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Title of Protocol:
A pilot single arm trial with Sacituzumab Govitecan as neoadjuvant therapy in pts with non-urothelial muscle invasive bladder cancer

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Sponsor: Petros Grivas, MD, PhD
IND number: IND exempt
Institutional Protocol Number: RG1122399
Investigational agent: **Sacituzumab Govitecan (SG)**

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Gilead Sciences

Table of Contents

ABBREVIATIONS

AE	*Adverse Event*
ADA	*Anti-drug Antibodies*
ADC	*Antibody-Drug Conjugate*
ALT	*Alanine Aminotransferase*
ANC	*Absolute Neutrophil Count*
aPTT	*Activated Partial Thromboplastin Time*
AST	*Aspartate Aminotransferase*
AUC	*Area Under the Curve*
BUN	*Blood Urea Nitrogen*
CBC	*Complete Blood Count*
CBR	*Clinical Benefit Rate*
CFR	*Code of Federal Regulation*
CI	*Confidence Interval*
Cl	*Chlorine*
CL2A	*Coupled by a Proprietary Hydrolysable Linker 2*
CNS	*Central Nervous System*
CO2	*Carbon dioxide*
CR	*Complete Response*
CRF	*Case Report Form*
CT	*Computed Tomography Imaging*
CTCAE	*Common Terminology Criteria for Adverse Events*
DLT	*Dose-Limiting Toxicity*
DN	*A Deoxyribonucleic Acid*
DOR	*Duration of Response*
EC	*Ethics Committee*
ECG	*Electrocardiogram*
ECOG	*Eastern Cooperative Oncology Group*
eCRF	*Electronic Case Report Form*
EOT	*End of Treatment*
FDA	*Food and Drug Administration*
GCP	*Good Clinical Practice*
GCSF	*Granulocyte Colony-Stimulating Factor*
GI	*Gastrointestinal*
HIPAA	*Health Insurance Portability and Accountability Act of 1996*
IB	*Investigator Brochure*
ICH	*International Committee on Harmonization*
IEC	*Independent Ethics Committee*
IgG	*Immunoglobulin G*
IMP	*Investigational Medicinal Product*
IND	*Investigational New Drug*
INR	*International Normalized Ratio*
IRB	*Institutional Review Board*
iRECIST	*Modified RECIST 1.1 for Immune-Based Therapeutics*
IRR	*Infusion-Related Reaction*
IV	*Intravenous*
K	*Potassium*
LDH	*Lactate Dehydrogenase*
mAb	*Monoclonal Antibody*
MedDRA	*Medical Dictionary for Regulatory Activities*
MRI	*Magnetic Resonance Imaging*
MIBC	*Muscle invasive bladder cancer*
mUC	*Metastatic Urothelial Cancer*
Na	*Sodium*
NCI	*National Cancer Institute*

NSCLC	*Non-Small Cell Lung Cancer*
ORR	*Objective Response Rate*
OS	*Overall Survival*
pCR	*Pathologic complete response*
PD	*Progressive Disease*
PD-1	*Anti-programmed Cell Death Protein 1*
PD-L1	*Anti-programmed Death Ligand 1*
PFS	*Progression-Free Survival*
PK	*Pharmacokinetics*
PR	*Partial Response*
PT	*Prothrombin time*
RECIST 1.1	*Response Evaluation Criteria in Solid Tumors Version 1.1*
RP2D	*Recommended Phase 2 Dose*
SAE	*Serious Adverse Event*
SCLC	*Small Cell Lung Cancer*
SD	*Stable Disease*
SG	*Sacituzumab Govitecan*
SmPC	*Summary of Product Characteristics*
SOC	*System Organ Class*
StD	*Standard Deviation*
SUSAR	*Suspected Unexpected Serious Adverse Reaction*
Trop-2	*Trophoblastic Cell-Surface Antigen*
TNBC	*Triple-Negative Breast Cancer*
TSH	*Thyroid-Stimulating Hormone*
TURBT	*Transurethral Resection of Bladder Tumor*
UC	*Urothelial Cancer*
UGT1A1	*Uridine diphosphate-glucuronosyltransferase 1A1*
ULN	*Upper Limit of Normal*
WBC	*White Blood Cells*

1.0 GENERAL INFORMATION

Protocol Title	**A pilot single arm trial with Sacituzumab Govitecan as neoadjuvant therapy in pts with non-urothelial muscle invasive bladder cancer**
Protocol Number	*RG1122399*
Protocol Sponsor	*Petros Grivas, MD, PhD*
Trial Type	*Pilot trial*
Clinical Indication	*Neoadjuvant therapy for non-UC MIBC*
Trial Blinding	*None*
Type of control	*None*
Investigation Drug	*Sacituzumab Govitecan*
Route of administration	*IV*
Treatment Groups	*Single arm of patients with new diagnosis of non-UC MIBC*
Treatment Schedule	*Days 1 & 8 every 21 days x 3 cycles*
Primary Endpoint	*pCR*
Number of trial subjects	*14-18*
Estimated enrollment period	*18-27 months*
Estimated duration of trial	*2-3 years*
Duration of Participation	*9 weeks of neoadjuvant treatment (up to 2 years follow-up)*

2.0 INTRODUCTION TO THE PROTOCOL

2.1 Introduction

2.1.1 Bladder Cancer

Bladder cancer (BC) is one of the most common malignancies in the US with an estimated number of 81,400 new cases and 17,980 deaths in 2020. It is the 4th most common cancer in men and there are >500,000 patients with BC alive in the US; it accounts for about 5% of all new cancers in the US.[1] Almost 25% of patients with BC present with MIBC; among the ~65% of patients presenting with non-muscle-invasive bladder cancer (NMIBC), up to 15-20% may progress to MIBC every year.[2] Radical cystectomy (RC) is the standard of care for MIBC, however it results in a 5-year recurrence-free survival rate of 68% (only 35% for those with lymph node involvement). This is probably due to micro-metastases present at the time of surgery considering the high rate of distant recurrence.[3,4] Systemic cisplatin-based combination neoadjuvant chemotherapy prior to RC results in high pathologic complete response (pCR) rates of (30-35%) and clinically meaningful increases in overall survival (OS) compared to local therapy alone and has become the standard of care for cisplatin-eligible patients with urothelial histology MIBC.[5-7]

2.1.2 Variant Histology Bladder Cancer

Most MIBC cases, approximately 75-80%, consist of pure or predominantly urothelial carcinoma (UC), while the remaining 20-25% consist of predominantly other histologic variants.[8] The presence of squamous cell or glandular features admixed with predominant UC histology pattern did not compromise pCR rates with neoadjuvant cisplatin-based combination chemotherapy.[9] However, the data are not that clear with other histologic variants, and also when non-UC variants represent the predominant histology of MIBC. Several different types of urothelial and non-urothelial variants have been described and are comprised of a variety of histologic types, including pure squamous cell carcinoma, pure adenocarcinoma/glandular features, small cell/neuroendocrine carcinoma,

sarcomatoid, micropapillary, plasmacytoid, nested, among others.[8] These non-UC variants may not respond well to cisplatin-based chemotherapy as discussed below and represent a major unmet clinical need.

2.1.3 Neoadjuvant therapy options for non-UC MIBC

Currently, there is no standard of care systemic neoadjuvant therapy for these variant cases. Our retrospective analyses showed that pathologic complete response (pCR) to cisplatin-based neoadjuvant chemotherapy in plasmacytoid urothelial carcinoma (PUC) compared to conventional urothelial carcinoma (CUC) was 10% vs 33%, respectively (p=0.03)[10], pCR to cisplatin-based NAC of sarcomatoid urothelial carcinoma compared to CUC was 6% vs 35% (p=0.02)[11]. A retrospective study of patients with squamous cell BC treated at Cleveland Clinic showed no pCR (out of five cases) to cisplatin-based NAC.[12] Several studies showed the relatively poor response of variant histology to cisplatin-based neoadjuvant chemotherapy.[10-12] This represents a major unmet clinical need for novel neoadjuvant regimens that can eradicate micro-metastasis, downstage the primary tumor and improve outcomes. A moderately effective agent could even lead to an accelerated approval of safe compounds in this challenging "orphan disease" setting of non-urothelial variant bladder cancer.

2.1.4 TROP-2 Expression in Bladder Cancer

Human trophoblast cell surface antigen 2 (Trop-2), a 40-kDa protein encoded by the *TACSTD2* gene, is a transmembrane glycoprotein that is highly expressed on the surface of most epithelial cancer cells.[13-15] This transmembrane glycoprotein functions in a variety of cell signaling pathways and was first elucidated as a transducer of an intracellular calcium signal.[16] Trop-2 expression has been demonstrated to depend on a large variety of transcription factors. Trop-2 also plays a key role in cell transformation and proliferation. Elevated Trop-2 expression is associated with poor prognosis for several cancer types, including mUC.[17-22] Trop-2 expression was found to be significantly higher in invasive BCs compared both with normal bladder and with non-invasive BCs, thus positively correlating is associated with cancer severity.[17]

The current literature evaluating TROP2 expression in variant histology bladder cancer is limited. However, TCGA BLCA provided data of TACSTD2 (TROP2) gene expression across several bladder cancer variant histologic types (Figure 1). While the sample sizes are relatively small, several variants have notable TROP2 gene expression, such as plasmacytoid, squamous cell and micropapillary. Neuroendocrine/small cell variant did not seem to exhibit any TROP2 gene expression. Interestingly, in collaboration with the University of Wisconsin, we have shown notable expression of TROP2 protein in circulating tumor cells from patients with metastatic bladder cancer, also in the context of tumor heterogeneity.[23] Within our own institution, evaluation of TMA expression in variant histology tissues, specifically squamous cell and plasmacytoid urothelial variants, revealed similar membrane and cytoplasmic expression of TROP2 (based on H-score) as compared to conventional UC, as shown in Table 1. The exception to this is neuroendocrine variant, which had lower expression of TROP2, consistent with other known studies.[24] A pooled analysis from cohorts 1 and 2 of the TROPHY-U-01 trial evaluated Trop-2 protein expression measured by H-score (specific staining intensity; intensity multiplied by proportion of cells with Trop-2 staining) and its association with outcomes with sacituzumab govitecan (SG) monotherapy in patients with previously treated mUC. Out of 92 patients with evaluable staining, 61 had high, 26 had medium and only 5 had low Trop-2 expression. Patients with high Trop-2 expression (H-score >200-300) had ORR 34% (95%CI 23-48), those with medium expression (H-score ≥100-200) had ORR 27% (95% CI 12-48) and those with low expression (H-score <100) had ORR 20% (95% CI 1-72). A patient with low H-score (15) achieved partial response. PFS and OS appeared similar between patients with medium and high Trop-2 expression. Overall, the analysis from this phase II clinical trial showed that activity of SG was noted across Trop-2 protein expression level, including low expression (H-score <100).[25]

Figure 1. TCGA BLCA data of TROP2 gene expression across several bladder cancer variant histologic types

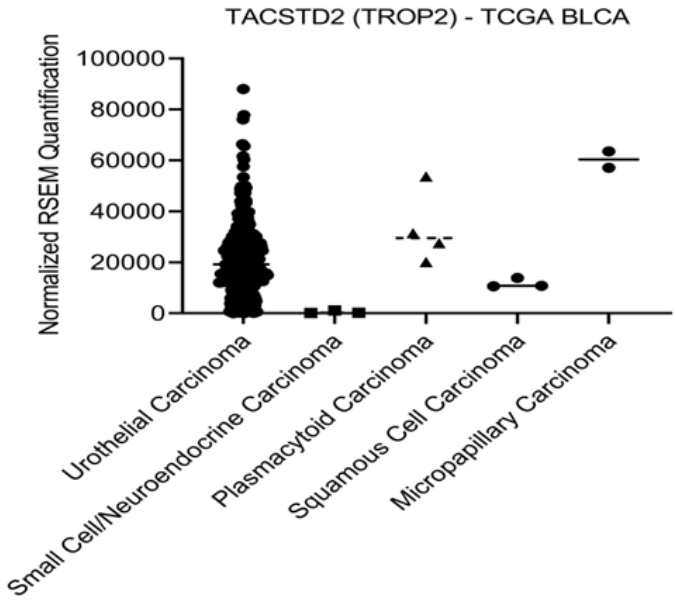


Figure 2. Preliminary TAN TMA for TROP 2 expression[24]

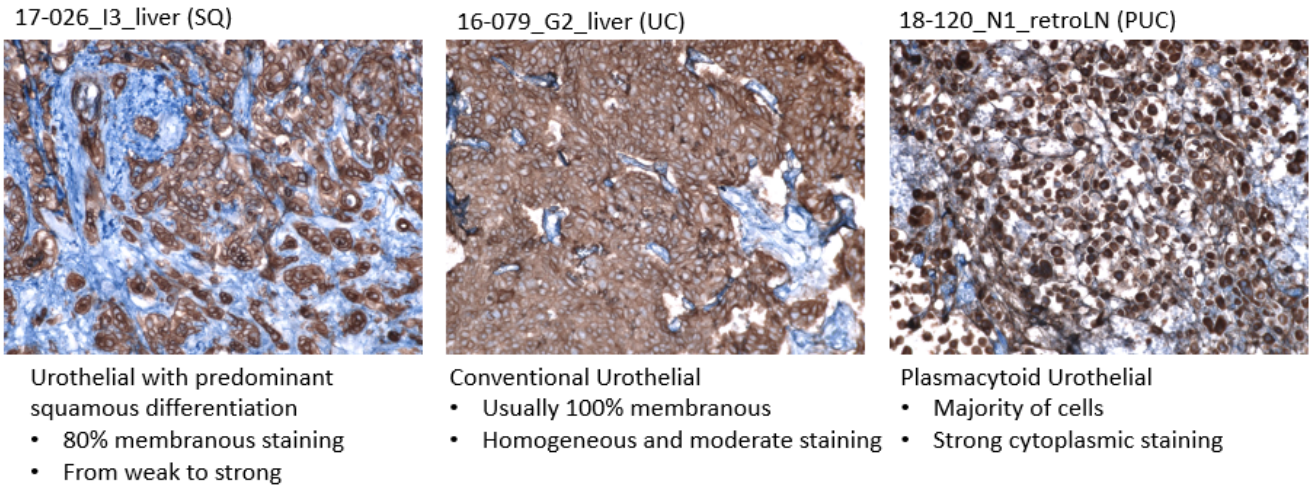


Table 1: UW TAN TMA expression scores for BC histologic variants[24]

Histologic Variant	Tissue	Membrane H-Score (median)	Cytoplasm H-Score (median)
Plasmacytoid	Liver	240	250
Plasmacytoid	L4	190	170
Plasmacytoid	LN	160	200
Plasmacytoid	Primary	200	125
Plasmacytoid	Mesentery	230	210
Plasmacytoid	Primary	160	120
Plasmacytoid	Vena Cava	300	200

Plasmacytoid	Omentum	220	200
Plasmacytoid	Rectal	30	300
Plasmacytoid	Primary	270	250
Plasmacytoid	LN1	15	280
Plasmacytoid	RP LN	110	200
Plasmacytoid	Iliac LN	30	220
Plasmacytoid	Primary	60	250
Squamous cell	Liver	120	180
Squamous cell	LN	200	200
Squamous cell	Lung	200	80
Squamous cell	L2	250	200
Squamous cell	Liver	300	120
Squamous cell	Omentum	240	70
Squamous cell	Adrenal	200	210
Squamous cell	Primary	280	30
Squamous cell	Small bowel	275	145
Squamous cell	Liver	180	100
Squamous cell	Liver	220	200
Squamous cell	Peri colonic	160	110
Squamous cell	LN	220	200
Squamous cell	Primary	160	200
Squamous cell	Primary	150	95
Squamous cell	Adrenal	290	220
Neuroendocrine	LN	0	15
Neuroendocrine	Lung	0	5
Neuroendocrine	LN	0	90
Neuroendocrine	Liver	0	30
Neuroendocrine	Pancreas	0	45

2.1.5 Sacituzumab Govitecan

Sacituzumab govitecan is a novel Trop-2 directed antibody-drug conjugate (ADC) composed of the following three compounds: 1)The humanized monoclonal antibody, hRS7 IgG1k, which binds to Trop-2 (trophoblastic cell surface antigen, also known as EGP-1, epithelial glycoprotein-1) 2) The camptothecin-derived agent, SN-38, a topoisomerase 1 inhibitor, with a high drug to antibody ratio (7.6 molecules of SN-38 per antibody) 3) A hydrolysable, proprietary linker, CL2A, which binds SN-38 to the antibody.

SG is prepared at a ratio of 7 to 8 molecules of SN-38 per molecule of the anti-Trop-2 antibody, hRS7.[26] For clinical use, 10 mg/mL are formulated in 25 mM MES buffer, pH 6.5, together with the other excipients (25 mM trehalose, 0.01% Polysorbate 80). It is then lyophilized at 200 mg/vial. IMMU-132 is supplied in 50-mL clear glass vials to be stored under refrigerated conditions (2-8C) until used. Since the formulated drug product contains no preservative, vials should be used only once and within eight hours of reconstitution.

Internalization of Trop-2–bound SG delivers SN-38 inside tumor cells, thereby killing the tumor cells[27] while the hydrolysable linker enables SN-38 to be released into the tumor microenvironment, killing adjacent tumor cells (bystander effect). Various mechanisms conferring resistance to SG have been proposed and are currently being investigated, including parallel genomic alterations in both antibody and payload targets. A study demonstrated the T256R missense mutation in TACSTD2/TROP2 confers SG resistance via defective plasma membrane localization and reduced cell surface binding by hRS7.[27]

2.2 Preclinical Data

The anti-tumor activity of the hRS7-SN-38 conjugate was examined in mice bearing a variety of human epithelial cancer xenografts, where it was shown to have significant anti-tumor effects compared to free SN-38, irinotecan, or an irrelevant IgG-SN-38 conjugate.[26,28,29] There were significant changes in liver transaminases that were returning to normal levels 2 weeks after treatment in mice.[28]

While Trop-2 is highly conserved among various species, the hRS7 antibody does not bind to murine Trop-2, but an immunohistology study of cynomolgus monkey tissues showed hRS7 bound to Trop-2 and had a similar distribution as found in humans.[28] Thus, a toxicology study was performed with IMMU-132 in monkeys with the purpose to assess the dose-limiting toxicity and to determine if Trop-2 expressing normal tissues would limit the use of this conjugate. Two doses were administered within one week (3 days apart) at a cumulative amount of 120 and 240 mg/kg (1.92 and 3.84 mg/kg of SN-38; human equivalent dose of the conjugate was 38.7 and 77.4 mg/kg). Pharmacokinetic analysis of the clearance of the hRS7 IgG and SN-38 (as free SN-38, total SN-38, and a derived IgG-bound SN-38) showed the IgG cleared at what appeared to be an expected rate (e.g., 30% cleared over 1 day, terminal half-life ~5 days). The clearance parameters for the total and IgG-bound SN-38 were very similar, with a half-life of ~13 h, which reflects the clearance of the IgG and the fact that the SN-38 is released from the conjugate at a rate of ~50% every 20 h. As expected, the AUC for the total and IgG-bound SN38 was nearly 15-times higher than the free SN-38, which had a half-life of ~25 h, similar to the rate reported for SN-38 released from irinotecan. Thus, the conjugate is capable of liberating low concentrations of SN-38 in the serum at a slow and sustained rate.

In cynomolgus monkeys administered the cumulative dose of 120 mg/kg, there was evidence of myelosuppression within 3 days, but the decrease in counts did not achieve gradable levels and were completely restored within 10-14 days. No significant change in serum chemistries or tissue pathology was noted. At 240 mg/kg, severe gastrointestinal (GI) and hematological toxicities occurred; therefore, the maximum tolerated dose had been exceeded. The conjugate displayed a similar toxicity profile as irinotecan, with major GI and hematological toxicity. Importantly, there were no serious histopathological changes to tissues with known hRS7 binding in the monkey, with the exception of mild to moderate hemorrhage of the endometrium and atrophy of the endometrial glands that were showing recovery at the time the study was terminated. (See Investigator Brochure for additional information on IMMU-132)

In vitro studies in 2 triple-negative breast cancer cell lines, one expressing Trop-2 and another being Trop-2-negative, showed that double-stranded DNA breaks only occurred in the Trop-2- expressing cell line after a brief exposure (1 h) with IMMU-132.[26] This study confirmed that SN-38 delivery by the specific IMMU-132 conjugate was necessary for the therapeutic effect, with a non-targeting conjugate having nearly 3-fold less double-stranded DNA breaks than IMMU-132-treated Trop-2-expressing cells. In mice bearing Trop-2-expressing human tumor xenografts that were given irinotecan or IMMU-132, SN-38 levels in the tumors exposed to IMMU-132 were as much as 130-fold more than in the irinotecan-treated animals, illustrating the superior targeting of the conjugate to deliver SN-38 to tumors.[30]

2.3 Clinical Data to Date

2.3.1 Study of IMMU-132-01

IMMU-132-01 was a single-arm, multicenter Phase I/II trial of SG comprising a dose-escalation and cohort-expansion phase. The study was designed as a basket trial for subjects with advanced relapsed/refractory metastatic epithelial cancers, including triple negative breast cancer (TNBC), as well as ovarian, prostate (castrate-resistant), lung (non-small cell and small-cell lung cancer [NSCLC, SCLC]), head and neck (squamous cell), esophageal, gastric, colorectal, pancreatic, hepatocellular, renal (clear cell) and urinary bladder cancers. In the completed Phase I part of the trial, the maximum tolerated dose of SG as well as a maximum acceptable dose were established.

2.3.1.1 Phase I Study of IMMU-132-01

At the starting dose level of 8.0 mg/kg, 3 patients were treated without DLT, dose delays, or reductions. At the next dose level of 12 mg/kg, 9 patients were enrolled because protocol-required delays in administering the second dose were encountered. All but one of these patients received 12 mg/kg as their second dose. Four of the 9 patients at the 12 mg/kg dose level had the third dose that started the second cycle decreased to 9 mg/kg, and the second cycle was delayed an additional week in 3 patients. None of the 9 patients had a dose-limiting event during the first cycle and therefore accrual to the 18 mg/kg dose level was allowed.

Here, all 3 patients had dose delays after their first treatment, with only one patient receiving the second treatment at 18 mg/kg. Two patients had dose-limiting grade 4 neutropenia, 1 after the first dose, the other after the second 18 mg/kg dose, with this latter patient also experiencing grade 2 diarrhea. Therefore, with 0 of 9 patients having DLT in the first cycle at 12 mg/kg, this level was declared the MTD for a single-cycle regimen.

Additional dose-finding studies continued to refine the dose level that would allow multiple cycles to be given with minimal delay between treatments/cycles. Therefore, 4 more patients were enrolled at the 8 mg/kg dose level, and a new intermediate level of 10 mg/kg was opened. Of the initial three patients enrolled at 8 mg/kg, two patients continued treatment at 8 mg/kg for a total of 31 and 11 treatments, while another received three 8 mg/kg doses before dose reduction to 6 mg/kg. The additional 4 patients received 3 to 9 doses of 8 mg/kg before withdrawing with disease progression. Two of these patients received only a dose before a protocol-required reduction to 6 mg/kg, because of a grade 2 rash and neutropenia.

Five of the 6 patients enrolled at 10 mg/kg received 6 to 30 doses without reduction before withdrawing due to disease progression. One patient developed grade 3 febrile neutropenia after receiving 1 dose. Ultimately, the patient had rapid deterioration and died 4 weeks from the first dose.

Thus, while the overall results supported 12 mg/kg as the maximum tolerated dose (MTD), this dose was associated with dose delays and reductions in several subjects. A SG dose of 10 mg/kg was found to be safe and efficacious and is the recommended starting dose for subjects with normal hepatic function and mild hepatic impairment.[31-32]

2.3.1.2 Phase II Study of IMMU-132-01

The Phase II part of the study was designed to investigate the safety and activity of SG in the above-listed cancers. In the TNBC cohort, 69 patients with heavily treated disease, the confirmed objective response rate of SG was 30% (partial response, n = 19; complete response, n = 2), median response duration was 8.9 (95% CI, 6.1 to 11.3) months, and the clinical benefit rate (complete response + partial response + stable disease ≥ 6 months) 46%. These responses occurred early, with median onset of 1.9 months. Median progression-free survival was 6.0 (95% CI, 5.0 to 7.3) months, and median overall survival was 16.6 (95% CI, 11.1 to 20.6) months. SG induced early and durable responses in heavily pretreated patients with metastatic TNBC.[33] This led to Food and Drug Administration (FDA) granting Breakthrough Therapy Designation to SG for the treatment of subjects with relapsed/refractory metastatic TNBC who have received at least 2 prior therapies for metastatic disease

In the mUC cohort, 44 cases were enrolled and assigned to the 10 mg/kg dose, of which 42 had progression after platinum-based therapies, and 15 had progression after anti-PD-1/PD-L1 as well. The overall response rate was 32% with median PFS of 7.2 months. Subjects who were treated in second line (17 cases) or third line (11 cases) had 41% and 36% response rate with median PFS of 9.5 and 7 months, respectively. Subjects previously treated with platinum-based chemotherapy and anti-PD-1/PD-L1 had received 3 to 7 lines and had 25% response rate (16 cases). Median response duration was 6 months with follow-up still ongoing at that time. This showed clinically significant durable responses even in a population who had received 2 prior treatments.[34]

The two most notable AE were neutropenia and diarrhea. Dose reduction occurred because of neutropenia or febrile neutropenia in 9/44 (20%) cases, 8 times within the first 6 cycles, generally the 2nd or 3rd, and a second

dose reduction was needed in 3 cases. In all other cases (6) dose reduction was related to general tolerance probably related to the subjects' advanced disease, except a case related to liver function test elevation. Diarrhea occurred in 40 out of 44 subjects, however, when evaluated at Day 1 or Day 8 of cycle, diarrhea occurred after 317 of 860 (37%) injections and was Grade 1 in 95% of the occurrences. In half of the subjects, diarrhea was described as "continuing" i.e., recurring from an administration to the next. More severe diarrhea was always delayed: 8 Grade 2 diarrhea events were reported at an average of 9 days after the injection and lasted on average 3 days, and 5 occurrences of Grade 3 events of diarrhea started on average 6 days later with a duration of 2.5 days. These episodes occurred in 9 subjects (of 44), 5 had a single episode, and 4 had 2 consecutive episodes. All occurred within the first 6 cycles with no particular pattern. Thus, subjects should be educated about this possible AE and advised to promptly take antidiarrheal medication.[32]

2.3.2 TROPHY-U-01 Trial

TROPHY-U-01 is a phase II trial assessing the activity of SG in patients with locally advanced unresectable or mUC. SG 10 mg/kg was administered intravenously on days 1 and 8 in a 21-day treatment cycle, until unacceptable toxicity, loss of clinical benefit, or withdrawal of consent. This phase II trial includes 5 cohorts and data have been presented from cohorts 1 (full cohort) and 2 (interim) so far.

Cohort 1:
A total of 113 subjects with baseline tumor assessment (78% men; median age 66 [range: 33 - 90 years], 66% visceral metastases; 34% liver metastases; received a median of 3 [range: 1 to 8] prior therapies) were treated with SG starting at 10 mg/kg IV on Days 1 and 8 of a 21-day cycle. Central review confirmed ORR 27% (31/113) with 6 complete responses (CRs) and 25 partial responses (PRs). Most subjects (77% [72/94]) had target lesion reduction. With median follow-up duration of 9.1 months (range: 0-19.9 months), median DOR was 7.2 months and CBR 37%; median PFS and OS were 5.4 months and 10.9 months, respectively. Key Grade ≥3 treatment-related AEs were neutropenia (35%), anemia (14%), febrile neutropenia (10%), and diarrhea (10%), there was 1 treatment-related death (neutropenic sepsis).[35] Based on the data, SG received accelerated FDA approval in April 2021 in patients with mUC whose cancer had progression after platinum-based chemotherapy and anti-PD1/PDL1 therapy.[36] A phase 3 (TROPiCS-04) trial has been launched comparing SG to either taxane or vinflunine in patients with mUC and progression after chemotherapy and anti-PD1/PDL1.[37]

Cohort 2:
Twenty-one subjects with baseline tumor assessment (52% men; median age 76 years [range: 57 to 87 years], 67% visceral metastases; 24% liver metastases; received a median of 2 [range: 1 to 5] prior therapies) have been treated with SG at 10 mg/kg IV on Days 1 and 8 of a 21-day cycle. At a median follow-up of 6.8 months, ORR was 29% (6/21) with 6 confirmed PRs. The median PFS was 5.5 months and the OS rates at 6 and 12 months were 76.4% and 43.0%, respectively. Most subjects (62% [13/21]) had target lesion reduction. Key Grade ≥3 treatment-related AE were neutropenia (39%), fatigue (33%), diarrhea (28%), leukopenia (22%), anemia (17%), and febrile neutropenia (11%). No event of interstitial lung disease, ocular toxicities, or Grade >2 neuropathy was reported. There were no treatment related deaths.[38]

2.4 Dose Rationale

Most patients in IMMU-132-01 study have been treated with 8 or 10 mg/kg doses. There was no major difference in safety between these two doses, but there was a trend for better efficacy with 10 mg/kg dosing, which was therefore selected for the phase II studies. 10 mg/kg dosing is the FDA-approved dosage for treatment use.

2.5 Risks/Benefits

Based on the efficacy (ORR, DOR, PFS, OS), manageable safety/toxicity profile of SG across clinical trials and its FDA approval in mUC, as well as the robust gene and protein Trop-2 expression data in variant histologic types of BC, the benefits of 3 cycles of SG clearly outweigh possible risks as neoadjuvant therapy in variant histology BC that represents an "orphan" disease and major unmet clinical need with very challenging prognosis so far.

3.0 OVERVIEW OF CLINICAL TRIAL

3.1 Study Objectives

3.1.1 Primary Objectives

To evaluate the antitumor efficacy of neoadjuvant SG as measured by the rate of patients achieving pCR (defined as ypT0N0) at radical cystectomy (RC) in non-UC variant histologic types.

3.1.2 Secondary and Exploratory Objectives

1. To assess the frequency and severity of adverse events according to Common Terminology Criteria for Adverse Events version 5.0 (CTCAE v5.0) in patients treated with SG
2. To assess the 2-year recurrence-free survival (RFS) rate, defined as time from trial enrollment to recurrence or death at the 2-year landmark time point
3. To assess overall survival (OS) rate at the two-year landmark time point in patients treated with SG
4. To assess the rate of pathologic downstaging to <ypT2N0 stage at time of radical cystectomy
5. To assess putative biomarkers (if possible), such as Trop-2 protein expression, tumor mutational burden, MSI status, PD-L1, TILs, gene expression and signatures, intrinsic molecular subtypes, homologous recombination deficiency, loss of heterozygosity, DNA damage response gene alterations, and their relationship with pCR, 2-years RFS and 2-year OS rates
6. To describe (if possible) the relationship between pre-treatment microbiome and microbiome change, pathologic partial and complete response as well as 2-year RFS and 2-year OS rates

3.2 Study Population: Patients with muscle invasive bladder cancer with non-urothelial variant histology
3.3 Study Design: Single center, single arm, unblinded, with neoadjuvant SG; primary endpoint: pCR (ypT0N0)
3.4 Estimated Accrual: 14-18 patients
3.5 Name of Sponsor Investigator: Petros Grivas, MD, PhD
3.6 Funding Source: Gilead Sciences

4.0 SAFETY CONSIDERATIONS

4.1 Stopping Rules

Due to the very small sample size and existing safety data no interim stopping rules were included in the statistical considerations (see statistical section 18.3). However, the experienced research team, statistician and PI will monitor very closely the trial, which will comply with the standard guidelines set forth by review committees and other institutional, state, and federal guidelines. Protocols are reviewed at least annually and as needed by the Consortium Data and Safety Monitoring Committee (DSMC), FHCC Scientific Review Committee (SRC) and the FHCC/University of Washington Cancer Consortium Institutional Review Board (IRB). The review committees evaluate accrual, adverse events and adherence to the applicable data and safety monitoring plan for studies actively enrolling or treating subjects. The IRB reviews the study progress and safety information to assess continued acceptability of the risk-benefit ratio for human subjects.

5.0 SUBJECT ELIGIBLITY

5.1 Inclusion Criteria

Participants are eligible to be included in the study only if all the following criteria apply:

1. Participants must be at least 18 years of age on the day of signing informed consent. Participant (or legally acceptable representative if applicable) provides written informed consent for trial.

2. Participants must have either histologically or by clinical consensus (based on imaging and/or exam under anesthesia) confirmed diagnosis of muscle invasive bladder cancer (cT2-T4aN0-N1M0 or cT1-4aN1M0 clinical stage per American Joint Commission on Cancer [AJCC]). Patients with clinical node-positive (N1) stage are eligible provided the lymph node (LN) is confined to the true pelvis and is within the planned surgical LN dissection template; cN1 is defined as a lymph node with ≥ 15mm short axis or biopsy-positive for carcinoma.

3. Must have clinical non-metastatic bladder cancer (M0) determined by cross-sectional CT CAP or MRI.

4. Review of pathology by local expert GU pathologist is required. Any component (%) of non-conventional urothelial (variant histology) noted on TURBT is allowed for histologic types not listed below. However, for the variant histologic types listed below, the following parameters need to be met:
   - Squamous cell carcinoma / squamous cell features need to be pure or predominant (≥ 1 variant histology with total non-conventional urothelial component >50%).
   - Adenocarcinoma / glandular features need to be pure or predominant (≥ 1 variant histology with total non-conventional urothelial component >50%).
   - Any % of neuroendocrine / small cell histology is excluded.

5. Patients must be considered unfit for cisplatin based on the Galsky et al.[39] criteria or refuse cisplatin despite adequate counseling in cisplatin-fit patients. Especially, for tumors containing squamous cell or glandular features, every effort should be made to discuss the benefit of neoadjuvant cisplatin-based chemotherapy shown in the S8710 trial. [40]

6. Participants must be deemed eligible for radical cystectomy (RC) and pelvic lymph node dissection (PLND) by both urologist and medical oncologist.

7. TURBT that showed muscularis propria (or lamina propria for cT1N1 tumors) invasion should be within 12 weeks prior to beginning trial therapy. Patients must have available tumor tissue from either initial or repeat TURBT, prior to starting SG therapy. Archival and/or fresh tumor tissue sample of a tumor lesion (TURBT specimen) should be provided and must contain muscle invasive component, at least ≥T2 tumor (for ≥cT2 tumors), unless clinical stage is cT1N1M0 (in that case muscle invasive component is not necessary). Formalin-fixed, paraffin embedded (FFPE) tissue blocks are preferred to slides. If submitting unstained cut slides, newly cut slides should be submitted to the testing laboratory, preferably within 14 days from the date slides are cut if possible. Patient must be willing to provide tumor tissue for research. Research samples will not be used for unrelated studies.

8. A male participant must agree to use a contraception as detailed in Appendix 2 of this protocol during the treatment period and for at least 180 days after the last dose of study treatment and refrain from donating sperm during this period.

9. A female participant is eligible to participate if she is not pregnant (see Appendix 2), not breastfeeding, and at least one of the following conditions applies:

   a.) Not a woman of childbearing potential (WOCBP)
   OR

b.) A WOCBP who agrees to follow the contraceptive guidance during the treatment period and for at least 180 days after the last dose of study treatment.

10. Have an Eastern Cooperative Oncology Group (ECOG) performance status (PS) of 0-2. Patients with ECOG PS 2 may be permitted only after discussion with the trial PI (Dr. Grivas). Evaluation of ECOG PS is to be performed within 7 days prior to the date of enrolment.

11. Have adequate organ function as defined in the following Table 2. Specimens must be collected within 10 days prior to the start of study treatment.

12. Patients must agree to undergo curative intent surgery.

Table 2. Adequate Organ Function Laboratory Values

System	Laboratory Value
Hematological	
Absolute neutrophil count (ANC)	≥1500/µL
Platelets	≥100 000/µL
Hemoglobin	≥9.0 g/dL or ≥5.6 mmol/L[a]
Renal	
Serum Creatinine Measured or calculated creatinine clearance (GFR can be used in place of creatinine clearance; 24-hour urine collection can be used for more accurate estimate as needed)	≤1.5 x ULN OR calculated creatinine clearance[b] (GFR can be used in place of creatinine or creatinine clearance) ≥30 ml/min (Gilbert's disease is an exclusion)
Hepatic	
Total bilirubin	≤1.5 × ULN OR direct bilirubin ≤ULN for participants with total bilirubin level >1.5 × ULN
AST (SGOT) and ALT (SGPT)	≤2.5 × ULN
Coagulation	
International normalized ratio (INR) OR prothrombin time (PT) Activated partial thromboplastin time (aPTT)	≤1.5 × ULN unless participant is receiving anticoagulant therapy

ALT (SGPT)=alanine aminotransferase (serum glutamic pyruvic transaminase); AST (SGOT)=aspartate aminotransferase (serum glutamic oxaloacetic transaminase); GFR=glomerular filtration rate; ULN=upper limit of normal.

[a] Criteria must be met without erythropoietin dependency and without packed red blood cell (pRBC) transfusion within last 2 weeks prior to study drug treatment

[b] Creatinine clearance (CrCl) should be calculated per institutional standard.

Note: This table includes eligibility-defining laboratory value requirements for treatment; laboratory value requirements should be adapted according to local regulations and guidelines for the administration of specific chemotherapies.

5.2 Exclusion Criteria

Participants are excluded from the study if any of the following criteria apply:

1. Patients with any % of neuroendocrine / small cell histology.

2. Patients considered to be medically unfit for SG, TURBT or radical cystectomy (per Investigator discretion).

3. Prior systemic anti-cancer therapy, including investigational agent/device within 4 weeks or prior radiation therapy within 2 weeks. Intravesical therapies are allowed without specified treatment interval.

4. Known locally advanced (unresectable, e.g. cT4b) or metastatic (cN2-3, M1) cancer on baseline radiographic imaging (CT or MRI) obtained within 28 days prior to registration.

5. Active infection requiring systemic antibiotic therapy at the time of trial therapy initiation. HIV-positive patients on active therapy are eligible as long as their viral load is undetectable and CD4 count is within normal parameters during the time of trial therapy initiation.

6. Known history of active Hepatitis B (defined as Hepatitis B surface antigen [HBsAg] detected or positive HBV PCR test) or known active Hepatitis C virus (defined as HCV RNA [qualitative] detected) infection. Note: no testing for Hepatitis B and Hepatitis C is required if there is no clinical suspicion of such infection.

7. History or current evidence of any condition, therapy, or laboratory abnormality that might confound the results, interfere with the subject's participation for the full duration of the trial, or is not in the best interest of the subject to participate, in the opinion of the treating investigator.

8. Known personality, psychiatric or substance abuse disorder that would interfere with cooperation with the requirements of the trial.

9. Patients may not have concurrent upper urinary tract (i.e., ureter, renal pelvis) invasive urothelial carcinoma. Patients with history of non-invasive (Ta, Tis) upper tract urothelial carcinoma that have been definitively treated with at least one post-treatment disease assessment (i.e., cytology, biopsy, imaging) that demonstrated no evidence of residual disease are eligible. Previously treated or concurrent non-invasive (Ta, Tis) urethra carcinoma is allowed, but history of or concurrent invasive urethra carcinoma is excluded.

10. Patients may not have another malignancy that could interfere with the evaluation of safety or efficacy of SG. Patients with prior malignancy will be allowed without PI approval in the following circumstances:
    a. Not currently active and completed therapy at least 2 years prior to the date of registration.
    b. Non-invasive cancer, such as low risk cervical cancer or any *carcinoma in situ*.
    c. Localized (early stage) cancer treated with curative intent (without evidence of recurrence and intent for further therapy), and in which no systemic chemotherapy was indicated (e.g., low/intermediate risk prostate cancer, non-melanoma skin cancer, etc.). Low/intermediate risk prostate cancer on active surveillance or watchful waiting is allowed. Other cancers not meeting these criteria must be discussed with trial PI (Dr. Grivas).

11. Patients may not have undergone major surgery (e.g., intra-thoracic, intra-abdominal or intra-pelvic), open biopsy or significant traumatic injury ≤ 3 weeks prior to starting SG, or who have not recovered from side effects of such procedure or injury.

12. Have active (based on symptoms, endoscopic or biopsy findings) chronic inflammatory bowel disease (ulcerative colitis, Crohn's disease) at time of trial therapy initiation or history of GI perforation within 6 months of enrollment.

13. Patients may not have clinically significant cardiac diseases, including any of the following:
    a. History or presence of serious uncontrolled ventricular arrhythmias.
    b. Any of the following within 6 months prior to starting study drug: myocardial infarction (MI), severe/unstable angina, Coronary Artery Bypass Graft (CABG), NYHA Class III or greater Congestive Heart Failure (CHF) or left ventricular ejection fraction of < 40%, Cerebrovascular Accident (CVA), Transient Ischemic Attack (TIA).

14. Patients unwilling or unable to comply with the protocol or with known allergy to SG, its analogues, or excipients in the various formulations.

15. Patients may not participate in any other therapeutic clinical trials, including those with other investigational agents not included in this trial before radical cystectomy.

16. WOCBP with positive urine pregnancy test within 72 hours prior to enrolment. If the urine test is positive or cannot be confirmed as negative, a serum pregnancy test will be required for eligibility. Active lactation is an exclusion.

17. History of allogeneic solid visceral organ transplant, Gilbert's disease, prior systemic irinotecan or topotecan or other topoisomerase-1 inhibitor, or concomitant medications that significantly interfere with ABCA1 transporter or *UGT1A1* with no alternate option available.

6.0 SUBJECT REGISTRATION

Subjects will be registered by the Fred Hutch/UW Study Coordinator and entered into OnCore. A complete, signed, study consent and HIPAA consent are required for registration.

7.0 TREATMENT PLAN

Table 3. Treatment schedule outline

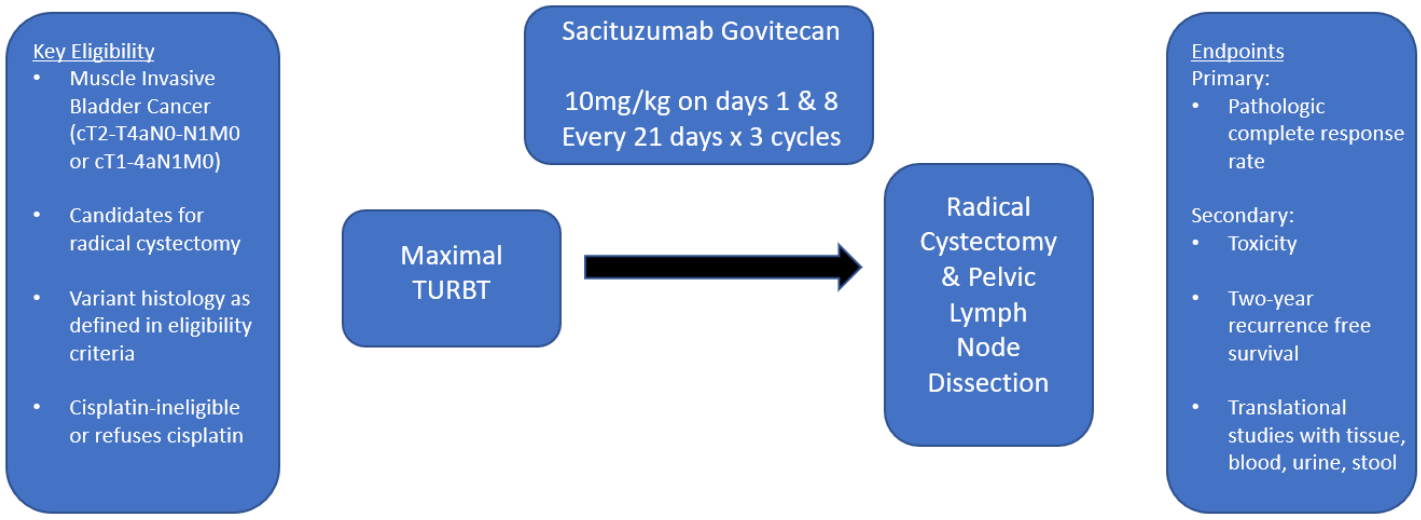
Outline Treatment Schedule	
Day*	***Treatment****
1 (Cycle 1 Day 1)	*SG 10mg/kg*
8 (Cycle 1 Day 8)	*SG 10mg/kg*
15 (Cycle 1 Day 15)	*Break*
22 (Cycle 2 Day 1)	*SG 10mg/kg*

29 (Cycle 2 Day 8)	*SG 10mg/kg*
36 (Cycle 2 Day 15)	*Break*
43 (Cycle 3 Day 1)	*SG 10mg/kg*
50 (Cycle 3 Day 8)	*SG 10mg/kg*
57 (Cycle 3 Day 15)	*Break*
	Proceed to radical cystectomy and pelvic lymph node dissection ideally within 2-6 weeks of the last dose of SG

*Refer to section 8.1 for details on window for treatment delays

[+]Strongly recommend primary prophylaxis with GCSF. Either pegfilgrastrim or filgrastim or their biosimilars are allowed

7.1 Treatment Plan Overview



7.2 Duration of Therapy

Treatment period is a 9-week neoadjuvant treatment with SG prior to RC. Treatment schedule will include SG on days 1 and 8 every 3 weeks, see above table 3.

7.3 Duration of Follow-Up

Patients will be followed clinically and radiographically for at least 2 years post-cystectomy as standard of care. First surveillance scan will be approximately 1-3 months after RC and then will continue every 3-6 months for 2 years as per the treating physician and standard of care practice. Date of diagnosis for progression, first subsequent therapy and survival shall be collected. Phone follow-up may also be done for patients unable or unwilling to return for follow-up evaluations.

7.4 Dosing Delays/Dose Modifications

7.4.1 Dose Delays

SG is to be administered in 21-day cycles on Day 1 and Day 8. Visit windows of 4 days prior to and/or after the scheduled infusion are permitted. The scheduled Day 1 infusion may be delayed for up to 1 week for treatment-related toxicities. Instructions for dose delays and dose reductions for specific toxicities are summarized below. For toxicities not specifically addressed in the toxicity management table, dosing may be delayed for >Grade 2 toxicities for a maximum of 1 week. If the toxicity has improved to ≤Grade 2, the dose should be administered at that time, if possible, at the discretion of the treating provider (this should ideally be discussed with PI: Dr. Grivas). For a toxicity that delays Day 8 dosing, if the toxicity has not resolved to ≤Grade 2 within 4 days, dosing should resume with the next scheduled cycle, i.e., the next dose will be Day 1 of the following cycle. There can be a maximum of 28 days between doses due to toxicities. If the reason for delay is due to a required procedure, the

maximal interval between two doses can be 35 days. Treatment interruptions for reasons other than resolution of toxicities or required procedures are not permitted outside of the permitted visit windows. Alopecia and non-clinically significant lab abnormalities are excluded from the need of dose delays noted above.

7.4.2 Dose Modifications and Discontinuation

The major toxicities of SG are expected to be GI symptoms and hematologic suppression. SG dose reductions and interruptions will be managed based on toxicity severity, as assessed by NCI CTCAE v5.0. Leukopenia or lymphopenia in the absence of neutropenia will not require dose delay or dose modification. SG dose must not be re-escalated following dose reduction. Table below summarizes recommendations for SG dose reduction and discontinuation for treatment-related toxicities. Patients will also be monitored for infusion related reactions (IRR), additional details of recommended treatment of IRRs are described in Section 10.3

TABLE 4: Recommended Dose Reduction Schedule for SG

Adverse Reaction	Occurrence	Dose Modification
Severe Neutropenia		
Grade 4 neutropenia ≥ 7 days or less if clinically indicated, OR Grade 3-4 febrile neutropenia, OR At time of scheduled treatment, Grade 3-4 neutropenia which delays dosing by 2 or 3 weeks for recovery to ≤ Grade 1	First	Administer granulocyte-colony stimulating factor (G-CSF) as soon as clinically indicated
	Second	25% dose reduction; administer G-CSF as soon as clinically indicated
	Third	50% dose reduction; administer G-CSF as soon as clinically indicated
	Fourth	Discontinue treatment; administer G-CSF as soon as clinically indicated
At time of scheduled treatment, Grade 3-4 neutropenia which delays dosing beyond 3 weeks for recovery to ≤ Grade 1	First	Discontinue treatment; administer G-CSF as soon as clinically indicated
Severe Non-Neutropenic Toxicity		
Grade 4 non-hematologic toxicity of any duration, OR Any Grade 3-4 nausea, vomiting or diarrhea due to treatment that is not controlled with antiemetics and anti-diarrheal agents, OR Other Grade 3-4 non-hematologic toxicity persisting > 48 hours despite optimal medical management, OR At time of scheduled treatment, Grade 3-4 non-neutropenic hematologic or non-hematologic toxicity, which delays dose by 2 or 3 weeks for recovery to ≤ Grade 1	First	25% dose reduction
	Second	50% dose reduction
	Third	Discontinue treatment
In the event of Grade 3-4 non-neutropenic hematologic or non-hematologic toxicity, Grade 3 nausea or Grade 3-4 vomiting, which does not recover to ≤ Grade 1 within 3 weeks	First	Discontinue treatment

7.5 End of Treatment (EOT) Visit Schedule and Procedures

In participants who discontinue SG prior to its intended completion, tumor imaging should ideally be performed within 4 weeks or at the earliest convenience per provider discretion. If previous imaging was obtained within 4 weeks prior to the date of SG discontinuation, then imaging at the time of SG discontinuation is not mandatory. In participants who discontinue (without completing) SG without documented progression, every effort should be made to continue monitoring disease status by tumor imaging using ideally the same imaging used while on treatment to monitor disease status until the start of a new anticancer treatment, progression, pregnancy, death, withdrawal of consent, or the end of the study, whichever occurs first.

Other Procedures to be performed during the EOT Visit include:

- History and Physical Exam
- Vital Signs
- Performance Status
- Labs:
  - CBC and Differential
  - Blood Chemistry - Comprehensive metabolic panel, including electrolyte balance, and hepatic and renal functions
  - Specific Research Labs
- AE Assessment
- Concomitant Medications

8.0 SUBJECT EVALUATION

8.1 Trial Flow Chart

The Investigator or qualified designee will perform a complete physical exam during the screening period. Clinically significant abnormal findings should be recorded as medical history. A full physical exam should be performed during screening. Further clinical evaluations, including vitals, physical exam, performance status, imaging, labs, and research labs as per study flow chart below.

Trial Period		**Treatment Cycles of sacituzumab govitecan***								
Treatment Cycle/Title:	Screening	**Cycle 1**		**Cycle 2**		**Cycle 3**		Surgery[7]	End of Treatment /Discontinuation[9]	Survival Follow-Up[10]
		1	**8**	**1**	**8**	**1**	**8**			
Scheduling Window (Days):	-28 to -1	±4	±4	±4	±4	±4	±4	At time of pre-op for RC	1-month (+/- 2weeks) post-op of RC or after discontinuation[14]	SOC
Informed Consent[1]	X	X								
Inclusion/Exclusion Criteria	X	X								
Demographics and Medical History	X	X								
Prior and Concomitant Medication Review	X	X	X	X	X	X	X	X	X	

Pulse Oximetry first and then trial Treatment Administration		X	X	X	X	X	X			
Post-study anticancer therapy status								X	X	X
Survival Status		X	X	X	X	X	X	X	X	X
Review Adverse Events[12]		X	X	X	X	X	X	X	X	
Full Physical Examination	X	X	X	X	X	X	X	X	X	X (SOC)
Vital Signs and Weight	X	X	X	X	X	X	X	X	X	X (SOC)
ECOG Performance Status	X	X	X	X	X	X	X	X	X	X (SOC)
Pregnancy Test: Urine or Serum β-HCG for WOCBP	X									
PT/INR and aPTT	X							X		
CBC with Differential	X	X	X	X	X	X	X	X	X	X (SOC)
Comprehensive Serum Chemistry Panel	X	X	X	X	X	X	X	X	X	X (SOC)
EKG[13]	X							X		
Hepatitis B & C only if clinically indicated[2]	X									
Tumor Imaging[3]	X							X[8]	X[15]	X(SOC)
Archival and/or Fresh Tissue Collection[4]	X							X		X[11]
Correlative Studies: Blood Collection[5]		X				X		X	X	X[11]
Correlative Studies: Urine Collection[5]		X				X		X	X	X[11]
Correlative Studies: Stool Collection[6]		X				X				X[11]

***Scheduled Visits:** +/- 4 day window is allowed for scheduled SG therapy, required tests and/or visits except as otherwise noted. Delay only due to holidays, weekends, bad weather or other unforeseen circumstances will be permitted up to +/-7 days from target date. Study procedures, e.g., history, physical exam, pulse oximetry, blood, urine, stool collection, at each time point should be performed prior to SG administration. Strongly recommend primary prophylaxis with G-CSF. Either pegfilgrastrim or filgrastim or their biosimilars are allowed.

1. Cystoscopy with TURBT showing muscularis propria invasion should be performed within 12 weeks (84 days) of starting SG (C1D1). Patients will complete all other screening studies (i.e., labs, imaging, history, exam, etc.) within 28 days of starting SG.
2. Hepatitis B surface antigen (HBsAg) and Hepatitis C (HCV) testing is not required unless clinically indicated (as standard of care). These tests may be repeated during the course of the study, if clinically indicated (as standard of care).
3. For abdomen/pelvis, CT with IV contrast is the preferred method (IV contrast is suggested but not required for CT chest). MRI abdomen/pelvis (preferably with gadolinium if possible) can be performed, if CT with IV contrast cannot be obtained. CT abdomen/pelvis without IV contrast is allowed if IV contrast cannot be given and MRI cannot be performed. Follow-up scans will be done as clinically indicated based on pathologic stage at time of RC (as standard of care).
4. TURBT and RC tumor tissue (any number of block or slides are OK for eligibility).
Time points (up to three): TURBT, radical cystectomy, and at the time of recurrence (if possible)
-FFPE: Representative blocks preferred or 1 H&E slide plus minimum of 20 unstained slides (30 preferred if available)
-Fresh frozen OCT (and extra fresh if possible): block/tissue to be collected and stored after FFPE (stored samples will not be used for any studies unrelated to this trial)
5. Research blood & urine, samples shall be obtained at time points noted below. (Stored samples will not be used for any studies unrelated to this trial): Prior to cycle 1 day 1 infusion, prior to cycle 3 day 1 infusion, time of radical cystectomy, and end of treatment visit, and at recurrence, if feasible- up to 5 time points. Research blood for the

Hematopathology lab for peripheral blood biomarker analysis (e.g. TSCNK testing, lymphocytes) will be collected before cycle 1 day 1 infusion, prior to cycle 3 day 1 infusion, and at recurrence, if feasible, up to 3 time points.
-Peripheral blood: two 10 mL purple top tubes, one 5 mL purple top tubes, and one 10 mL Streck DNA tube
-Urine: At least 30 mL in standard urine cup (see section 9.2.11 for more details)
6. Research stool samples shall be obtained any time between enrollment and cycle 1, day 1 SG dose, any time after the cycle 2, day 8 SG dose (or after the most recent prior dose if cycle 2, day 8 dose is not given at all) and prior to cycle 3 day 1 infusion, and at the time of recurrence (if possible)). Stored samples will not be used for any studies unrelated to this trial.
7. Radical cystectomy (RC) with bilateral (standard or extended) pelvic lymph node dissection to be performed as soon as deemed safe, and ideally 2-6 weeks, after the last neoadjuvant infusion (RC is standard of care).
8. CT (or MRI as noted above) after final dose of study therapy and before surgery (standard of care)
9. If patient is removed from treatment for reason(s) other than progression, follow with regular tumor assessments as per standard of care until progression or start of new treatment. Adverse events should be reviewed at that post-RC safety visit, while phone follow-up may also be done as clinically needed to review potential subsequent adverse events; if those are felt to be at least possibly related to SG, they should be recorded as such.
10. Patients will be followed clinically and radiographically for at least 2 years post-cystectomy or when study-wide follow-up ends as per standard of care. First surveillance scan will be approximately 1-3 months after RC and then will continue every 3-6 months for 2 years as per treating physician and standard of care practice. Date of diagnosis for progression, first subsequent therapy and survival shall be reported. Phone follow-up may also be done for patients unable or unwilling to return for follow-up evaluations.
11. At time of recurrence, if feasible.
12. All AEs up until 30 days post initiation of SG and SAEs up until 90 days post initiation of SG or 30 days if participant starts new anticancer therapy, whichever is earlier, should be collected. Refer to Section 13.8.1
13. EKG to be done also during treatment if clinically indicated as per standard of care.
14. For patients who discontinue SG, scheduling the end of treatment visit will be at earliest convenience per provider discretion.
15. Tumor imaging is only necessary for those participants who discontinue SG. For those who complete SG and RC, tumor imaging should be per SOC survival follow-up.

9.0 TRIAL PROCEDURES

The Trial Flow Chart, Section 8.1, summarizes the trial procedures to be performed at each visit. Individual trial procedures are described in detail below. It may be necessary to perform these procedures at unscheduled time points if deemed clinically necessary by the Investigator or treating physician. Furthermore, additional evaluations/testing may be deemed necessary by the Investigator or treating physician.

9.1 Administrative Procedures

9.1.1 Informed Consent

The Investigator or qualified designee must obtain documented consent from each potential participant prior to participating in a clinical trial.

9.1.1.1

Consent must be documented by the participant's dated signature or by the participant's legally acceptable representative's dated signature on a consent form along with the dated signature of the person conducting the consent discussion.

A copy of the signed and dated consent form should be given to the participant before participation in the trial.

The initial informed consent form, any subsequent revised written informed consent form and any written information provided to the participant must receive the IRB/ERC's approval/favorable opinion in advance of use. The participant or his/her legally acceptable representative should be informed in a timely manner if new information becomes available that may be relevant to the participant's willingness to continue participation in the trial. The communication of this information will be provided and documented via a revised consent form or addendum to the original consent form that captures the participant's dated signature or by the participant's legally acceptable representative's dated signature. Specifics about a trial and the trial population will be added to the consent form template at the protocol level. The informed consent will adhere to IRB/ERC requirements, applicable laws, and regulations requirements.

9.1.2 Inclusion/Exclusion Criteria

All inclusion and exclusion criteria will be reviewed by the Investigator or qualified designee to ensure that the participant qualifies for the trial.

9.1.3 Medical History

A medical history will be obtained by the Investigator or qualified designee. Medical history will include all active conditions, and any condition diagnosed within the prior 10 years that are considered clinically significant by the Investigator. Details regarding the disease for which the participant has enrolled in this study will be recorded separately and not listed as medical history.

9.1.4 Prior and Concomitant Medications Review

9.1.4.1 Prior Medications

The Investigator or qualified designee will review prior medication use, including any protocol-specified washout requirement, and record prior medication taken by the participant within 28 days before starting the trial (for antibiotics taken within 90 days if it is possible). Treatment for the disease for which the participant has enrolled in this study will be recorded separately and not listed as a prior medication

9.1.4.2 Concomitant Medications

The Investigator or qualified designee will record medication, if any, taken by the participant during the trial. All medications related to reportable SAEs should be recorded as defined in Section 13.4.

9.1.5 Disease Details and Treatments

9.1.5.1 Disease Details

The Investigator or qualified designee will obtain prior and current details regarding disease status.

9.1.5.2 Prior Treatment Details

The Investigator or qualified designee will review all prior cancer treatments including systemic treatments, radiation, and surgeries.

9.1.5.3 Subsequent Anti-Cancer Therapy Status

The Investigator or qualified designee will review all new anti-neoplastic therapy initiated after the last dose of trial treatment if possible. If a participant initiates a new anti-cancer therapy after the last dose of trial treatment, the safety follow-up visit should ideally occur before the first dose of the new therapy. Once new anti-cancer therapy has been initiated the participant will move into survival follow-up.

9.1.6 Assignment of Screening Number

Patients will be assigned a screening number after they sign informed consent

9.1.7 Assignment of Enrollment Number

Patients will be enrolled locally into the study based on local procedures based on eligibility criteria signed off by investigator or designee.

9.1.8 Trial Compliance (Medication/Diet/Activity/Other)

All study procedures should be followed per protocol

9.2 Clinical Procedures/Assessments

9.2.1 Adverse Event (AE) Monitoring

The Investigator or qualified designee will assess each participant to evaluate for potential new or worsening AEs as specified in the Trial Flow Chart and more frequently if clinically indicated. AEs will be graded and recorded throughout the study and during the follow-up period according to NCI CTCAE Version 5.0 (the descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 will be utilized for adverse event reporting; https://ctep.cancer.gov/protocoldevelopment/electronic_applications/ctc.htm#ctc_50).

Toxicities will be characterized in terms regarding seriousness, causality, severity grading, and action taken regarding trial treatment. Please refer to section 13 for detailed information regarding the assessment and recording of AEs.

9.2.2 Full Physical Exam

The Investigator or qualified designee will perform a complete physical exam during the screening period. Clinically significant abnormal findings should be recorded as medical history. A full physical exam should be performed during screening.

9.2.3 Vital Signs

The Investigator or qualified designee will take vital signs at screening, prior to the administration of each dose of trial treatment and at treatment discontinuation as specified in the Trial Flow Chart (Section 8.0). Vital signs should include temperature, pulse, respiratory rate, weight, and blood pressure. Height will be measured at screening only.

9.2.4 Eastern Cooperative Oncology Group (ECOG) Performance Scale

The Investigator or qualified designee will assess ECOG status (see Appendix 1) at screening, prior to the administration of each dose of trial treatment and discontinuation of trial treatment as specified in the Trial Flow Chart.

9.2.5 Tumor Imaging and Assessment of Disease

Tumor imaging is strongly preferred to be acquired by computed tomography (CT) with IV contrast. For the abdomen / pelvis, magnetic resonance imaging (MRI) may be used when CT with IV contrast is contraindicated, or when local practice mandates it. CT abdomen / pelvis without IV contrast is acceptable if IV contrast is contraindicated and MRI cannot be done. MRI is the strongly preferred modality for imaging the brain (as clinically indicated). The same imaging technique regarding modality, ideally the same scanner, and the use of contrast should ideally be used in a participant throughout the study to optimize the reproducibility of the assessment of existing and new tumor burden and improve the accuracy of the assessment of response or progression based on imaging.

9.2.6 Tumor Imaging

Initial tumor imaging at screening must be performed within 28 days prior to the date of enrollment. The site study team must review screening images to confirm the participant does not have metastatic disease.

Tumor Imaging During the Study
The first on-study imaging assessment should be performed at the completion of neoadjuvant therapy and prior to radical cystectomy as SOC. Subsequent tumor imaging should be performed post-operatively based on pathologic stage at time of radical cystectomy as SOC. Tumor imaging intervals will be based on NCCN and other practice guidelines for given pathologic stage.

End of Treatment and Follow-up Tumor Imaging
In participants who discontinue SG prior to trial completion, tumor imaging should ideally be performed within 4 weeks or at the earliest convenience per provider discretion. If previous imaging was obtained within 4 weeks prior to the date of SG discontinuation, then imaging at SG discontinuation is not mandatory.

In participants who discontinue SG without documented progression, every effort should be made to continue monitoring their cancer status by tumor imaging using ideally the same imaging modality used while on treatment to monitor cancer status until the start of a new anticancer treatment, progression, pregnancy, death, withdrawal of consent, or the end of the study, whichever occurs first.

9.2.7 Tumor Tissue Collection and Correlative Studies Blood Sampling

9.2.7.1 Tumor Tissue Assessments

- Pre-treatment TURBT (archived tissue may be used)
- Radical Cystectomy
- At the time of recurrence, if feasible

9.2.7.2 Tissue Banking

Biospecimens from TURBT, cystectomy and biopsy of tumor recurrence may be banked locally at UWMC GU Research Lab (Dr. Ming Lam). Stored biospecimens will not be used for any studies unrelated to this trial. Diagnostic and research slides from FFPE may be digitized and resulting images may be further analyzed using digital pathology applications to assess prognostic and predictive value, e.g. relationship with clinical endpoints.

Intraoperative RC specimens will be collected directly from the operating room. Tissue necessary for standard of care pathology evaluation will be sent to the UWMC pathology department and additional specimens will be sent directly to UWMC lab.

Tissue sorting priority will be the following:

(1) Pathology/Clinical Evaluation

(2) Formalin-fixed paraffin-embedded blocks

(3) Fresh and fresh frozen tissue

If tumor is present, collect from viable areas for FFPE and frozen (if there is sufficient tumor).
If no tumor is grossly visible, collect from the scar or ulcer or tumor resection bed area for FFPE.

9.2.7.3 Tumor Infiltrating CD8+ T-Cell Density

In pre- and post-treatment tumor tissue specimens, CD8+ T-cell density, if feasible, may be examined by IHC performed on whole tissue histology sections. $CD8^+$ T cell density may be assessed using a fluorescent tryamide-based immunohistochemistry (mIHC) approach to identify CD8 and tumor (pancytokeratin AE1/AE3). The assays will be performed on representative slides from formalin-fixed paraffin-embedded pre-treatment TURBT and

post-treatment cystectomy tissue preferably in the CLIA-regulated Integrated Clinical Trials Pathology Lab (ICTPL) at the Fred Hutchinson Cancer Research Center.

9.2.7.4 Multiplex Immunohistochemistry mIHC on tumor microarrays (TMAs)

For additional exploratory analysis, tissue microarrays (TMAs) of pre- and post-treatment tumor tissues, if feasible, may be constructed using available tumor FFPE blocks. The TMAs will be constructed using three 1.0 mm diameter tumor core tissues containing at least 100 malignant cells representing the greatest extent of infiltration of lymphocytes. Also, a minimum of 20 (30 preferred) unstained 5-micron thick sections from both the pre- and post-treatment tumor samples may be cut into slides for additional correlative investigations. A multi-color mIHC may be performed).

9.2.7.5 Additional Analysis

Other analysis that may be performed depending on resources, tissue quality and availability may include the following:

Additional IHC Analyses

As tissue availability permits, additional IHC and immunofluorescence (IF) staining, if feasible, is planned to further characterize functional immune phenotypes and targets of interest for future drug development. Utilizing the TMAs constructed from the pre- and post-treatment archived tumor specimens, additional IHC stains planned, but not limited to Trop-2 expression. Exact list of IHC biomarkers may be modified according to new target identification and emerging translational science.

Immune Gene Expression Analysis

Baseline and post-treatment tumor cells, if feasible, may be isolated and enriched from patient tumor slides by macro-dissection. RNA will be extracted and assessed for quality control utilizing standard manufacturer RNA extraction kits and per manufacturer instructions. Extracted RNA from each sample passing quality control will be analyzed for the expression of immune mediating genes by standard quantitative PCR.

Nanostring

Nanostring-based transcriptional analysis, if feasible, may be performed on serial sections from the same FFPE specimens utilized for $CD8^+$ T cell density measurements (see above). In brief, RNA may be extracted from unstained FFPE samples from microscope slides and transcripts of immunological interest may be quantified using the established panels from Nanostring using Counter's Advance analyses technology. Up to 30 transcripts of particular interest for bladder cancer biology may be simultaneously measured using custom "add-in" probe set.

T-Cell Receptor Repertoire Analysis

Pre- and post-treatment tumor cells, if feasible, may be isolated and enriched from patient pre- and post-treatment tumor slides by macro-dissection. DNA may be extracted and assessed for quality control utilizing standard manufacturer DNA extraction kits and per manufacturer instructions. Extracted DNA from each sample may be analyzed for T-cell and B-cell receptor mutations by next generation sequencing platform, statistical and bioinformatics analysis.

RNASeq

RNA from frozen pre- and post- treatment tissues, if feasible, may be isolated by the UW GU research laboratory and generated into RNASeq libraries through the FHCC Genomics Core. Sequencing alignment and analysis may be conducted by the Hsieh Lab using standard platforms including EdgeR and DESeq2.

Exome Sequencing

Baseline tumor, post-treatment tumor, and germline DNA, if feasible, may be extracted from patient tissues (punches from FFPE or fresh frozen tissues). Extracted DNA from each sample may be analyzed for somatic

mutations, copy number alterations, and mutational load estimate. Additional DNA based investigations on tumor DNA may be performed as tumor DNA availability and emerging analysis platforms permit.

9.2.8 Correlative Studies: Mandatory Peripheral Blood Samples and Analysis (processing may vary as per lab practice and recommendations)

9.2.8.1 Peripheral Blood Assessments

- **Before First (C1D1) and Third (C3D1) Neoadjuvant Cycle (same day as treatment)**
  - Two 10 mL and one 5mL purple top tubes
  - One 10 mL Streck DNA tube
- **Pre-RC**
  - Two 10 mL top tubes
  - One 10 mL Streck DNA tube
- **Safety Follow Up/End of treatment Visit (after RC)**
  - Two 10 mL top tubes
  - One 10 mL Streck DNA tube
- **At time of recurrence, if feasible**
  - Two 10 mL and one 5mL purple top tubes
  - One 10 mL Streck DNA tube

9.2.8.2 ctDNA Analysis

Cell-free ctDNA analysis may be performed on Streck DNA tube samples at time points of collection. Germline DNA can be extracted from “buffy coat”.

9.2.8.3 Plasma TROP2 ELISA

Plasma from baseline and subsequent treatments may be analyzed for soluble TROP2 in the plasma by a TROP2 ELISA assay. The level of soluble TROP2 in the plasma will be correlated with clinical response.

9.2.9 Correlative Studies: Mandatory Urine Samples and Analysis

9.2.9.1 Urine Assessment and Sample Collection

- **Before First (C1D1) and Third (C3D1) Neoadjuvant Cycle**
  - **Pre-RC**
  - **Safety Follow Up/End of treatment Visit (after RC)**
  - **At time of recurrence, if feasible**

Urine should be collected in standard analysis cup and 30mL aliquoted into two 15mL falcon tubes.

9.2.9.2 Banking for Future Research: Urine Biomarker Analysis

Urine samples will be banked and stored for future correlative work related to this study. Future analyses may be done as resources and specimen availability permit. The exact list of urine biomarkers may be modified according to new target identification and emerging translational science. Stored samples will not be used for any studies unrelated to this trial.

9.2.10 Correlative Studies: Stool Sample Collection

9.2.10.1 Stool Assessment and Sample Collection, if feasible

- Before cycle 1, day 1 SG Dose (any time between enrollment and first treatment dose)
- Before cycle 3, day 1 [any time after the cycle 2, day 8 SG dose (or after the most recent prior dose if cycle 2, day 8 dose is not given at all) and prior to cycle 3, day 1 infusion]
- At time of recurrence, if feasible

Two stool samples per time point should be collected in standard analysis tubes, one tube with 5 mL RNA later and one tube without preservative. Samples should be stored at -80 °C until analysis. Plan to record the stool collection date and any antibiotic taken within 90 days prior to trial therapy initiation if possible.

9.2.10.2 Banking for Future Research

Stool samples will be banked and stored. Future analyses will include microbiome diversity, metagenomic, and meta-transcriptomic evaluation and bacterial culturing depending on availability of resources. Stored samples will not be used for any studies unrelated to this trial.

9.2.11 Research Samples Processing

All tissue, peripheral blood and urine samples collected will be labeled with a unique numeric identifier that will be coded for patient privacy protection. All specimens will be processed and stored at the GU Research Lab (Dr. Ming Lam). Stored samples will not be used for any studies unrelated to this trial

9.2.11.1 Tissue Block or Slide Samples

Tissue will be formalin-fixed, and paraffin embedded (FFPE). Five-µm sections may be cut and put on slides for H&E and IHC analysis. For potential frozen tissue processing (if ≥1 FFPE has been fulfilled), a cryomold may be prefilled with OCT halfway, then the tissue may be placed in the middle of the OCT followed by filling the rest of the cryomold with OCT. The cryomold may be immediately put into an isopentane bath on dry ice for freezing. The frozen OCT-embedded tissue may be stored in -80°C. Stored samples will not be used for any studies unrelated to this trial.

9.2.11.2 Peripheral Blood Samples

Peripheral blood should be collected as noted above.

One 5ml purple top may possibly be sent to FHCC Hematopathology **Testing Services** for peripheral blood biomarker analysis (if it is possible), before C1D1, before C3D1 and at recurrence, for up to 3 time points
Two 10ml purple top and 1 Streck DNA tube will be sent to the GU Research Lab (Dr. Ming Lam) for processing (up to 5 time points, as noted in the study calendar above).

<u>Purple Top Tubes Processing for Plasma and Buffy Coat (this may vary as per lab practice)</u>

****Process samples ideally within 120 minutes post-collection (record the blood draw time)****

- Gently mix each blood sample by inversion 10 times (do not shake).
- Place tubes immediately on wet ice for five minutes
- Centrifuge at 1500 RPM for 15 minutes at 4°C.

  After centrifugation, the plasma layer will be at the top half of the tube and the nucleated cells (WBC) will be in a whitish layer called the “buffy coat”, just under the plasma and above the red blood cells.

  Plasma Preparation

- Using a transfer pipette for each tube take the top two-thirds of the plasma and transfer plasma into

a 15-mL conical centrifuge tube, be careful not to disturb the buffy coat layer in each purple top tube (**NOTE:** see below for buffy coat processing instructions).

- Transfer equal amounts of plasma from each tube into two labeled polypropylene tubes for cryopreservation.
- Store the two aliquots of plasma samples from each tube in a freezer at ≤ -70°C or colder. DO NOT ALLOW SAMPLES TO THAW.
- Stored samples will not be used for any studies unrelated to this trial.

Buffy Coat Preparation

- From each purple top tube remove and aliquot the "buffy coat"; be careful not to disturb the layer of red blood cells.
- Store the aliquot of cells from each tube in one labeled polypropylene tube for cryopreservation.
- Store the samples in the freezer at ≤ -70°C or colder. DO NOT ALLOW SAMPLES TO THAW.
- Stored samples will not be used for any studies unrelated to this trial.

Streck Tube Processing (this may vary as per lab practice)

. Fill tube completely. IMMEDIATELY mix the blood sample by gentle inversion 8-10 times (do not shake). One inversion is a complete turn of the wrist (180 degrees and back). Store at ambient temperature (15-30°C). Follow double-spin protocol from Streck:

- Centrifuge whole blood at 300xg for 20min at room temperature.
- Transfer upper plasma layer to a new 15ml conical tube.
- Centrifuge plasma at 5000xg for 10min.
- Collect the double-spin supernatant, aliquot for storage at -80C (max. 6 x0.5mL tubes).

9.2.11.3 Urine Sample Processing (this may vary as per lab practice**)**

Sites should collect urine samples as noted in Section 9.2.9.1

- Centrifuge the two 15 mL urine samples for 10 minutes at 3500 RPM.
- The supernatant from each tube should be transferred into three 5 mL labeled polypropylene tube for cryopreservation.
- The cell pellet from each tube should be re-suspended in 1 mL phosphate-buffered saline (PBS) and individually transferred into one labeled polypropylene tube. Centrifuge polypropylene tube at 3500 RPM for 10 minutes then aspirate the supernatant. The remaining cell pellet will be cryopreserved.
- Store the samples in the freezer at ≤ -70°C or colder. DO NOT ALLOW SAMPLES TO THAW.
- Stored samples will not be used for any studies unrelated to this trial.

9.3 Laboratory Procedures/Assessments

Details regarding specific laboratory procedures/assessments to be performed in this trial are provided above.

Laboratory tests for screening should be performed within 10 days prior to the first dose of treatment. After Cycle 1, pre-dose safety laboratory procedures can be conducted up to 72 hours prior to dosing. Results must be

reviewed by the Investigator or qualified designee and found to be acceptable prior to each dose of trial treatment.

9.4 Other Procedures

9.4.1 Withdrawal/Discontinuation

When a participant discontinues/withdraws prior to trial completion, all applicable activities scheduled for the final trial visit should be performed at the End of Treatment visit (which should be scheduled at earliest convenience per provider discretion, ideally within 4 weeks of discontinuation, if possible). Any adverse events which are present at the time of discontinuation/withdrawal should be followed in accordance with the safety requirements outlined in Section 13 - Assessing and Recording Adverse Events.

9.4.2 Participant Replacement Strategy

A patient will be replaced if, following TURBT and registration into the study, they do not meet any of the following criteria:

- Begin neoadjuvant SG within 12 weeks from TURBT that showed muscularis propria invasion (or lamina propria invasion for cT1N1 tumors)
- Presence of non-conventional UC histology in any TURBT meeting the criteria outlined in inclusion and exclusion criteria (Section 5.1 and 5.2).

If a patient withdraws informed consent or is taken off SG therapy per investigator discretion any time before radical cystectomy or refuses radical cystectomy (but not due to SG-related toxicity or cancer progression as the reason for any of the above-stated scenario), they may be replaced and may not be considered evaluable for efficacy assessment.

9.5 Visit Requirements

Visit requirements are outlined in Section 8.0 - Trial Flow Chart. Specific procedure-related details are provided above in Section 8.1 - Trial Procedures.

9.5.1 Screening

Screening is performed after attaining informed consent and within 12 weeks of diagnosis of MIBC by TURBT.

9.5.1.1 Screening Period

Screening should be completed as soon as possible and no later than 28 days from screening initiation.

9.5.2 Treatment Period

Treatment period is a 9-week neoadjuvant treatment after diagnosis of MIBC by TURBT and prior to RC. Treatment schedule will include SG on days 1 and 8 every 21 days for 3 cycles.

9.5.3 Post-Treatment Visits

Post treatment visits will follow standard of care guidelines per pathologic stage at time of RC.

9.5.4 Safety Follow-Up Visit

The mandatory Safety Follow-Up Visit should be conducted after RC or before the initiation of a new anti-cancer treatment, whichever comes first. All AEs that occur prior to the Safety Follow-Up Visit should be recorded. Participants with an AE of Grade > 1 will be followed until the resolution of the AE to Grade 0-1 or until the beginning of a new anti-cancer therapy, whichever occurs first. Study treatment-related SAEs that occur after the last dose of treatment or before initiation of a new anti-cancer treatment should also be recorded, if known.

9.5.5 Follow-up Visits

Participants who discontinue study treatment for a reason other than disease progression will move into the Follow-Up Phase and should be assessed per standard of care. Every effort should be made to collect information regarding disease status until the start of new anti-cancer therapy, disease progression, death, or end of the study.

9.5.6 Survival Follow-up

Participants who experience confirmed disease progression or start a new anticancer therapy, will move into the Survival Follow-Up Phase, and should be contacted by telephone if needed to assess for survival status until death, withdrawal of consent, or the end of the trial, whichever occurs first.

10.0 TOXICITY MONITORING AND MANAGEMENT

Instructions for the infusion of SG are provided in the Pharmacy Manual. The following sections provide guidance for SG administration and management of treatment-related toxicities, including modification of dosing and treatment discontinuation. Toxicities should be assessed and managed in accordance with standard clinical and institutional practices and accepted treatment guidelines.

10.1 Preventative Medications

Infusion-Related Reactions (IRR): Premedication for prevention of IRR with antipyretics and H1 and H2 blockers should be administered before each SG infusion. Corticosteroids (hydrocortisone 50 mg or equivalent oral or IV) may be administered prior to subsequent infusions if the subject had experienced an IRR with a previous infusion. Additional details of recommended treatment of IRRs are described in Section 10.3.

Nausea, Vomiting: SG is considered to be moderately emetogenic. Premedication with a 2-drug antiemetic regimen is recommended. If nausea and vomiting are persistent, a 3-drug regimen may be used, including a 5-HT3 inhibitor (ondansetron or palonosetron, or other agents according to local practices), an NK1-receptor antagonist (fosaprepitant or aprepitant), and dexamethasone (10 mg oral or IV). Anticipatory nausea can be treated with olanzapine.

10.2 Management of SG

NCI CTCAE v5.0 is used to grade the severity of all AEs. The guidelines for management of toxicities associated with SG are based on the assessment of severity according to these criteria. Toxicities should be managed in accordance with standard medical practice and treatment guidelines. All clinically appropriate imaging or laboratory testing should be utilized to fully assess toxicity to determine the appropriate treatment. Appropriate follow-up studies should be utilized to follow all toxicities to resolution. Subjects with known UGT1A1*28 polymorphisms may have a higher risk of developing treatment-related toxicities. Additional monitoring may be required in those subjects. Instructions for SG dose reduction for treatment-related toxicities are provided in Section 7.4.2.

10.3 Infusion-related Toxicities

Infusion-related reactions are defined as symptoms that occur during and within the first 6 hours after the infusion of SG and can occur at any cycle. Symptoms can include fever, chills, rigors, arthralgias, myalgias, urticaria, pruritus, rash, diaphoresis, hypotension, dizziness, syncope, hypertension, dyspnea, cough, and wheezing, as well as severe hypersensitivity reactions including anaphylactic reactions. Infusion-related reactions should be treated in accordance with best clinical practices and standard institutional guidelines. Because of the potential for life-threatening IRR, SG should only be administered in a setting in which appropriately trained medical staff, emergency equipment, and medications are available in the event that resuscitation is required. NCI CTCAE v5.0 is used to grade the severity of all infusion-related-AEs. Premedication for the prevention of IRR is described in Section 10.1.

<u>Grade 3 and Grade 4</u>

Events Grade 3 and Grade 4 IRRs can include severe or clinically significant cardiopulmonary events and severe allergic reactions such as symptomatic bronchospasm and anaphylactic reactions. Grade 3 IRRs are defined as those which are prolonged and do not improve with symptomatic treatment and/or brief interruption of treatment, reactions that recur following treatment, and reactions that require hospitalization. Grade 4 reactions include potentially life-threatening reactions, requiring urgent intervention. Severe allergic and anaphylactic reactions should be treated in accordance with best clinical practices and standard institutional guidelines. If Grade 3 or Grade 4 IRRs occur, SG should be permanently discontinued.

Grade 2 Events

Grade 2 IRRs are defined as those that require infusion interruption and respond to symptomatic treatment; prophylactic medications are indicated for ≤24 hours. For Grade 2 IRRs, the infusion should be interrupted until symptoms resolve. After symptoms resolve, the infusion should be resumed at a slower infusion rate determined by the managing physician. Recommended infusion rates are provided in the Pharmacy Manual. For recurrent Grade 2 IRRs that fail to recover within 6 hours, despite optimal management, permanently discontinue SG.

10.4 Gastrointestinal Toxicities

Nausea, vomiting, and diarrhea are frequent SG-associated toxicities. Appropriate treatment, including, as needed, fluid and electrolyte replacement, is required to minimize the risk of serious consequences such as dehydration. Instructions for SG dose reduction for treatment-related GI toxicities are provided in Section 7.4.2.

Nausea and Vomiting

Instructions for the use of premedications for prophylactic treatment of nausea and vomiting and anticipatory nausea are provided in Section 10.1. Do not hold the dose of SG for Grade 3 nausea unless Grade 3 nausea persists despite maximal optimal medical management. Subjects maybe treated for delayed nausea and vomiting on Days 2 and 3 with 5-HT3 receptor antagonist (ondansetron or palonosetron) monotherapy and other agents if needed. Steroids may be added if symptoms do not resolve with these other agents. Consider olanzapine for persistent or anticipatory nausea; an olanzapine dose of 2.5 mg or 5 mg at bedtime is recommended. NK1 receptor antagonists (fosaprepitant and aprepitant) may be administered.

Diarrhea

Dietary modification should be recommended for the management of diarrhea, including a bland diet, small frequent meals, adequate fluid intake of clear liquids to maintain hydration, and discontinuation of lactose-containing foods and drinks and alcohol. Loperamide may be administered at the onset of treatment-related Grade 1 or Grade 2 diarrhea, at an initial dose of 4 mg, followed by 2 mg with every episode of diarrhea to a maximum dose of 16 mg/day. If diarrhea is not resolved after 24 hours, consider adding diphenoxylate/atropine, or opium tincture as clinically indicated.

Consider adding octreotide 100 to 150 mcg subcutaneous 3 times per day if diarrhea persists. For Grade 3 or Grade 4 diarrhea, the subject may be hospitalized and treated with IV fluids and octreotide. Antibiotics can be administered as clinically indicated.

Subjects who exhibit an excessive cholinergic response to treatment with SG (e.g., abdominal cramping, diarrhea, salivation, etc.) can receive appropriate premedication (e.g., atropine) for subsequent treatments

10.5 Neutropenia

Complete blood counts must be obtained prior to each SG infusion and should be administered only if ANC meet the following criteria:

- Day 1: ANC≥1500/mm3
- Day 8: ANC≥1000/mm3

Refer to section 7.4 addressing dose delays and dose modifications for neutropenia. The routine prophylactic use of growth factor (G-CSF) is not mandatory, but is strongly recommended as primary prophylaxis (with the first cycle) to reduce the risk of neutropenia. G-CSF may also be administered as secondary prophylaxis and in the setting of neutropenia in subjects at high risk of poor clinical outcomes, including those with prolonged neutropenia.

11.0 SUBJECT DISCONTINUATION OF ACTIVE TREATMENT

Subjects may be removed from this study at any time at their discretion. Subjects may also be removed from this protocol if they develop any untoward side effects from the study medications.

If a subject withdraws consent to participate in the study or aspects of the study, attempts should be made to obtain permission to record survival data up to the protocol-described end of the subject follow-up period. Survival data are important to the integrity of the final study analysis. Documentation in the medical record should state that the subject is withdrawing from the study and what, if any, selected data the subject will permit the investigator to obtain.

When a participant discontinues/withdraws prior to trial completion, all applicable activities scheduled for the final trial visit should be performed at the End of Treatment visit (which should be scheduled at earliest convenience per provider discretion).

A participant must be discontinued from study treatment for any of the following reasons:
• The participant or participant's legally acceptable representative requests to discontinue study treatment
• Confirmed radiographic cancer progression
• Any progression of malignancy, or any occurrence of another malignancy that requires active treatment
• Unacceptable adverse events
• The participant has a medical condition or personal circumstance which, in the opinion of the investigator, places the participant at unnecessary risk from continued administration of study treatment.
• The participant has a confirmed positive serum pregnancy test or breastfeeds
• Noncompliance with study treatment or procedure requirements
• The participant is lost to follow-up or died
• Administrative/logistical reasons

12.0 CONCOMITANT MEDICATIONS

12.1 Concomitant Medications/Vaccinations (allowed & prohibited)

Medications or vaccinations specifically prohibited in the exclusion criteria are not allowed during the ongoing trial. If there is a clinical indication for one of these or other medications or vaccinations specifically prohibited during the trial, discontinuation from trial therapy or vaccination may be required. The final decision on any supportive therapy or vaccination rests with the Investigator and/or the participant's treating physician. There are no substantial safety data regarding the concurrent administration of the coronavirus disease 2019 (COVID-19) vaccine and SG. Subjects are allowed to receive the COVID-19 vaccine to reduce the risk and complications of COVID-19 infection. The study visits should continue as planned if vaccination occurs while the subject is on the study.

12.2 Acceptable Concomitant Medications

All treatments that the Investigator considers necessary for a participant's welfare may be administered at the discretion of the Investigator in keeping with the community standards of medical care. All concomitant medication will be recorded on the case report form (CRF) including all prescription, over the counter (OTC), herbal supplements, and IV medications and fluids. If changes occur during the trial period, documentation of drug dosage, frequency, route, and date may also be included on the CRF. All concomitant medications received within 28 days before the first dose of trial treatment and 30 days after the last dose of trial treatment should be

recorded. Concomitant medications administered after 30 days after the last dose of trial treatment should be recorded only for SAEs and ECIs.

12.3 Prohibited Concomitant Medications

Participants are prohibited from receiving the following therapies during the Screening and Treatment Phase of this trial:

- Antineoplastic systemic therapy, e.g., chemotherapy, immunotherapy, targeted or biological therapy
- Investigational agents other than sacituzumab govitecan
- Radiation therapy

12.4 Drug Interactions

No formal drug-drug interaction studies with SG have been conducted. Concomitant administration of strong inhibitors or inducers of UGT1A1, with SG, should be avoided due to the potential to either increase (inhibitors) or decrease (inducers) the exposure to SN-38.

Strong UGT1A1 Inhibitors

Coadministration of SG with strong inhibitors of UGT1A1 (e.g., atazanavir, gemfibrozil, indinavir) may increase systemic exposure to the active metabolite, SN-38. Do not administer strong UGT1A1 inhibitors with SG unless there are no therapeutic alternatives.

Table 5A: List of strong inhibitors of UGT1A1

Adenine	Propofol	Nilotinib	Pazopanib
Regorafenib	Flunitrazepam	Erlotinib	Sorafenib
Enasidenib	Pibrentasvir	Glecaprevir	Rucaparib
Ertugliflozin	Fostamatinib	Ketoconazole	Indinavir
Valproic acid	Flurbiprofen	Eltrombopag	Silibinin
Sodium aurothiomalate	Atazanavir	Gemfibrozil	Ombitasvir
Defarasirox	Dacomitinib	Probenecid	Amitriptyline
Dasabuvir	Paritaprevir	Indomethacin	Pexidartinib
Ubrogepant	Belumosudil		

Strong UGT1A1 Inducers

Exposure to SN-38 may be reduced in patients concomitantly receiving UGT1A1 enzyme inducers. Do not administer strong UGT1A1 inducers SG unless there are no therapeutic alternatives.

Table 5B: List of strong inducers of UGT1A1

Carbamazepine	Testosterone propionate	Primidone
Phenytoin	Nelfinavir	Zidovudine
Phenobarbital	Ritonavir	Ethinylestradiol
Tipranavir	Efavirenz	Desogestrel
Rifampicin	Lamotrigine	

Participants who, in the assessment by the investigator, require the use of any of the aforementioned treatments for clinical management should be removed from the study. All treatments that the Investigator considers necessary for a participant's welfare may be administered at the discretion of the Investigator in keeping with the community standards of medical care. There are no prohibited therapies during the Post-Treatment Follow-up Phase.

13.0 ASSESSING AND RECORDING ADVERSE EVENTS

13.1 Adverse Event

According to ICH guidelines (Federal Register. 1997; 62(90):25691-25709) and 21 CFR 312.32, IND Safety Reports, and ICH E2A, Definitions and Standards for Expedited Reporting, an adverse event is defined as follows:

An adverse event is any untoward medical occurrence in a clinical investigation subject administered a medicinal product and which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product.

Abnormal laboratory values for laboratory parameters specified in the study should not be recorded as an adverse event unless an intervention is required (repeat testing to confirm the abnormality is not considered intervention), the laboratory abnormality results in a serious adverse event or the adverse event results in study termination or interruption/discontinuation of study treatment.

Medical conditions present at screening (i.e., before the study treatment is administered) are not adverse events and should not be recorded on adverse event pages of the CRFs. These medical conditions should be adequately documented on the subject chart. However, medical conditions present at baseline that worsen in intensity or frequency during the treatment or post-treatment periods should be reported and recorded as adverse events.

13.2 Serious Adverse Event

An adverse event should be classified as an SAE if it meets at least one of the following criteria:

Fatal	Adverse event results in death.
Life threatening:	The adverse events placed the subject at immediate risk of death. This classification did not apply to an adverse event that hypothetically might cause death if it were more severe.
Hospitalization:	It required or prolonged inpatient hospitalization. Hospitalizations for elective medical or surgical procedures or treatments planned before enrollment in the treatment plan or routine check-ups are not SAEs by this criterion. Admission to a palliative unit or hospice care facility is not considered to be a hospitalization.
Disabling/incapacitating	Resulted in a substantial and permanent disruption of the subject's ability to carry out normal life functions.
Congenital anomaly or birth defect:	An adverse outcome in a child or fetus of a subject exposed to the molecule or treatment plan regimen before conception or during pregnancy.
Medically significant:	The adverse event did not meet any of the above criteria but could have jeopardized the subject and might have required medical or surgical intervention to prevent one of the outcomes listed above.

13.3 Unexpected Adverse Event

An unexpected adverse event is defined as an event that has a nature or severity, or frequency that is not consistent with the applicable investigator brochure, or the prior medical condition of the subject or other treatment given to the subject. "Unexpected," as used in this definition, refers to an adverse drug experience that has not been previously observed and reported in preclinical or clinical studies rather than an experience that has not been anticipated based on the pharmacological properties of the study drug.

13.4 Monitoring and Recording Adverse Events

All AEs will be assessed by the investigator or qualified designee and recorded in the CRFs. The investigator should attempt to establish a diagnosis of the event on the basis of signs, symptoms and/or other clinical information. In such cases, the diagnosis should be documented as the adverse event or serious adverse event and not described as the individual signs or symptoms. The following information should be recorded:

- Description of the adverse event using concise medical terminology
- Description as to whether or not the adverse event is serious, noting all criteria that apply
- The start date (date of adverse event onset)
- The stop date (date of adverse event resolution), if applicable
- The severity (grade) of the adverse event
- A description of the potential relatedness of the adverse event to study drug, a study procedure, or other causality
- The action taken due to the adverse event
- The outcome of the adverse event

13.5 Grading Adverse Event Severity

All AEs will be graded in severity according to the NCI Common Terminology Criteria for Adverse Events (CTCAE) Version 5.0. If a CTCAE criterion does not exist, the investigator should use the following grades or adjectives: Grade 1 (mild), Grade 2 (moderate), Grade 3 (severe), Grade 4 (life-threatening), or Grade 5 (fatal) to describe the maximum intensity of the adverse event. Grading of adverse event severity as described in Table 6.

Table 6: Grading of Adverse Event Severity

Grade	Adjective	Description
Grade 1	Mild	Does not interfere with subject's usual function
Grade 2	Moderate	Interferes to some extent with subject's usual function
Grade 3	Severe	Interferes significantly with subject's usual function
Grade 4	Life-Threatening	Results in a threat to life or in an incapacitating disability
Grade 5	Death	Results in death (should be reported as a SAE)

SAE=serious adverse event.

13.6 Attribution of an Adverse Event

Association or relatedness to the study agent will be assessed by the investigator as follows:

- **Definite:** The event follows a reasonable temporal sequence from exposure to the investigational agent, has been previously described in association with the investigational agent, and cannot reasonably be attributed to other factors such as the subject's clinical state, other therapeutic interventions or concomitant medications; AND the event disappears or improves with withdrawal of the investigational agent and/or re-appears on re-exposure (e.g., in the event of an infusion reaction).
- **Probable:** The event follows a reasonable temporal sequence from exposure to the investigational agent and has previously been described in association with the investigational agent OR cannot reasonably be attributed to other factors such as the subject's clinical state, other therapeutic interventions or concomitant medications.
- **Possible**: The event follows a reasonable temporal sequence from exposure to the investigational agent but could be attributable to other factors such as the subject's clinical state, other therapeutic interventions or concomitant medications.

- **Unlikely:** Toxicity is doubtfully related to the investigational agent(s). The event may be attributable to other factors such as the subject's clinical state, other therapeutic interventions, or concomitant medications.
- **Unrelated:** The event is clearly related to other factors such as the subject's clinical state, other therapeutic interventions, or concomitant medications.

For general AE assessment, an AE is considered related if it is assessed as definitely, probably, or possibly related; unrelated if it is assessed as unlikely related or unrelated.

13.7 Adverse Event Recording Period

AEs will be monitored and recorded in study-specific case report forms (CRFs) from the time of first exposure to an investigational product in this study. AEs with an onset date prior to the first exposure to an investigational product will not be recorded, except in the case of clinically significant worsening of the AE during the specified AE monitoring time frame.

13.8 Adverse Event Reporting Requirements

13.8.1 Recording AE in Study Record

Any adverse event (AE) that occurs after the initial dose of study treatment, during treatment, or within 30 days of the last dose of treatment will be recorded in REDCap. Investigators are to report all AEs directly observed or spontaneously reported by subjects using concise medical terminology. If possible, a specific diagnosis rather than individual signs and symptoms should be reported as AEs. Each subject will be questioned about AEs at each clinic or evaluation visit, asking, for example, "Since your last clinic visit, have you had any health problems?"

All AEs will be reported from the time of SG initiation through 30 days following the last day of administration of study drug(s) or initiation of alternate therapy. SAEs will only be followed up during the trial and up to 90 days after last study drug(s) dose. During such follow -ups, subjects who discontinued therapy for reasons other than disease progression, any SAE determined within the 90 days of last dose of SG by the Investigator to be potentially related to study drug(s) must also be reported. All AEs must be reported in REDCap, whether or not considered related to study medication.

13.8.2 AE Reporting to Gilead

In the event of an unanticipated problem, life-threatening complications or pregnancy, treating investigators must also immediately notify Gilead Sciences, Inc. using the following information below in Table 7.

Table 7: Required Timelines for AE Reporting to Gilead

Case Type	Timeframe	Format	Method
Serious Adverse Event	24 hours of awareness	CRF	Email to Safety_fc@gilead.com and cc PI/study team designee
Pregnancy/Exposure During Pregnancy	24 hours of awareness	CRF	Email to Safety_fc@gilead.com and cc PI/study team designee

13.8.3 Reporting to IRB

The investigator or designee must report events to the FHCC IRB in accordance with the policies of the IRB.

13.8.4 FDA Reporting Requirements

The sponsor-investigator assumes responsibility for IND safety reporting to the FDA, in accordance with regulations under 21 CFR 312.32.

The sponsor-investigator will assess each reported event for seriousness, expectedness, and the relationship to the investigational product.

For determination of IND safety reporting, AE attribution will be assessed according to the suspected adverse reaction definition in 21 CFR 312.32, as an AE for which there is a reasonable possibility that the drug caused the adverse event, where "reasonable possibility" means there is evidence to suggest a causal relationship between the drug and the AE. Suspected adverse reactions that are serious and unexpected will be reported to the FDA as an IND safety report.

SAEs that do not meet IND Safety Report criteria will be reported to the FDA as part of annual reporting responsibilities described under 21 CFR 312.33.

14.0 CRITERIA FOR ENDPOINT EVALUATIONS

14.1 Disease Assessment Prior to Enrollment:

Outlined in Section 8.0 Trial Procedures

14.2 Definition of Progression After Enrollment:

Clinical or radiology progression (RECIST 1.1). If cancer is deemed unresectable at time of RC, this would count as progression. If the patient cannot get RC due to toxicity, personal choice, or any other reason, this will be captured, and the patient will count only towards the denominator for the calculation of the primary endpoint. If an enrolled patient does not start neoadjuvant therapy, they can be replaced.

15.0 DATA AND SAFETY MONITORING PLAN

Institutional support of trial monitoring will be in accordance with the FHCC/University of Washington Cancer Consortium Institutional Data and Safety Monitoring Plan (DSMP). Under the provisions of this plan, FHCC Clinical Research Support (CRS) coordinates data and compliance monitoring conducted by consultants, contract research organizations, or FHCC employees unaffiliated with the conduct of the study. Independent monitoring visits occur at specified intervals determined by the assessed risk level of the study and the findings of previous visits per the institutional DSMP.

In addition, protocols are reviewed at least annually and as needed by the Consortium Data and Safety Monitoring Committee (DSMC), FHCC Scientific Review Committee (SRC) and the FHCC/University of Washington Cancer Consortium Institutional Review Board (IRB). The review committees evaluate accrual, adverse events, stopping rules, and adherence to the applicable data and safety monitoring plan for studies actively enrolling or treating subjects. The IRB reviews the study progress and safety information to assess continued acceptability of the risk-benefit ratio for human subjects. Approval of committees as applicable is necessary to continue the study. The trial will comply with the standard guidelines set forth by these regulatory committees and other institutional, state, and federal guidelines.

16.0 DATA MANAGEMENT/CONFIDENTIALITY

The investigator will ensure that data collected conform to all established guidelines. Each subject is assigned a unique subject number to protect subject confidentiality. Subjects will not be referred to by this number, by name, or by any other individual identifier in any publication or external presentation. The licensed medical records department, affiliated with the institution where the subject receives medical care, maintains all original inpatient and outpatient chart documents. Subject research files are stored in a secure place (or database). Access is restricted to authorized personnel.

17.0 STATISTICAL CONSIDERATIONS

17.1 Study Design

Single center, single arm, unblinded study of neoadjuvant SG for patients with muscle invasive bladder cancer with non-urothelial variant histology. Primary endpoint is pCR.

17.2 Primary/Secondary Endpoints/Hypotheses and Analytical Methods

The primary objective of this study is to evaluate the pCR rate at RC with neoadjuvant SG in non-UC histologic variants. Based on historical information, a pCR rate of 8% or less was considered of insufficient activity based on historical data for TURBT alone; pCR rate along with the 95%CI based on the Clopper-Pearson method will be estimated.

Secondary objectives include an evaluation of the frequency and severity of toxicity of SG, the number and percentage of patients will be summarized. For analysis of recurrence-free survival and overall survival at 2 years, the KM method will be used to estimate the 2-year RFS, OS, and their corresponding 95%CIs, and the KM plots will be provided, when appropriate.

17.3 Sample Size and Power

A single stage design with 18 patients has 90% power to rule out an 8% pCR rate if the true pCR rate were 35%, using a 1-sided 5% level binomial test. The observation of at least 4 patients with pCR (22%) would be considered evidence to rule out the 8% pCR rate. With 18 patients, binary proportions can be estimated to within 24% with 95% confidence. Any toxicity with true prevalence of 10% or greater is likely to be observed with 84% chance. The estimated duration of accrual is 18-27 months (1 patient per 30-45 days).

However, if after the study has been active for 18 months and ≤8 patients have been enrolled, the accrual goal may be modified target a total of 14 patients. If accrual is reduced to 14 patients, the design has 96% power to rule out an 8% pCR rate if the true pCR rate were 40%, using a 1-sided 10% level binomial test. The observation of ≥3 patients with pCR (21%) would be considered evidence to rule out the 8% pCR rate.

17.4 Additional Efficacy Hypotheses, Outcome Measures, and Statistical Methods

The feasibility of the regimen will be evaluated by the percentage of patients able to undergo RC ideally 2-6 weeks from end of SG therapy. This proportion along with 95%CI will be evaluated. The relationship (or lack thereof) between any delay of RC beyond 6 weeks (from end of SG therapy) and SG therapy will be described. Distributions of time-to-event outcomes will be estimated using the method of Kaplan-Meier. The rates at specified time points will use these estimates and the associated 95%CI. Continuous outcomes will be summarized by means, medians, and quantiles.

18.0 INVESTIGATOR OBLIGATIONS

The PI is responsible for the conduct of the clinical trial at the site and is responsible for personally overseeing the treatment of all study subjects. The PI must assure that all study site personnel, including sub-Investigators and

other study staff members, adhere to the study protocol and to all applicable regulations and guidelines regarding clinical trials both during and after study completion. All subjects are informed of the nature of the program, its possible hazards, and their right to withdraw at any time, and each subject signs a form indicating their consent to participate prior to receiving any study-related procedures.

19.0 ADMINISTRATIVE AND REGULATORY CONSIDERATIONS

19.1 Documentation

The documentation of clinical data must be stored according to legal requirements. The Sponsor-Investigator and study staff have responsibility for maintaining a comprehensive and centralized filing system containing all study-related documentation. These files must be suitable for inspection by the FDA and/or other applicable regulatory agencies/competent authorities at any time, and should consist of the following elements: subject files (complete medical records, laboratory data, supporting source documentation, and the Informed Consent); study files (the protocol with all amendments, copies of all pre-study documentation, and all correspondence between the FDA, IRB, and and Sponsor-Investigator); and drug accountability files, containing a complete account of the receipt and disposition of the study drug.

19.2 Access to Source Data

All source data and study records will be made available for study monitoring/auditing in accordance with the Fred Hutch/UW Cancer Consortium Data and Safety Monitoring Plan and for inspection by representatives of the FDA or other regulatory agencies.

19.3 Data Collection

Study data will be collected and managed using Research Electronic Data Capture (REDCap) electronic data capture tools hosted at the Institute of Translational Health Sciences. REDCap is a secure web-based designed to support data capture of research studies.

19.4 Protocol Interpretation and Compliance

The procedures defined in the protocol are carefully reviewed by the PI and his/her staff prior to the time of study initiation to ensure accurate representation and implementation. Protocol amendments, if any, are reviewed and implemented promptly following IRB approval and submission to the FDA. The Sponsor-Investigator is responsible for submitting protocol amendments to the FDA as described in 21 CFR § 312.30 (Protocol Amendments).

19.5 Disclosure of Data/Publication

Individual subject medical information obtained as a result of this study is considered confidential and disclosure to third parties other than those noted below is prohibited. Such medical information may be given to the subject's personal physician or to other appropriate medical personnel responsible for the subject's welfare. Data generated as a result of this study are to be available for inspection on request by the FDA or other regulatory agencies and by the IRB/EC.

It is anticipated that the final results of this study will be submitted to a peer-reviewed scientific journal.

19.6 Ethical Considerations

The Investigator agrees to conduct this study in accordance with applicable United States FDA clinical trial regulations and guidelines, applicable United States FDA clinical trial regulations and guidelines, the ICH (E6) GCP guidelines, the IRB/EC, and local legal requirements and with the Declaration of Helsinki (1989). The Investigator will conduct all aspects of this study in accordance with all national, state, and local laws of the applicable regulatory agencies.

19.7 Informed Consent

The PI assumes the responsibility of obtaining written Informed Consent for each subject or the subject's legally authorized representative before any study-specific procedures are performed.

Subjects meeting the criteria set forth in the protocol will be offered the opportunity to participate in the study. To avoid introduction of bias, the Investigator must exercise no selectivity with regard to offering eligible subjects the opportunity to participate in the study. Subjects or parents/legal guardians of all candidate subjects will receive a comprehensive explanation of the proposed treatment, including the nature of the therapy, alternative therapies available, any known previously experienced adverse reactions, the investigational status of the study drug, and other factors that are part of obtaining a proper Informed Consent. Subjects will be given the opportunity to ask questions concerning the study, and adequate time to consider their decision to or not to participate.

Informed Consent will be documented by the use of a written Consent Form that includes all the elements required by FDA regulations and ICH guidelines. The form is to be signed and dated by the subject or subject's legally authorized representative and by the person who administers the consent process. A copy of the signed form will be given to the person who signed it, the original signed Consent Form will be filed with the subject's medical records, and copy maintained with the subject's study records. The date and time of time of the Informed Consent must be recorded in the source documents.

If an amendment to the protocol changes the subject participation schedule in scope or activity, or increases the potential risk to the subject, the Informed Consent Form must be amended. Any amended Informed Consent must be approved by the IRB/EC prior to use. The revised Informed Consent Form must be used to obtain re-consent from any subjects currently enrolled in the study if the subject is affected by the amendment and must be used to document consent from any new subjects enrolled after the approval date of the amendment.

19.8 Institutional Review Board/Ethics Committee

The PI will assure that an appropriately constituted IRB/EC that complies with the requirements of 21 CFR Section 56 or written assurance of compliance with ICH (E6) guidelines will be responsible for the initial and continuing review and approval of the clinical study. Before initiation of the study, the PI or designee will forward copies of the protocol and Consent Form to be used for the study to the IRB/EC for its review and approval.

The PI or designee will also assure that all changes in the research activity and all unanticipated problems involving risks to human subjects or others will be reported promptly to the IRB/EC, and that no changes will be made to the protocol without prior Sponsor and IRB/EC approval, except where necessary to eliminate apparent immediate hazards to human subjects.

The PI or designee must comply with the policies of the IRB of record for IRB notification of any SAE occurring at the site and of any safety reports (e.g., IND Safety Reports).

The Investigator or designee will be responsible for submitting periodic progress reports to the IRB/EC at intervals appropriate to the degree of subject risk involved in the study, but not less than once per year and at the completion or termination of the study.

20.0 LABELING, PACKAGING, STORAGE AND RETURN OF CLINICAL SUPPLIES

20.1 Investigational Product

The Sponsor- Investigator and designees shall take responsibility for and shall take all steps to maintain appropriate records and ensure appropriate supply, storage, handling, distribution and usage of investigational product in accordance with the protocol and any applicable laws and regulations.

20.2 Product Description

Product Name & Potency	Dosage Form

Sacituzumab govitecan 10mg/kg	IV

20.3 Packaging and Labeling Information
Supplies will be labeled in accordance with regulatory requirements. See Package Insert for packaging and labeling information

20.4 Storage and Handling Requirements
Clinical supplies must be stored in a secure, limited-access location under the storage conditions specified in the Package Insert.

Clinical supplies may not be used for any purpose other than stated in the protocol

20.5 Returns and Reconciliation
At the end of the study, following authorization by study management, study medication may be destroyed at the site as dictated by the appropriate standard operating procedures. Destruction must be documented with signature by the institution's pharmacist or delegate. Alternatively, after notification, all unused products will be returned to Gilead Inc., or designee

20.6 Sacituzumab Govitecan

20.6.1 Other Names
TRODELVY®

20.6.2 Classification
Antibody-drug conjugate (ADC)

20.6.3 Mode of Action
Sacituzumab govitecan is a Trop-2 directed antibody-drug conjugate (ADC) composed of the following three compounds: 1)The humanized monoclonal antibody, hRS7 IgG1k, which binds to Trop-2 (trophoblastic cell surface antigen, also known as EGP-1, epithelial glycoprotein-1) 2) The camptothecin-derived agent, SN-38, a topoisomerase 1 inhibitor, with a high drug to antibody ratio (7.6 molecules of SN-38 per antibody) 3) A hydrolysable, proprietary linker, CL2A, which binds SN-38 to the antibody

Internalization of Trop-2–bound SG delivers SN-38 inside tumor cells, interacting with topoisomerase I thus preventing re-ligation of topoisomerase I-induced single stand breaks, thereby killing the tumor cells[27] while the hydrolysable linker enables SN-38 to be released into the tumor microenvironment, killing adjacent tumor cells (bystander effect).

20.6.4 Preparation and Administration
Sacituzumab should be prepared and administered per pharmacy institutional policy. Please see Package Insert for full details on preparation and administration.

The recommended dose of SG is 10 mg/kg administered as IV infusion on Days 1 and 8 of continuous 21-day cycles. C1D1 weight will be used for each infusion unless there is a **≥ (> =)**10% change in weight. Weight will be measured prior to each dose. Do not administer SG at dose greater than 10 mg/kg. Administer SG as an IV infusion only; do not administer as IV push or bolus. SG is a cytotoxic drug. Follow applicable special handling and disposal procedures.
• Administer the first infusion over 3 hours. Monitor the subject clinically during and for at least 30 minutes after infusion (post-infusion monitoring is recommended at least for the first infusion) Subsequent infusions may be administered over 1 to 2 hours if previous infusions were well tolerated (post-infusion monitoring is not needed if previous infusions were well tolerated).

• Protect the infusion bag from light.
• An infusion pump may be used.
• Compatibility with polypropylene infusion bags has been confirmed.
• Do not mix SG, or administer as an infusion, with other medicinal products. Upon completion of the infusion, flush the IV line with 20 mL 0.9% Sodium Chloride Injection, United States Pharmacopeia.

Shipment address for this study:
Attn: IDS RG1122399
Pharmacy G5-920
Fred Hutchinson Cancer Center
825 Eastlake Ave East
Seattle, WA 98109

20.6.5 Storage and Stability

- See Package Insert for full details on storage instructions
- Store vials in a refrigerator at 2°C to 8°C (36°F to 46°F) in the original carton to protect from light until time of reconstitution. Do not freeze
- After reconstitution, the infusion bag containing SG solution can be stored in a refrigerator at 2°C to 8°C (36°F to 46°F) for up to 4 hours
- SG is a cytotoxic drug. Follow institution guidelines on handling and disposal procedures

20.6.6 Drug Interactions

See Section 12.4 and Package Insert for full details on drug interactions

20.6.7 Agent Ordering

Pharmacy will be responsible for ordering per institutional policy.

20.6.8 Agent Accountability

Sacituzumab govitecan will be stored and accessed per pharmacy institutional policy.

20.6.9 Side Effects

See Package Insert for full summary of side effects.

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22.0 APPENDICES

Appendix 1: ECOG Performance Status

GRADE	SCALE
0	Fully active, able to carry out all pre-disease performance without restriction
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light housework, office work
2	Ambulatory and capable of all self-care but unable to carry out work activities. Up and about more than 50% of waking hours.
3	Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.
4	Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.
5	Dead

Appendix 2: Contraceptive Guidance and Pregnancy Testing

Woman of Childbearing Potential (WOCBP)

A woman is considered fertile following menarche and until becoming post-menopausal unless permanently sterile (see below)

Women in the following categories are not considered WOCBP:

- Premenarchal
- Premenopausal female with 1 of the following:
    - Documented hysterectomy
    - Documented bilateral salpingectomy
    - Documented bilateral oophorectomy

Note: Documentation can come from the site personnel's review of the participant's medical records, medical examination, or medical history interview.

- Postmenopausal female
    - A postmenopausal state is defined as no menses for 12 months without an alternative medical cause.
        - A high follicle stimulating hormone (FSH) level in the postmenopausal range may be used to confirm a postmenopausal state in women not using hormonal contraception or hormonal replacement therapy (HRT). However, in the absence of 12 months of amenorrhea, confirmation with two FSH measurements in the postmenopausal range is required.
    - Females on HRT and whose menopausal status is in doubt will be required to use one of the non-hormonal highly effective contraception methods if they wish to continue their HRT during the study. Otherwise, they must discontinue HRT to allow confirmation of postmenopausal status before study enrollment.

Contraception Requirements

Male Participants:
Male participants with female partners of childbearing potential are eligible to participate if they agree to one of the following during the protocol defined time frame in section 8:

- Be abstinent from penile-vaginal intercourse as their usual and preferred lifestyle (abstinent on a long term and persistent basis) and agree to remain abstinent

- Use a male condom plus partner use of a contraceptive method with a failure rate of <1% per year as described in Table 8 below when having penile-vaginal intercourse with a woman of childbearing potential who is not currently pregnant.
    - Note: Men with a pregnant or breastfeeding partner must agree to remain abstinent from penile-vaginal intercourse or use a male condom during each episode of penile penetration.

Female Participants:
Female participants of childbearing potential are eligible to participate if they agree to use a highly effective method of contraception that has a low user dependency consistently and correctly as described in Table 9 during the protocol-defined time frame in the protocol.

Table 8. Highly Effective Contraceptive Methods That Have Low User Dependency

Highly Effective Methods That Have Low User Dependency *Failure rate of <1% per year when used consistently and correctly.*
• Progestogen- only contraceptive implant [a, b] • Intrauterine hormone-releasing system (IUS) [b] • Intrauterine device (IUD) • Bilateral tubal occlusion
• **Vasectomized partner** A vasectomized partner is a highly effective contraception method provided that the partner is the sole male sexual partner of the WOCBP and the absence of sperm has been confirmed. If not, an additional highly effective method of contraception should be used.
• **Sexual abstinence** Sexual abstinence is considered a highly effective method only if defined as refraining from heterosexual intercourse during the entire period of risk associated with the study treatment. The reliability of sexual abstinence needs to be evaluated in relation to the duration of the study and the preferred and usual lifestyle of the participant.

WOCBP should only be included after a negative highly sensitive urine or serum pregnancy test. Pregnancy testing will be done whenever an expected menstrual cycle is missed or when pregnancy is otherwise suspected.