Background Sacituzumab govitecan (SG, Trodelvy®) is a TROP-2-directed antibody-drug conjugate (ADC) coupled to an active form of irinotecan (SN-38) via novel hydrolysable linker (CL2A). SG was approved for adult patients with locally advanced or metastatic urothelial cancers after 2 lines of therapy. TROP-2 is also highly expressed by non-muscle invasive bladder cancers (NMIBC) and treatment options remain limited for BCG-unresponsive NMIBC. We developed model systems to address SG biology and efficacy during short cell exposure and to evaluate SG intravesicular instillations as a treatment option for high-risk NMIBC patients.

Methods Binding affinity of SG and its naked antibody moiety hRS7, was determined by Surface Plasmon Resonance. SN38 release from SG was measured in buffers (pH 6-8) and human urine. SG internalization was visualized by confocal microscopy and Incucyte. SG cytotoxicity after continuous vs. pulse exposure of NMIBC cells was assayed by proliferation assays and DNA damage quantification (γH2Ax staining). SG efficacy was measured by in vivo imaging in sub-cutaneous vs. orthotopic NMIBC xenografts (UM-UC3-hTROP-2^+/-luc^+) after intraperitoneal injections vs. intravesicular instillations, respectively.

Results hRS7 and SG bound mammalian-expressed human TROP-2 protein with similar affinity (K_D=0.33 and 0.36 nM, respectively). Free SN-38 conversion from SG was below 3.5% after 2 hours in buffer pH 6 to 8 and in human urine at 37°C. Loss of cell viability and enhanced DNA damage were demonstrated in TROP-2^high NMIBC cells after both continuous and short exposures to SG. In TROP-2^high cells, SG 2-fold more potent than control ADC after 2h-pulsed exposure (EC_50, p = 0.0223) and SG mediated more DNA damage than control ADC after 30 min-pulsed exposure (p < 0.0001). Moreover, SG rapidly internalizes in TROP2-positive cells (Incucyte) and, after 1-hr incubation at 37°C of RT112, 5637, and TROP-2-transduced UM-UC-3 NMIBC cells, confocal analysis demonstrated diminishing SG signal at the cell surface and appearance of SG-positive lysosomes (LAMP1^+) along with vesicles positive for both TROP2 and SG. Finally, SG produced antitumor effects in both subcutaneous and orthotopic xenografts models (vehicle vs. SG p <0.0001).

Conclusions SG colocalization with intracellular TROP-2 and LAMP1 is consistent with prompt SG internalization and SN-38 intracellular release and suggests an additional mechanism of action of SG via TROP-2-dependent endocytosis. Altogether, our results support the hypothesis that intravesicularly-delivered SG can release SN-38 payload in bladder tumor before voiding and indicate that SG instillations in bladder may offer a therapeutic option to high-risk NMIBC patients.

Ethics Approval All animal experiments were approved by the Institutional Animal Care and Use Committee of Gilead Sciences Inc. and carried out by certified staff in an Association for Assessment and Accreditation of Laboratory Animal Care International (AAALACI) accredited facility, in compliance with the institution’s Guide for the Care and Use of Laboratory Animals and applicable laws (including the Animal Welfare Act of 1966 and, if applicable, the California Laboratory Animal Use Approval Program).