On April 22, 2020, the Food and Drug Administration (FDA) granted accelerated approval to sacituzumab govitecan-hziy (TRODELVY, Immunomedics, Inc.) (SG) for patients with metastatic triple-negative breast cancer (TNBC), who had received at least two prior therapies for metastatic disease (1). SG is an antibody-drug conjugate (ADC) of a humanized anti-Trop-2 monoclonal antibody (mAb), HuRS7, linked to ≈8 molecules of SN-38—the active metabolite of irinotecan and a potent inhibitor of topoisomerase 1 (2). Notably, until SG no topoisomerase I inhibitors had been used in metastatic TNBC, and SG effectively constitutes a new cytotoxic drug, for treating a disease that still heavily depended on chemotherapy.

Efficacy of SG in TNBC was validated in the ASCENT randomized Phase III clinical trial (NCT02574455; EudraCT number, 2017-003019-21), as compared with single-agent chemotherapy of physician’s choice (eribulin, vinorelbine, capecitabine, or gemcitabine) (3), on 468 patients at primary end-point analysis (i.e., excluding patients with baseline brain metastases). The primary end-point was progression-free survival (PFS). The median PFS was 5.6 mo with SG and 1.7 mo with chemotherapy. Remarkably, the median overall survival (OS) was 12.1 mo with SG versus 6.7 mo with chemotherapy. The patients’ overall response rates (ORR), assessed using RECIST 1.1 quantitative criteria, was 35% with SG (72 partial responses; 10 complete responses) and 5% with chemotherapy (9 partial responses; 2 complete responses).

Immunohistochemistry analysis showed Trop-2 expression in ≈75% of unselected breast cancers (4), and in 88% (1), and 77–82% (5) TNBC. To be remarked, biochemical analysis revealed Trop-2 expression in essentially all breast cancer cases, though at broadly different expression levels (6). Hence, Trop-2 expression was not adopted as a selection criterium in SG efficacy studies. An exploratory post-hoc analysis of the ASCENT trial indicated that SG mostly benefited TNBC patients with Trop-2 high (6.9 mo PFS) and medium (5.6 mo PFS) expression, as determined by immunohistochemistry. SG showed considerably less impact on Trop-2 low cases (2.7 mo PFS) (5), although SG benefit was not ruled out. Corresponding findings were obtained for OS, whereby Trop-2 high (14.2 mo OS) and medium (14.9 mo OS) TNBC patients profited the most, whereas Trop-2 low cases showed lower survival advantage (9.3 mo OS) versus chemotherapy (7.6 mo OS) (5). ORR followed a similar gradient, with 44% responses for Trop-2 high cases, 38% for Trop-2 medium, and 22% for Trop-2 low patients (5). These findings supported the clinical efficacy of SG in TNBC through the specific targeting of Trop-2, although the post-hoc nature limited the power of this analysis.

The ASCENT trial had followed a first, single-arm, multicenter, Phase I/II trial (NCT01631552), which had enrolled 515 patients, bearing cervical, colorectal, endometrial, ovarian, esophageal, gastric adenocarcinoma, glioblastoma multiforme, head and neck cancer,
hepatocellular, prostate, non-small cell lung cancer (NSCLC), SCLC, pancreatic, TNBC and other breast cancers, and metastatic urothelial cancer. Among these, 69 patients with relapsed/refractory metastatic TNBC received 10 mg/kg SG on days 1 and 8 of 21-day repeated cycles. The primary end-points were safety and ORR; secondary end-points were PFS and OS (7). The confirmed ORR was 30% (19 partial responses; 2 complete responses), the median response duration was 8.9 mo. Responses to SG therapy occurred early, with a median onset of 1.9 mo. Median PFS was 6.0 mo, median OS was 16.6 mo. An expansion of this trial was conducted on 108 patients with metastatic TNBC. An ORR of 33.3% was found, with a median response duration of 7.7 mo, a PFS of 5.5 mo, and a median OS of 13.0 mo (1).

SG showed efficacy in Trop-2-expressing urothelial carcinomas (8), and on April 13, 2021 the FDA granted accelerated approval of SG for patients with advanced bladder cancer. SG showed efficacy also in metastatic endometrial cancer (9), SCLC (10), and NSCLC (11). Among the 54 NSCLC patients treated with SG, the objective response rate was 17%, the ORR was 43%. PFS was 5.2 mo, the median OS was 9.5 mo (11). More than 90% of 26 assessable archival tumor specimens were highly positive (2+/3+) for Trop-2 by immunohistochemistry (11), supporting SG specificity for Trop-2-expressing cancers, and consolidating SG as a breakthrough therapy in advanced/metastatic tumors.

SG was shown to associate to a distinct toxicity pattern. In the first NCT01631552 Phase I/II basket trial, grade 3 or higher adverse events in TNBC patients included neutropenia (39%), leukopenia (16%), anemia (14%), and diarrhea (13%); the incidence of febrile neutropenia was 7% (7). In the expansion trial to 108 patients, grade 3 or higher adverse events were observed, that included neutropenia (28%), diarrhea (7%), nausea (7%) (1). Treatment-related adverse events of grade 3 or higher in the ASCENT trial were neutropenia (51% with SG versus 33% with chemotherapy), leukopenia (10% versus 5%), diarrhea (10% versus <1%), anemia (8% versus 5%), and febrile neutropenia (6% versus 2%) (3). A post-hoc analysis of the ASCENT trial conducted for correlation with Trop-2 expression levels, revealed neutropenia in 63% of Trop-2 high cases, diarrhea in 68% and nausea in 57%. Comparable findings were obtained in Trop-2 medium cases (neutropenia, 59%; diarrhea, 53%; nausea, 63%), and in Trop-2 low patients (neutropenia, 71%; diarrhea, 54%; nausea, 64%). Hence, systemic adverse events did not appreciably vary according with the Trop-2 status of TNBC (5).

In the NSCLC trial the most common adverse reactions (≥25% of patients) were nausea, neutropenia, diarrhea, fatigue, anemia, vomiting, alopecia (11). Limited, if any, skin rash and mucosal inflammation were observed both in the ASCENT TNBC trial (3) and in the NSCLC trial (11). This is in stark contrast with skin rash and mucosal inflammation being dose-limiting toxicities in the clinical trial on the PF-06664178 anti-Trop-2 antibody (12). These appeared as on-target/off-cancer effects, related to the expression of Trop-2 by normal tissues (4,13,14), although it should be noted that the PF-06664178 payload is a Dolastatin 10 analogue, which is akin to the more potent auristatin microtubule inhibitors. Systemic release of the SN38 payload, rather than on-target/off-cancer binding, was proposed to induce a considerable fraction of SG side effects (15). However, dose-limiting neutropenia after a first cycle of SG in patients was not found to correlate with SN-38 levels in serum (16), and most SN-38 was found still bound to SG at 6 h after a single dose (0.5 mg) of SG, although at this time only a very limited amount of SG was detectable in the tumor (17).

SN-38 is a hydrophobic payload. A carbamate bond on the dipiperidino side chain was added to SN-38 to improve solubility before conjugation to HuRS7. The carbamate bond is subject to cleavage by carboxylesterases and to subsequent glucuronidation by UGT1A1. Polyethylene glycol groups were added to SN-38 to increase its solubility. A protease site was inserted in the SG linker to allow cleavage by lysosomal enzymes, and intracellular release of SN-38 upon SG internalization in tumor cells. A maleimide group was inserted to bind the sulfhydryl groups generated by reduction of mAb inter-chain disulphide bridges (18). This procedure is led to completion, and removes all covalent bonds holding together the antibody constituent chains (19).

Since SN-38 is active in the S-phase of the cell-cycle, maintaining low concentrations of SN-38 in tumors over prolonged periods was considered advantageous to improve potency (18). However, the tumor uptake of 131I-RS7 only is 7–16% of the administered dose, a ~2-fold higher rate than an irrelevant control 131I-mAb (20). Further, the linker chemical modifications reduce the half-life of SG to 11–14 hours in plasma (16). Thus, administration of SG was indicated to lead to considerable systemic release of SN-38, rather than to a sustained release of the drug inside the targeted tumor (15).

The SG-targeted epitope in Trop-2 may further limit its efficacy. The RS7 mAb was shown to bind the same epitope...
as the T16, 162-46.2 (13,21) and E1 mAb (6). Hence, RS7 adds up to the list that includes most anti-Trop-2 antibodies, among them MOv16 (13), cAR47A6.4.2 (22), 77220, MM0588, YY-01 (21), which were shown to bind an immunodominant epitope (13,21), located in the N-terminal region of the stem domain of Trop-2 (D146-T274) (21). This epitope was shown to be equally accessible in cancer cells and in normal tissues (4,13,14,23), thus raising issues on a lack of cancer specificity (24). The Rinat-Pfizer RN926 anti-Trop-2 mAb, which was developed in the PF-06664178/Aur0101 ADC, was also shown to bind this immunodominant region of Trop-2 (domain 3, residues 152-206, and domain 4, residues 209-274; WO 2013/068946). PF-06664178 had shown early promise (25). A Phase I, open-label, dose-escalation study of PF-06664178 was conducted in patients with advanced solid tumors. Doses of 3.60, 4.2 and 4.8 mg/kg were found to be intolerable, due to skin rash, mucosal lesions and neutropenia. PF-06664178 showed modest antitumor activity, and was ultimately discontinued (12). Hence, exposure of normal tissues to anti-Trop-2 ADCs bearing high-potency payloads can lead to unmanageable toxicity.

In contrast with PF-06664178, SG was suggested to possess an appropriate compromise between a moderately-stable linker, with a short half-life in the circulation, a high drug-antibody ratio, and a payload with relatively low toxicity (\( \text{in vitro IC}_{50} \approx 1.0–6.0 \, \text{nM} \)), which allowed repeated, high dosing to patients (18). However, this design leads to a much more rapid linker hydrolysis and toxic payload release for SG than for other ADCs (26). Hence, lower toxicity toward normal tissues may come at the price of lower potency of SG (18). Experimental evidence on the Datopotamab deruxtecan ADC (a humanized anti-Trop-2 mAb conjugated to a different, camptothecin-derived, topoisomerase I inhibitor) (27) may contribute shedding light on some of these issues.

Taken together, these findings suggest that fundamental improvements in anti-Trop-2 therapy require better selectivity for/preferential targeting of Trop-2 in cancer cells. An interesting lead was recently provided by evidence that Trop-2 is activated by proteolysis by ADAM10. This then drives colon cancer malignant progression (6), through cleavage of E-cadherin and inhibition of cell-cell adhesion (28). Trop-2 cleavage by ADAM10 does not occur in normal tissues (6,28). Recent experimental findings support this lead, to allow for selective targeting of Trop-2 in cancer cells, while sparing toxicity towards normal cells (29,30).

**Conclusions**

SG has paved the way for a broad utilization of Trop-2-targeting in multiple Trop-2-expressing cancers. However, SG has a short half-life in patients (16), and frequent dosing is required. The down-side of this approach is induction of side effects, such as neutropenia and diarrhea, which have been suggested to be due to the release of SN38 as a free drug in the circulation (15).

Follow-up anti-Trop-2 therapies are now underway, that plan to exploit different ADC linker structure and conjugation chemistry and more potent payloads. Further down-the-line approaches are expected to exploit strategies for preferential targeting of cancer cleaved/activated Trop-2 versus the native form expressed in normal cells (6,28).

In summary, SG effectiveness has shown that Trop-2 targeting is a valuable therapeutic strategy in advanced/metastatic cancer. However, critical pitfalls remain in current targeting approaches. Novel insight into Trop-2 biology may provide the rationale for next-generation ADC design, and improved impact of Trop-2-targeting therapy.

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appropriately investigated and resolved.

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