2-directed ADC molecule at preclinical stage equipped with potent cytotoxin payload monomethyl auristatin E (MMAE) and Cathepsin B-cleavable linker maleimide (Mal)-Valine (V)-Lysine (K) (mPEG₂₄)-PAB (unpublished). In animal models, it showed more than 200× therapeutic window. Since MMAE has excellent enhanced permeation and retention (EPR) and “bystander” effects, DS001 could potentially become strong candidate for solid tumors that require effective tissue penetration and “bystander” effects, such as pancreatic cancer. In this study, we used DS001 as a valuable tool to explore the value of TROP2 as a patient-tailoring biomarker for TROP2-directed ADCs with stable linkers and potent payloads.

We first measured the expression levels of TROP2 in Chinese BC, including TNBC, patients and analyzed their correlations with the clinicopathological factors and the overall survival rates. The impact of TROP2 expression on the therapeutical effects of DS001 was also investigated in tumor cell lines and patient-derived organoids (PDOs). Our results suggest that TROP2 expression does not correlate with unfavorable prognosis of BC, but can serve as a valuable patient-tailoring biomarker for TROP2-directed therapeutics.

Materials and Methods

Patients and Tissue Microarray (TMA) Specimens

Formalin-fixed paraffin-embedded tissue blocks including BC tumors and normal tissues adjacent to tumors (with a spatial distance of 2 cm away from the tumors) were collected from Shanghai General Hospital, China. Tissues from 2004 to 2014 were obtained from patients with primary BC who received no chemotherapy or radiotherapy. For tissue microarray construction, all specimens were re-evaluated and checked by HE staining and the representative areas were selected and constructed into 2.0 mm tissue cores. In this study, a total of 404 cases, including 241 BC tissues and 163 normal tissues adjacent to tumors were analyzed. Clinical information, including age, tumor size, expression of ER, PR, HER2 and Ki-67, lymph node status, morphologic subtypes and follow-up information for overall survival (OS) rates were retrieved from the patients' electronic medical record. Clinicopathological classification and staging were assigned according to the 8th edition of American Joint Committee on Cancer (AJCC) staging system. All of the research was reviewed and approved by the Ethics Committee of Shanghai General Hospital (#2018KY153).

Cell Culture

MDA-MB-468, SKBR3, HCC1806, MDA-MB-231, MDA-MB-453, MCF7, MCF-10A and SUM159 cells were purchased from Cell Bank/Stem Cell Bank of the Chinese Academy of Sciences (Shanghai, China) and cultured as recommended by the suppliers.

PDOs Culture and Section Preparation

Organoids were cultured as previously described.¹³ Fresh BC tissues cut into 1–3 mm³ pieces were digested in BC tissue digest medium (Table S1) and incubated on orbital shaker at 37°C for 40 min. The digested tissue suspension was shaken vigorously to disperse and then filtered through 70 µm cell strainers (Corning, Upstate, NY). FBS (2% final concentration) was added to the filtered suspension to stop digesting followed by centrifugation at 400 g. After washing twice with organoids washing medium (Table S2), the pellet was resuspended by Cultrex growth factor reduced BME type 2 (Trevigen, Gaithersburg, MD). BME-cell suspension drops (40 µL) were allowed to solidify on 24-well suspension culture plates (Greiner, Monroe, NC) at 37°C. BC organoid medium (600 µL, Table S3) was added to each well and incubated at 37°C with 5% CO₂. Medium was changed every 3 days and the organoids were passaged every 1–4 weeks. To prepare sections for immunohistochemistry (IHC), PDOs seeded in plates were scraped off and centrifuged at 400 g. After washing twice with PBS, PDO pellets were fixed in formalin at room temperature and then subjected to routine processing of paraffin embedding.

Immunohistochemistry (IHC)

For IHC analysis, 3 µm-thick TMAs and PDOs sections were dewaxed in xylene and rehydrated by graded ethanol solutions. Antigens were retrieved by high-pressure heat method with citrate solution (pH = 6). The sections were immersed in 3% hydrogen peroxide (Sigma, St Louis, MO) solution for 15 min and blocked with normal goat serum (Gibco, Grand Island, NY) for another 15 min at room temperature. Samples were then incubated with mouse anti-TROP2 monoclonal antibody (Clone 01, 1:200, Enzo Life Sciences, Raamsdonksveer, The Netherlands) at 4°C overnight, followed by detection with mouse/rabbit streptavidin-biotin detection kit (ZS BIO, Beijing, China). The sections were then stained by 3,3-diaminobenzidine (DAB), counterstained with hematoxylin, dehydrated through graded ethanol solutions, cleared in xylene, and mounted by neutral resins. Tris-buffered saline with Tween-20 (Sigma, St Louis, MO) was used for rinsing sections between steps.

Interpretation and Evaluation of IHC Results

TMA slides were digitally scanned using Vectra Polaris Pathology Imaging System and images were visualized by Inform software (PerkinElmer, Waltham, MA). PDO slides were captured with an Olympus optical microscope. For analysis, both the staining intensity and percentage of positive tumor cells were graded and multiplied to obtain an overall staining score as described previously.¹⁴ Staining intensity was scored on a scale of 0–3 as follows: 0 (negative), 1 (weak), 2 (medium) and 3 (strong). Percentage of positive tumor cells was categorized into five semi-quantitative classes: 0 (≤5% positive cells), 1 (6–25% positive cells), 2 (26–50% positive cells), 3 (51–75% positive cells) and 4 (>76% positive cells). Overall staining scores greater than 4 were defined as high expression of TROP2. All scoring and grading were performed independently by two experienced pathologists who were not informed of the patients' clinical history and any discrepancies were resolved by jointed discussion.

Kaplan–Meier Plotter Database Analysis

The Kaplan Meier plotter (<http://kmpplot.com/analysis/>) is a free online meta-analysis tool capable of assessing the effect of 54k genes (mRNA, miRNA, protein) on patient survival rates in 21 cancer types, including BC (n = 7830). It was used in our study to validate the prognostic value of TROP2 expression on patient overall survival using protein database *Tang_2018*¹⁵ containing 65 BC patients, and database *Liu_2014*¹⁶ containing 116 BC patients. All patients were separated into TROP2-high and TROP2-low groups based on their median values. Kaplan–Meier survival plots were generated and significance by log-rank *P* value was computed by SPSS.

Flow Cytometry

Cell surface expression of TROP2 was determined by immunofluorescence staining and flow cytometric analysis (CytoFLEX, Beckman Coulter, CA). Cells were incubated with hRS7 for 30 min on ice. After washing thoroughly with staining media, the cells were stained with PE-labeled goat anti-human IgG antibody (Biolegend, San Diego, CA)

for 30 min on ice. Cells were washed and resuspended in the staining media, then tested by flow cytometry. Isotype monoclonal antibodies were used as negative control. The relative TROP2 protein expression was defined as median fluorescence intensity (MFI) after subtracting negative control.

Preparation of DS001, a TROP2-Directed ADC Molecule

Humanized monoclonal TROP2 antibody hRS7 was prepared according to the US patent 9,931,417 B2. Drug-linker Mal-Valine-Lysine (mPEG₂₄)-PAB-MMAE was synthesized by Sorrento Therapeutics (San Diego, CA). To conjugate, hRS7 was reduced using 6 molar equivalents of TCEP (Pierce, Rockford, IL) in PBS, pH 7.4 at 37 °C for 2 hrs, followed by adding 12 molar equivalents of drug-linker to incubate at 4 °C for 16 hrs. Extra drug-linkers were removed by ultrafiltration using Amicon Ultracontainer (50,000 MWCO, Millipore Corporation, Billerica, MA). The concentration of DS001 was measured by Nanodrop 2000 UV detector (Thermo Fisher Scientific, Frederick, MA) at 280 nm. The final products were analyzed by reverse phase (RP)-HPLC (Agilent Technologies Inc, Santa Clara, CA) and Q-TOF LC-MS/MS equipment (Waters, Milford, MA).

Cell Viability Assay

Cell viability was assayed as previously described.¹⁷ In brief, 3000 to 5000 cells/well were seeded into 96-well plates and incubated overnight. The next day, cells were treated with different concentrations of DS001 or MMAE alone (3-fold serial dilutions starting at 300 nM) for 96 hrs. CCK8 agent (Dojindo, Kumamoto, Japan) was added according to the manufacturer's instructions and incubated for 1–2 h. Growth inhibition was measured as percentage of growth relative to untreated cells. Dose-response curves were generated from the means of triplicate determinations, IC₅₀ (half maximal inhibitory concentration) values and I_{max} (%) values (maximal inhibition rate) were calculated by nonlinear regression (four-parameter) using Prism GraphPad Software. I_{max} (%) values were calculated using the formula: [1-minimum viability (%)] × 100.

PDOs Viability Assay

PDOs viability was measured as previously described.¹⁸ Organoids were harvested and resuspended in growth media containing 5% BME (Trevigen, Gaithersburg, MD). 384-well plates (Corning, Upstate, NY) were coated with 10 µL of BME before 30 µL of organoid suspensions were added into each well. After incubation overnight, serially diluted drugs were added. After 6 days of incubation, 40µL of Cell Titer-Glo 3D Reagent (Promega, Madison, WI) per well were added to measure cell viability. The plates were shaken for 30 min at room temperature and read on SpectraMax microplate reader (Molecular Devices, San Francisco, CA). Dose-response curves were generated from the means of triplicated or duplicated determinations. IC₅₀ and I_{max} values were calculated by Prism GraphPad Software.

Statistical Analysis

Chi-squared test was employed to analyze the correlations between TROP2 expression and patient clinical parameters. Kaplan-Meier analysis was performed to estimate the survival curve. Statistical significance was determined using the Log rank test. Correlation values reported were Pearson correlation coefficients. The aforementioned analysis was carried out with the statistical software SPSS 20.0 (SPSS, Chicago, IL). The viability curves were performed using GraphPad Software. Statistical significance was defined as two-tailed $P < 0.05$.

Results

TROP2 Was Overexpressed in BC and TNBC

To measure the expression level of TROP2 in BC, we performed IHC on tissue microarrays containing 241 BC and 163 normal adjacent tissues. As shown in Figure 1, TROP2 was expressed both on the cell membrane and in the cytoplasm. Samples could be divided into TROP2-high and TROP2-low groups according to the scoring criteria described in Materials and Methods. Statistical analysis (Table 1) indicated that significantly more ($P = 0.046$) BC tissues were scored as TROP2-high (41.08%, 99/241) than matched normal adjacent tissues (31.29%, 51/163).

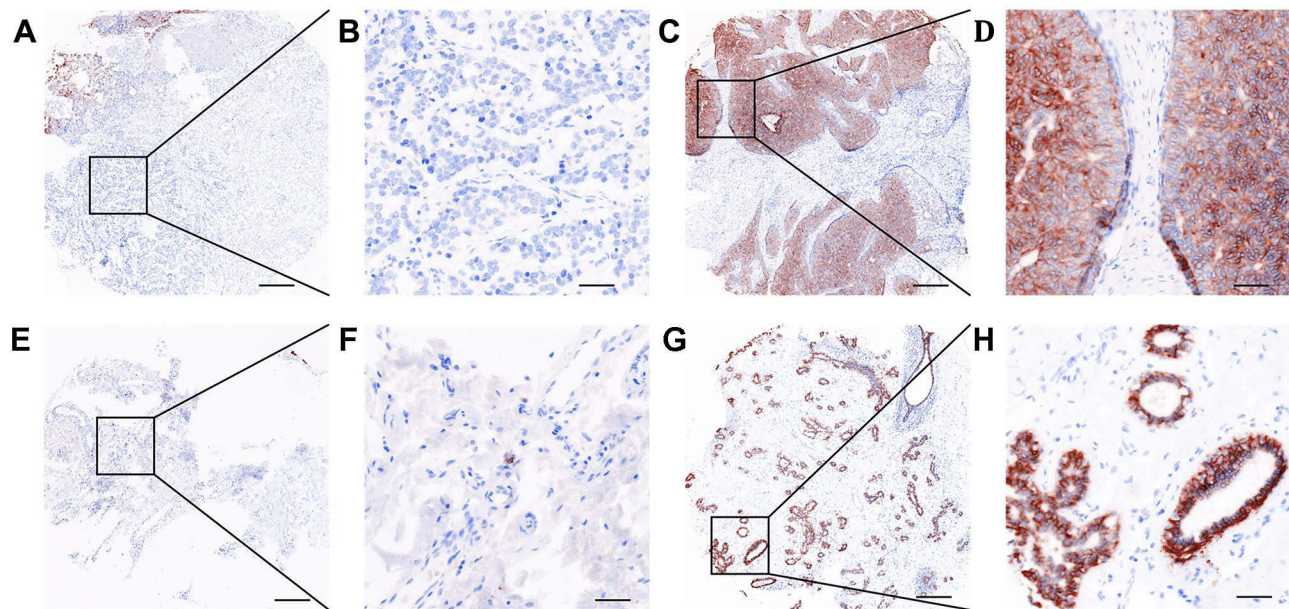


Figure 1 IHC staining of TROP2 in BC tissues on tissue microarray sections. Positive staining was observed as dark brown color. (A and E) Low expression of TROP2 in BC tissues and matched adjacent tissues, respectively. (C and G) High expression of TROP2 in BC tissues and matched adjacent tissues, respectively. (A), (C), (E) and (G) were scanned by Vectra Polaris Pathology Imaging System at $\times 10$ magnification (bar, 500 μm), (B), (D), (F) and (H) were shown at original $\times 50$ magnification (bar, 100 μm). **Abbreviations:** IHC, Immunohistochemistry; BC, breast cancer.

Notably, TNBC tissues (59.38%, 19/32) had significantly more ($P = 0.024$) TROP2-high scores than other types of BC (38.28%, 80/209). Therefore, TROP2 is overexpressed in BC, especially in TNBC.

Correlation Between TROP2 Expression Level and Patient Prognosis

The correlation between TROP2 expression and patient prognosis in 241 BC patients was explored. The expression level of TROP2 showed statistically significant inverse correlation with the expression level of ER ($P = 0.022$) in ER⁺ patients, but not with those of PR, HER2 or Ki-67, nor with age, tumor size, lymph node status, morphologic subtypes or TNM stage (Table 2). Furthermore, statistically significant correlation was not reached ($P = 0.119$) between TROP2 expression level and the patient overall survival rate as indicated by Kaplan-Meier analysis with Log rank test based on clinical follow-up information (Figure 2A). We also assessed the effect of TROP2 expression on BC patient survival using the Kaplan Meier plotter meta-analysis tool.¹⁹ All protein databases containing TROP2 and survival data were selected, which were Tang_2018¹⁵ and Liu_2014.¹⁶ One hundred and eighty-one selected BC patients were separated into TROP2-high and TROP2-low groups based on their median values. As shown in Figure 2B, no significant correlation ($P = 0.376$) was observed between TROP2

Table 1 The Expression Level of TROP2 in Breast Tissues

Characteristic	Cases	TROP2 Expression (%)		Pearson χ^2	P-value
		Low	High		
Total cases	404				
BC tissue	241	142 (58.92)	99 (41.08)	3.993	0.046
Matched adjacent tissue	163	112 (68.71)	51 (31.29)		
Total cases	241				
TNBC tissue	32	13 (40.62)	19 (59.38)	5.103	0.024
Other BC tissue	209	129 (61.72)	80 (38.28)		

Abbreviations: BC, breast cancer; TNBC, triple negative breast cancer.

Table 2 Correlation Between the TROP2 Expression Levels and Clinicopathologic Features in BC Patients

Clinicopathologic Parameters	Cases	TROP2 Expression		Pearson χ^2	P-value
		Low	High		
Total cases	241				
Age				2.086	0.149
≤50	88	53	35		
>50	153	89	64		
ER				5.224	0.022
Negative	64	30	34		
Positive	177	112	65		
PR				3.336	0.068
Negative	79	40	39		
Positive	162	102	60		
HER2				0.814	0.367
Negative	75	41	34		
Positive	166	101	65		
Tumor size (cm)				1.146	0.564
≤2	82	52	30		
>2 and ≤5	139	78	61		
>5	20	12	8		
Ki-67				0.023	0.879
Low	113	66	47		
High	128	76	52		
Lymph node status				1.399	0.237
N0	155	87	68		
N1+N2+N3	86	55	31		
TNM stage				5.755	0.056
I	55	35	20		
II	140	74	66		
III	46	33	13		
Morphologic subtypes				1.267	0.737
Ductal carcinoma in situ	20	10	10		
Invasive ductal carcinoma	198	119	79		
Invasive lobular carcinoma	11	7	4		
Mucinous adenocarcinoma	12	6	6		

Abbreviations: BC, breast cancer; ER, estrogen receptor; PR, progesterone receptor; HER2, human epidermal growth factor receptor 2.

expression and the overall survival rates of patients from these two databases. Taken together, our results suggested that TROP2 expression might play limited role in BC disease progression and is not suitable as an independent prognostic biomarker of BC.

TROP2 Expression Level Predicated the Responsiveness of DS001 in BC Cell Lines and PDOs

A panel of eight BC cell lines was divided into TROP2-high (MDA-MB-468, MCF-10A, SK-BR-3, MCF7 and HCC1806) and TROP2-low (MDA-MB-231, MDA-MB-453 and SUM159) groups according to their expression levels of TROP2 determined by flow cytometry (Table 3, Figure S1). After incubation with serial dilutions of DS001 or MMAE, cell viabilities were assayed. All cell lines were sensitive to MMAE (Figure 3A), but differently responded to DS001 (Figure 3B, Table 3), TROP2-high cell lines were more responsive to DS001 than TROP2-low cell lines. As shown in Figure 3C and D, statistically significant inverse correlation was demonstrated between the TROP2 expression levels and the IC₅₀ values of DS001 ($r = -0.777$, $P = 0.023$), but not with the IC₅₀ values of MMAE ($r = -0.074$, $P = 0.816$). The correlation between TROP2 expression level and I_{max} (%) values of MMAE or DS001 were not statistically

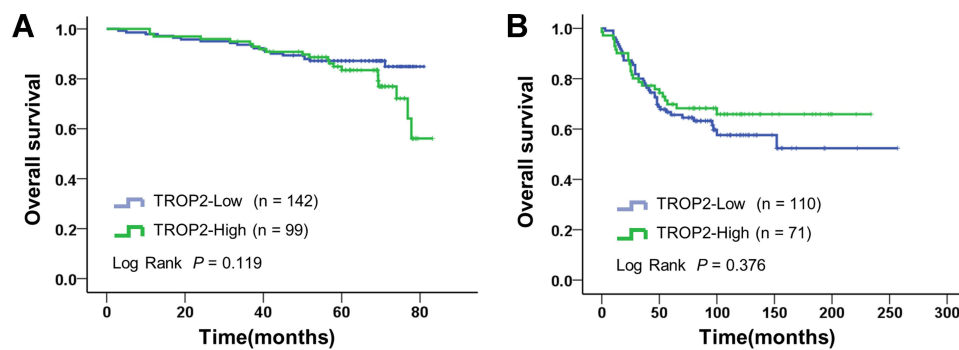


Figure 2 Survival analysis based on TROP2 expression in BC tissues by Kaplan–Meier method and Log rank test. **(A)** Survival analysis in 241 BC patients collected from Shanghai General Hospital, China. **(B)** Survival analysis in 181 BC patients from *Tang_2018* and *Liu_2014* online protein databases (Kaplan–Meier Plotter).

Abbreviation: BC, breast cancer.

significant (Figure 3E and F, $P = 0.545$ or $P = 0.193$, respectively), but DS001 had no inhibition on SUM159 cells, which express TROP2 at the lowest level (Figure 3B and F). It seemed that when the expression level of TROP2 fell below a cut-off value, the cells stopped responding to DS001 treatment, but once it reached the cut-off value, the impact of TROP2-expression level on DS001 efficacy could be overcome by increasing the concentration of DS001.

PDOs are considered as predictive preclinical models for treatment response in cancer patients due to their ability to recapitulate the histological and genetic characteristics of primary tumors.^{20–22} In this study, six PDOs were successfully established and treated with serial dilutions of DS001. Concurrently these PDOs were made into formalin fixed paraffin embedded sections for IHC to detect the expression of TROP2. Seen in Figure 4A and B, three PDOs (0713T2, 0407T3 and 0407T2) were divided into TROP2-high group and three PDOs (0331T2, 0414T1 and 0713T1) were divided into TROP2-low group according to IHC staining. As expected, TROP2-high PDOs showed stronger response to DS001 with greater I_{max} (%) and smaller IC_{50} values than TROP2-low PDOs (Figure 4C–E). These results confirmed the correlation between the TROP2 expression level and PDO responsiveness to ADC treatment.

Taken together, TROP2 expression levels were correlated the responsiveness of DS001, a TROP2-directed ADC molecule with stable linker and potent payload.

Discussion

Target-based patient tailoring is normally a prerequisite for target-directed therapies to maximize the therapeutic window and avoid unnecessary medication. Previous research has explored the possibility of using TROP2 as a tailoring biomarker for SG but the results were conflicting.²³ First, mice bearing xenografts from wild-type MDA-MB-231 cells, a tumor cell line with

Table 3 Summary of the TROP2 Relative Expression Levels, IC_{50} and I_{max} Values of Diverse BC Cell Lines

Cell	Subtype	TROP2 Relative Expression	IC_{50} (nM)		I_{max} (%)	
			MMAE	DS001	MMAE	DS001
MDA-MB-468	TNBC	57098	0.1102	0.3019	67.35	66.65
SK-BR-3	HER2+	48,206	0.0489	0.0245	75.64	74.83
MCF-10A	Fibroadenoma	41,671	0.3391	0.47	82.22	64.33
MCF7	Luminal A	30490	1.592	0.4261	61.63	27.25
HCC1806	TNBC	19182	0.0958	0.2512	74.46	71.31
MDA-MB-231	TNBC	5718	0.3787	145	61.99	59.68
MDA-MB-453	TNBC	1971	0.0715	117.5	65.75	55.64
SUM159	TNBC	7	0.5506	>300	75.42	–26.1

Abbreviations: BC, breast cancer; MMAE, monomethyl auristatin E; IC_{50} , half maximal inhibitory concentration; I_{max} , maximal inhibition rate.

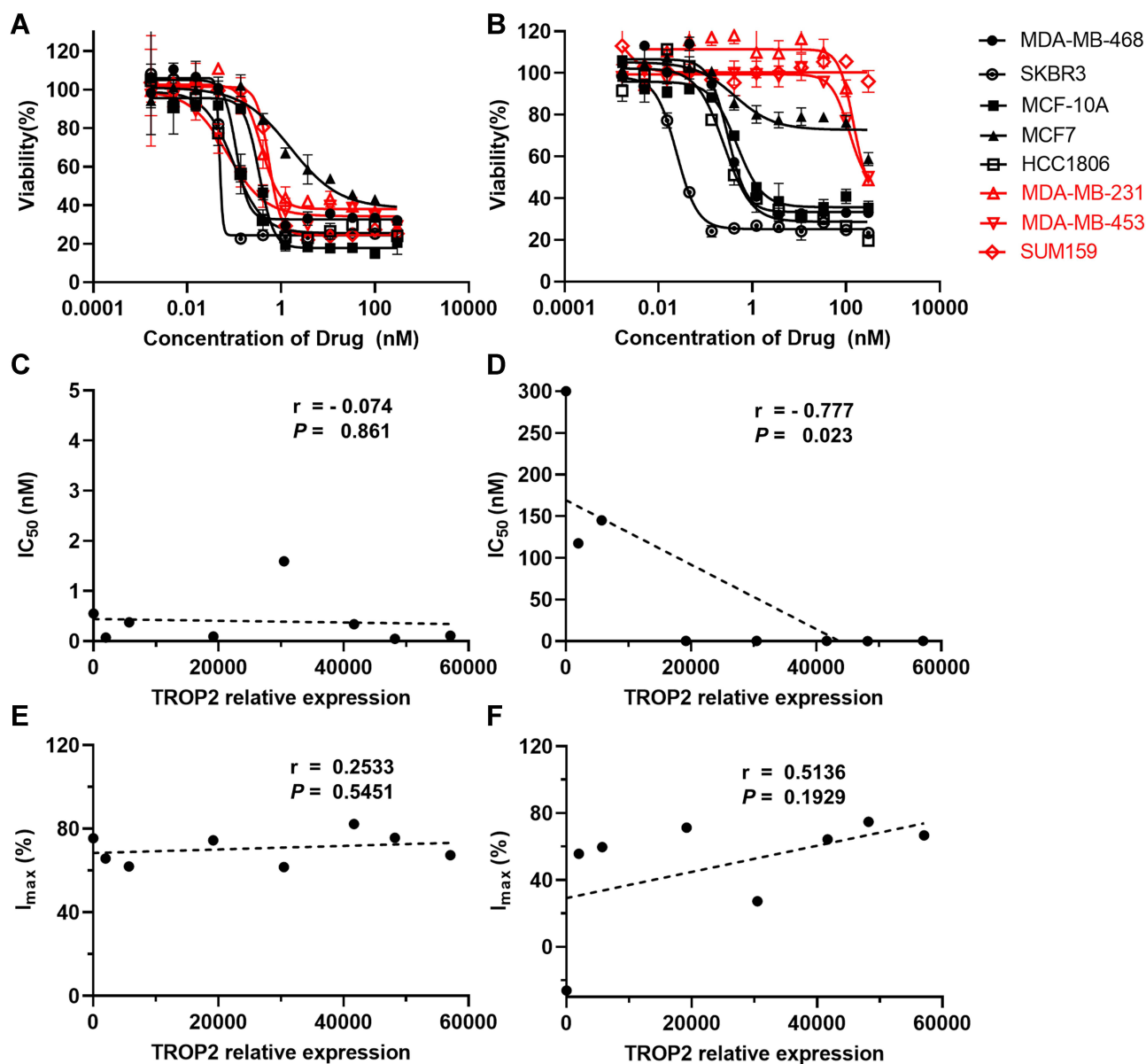


Figure 3 The expression levels of TROP2 were significantly correlated with the anti-tumor effects of DS001 in diverse BC cell lines. Dose-response curves generated after 96 h treatment with (A) MMAE and (B) DS001. Data were represented as mean \pm SD ($n = 3$). Correlation between the TROP2 expression levels and IC₅₀ values for (C) MMAE and (D) DS001. Correlation between the TROP2 expression levels and the I_{max} (%) values for (E) MMAE and (F) DS001. Pearson correlation coefficients were shown.

Abbreviations: BC, breast cancer; MMAE, monomethyl auristatin E; IC₅₀, half maximal inhibitory concentration; I_{max}, maximal inhibition rate.

low level of TROP2 expression, were unresponsive to SG, whereas mice bearing xenografts from the same cells ectopically overexpressing TROP2 showed strong response to SG.²⁴ It suggested that higher TROP2 expression was associated with better therapeutical efficacy. However, other preclinical studies showed that SG could mediate anti-tumor responses in tumor types with different levels of TROP2 expression.^{25–27} Clinically, all TNBC patient subgroups with variable TROP2-expression levels could benefit from SG treatment, but in different degrees.⁶ Thus, it might not be necessary to have TROP2-based patient tailoring as a prerequisite for SG treatment, but it is still useful to predict patient responsiveness.

The conclusion from the SG studies might not be applied directly to all TROP2-directed ADCs. SG adopted a weak payload SN-38, which is the active metabolite of the approved drug irinotecan for several cancers,^{28,29} and an unstable linker CL2A, which is pH-sensitive hydrolysable and may release payloads both in and out of tumor microenvironments.^{25,26} In fact, the half-life of SG is only about 14 hours in human³⁰ and, in theory, cannot sustain

once a week administration regime for SG. Thus, the slow release of SN-38 from the ADC molecules and hRS7-triggered antibody-dependent cellular cytotoxicity (ADCC) might contribute significantly to the therapeutic effect of SG. Therefore, off-target and on-target toxicities may not be a serious concern for SG due to its unique molecule design.

The success of SG has ignited fierce competition in TROP2-directed ADCs in China. There are more than five TROP2-directed ADC molecules in clinical trials (ESG-401 (CTR20212074), SKB-264 (NCT04152499), FDA018 (CXSL2101031), BAT-8003 (NCT03884517), DAC-002 (CTR20201860)) and many others in preclinical studies. Most of these ADCs adopted stable linkers and some of them used potent cytotoxic payloads. Previously, two TROP2-directed ADC molecules that adopted both stable linker and potent payloads, namely PF-06664178 from Pfizer³¹ and BAT8003 from Bio-Thera (Guangzhou, China), failed to achieve sufficient therapeutic window in Phase I clinical trials (NCT02122146). PF-06664178 has AcLys-VC-PABC linker and cytotoxic payload Aur0101,³² which is even more potent than MMAE. At doses higher than 2.40 mg/kg, PF-06664178 was intolerable due to toxicities in skin rash, mucosa and neutropenia. Skin rash and mucosa are considered on-target toxicities, which could be limited by selecting patients

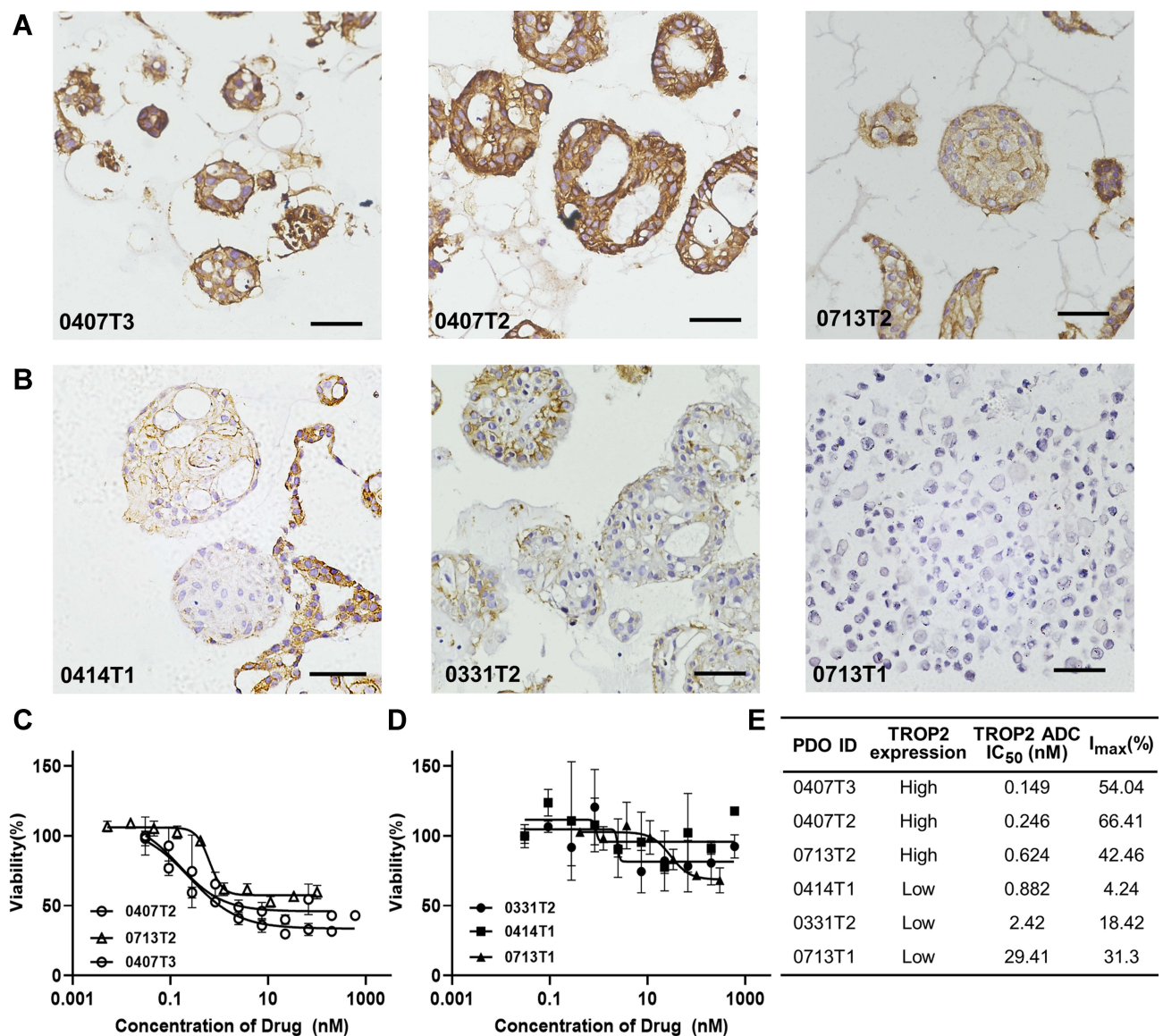


Figure 4 The expression levels of TROP2 were correlated with the anti-tumor effects of DS001 in PDOs. **(A and B)** IHC images of BC PDOs with high and low expression levels of TROP2, respectively. Images were captured by an Olympus optical microscope at 400× magnification (bar, 50 μm). **(C and D)** Dose-response curves of DS001 in PDOs with high and low TROP2 expression levels, respectively. **(E)** Calculated IC₅₀ and I_{max} values by nonlinear regression (four-parameter).

Abbreviations: BC, breast cancer; PDO, patient-derived organoid; IHC, Immunohistochemistry; IC₅₀, half maximal inhibitory concentration; I_{max}, maximal inhibition rate.

with highest TROP2 expression levels in tumor tissues. Therefore, target-based tailoring might be crucial for the clinical success of TROP2-directed ADC molecules with both stable linkers and potent payloads.

In our study, we first confirmed that TROP2 was significantly overexpressed in BC especially in TNBC tissues when compared to normal adjacent tissues. The level of TROP2 expression was correlated inversely with the ER expression level in ER⁺ BC patients, but whether TROP2 expression is negatively regulated by ER or vice versa is not clear. Clinically SG showed a promising therapeutic activity in patients with ER⁺ subtype of breast cancer,³³ which indicated although TROP2 was inversely correlated with ER status, some ER⁺ breast cancers may overexpress TROP2 and can be responsive to TROP2-based ADCs. Previous research had detected TROP2 overexpression in breast neuroendocrine carcinomas (NECs),³⁴ which suggested a proportion of breast NECs may be amenable to treatment with TROP2-based ADCs. It was reported that TROP2⁺ and E-cadherin⁻ BC patients had poor overall survival rates.³⁵ Our data showed that the expression level of TROP2 alone could not predicate the overall survival rate ($P > 0.05$), consistent with the Kaplan–Meier plotter analysis of protein databases obtained from BC patients. Therefore, TROP2 might not be an independent prognostic marker for survival of BC patients.

We then assessed the patient-tailoring and outcome-predictive value of TROP2 in response to DS001 by using various BC cell lines and PDO models. The expression level of TROP2 was correlated with DS001 potency (IC₅₀), in both BC cell lines and PDOs. No statistically significant correlation was identified between TROP2 expression level and DS001 efficacy (I_{max}) in cell lines, but DS001 was not effective in a cell line expressing TROP2 at very low level. It seemed that when TROP2 expression level was below the cut-off value, the tumor cells did not respond to DS001. Therefore, patients do not express TROP2 at certain level in their tumor tissues should not be treated with DS001 or other TROP2-directed ADCs. However, once TROP2 expression level reached the cut-off value, its impact on efficacy could be overcome by increasing the concentration of DS001 in vitro. In PDOs, the expression level of TROP2 affected the efficacy of DS001, but statistical analysis was not performed due to limited samples. In clinic, the doses of ADCs are usually limited by the dose-limiting toxicities (DLTs). Thus, it is the potencies, but not the efficacies obtained from the highest concentration of ADCs in vitro, that are relevant in human. The correlation between TROP2 expression and the therapeutic effect of DS001 might be due to the important role played by receptor-mediated endocytosis in the mode of action of DS001. The more TROP2 molecules expressed on the tumor cells, the faster DS001 could be endocytosed into cells and cleaved by cathepsin B, which would be translated into therapeutical potency of DS001.

Due to the limitations of semiquantitative method of IHC and the small number of PDO samples, our conclusion should be carefully evaluated in further investigations with more clinical samples.

Conclusion

In conclusion, our results suggested that TROP2 could serve as a patient-tailoring and outcome predictive biomarker for TROP2-directed ADC molecules, but not as a general prognostic biomarker to predicate patient survival. Although the cut-off value of TROP2 expression level for tumor cells to respond to ADC treatment was not determined, our result suggested that BC patients do not express TROP2 in their tumor tissue at certain level should not be treated by TROP2-directed ADCs.

Abbreviations

TROP2, trophoblast cell surface antigen 2; ADC, antibody drug conjugate; TNBC, triple-negative breast cancer; BC, breast cancer; TMA, tissue microarray; PDO, patient-derived organoid; ER, estrogen receptor; PR, progesterone receptor; HER2, human epidermal growth factor receptor 2; PD-L1, programmed death-ligand 1; EGP1, epithelial glycoprotein 1; SG, sacituzumab govitecan-hziy; CDx, companion diagnostics; MMAE, monomethyl auristatin E; EPR, enhanced permeation and retention; IHC, immunohistochemistry; MFI, median fluorescence intensity; IC₅₀, half maximal inhibitory concentration; I_{max}, maximal inhibition rate.

Data Sharing Statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics Approval and Informed Consent

The studies involving human participants were reviewed and approved by the Ethics Committee of Shanghai General Hospital affiliated to the Shanghai Jiao Tong University (#2018KY153). The procedures used in this study adhere to the tenets of the Declaration of Helsinki. Informed consent was obtained from all individual participants included in the study.

Consent for Publication

All authors agree to the publication of all texts, images and tables in this study.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

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References

1. Sung H, Ferlay J, Siegel RL, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin.* 2021;71(3):209–249. doi:10.3322/caac.21660
2. Britt KL, Cuzick J, Phillips KA. Key steps for effective breast cancer prevention. *Nat Rev Cancer.* 2020;20(8):417–436. doi:10.1038/s41568-020-0266-x
3. Foulkes WD, Smith IE, Reis-Filho JS. Triple-negative breast cancer. *N Engl J Med.* 2010;363(20):1938–1948. doi:10.1056/NEJMra1001389
4. Schafer JM, Lehmann BD, Gonzalez-Ericsson PI, et al. Targeting MYCN-expressing triple-negative breast cancer with BET and MEK inhibitors. *Sci Transl Med.* 2020;12(534). doi:10.1126/scitranslmed.aaw8275
5. Weiss J, Glode A, Messersmith WA, Diamond J. Sacituzumab govitecan: breakthrough targeted therapy for triple-negative breast cancer. *Expert Rev Anticancer Ther.* 2019;19(8):673–679. doi:10.1080/14737140.2019.1654378
6. Bardia A, Tolaney SM, Punie K, et al. Biomarker analyses in the phase III ASCENT study of sacituzumab govitecan versus chemotherapy in patients with metastatic triple-negative breast cancer. *Ann Oncol.* 2021;32(9):1148–1156. doi:10.1016/j.annonc.2021.06.002
7. Bardia A, Mayer IA, Vahdat LT, et al. Sacituzumab Govitecan-hziy in refractory metastatic triple-negative breast cancer. *N Engl J Med.* 2019;380(8):741–751. doi:10.1056/NEJMoa1814213
8. Lipinski M, Parks DR, Rouse RV, Herzenberg LA. Human trophoblast cell-surface antigens defined by monoclonal antibodies. *Proc Natl Acad Sci U S A.* 1981;78(8):5147–5150. doi:10.1073/pnas.78.8.5147
9. Zaman S, Jadid H, Denson AC, Gray JE. Targeting Trop-2 in solid tumors: future prospects. *Onco Targets Ther.* 2019;12:1781–1790. doi:10.2147/ott.S162447
10. Zeng P, Chen MB, Zhou LN, Tang M, Liu CY, Lu PH. Impact of TROP2 expression on prognosis in solid tumors: a systematic review and meta-analysis. *Sci Rep.* 2016;6:33658. doi:10.1038/srep33658
11. Trerotola M, Cantanelli P, Guerra E, et al. Upregulation of Trop-2 quantitatively stimulates human cancer growth. *Oncogene.* 2013;32(2):222–233. doi:10.1038/onc.2012.36
12. Wahby S, Fashoyin-Aje L, Osgood CL, et al. FDA approval summary: accelerated approval of sacituzumab govitecan-hziy for third-line treatment of metastatic triple-negative breast cancer. *Clin Cancer Res.* 2021;27(7):1850–1854. doi:10.1158/1078-0432.Ccr-20-3119

13. Sachs N, de Ligt J, Kopper O, et al. A living biobank of breast cancer organoids captures disease heterogeneity. *Cell*. 2018;172(1–2):373–386.e10. doi:10.1016/j.cell.2017.11.010
14. Luo D, Li H, Hu J, et al. Development and validation of nomograms based on gamma-glutamyl transpeptidase to platelet ratio for hepatocellular carcinoma patients reveal novel prognostic value and the ratio is negatively correlated with P38MAPK expression. *Front Oncol*. 2020;10:548744. doi:10.3389/fonc.2020.548744
15. Tang W, Zhou M, Dorsey TH, et al. Integrated proteotranscriptomics of breast cancer reveals globally increased protein-mRNA concordance associated with subtypes and survival. *Genome Med*. 2018;10(1):94. doi:10.1186/s13073-018-0602-x
16. Liu NQ, Stingl C, Look MP, et al. Comparative proteome analysis revealing an 11-protein signature for aggressive triple-negative breast cancer. *J Natl Cancer Inst*. 2014;106(2):djt376. doi:10.1093/jnci/djt376
17. Shao T, Chen T, Chen Y, et al. Construction of paclitaxel-based antibody-drug conjugates with a PEGylated linker to achieve superior therapeutic index. *Signal Transduct Target Ther*. 2020;5(1):132. doi:10.1038/s41392-020-00247-y
18. Broutier L, Mastrogianni G, Versteegen MM, et al. Human primary liver cancer-derived organoid cultures for disease modeling and drug screening. *Nat Med*. 2017;23(12):1424–1435. doi:10.1038/nm.4438
19. Á Ó, Lánczky A, Gyórfy B. Survival analysis in breast cancer using proteomic data from four independent datasets. *Sci Rep*. 2021;11(1):16787. doi:10.1038/s41598-021-96340-5
20. Bradley CA. Gastrointestinal cancer: organoids predict clinical responses. *Nat Rev Gastroenterol Hepatol*. 2018;15(4):189. doi:10.1038/nrgastro.2018.25
21. de Witte CJ, Espejo Valle-Inclan J, Hami N, et al. Patient-derived ovarian cancer organoids mimic clinical response and exhibit heterogeneous inter- and inpatient drug responses. *Cell Rep*. 2020;31(11):107762. doi:10.1016/j.celrep.2020.107762
22. Vlachogiannis G, Hedayat S, Vatsiou A, et al. Patient-derived organoids model treatment response of metastatic gastrointestinal cancers. *Science*. 2018;359(6378):920–926. doi:10.1126/science.aao2774
23. Vranic S, Gatalica Z. Trop-2 protein as a therapeutic target: a focused review on Trop-2-based antibody-drug conjugates and their predictive biomarkers. *Bosn J Basic Med Sci*. 2021. doi:10.17305/bjbm.2021.6100
24. Cardillo TM, Rossi DL, Zalath MB, et al. Predictive biomarkers for sacituzumab govitecan efficacy in Trop-2-expressing triple-negative breast cancer. *Oncotarget*. 2020;11(43):3849–3862. doi:10.18632/oncotarget.27766
25. Cardillo TM, Govindan SV, Sharkey RM, et al. Sacituzumab Govitecan (IMMU-132), an Anti-Trop-2/SN-38 antibody-drug conjugate: characterization and efficacy in pancreatic, gastric, and other cancers. *Bioconjug Chem*. 2015;26(5):919–931. doi:10.1021/acs.bioconjchem.5b00223
26. Goldenberg DM, Cardillo TM, Govindan SV, Rossi EA, Sharkey RM. Trop-2 is a novel target for solid cancer therapy with sacituzumab govitecan (IMMU-132), an antibody-drug conjugate (ADC). *Oncotarget*. 2015;6(26):22496–22512. doi:10.18632/oncotarget.4318
27. Cardillo TM, Govindan SV, Sharkey RM, Trisal P, Goldenberg DM. Humanized anti-Trop-2 IgG-SN-38 conjugate for effective treatment of diverse epithelial cancers: preclinical studies in human cancer xenograft models and monkeys. *Clin Cancer Res*. 2011;17(10):3157–3169. doi:10.1158/1078-0432.Ccr-10-2939
28. Bailly C. Irinotecan: 25 years of cancer treatment. *Pharmacol Res*. 2019;148:104398. doi:10.1016/j.phrs.2019.104398
29. de Man FM, Goey AKL, van Schaik RHN, Mathijssen RHJ, Bins S. Individualization of irinotecan treatment: a review of pharmacokinetics, pharmacodynamics, and pharmacogenetics. *Clin Pharmacokinet*. 2018;57(10):1229–1254. doi:10.1007/s40262-018-0644-7
30. Ocean AJ, Starodub AN, Bardia A, et al. Sacituzumab govitecan (IMMU-132), an anti-Trop-2-SN-38 antibody-drug conjugate for the treatment of diverse epithelial cancers: safety and pharmacokinetics. *Cancer*. 2017;123(19):3843–3854. doi:10.1002/encr.30789
31. Strop P, Tran TT, Dorywalska M, et al. RN927C, a site-specific Trop-2 Antibody-Drug Conjugate (ADC) with enhanced stability, is highly efficacious in preclinical solid tumor models. *Mol Cancer Ther*. 2016;15(11):2698–2708. doi:10.1158/1535-7163.Mct-16-0431
32. King GT, Eaton KD, Beagle BR, et al. A Phase 1, dose-escalation study of PF-06664178, an anti-Trop-2/Aur0101 antibody-drug conjugate in patients with advanced or metastatic solid tumors. *Invest New Drugs*. 2018;36(5):836–847. doi:10.1007/s10637-018-0560-6
33. Kalinsky K, Diamond JR, Vahdat LT, et al. Sacituzumab govitecan in previously treated hormone receptor-positive/HER2-negative metastatic breast cancer: final results from a phase I/II, single-arm, basket trial. *Ann Oncol*. 2020;31(12):1709–1718. doi:10.1016/j.annonc.2020.09.004
34. Vranic S, Palazzo J, Sanati S, et al. Potential novel therapy targets in neuroendocrine carcinomas of the breast. *Clin Breast Cancer*. 2019;19(2):131–136. doi:10.1016/j.clbc.2018.09.001
35. Zhao W, Kuai X, Zhou X, et al. Trop2 is a potential biomarker for the promotion of EMT in human breast cancer. *Oncol Rep*. 2018;40(2):759–766. doi:10.3892/or.2018.6496

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