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ORIGINAL ARTICLE

Biomarker analyses in the phase III ASCENT study of sacituzumab govitecan versus chemotherapy in patients with metastatic triple-negative breast cancer☆

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Background: The pivotal phase III ASCENT trial demonstrated improved survival outcomes associated with sacituzumab govitecan (SG), an anti-trophoblast cell-surface antigen 2 (anti-Trop-2) antibody-drug conjugate linked with the topoisomerase-inhibitor SN-38, over single-agent chemotherapy treatment of physician's choice (TPC) in previously treated metastatic triple-negative breast cancer (mTNBC). This prespecified, exploratory biomarker analysis from the ASCENT trial evaluates the association between tumor Trop-2 expression and germline *BRCA1/2* mutation status with clinical outcomes.

Patients and methods: Patients with mTNBC refractory to or progressing after two or more prior chemotherapies, with one or more in the metastatic setting, were randomized to receive SG (10 mg/kg intravenously days 1 and 8, every 21 days) or TPC (capecitabine, eribulin, vinorelbine, or gemcitabine) until disease progression/unacceptable toxicity. Biopsy or surgical specimens were collected at study entry to determine Trop-2 expression level using a validated immunohistochemistry assay and histochemical scoring. Germline *BRCA1/2* mutation status was collected at baseline.

Results: Of 468 assessable patients, 290 had Trop-2 expression data [64% ($n = 151$ SG) versus 60% ($n = 139$ TPC)] and 292 had known *BRCA1/2* mutation status [63% ($n = 149$ SG) versus 61% ($n = 143$ TPC)]. Median progression-free survival in SG- versus TPC-treated patients was 6.9, 5.6, and 2.7 months versus 2.5, 2.2, and 1.6 months for high, medium, and low Trop-2 expression, respectively. Median overall survival (14.2, 14.9, and 9.3 months versus 6.9, 6.9, and 7.6 months) and objective response rates (44%, 38%, and 22% versus 1%, 11%, and 6%) were numerically higher with SG versus TPC in patients with high, medium, and low Trop-2 expression, respectively. Efficacy outcomes were numerically higher with SG versus TPC in patients with and without germline *BRCA1/2* mutations.

Conclusions: SG benefits patients with previously treated mTNBC expressing high/medium Trop-2 compared with standard-of-care chemotherapy and regardless of germline *BRCA1/2* mutation status. The small number of patients with low Trop-2 expression precludes definitive conclusions on the benefit of SG in this subgroup.

Key words: triple-negative breast cancer, trophoblast cell-surface antigen 2, BRCA

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INTRODUCTION

Precision medicine has gained momentum in recent years, with increasing attention given to the identification of key biomarkers that predict response to treatment. Triple-negative breast cancer (TNBC) is a heterogeneous disease with distinct molecular subtypes, resulting in unique drug sensitivities.[1,2] A potential biomarker for response in TNBC is trophoblast cell-surface antigen 2 (Trop-2), a transmembrane glycoprotein calcium signal transducer expressed in human epithelial cells.[3] Increased expression of Trop-2 is associated with tumor growth in a variety of solid epithelial tumors, including TNBC and other breast cancer subtypes.[3-5] Elevated levels of membrane Trop-2 are associated with poor prognosis and increased tumor growth in breast cancer, including decreased survival.[3,5]

Sacituzumab govitecan (SG) is a novel, Trop-2-directed antibody—drug conjugate comprising a humanized anti-Trop-2 IgG_1 kappa antibody coupled to an SN-38 payload, the active metabolite of the topoisomerase 1 inhibitor irinotecan, via a proprietary, hydrolysable linker. SG is distinct from other antibody—drug conjugates due to its high antibody specificity for Trop-2, a high ratio of drug to antibody (7.6: 1),[6] and delivery of SN-38 in its most active, nonglucuronidated form.[7] Following SG administration, the anti-Trop-2 monoclonal antibody binds to Trop-2 expressed on the tumor cell surface, allowing internalization and targeted delivery of SN-38 to tumor cells.[6,8-10] Its proprietary linker also allows SN-38 to be liberated in the tumor microenvironment, enabling antitumor effects (bystander effect) without prerequisite internalization and enzymatic cleavage of SN-38 from the anti-Trop-2 antibody.[6,8,11]

As *BRCA1* or *BRCA2* (*BRCA1/2*) confer a deficiency in homologous recombination repair of double-stranded DNA breaks, there is interest in *BRCA1/2* as a potential biomarker of response for therapy regimens that target DNA damage, particularly for TNBC.[12] Approximately 15% of patients with TNBC have germline *BRCA* mutations, a higher prevalence compared with other breast cancer subtypes.[13-15] Topoisomerase I inhibitors like SN-38, the payload in SG, have been shown to increase double-stranded DNA breaks, regardless of *BRCA* mutation status.[14] SG has demonstrated inhibition of tumor growth in translational models of *BRCA*-mutated TNBC and may confer synthetic lethality to TNBC tumors.[14]

A phase I/II single-arm basket study (NCT01631552) was conducted of SG in patients with metastatic, epithelial cancers. In the cohort of 108 patients with heavily pretreated metastatic TNBC (mTNBC), an objective response rate (ORR) of 33%, median progression-free survival (PFS) of 5.5 months, and median overall survival (OS) of 13.0 months were observed.[16] In this cohort of patients with mTNBC, 88% of 48 primary or mTNBC tumors had moderate to strong Trop-2 staining, with the majority expressing Trop-2 in >50% of tumor cells. All responders had moderate to strong Trop-2 staining, demonstrating the potential for greater benefit of SG in tumors with relatively high Trop-2 expression;[17] however, the number of samples with low or no Trop-2 staining were limited ($n = 6$), highlighting the need for further evaluation of Trop-2 in a larger dataset. The safety and efficacy results from this trial led to an accelerated approval by the United States Food and Drug Administration (FDA), with full approval received based on the results of the phase III ASCENT trial (NCT02574455).

The randomized phase III ASCENT trial of SG versus single-agent chemotherapy treatment of physician's choice (TPC; eribulin, vinorelbine, gemcitabine, or capecitabine) in 468 patients with chemotherapy-pretreated mTNBC confirmed the initial findings from the phase I/II study. Treatment with SG was associated with a significant survival benefit compared with TPC with a median PFS of 5.6 versus 1.7 months {hazard ratio (HR) 0.41 [95% confidence interval (CI) 0.32-0.52]; $P < 0.001$} and a median OS of 12.1 versus 6.7 months [HR, 0.48 (95% CI 0.38-0.59); $P < 0.001$], along with a tolerable safety profile. Here, we present a prespecified biomarker assessment of the potential association between tumor membrane Trop-2 expression or germline *BRCA1/2* mutation status on efficacy of SG versus TPC in the phase III ASCENT study.

METHODS

Study design

The study design for ASCENT (NCT02574455) has been described previously.[18] Briefly, ASCENT was an international, multicenter, randomized, phase III study comparing the efficacy and safety of SG versus TPC in patients in the second-line or greater mTNBC setting. Patients were stratified at randomization by the number of prior chemotherapy regimens for advanced disease (2-3 versus >3), presence of known brain metastases at baseline (yes versus no), and geography (North America versus rest of world).

The primary endpoint was PFS (by blinded independent central review) in patients without known baseline brain metastases (measured by computed tomography or magnetic resonance imaging per RECIST version 1.1).[19] Secondary endpoints included investigator-assessed PFS for the full population, including all randomized patients with and without brain metastases, OS, ORR, duration of response, time to response, and safety. Exploratory endpoints included biomarker assessment.

The ASCENT trial was conducted and approved by each investigational site institutional review board/ethics committee before initiation, and in accordance with the Declaration of Helsinki, International Council for Harmonisation Guidelines for Good Clinical Practice, FDA Code of Federal Regulations, national and local drug and data protection laws, and other applicable regulatory requirements. All patients provided written informed consent before enrollment.

Patients

Patients had mTNBC that had progressed following two or more prior standard chemotherapy regimens (no upper limit) for unresectable, locally advanced, or metastatic disease, and included a taxane (any setting). Per protocol, patients were also eligible after only one prior regimen in the metastatic setting if their disease recurred within 12 months of completing (neo)adjuvant therapy. Eligible patients had TNBC according to standard American Society of Clinical Oncology/College of American Pathologists criteria.[20] Patients with stable brain metastases for at least 4 weeks before treatment were eligible, but were excluded from evaluation of the primary endpoint and this exploratory analysis.

Sample collection and assay procedure

Primary or metastatic archival biopsy or surgical specimens were requested at study entry to determine tumor Trop-2 expression; however, known Trop-2 expression was not required to determine patient eligibility. Trop-2 expression was determined by using a validated immunohistochemistry (IHC) assay (OptiVIEW DAB detection kit; Roche Diagnostics, Indianapolis, IN) as specified according to manufacturer instructions, including quality control methods and by using Trop-2 mouse monoclonal antibody (ENZ ABS380-0100; ENZO Life Sciences, Farmingdale, NY). IHC was carried out centrally (Laboratory Corporation of America, Research Triangle Park, NC), and interpretation of Trop-2 staining was carried out by a qualified pathologist.

Tumor cell membrane Trop-2 expression was categorized based on a histochemical score (H-score), a numerical value represented by a weighted summation of percent staining which accounts for both the staining intensity and the percentage of cells at that intensity. H-scores were calculated using the following formula: H-score = (3 × % cells with strong intensity staining) + (2 × % cells with moderate intensity staining) + (1 × % cells with mild intensity staining).[21] The categories were selected based on the distribution of the H-score (range from 0 to 300) to divide the population into low, medium, and high groups. The following Trop-2 expression categories were used: H-score 0 to <100: Trop-2 low; H-score 100-200: Trop-2 medium; H-score >200-300: Trop-2 high. The status of germline *BRCA1/2* mutations was collected at baseline, if known.

Statistical analyses

Subgroup analyses of PFS, OS, and ORR by biomarker were carried out. Efficacy analyses were based on data from the primary study analysis (cutoff date 11 March 2020) of the brain metastases negative patient population.[18] Only patients with known Trop-2 or *BRCA1/2* results are included in the analysis. The analyses were exploratory in nature, with no adjustment for multiple testing and no formal testing of benefit of SG versus TPC subgroups.

PFS was defined as the time from randomization until objective tumor progression or death or censored at the last radiographic assessment for patients without progression or death. PFS and OS were analyzed using the Kaplan–Meier method, with medians and corresponding 95% CIs determined according to the Brookmeyer and Crowley method with log-log transformation. CIs for ORR were calculated by the Clopper-Pearson method.

RESULTS

Patients

As reported previously, 529 patients were enrolled in ASCENT (Figure 1); 61 patients had brain metastases at baseline and 468 patients had no history of brain metastases. Of the patients who were negative for brain metastases (primary efficacy dataset) and included in this analysis, 235 patients were randomized to receive SG, and 233

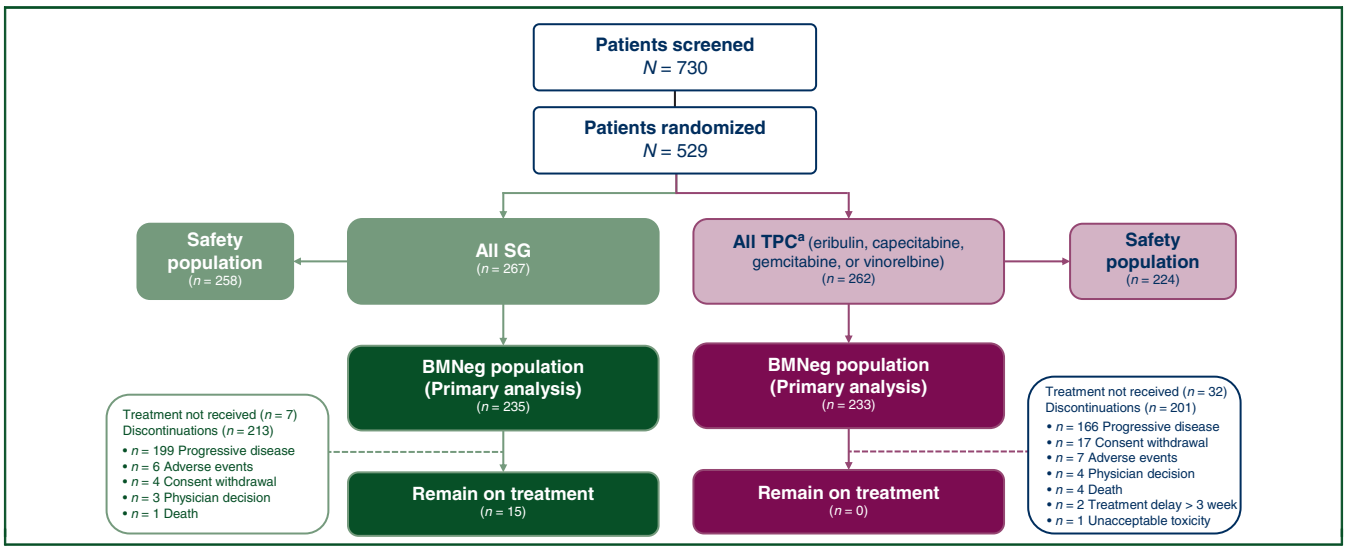

Figure 1. CONSORT diagram: enrollment, intent-to-treat and safety populations, and patient withdrawals.

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BMNeg, brain metastases negative; SG, sacituzumab govitecan; TPC, treatment of physician's choice.

[a] Patients in the TPC arm were randomized to eribulin (*n* = 139), vinorelbine (*n* = 52), gemcitabine (*n* = 38), or capecitabine (*n* = 33).

patients were randomized to receive TPC (54% eribulin, 20% vinorelbine, 13% capecitabine, and 12% gemcitabine). Patients in the SG- versus TPC-treated cohorts had a median age of 54 years (range, 29-82 years) and 53 years (range, 27-81 years; Table 1), respectively. The most common prior chemotherapy received in the SG- versus TPC-treated cohorts, respectively, was cyclophosphamide (82% each), carboplatin (63% versus 69%), and capecitabine (63% versus 68%); all patients received a prior taxane. The median number of prior anticancer regimens was 4 (range, 2-17).

Demographics and baseline characteristics of patients with known and unknown Trop-2 expression and those of patients with known or unknown *BRCA1/2* mutational status are presented in Supplementary Tables S1 and S2, respectively, available at https://doi.org/10.1016/j.annonc.2021.06.002. Demographics and baseline characteristics of patients included in the biomarker analyses were well-balanced across treatment arms. Patients who were not included in the biomarker analyses due to unknown Trop-2 expression or *BRCA1/2* mutational status generally had similar characteristics as those who were included. Compared with patients who were included in the analyses, minor differences were observed for prior use of checkpoint inhibitors in patients who were not included in the Trop-2 analysis, and for median age and prior use of checkpoint inhibitors in patients who were not included in the *BRCA1/2* analysis. Trop-2 expression levels in patients who did not have TNBC at initial breast cancer diagnosis were broadly similar to those of patients with TNBC at initial breast cancer diagnosis (data not shown).

Among 235 patients who were treated with SG, 151 (64%) had archival tumors evaluated for Trop-2 expression. Of these, the majority of patients had a high H-score [n = 85 (56%)], with a medium and low H-score found in 26% (n = 39) and 18% (n = 27) of patients, respectively. In the SG arm, there were seven patients in the low H-score group who had no Trop-2 expression. A similar distribution of Trop-2 expression was observed in patients who received single-agent chemotherapy with high, medium, and low H-scores of 52%, 25%, and 23%, respectively. In the TPC arm, there were four patients in the low H-score group who had no Trop-2 expression. Data on Trop-2 expression in primary versus metastatic tumors were not collected.

Germline *BRCA1/2* mutational status was known in 149 (63%) patients who received SG and in 143 (61%) of patients who received TPC. Of those with known germline *BRCA1/2* status, there were 16 of 149 patients (11%) who were *BRCA1/2*-positive (germline pathogenic variants in either *BRCA1* or *BRCA2*) and 133 of 149 patients (89%) who were *BRCA1/2*-negative (also considered to be germline *BRCA* wild type) in the SG arm, and 18 of 143 (13%) *BRCA1/2*-positive and 125 of 143 (87%) *BRCA1/2*-negative patients in the TPC arm.

Table 1. Demographics assessed in patients who were negative for brain metastases

	SG (n = 235)	TPC (n = 233)
Median age (range), years	54 (29-82)	53 (27-81)
Female, *n* (%)	233 (99)	233 (100)
Race or ethnic group, *n* (%)		
White	188 (80)	181 (78)
Black	28 (12)	28 (12)
Asian	9 (4)	9 (4)
Other or not specified	10 (4)	15 (6)
ECOG PS, *n* (%)		
0	108 (46)	98 (42)
1	127 (54)	135 (58)
BRCA1/2 mutational status, *n* (%)	149 (63)	143 (61)
Positive	16 (7)	18 (8)
Negative	133 (57)	125 (54)
Trop-2 expression, *n* (%)	151 (64)	139 (60)
(High) H-score >200-300	85/151 (56)	72/139 (52)
(Medium) H-score 100-200	39/151 (26)	35/139 (25)
(Low) H-score 0 to <100[a]	27/151 (18)	32/139 (23)
Initial diagnosis of TNBC,[b] *n* (%)		
Yes	165 (70)	157 (67)
No	70 (30)	76 (33)
Median previous anticancer regimens,[c] *n* (range)	4 (2-17)	4 (2-14)
Most common prior chemotherapy, *n* (%)		
Taxane[d]	235 (100)	233 (100)
Cyclophosphamide	192 (82)	192 (82)
Carboplatin	147 (63)	160 (69)
Capecitabine	147 (63)	159 (68)
Previous PARP inhibitor, *n* (%)	17 (7)	18 (8)
Previous use of checkpoint inhibitors, *n* (%)	67 (29)	60 (26)
Most common sites of disease,[e] *n* (%)		
Lung only	108 (46)	97 (42)
Liver	98 (42)	101 (43)
Bone	48 (20)	55 (24)

BRCA, breast cancer gene; ECOG PS, Eastern Cooperative Oncology Group performance status; H-score, histochemical score; PARP, poly (ADP-ribose) polymerase; SG, sacituzumab govitecan; TNBC, triple-negative breast cancer; TPC, treatment of physician's choice; Trop-2, trophoblast cell-surface antigen 2.

[a] In this H-score group, seven and four patients in the SG and TPC arms, respectively, had no Trop-2 expression.

[b] Patients in study either had TNBC at initial diagnosis or had hormone receptor-positive disease that converted to hormone-negative at time of study entry.

[c] Anticancer regimens refer to any treatment regimen that was used to treat breast cancer in any setting.

[d] Includes paclitaxel, paclitaxel albumin, and docetaxel.

[e] Based on independent central review of target and non-target lesions at baseline.

Trop-2 expression and efficacy outcomes

SG-treated patients with high, medium, and low Trop-2 H-scores had median PFS of 6.9 months (95% CI 5.8-7.4 months), 5.6 months (95% CI 2.9-8.2 months), and 2.7 months (95% CI 1.4-5.8 months), respectively. Compared with SG, TPC-treated patients had numerically lower median PFS across high (2.5 months; 95% CI 1.5-2.9 months), medium (2.2 months; 95% CI 1.4-4.3 months), and low (1.6 months; 95% CI 1.4-2.7 months) Trop-2 H-scores (Figure 2A). Median OS with SG treatment was 14.2 months (95% CI 11.3-17.5 months), 14.9 months (95% CI 6.9 months to not evaluable), and 9.3 months (95% CI 7.5-17.8 months) in patients with high, medium, and low Trop-2 scores, respectively. In patients with who received TPC, median OS was 6.9 months (95% CI 5.3-8.9 months), 6.9 months (95% CI 4.6-10.1 months), and 7.6 months (95% CI 5.0-9.6 months) for high, medium, and low Trop-2 H-scores, respectively; Figure 2B).

The ORR in SG-treated patients with high, medium, and low Trop-2 H-scores was 44%, 38%, and 22%, respectively. In comparison, the ORR in the TPC arm was 1%, 11%, and

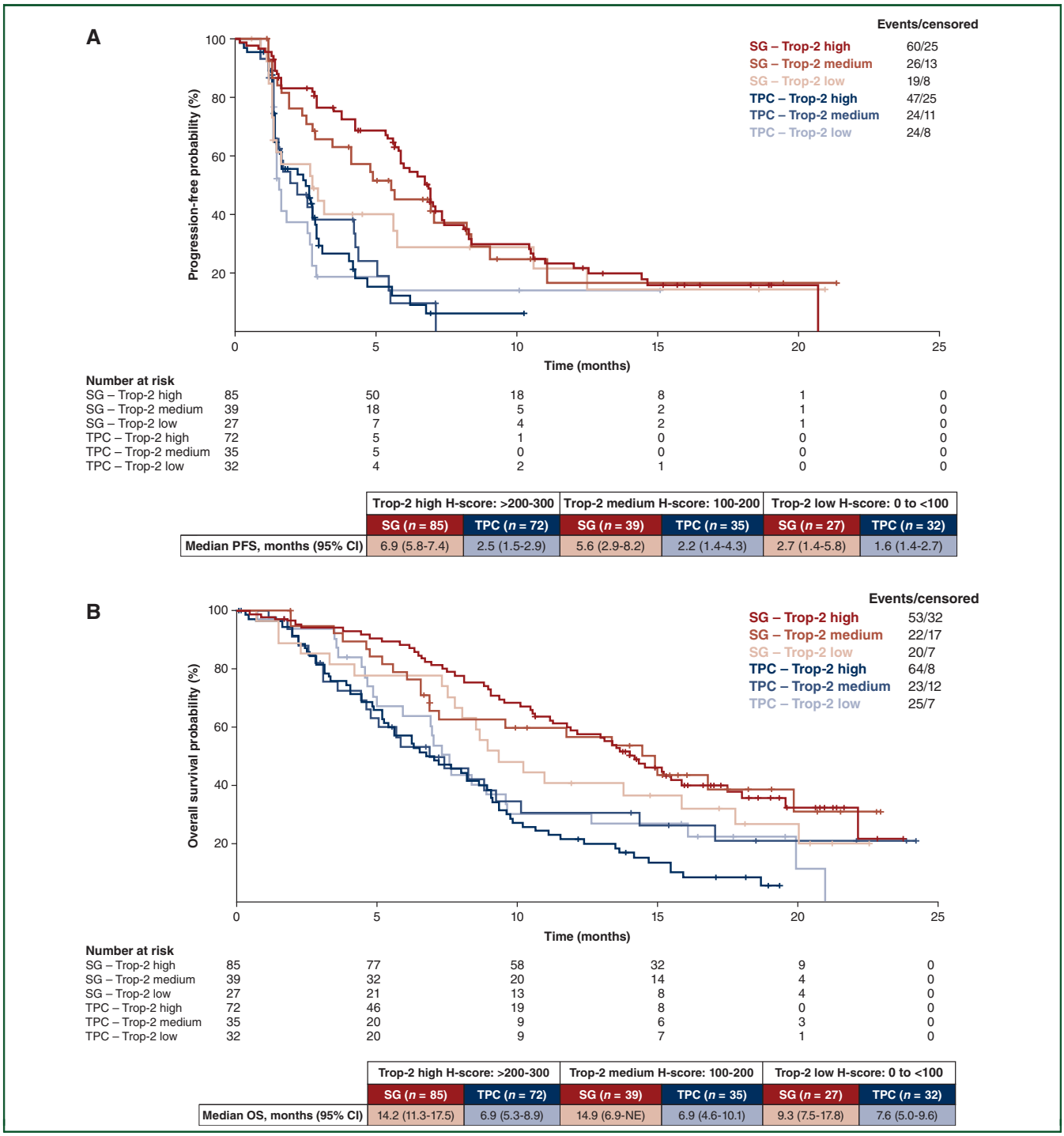
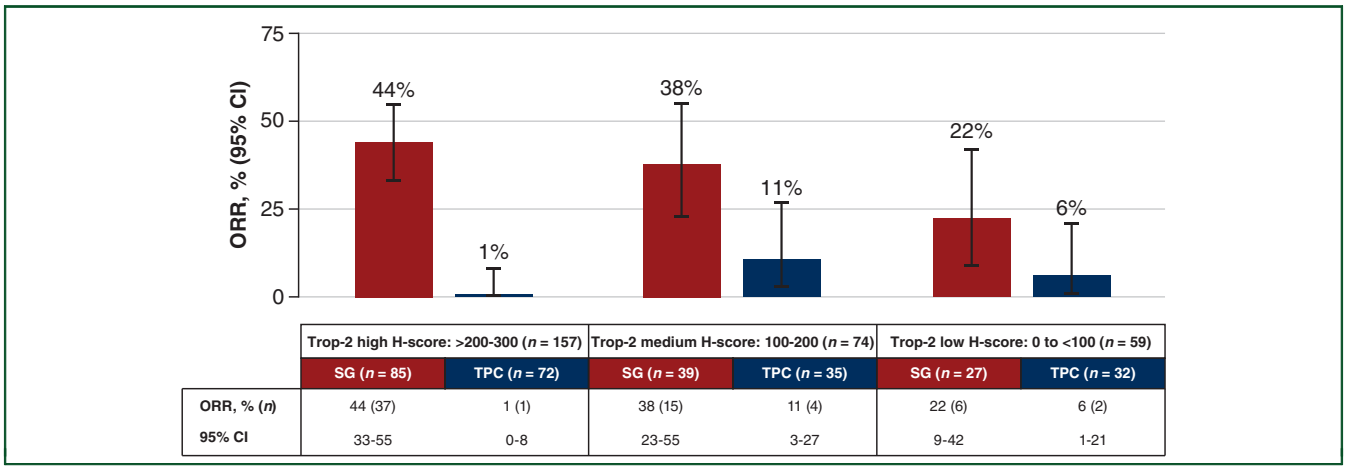


Figure 2. (A) Progression-free survival by Trop-2 expression. (B) Overall survival by trophoblast cell-surface antigen 2 (Trop-2) expression.
CI, confidence interval; H-score, histochemical score; OS, overall survival; PFS, progression-free survival; SG, sacituzumab govitecan; TPC, treatment of physician's choice; Trop-2, trophoblast cell-surface antigen 2.

6% in patients with high, medium, and low Trop-2 H-scores, respectively (Figure 3).

BRCA mutational status and efficacy outcomes

Compared with TPC, treatment with SG resulted in numerically higher median PFS, median OS, and response outcomes, regardless of germline *BRCA1/2* mutation status at study entry (Table 2). In *BRCA1/2*-positive patients, the median PFS was 4.6 versus 2.5 months in SG-treated versus TPC-treated patients, respectively; median OS was 15.6 versus 4.4 months in SG-treated versus TPC-treated patients, respectively. In *BRCA1/2*-negative patients, the median PFS was 4.9 and 1.6 months in SG-treated versus TPC-treated patients, respectively; median OS was 10.9 versus 7 months in SG-treated versus TPC-treated patients,



	Trop-2 high H-score: >200-300 (*n* = 157)		Trop-2 medium H-score: 100-200 (*n* = 74)		Trop-2 low H-score: 0 to <100 (*n* = 59)	
	SG (*n* = 85)	TPC (*n* = 72)	SG (*n* = 39)	TPC (*n* = 35)	SG (*n* = 27)	TPC (*n* = 32)
ORR, % (*n*)	44 (37)	1 (1)	38 (15)	11 (4)	22 (6)	6 (2)
95% CI	33-55	0-8	23-55	3-27	9-42	1-21

Figure 3. Objective response rate by Trop-2 expression.
CI, confidence interval; H-score, histochemical score; ORR; objective response rate; SG, sacituzumab govitecan; TPC, treatment of physician's choice; Trop-2, trophoblast cell-surface antigen 2.

Table 2. ORR, PFS, and OS summary by germline *BRCA1/2* status

	Germline *BRCA1/2*-positive		Germline *BRCA1/2*-negative	
	SG (*n* = 16)	TPC (*n* = 18)	SG (*n* = 133)	TPC (*n* = 125)
ORR, *n* (%)	3 (19)	1 (6)	44 (33)	7 (6)
Odds ratio (95% CI)	3.9 (0.4-42.2)		8.3 (3.6-19.4)	
Median PFS, months (95% CI)	4.6 (1.3-10.3)	2.5 (0.8-5.5)	4.9 (3.8-5.9)	1.6 (1.5-2.5)
HR (95% CI)	0.6 (0.2-1.6)		0.4 (0.3-0.6)	
Median OS, months (95% CI, months)	15.6 (6.2-NE)	4.4 (3.6-9.7)	10.9 (9.6-13.4)	7.0 (5.6-8.2)
HR (95% CI)	0.4 (0.2-0.9)		0.5 (0.4-0.7)	

BRCA, breast cancer gene; CI, confidence interval; HR, hazard ratio; NE, not evaluable; ORR, objective response rate; OS, overall survival; PFS, progression-free survival; SG, sacituzumab govitecan; TPC, treatment of physician's choice.

respectively. In *BRCA1/2*-positive patients, ORR was 19% versus 6% in SG-treated versus TPC-treated patients, respectively. In *BRCA1/2*-negative patients, the ORR was 33% versus 6% in SG-treated versus TPC-treated patients, respectively.

DISCUSSION

In the pivotal phase III ASCENT study, SG demonstrated improved outcomes compared with TPC (eribulin, vinorelbine, gemcitabine, or capecitabine), thereby becoming the first Trop-2-directed antibody—drug conjugate to show a significant PFS and OS benefit compared with standard-of-care chemotherapy in the second-line or greater mTNBC setting.[18] These efficacy data, however, were based on a patient population unselected for Trop-2 expression. In this prespecified biomarker analysis, we assessed efficacy outcomes by tumor membrane Trop-2 expression and *BRCA1/2* mutational status. Our results show the SG arm had numerically higher efficacy outcomes in high and medium Trop-2 expression subgroups compared with those in the TPC arm. The benefit of SG over TPC was also similar in *BRCA1/2*-positive and -negative patients.

Although Trop-2 is overexpressed across a variety of epithelial cancers, overexpression is more common in TNBC compared with other breast cancer subtypes.[22] Preclinical studies of TNBC demonstrated that expression levels of Trop-2 and homologous recombination repair deficiency can influence the antitumor activity of SG; SG provided higher activity in preclinical models with higher tumor Trop-2 expression and homologous recombination deficiency.[23] In addition, the presence of intratumoral heterogeneity in Trop-2 expression in clinical breast cancer samples and the possibility for Trop-2 plasticity in the tumor microenvironment highlight the need to assess the clinical impact of SG in TNBC tumors with varying Trop-2 expression.[24] The availability of the ASCENT clinical dataset allowed for assessment of SG efficacy in patients with varying tumor Trop-2 expression and the potential for Trop-2 as a biomarker of response in TNBC.

In this study, outcomes among Trop-2 subgroups were numerically higher with SG versus TPC treatment in patients with high and medium Trop-2 expression; SG-treated patients with high/medium Trop-2 expression had similar median PFS, median OS, and response rates as SG-treated patients in the overall primary analysis from ASCENT.[18] As the majority of breast cancers express Trop-2,[3,4] the number of patients with low Trop-2-expression tumors was small. These results suggest evaluating Trop-2 expression may not be needed to predict which patients are unlikely to derive benefit from SG versus TPC, but the small numbers of patients in the low Trop-2 expression groups and absence of formal testing limit definitive conclusions on the benefit of

SG (or lack thereof) in patients with low Trop-2 expression. Additional studies are needed to address whether higher Trop-2 expression is predictive of better response to SG and if primary tumor Trop-2 expression is predictive of response to SG in mTNBC. Finally, although high Trop-2 expression has previously been associated with poor prognosis in breast cancer,[3,5] this study did not suggest an adverse prognostic effect of high Trop-2 expression in either treatment arm.

Because the *BRCA1* and *BRCA2* genes encode proteins involved in double-stranded DNA break repair, it is hypothesized that cancers with pathogenic variants in *BRCA1/2* may be more sensitive to chemotherapies that cause DNA damage, such as platinum-based regimens.[25-27] Due to the strong association of TNBC with germline *BRCA1/2* mutations,[13,14,28] we assessed whether TNBC with germline pathogenic variants in *BRCA1/2* had increased sensitivity to or was a predictive biomarker for response to SG, which primarily exerts its antitumor activity by eliciting DNA damage. In our analysis, there did not appear to be a difference in the effect size of SG between *BRCA1/2*-positive and -negative patients. Due to the small numbers of patients who were *BRCA1/2*-positive, further comparison of efficacy outcomes with *BRCA1/2*-negative patients was not carried out. However, DNA repair may be altered through other mechanisms beyond germline mutations, and additional research is needed into biomarkers of response and resistance for SG. As we are unable to exclude secondary reversion mutations and due to the advanced setting of the ASCENT trial, these data are not sufficient to draw definitive conclusions on the predictive value of germline *BRCA* mutations for SG in advanced TNBC.

The primary limitations of this study are that ASCENT was not powered to detect predictive effects, and there was no formal testing carried out between treatment arms in this subanalysis. In addition, the number of patients in both the low Trop-2 expression subgroup and the germline *BRCA1/2*-positive subgroup were small, which limited meaningful interpretation of the data when compared with other subgroups. Formal statistical analysis of Trop-2 expression and various baseline patient characteristics to determine a potential correlation was not carried out, due to the variability of patient characteristics and methodological concerns about exploratory non-hypothesis-based *post hoc* analyses. These data also reflect the current measurement of Trop-2 expression by IHC, and future assays may be developed to better quantify its expression. An additional limitation is that these data did not assess somatic *BRCA1/2* mutations in germline *BRCA1/2*-negative patients or mutations in other genes associated with homologous recombination, such as *PALB2.* Finally, differences in Trop-2 expression in primary breast cancers compared with metastatic lesions were not assessed and could be different. Most samples were archival tumor samples, and the biopsies were not from baseline when patients started therapy, so it is uncertain if the Trop-2 expression was reflective of the baseline state when the patient went on study, especially as Trop-2 may be a dynamic biomarker.

Conclusion

The collective results from the current study suggest SG benefits patients with previously treated mTNBC expressing high/medium Trop-2 compared with standard-of-care single-agent chemotherapy and regardless of germline *BRCA1/2* mutation status. Further studies are warranted to fully elucidate outcomes among these patient populations and those with low Trop-2 expression. Currently, studies are ongoing to further evaluate SG in earlier-stage TNBC and human epidermal growth factor receptor 2 (HER2)-negative breast cancer (NeoSTAR, NCT04230109; SASCIA, NCT04595565), in combination with other agents for the treatment of mTNBC (SEASTAR, NCT03992131; MORPHEUS-TNBC, NCT03424005; NCT04468061), and for the treatment of hormone receptor-positive/HER2-negative metastatic breast cancer (TROPiCS-02, NCT03901339). Biomarker data from these studies will provide further insight into the relationship of Trop-2 expression and germline *BRCA1/2* mutation status as a potential predictive biomarker of response to SG in breast cancer.

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DATA SHARING

Immunomedics, Inc., a subsidiary of Gilead Sciences, Inc. will provide the study protocol and statistical analysis plan with publication of this manuscript as well as post results on clinicaltrials.gov, as required.

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