DF/HCC Protocol #: 20-153

TITLE: Saci-IO HR+: Randomized phase II study of sacituzumab govitecan with or without pembrolizumab in hormone receptor-positive (HR+) / HER2- metastatic breast cancer (MBC)

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Industry-Supplied Agents:
Sacituzumab govitecan (IMMU-132, Trodelvy™), [redacted]
Pembrolizumab (MK-3475; Keytruda TM), [redacted]

IND #: 146973
IND Sponsor: Ana C. Garrido-Castro, MD

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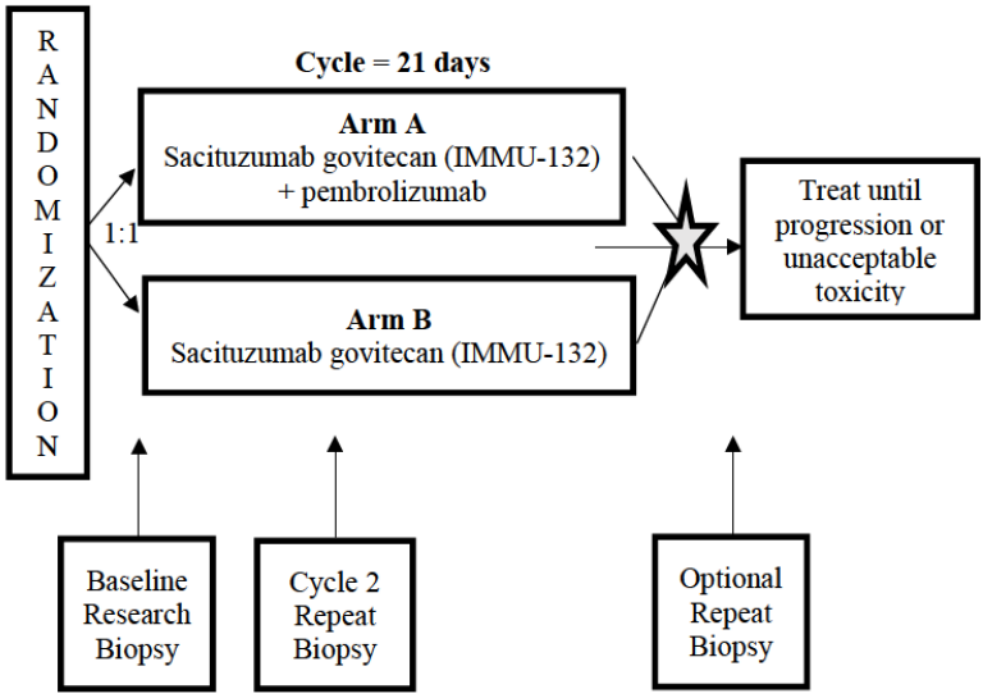
SCHEMA

Eligibility:

- HR + HER2- MBC: ER≥1% and/or PR≥1%, HER2-negative per ASCO CAP guidelines
- No restrictions on PD-L1 status
- Prior treatment with ≥ 1 hormonal therapy and 0-1 prior line of chemotherapy for MBC
- N = 110 patients



N = 110 patients for 80% power to detect an improvement in PFS from 5.5 months (Arm B) to 8.5 months (Arm A) with one-sided alpha of 0.1

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1 OBJECTIVES

1.1 Study Design

This is a randomized phase 2 open label study of sacituzumab govitecan +/- pembrolizumab for patients with hormone receptor-positive (HR+) / HER2- metastatic breast cancer (MBC). Patients will be randomized in a 1:1 ratio to sacituzumab govitecan (IMMU-132) with pembrolizumab (Arm A) or sacituzumab govitecan alone (Arm B) and will be treated until unacceptable toxicity or progression as defined by RECIST 1.1 criteria.

1.2 Primary Objectives

To compare the progression-free survival (PFS) of sacituzumab govitecan with pembrolizumab to that of sacituzumab govitecan alone in patients with HR+ / HER2- MBC.

1.3 Secondary Objectives

- To compare the PFS of sacituzumab govitecan with pembrolizumab to that of sacituzumab govitecan alone in the subgroup of patients with PD-L1-positive HR+ / HER2- MBC.
- To compare the efficacy of sacituzumab govitecan with pembrolizumab to that of sacituzumab govitecan alone by assessing other clinical outcome measures, including overall survival (OS), objective response rate (ORR) by RECIST 1.1, duration of response (DOR), time to objective response (TTOR), time to progression (TTP), and clinical benefit rate (CBR), in all-comers (regardless of PD-L1 status) and in the subgroup of patients with PD-L1-positive HR+ / HER2- MBC.
- To evaluate the safety and tolerability of sacituzumab govitecan and pembrolizumab compared to sacituzumab govitecan alone by monitoring adverse events, including immune-related adverse events.

1.4 Correlative/Exploratory Objectives for Enrolled Participants

1.4.1 To explore tissue biomarkers of antitumoral immune activity and tumor genomic instability as predictors of response and resistance to sacituzumab govitecan plus pembrolizumab compared to sacituzumab govitecan alone in patients with HR+ / HER2- MBC.

- To characterize baseline Trop-2 expression by histological assessment and correlate with disease response to treatment (PFS, ORR by RECIST 1.1, OS).
- To assess changes in Trop-2 staining, from baseline to on-treatment and at-progression, and correlate with disease response to treatment (PFS, ORR by RECIST 1.1, OS).

- To characterize tumor-infiltrating lymphocytes (TILs), by histological assessment, at baseline and correlate with disease response to treatment (PFS, ORR by RECIST 1.1, OS).
- To characterize the expression of markers of immune cell subsets (i.e., CD8 for cytotoxic T cells, CD68 for macrophages), inhibitory checkpoint pathway molecules (i.e., PD-1, PD-L1, TIM3, LAG3), and co-stimulatory pathway molecules (i.e., GITR, OX40) by immunohistochemistry (IHC) and/or immunofluorescence (IF).
- To explore whether immunosuppressive and/or immune-stimulating immune marker profiles at baseline correlate with disease response to treatment (PFS, ORR by RECIST 1.1, OS).
- To characterize mutational load and neoantigen burden at baseline and correlate with disease response to treatment (PFS, ORR by RECIST 1.1, and OS).
- To characterize RNA expression signatures of immune pathway activation and DNA damage repair deficiency at baseline and correlate with disease response to treatment (PFS, ORR by RECIST 1.1, and OS).
- To explore whether changes in TILs, immunosuppressive and/or immune-stimulating immune marker profiles, mutational load, neoantigen burden, and RNA expression signatures, between paired biopsies from baseline and after 2 cycles of treatment, correlate with disease response to treatment (PFS, ORR by RECIST 1.1, OS).
- To explore mechanisms of resistance to sacituzumab govitecan plus pembrolizumab compared to sacituzumab govitecan alone in paired biopsies from baseline and at time of progression.
- To explore whether the number and/or type of mutations identified using a next generation sequencing (NGS) panel is correlated with patient outcomes (PFS, ORR by RECIST 1.1, and OS). This will be performed on DFCI participants only.

1.4.2 To explore blood biomarkers of antitumoral immune activity as predictors of response or resistance to sacituzumab govitecan plus pembrolizumab compared to sacituzumab govitecan alone in patients with HR+ / HER2- MBC.

- To characterize serial changes in immune marker profile in peripheral blood mononuclear cells (PBMCs) and in plasma over the course of study treatment.
- To explore whether induction of changes in the immunosuppressive and/or immune-stimulating marker profile in PBMCs correlates with disease response to therapy (PFS, ORR by RECIST 1.1, OS).
- To investigate whether there is an immune marker (i.e., PD-L1) in circulating PBMCs that correlates to tumor infiltrating lymphocyte (TIL) percentage in baseline tumor.
- To characterize serial changes of neoantigen burden in circulating tumor DNA and correlate with disease response to treatment (PFS, ORR by RECIST 1.1, OS).
- To explore serial changes in blood biomarkers as mechanisms of resistance to sacituzumab govitecan plus pembrolizumab compared to sacituzumab govitecan alone.

1.4.3 To explore the structure and function of the gut microbiome as predictors of response or resistance to sacituzumab govitecan plus pembrolizumab compared to sacituzumab govitecan alone in patients with HR+ / HER2- MBC.

- To characterize structure and function of the gut microbiome at baseline and correlate with disease response to treatment (PFS, ORR by RECIST 1.1, OS).
- To explore whether changes in the overall diversity of gut microbiome, estimated by Shannon index, correlate with disease response to treatment (PFS, ORR by RECIST 1.1, OS).
- To explore correlates of resistance to sacituzumab govitecan plus pembrolizumab compared to sacituzumab govitecan alone in paired samples from baseline and at time of progression.

1.4.4 To explore patient reported outcomes as measured by the following:

- To assess and compare the impact of treatment on Health-Related Quality of Life (HRQoL) between treatment arms, using European Organization for the Research and Treatment of Cancer (EORTC) quality of life (QOL) questionnaire version 30 (QLQ-C30) and the European Quality of Life (EuroQOL) EQ-5D-5L instruments.
- To assess and compare the impact of treatment-related symptoms using a set of 16 relevant symptom concepts from the Patient-Reported Outcomes (PRO) version of the Common Terminology Criteria for Adverse Events (PRO-CTCAE™) item library (Decreased appetite, Nausea, Vomiting, Constipation, Diarrhea, Abdominal pain, Shortness of breath, Cough, Wheezing, Rash, Hair loss, Itchy skin, Hives, Muscle Aches, Joint Aches, and Fatigue).

1.5 Correlative Objectives for Pre-Screened Participants (prior to Sponsor Amendment 3, PD-L1 eligibility amendment)

1.5.1 To explore tissue biomarkers that characterize the immune microenvironment of HR+/HER2- MBC

- To characterize tumor-infiltrating lymphocytes (TILs) by histological assessment.
- To characterize the expression of various biomarkers by immunohistochemistry (IHC) and/or immunofluorescence (IF).
- To characterize the expression of markers of immune cell subsets (i.e., CD8 for cytotoxic T cells, CD68 for macrophages), inhibitory checkpoint pathway molecules (i.e., PD-1, PD-L1, TIM3, LAG3), and co-stimulatory pathway molecules (i.e., GITR, OX40) by immunohistochemistry (IHC) and/or immunofluorescence (IF).
- To characterize mutational load and neoantigen burden.
- To characterize RNA expression signatures of immune pathway activation and DNA damage repair deficiency
- To explore the number and/or type of mutations identified using a next generation sequencing (NGS) panel or whole exome sequencing.

2 BACKGROUND

2.1 HR+ / HER2- Metastatic Breast Cancer (MBC)

Worldwide, breast cancer is the most common non-cutaneous cancer and the leading cause of cancer death in women.[1] In the United States, breast cancer accounts for nearly one-third of all new cancer diagnoses among women with over 250,000 new cases per year.[2] Metastatic breast cancer (MBC) remains incurable and is a major cause of mortality in the United States with over 40,000 American women dying of breast cancer in 2019 alone.[2] Hormone receptor-positive (HR+) / human epidermal growth factor receptor 2-negative (HER2-) breast cancer, defined by the presence of estrogen and/or progesterone receptors and the absence of HER2, accounts for the majority of breast cancers, approximately 70%.[3] Although initially sensitive to endocrine-based therapies, including CDK4/6 inhibitors, it eventually develops resistance to hormone therapy in the metastatic setting.[3] Pre-treated HR+ / HER2- MBC that has progressed on hormone therapy typically displays low response rates to chemotherapy with short median progression free survival (PFS) of 4 to 5 months and median overall survival (OS) around 18 months.[4] Therefore, improved therapies are urgently needed for patients with pre-treated HR+ / HER2- MBC that has progressed on hormone therapy.

2.2 Immune Checkpoint Inhibitors in Breast Cancer

Immune checkpoint inhibitors function by blocking receptors, such as cytotoxic T lymphocyte antigen-4 (CTLA-4, CD152) and programmed cell death-1 (PD-1, CD279), to re-activate cytotoxic T cell responses. PD-1, which is induced following initial T cell activation to regulate T cells,[5] binds the ligands PD-L1 and PD-L2 to attenuate T cell receptor signaling,[6] thereby leading to decreased T cell proliferation, cytotoxicity, and cytokine production.[7] Immune checkpoint inhibitors block these checkpoints to improve the cytotoxicity and proliferative capacity of tumor infiltrating lymphocytes. Immune checkpoint inhibitors, including monoclonal antibodies against PD-1 (i.e., pembrolizumab), PD-L1 (i.e., atezolizumab), and CTLA-4 (i.e., ipilimumab), have generated durable responses across many tumor types.[8-15]

However, few patients with breast cancer respond to immune checkpoint inhibitors as single agents.[16] These therapies have therefore been investigated in combination with other agents, including chemotherapy, targeted therapy, and immunotherapeutic agents. This approach has proved most successful in TNBC, where the combination of the chemotherapy nab-paclitaxel and the PD-L1 monoclonal antibody atezolizumab led to a statistically significant improvement in PFS and a clinically relevant improvement in overall survival for patients with metastatic PD-L1-positive TNBC in the IMpassion130 trial (Table 1).[17] Based on these findings, the FDA granted accelerated approval for atezolizumab in combination with nab-paclitaxel for the first-line treatment of advanced or metastatic PD-L1-positive TNBC in March 2019. Nevertheless, effective immune checkpoint regimens in PD-L1-negative TNBC, hormone receptor-positive breast cancer, and HER2-positive breast cancer have yet to be established, so the majority of patients with breast cancer do not benefit from immune checkpoint inhibitors.

Table 1: Progression-Free Survival and Overall Survival in the IMpassion130 Study[17,18]

	Progression-free Survival	Overall Survival

	Atezolizumab + nab-paclitaxel (N = 451)	**Placebo + nab-paclitaxel (N = 451)**	**Hazard ratio (95% CI) p-value**	**Atezolizumab + nab-paclitaxel (N = 451)**	**Placebo + nab-paclitaxel (N = 451)**	**Hazard ratio (95% CI) p-value**
ITT	7.2 months	5.5 months	0.80 (0.69 - 0.92) p = 0.0025	21.0 months	18.7 months	0.86 (0.72 - 1.02) p = 0.08
PD-L1+[a]	7.5 months	5.0 months	0.62 (0.49 - 0.78) p < 0.001	25.0 months	18.0 months	0.71 (0.54 - 0.93) NA[b]

ITT = intent to treat; OS = overall survival; PD-L1 = programmed cell death ligand 1 positive

[a] PD-L1+ was defined as ≥ 1% of immune cells in the microenvironment stained positive for PD-L1 using the SP142 antibody

[b] Per the statistical design, because the OS difference in the ITT population was not significant, there was no formal statistical testing of the difference in OS in the PD-L1+ population

2.3 Pembrolizumab

2.3.1 Mechanism of Action of Pembrolizumab

Pembrolizumab is a potent and highly selective humanized monoclonal antibody (mAb) of the IgG4/kappa isotype designed to directly block the interaction between PD-1 and its ligands, PD-L1 and PD-L2.[19] Keytruda™ (pembrolizumab) is approved in the United States for the treatment of numerous cancers including melanoma, non-small cell lung cancer, head and neck squamous cell cancer, classical Hodgkin lymphoma, primary mediastinal large B-cell lymphoma, urothelial carcinoma, microsatellite instability-high cancer, gastric cancer, cervical cancer, hepatocellular carcinoma, and merkel cell carcinoma.[19] Please refer to the Full Prescribing Information for pembrolizumab for complete prescribing and safety information.[19]

2.3.2 Nonclinical Studies of Pembrolizumab

Animal models demonstrated that PD-1 inhibition increased the severity of some infections and enhanced inflammatory responses.[19] M. tuberculosis-infected PD-1 knockout mice exhibited markedly decreased survival compared with wild-type controls, which correlated with increased bacterial proliferation and inflammatory responses.[19] PD-1 knockout mice have also shown decreased survival following infection with lymphocytic choriomeningitis virus.[19] Administration of pembrolizumab in chimpanzees with naturally occurring chronic hepatitis B infection resulted in two out of four animals with significantly increased levels of serum ALT, AST, and GGT, which persisted for at least 1 month after discontinuation of pembrolizumab.[19]

Pembrolizumab has not undergone preclinical fertility studies.[19] No effects on male and female reproductive organs were observed in 1-month and 6-month repeat-dose toxicology studies in monkeys, although most of these animals were not sexually mature.[19]

2.3.3 Pharmacokinetics of Pembrolizumab

The first-in-human phase I study (PN001, NCT01295827) evaluated the safety and clinical activity of pembrolizumab as a monotherapy. Part A of this study was a 3+3 dose escalation study in subjects with solid tumors to evaluate safety, tolerability, pharmacokinetics (PK), and pharmacodynamics, and to determine a maximum tolerated dose (MTD) or preliminary recommended phase 2 doses (RP2Ds).[19] Doses were 1, 3, and 10 mg/kg every 2 weeks (Q2W); doses of either 2 mg/kg or 10 mg/kg were also administered every 3 weeks (Q3W).[19] All 3 dose levels were well tolerated and no dose-limiting toxicities (DLTs) were observed; therefore, the MTD was not determined.[19] The RP2D was determined based on safety, PK, and pharmacodynamic measurements, along with the strength of antitumor activity signals observed.[19]

The half-life ($t_{1/2}$) of pembrolizumab is approximately 4 weeks and there is no indication of dose dependency or half-life in the three dose groups (1, 3, and 10 mg/kg).[19] The long $t_{1/2}$ supports a dosing interval of every 2 or 3 weeks.[19] There was a dose-related increase in exposure from 1 to 10 mg/kg.[19] Serum concentrations of pembrolizumab were lower by a factor of approximately 5 in patients receiving 2 mg/kg Q3W than in those receiving 10 mg/kg Q3W.[19] Steady-state trough concentrations were 20% greater in the patients receiving 10 mg/kg Q2W than in those receiving the same dose Q3W.[19]

A population pharmacokinetic analysis was performed using serum concentration time data from 476 patients.[19] Within the resulting population pharmacokinetic model, clearance and volume parameters of pembrolizumab were found to be dependent on body weight.[19] The relationship between clearance and body weight, with an allometric exponent of 0.59, is within the range observed for other antibodies and would support both body weight normalized dosing or a fixed dose across all body weights.[19] Pembrolizumab has been found to have a wide therapeutic range, and the population pharmacokinetic evaluation revealed that there was no significant impact of tumor burden or cancer type on exposure.[19]

The choice of the 200 mg Q3W as an appropriate dose for the switch to fixed dosing was based on simulations performed using the population pharmacokinetic model of pembrolizumab showing that the fixed dose of 200 mg every 3 weeks provided exposures that 1) were optimally consistent with those obtained with the 2 mg/kg dose every 3 weeks, 2) maintained individual patient exposures in the exposure range associated with maximal efficacy response and 3) maintained individual patient's exposure in the exposure range established as well tolerated and safe.[19] A fixed dose regimen simplified the dosing regimen to be more convenient for physicians, minimizing the potential for dosing errors, and reduced complexity in the logistical chain at treatment facilities, decreasing wastage.[19]

The pharmacokinetic properties of pembrolizumab were further characterized in 2993 patients with various cancers who received pembrolizumab.[19] Peak and trough concentrations increased dose proportionally in the dose range of 2 to 10 mg/kg every 3 weeks, and steady-state concentrations were reached by 16 weeks of repeated dosing with an every 3-week dosing regimen.[19] Pembrolizumab does not rely on metabolism for clearance and instead undergoes catabolism to small peptides and single amino acids via general protein degradation routes.[20] Clearance is 23% lower at steady state than after the first dose.[19] Age, sex, race, tumor burden, renal impairment (eGFR $\geq$ 15 mL/min/1.73 m2), and mild hepatic impairment (total bilirubin $\leq$

upper limit of normal (ULN) and AST > ULN or total bilirubin between 1 and 1.5 times ULN and any AST) had no clinically meaningful effect on the clearance of pembrolizumab.[19]

2.3.4 Clinical Studies of Pembrolizumab

Pembrolizumab has demonstrated efficacy across a range of cancer types. Discussed here are the efficacy results for melanoma and non-small cell lung cancer as two representative examples. When treated with pembrolizumab monotherapy, the ORR for patients with ipilimumab-naïve and ipilimumab-refractory metastatic melanoma was 33% or 34% and 27% or 29% with every 3 or 2 week dosing, respectively.[19] The majority of responses were seen in patients with melanoma by 16 weeks of therapy; however, some responses were reported after 24 weeks or more of therapy with pembrolizumab.[19] In some patients, a RECIST-defined progression followed by response was observed.[19] The ORR for patients with treatment-naïve metastatic non-small cell lung cancer treated with pembrolizumab was 27% as monotherapy in PD-L1 positive tumors and 48-58% in combination with chemotherapy.[19]

In these studies, the most frequent treatment-related adverse events (AEs) were fatigue, nausea, cough, pruritis, diarrhea, and rash.[19] Most AEs were not considered serious. The most commonly-reported immune-related AEs were rash, pruritis, vitiligo, hypothyroidism, arthralgia, diarrhea, and pneumonitis.[19] Important identified risks include: pneumonitis, thyroid disorders (hypothyroidism and hyperthyroidism), colitis, diarrhea, hepatitis, nephritis, uveitis, rash/pruritis, and neuropathy.[19]

2.3.5 Pembrolizumab in Metastatic Breast Cancer

Recent trials have reported the safety of pembrolizumab in metastatic TNBC. In the phase Ib KEYNOTE-012 trial of pembrolizumab in 32 patients with advanced TNBC, common toxicities were mild, occurred in 56% of patients, and were similar to those observed in other tumor cohorts, such as arthralgia (19%), fatigue (19%), myalgia (19%), and nausea (16%).[21] A total of five patients (16%) experienced grade 3 or higher toxicity, including anemia, aseptic meningitis, lymphopenia, pyrexia, and headache, and one patient (3%) experienced a treatment-related death due to disseminated intravascular coagulation.[21]

However, the efficacy of pembrolizumab monotherapy in TNBC has been low. In the phase Ib KEYNOTE-012 trial, 32 patients with pre-treated metastatic TNBC had an ORR of 18.5%.[21] In the phase 2 KEYNOTE-086 trial, 170 patients with pre-treated metastatic TNBC had an ORR of 5%.[16] The ORR increased to 23% in Cohort B of this trial, which enrolled 52 patients with treatment-naïve, PD-L1-positive metastatic TNBC.[16]

The efficacy of pembrolizumab in combination with chemotherapy has been similarly low in initial trials of unselected patients with TNBC. In a phase 1b/2 trial of pembrolizumab and eribulin, the ORR was 29% for 65 patients with treatment-naïve metastatic TNBC and 22% for 41 patients with pretreated metastatic TNBC.[22] The median overall survival for these patients was 17.7 months and 16.3 months, respectively.[22] A large phase III trial of pembrolizumab in combination with multiple different chemotherapy agents for treatment-naïve metastatic TNBC showed that pembrolizumab plus chemotherapy resulted in a significant and clinically

meaningful improvement in PFS only among patients with highly PD-L1 positive disease, measured as Combined Positive Score (CPS) ≥ 10 by the PD-L1 IHC 22C3 assay (NCT02819518).[119]

Compared to metastatic TNBC, HR+ / HER2- MBC has demonstrated lower response rates to immune checkpoint inhibitors, including pembrolizumab. In the phase Ib KEYNOTE-028 trial, the small cohort of 25 patients with PD-L1-positive pretreated HR+ / HER2- MBC demonstrated an ORR of 12% with pembrolizumab monotherapy.[23] This ORR in PD-L1+ HR+ MBC was higher than the ORR of 2.8% observed in PD-L1-unselected HR+ / HER2- MBC treated with the PD-L1 inhibitor avelumab in the phase I JAVELIN trial.[24] Similarly, the phase II study of eribulin with or without pembrolizumab showed no difference in PFS with the addition of pembrolizumab to eribulin compared to eribulin alone in 88 patients with pre-treated HR+ / HER2- MBC.[25] The mechanisms underlying the lower response rates of HR+ / HER2- MBC to immune checkpoint inhibitors compared to TNBC are largely unknown, though may be related to lower levels of tumor infiltrating lymphocytes, lower tumor mutation burden, and lower PD-L1 expression.[26,27]

2.4 Sacituzumab Govitecan (IMMU-132)

2.4.1 Mechanism of Action of Sacituzumab Govitecan

Sacituzumab govitecan is an antibody-drug conjugate (ADC) composed of hRS7, a humanized IgG1κ monoclonal antibody, SN-38, a camptothecin analog, and CL2A, a linker which couples SN-38 to hRS7. Trophoblastic cell-surface antigen-2 (Trop-2) is a cell surface antigen overexpressed in many epithelial cancers and has been linked to aggressive disease and poor prognosis. The antibody component binds to Trop-2 such that the ADC concentrates on tumor cell surfaces. SN-38 is the active metabolite of irinotecan and a topoisomerase I inhibitor that induces single-stranded DNA breaks during replication. If unrepaired, these breaks progress to double-stranded DNA breaks and results in cell death. The CL2A linker is unique in that it is subject to pH-dependent hydrolysis. Following internalization of the ADC-antigen complex, SN-38 is released in the acidic lysosome and kills the target cells. In addition, SN-38 can be hydrolyzed from extracellular, surface-bound ADC. The released drug diffuses into neighboring cells in an antigen-independent manner and this leads to death of cells in the immediate proximity of the target-expressing cells. These two mechanisms of action combine to enable sacituzumab govitecan to target and kill cancer cells that over-express Trop-2 as well as other cells in the tumor with low or no Trop-2 expression.

2.4.2 Nonclinical Studies of Sacituzumab Govitecan

Pharmacology Studies

In vitro cytotoxicity of sacituzumab govitecan was assessed in 6 different cell lines representative of several different epithelial tumors; PC-3 (prostate), Calu-3 (NSCLC), COLO 205 (colon), Capan 1 (pancreatic), SK-MES-1 (squamous cell lung), and BxPC-3 (pancreatic). In general, the IC50 values ranged from 1.95 nM to 23.14 nM.[28]

The antitumor activity of the hRS7-SN-38 conjugate was examined *in vivo* in mice bearing a variety of human epithelial cancer xenografts, where it was shown to have significant antitumor effects compared to free SN-38, irinotecan, or an irrelevant IgG-SN-38 conjugate.[28-30]

Pharmacokinetics

Toxicokinetic analysis of the 3-month repeat-dose study in Cynomolgus monkeys using validated assays showed a dose-proportional exposure (Cmax and AUC) to sacituzumab govitecan, total antibody, total SN-38 and free SN-38 with no relevant gender differences after the first dose. Serum half-life was estimated in recovery animals and ranged from 67.3 to 152 hours. On average the amount of free SN-38 in circulation was low, <3% free SN-38 as compared to the total SN-38 bound to sacituzumab govitecan. This confirms that the majority of SN-38 administered as sacituzumab govitecan remains bound to the ADC in the serum and is not circulating as the free cytotoxic payload. The inactive metabolite, SN-38G, readily appeared in serum with mean Tmax ranging from 4.0 to 12 hours.

Toxicology

In acute toxicity studies in Swiss-Webster Mice, sacituzumab govitecan at doses of up to 750 mg/kg/dose (i.e., cumulative doses of up to 1500 mg/kg) caused minimal loss (<10%) in body weight. There was no evidence of hematological toxicity and no abnormal histology findings. Transient increases in hepatic transaminases were observed that returned to normal by the end of the study.

In Cynomolgus monkeys, sacituzumab govitecan administered 50 mg/kg/dose (human equivalent dose = 16 mg/kg/dose) for 4 treatment cycles (Days 1 and 8 of a 21-day cycle) was considered a no-observed-adverse-effect level, and 120 mg/kg/dose administered 3 days apart was associated with lethality. In monkeys, target organs included the female reproductive tract; skin (hair loss, pigmentation); kidney (periarteritis); lymphoid organs (lymphoid depletion); bone marrow (reduced cellularity) with concomitant reductions in red cells, white cells, and platelets; and the gastrointestinal tract (necrosis, erosions, inflammation, fibrosis, hemorrhage, edema).

Local tolerance was evaluated in the GLP-compliant monkey studies. Although changes were observed at the injection site, the study pathologist interpreted these findings as related to procedural trauma and not the study drug. These changes consisted of mild to moderate perivascular hemorrhage, moderate hemorrhage in the dermis and subcutis, and minimal to mild perivascular mixed cell infiltration.

SN-38 was negative for mutagenicity in a bacterial reverse mutation test and was found to be clastogenic in an *in vitro* mammalian cell micronucleus test. Neither the carcinogenicity, nor effects of sacituzumab govitecan on fertility, early embryonic development, or pre- and post-natal development have been assessed. However, SN-38 is a camptothecin and hence might be carcinogenic. Furthermore, SN-38 is a known developmental toxicant (Camptosar® Prescribing Information).

2.4.3 Pharmacokinetics of Sacituzumab Govitecan

The serum pharmacokinetics of sacituzumab govitecan and free SN-38 were evaluated in Phase 1/II study in a population of HR+ / HER2- MBC patients who received sacituzumab govitecan as a single agent at a dose of 10mg/kg. The pharmacokinetic parameters of sacituzumab govitecan and free SN-38 as determined by noncompartmental analysis are presented in the table below.

Table 2: Summary of Mean (± Standard Deviation) Sacituzumab govitecan and Free SN38

	Sacituzumab govitecan					**Free SN-38**		
	C_{max} [ng/mL]	**AUC_{0-168}** [h ng/mL]	**$t_{1/2}$** [h]	**Vz** [L/kg]	**Cl** [L/h/kg]	**C_{max}** [ng/mL]	**AUC_{0-168}** [h ng/mL]	**$t_{1/2}$** [h]
	n=43	n=42	n=42	n=42	n=42	n=43	n=37	n=37
Mean	243,000	5,210,000	15.6	0.0450	0.00202	127	3,900	17.7
SD	45,600	1,230,000	2.90	0.0114	0.000477	59.7	1,830	4.74

C_{max}: maximum plasma concentration
AUC 0-168: area under plasma concentration curve through 168 hours
$t_{½}$: terminal elimination half-life
Cl: clearance
Vz: volume of distribution of the terminal elimination phase
SD: standard deviation

Distribution

The maximum concentrations of sacituzumab govitecan and SN-38 occurred close to the end of infusion. The mean volume of distribution (Vz) for sacituzumab govitecan was 0.0450 L/kg.
SN-38 is highly protein bound to human plasma proteins (approximately 95%). The plasma protein to which SN-38 predominantly binds is albumin. The relative amount of free SN-38 compared to total SN-38 was small and was less than or equal to 16.9% for all time points, averaging below 7.14%.

Elimination

Following administration of 10 mg/kg sacituzumab govitecan the clearance of the sacituzumab govitecan was calculated by non-compartmental analysis to be 0.00202 L/h/kg. SN-38 appeared to follow metabolite kinetics, with the elimination of SN-38 appearing to be limited by its rate of release from sacituzumab govitecan.

Metabolism

No metabolism studies with sacituzumab govitecan have been conducted. SN-38 is known to be metabolized via the human UGT1A13.

Immunogenicity

As with all therapeutic proteins, there is potential for an immune response to sacituzumab govitecan. Based on available data in the safety population (n=420), the rate of treatment emergent and persistent ADA is very low and has been limited to 3 subjects (0.7%), with an additional 17 subjects (4%) having a transient ADA positive sample. None of the treatment emergent and confirmed positive patients had infusion-related hypersensitivity adverse events.

2.4.4 Clinical Studies of Sacituzumab Govitecan

Clinical Study IMMU-132-01 (US FDA IND #115621) is an ongoing, multi-center, US-only, trial evaluating the safety, toxicity, and PK of sacituzumab govitecan. Over 500 subjects have been enrolled in the trial, including 215 heavily pretreated patients with metastatic breast cancer (MBC), 54 of whom are ER+/HER2-. The study population includes subjects with relapsed/refractory, metastatic epithelial cancers. The starting dose for the Phase 1 portion was 8 mg/kg administered on Days 1 and 8 of a 21-day cycle. After evaluation at this and higher doses in a 3+3 Phase 1 dose-finding design, 8 and 10 mg/kg sacituzumab govitecan administered on Days 1 and 8 of a 21-day cycle were chosen for further Phase 2 evaluation in certain patient populations; namely, breast cancer, non-small-cell lung cancer, small-cell lung cancer, colorectal cancer, pancreatic cancer, urothelial cancer, endometrial cancer, and esophageal cancer.

Initially in Phase 2, subjects were recruited in a sequential manner to the 8 mg/kg dose and subsequently to the 10 mg/kg dose. An interim analysis was performed when 81 and 97 subjects with different tumor types had been treated at the 2 dose levels, respectively. There did not appear to be important differences in safety, and subjects receiving the 10 mg/kg dose achieved a similar duration of treatment as those receiving 8 mg/kg. Furthermore, data suggested that the 10 mg/kg dose was associated with slightly better objective (overall) response rate ORR and clinical benefit rate (CBR). Results of this study have been published.[31] Based on the evidence available, it was decided that further subject accrual would proceed at the 10 mg/kg dose level.

Sacituzumab govitecan has demonstrated safety and efficacy in multiple patient populations. Promising tumor responses have been observed in patients with advanced, relapsed/refractory TNBC (33% [36/108]),[32] urothelial carcinoma (31% [14/45]),[33] non-small lung cancer (19% [9/47]),[34] small-cell lung cancer (14% [7/50]),[35] and others. One of the more important safety observations from these studies is that it appears that the toxicity profile of sacituzumab govitecan is more tolerable than when SN-38 is administered as the parent compound, irinotecan, or with other non-tumor-targeting SN-38 products.

In the phase-3 study ASCENT patients with mTNBC treated with ≥2 prior lines of chemotherapy in the advanced/metastatic setting (prior taxane required) were randomized 1:1 to receive sacituzumab govitecan (10 mg/kg IV on d 1, 8 every 21 d) or treatment of physician's choice (TPC; capecitabine, eribulin, vinorelbine, or gemcitabine) until disease progression/unacceptable toxicity. Sacituzumab govitecan (n=235) compared with TPC (n=233) significantly improved mPFS (5,6 vs 1,7 mo; HR, 0,41; P<0,0001) and median OS (12,1 vs 6,7 mo; HR, 0,48; P<0,0001). ORR was 35% for sacituzumab govitecan vs 5% for TPC (P<0,0001).[120]

Two additional clinical trials using sacituzumab govitecan are ongoing. One of these trials is in breast cancer: the Phase 3 randomized TROPICS-02 trial, IMMU-132-09 (US FDA IND #122694, EudraCT 2018-004201-33) in relapsed/refractory HR+, HER2-negative metastatic breast cancer comparing sacituzumab govitecan to treatment of physician's choice (TPC). The second ongoing trial is in metastatic urothelial cancer: the IMMU-132-06 (US FDA IND #140084) Phase 2 trial after failure of a platinum-based regimen or anti-PD1-based immunotherapy.

2.4.5 Sacituzumab Govitecan in Metastatic Breast Cancer

In the phase 1/2 IMMU-132-01 trial, 108 patients with metastatic triple-negative breast cancer received sacituzumab govitecan as a third or higher line of therapy at 10 mg/kg on days 1 and 8 every 21 days until progression or unacceptable toxicity.[32] The age range of all patients was from 31 to 80 years old with a median age of 55 years old.[32] The patient population were heavily pre-treated with a median of 3 prior anticancer therapies. Some patients received up to 10 prior therapies and 69% of patients had received prior platinum agents for metastatic disease.[32]

The most common adverse events were neutropenia, nausea, and diarrhea (Table 3).[32] Most adverse events were managed with supportive medications or dose modifications, and the majority of patients were able to continue at the 10 mg/kg dose.[32] Only three patients (2.8%) discontinued treatment due to adverse events (treatment-related grade 3 transient infusion reaction and grade 2 fatigue; non-treatment related hypertension).[32] No treatment-related deaths occurred.

Table 3. Drug-related adverse events in TNBC patients

Body system	Adverse event (AE)	All grades	Grade 3 or 4
Hematologic	Neutropenia	64%	42%
	Anemia	50%	11%
	Leukopenia	21%	12%
Gastrointestinal	Nausea	67%	6%
	Diarrhea	62%	8%
	Vomiting	49%	6%
	Constipation	34%	1%
Other	Fatigue	55%	8%
	Alopecia	36%	0%
	Decreased appetite	30%	0%
	Hyperglycemia	24%	4%
	Hypomagnesemia	21%	1%
	Hypophosphatemia	15%	9%

Out of the 108 patients, three achieved complete responses and 33 partial responses, for an objective response rate of 33% (RECIST 1.1).[32] Clinical benefit rate (CR+PR+SD ≥6 months) was 45% (49/108).[32] The median time to onset of response was 2 months with a median duration of response of 7.7 months.[32] Importantly, 6 patients were progression free for more than 1 year from start of treatment.[32] The median PFS was 5.5 months (95% CI, 4.1 to 6.3), and the median OS was 13.0 months (95% CI, 11.2 to 13.7).[32] Patients benefited from sacituzumab govitecan treatment irrespective of age, onset of metastatic disease, number of prior regimens, or presence or absence of visceral metastases.[32]

Also in the phase 1/2 IMMU-132-01 trial, 54 patients with HR+ / HER2- MBC received sacituzumab govitecan at 10 mg/kg on days 1 and 8 every 21 days until progression or unacceptable toxicity.[36] All patients had received at least 2 prior lines of therapy in any setting, one of which was endocrine-based therapy.[36] The ORR for this population was 31.5% with median PFS of 5.5 months and median OS of 12 months.[36] Key grade ≥ 3 adverse events were neutropenia, anemia, and diarrhea.[36]

In April 2020, the FDA granted accelerated approval to sacituzumab govitecan for patients with metastatic triple negative breast cancer who received at least two prior therapies for metastatic

disease based on the result of the single-arm phase II trial. The ORR was 33.3% (95% CI: 24.6, 43.1). The median response duration was 7.7 months (95% CI: 4.9, 10.8).[121]

The rationale to support enrolling patients with pre-treated HR+ / HER2- MBC, who have progressed on any number of prior hormone therapies and up to one prior chemotherapy, on this randomized phase II study employing sacituzumab govitecan as the control arm is that these patients have short-lived responses to chemotherapy,[4,37,38] which is the usual next line of therapy after progression on hormone therapies. Specifically, the ORR and median PFS for these patients on standard-of-care chemotherapy agents as early line therapies are similar to the ORR of 31% and the median PFS of 6.8 months observed with sacituzumab govitecan as a later line therapy for heavily pre-treated patients with HR+ / HER2- MBC,[39] indicating that this ADC would likely perform at least as well as standard chemotherapies in an earlier line setting. Furthermore, some of these patients have already been exposed to chemotherapy agents with known clinical benefit, namely taxanes and anthracyclines, in the neoadjuvant or adjuvant setting, increasing the probability that their recurrent metastatic tumors are resistant to these therapies. Finally, and most importantly to patients, sacituzumab govitecan has lower rates of severe potentially permanent toxicity, such as neuropathy and heart failure,[32] than taxanes and anthracyclines.

2.5 Rationale for Combination of Pembrolizumab and Sacituzumab Govitecan

Patients with HR+ MBC refractory to hormone therapies and CDK4/6 inhibitors have short responses to chemotherapy with a median PFS of around 4 to 5 months.[4] Immune checkpoint inhibitors, although associated with durable responses in other tumors, have yielded disappointingly low monotherapy responses in HR+ MBC.[16] Efforts to boost responses by combining immunotherapy with chemotherapy have proved most successful in triple negative breast cancer.[17] However, the optimal agent to combine with immunotherapy to prolong survival in HR+ MBC remains urgently needed and unknown.

Sacituzumab govitecan, an anti-Trop-2-SN-38 ADC, may be an effective immunotherapy combination agent for patients with HR+ MBC. Targeting Trop-2, a transmembrane signaling receptor overexpressed in breast cancer, sacituzumab govitecan resulted in a median PFS of approximately 6 months in pre-treated HR+ MBC.[39] This ADC may boost anticancer immunity by binding immune cell receptors to promote antibody-dependent cellular cytotoxicity (ADCC).[29] In addition, the SN-38 payload of sacituzumab govitecan is the active metabolite of irinotecan, which has been shown to deplete regulatory T cells, upregulate MHC class I and PD-L1 expression, and augment the antitumor activity of anti-PD-1/L1 antibodies in murine tumor models.[40] Furthermore, the irinotecan analogue camptothecin enhances CD8+ cytotoxic T cell effector functions and antitumor immune responses by inhibiting NR4A transcription factors,[41] which have recently been shown to a play central role in inducing the T cell dysfunction associated with chronic antigen stimulation in solid tumors.[42,43] Finally, ADCs have enhanced the effects of immunotherapy in other breast cancer subtypes, as demonstrated by the promising preliminary results of the ADC trastuzumab emtansine (TDM-1) in combination with atezolizumab in patients with advanced PD-L1+ HER2+ breast cancer in the KATE2 trial.[44]

Thus, multiple lines of evidence suggest that sacituzumab govitecan will enhance the antitumor immune effects of pembrolizumab in HR+ HER2- MBC. We hypothesize that sacituzumab

govitecan will enhance T cell infiltration into tumors by selectively targeting breast cancer cells and exposing tumor neoantigens. This trial therefore aims to answer the question of whether pembrolizumab added to sacituzumab govitecan will extend survival for HR+ MBC patients. Considering the potential immunomodulatory effects of sacituzumab govitecan described above, we hypothesize that patients with HR+ MBC tumors may derive benefit from pembrolizumab when given in combination with sacituzumab govitecan, and that this benefit will be observed among all patients with HR+ tumors regardless of baseline PD-L1 expression. We will also explore differences in the efficacy of sacituzumab govitecan plus pembrolizumab vs. sacituzumab govitecan alone in the subgroup of patients with PD-L1-positive tumors, as determined by CPS ≥1 using the PharmDx 22C3 assay, which represents approximately 20-25% of HR+ / HER2- metastatic tumors.

2.6 Correlative Studies Background

2.6.1 Blood and Tissue Analysis

The importance of anticancer immunity has become apparent over the last few years, as immune checkpoint inhibitors have improved survival across many cancer types.[45] However, less than half of patients with solid tumors derive benefit from these drugs,[15,46] and few patients with breast cancer benefit.[16] Thus, elucidating the mechanisms underlying immune evasion is crucial to develop new therapeutic strategies that enhance the efficacy of immunotherapy in breast cancer.

Growing evidence suggests that patients with advanced solid tumors show differences in tumor microenvironment regarding the presence or absence of a gene expression profile indicative of a pre-existing T-cell–inflamed tumor microenvironment.[47] Tumors classified as T-cell inflamed harbor a significant infiltration of CD8+ T cells and a type-I interferon (IFN) signature. In this group, the main mechanisms of immune evasion may be the overexpression of immunosuppressive molecules in the tumor microenvironment, including immune checkpoint molecules (CTLA-4, PD-1/PD-L1, TIM-3, LAG-3), indoleamine-2,3-dioxygenase (IDO), and FoxP3, characteristically expressed on regulatory T cells. Interestingly, these immunosuppressive molecules are stimulated by a type-I IFN antitumor response, resulting in T-cell exhaustion and adaptive immune resistance.[47,48] In contrast, other tumors are characterized by low or absent intratumoral CD8 T cells and no evidence of a type-I IFN transcriptional signature; this tumor phenotype is non-T-cell-inflamed.[47]

The T-cell inflamed phenotype has positive prognostic value for several types of cancer, including breast cancer,[49,50] suggesting that an attempt by the host to generate an anti-tumor immune response reflects a biologic process associated with improved clinical outcomes.[47] In breast oncology, different groups have demonstrated that the amount of tumor infiltrating lymphocytes (TILs) in a tumor specimen, commonly assessed by histological evaluation of a standard hematoxylin and eosin-stained slide by a trained pathologist, is a significant predictor of both response to therapy and overall disease outcomes in the neoadjuvant and adjuvant settings.[51-57] Recently, more in-depth methods of immunologic profiling have been explored in breast cancer, for example, mRNA expression of immune-activating and immunosuppressive factors, and these additional immune profiles also appear to have prognostic significance.[49] Furthermore, in the metastatic setting, the T-cell-inflamed phenotype appears to be associated with clinical response

to several immunotherapies, including checkpoint blockade.[58] Thus, one of the main objectives of the correlative science in this trial is the characterization of a broad array of immune markers in HR+ / HER2- MBC to investigate whether these markers predict response to immunotherapy.

Considering the mechanism of action of anti-PD-1/anti-PD-L1 drugs, the absence of significant T-cell infiltrate, in addition to low expression of immune checkpoint molecules, may correlate with a non-inflamed tumor phenotype that is associated with *de novo* resistance to these agents. For this group of patients, therapeutic strategies that promote an increase in cytotoxic T-cell infiltration, such as antibody-drug conjugates, may be crucial to successfully overcoming T-cell exclusion and improve the likelihood of benefit of PD-1 blockade.

Additionally, as part of the correlative work in this study, we will characterize the immune marker profile of peripheral blood mononuclear cells (PBMCs) in enrolled patients. Furthermore, given the demonstrated clinical significance of TILs in breast cancer specimens, we will evaluate whether there is a peripheral marker whose level corresponds to TIL infiltration. We will also evaluate whether there is a correlation between changes in PBMC immune profiles and response to treatment. Evidence of an association would be of significant interest, as it would indicate a potential biomarker of response or resistance in the peripheral blood.

Finally, sacituzumab govitecan selectively targets breast cancer cells by binding to Trop-2 for targeted delivery of SN-38 directly to tumor cells while minimizing systemic exposure of SN-38 to decrease host toxicity. However, the role of Trop-2 expression as a potential predictive biomarker is unknown. Therefore, we will evaluate the association between baseline tumor tissue Trop-2 expression and response.

2.6.2 Genomic Analysis

In addition to the immune microenvironment, intrinsic tumor factors may be associated with response to immune checkpoint inhibitors. Although some of the mechanisms related to de novo or acquired resistance to immunotherapy have recently been described in other tumors, including loss of function in beta-2-microglobulin or defects in the interferon signaling pathway,[59,60] resistance pathways in breast cancer remain largely unknown. Several gene pathways have been described as candidate resistance mechanisms in other tumors, including MYC amplification,[61] WNT-β-catenin upregulation,[62] MAPK activation, and PTEN loss.[63-65] Emerging genomic correlates of response to immunotherapy in other tumors include mutational load,[66,67] tumor aneuploidy,[68] and mismatch repair defects.[69] Notably, there is little data on genomic correlates of response to anti-PD-1 therapy in patients with breast cancer.

Therefore, as a correlative study in this trial, we will explore whether the number and/or type of mutations identified using a targeted next generation sequencing (NGS) panel, OncoPanel, as well as more comprehensive sequencing in a subset of patients, correlates with clinical outcomes (PFS, ORR, CBR, and OS). OncoPanel is a cancer genomic assay to detect somatic mutations, copy number variations, and structural variants in tumor DNA extracted from fresh, frozen, or formalin-fixed paraffin-embedded samples. The OncoPanel assay surveys exonic DNA sequences of 447 cancer genes and 191 regions across 60 genes for rearrangement detection. DNA is isolated from tissue containing at least 20% tumor nuclei and analyzed by massively parallel sequencing using a solution-phase Agilent SureSelect hybrid capture kit and an Illumina HiSeq 2500

sequencer. This CLIA-approved NGS assay, which has been extensively validated,[70] will be performed at the Center for Advanced Molecular Diagnostics in the Department of Pathology at Brigham and Women's Hospital, while more comprehensive sequencing, such as whole exome or whole genome sequencing, will be performed at the Broad Institute of the Massachusetts Institute of Technology.

2.6.3 Microbiome Analysis

The gut microbiome modulates immune system development,[71] but the microbial populations of healthy individuals vary markedly in composition.[72,73] The diversity of intestinal microbiota represents a careful balance between immune tolerance of beneficial microbes and inflammatory responses against pathogens. Alterations in the gut microbiota and their resulting interactions with intestinal epithelium and the host immune system are associated with many diseases, including cancer.[74] Preclinical studies have provided strong evidence for the role of gut microbiota in modulating response and resistance to immune checkpoint inhibitors, raising the possibility that stool microbiota could be used as a response biomarker for immunotherapy.[75,76] Interestingly, postmenopausal women with breast cancer have altered composition and low diversity of their gut microbiota compared to healthy controls, as measured by the Shannon index.[77] Identification of biomarkers that predict response to immunotherapy could spare *de novo* resistant patients from the unnecessary risk of immune-related adverse events. In addition, the identification of bacterial species associated with response could open new strategies to maximize the clinical benefit of cancer immunotherapy through the modulation of gut microbiota.

Emerging evidence suggests that the gut microbiome may influence response to checkpoint inhibitors in a number of malignancies.[78-81] Preclinical studies in murine models have demonstrated that microbiome composition is associated with response to PD-L1 inhibitors, with mice exhibiting a "favorable" microbiome having a greater likelihood of responding to treatment as compared to mice with an "unfavorable" microbiome. Studies also demonstrate that transplanting feces from mice with favorable microbiota to those with unfavorable microbiota increases response rates, suggesting that modification of the microbiome could have a therapeutic effect in patients receiving checkpoint inhibitors.[79] Translational work demonstrates that fecal transplants from patients responding to checkpoint inhibitors to nude mice increases the likelihood of response to immunotherapy in these animals, whereas transplants from human non-responders leads to a lower likelihood of response.[79,80] The evidence linking the gut microbiome to checkpoint inhibitor efficacy in humans has been relatively limited to date, but a number of recent publications have demonstrated a relationship between composition of the microbiome and response to immunotherapy in melanoma, non-small cell lung carcinoma and renal cell cancer.[78-81] Notably, the bacterial species associated with a favorable response to treatment have differed across disease sites and studies.[78,81]

Microbiome composition is influenced by many factors, including age, genetics, and use of antibiotics and probiotics.[82-84] Studies demonstrate that dietary composition is one of the primary drivers of microbiome diversity and taxa.[82,83] Other factors related to energy balance, the balance of calories ingested versus expended, such as physical activity and body weight also impact microbiome composition.[83,84] Limited data in humans suggest that factors impacting microbiome composition may also be related to checkpoint inhibitor response. An analysis of 113 patients with

metastatic melanoma found significant associations between dietary factors (ingestion of red meat [p=0.006], sugar-sweetened beverages [p=0.048] and fruits/vegetables [p=0.049]) and microbiome composition.[85] Use of antibiotics (p=0.05) and probiotics (p=0.02) was also associated with lower microbiome α-diversity. Exploratory analyses of 46 of these patients who were initiating treatment with anti-PD-1 therapy suggested that individuals in this cohort with a higher consumption of dietary fiber had a 5-fold likelihood of response to anti-PD-1 therapy as compared to individuals with low fiber consumption (personal communication, Wargo). These findings are provocative but were based on a dietary screening tool that provides a relative crude assessment of dietary intake. The study also did not assess other modifiable factors that can influence microbiome composition, such as physical activity, precluding identification of an optimal strategy of lifestyle modification to enhance immunotherapy response.

In an effort to identify predictive biomarkers of response or resistance to immune checkpoint inhibitors, stool samples will be collected to determine whether baseline characteristics of the structure of the gut microbiome or changes in the overall diversity of gut microbiome are associated with efficacy of pembrolizumab in combination with sacituzumab govitecan in patients with HR+ / HER2- MBC. Additionally, to identify potentially modifiable drivers of microbiome diversity and composition, participants will undergo assessment of dietary composition, physical activity and body mass index at the same timepoints.

2.6.4 Pre-Screening Participants (prior to Sponsor Amendment 3, PD-L1 eligibility amendment)

The mechanisms underlying the lower response rate of HR+ MBC to immune checkpoint inhibitors are largely unknown. Lower mutation and neoantigen burden, lower levels of TILs, and lower PD-L1 expression have all been postulated as potential mechanisms but have not been systematically characterized in large numbers of patients with HR+ MBC. The archival tumor tissue required to determine the PD-L1 status of pre-screening participants presents a rich opportunity to define the immune landscape of HR+ MBC. In particular, rates of different immune cell subsets beyond TILs have been less well characterized in HR+ MBC.

Therefore, we will explore a variety of tissue biomarkers in the tumor samples from pre-screening participants, including immune cell subsets, inhibitory and costimulatory checkpoint pathway molecules, tumor mutation and neoantigen burden, and immune-related transcriptomic signatures and genomic pathway alterations. We will also define rates of PD-L1 positivity by different PD-L1 antibodies and compare rates of positivity.

3 PARTICIPANT SELECTION

Eligibility will be assessed as part of the screening procedures for all patients.

3.1 Eligibility Criteria

3.1.1 Participants must have histologically or cytologically confirmed invasive breast cancer with unresectable locally advanced or metastatic disease. Participants without pathologic or cytologic confirmation of metastatic disease should have unequivocal evidence of metastasis from physical examination or radiologic evaluation, i.e. visible chest wall disease or metastases on imaging meeting standard radiology criteria (i.e., lymph nodes larger than 1 cm in the short axis diameter).

3.1.2 Participants must have HR-positive, HER2-negative breast cancer (ER≥1% and/or, PR≥1%, HER2-negative per ASCO CAP guidelines) on local pathology review. If a patient has more than one histological result, the most recent sample will be considered for inclusion.

3.1.3 Participants must have either progressed on or within 12 months of adjuvant endocrine therapy within the last 12 months or have progressed on at least one line of endocrine therapy for metastatic disease and be considered appropriate candidates for chemotherapy.

3.1.4 Participants must have evaluable or measurable disease per RECIST 1.1. See Section 11 for the assessment of measurable disease. For instance, patients with bone only disease will be allowed to participate.

3.1.5 Participants must agree to undergo a research biopsy, if tumor is safely accessible, at baseline. Previously collected archival tissue will also be requested, if available, for all participants. A source of archival tumor tissue should be identified at time of registration (see Section 9 for more details). Participants must agree to a mandatory repeat biopsy 3-6 weeks after starting treatment, if tumor is safely accessible.

3.1.6 Prior chemotherapy: Participants may have received 0-1 prior chemotherapeutic regimens for metastatic breast cancer and must have been off treatment with chemotherapy for at least 14 days prior to study treatment initiation. If a prior chemotherapy was given for less than 1 cycle, it will not be counted as a prior line. No prior irinotecan or topoisomerase I-containing antibody drug conjugates in the metastatic or neo/adjuvant setting are allowed. All toxicities related to prior chemotherapy must have resolved to CTCAE v5.0 grade 1 or lower, unless otherwise specified in 3.1.15, except alopecia can be any grade and neuropathy can be grade 2 or lower.

3.1.7 Prior biologic therapy: Patients must have discontinued all biologic therapy at least 14 days prior to study treatment initiation. All toxicities related to prior biologic therapy must have resolved to CTCAE v5.0 grade 1 or lower, unless otherwise specified in 3.1.15.

3.1.8 Prior targeted therapy: Targeted therapy (e.g., prior CDK 4/6 inhibitors) must have been discontinued ≥ 14 days prior to initiation of study therapy. All toxicities related to prior targeted therapy must have resolved to CTCAE v5.0 grade 1 or lower, unless otherwise specified in 3.1.15.

3.1.9 Prior investigational agents for treatment of cancer: Investigational agents must have been discontinued ≥ 14 days prior to initiation of study therapy. All toxicities related to prior investigational agents must have resolved to CTCAE v5.0 grade 1 or lower, unless otherwise specified in 3.1.15.

3.1.10 Prior radiation therapy: Patients may have received prior radiation therapy. Radiation therapy must be completed at least 7 days prior to the initiation of study treatment (at least 7 days for SRS), and all toxicities related to prior radiation therapy must have resolved to CTCAE v5.0 grade 1 or lower, unless otherwise specified in 3.1.15. A 1-week washout is permitted for palliative radiation (≤2 weeks of radiotherapy) to non-CNS disease.

3.1.11 Previously treated brain metastases are permitted, with the following provisions:

- Prior SRS should complete ≥ 7 days before study treatment initiation
- Prior WBRT should complete ≥ 7 days before study treatment initiation.
- Any corticosteroid use for brain metastases must have been discontinued for ≥ 7 days prior to study treatment initiation.

3.1.12 Participants on bisphosphonates or RANK ligand inhibitors may continue receiving therapy during study treatment and also may initiate therapy with these agents on study if clinically indicated.

3.1.13 The subject is ≥ 18 years old.

3.1.14 ECOG performance status 0-1 (Karnofsky > 60%, see Appendix A).

3.1.15 Participants must have normal organ and marrow function as defined below:

• Absolute neutrophil count	≥1,000/mcL
• Platelets	≥100,000/mcL
• Hemoglobin	≥ 9.0 g/dl
• INR/PT/aPTT	≤1.5 × ULN unless participant is receiving anticoagulant therapy as long as PT or aPTT is in therapeutic range of anticoagulant
• Total bilirubin	≤1.5 × institutional upper limit of normal (ULN) (or ≤2.0 x ULN in patients with documented Gilbert's Syndrome)
• AST(SGOT)/ALT(SGPT)	≤2.5 × institutional ULN or ≤5 × institutional ULN for participants with documented liver metastases
• Serum creatinine	≤1.5 × institutional ULN OR creatinine

clearance ≥ 30 mL/min/ 1.73m^2 for participants with creatinine levels above institutional ULN.

3.1.16 Female subjects of childbearing potential must have a negative serum or urine pregnancy test within 2 weeks prior to study treatment initiation.

Childbearing potential is defined as participants who have not reached a postmenopausal state (≥ 12 continuous months of amenorrhea with no identified cause other than menopause) and have not undergone surgical sterilization (removal of ovaries and/or uterus).

3.1.17 Women of childbearing potential (WOCBP) must agree to use an adequate method of contraception. Contraception is required starting with the first dose of study medication through 180 days (6 months) after the last dose of study medication. Examples of contraceptive methods with a failure rate of < 1% per year include bilateral tubal ligation, male sterilization, and copper intrauterine devices. Periodic abstinence (e.g., calendar, ovulation, symptothermal, or post-ovulation methods) and withdrawal are not acceptable methods of contraception. Hormonal contraceptives are contraindicated for HR+ breast cancer (Appendix B).

3.1.18 Males who are sexually active with WOCBP must agree to follow instructions for method(s) of contraception for the duration of study treatment with pembrolizumab and 3 months after the last dose of study treatment (Appendix B).

3.1.19 The participant must be capable of understanding and complying with the protocol and willing to sign a written informed consent document.

3.2 Exclusion Criteria

3.2.1 Prior therapy with any anti-PD-1, PD-L1, or PD-L2 agent or sacituzumab govitecan (IMMU-132). Prior therapy with irinotecan or topoisomerase I-containing antibody drug conjugates at any time for early stage or metastatic disease.

3.2.2 Prior hypersensitivity to the excipients of pembrolizumab or sacituzumab govitecan (IMMU-132) therapy.

3.2.3 Known history of UDP-glucuronosyltransferase 1A1 (UGT1A1) *28 allele homozygosity, which is associated with increased risk for neutropenia and diarrhea related to irinotecan.[86]

Note: Concurrent administration of strong UGT1A1 inhibitors or inducers is not allowed during the course of the study (See Section 5.6.2).

3.2.4 Known brain metastases that are untreated, symptomatic, or require therapy to control symptoms.

3.2.5 Participant has known leptomeningeal disease.

3.2.6 Major surgery within 2 weeks prior to study treatment initiation. Patients must have recovered from any effects of any major surgery.

3.2.7 Uncontrolled, significant intercurrent or recent illness including, but not limited to, ongoing or active infection, uncontrolled non-malignant systemic disease, uncontrolled seizures, or psychiatric illness/social situation that would limit compliance with study requirements in the opinion of the treating investigator.

3.2.8 Participant has a medical condition that requires chronic systemic steroid therapy (> 10 mg of prednisone daily or equivalent) or any other form of immunosuppressive medication (including disease modifying agents) and has required such therapy in the last 2 years. Replacement therapy (e.g. thyroxine, insulin, or physiologic corticosteroid replacement therapy for adrenal or pituitary insufficiency, etc.) is not considered a form of systemic therapy.

3.2.9 Participant has documented history of autoimmune disease or syndrome that currently requires systemic steroids or immunosuppressive agents.

3.2.10 History of (non-infectious) pneumonitis/interstitial lung disease that required steroids or current pneumonitis/interstitial lung disease.

3.2.11 Individuals with a history of a second malignancy are ineligible except for the following circumstances. Individuals with a history of other malignancies are eligible if they have been disease-free for at least 3 years or are deemed by the investigator to be at low risk for recurrence of that malignancy. Individuals with the following cancers that have been diagnosed and treated within the past 3 years are eligible: cervical/prostate carcinoma *in situ*, superficial bladder cancer, non-melanoma cancer of the skin. Patients with other cancers diagnosed within the past 3 years and felt to be at low risk of recurrence should be discussed with the study principal investigator to determine eligibility.

3.2.12 Participant has a known history of human immunodeficiency virus (HIV), Hepatitis B (defined as Hepatitis B surface antigen [HBsAg] reactive) or known active Hepatitis C virus (defined as detected HCV RNA [qualitative]) infection. HIV-positive participants are ineligible due to the potential for pharmacokinetic interactions of combination antiretroviral therapy with study drugs and the increased risk of fatal infections. Note: No testing for HIV, Hepatitis B, or Hepatitis C is required unless mandated by local health authority.

3.2.13 The participant has received a live vaccine within 28 days prior to study treatment initiation. Examples of live vaccines include, but are not limited to, the following: measles, mumps, rubella, varicella/zoster, yellow fever, rabies, BCG, and typhoid vaccine. The use of the inactivated seasonal influenza vaccine is allowed.

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

3.2.15 It is unknown whether pembrolizumab is excreted in human milk. Since many drugs are excreted in human milk, and because of the potential for serious adverse reactions in the nursing infant, participants who are breast-feeding are not eligible for enrollment.

3.3 Retreatment Eligibility Criteria

In addition to the above criteria aside from prior treatment with sacituzumab govitecan and pembrolizumab, the following eligibility criteria are required:

3.3.1 The participant stopped initial treatment with sacituzumab govitecan and/or pembrolizumab after attaining an investigator-assessed CR according to RECIST 1.1, confirmed in at least two consecutive restaging scans, was treated for at least 24 weeks with pembrolizumab and/or sacituzumab govitecan before discontinuing therapy, and received at least three cycles (with pembrolizumab and sacituzumab govitecan (Arm A) or sacituzumab govitecan alone (Arm B)) beyond the date when the initial CR was declared.

3.3.2 The participant experienced an investigator-assessed or radiographic disease progression after stopping their initial treatment with sacituzumab govitecan and/or pembrolizumab (Arm A) or sacituzumab govitecan (Arm B).

3.3.3 The participant did not receive any anti-cancer treatment other than sacituzumab govitecan (or pembrolizumab) since the last dose of pembrolizumab (or sacituzumab govitecan) for Arm A; the participant did not receive any anti-cancer treatment since the last dose of sacituzumab govitecan for Arm B.

3.3.4 Participants must agree to undergo a research biopsy, if tumor is safely accessible, prior to retreatment. Participants must agree to a mandatory repeat biopsy at the time of disease progression on retreatment, if a complete or partial response, or stable response for ≥ 6 months was achieved during the retreatment phase.

3.3.5 Prior radiation therapy: Patients may have received prior radiation therapy. Radiation therapy must be completed at least 7 days prior to retreatment initiation (at least 7 days for SRS), and all toxicities related to prior radiation therapy must have resolved to CTCAE v5.0 grade 1 or lower, unless otherwise specified in 3.3.8.

3.3.6 Stable, previously treated brain metastases are permitted, with the following provisions:
- Prior SRS should complete ≥ 7 days before study treatment initiation
- Prior WBRT should complete ≥ 7 days before study treatment initiation
- Any corticosteroid use for brain metastases must have been discontinued for ≥ 7 days prior to retreatment initiation.

3.3.7 ECOG performance status 0-1 (Karnofsky >60%, see Appendix A).

3.3.8 Participants must have normal organ and marrow function as defined below:

• Absolute neutrophil count	≥1,000/mcL
• Platelets	≥100,000/mcL
• Hemoglobin	≥ 9.0 g/dl
• INR/PT/aPTT	≤1.5 × ULN unless participant is receiving anticoagulant therapy as long as PT or aPTT is in therapeutic range of anticoagulant
• Total bilirubin	≤1.5 × institutional upper limit of normal (ULN) (or ≤2.0 x ULN in patients with documented Gilbert's Syndrome)
• AST(SGOT)/ALT(SGPT)	≤2.5 × institutional ULN or ≤5 × institutional ULN for participants with documented liver metastases
• Serum creatinine	≤1.5 × institutional ULN OR creatinine clearance ≥ 30 mL/min/ 1.73m^2 for participants with creatinine levels above institutional ULN.

3.3.9 Female subjects of childbearing potential must have a negative serum or urine pregnancy test within 2 weeks prior to study treatment initiation.

Childbearing potential is defined as: participants who have not reached a postmenopausal state (≥ 12 continuous months of amenorrhea with no identified cause other than menopause) and have not undergone surgical sterilization (removal of ovaries and/or uterus).

3.3.10 Women of childbearing potential (WOCBP) must agree to use an adequate method of contraception. Contraception is required starting with the first dose of study medication through 180 days (6 months) after the last dose of study medication. Examples of contraceptive methods with a failure rate of < 1% per year include bilateral tubal ligation, male sterilization, established and proper use of hormonal contraceptives that inhibit ovulation, hormone-releasing intrauterine devices, and copper intrauterine devices. Periodic abstinence (e.g., calendar, ovulation, symptothermal, or post-ovulation methods) and withdrawal are not acceptable methods of contraception (Appendix B).

3.3.11 Males who are sexually active with WOCBP must agree to follow instructions for method(s) of contraception for the duration of study treatment with pembrolizumab and 3 months after the last dose of study treatment (Appendix B).

3.3.12 Participants on bisphosphonates or RANK ligand inhibitors may continue receiving therapy during study treatment and also may initiate therapy with these agents on study if clinically indicated.

3.4 Retreatment Exclusion Criteria

3.4.1 Major surgery within 2 weeks prior to study treatment initiation. Patients must have recovered from any effects of any major surgery.

3.4.2 Uncontrolled, significant intercurrent or recent illness including, but not limited to, ongoing or active infection, uncontrolled non-malignant systemic disease, uncontrolled seizures, or psychiatric illness/social situation that would limit compliance with study requirements in the opinion of the treating investigator.

3.4.3 For patients receiving retreatment with pembrolizumab: Participant has a medical condition that requires chronic systemic steroid therapy (> 10 mg of prednisone daily or equivalent) or any other form of immunosuppressive medication (including disease modifying agents) and has required such therapy in the last 2 years. Replacement therapy (e.g. thyroxine, insulin, or physiologic corticosteroid replacement therapy for adrenal or pituitary insufficiency, etc.) is not considered a form of systemic therapy.

3.4.4 For patients receiving retreatment with pembrolizumab: Participant has documented history of autoimmune disease or syndrome that currently requires systemic steroids or immunosuppressive agents. A history of autoimmune disease related to prior checkpoint inhibitor therapy is permitted on a case-by-case basis upon review by the principal investigator.[87]

3.4.5 History or evidence of active, non-infectious pneumonitis or interstitial lung disease. A history of pneumonitis related to prior checkpoint inhibitor therapy is permitted on a case-by-case basis upon review by the principal investigator.[87]

3.4.6 It is unknown whether pembrolizumab is excreted in human milk. Since many drugs are excreted in human milk, and because of the potential for serious adverse reactions in the nursing infant, participants who are breast-feeding are not eligible for enrollment.

3.4.7 The participant has received a live vaccine within 28 days prior to study treatment initiation. Examples of live vaccines include, but are not limited to, the following: measles, mumps, rubella, varicella/zoster, yellow fever, rabies, BCG, and typhoid vaccine. The use of the inactivated seasonal influenza vaccine is allowed.

3.5 Inclusion of Women and Minorities

Both men and women of all races and ethnic groups are eligible for this trial.

4 REGISTRATION AND RANDOMIZATION PROCEDURES

4.1 General Guidelines for DF/HCC Institutions

DF/HCC Institutions will register eligible participants in the Clinical Trials Management System (CTMS) OnCore. Registrations must occur prior to the initiation of any protocol-specific therapy or intervention. Any participant not registered to the protocol before protocol-specific therapy or intervention begins will be considered ineligible and registration will be denied. An investigator will confirm eligibility criteria and a member of the study team will complete the protocol-specific eligibility checklist.

The eligibility checklist(s) and all pages of the consent form(s) will be emailed to the Office of Data Quality (ODQ) at qact@partners.org. The ODQ will (a) review the eligibility checklist, (b) register the participant on the protocol, and (c) randomize the participant. Randomization can only occur during ODQ business hours (8:30am - 5pm Eastern Time, Monday through Friday excluding holidays). An email confirmation of the registration and/or randomization will be sent to the Sponsor-Investigator, study coordinator(s) from the Lead Site, treating investigator and registering person immediately following randomization.

Following registration, participants may begin protocol-specific therapy and/or intervention. Issues that would cause treatment delays should be discussed with the Sponsor-Investigator. If the subject does not receive protocol therapy following registration, the subject must be taken off-study in the CTMS (OnCore) with an appropriate date and reason entered.

4.2 Registration Process for DF/HCC Institutions

Applicable DF/HCC policy (REGIST-OP-1) must be followed.

4.3 General Guidelines for Other Investigative Sites

Eligible participants will be entered on study centrally at the Dana-Farber Cancer Institute through the Project Manager. Sites may email the Project Manager to verify slot level availabilities. The required forms in Section 4.4 should be emailed to the Project Manager. Following registration, participants should begin protocol therapy within 7 days. Issues that would cause treatment delays should be discussed with the Sponsor-Investigator. If the subject does not receive protocol therapy following registration, the subject must be taken off study in the CTMS (OnCore) with an appropriate date and reason entered.

4.4 Registration Process for Other Investigative Sites

To register a participant, the following documents should be completed by the participating site and emailed to the Project Manager at CTOPM@dfci.harvard.edu:

- Completed DF/HCC Eligibility Checklist

- Signed participant consent form
- HIPAA authorization form (if separate from the main consent form)
- Required laboratory test results including Hematology (CBC with differential), serum chemistries (creatinine and/or creatinine clearance, bilirubin, ALT and AST, magnesium and phosphorous), thyroid function tests, coagulation, and pregnancy test (if applicable)
- Tumor Assessments (CT/CAP, MRI)
- Pathology report confirming diagnosis and ER, PR, and HER2 status
- Status of required research biopsy
- Clinic visit note documenting medical history, physical exam, vital signs, and ECOG

To complete the registration process, the Project Manager will:

- Follow the DF/HCC Standard Operating Procedure for Human Subject Research Titled *Subject Protocol Registration* (SOP #: REGIST-OP-1) and register the participant on the protocol
- Email the study team at the participating site with the participant study number, treatment assignment and to confirm registration/randomization

NOTE: Participants MUST be registered and randomized prior to the start of protocol treatment. Registration and randomization can only be conducted during the business hours of 8:00 AM and 5:00 PM Eastern Time Monday through Friday. Same day treatment registrations will only be accepted with prior notice and discussion with the DF/HCC Project Manager.

5 TREATMENT PLAN

5.1 Treatment Regimen

This is an open-label, randomized, phase II study of sacituzumab govitecan 10 mg/kg IV on days 1 and 8 of each 21-day cycle and pembrolizumab 200 mg IV on day 1 of each 21-day cycle. One hundred and ten participants will be enrolled in the study to compare the efficacy of the combination to sacituzumab govitecan alone, as defined by PFS, in patients with HR+ / HER2- MBC.

Treatment will be administered on an outpatient basis. For patients randomized to combination therapy on Arm A, sacituzumab govitecan will be administered prior to pembrolizumab as shown below. The time interval between the end of the sacituzumab govitecan infusion and the start of the start of the pembrolizumab infusion should be documented each time. Reported adverse events and potential risks are described in Section 7. Appropriate dose modifications are described in Section 6. No investigational or commercial agents of therapies other than those described below may be administered with the intent to treat the participant's malignancy.

Sacituzumab govitecan (IMMU-132, Trodelvy™), [redacted]
Pembrolizumab (MK-3475; Keytruda TM), [redacted]

Regimen Description							
Agent	**Premedication; Precautions**	**Dose**	**Route**	**Schedule**	**Cycle length**	**Infusion**	**Observation period post-infusion**
Arm A							
Sacituzumab govitecan (IMMU-132)	Antipyretics, H_1 and H_2 blockers, 5-HT3 or NK_1 receptor antagonist (See Section 5.3)	10 mg/kg	IV	Days 1 and 8	21 days (3 weeks)	Slow infusion (See Section 5.3)	30 min (+/- 10 min) after 1st dose; per institutional guidelines for subsequent doses
Pembrolizumab (MK-3475; Keytruda TM)	Not routinely necessary unless prior infusion reaction	200 mg	IV	Day 1	21 days (3 weeks)	Over 30 min (+/- 10 min)	30 min (+/- 10 min)
Arm B							
Sacituzumab govitecan (IMMU-132)	Antipyretics, H_1 and H_2 blockers, 5-HT3 or NK_1 receptor antagonist (See Section 5.3)	10 mg/kg	IV	Days 1 and 8	21 days (3 weeks)	Slow infusion (See Section 5.3)	30 min (+/- 10 min) after 1st dose; per institutional guidelines for subsequent doses

5.2 Pre-Treatment Criteria

5.2.1 Cycle 1, Day 1

Participants do not need to re-meet eligibility criteria on Cycle 1, Day 1.

- Absolute neutrophil count ≥ 1,000/mcL
- Platelets ≥ 100,000/mcL
- Total bilirubin ≤ 1.5 × institutional upper limit of normal (ULN) (or ≤ 2.0 x ULN in patients with documented Gilbert's Syndrome)
- AST(SGOT)/ALT(SGPT) ≤ 2.5 × institutional ULN or ≤ 5 × institutional ULN for participants with documented liver metastases
- Creatinine ≤1.5 × institutional ULN OR creatinine clearance ≥ 30 mL/min/1.73 m^2 for participants with creatinine levels above institutional ULN.

5.2.2 Day 8 and Day 1 of subsequent cycles

- Absolute neutrophil count ≥ 1,000/mcL
- Platelets ≥ 75,000/mcL
- Total bilirubin ≤ 1.5 × institutional ULN (or ≤ 2.0 x ULN in patients with documented Gilbert's Syndrome)
- AST(SGOT)/ALT(SGPT) ≤ 3.0 × institutional ULN or ≤ 5 × institutional ULN for participants with documented liver metastases
- Creatinine ≤2.0 × institutional ULN OR creatinine clearance ≥ 30 mL/min/1.73 m^2 for participants with creatinine levels above institutional ULN.

Please see Section 6 regarding dose delays and discontinuation.

5.3 Agent Administration

5.3.1 Preventative Medications

Infusion-Related Reactions: Premedication for prevention of infusion-related reactions with antipyretics and H1 and H2 blockers should be administered before each sacituzumab govitecan infusion. Corticosteroids (hydrocortisone 50 mg or equivalent PO or IV) may be administered prior to subsequent infusions if the subject had experienced an infusion-related reaction with a previous infusion. Additional details of recommended treatment of infusion-related reactions are described in Section 6.3.1.

Nausea, Vomiting: Sacituzumab govitecan is considered to be highly 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 PO or IV). Anticipatory nausea can be treated with olanzapine. The recommended treatment of delayed nausea and vomiting is described in Section 6.3.2.

5.3.2 Pembrolizumab

Pembrolizumab will be administered by trained medical personnel at the investigational site. Treatment compliance will be monitored through documentation of study treatment administration in the subject's medical record.

Pembrolizumab will be administered in clinic on day 1 (+/- 4 days) of each cycle. It will be administered as a 30-minute IV infusion. Sites should make every effort to target infusion timing to be as close to 30 minutes as possible. However, given the variability of infusion pumps from site to site, a window of -10 minutes and +10 minutes is permitted (i.e., infusion time is 20-40min).

Sacituzumab govitecan infusion and observation period after sacituzumab govitecan should be completed prior to the administration of pembrolizumab when both drugs are administered on the same day of each cycle. There should be no overlap in timing of the two administrations.

Completion of 35 treatments (approximately 2 years) with pembrolizumab requires stopping pembrolizumab. The number of treatments is calculated starting with the first dose.

5.3.3 Sacituzumab govitecan

Sacituzumab govitecan is administered intravenously as a slow infusion as described below. Do not administer it as an IV push or bolus.

Intravenous access must be well established prior to initiating infusion. Sacituzumab govitecan may be administered via a peripheral or central line. At the time of dosing, the IV line will be connected to an infusion container containing the prepared volume of sacituzumab govitecan. Either gravity or an infusion pump may be used. Only normal saline should be used as the infusion base solution, since the compatibility of sacituzumab govitecan with other infusion diluents has not been determined.

The initial infusion should proceed slowly. Administer the **first** infusion over 3 hours (+/- 10 minutes). For the first infusion, monitor the patient during the infusion and for at least 30 minutes after infusion. Subsequent infusions may be administered over 1-2 hours if previous infusions were well tolerated, and patient may be monitored per institutional guidelines. No specific infusion rate is prescribed, the conduct of the infusion must be patient specific determined as appropriate by the managing physician. The intravenous line should be flushed slowly with 20 mL normal saline and the end of infusion time recorded. In the event of infusion reactions or vital sign changes, the infusion rate may be slowed, interrupted or terminated, as considered appropriate by the managing physician.

5.4 Definition of Dose-Limiting Toxicity (DLT)

We will perform a safety run-in analysis on the first 12 participants enrolled to the combination arm of the trial. If there are 4 or more dose-limiting toxicity (DLTs) in the first 12 participants, enrollment will be halted to discuss whether the study will be amended, with re-evaluation of the appropriate dosing schedule and study design or closed. Patients with DLTs that recover to ≤ Grade 1 may drop to a lower dose of sacituzumab govitecan (Section 6.3, Table 6) and continue treatment at the discretion of the treating physician.

A DLT is defined as an IP-related AE(s) (attributed to pembrolizumab and/or the combination of pembrolizumab plus sacituzumab govitecan) occurring during the DLT assessment period (the first 21-day cycle of concurrent sacituzumab govitecan and pembrolizumab), if judged by the investigator to be possibly, probably, or definitely related to study drug administration, that meets one of the following criteria:

- Any grade 5 toxicity
- Grade 3 thrombocytopenia if associated with clinically significant bleeding requiring

medical intervention or

- Grade 4 thrombocytopenia of any duration
- Grade 4 neutropenia lasting ≥ 7 days after appropriate administration of G-CSF
- ≥ Grade 3 febrile neutropenia
- Any other grade 4 hematologic toxicity lasting ≥ 14 days, unless deemed by the investigator to be clinically insignificant
- ≥ Grade 3 AST or ALT elevation lasting ≥ 7 days in patients with documented liver metastases
- Grade 2 bilirubin elevation (except bilirubin > 3 x ULN in patients with documented Gilbert´s syndrome)
- Cases of Hy's Law
- Any ≥ Grade 3 non-hematologic toxicity:
  Excluding:
  - ≥ Grade 3 electrolyte abnormalities that lasts <24 to 72 hours, are not clinically complicated, and resolve spontaneously or respond to conventional medical interventions
  - ≥ Grade 3 AST or ALT elevation that lasts < 72 hours, are not clinically complicated, and resolve spontaneously or respond to conventional medical interventions
  - ≥ Grade 3 amylase or lipase that is not associated with symptoms or clinical manifestations of pancreatitis
  - Grade 3 or 4 lymphopenia that is not associated with symptoms or clinical complications
  - Grade 3 nausea/vomiting or diarrhea < 72 hours with adequate antiemetic and other supportive care
  - Grade 3 fatigue < 7 days
  - Alopecia of any grade
- Any Grade 2 treatment-related pneumonitis or interstitial lung disease that did not resolve with dose delay and systemic steroids to ≤ Grade 1 within 14 days.
- ≥ Grade 3 other non-laboratory toxicity lasting > 5 days despite optimal supportive care, excluding alopecia of any grade

Management and dose modifications associated with the above adverse events are outlined in Section 6.

5.5 Definition of Potential Drug Induced Liver Injury (DILI)

Definition of drug induced liver injury (DILI) criteria is mandatory for all pre-marketed asset protocols enrolling participants without known abnormalities in liver function at baseline AND for protocols involving participants with known liver abnormalities at baseline or with other clinical confounders where asset specific criteria for potential drug induced liver injury have been defined. All occurrences of potential DILIs, meeting the defined criteria, must be reported as SAEs.

In participants without known abnormalities in liver function at baseline, potential DILI is defined as:

1) ALT or AST elevation > 3 times upper limit of normal (ULN)

AND
2) Total bilirubin > 2 times ULN, without initial findings of cholestasis (elevated serum alkaline phosphatase)
AND
3) No other immediately apparent possible causes of AST or ALT elevation and hyperbilirubinemia, including, but not limited to, viral hepatitis, pre-existing chronic or acute liver disease, or the administration of other drug(s) known to be hepatotoxic.

In participants with known abnormalities in liver function at baseline (e.g., liver metastases), or with other clinical confounders (e.g., Gilbert´s disease), potential DILI is defined as:
1) ALT or AST elevation > 5 times upper limit of normal (ULN)
AND
2) Total bilirubin > 3 times ULN, without initial findings of cholestasis (elevated serum alkaline phosphatase)
AND
3) No other immediately apparent possible causes of increase in AST or ALT elevation and hyperbilirubinemia, including, but not limited to, viral hepatitis, pre-existing chronic or acute liver disease, or the administration of other drug(s) known to be hepatotoxic.

5.6 General Concomitant Medication and Supportive Care Guidelines

5.6.1 Concomitant Medication Guidelines

Medications or vaccinations specifically prohibited in the exclusion criteria (3.2.13) 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 may be required. The investigator should discuss any questions regarding this with the Sponsor-Investigator.

Acceptable Concomitant Medications
All treatments that the investigator considers necessary for a subject's welfare may be administered at the discretion of the investigator in keeping with the community standards of medical care and documented in the medical record. All prior treatment or medication administered during the 28 days preceding the first dose of trial treatment and any concomitant therapy administered to the subject throughout the study until 30 days after the last dose of trial treatment, including medication name and indication, will be recorded in the eCRFs.

Palliative and/or supportive medications, such as pain medications, bone-modifying medications (bisphosphonates or denosumab), anti-emetics or anti-diarrheal medications, transfusions and growth factor support are allowed at the investigator's discretion. Palliative radiotherapy is permitted, but presence of new or worsening metastases will be considered progression. However, if there is clear evidence of clinical benefit, treatment may be continued after completion of palliative radiotherapy. In this case, sacituzumab govitecan +/- pembrolizumab administration should be interrupted one week before the procedure and reinstated no earlier than one week after the procedure. Similarly, if patients require surgery for reasons related to their cancer or other reasons, sacituzumab govitecan +/- pembrolizumab administration should be interrupted one week before the procedure and reinstated no earlier than two weeks after the procedure.

Prohibited Concomitant Medications

Subjects are prohibited from receiving the following therapies during the Screening (14 days prior to study treatment initiation) and Treatment Phase of this trial:

- Antineoplastic systemic chemotherapy or biological therapy
- Immunotherapy not specified in this protocol
- Investigational agents other than pembrolizumab or sacituzumab govitecan
- Live vaccines within 28 days prior to the first dose of trial treatment and while participating in the trial. Examples of live vaccines include, but are not limited to, the following: measles, mumps, rubella, varicella/zoster, yellow fever, rabies, BCG, and typhoid vaccine. The use of the inactivated seasonal vaccine is allowed.

- Systemic glucocorticoids should be avoided for any purpose other than to modulate symptoms from radiation, prevent hypersensitivity reactions, or to treat an event of clinical interest of suspected immunologic etiology. If corticosteroids are required for this purpose, the minimum effective dose should be used. The use of physiologic doses of corticosteroids (10 mg prednisone daily or equivalent) can be used without principal investigator (PI) authorization. For participants receiving sacituzumab govitecan, doses of corticosteroids >10mg prednisone daily or equivalent may be used with PI authorization; pembrolizumab may not be given while on higher doses of corticosteroids (>10mg prednisone daily or equivalent). The use of dexamethasone for nausea and vomiting attributed to sacituzumab govitecan refractory to other medications (such as a 5-HT3 inhibitor, i.e., ondansetron or palonosetron; an NK1-receptor antagonist, i.e., fosaprepitant or aprepitant; olanzapine 10 mg daily for days 1 to 4; or other agents according to local practices) is permitted.

- Subjects may receive other medications that the investigator deems to be medically necessary.

There are no prohibited therapies during the Post-Treatment Follow-up Phase.

5.6.2 Supportive Care Guidelines – General Medications

The following treatments are permitted throughout the duration of the study Treatment Phase and during Follow-up:

- Standard therapies for pre-existing medical conditions unless listed as prohibited therapy. Any medication intended solely for supportive care (e.g., analgesics, anti-diarrheal, anti-depressants) may be used at the investigator's discretion. At the discretion of the investigator, prophylactic antiemetic and anti-diarrheal medication(s) may be used as per standard clinical practice before doses of study drugs or before, during or after radiation treatment.
- Bisphosphonate or denosumab therapy to be used in accordance with the approved labeled indication and/or nationally recognized treatment guidelines. Participants already receiving bisphosphonate/denosumab at the time of study entry can continue the treatment.

Participants may initiate treatment with bisphosphonate/denosumab after study entry with physician discretion.

- Pain medications administered per standard clinical practice are acceptable while the patient is enrolled in the study.

Patients who experience toxicities should be treated symptomatically as clinically indicated. Medications that are considered necessary for the subject's welfare and that are not expected to interfere with the evaluation of study treatment or be restricted may be given at the discretion of the investigator. Ancillary treatments will be given as medically indicated.

Participants should receive appropriate supportive care measures as deemed necessary by the treating investigator. Suggested supportive care measures for the management of AEs with potential immunologic etiology are outlined along with the dose modification guidelines in Section 6 [Table 4-6]. Where appropriate, these guidelines include the use of oral or IV treatment with corticosteroids, as well as additional anti-inflammatory agents if symptoms do not improve with administration of corticosteroids. Note that several courses of steroid tapering may be necessary as symptoms may worsen when the steroid dose is decreased. For each disorder, attempts should be made to rule out other causes such as metastatic disease or bacterial or viral infection, which might require additional supportive care. The treatment guidelines are intended to be applied when the Investigator determines the events to be related to pembrolizumab.

Note: If after the evaluation of the event, it is determined not to be related to pembrolizumab, the Investigator does not need to follow the treatment guidance. Refer to Table 4-6 in Section 6 for guidelines regarding dose modification and supportive care.

It may be necessary to perform conditional procedures such as bronchoscopy, endoscopy, or skin photography as part of evaluation of the event.

Potential Drug Interactions:

No formal pharmacokinetic drug-drug interaction studies have been conducted with pembrolizumab or sacituzumab govitecan. SN-38 (the active metabolite of sacituzumab govitecan) is metabolized via human UDP-glucuronosyltransferase 1A1(UGT1A1). Concomitant administration of strong inhibitors or inducers of UGT1A1, with sacituzumab govitecan, should be avoided due to the potential to either increase (inhibitors) or decrease (inducers) the exposure to SN-38. If a patient requires a strong UGT1A1 inhibitor or inducer, they should not enroll onto this study or should stop study treatment if enrolled.

Strong UGT1A1 Inhibitors

Co-administration of sacituzumab govitecan 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 sacituzumab govitecan unless there are no therapeutic alternatives.

Strong UGT1A1 Inducers

Exposure to SN-38 may be substantially reduced in patients concomitantly receiving UGT1A1 enzyme inducers (e.g., carbamazepine, phenytoin, phenobarbital, tipranavir, rifampicin, nelfinavir, ritonavir, efavirenz, lamotrigine, testosterone propionate). Do not administer strong UGT1A1 inducers with sacituzumab govitecan unless there are no therapeutic alternatives.

5.7 Criteria for Taking a Participant Off Protocol Therapy

Duration of therapy will depend on individual response, evidence of disease progression and tolerance. In the absence of treatment delays due to adverse event(s), treatment may continue until one of the following criteria applies:

1. Disease progression by RECIST 1.1 criteria:

   A. Participants who have attained a confirmed complete response (CR) that have been treated for at least 24 weeks on protocol therapy and had at least three cycles (with pembrolizumab and sacituzumab govitecan (Arm A) or sacituzumab govitecan alone (Arm B)) beyond the date when the initial CR was declared may be eligible for additional sacituzumab govitecan and/or pembrolizumab therapy if they progress after stopping study treatment. This retreatment is termed the Second Course Phase Therapy. See additional details below.

2. Dose reduction beyond 50% of sacituzumab govitecan requires stopping sacituzumab govitecan. The patient may continue on treatment with pembrolizumab until progression or up to 35 doses of pembrolizumab are completed, whichever comes first.

3. Intercurrent illness that prevents further administration of treatment.

4. Unacceptable adverse event(s).

5. Participant demonstrates an inability or unwillingness to comply with the medication regimen and/or documentation requirements.

6. Participant decides to withdraw from the protocol therapy.

   Note: Participants on either treatment arm (Arm A or Arm B) may elect to stop therapy (sacituzumab govitecan and pembrolizumab OR sacituzumab govitecan only on either arm) with confirmed CR after at least 24 weeks of treatment. These participants are still required to undergo regular disease restaging every 9-12 weeks. Note: for participants on Arm A, if only sacituzumab govitecan is discontinued, Day 8 assessments (AE assessment, labs, vitals, etc.) are not needed.

7. General or specific changes in the participant's condition render the participant unacceptable for further treatment in the judgment of the treating investigator.

Participants will be removed from the protocol therapy when any of these criteria apply. The reason for removal from protocol therapy, and the date the participant was removed, must be documented in the case report form (CRF) and in the CTMS system (OnCore). Alternative care options will be discussed with the participant.

In the event of unusual or life-threatening complications, treating investigators must immediately notify the Sponsor-Investigator, Ana Garrido-Castro, MD, phone: 617-632-3800, or email: ana_garrido-castro@dfci.harvard.edu .

Second Course Phase Therapy:

Retreatment Phase (available to both Arms)
Participants who stop sacituzumab govitecan and/or pembrolizumab with CR after at least 24 weeks of treatment may be eligible for additional pembrolizumab and/or sacituzumab govitecan therapy if they progress after stopping study treatment. For participants randomized to the combination arm (Arm A): if both sacituzumab govitecan and pembrolizumab are discontinued and participant experiences progression, both sacituzumab govitecan and pembrolizumab can be restarted at physician's discretion; or if sacituzumab govitecan is discontinued and pembrolizumab is continued and participant experiences progression, sacituzumab govitecan can be added to the pembrolizumab at physician's discretion. For participants randomized to sacituzumab govitecan monotherapy (Arm B): if sacituzumab govitecan is discontinued and participant experiences progression, sacituzumab govitecan can be restarted at physician's discretion. This retreatment is termed the Second Course Phase of this study and is only available if the study remains open and the subject meets the following conditions:

- Stopped initial treatment with sacituzumab govitecan and/or pembrolizumab after attaining an investigator-assessed CR according to RECIST 1.1, confirmed in at least two consecutive restaging scans, was treated for at least 24 weeks with pembrolizumab and/or sacituzumab govitecan before discontinuing therapy, and received at least three cycles (with pembrolizumab and sacituzumab govitecan (Arm A) or sacituzumab govitecan alone (Arm B)) beyond the date when the initial CR was declared

AND

- Experienced an investigator-assessed or radiographic disease progression after stopping their initial treatment with sacituzumab govitecan and/or pembrolizumab (Arm A) or sacituzumab govitecan (Arm B)

- Did not receive any anti-cancer treatment other than:

  - Arm A: Sacituzumab govitecan (or pembrolizumab) since the last dose of pembrolizumab (or sacituzumab govitecan)

  - Arm B: No other anti-cancer treatment since the last dose of sacituzumab govitecan

- Participants who stop pembrolizumab after receiving 35 doses may be eligible for retreatment if they progress after stopping study treatment, provided they meet the requirements detailed in Sections 3.3 and 3.4. Participants may be retreated in the Second Course Phase (Retreatment) for up to an additional 17 cycles (approximately 1 year).

The retreatment section of the eligibility checklist should be completed and signed by the treating investigator. DF/HCC sites will re-send the checklist to ODQ for processing. Non-DF/HCC sites will email documentation to the Coordinating Center at ctopm@dfci.harvard.edu for review. Provided the patient meets criteria per protocol for retreatment, the Coordinating Center will process the checklist and the participating site will be notified that the patient may proceed to retreatment.

Subjects who restart treatment will be retreated at the same dose and dose interval as when they last received pembrolizumab and/or sacituzumab govitecan. Visit requirements are as outlined for subjects on the initial treatment phase of the trial. These participants may resume the scan schedule they had previously during the treatment break, i.e., every 9 weeks.

5.8 Duration of Follow Up

Participants will be followed on-study until death. Participants removed from protocol therapy for unacceptable adverse event(s) will be followed until resolution or stabilization of the adverse event. An additional adverse event assessment will be performed 90 days (-15/+30 days) after the last dose of treatment for participants who receive pembrolizumab, including participants who stopped early for CR, are candidates for retreatment, and have not yet restarted pembrolizumab. This may be conducted by phone.

Participants who discontinued therapy for reasons other than progression will be followed for first disease progression or until the start of another anti-cancer therapy. Tumor assessments should continue to be performed every 9-12 weeks on these participants until first disease progression event, start of another anti-cancer therapy, or death, whichever occurs first. Research bloods are optional at the time of tumor assessments. At either the time of disease progression or start of another anti-cancer therapy, participants will then be followed for survival.

Participants will be followed or contacted every 6 months for survival.

5.9 Criteria for Taking a Participant Off Study

Participants will be removed from study when any of the following criteria apply:

- Lost to follow-up
- Withdrawal of consent for data submission
- Death

The reason for taking a participant off study, and the date the participant was removed, must be documented in the case report form (CRF). In addition, the study team will ensure Off

Treatment/Off Study information is updated in OnCore in accordance with DF/HCC policy REGIST-OP-1.

6 DOSING DELAYS/DOSE MODIFICATIONS

Dose delays and modifications will be made as indicated in the following table(s). The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 will be utilized for dose delays and dose modifications. A copy of the CTCAE version 5.0 can be downloaded from the CTEP website: http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

6.1 Dosing Delays/Omission/Modifications

Dosing interruptions within a 7-day window are permitted in the case of medical / surgical events or logistical reasons not related to study therapy (e.g., elective surgery, unrelated medical events, patient vacation, and/or holidays). Participants with doses held for these reasons for more than 7 days require prior approval from the Sponsor-Investigator and should resume therapy within 6 weeks of the scheduled interruption. The reason for interruption should be documented in the participant's study record. If a dose is held on day 1 of a cycle, the cycle restarts when the participant meets criteria to start; the assessment schedule should be adjusted accordingly.

In the absence of an unacceptable therapy-related toxicity and/or clinical signs of disease progression, subjects may continue treatment at the discretion of the investigator. Subjects must be instructed to notify their physician immediately for any and all toxicities.

Guidelines for the management of AEs (i.e., dose interruptions and dose reductions) and recommendations for treating expected toxicities are presented in the next sections. Consultation with a medical or surgical specialist, especially prior to an invasive diagnostic or therapeutic procedure, is recommended. For management of AEs which can be clearly attributed to sacituzumab govitecan or pembrolizumab, independent dose modification for either agent is allowed. For AEs without clear attribution to either study treatment, management of toxicity should include dose modifications of both agents.

6.1.1 Management of Dosing Delays/Interruptions/Modifications attributable to Pembrolizumab or Sacituzumab Govitecan:

Pembrolizumab should be held (delayed) or omitted for toxicities that are considered possibly, probably or definitely related to pembrolizumab (per Section 6.2 guidance). Sacituzumab govitecan should be held (delayed), dose reduced, or omitted for toxicities that are considered possibly, probably or definitely related to sacituzumab govitecan (per Section 6.3 guidance). If on arm A (first course or retreatment), either drug may be discontinued, while the other drug continues.

- If a subject on Arm A does not meet criteria for treatment (Section 5.2) due to a toxicity that is considered attributable to only one of the drugs of the combination (e.g., platelets 60,000/mm^3 attributed to sacituzumab govitecan), the drug causing the toxicity should be

held, and the other study drug may be administered (unless specified in the corresponding AE table of that study drug).

- For subjects on Arm A, if one study drug is delayed but the other study drug is given, then the study drug that was held should be re-initiated if appropriate at the next regularly scheduled administration time, i.e. if held on day 1 of the cycle, then sacituzumab govitecan could be restarted on day 8 of the cycle or day 1 of the next cycle (± 2 days for Day 1) and pembrolizumab could be restarted on day 1 of the next cycle (± 4 days).

- There must be a minimum of 14 days (± 2 days) between Day 8 and Day 1 of the next cycle of sacituzumab govitecan. There should be a minimum of 21 days ± 4 days between administration of each dose of pembrolizumab.

- If treatment with sacituzumab govitecan is held or omitted due to an AE and the patient cannot be retreated with sacituzumab govitecan within 6 weeks from the last dose, sacituzumab govitecan should be permanently discontinued. Pembrolizumab can be continued per protocol (if the AE is considered unrelated to pembrolizumab).

- If treatment with pembrolizumab is held or omitted due to an AE and the patient cannot be retreated with pembrolizumab within 9 weeks from the last dose, pembrolizumab should be permanently discontinued. Sacituzumab govitecan can be continued per protocol (if the AE is considered unrelated to sacituzumab govitecan).

- For subjects on Arm A, if sacituzumab govitecan is discontinued and pembrolizumab is continued, Day 8 assessments (AE assessment, labs, vitals, etc.) are not needed.

6.1.2 Management of Diarrhea

Diarrhea requires special attention, as it can be caused by either study drug. See Section 6.2 and 6.3 for additional diarrhea management guidelines for pembrolizumab and sacituzumab govitecan, respectively. At enrollment, patients should receive instructions on the management of diarrhea. In the event of diarrhea, supportive measures should be initiated as early as possible. These include the following:

- At the first sign of loose stools, the patient should initiate anti-diarrheal therapy (for example, loperamide) and notify the investigator/site for further instructions and appropriate follow-up.
- Patients should also be encouraged to drink fluids (for example, 8 to 10 glasses of hydrating liquids per day).
- Site personnel should assess response within 24 hours.
- If diarrhea does not resolve with anti-diarrheal therapy within 24 hours to either baseline or Grade 1, both study drugs should be suspended until diarrhea is resolved to baseline or Grade 1.
- When sacituzumab govitecan recommences, dosing should be adjusted as outlined in Table 6 in Section 6.3.

In cases of significant diarrhea, grade 2 through 4, which has not responded to the above interventions, and which has not been determined to be related to another cause (i.e. infection such as C. difficile), an endoscopic procedure to document colitis prior to initiating steroids is strongly recommended if the addition of steroids to treat potential colitis is being considered.

6.2 Management of Toxicities Attributable to Pembrolizumab

AEs associated with pembrolizumab exposure may represent an immunologic etiology. These immune-related AEs (irAEs) may occur shortly after the first dose or several months after the last dose of pembrolizumab treatment and may affect more than one body system simultaneously. Therefore, early recognition and initiation of treatment is critical to reduce complications. Based on existing clinical study data, most irAEs were reversible and could be managed with interruptions of pembrolizumab, administration of corticosteroids and/or other supportive care. For suspected irAEs, ensure adequate evaluation to confirm etiology or exclude other causes. Additional procedures or tests such as bronchoscopy, endoscopy, skin biopsy may be included as part of the evaluation.

Based on the severity of irAEs, withhold or permanently discontinue pembrolizumab and administer corticosteroids. Dose modification and toxicity management guidelines for irAEs associated with pembrolizumab are provided in Table 4.
Key dose modification management steps to highlight include:

- Permanently discontinue pembrolizumab for recurrent grade 2 pneumonitis.
- Permanently discontinue pembrolizumab for any instance of myocarditis, regardless of grade.

Subjects receiving corticosteroids for treatment of drug-related adverse events must be at ≤ 10 mg/day prednisone or equivalent prior to re-initiation of pembrolizumab.

Table 4. Dose modification and toxicity management guidelines for immune-related AEs associated with pembrolizumab

General instructions:

1. Severe and life-threatening irAEs should be treated with IV corticosteroids followed by oral steroids. Other immunosuppressive treatment should begin if the irAEs are not controlled by corticosteroids.
2. Pembrolizumab must be permanently discontinued if the irAE does not resolve or the corticosteroid dose is not ≤10 mg/day within 9 weeks of the last pembrolizumab treatment.
3. The corticosteroid taper should begin when the irAE is ≤ Grade 1 and continue at least 4 weeks.
4. If pembrolizumab has been withheld, pembrolizumab may resume after the irAE decreased to ≤ Grade 1 after corticosteroid taper.

irAEs	Toxicity grade (CTCAE V5.0)	Action with pembrolizumab	Corticosteroid and/or other therapies	Monitoring and follow-up
Pneumonitis	Grade 2	Withhold	• Administer corticosteroids (initial dose of 1 - 2 mg/kg prednisone or equivalent) followed by taper	• Monitor participants for signs and symptoms of pneumonitis • Evaluate participants with suspected pneumonitis with radiographic imaging and initiate corticosteroid treatment
	Grade 3 or 4, or recurrent Grade 2	Permanently discontinue		

			• Add prophylactic antibiotics for opportunistic infections	
Diarrhea / Colitis	Grade 2 or 3	Withhold	• Administer corticosteroids (initial dose of 1 - 2 mg/kg prednisone or equivalent) followed by taper	• Monitor participants for signs and symptoms of enterocolitis (ie, diarrhea, abdominal pain, blood or mucus in stool with or without fever) and of bowel perforation (ie, peritoneal signs and ileus) • Participants with ≥Grade 2 diarrhea suspecting colitis should consider GI consultation and performing endoscopy to rule out colitis • Participants with diarrhea/colitis should be advised to drink liberal quantities of clear fluids. If sufficient oral fluid intake is not feasible, fluid and electrolytes should be substituted via IV infusion
	Grade 4 or recurrent Grade 3	Permanently discontinue		
AST or ALT elevation or Increased Bilirubin	Grade 2 [a]	Withhold	• Administer corticosteroids (initial dose of 0.5 - 1 mg/kg prednisone or equivalent) followed by taper	• Monitor with liver function tests (consider weekly or more frequently until liver enzyme value returned to baseline or is stable)
	Grade 3 [b] or 4 [c]	Permanently discontinue	• Administer corticosteroids (initial dose of 1 - 2 mg/kg prednisone or equivalent) followed by taper	
Type 1 diabetes mellitus (T1DM) or Hyperglycemia	New onset T1DM or Grade 3 or 4 hyperglycemia associated with evidence of β-cell failure	Withhold [d]	• Initiate insulin replacement therapy for participants with T1DM • Administer anti-hyperglycemic in participants with hyperglycemia	• Monitor participants for hyperglycemia or other signs and symptoms of diabetes
Hypophysitis	Grade 2	Withhold	• Administer corticosteroids and initiate hormonal replacements as clinically indicated	• Monitor for signs and symptoms of hypophysitis (including hypopituitarism and adrenal insufficiency)
	Grade 3 or 4	Withhold or permanently discontinue [d]		

Hyperthyroidism	Grade 2	Continue	• Treat with non-selective beta-blockers (e.g., propranolol) or thionamides as appropriate	• Monitor for signs and symptoms of thyroid disorders
	Grade 3 or 4	Withhold or permanently discontinue [d]		
Hypothyroidism	Grade 2, 3, or 4	Continue	• Initiate thyroid replacement hormones (e.g., levothyroxine or liothyronine) per standard of care	• Monitor for signs and symptoms of thyroid disorders
Nephritis: grading according to increased creatinine or acute kidney injury	Grade 2	Withhold	• Administer corticosteroids (prednisone 1 – 2 mg/kg or equivalent) followed by taper	• Monitor changes of renal function
	Grade 3 or 4	Permanently discontinue		
Neurological Toxicities	Grade 2	Withhold	• Based on severity of AE administer corticosteroids	• Ensure adequate evaluation to confirm etiology and/or exclude other causes
	Grade 3 or 4	Permanently discontinue		
Myocarditis	Grade 1	Withhold	• Based on severity of AE administer corticosteroids	• Ensure adequate evaluation to confirm etiology and/or exclude other causes
	Grade 2, 3 or 4	Permanently discontinue		
Exfoliative Dermatologic Conditions	Suspected SJS, TEN, or DRESS	Withhold	• Based on severity of AE administer corticosteroids	• Ensure adequate evaluation to confirm etiology and/or exclude other causes
	Suspected SJS, TEN, or DRESS	Permanently Discontinue		
Primary adrenal insufficiency	Grade 2	Withhold	• Initiate hormonal replacement as clinically indicated.	• Monitor for signs and symptoms of adrenal insufficiency
	Grade 3 or 4	Withhold or permanently discontinue[d]		
All Other immune-related AEs (irAEs)	Intolerable/persistent Grade 2	Withhold	• Based on severity of AE administer corticosteroids	• Ensure adequate evaluation to confirm etiology or exclude other causes
	Grade 3	Withhold or discontinue based on the event [e].		
	Grade 4 or recurrent Grade 3	Permanently discontinue		

[a] AST/ALT: >3.0 – 5.0 x ULN if baseline normal; >3.0 - 5.0 x baseline, if baseline abnormal; bilirubin:>1.5 - 3.0 x ULN if baseline normal; >1.5 - 3.0 x baseline if baseline abnormal

[b] AST/ALT: >5.0 to 20.0 x ULN, if baseline normal; >5.0 - 20.0 x baseline, if baseline abnormal; bilirubin:>3.0 - 10.0 x ULN if baseline normal; >3.0 - 10.0 x baseline if baseline abnormal

[c] AST/ALT: >20.0 x ULN, if baseline normal; >20.0 x baseline, if baseline abnormal; bilirubin: >10.0 x ULN if baseline normal; >10.0 x baseline if baseline abnormal

[d] The decision to withhold or permanently discontinue pembrolizumab is at the discretion of the investigator or treating physician. For participants with Grade 3 or 4 immune-related endocrinopathy where withhold of pembrolizumab is required,

pembrolizumab may be resumed when AE resolves to ≤ Grade 2 and is controlled with hormonal replacement therapy or achieved metabolic control (in case of T1DM)

[e] Events that require discontinuation include but are not limited to encephalitis and other clinically important irAEs (e.g., vasculitis and sclerosing cholangitis)

6.2.1 Pembrolizumab Infusion-Related Reactions

Pembrolizumab may cause severe or life-threatening infusion-reactions including severe hypersensitivity or anaphylaxis. Signs and symptoms usually develop during or shortly after drug infusion and generally resolve completely within 24 hours of completion of infusion. Dose modification and toxicity management guidelines on pembrolizumab associated infusion reaction are provided in Table 5.

Table 5: Pembrolizumab Infusion Reaction Dose modification and Treatment Guidelines

NCI CTCAE Grade	Treatment	Premedication at Subsequent Dosing
Grade 1 Mild reaction; infusion interruption not indicated; intervention not indicated	Increase monitoring of vital signs as medically indicated until the participant is deemed medically stable in the opinion of the investigator.	None
Grade 2 Requires therapy or infusion interruption but responds promptly to symptomatic treatment (e.g., antihistamines, NSAIDs, narcotics, IV fluids); prophylactic medications indicated for ≤24 hrs	**Stop Infusion.** Additional appropriate medical therapy may include but is not limited to: IV fluids Antihistamines NSAIDs Acetaminophen Narcotics Increase monitoring of vital signs as medically indicated until the participant is deemed medically stable in the opinion of the investigator. If symptoms resolve within 1 hour of stopping drug infusion, the infusion may be restarted at 50% of the original infusion rate (e.g., from 100 mL/hr to 50 mL/hr). Otherwise, dosing will be held until symptoms resolve and the participant should be premedicated for the next scheduled dose. **Participants who develop Grade 2 toxicity despite adequate premedication should be permanently discontinued from further study drug treatment**	Participant may be premedicated 1.5h (± 30 minutes) prior to infusion of pembrolizumab with: Diphenhydramine 50 mg po (or equivalent dose of antihistamine). Acetaminophen 500-1000 mg po (or equivalent dose of analgesic).
Grades 3 or 4 Grade 3: Prolonged (i.e., not rapidly responsive to symptomatic medication and/or brief interruption of infusion); recurrence of symptoms following initial improvement; hospitalization indicated for other clinical sequelae (e.g., renal impairment, pulmonary infiltrates) Grade 4: Life-threatening; pressor or ventilatory support indicated	**Stop Infusion.** Additional appropriate medical therapy may include but is not limited to: Epinephrine** IV fluids Antihistamines NSAIDs Acetaminophen Narcotics Oxygen Pressors Corticosteroids Increase monitoring of vital signs as medically indicated until the participant is deemed medically stable in the opinion of the investigator. Hospitalization may be indicated. **In cases of anaphylaxis, epinephrine should be used immediately. **Participant is permanently discontinued from further study drug treatment.**	No subsequent dosing
Appropriate resuscitation equipment should be available at the bedside and a physician readily available during the period of drug administration. For further information, please refer to the Common Terminology Criteria for Adverse Events v4.0 (CTCAE) at http://ctep.cancer.gov		

6.2.2 Non-Immune Related Toxicities associated with Pembrolizumab

Non-irAEs will be managed as appropriate, following clinical practice recommendations. For any intolerable or persistent Grade 2 toxicities and Grade 3 toxicities (excluding endocrinopathy) lasting 12 weeks or longer after the last dose of pembrolizumab, pembrolizumab should be permanently discontinued.

6.3 Management of Toxicities Attributable to Sacituzumab Govitecan

NCI-CTCAE v5.0 is used to grade the severity of all AEs. The guidelines for management of toxicities associated with sacituzumab govitecan 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 a toxicity to determine the appropriate treatment. Appropriate follow-up studies should be utilized to follow all toxicities to resolution. Patients with known UGT1A1 *28 polymorphism may have a higher risk of developing treatment related toxicities. Additional monitoring may be required in those patients.

Dose Delays – Sacituzumab Govitecan

Sacituzumab govitecan is to be administered in 21-day cycles on Day 1 and Day 8; the next cycle should start 14 days (± 2 days) after the Day 8 dose (i.e., the Day 8 infusion will be counted as the first day of that 14-day period). Visit windows of ± 2 days from the scheduled infusion are permitted. The scheduled Day 1 and Day 8 infusions may be delayed for treatment-related toxicities. For Day 8 dosing, if the toxicity has not resolved to ≤ Grade 2 within 1 week, Day 8 dosing of that cycle should be omitted and dosing should resume with the Day 1 of the following cycle. There should be a minimum of 7 days (± 2 days) between the Day 1 and the Day 8 infusions. There should be a minimum of 14 days (± 2 days) between the Day 8 infusion and the Day 1 infusion of the next cycle.

For patients randomized to the combination arm, if drugs are administered on different days in the same 21-day cycle, every effort should be made to administer both drugs on the same day in subsequent cycles (all assessments being adjusted accordingly), provided that criteria are met per protocol and that 14 days (± 2 days) have elapsed since the administration of the previous dose of sacituzumab govitecan and 21 ± 4 days since the administration of the previous dose of pembrolizumab. If Day 8 sacituzumab govitecan dosing is delayed, causing adjustment to Day 1 of the following cycle, it is recommended to administer pembrolizumab on the same day as Day 1 of the next cycle of sacituzumab govitecan.

Dose Reductions and Discontinuation

The major toxicities of sacituzumab govitecan are expected to be gastrointestinal symptoms and hematologic suppression. All subjects will be closely monitored over the course of their treatment and aggressively medically managed, including dose reduction and interruption, in order to prevent the need for treatment discontinuation and serious complications of these toxicities. Sacituzumab dose reductions and interruptions will be managed based on toxicity severity, as assessed by NCI CTCAE v5.0. The sacituzumab govitecan dose must not be re-escalated following a dose reduction. The table below summarizes recommendations for sacituzumab govitecan dose reductions and discontinuations for treatment-related toxicities.

Dose holds for anemia are not mandated but can be applied as clinically indicated. Supportive care (i.e., red blood cell transfusions) may be managed according to institutional guidelines.

Table 6: Recommended Dose Reduction Schedule for Sacituzumab Govitecan

Event NCI CTCAE v5.0	**Occurrence**	**Recommended dose reduction or action**
Severe Neutropenia		
Grade 4 neutropenia ≥ 7 days 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	25% dose reduction and administer granulocyte-colony stimulating factor (G-CSF)*
	Second	50% dose reduction and administer G-CSF*
	Third	Discontinue treatment and administer G-CSF*
At time of scheduled treatment, Grade 3-4 neutropenia which delays dosing beyond 3 weeks for recovery to ≤ Grade 1	First	Discontinue treatment and administer G-CSF*
Severe Non-Neutropenic Toxicity		
Grade 4 non-hematologic toxicity of any duration, OR Any ≥Grade 3 nausea, vomiting or diarrhea due to treatment that is not controlled with antiemetics and anti-diarrheal agents**, OR Other ≥Grade 3 non-hematologic toxicity persisting >48 hours despite optimal medical management, OR At time of scheduled treatment, ≥Grade 3 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
≥Grade 3 non-neutropenic hematologic or non-hematologic toxicity, which does not recover to ≤ Grade 1 within 3 weeks	First	Discontinue treatment

*Prophylactic administration of growth factors may be considered if clinically indicated (See Section 6.3.3)
**Sacituzumab govitecan may be dose reduced for recurring Grade 2 diarrhea that is not controlled with antidiarrheal agents.

6.3.1 Sacituzumab Govitecan Infusion-Related Reactions

Infusion-related reactions are defined as symptoms that occur during and within the first 6 hours after the infusion of sacituzumab govitecan. 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 infusion-related reactions, sacituzumab govitecan 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 adverse events.

Grade 3 and Grade 4 Events

Grade 3 and Grade 4 infusion-related reactions can include severe or clinically significant cardiopulmonary events and severe allergic reactions, such as symptomatic bronchospasm and anaphylactic reactions. Grade 3 infusion-related reactions 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. Sacituzumab govitecan should be permanently discontinued with the first occurrence of Grade 4 infusion-related reaction or recurrent Grade 2 or Grade 3 infusion-related reaction despite optimal management.

Grade 2 Events

Grade 2 infusion-related reactions are defined as those that require infusion interruption and respond to symptomatic treatment. For Grade 2 infusion-related reactions, the infusion should be interrupted for at least 15 minutes until symptoms resolve. After symptoms resolve, the infusion should be resumed at a slower infusion rate determined as appropriate by the managing physician. For recurrent Grade 2 infusion reactions that fail to recover within 6 hours, despite optimal management, sacituzumab govitecan should be permanently discontinued.

6.3.2 Sacituzumab Govitecan Gastrointestinal Toxicities

Nausea, vomiting, and diarrhea are frequent sacituzumab govitecan-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 Sacituzumab Govitecan dose reduction for treatment-related gastrointestinal toxicities are provided above in Table 6.

Nausea and Vomiting

Instructions for the use of pre-medications for prophylactic treatment of nausea and vomiting and anticipatory nausea are provided in Section 5.3.1. Do not hold the dose of sacituzumab govitecan for Grade 3 nausea unless Grade 3 nausea persists despite maximal optimal medical management. Subjects should be treated for delayed nausea and vomiting on Days 2 and 3 with 5-HT_3 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 containing alcohol. Loperamide should be administered at the onset of treatment-related 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, add diphenoxylate/atropine or opium tincture as clinically indicated.

Add octreotide 100-150 mcg subcutaneously 3 times per day (tid) if diarrhea persists. For Grade 4 diarrhea, consider subject hospitalization and treatment with IV fluids and octreotide. Antibiotics can be administered as clinically indicated.

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

6.3.3 Sacituzumab Govitecan Neutropenia

Complete blood counts must be obtained prior to each sacituzumab govitecan infusion, and sacituzumab govitecan should be administered if ANC meets the following criteria:

- Day 1: ANC $\geq 1000/mm^3$
- Day 8: ANC $\geq 1000/mm^3$

The routine prophylactic use of growth factors is not recommended however, they may be used per investigator discretion; this includes but is not limited to administration in the in the setting of neutropenia in subjects at high risk of poor clinical outcomes, including those with prolonged neutropenia, ANC$<1000/mm^3$, febrile neutropenia, and serious infections.

7 ADVERSE EVENTS: LIST AND REPORTING REQUIREMENTS

Adverse event (AE) monitoring and reporting is a routine part of every clinical trial. The following list of reported and/or potential AEs (Section 7.1) and the characteristics of an observed AE (Section 7.2) will determine whether the event requires expedited reporting **in addition** to routine reporting.

7.1 Adverse Events List

7.1.1 Expected Adverse Events for Pembrolizumab

In the pembrolizumab monotherapy trials (P001/P002, P012, P013, and P028, plus the P011 monotherapy arm), the overall incidence of AEs ranged from 83.0% (73 of 88 subjects in P012) to 100% (10 of 10 subjects in P011). The most commonly reported AEs included fatigue, diarrhea, decreased appetite, nausea, and anemia. The incidence of drug-related AEs (DRAEs) ranged from 39.8% (35 of 88 subjects in P013) to 80.0% (8 of 10 subjects in P011). The most commonly reported DRAEs across all studies were nausea, fatigue, and diarrhea. The incidence of Grade 3-5 DRAEs across studies ranged from 6.8% (6 of 88 in P013) to 12.0% (187 of 1562 subjects) in P001/P002. The most commonly reported Grade 3-5 DRAEs were anemia, elevated alanine aminotransferase, and elevated aspartate aminotransferase. Most subjects who experienced an AE continued in the study: the incidence of AEs leading to discontinuation ranged from 1.9% (8 of 430 subjects in P028) to 12.3% (192 of 1562 subjects in P001/P002). The majority of AEs leading to discontinuation were not considered to be related to pembrolizumab. Discontinuations due to a DRAE were infrequent and ranged from 0% (no subjects in P011) to 4.5% (4 of 88 subjects in P013). The most commonly reported DRAEs leading to discontinuation were pneumonitis, elevated alanine aminotransferase, and elevated aspartate aminotransferase.

List of AEs considered expected:

- Cardiac disorders: Myocarditis
- Endocrine disorders: Adrenal insufficiency, Hyperthyroidism, Hypophysitis, Hypopituitarism, Hypothyroidism, Secondary adrenal insufficiency, Thyroid disorder
- Eye disorders: Uveitis
- Gastrointestinal disorders: Abdominal pain, Colitis, Diarrhea, Pancreatitis
- General disorders and administration site conditions: Asthenia, Pyrexia
- Hepatobiliary disorders: Autoimmune hepatitis, Hepatitis, Sclerosing cholangitis
- Infusion related reaction
- Metabolism and nutrition disorders: Diabetic ketoacidosis, Hyponatremia, Type 1 diabetes mellitus
- Musculoskeletal and connective tissue disorders: Arthralgia, Back pain, Myositis, Vasculitis
- Nervous system disorders: Guillain-Barré syndrome, Myelitis
- Renal and urinary disorders: Nephritis
- Respiratory, thoracic and mediastinal disorders: Cough, Pneumonitis
- Skin and subcutaneous tissue disorders: Pruritis, Rash, Severe skin reaction, Vitiligo

7.1.2 Expected Adverse Events for Sacituzumab Govitecan

The safety profile of sacituzumab govitecan was initially characterized based on 420 patients included in the overall safety population in the IMMU-132-01 study. These patients were treated with doses of 8 mg/kg, 10 mg/kg, 12 mg/kg, and 18 mg/kg. This population included 164 patients with breast cancer (including 108 patients with metastatic triple negative breast cancer treated with

10 mg/kg), 45 patients with urothelial cancer, 110 patients with lung cancer (small cell lung cancer and non-small cell lung cancer), and 101 patients with other epithelial cancers (including cervical, colorectal, endometrial, epithelial ovarian, esophageal, gastric, glioblastoma multiforme, head and neck, hepatocellular, prostate, pancreatic, and renal). The mean treatment duration in these patients was 160 days (7.7 cycles). The following is a summary of the safety profile; additional details can be found in the Investigator's Brochure.

Nearly all patients experienced at least one adverse event; approximately 75% of patients experienced one ≥NCI CTCAE Grade 3 AE; 41% experienced one or more SAEs. The protocol provided instructions for dose reduction and interruption, and treatment discontinuation based on toxicities. AEs led to treatment interruption in 48% of patients; however, only 11% of patients discontinued due to adverse events. Fatal adverse events (within 30 days of last dose) occurred in 6.7% of patients; however nearly all of these deaths were in the setting of disease progression.

The most frequent adverse events were gastrointestinal (nausea, vomiting, and diarrhea) and myelosuppressive (neutropenia and anemia). Nausea occurred in 67% (5% ≥Grade 3); vomiting occurred in 44% (4% ≥Grade 3), and diarrhea occurred in 62% (9% ≥Grade 3) of patients. All patients were administered prophylactic antiemetic treatments and nausea, vomiting, and diarrhea were managed according to standard treatment guidelines. With aggressive management of these toxicities, few patients required treatment discontinuation.

The most frequent myelosuppressive adverse events were neutropenia (41%, ≥Grade 3 28%) and anemia (41%, ≥Grade 3 11%). Neutrophil count decreased occurred in 18% of patients (13% ≥Grade 3) and febrile neutropenia occurred in 6%. Although treatment was interrupted due to neutropenia in 21% of patients, only one patient discontinued due to this toxicity.

Infections occurred in 43% of patients (10% ≥Grade 3); the most frequent were common, community-acquired, and less severe, such as urinary tract infections (12%) and upper respiratory tract infections (10%); pneumonia occurred in 5% (3% ≥Grade 3). Sepsis occurred in 6 patients (1.4%) and septic shock occurred in 2 patients (0.5%).

Other frequent adverse events included fatigue (53%) and alopecia (42%). Only 2 patients had infusion-related hypersensitivity reactions requiring permanent discontinuation of treatment (anaphylaxis in one patient and wheezing, cough, and nasal congestion in a second patient). Preliminary data from this study suggest that sacituzumab govitecan is associated with a low rate of immunogenicity. Persistent positive anti-drug antibodies developed in 3 patients.

SN-38 (the active metabolite of irinotecan) is metabolized by UGT1A1. Irinotecan-treated patients who are homozygous for the UGT1A1 *28 allele are at increased risk for neutropenia and diarrhea.[86] Preliminary results from the IMMU-132-01 study suggest that the frequency of some exposure-related adverse events (i.e., neutropenia and febrile neutropenia) may be higher in patients homozygous for the *28 allele as well; however, the frequencies of neutropenia and other treatment-related adverse events, notably diarrhea, did not differ among the subgroups.

Potential AEs considered expected (for a comprehensive list of expected AEs, please refer to the Investigator's Brochure):

- Nausea, vomiting, diarrhea, constipation, and abdominal pain
- Neutropenia, anemia requiring blood transfusion, thrombocytopenia
- Bacterial and viral infections, including urinary tract infections and pneumonia

- Fever and febrile neutropenia
- Fatigue and headache
- Anorexia, dehydration, and weight loss
- Dysgeusia, hypogeusia, ageusia
- Alopecia
- Rash and pruritus
- Appendicitis, ileitis, or colitis (rare)
- Sepsis
- Anaphylaxis (rare)

7.2 Adverse Event Characteristics

- **CTCAE term (AE description) and grade:** The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 5.0. A copy of the CTCAE version 5.0 can be downloaded from the CTEP web site http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

- **For expedited reporting purposes only:**
  - AEs for the agent(s) that are listed above should be reported only if the adverse event varies in nature, intensity or frequency from the expected toxicity information which is provided.

- **Attribution** of the AE:
  - Definite – The AE *is clearly related* to the study treatment.
  - Probable – The AE *is likely related* to the study treatment.
  - Possible – The AE *may be related* to the study treatment.
  - Unlikely – The AE *is doubtfully related* to the study treatment.
  - Unrelated – The AE *is clearly NOT related* to the study treatment.

- **Expectedness** of the AE:
  Adverse events can be 'Expected' or "Unexpected'

  - Expected adverse event

  Expected adverse events are those that have been previously identified as resulting from administration of the agent. For the purposes of this study, an adverse event is considered expected when it appears in the current adverse event list, the Investigator's Brochure, the package insert or is included in the informed consent document as a potential risk.

  - Unexpected adverse event

  For the purposes of this study, an adverse event is considered unexpected

when it varies in nature, intensity or frequency from information provided in the current adverse event list, the Investigator's Brochure, the package insert or when it is not included in the informed consent document as a potential risk.

Investigators **must** report to the Sponsor-Investigator any serious adverse event (SAE) that occurs after the initial dose of study treatment, during treatment, or within 30 days of the last dose of treatment on the local institutional SAE form (90 days for pembrolizumab).

In the event of an unanticipated problem or life-threatening complications treating investigators must immediately notify the Sponsor-Investigator.

All occurrences of potential DILIs, meeting the defined criteria in Section 5.5, must be reported as SAEs.

For Multi-Center Trials where a DF/HCC investigator is serving as the Sponsor, each participating institution **must** abide by the reporting requirements set by the DF/HCC (listed in Section 7.3). The Sponsor-Investigator and Coordinating Center should be notified of SAEs within 1 business day of learning of the event.

7.3 Expedited Adverse Event Reporting

The following adverse events must be reported to the DFCI IRB according to the expedited reporting guidelines:

- All locally occurring Adverse Events that are *Serious*, *Unexpected*, and there is a *Reasonable Possibility* the Adverse Event is related to the study intervention should be reported to the IRB.

Investigative sites within DF/HCC will report AEs directly to the DFCI Office for Human Research Studies (OHRS) per the DFCI IRB reporting policy.

- All life threatening and fatal Serious Adverse Events (grade 4 and 5) considered serious, unexpected, and related, must be submitted via a written adverse event report to OHRS **within 5 working days** from notification of the event.
- For all other Serious Adverse Events considered serious, unexpected, and related, a full written adverse event report must be submitted to OHRS **within 10 working days** from notification of the event.
- Any unrelated adverse event does not require reporting to OHRS except grade 5 events which must be reported at the time of continuing review.

Other investigative sites will report SAEs to their respective IRB according to their local IRB's policies and procedures for reporting adverse events. A copy of the submitted institutional AE form will be forwarded to the Coordinating Center. The Coordinating Center will submit AE reports from outside institutions to the DFCI IRB according to DFCI IRB policies and procedures in reporting adverse events.

7.4 Reporting to the Food and Drug Administration (FDA)

The Sponsor-Investigator, as study sponsor, will be responsible for all communications with the FDA. The Sponsor-Investigator will report to the FDA, regardless of the site of occurrence, any serious adverse event that meets the FDA's criteria for expedited reporting following the reporting requirements and timelines set by the FDA.

7.5 Reporting to Hospital Risk Management

Participating investigators will report to their local Risk Management office any participant safety reports, sentinel events or unanticipated problems that require reporting per institutional policy.

7.6 Expedited Reporting to [redacted]

A serious adverse event is any adverse event occurring at any dose or during any use of [redacted] product (i.e., Arm A only) that:

- Results in death;
- Is life threatening;
- Results in persistent or significant disability/incapacity;
- Results in or prolongs an existing inpatient hospitalization;
- Is a congenital anomaly/birth defect;
- Is a new cancer (that is not a condition of the study);
- Is associated with an overdose;
- Is another important medical event

Any serious adverse event, or follow up to a serious adverse event, including death due to any cause other than progression of the cancer under study that occurs to any subject from the time the consent is signed through 90 days following cessation of pembrolizumab, or the initiation of new anti-cancer therapy, whichever is earlier, whether or not related to [redacted] product, must be reported within 24 hours to the Sponsor-Investigator and within 2 working days to [redacted] on a MedWatch 3500A Form.

Non-serious Events of Clinical Interest will be forwarded to [redacted] and will be handled in the same manner as SAEs. Medwatch 3500A Forms will be used for these reports.

Additionally, any serious adverse event, considered by an investigator who is a qualified physician to be related to [redacted] product that is brought to the attention of the investigator at any time outside of the time period specified in the previous paragraph also must be reported immediately to [redacted]

SAE reports and any other relevant safety information are to be forwarded to the [redacted]

Any SAE that is reportable to the FDA should also be transmitted to [redacted] at the

number above.

All subjects with serious adverse events must be followed up for outcome.

7.6.1 Events of Clinical Interest (ECIs)

Selected non-serious and serious adverse events are also known as Events of Clinical Interest (ECI) and must be reported within 24 hours to the Sponsor and within 2 working days to [redacted]

For the time period beginning when the consent form is signed until treatment allocation/randomization, any ECI, or follow up to an ECI, that occurs to any subject must be reported within 24 hours to the Sponsor and within 2 working days to [redacted] if it causes the subject to be excluded from the trial, or is the result of a protocol-specified intervention, including but not limited to washout or discontinuation of usual therapy, diet, placebo treatment or a procedure.

For the time period beginning at treatment allocation/randomization through 90 days following cessation of treatment, or 30 days following cessation of treatment if the subject initiates new anticancer therapy, whichever is earlier, any ECI, or follow up to an ECI, whether or not related to [redacted] product, must be reported within 24 hours to the Sponsor and within 24 hours to [redacted]

Events of clinical interest for this trial include:

1. Overdose of [redacted] product, as defined in - Definition of an Overdose for This Protocol and Reporting of Overdose to the Sponsor, that is not associated with clinical symptoms or abnormal laboratory results.
2. An elevated AST or ALT lab value that is greater than or equal to 3X the upper limit of normal and an elevated total bilirubin lab value that is greater than or equal to 2X the upper limit of normal and, at the same time, an alkaline phosphatase lab value that is less than 2X the upper limit of normal, as determined by way of protocol-specified laboratory testing or unscheduled laboratory testing.*

*Note: These criteria are based upon available regulatory guidance documents. The purpose of the criteria is to specify a threshold of abnormal hepatic tests that may require an additional evaluation for an underlying etiology.

7.6.2 Definition of an Overdose of Pembrolizumab for This Protocol and Reporting of Overdose to [redacted]

For purposes of this trial, an overdose of pembrolizumab will be defined as any dose of 1,000 mg or greater. No specific information is available on the treatment of overdose of pembrolizumab. Appropriate supportive treatment should be provided if clinically indicated. In the event of overdose, the subject should be observed closely for signs of toxicity. Appropriate supportive treatment should be provided if clinically indicated.

If an adverse event(s) is associated with ("results from") the overdose of a [redacted] product, the adverse event(s) is reported as a serious adverse event, even if no other seriousness criteria are met.

If a dose of [redacted]s product meeting the protocol definition of overdose is taken without any associated clinical symptoms or abnormal laboratory results, the overdose is reported as a non-serious Event of Clinical Interest (ECI), using the terminology "accidental or intentional overdose without adverse effect."

All reports of overdose with and without an adverse event must be reported within 24 hours to the Sponsor and within 2 working days hours to [redacted]

7.6.3 Reporting of Pregnancy and Lactation to [redacted]

Although pregnancy and lactation are not considered adverse events, it is the responsibility of investigators or their designees to report any pregnancy or lactation in a subject (spontaneously reported to them) that occurs during the trial.

Pregnancies and lactations that occur after the consent form is signed but before treatment allocation/randomization must be reported by the investigator if they cause the subject to be excluded from the trial or are the result of a protocol-specified intervention, including but not limited to washout or discontinuation of usual therapy, diet, placebo treatment or a procedure.

Pregnancies and lactations that occur from the time of treatment allocation/randomization through 120 days following cessation of pembrolizumab therapy, or 30 days following cessation of treatment if the subject initiates new anticancer therapy, whichever is earlier, must be reported by the investigator. All reported pregnancies must be followed to the completion/termination of the pregnancy. Pregnancy outcomes of spontaneous abortion, missed abortion, benign hydatidiform mole, blighted ovum, fetal death, intrauterine death, miscarriage and stillbirth must be reported as serious events (Important Medical Events). If the pregnancy continues to term, the outcome (health of infant) must also be reported.

Such events must be reported within 24 hours to the Sponsor and within 2 working days to [redacted]

7.7 Expedited Reporting to [redacted]

A serious adverse event is any adverse event occurring at any dose or during any use of [redacted] product that:

- Results in death;
- Is life-threatening;
- Results in persistent or significant disability/incapacity;
- Results in or prolongs an existing inpatient hospitalization;
- Is a congenital anomaly/birth defect;

- Is a new cancer (that is not a condition of the study);
- Is associated with an overdose;
- Is another important medical event

All SAEs and pregnancies must be reported to the Sponsor-Investigator within 24 hours of awareness of the event. These events should be reported to [redacted] within 15 working days of event awareness.

The initial SAE report should be as complete as possible; however, reporting should not be delayed in order to obtain more information. Follow-ups should be reported as appropriate. The study team is required to provide follow-up information in response to queries from [redacted] or their designee.

All SAEs from the time the subject signs the informed consent and until 30 days after the last dose of study drug must be reported. During the period after informed consent has been obtained and before the first dose of study drug has been administered, only SAEs caused by a protocol-mandated intervention (e.g., biopsy) should be reported. Any SAE that occurs 30 days after the last dose of study drug and is assessed as possibly related to study drug must be reported.

All SAEs from the time the subject signs the informed consent and until 30 days after the last dose of study drug must be reported. During the period after informed consent has been obtained and before the first dose of study drug has been administered, only SAEs caused by a protocol-mandated intervention (e.g., biopsy) should be reported. Any SAE that occurs 30 days after the last dose of study drug and is assessed as possibly related to study drug must be reported.

All SAEs and Pregnancies/Exposure During Pregnancies should be reported via email to: [redacted]

Case Type	Timeframe	Format	Method
Serious Adverse Event	15 working days of awareness	Medwatch 3500A	email to [redacted]
Pregnancy/Exposure During Pregnancy	15 working days of awareness	Medwatch 3500A	email to [redacted]

Any SAE that meets FDA reporting criteria will also be reported to [redacted] as per above.

7.8 Routine Adverse Event Reporting

All Grade 2 or higher Adverse Events (excluding non-clinically significant laboratory values) Adverse Events **must** be reported in routine study data submissions to the Sponsor-Investigator on the toxicity case report forms. **AEs reported through expedited processes (e.g., reported to the IRB, FDA, etc.) must <u>also</u> be reported in routine study data submissions.**

8 PHARMACEUTICAL INFORMATION

A list of the adverse events and potential risks associated with the investigational and other agents administered in this study can be found in Section 7.1.

8.1 Pembrolizumab

Please refer to the Investigator's Brochure for detailed agent information, and to the FDA label for additional information.

8.1.1 Description

Pembrolizumab is a humanized monoclonal antibody of the IgG4/kappa isotype, also called MK-3475 and Keytruda. Pembrolizumab blocks negative immune regulatory signaling by binding to the PD-1 receptor, inhibiting the interaction between PD-1 and its ligands.

The molecular weight of pembrolizumab is 148.9-149.5 KDa.

8.1.2 Form

Clinical supplies will be manufactured and provided by [redacted] as summarized in the following table.

Table: Product Description

Product Name & Potency	Dosage Form
Pembrolizumab 100 mg/ 4mL	Solution for Injection

8.1.3 Storage and Stability

Store intact vials between 2°C-8°C (36°F-46°F). Do not freeze. Protect from light by storing in the original box.

Administer prepared solutions immediately after preparation. If not administered immediately, prepared solutions may be stored refrigerated for up to 20 hours. Pembrolizumab solutions may be stored at room temperature for a cumulative time of up to 4 hours. This includes room temperature storage of liquid drug product solution in vials, room temperature storage of infusion solution in the IV bag, and the duration of infusion.

8.1.4 Compatibility

Compatible IV bag materials: PVC plasticized with DEHP, non-PVC (polyolefin), EVA, or PE lined polyolefin.

8.1.5 Handling

Qualified personnel, familiar with procedures that minimize undue exposure to themselves and the environment, should undertake the preparation, handling, and safe disposal of the immunotherapeutic agent in a self-contained and protective environment.

8.1.6 Availability

Pembrolizumab is an investigational agent and will be supplied free of charge from [redacted]

8.1.7 Preparation

Pembrolizumab solution for infusion must be diluted prior to administration. Allow the required number of vials to equilibrate to room temperature. Do not shake the vials. Do not use if opaque or extraneous particulate matter other than translucent to white proteinaceous particles is observed. Do not use if discolored. To prepare the infusion solution add the dose volume of Pembrolizumab to an infusion bag containing 0.9& Sodium Chloride Injection, USP of 5% Dextrose Injection, USP. Gently invert the bag 10-15 times to mix the solution. The final concentration must be between **1 mg/mL to 10 mg/mL.**

8.1.8 Administration

Route of administration: IV infusion only. Do not administer as an IV push or bolus injection.

Method of administration: Infuse over approximately 30 minutes (range: 20-40 minutes) using an infusion set containing a low-protein binding 0.2 to 5 m in-line filter make of polyethersulfone or polysulfone. Infusion rate should not exceed 6.7 mL/min. A central line is not required however if a subject has a central venous catheter in place, it is recommended that it be used for the infusion. Do not co-administer drugs through the same infusion line. Following the infusion, flush the IV line with normal saline.

8.1.9 Ordering

Pembrolizumab will be obtained directly from [redacted]

8.1.10 Accountability

The investigator, or a responsible party designated by the investigator, should maintain a careful record of the inventory and disposition of the agent using the NCI Drug Accountability Record Form (DARF) or another comparable drug accountability form. (See the NCI Investigator's Handbook for Procedures for Drug Accountability and Storage.)

8.1.11 Destruction and Return

At the end of the study, unused supplies of pembrolizumab should be destroyed according to institutional policies. Destruction will be documented in the Drug Accountability Record Form.

8.2 Sacituzumab govitecan

8.2.1 Description

Sacituzumab govitecan is an antibody-drug conjugate (ADC) composed of hRS7, a humanized IgG1κ monoclonal antibody which binds to Trop-2 (trophoblast cell-surface antigen-2), SN-38, a camptothecin analog which is an inhibitor of topoisomerase I, and CL2A, a pH-sensitive linker which couples SN-38 to hRS7 IgG1κ.

The molecular weight of sacituzumab govitecan is 1601.8 g/mol.

8.2.2 Form

Sacituzumab govitecan is formulated for investigational use only. Formulation consists of 10 mg/mL Sacituzumab govitecan formulated in 22 mM MES, pH 6.5, together with the following excipients (25 mM trehalose and 0.01% Polysorbate 80), which are then lyophilized. Glass vials of sacituzumab govitecan 180 mg as a sterile, non-pyrogenic, lyophilized powder are to be stored under refrigerated conditions (2-8ºC) and protected from light until used.

Each vial is labeled "Caution: New Drug-Limited by Federal (or United States) Law to Investigational Use. Sponsor: [redacted] and identified by study drug name, lot number, and dose. Since the formulated drug product contains no preservative, vials should be used only once. Sacituzumab govitecan will be supplied in cartons, each containing 1 vial.

8.2.3 Storage and Stability

Sacituzumab govitecan is photosensitive and should be protected from light during storage and use and must be stored under refrigerated conditions (2-8ºC) in a locked room that can be accessed only by the pharmacist, the study Investigator, or another duly authorized study/site personnel. The study medications must not be used outside of the context of this protocol. Under no circumstances should the Investigator or other site personnel supply study drug to other Investigators, subjects, or clinics, or allow supplies to be used other than as directed by this protocol without prior written authorization from [redacted]

If abnormalities of the drug vial, reconstituted product or specific adverse events are noted that are thought to be attributed to the study drug, under no circumstances are

additional testing or procedures to be performed on the affected study drug vial or infusion bag and study drug should not be discarded. Entire study drug should be retained at the site, and [redacted] should be notified immediately.

8.2.4 Compatibility

Only normal saline should be used as the infusion base solution, since the compatibility of sacituzumab govitecan with other infusion diluents has not been examined. Do not mix or administer as an infusion, with other medicinal products.

8.2.5 Handling

Qualified personnel, familiar with procedures that minimize undue exposure to themselves and the environment, should undertake the preparation, handling, and safe disposal of the antibody drug conjugate in a self-contained and protective environment.

8.2.6 Availability

Sacituzumab govitecan is an investigational agent and will be supplied free of charge from [redacted]

8.2.7 Preparation

Obtain the appropriate number of vials from 2-8°C storage and allow them to warm to room temperature prior to reconstitution. **The product vials, as well as reconstituted/diluted product in the infusion bag, must be protected from light following institutional guidelines.**

Reconstitute with normal saline and dilute immediately into infusion bags. Initiate the infusion within 1 hour of reconstitution/dilution. If infusion is delayed beyond 1 hour, refrigerate at 2-8°C for no more than 4 hours from reconstitution/dilution prior to infusion. The vial should remain in the box, protected from light, while warming up to room temp, and once reconstituted and diluted into the IV bag, a paper sleeve should be put over the IV bag during administration or if stored at 2-8°C to protect from light. If refrigerated, allow the diluted solution to come to room temperature prior to administration. If infusion is not initiated within 4 hours after reconstitution/dilution, dispose of the original preparation and prepare a new infusion bag by reconstituting and diluting from new vials. Discard any unused portion in the vial. The product does not contain a preservative.

Appropriate use of aseptic technique should be employed in preparing the dose. Allow the sacituzumab govitecan vials to warm to room temperature to allow faster dissolution. The lyophilized powder in each vial should be reconstituted using 20 mL of 0.9% sterile sodium chloride (normal saline). The reconstituted solution should be gently swirled (do not shake the vials) and allowed to dissolve for up to 15 minutes. Calculate the required dose (mg) based on the subject's body weight at Cycle 1, Day 1 (C1D1). The dose is to remain constant throughout the study unless there is a > 10% change in body weight from

C1D1. Modifications to the study drug doses administered should be made for a >10% change in body weight from C1D1 and according to local and regional prescribing standards. Dose modifications for changes in body weight <10% may be made according to local institutional guidelines. Weight may be rounded per local institutional practices.

The appropriate calculated amount should then be withdrawn from the supplied vials of study drug. A 21-gauge needle is recommended. Inject the solution into a glass or plastic infusion container slowly to minimize foaming and do not shake the contents. Adjust the volume in the infusion container as needed with normal saline to obtain a concentration of 1.1-3.4 mg/mL (total volume should not exceed 500 mL). Only normal sterile saline should be used since the stability of the reconstituted product has not been determined with other infusion-based solutions. The product does not contain a preservative; therefore, the prepared study drug should be refrigerated until used and all infusions must be initiated within 4 hours of the initial reconstitution.

8.2.8 **Administration**

Route of administration: slow IV infusion only. Do not administer as an IV push or bolus injection.

Method of administration: Intravenous access must be well established prior to initiating infusion. At the time of dosing, the IV line will be connected to an infusion container containing the prepared volume of sacituzumab govitecan. Either gravity or an infusion pump may be used. In-line filters and other ancillary infusion equipment is not recommended for use. The initial infusion should proceed slowly. Administer the **first** infusion over 3 hours. Monitor the patient during the infusion, and for at least 30 minutes after infusion. Subsequent infusions may be administered over 1-2 hours if previous infusions were well tolerated. No specific infusion rate is prescribed, the conduct of the infusion must be patient specific determined as appropriate by the managing physician. Following completion, the intravenous line should be flushed slowly with 20 mL normal saline and the end of infusion time recorded. In the event of infusion reactions or vital sign changes, the infusion rate may be slowed, interrupted or terminated, as considered appropriate by the managing physician.

8.2.9 **Ordering**

Sacituzumab govitecan will be obtained directly from [redacted]

8.2.10 **Accountability**

The investigator, or a responsible party designated by the investigator, should maintain a careful record of the inventory and disposition of the agent using the NCI Drug Accountability Record Form (DARF) or another comparable drug accountability form. (See the NCI Investigator's Handbook for Procedures for Drug Accountability and Storage.)

In case of a Sacituzumab govitecan temperature excursion, the following mailbox may be used for notification with documentation: [redacted]

8.2.11 Destruction and Return

At the end of the study, or if instructed during 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 site's pharmacist or delegate.

9 BIOMARKER, CORRELATIVE, AND SPECIAL STUDIES

All biomarker analyses completed on blood and tissue samples for correlative data will be exploratory.

Prior to Sponsor Amendment 3, the PD-L1 eligibility amendment: All patients who underwent pre-screening for the trial were required to submit an archival tissue sample for central PD-L1 testing. If the participant enrolled on the trial, no additional archival tissue was required. Additional tissue leftover after central testing will be used for tissue analyses listed in Section 9.3. Any tissue leftover after these analyses have been completed will be banked for future research.

After Sponsor Amendment 3, the PD-L1 eligibility amendment: Central PD-L1 testing will not be required for eligibility. Archival tissue will be requested for all patients for the purposes of retrospective central PD-L1 testing and other exploratory biomarker analyses as described in this section.

In all patients in whom tumor is safely accessible, baseline and on treatment tumor biopsies are required. We plan to use these tissues to perform immune profiling assays, including characterization based on histology (TILs), protein expression, and mRNA expression, as detailed below. We will perform targeted panel (OncoPanel) and whole exome sequencing to determine mutational load and identify specific genomic alterations associated with response. Additionally, we will bank specimens for possible future additional analyses.

Serial blood draws for correlative science are required on this trial; blood draws will be obtained every 9 weeks prior to the infusion of study drugs, at the end-of-treatment visit in patients who go off study for progressive disease, and all efforts will be made to obtain an additional blood draw at the time of progressive disease in patients who went off study for any reason other than progressive disease. On each blood draw, we will perform flow cytometry to characterize protein expression of immune mediators, as detailed below, and additional blood will be banked for future analyses.

Instructions for the collection, labeling and shipment of blood, tissue, and stool samples are outlined below and further detailed in the Lab Manual.

Please note that some of the downstream correlative plans for blood and tissue include whole exome sequencing, RNA sequencing, single cell sequencing, single-nucleus sequencing, tumor tissue spatial profiling, gene expression assays, and digital spatial profiling, as well as analysis of the data generated, which may be performed at DFCI, BWH, Harvard Medical School, Yale Cancer Center, the Broad Institute of MIT, Adaptive Biotechnologies, Foundation Medicine, Nanostring Technologies, Inc., Reveal Genomics, BostonGene, [redacted] and/or Ventana. All samples will be sent in a de-identified fashion. Only designated study personnel at DFCI will have access to PHI.

Specimen Collection Table

Specimen	Screening	C1D1	C2D1	C3D1	Subsequent Cycles Day 1	EOT/PD	Shipping Condition	Ship to
Lavender Top Tube (Section 9.3)		x					Ambient	DFCI T-Hub
Streck Tube (Section 9.3)		x			x[a]	x[c]	Ambient	DFCI T-Hub
CPT Tubes (Section 9.3)		x			x[a]	x[c]	Ambient	DFCI T-Hub
Archival tumor tissue (blocks or slides) (Section 9.1)	x						Ambient	BOC Translational Team
Required fresh tumor tissue biopsy[b] (Section 9.2)	x		x				FFPE, RNAlater: Ambient OCT: Frozen (dry ice) Flash Frozen w/o media [e]	DFCI T-Hub: OCT, RNAlater, FFPE/formalin CCG Team: Flash Frozen w/o media [e]
Optional fresh tumor tissue biopsy (Section 9.2)						x	FFPE, RNAlater: Ambient OCT: Frozen (dry ice)	DFCI T-Hub: OCT, RNAlater, FFPE/formalin
Stool sample collection (Section 9.4)	x		x			x[d]	Ambient	DFCI T-Hub

a. With Restaging (every 9 weeks, coinciding with day 1 of that cycle).
b. Fresh tumor biopsy of accessible metastatic lesion is mandatory before treatment begins and between C2D1 and C3D1. The baseline biopsy may be performed at screening or on Cycle 1 Day 1 prior to dosing. The on-treatment biopsy should take place after dosing on C2D1 and before dosing on C3D1. See section 9.2 below.
c. Blood samples are mandatory for patients with progressive disease. For patients who come off treatment for other reasons, blood samples are optional at end of treatment and every 9-12 weeks during follow up. See Section 10, Study Calendar.

d. Stool sample will be collected at time of progression if response was observed with treatment.
e. Only for subset of DFCI patients, who undergo single-nucleus RNA sequencing. See Lab Manual and Section 9.3.3.

9.1 Central PD-L1 Testing on Archival Tissue

Testing for PD-L1 positivity will be performed by Discovery Life Sciences (DLS)/QualTek Molecular Laboratories. PD-L1 protein expression will be determined by using the anti-PD-L1 antibody clone IHC 22C3 PharmDx commercial kits (Agilent). PD-L1 IHC 22C3 PharmDx is a qualitative immunohistochemical assay using Monoclonal Mouse Anti-PD-L1. Clone 22C3 is intended for use in the detection of PD-L1 protein in formalin-fixed, paraffin-embedded (FFPE) tissue. PD-L1 protein expression will be determined by using Combined Positive Score (CPS), which is the number of PD-L1 staining cells (tumor cells, lymphocytes, macrophages) divided by the total number of viable tumor cells, multiplied by 100. The specimen will be considered PD-L1-positive if CPS $\geq$ 1.

9.1.1 Collection

1 block or 25 unstained slides (4-5 um slide thickness) and 1 representative H&E of tumor tissue should be requested from a past procedure. Tumor tissue will be requested from the most recent specimen available, and metastatic archival tissue will be requested when available over primary archival tissue. Although bone tissue is not preferred, it is allowed (unless decalcified) if no other tumor tissue is available. See section 9.3 for additional information regarding planned/potential research using archival tissue samples.

9.1.2 Shipping

Archival tissue slides will be shipped to the DFCI translational team as described in the laboratory manual.

9.2 Fresh Tissue Biopsy

9.2.1 Objectives

The study will obtain paired tumor biopsies for patients enrolled (after sacituzumab govitecan monotherapy and after sacituzumab govitecan in combination with pembrolizumab). Paired biopsies will be used to evaluate changes in TILs, expression of immune markers (e.g., immune cell subsets, inhibitory and co-stimulatory pathway molecules by immunohistochemistry and/or immunofluorescence) and other relevant markers. Additionally, the biopsies will be subjected to whole exome sequencing (WES) to determine changes in mutational load, mutational signatures, and genomic alterations in antigen presentation and other immunotherapy-response pathways. A portion of the biopsies will be used to perform single-cell RNA sequencing to determine mRNA expression signatures in tumor and immune cells that correlate with response and resistance to immunotherapy.

9.2.2 Collection of Specimens

Biopsies will be performed at the below timepoints, if tumor is safely accessible for biopsy.

Mandatory:

- Baseline during the Screening Phase or on C1D1 prior to dosing
- After 3-6 weeks of treatment (any time from C2D1-C3D1)

Optional:

- At time of progression

Retreatment

For patients proceeding to the retreatment phase, biopsies will be performed at the below timepoints:

Mandatory:

- At time of progression, prior to restarting sacituzumab govitecan + pembrolizumab (Arm A) or sacituzumab govitecan (Arm B)
- At time of progression on retreatment, if a complete or partial response, or stable disease for ≥ 6 months was achieved during the retreatment phase

If dosing is delayed placing the biopsy outside of the allowable window, the biopsy should be rescheduled to be within the window. If not feasible, the biopsy should be obtained as close to within the window as possible. Tissue specimens will be collected from recurrent or metastatic lesions using standard institutional procedures. The amount of tissue collected will follow the guidelines listed below. If a participant has more than one site of disease, only one site needs to be biopsied in order to go on to the study and the site is left to the discretion of the patient and their treating physician. Fine need aspirates (FNA) are not allowed. Participants who undergo a research biopsy procedure for the purpose of this protocol, and in whom inadequate tissue is obtained, are still eligible and are not required to undergo a repeat biopsy in order to enter the study.

- *Breast*: A goal of 3-6 core biopsy specimens will be obtained using standard institutional guidelines for a diagnostic core biopsy of a breast mass.
- *Skin/chest wall*: A goal of 1-2 4-mm punch biopsies will be obtained.
- *Lymph node*: A goal of 3-6 core biopsy specimens will be obtained using an 18-gauge needle.
- *Liver*: A goal of 3-6 core biopsy specimens will be obtained using an 18-gauge needle.
- *Lung*: Because of the risk of pneumothorax associated with core needle biopsies of lung nodules, no core biopsies of lung nodules will be performed on this protocol, unless they are clinically indicated.
- *Bone*: Because the yield of malignant tissue from bone biopsies tends to be relatively low, no bone biopsies will be performed on this protocol, unless they are clinically indicated.

- *Pleural fluid*: A goal of 500 cc of pleural fluid will be obtained with a standard thoracentesis procedure, with or without image guidance, according to the clinical judgment of the treating physician and clinician performing the procedure. Less than the goal amount is acceptable and should be based upon the clinical judgment of the Investigator and the clinician performing the procedure. If more than the goal amount of fluid is obtained, then the entire specimen (with the exception of what is needed for clinical purposes, if applicable) will be stored in the tissue bank.
- *Ascites fluid*: A goal of 500 cc of ascites fluid will be obtained with a standard paracentesis procedure, with or without image guidance, according to the clinical judgment of the treating physician and clinician performing the procedure. Less than the goal amount is acceptable and should be based upon the clinical judgment of the Investigator and the clinician performing the procedure. If more than the goal amount of fluid is obtained, then the entire specimen (with the exception of what is needed for clinical purposes, if applicable) will be stored in the tissue bank.

Please note that the above are guidelines for the amount of tissue to be obtained at the baseline biopsy and are not meant to replace clinical judgment at the time the procedure is performed. Less than the goal quantity of tissue is accepted for each type of biopsy and will be left to the clinical judgment of the physician performing the procedure. If ascites or pleural fluid is to be used as the investigational biopsy specimen, consideration should be given to confirming the malignant nature of the ascites or pleural fluid prior to study entry.

If a patient is undergoing resection of a lesion for clinical reasons (i.e. wedge resection of a new lung lesion for confirmation of diagnosis or re-testing of hormone receptor or HER2 status; or, resection of a chest wall lesion; or, resection of a lymph node), then the patient may opt to have a portion of that tissue stored for research at the time of the procedure (provided that the tissue is collected and processed as specified in this section and the Lab Manual), in which case, the participant would not be required to undergo a separate research biopsy for entry into this protocol.

9.2.3 Shipping

Tissue will be shipped to the DFCI Breast Translational Hub-Smith (T-Hub)per the instructions in the laboratory manual.

9.3 Archival and Fresh Tissue – Potential Testing

9.3.1 Archival Tissue from Pre-Screening and Enrolled Participants

Prior to Sponsor Amendment 3, the PD-L1 eligibility amendment: After 5 unstained slides were sent to DLS/QualTek for PD-L1 testing, the remaining tissue (up to 10 unstained slides and 1 H&E) was sent to the DFCI Study Team to create a small tissue bank. Various assays described in Section 9.3.3 will be performed, in order to characterize the immune microenvironment of HR+/HER2- breast cancer. This work may be published separately from this trial. Any remaining tissue leftover after this work has been performed will be banked for future research.

After Sponsor Amendment 3, the PD-L1-eligibility amendment: A total of 15 unstained slides (4-5 micron thickness) from an archival FFPE tumor sample (metastatic or primary tumor) will be requested for the purposes of central PD-L1 testing and other planned assays as described in Section 9.3.3. Up to five slides may be sent to DLS/Qualtek for central PD-L1 testing, which will occur retrospectively. Any remaining tissue leftover after this work has been performed will be banked for future research.

9.3.2 Fresh Tissue Biopsies

Enrolled participants with accessible disease will undergo required paired research biopsies at baseline and on treatment and an optional biopsy at progression. In the case of retreatment, there may be additional biopsies performed at progression timepoints. Tissue collected from these biopsies will be used in accordance with section 9.3.3.

9.3.3 Potential Tissue Assays

Assay 1: Tumor infiltrating lymphocyte (TIL) percentage and determination of lymphocyte predominant breast cancer (LPBC)

- Paraffinized, hematoxylin and eosin-stained slides taken from 1-2 tissue planes will be derived from each biopsy and will be reviewed by certified pathologists. In the research setting, all cases are reviewed by two pathologists and any discordant results resolved by consensus review. The extent of lymphocytic infiltrate in tumor tissue will be assessed, and stromal TIL percentage will be determined. More detailed guidelines for the quantification of stromal TILs in breast cancer can be found in the recommendations from the International TILs Working Group.[88]

- After assessment of the TIL percentage, the pathologists will categorize the specimen as lymphocyte predominant breast cancer (LPBC), defined as a tumor that contains >60% stromal lymphocytes, or non-LPBC.

Assay 2: Immunohistochemistry and immunofluorescence assays

- Tissue will be collected and fixed by 10% neutral buffered formalin overnight, dehydrated, and paraffin embedded. Four micrometer-thick sections will be cut. The paraffin blocks and unstained slides will be stored at room temperature. Immunohistochemical staining will be performed in the Center for Immuno-Oncology Pathology Core at Dana-Farber/Harvard Cancer Center (DF/HCC) Specialized Histopathology Core.

- Unstained slides from a metastatic site or newly acquired biopsy may be sent to BWH Pathology, Yale Cancer Center, [redacted] or Ventana for exploratory assessment of Trop-2 expression and other biomarkers.

- Formalin-fixed, paraffin-embedded (FFPE) tumor slides will be prepared and H&E stained for assessment of TIL in pre- and post-treatment tumor samples. Subsets of different immune populations (effector/memory CD8 cells, T regulatory cells, dendritic cells, tumor

associated macrophages, and Tie-2 expressing monocytes (TEM)) will be identified using immunohistochemical (IHC) and/or immunofluorescence (IF) staining on FFPE tumor slides. Cyclic immunofluorescence (CyCIF) at Harvard Medical School may also be utilized to define immune cell subpopulations and single cells with this highly multiplexed immunostaining method.

- Central PD-L1 IHC testing may also be performed on the tumor tissue research biopsies collected on study. Up to five FFPE unstained slides (4-5 micron thickness) may be sent to DLS/Qualtek for central PD-L1 testing, which will occur retrospectively. This will allow characterization of changes in PD-L1 expression with exposure to therapy and will also enable comparison of PD-L1 results between metastatic research tumor biopsies and archival tumor samples (Section 9.1 and 9.3.1).

  Testing for PD-L1 positivity will be performed by Discovery Life Sciences (DLS)/QualTek Molecular Laboratories. PD-L1 protein expression will be determined by using the anti-PD-L1 antibody clone IHC 22C3 PharmDx commercial kits (Agilent). The specimen will be considered PD-L1-positive if CPS $\geq$ 1.

- Investigators at our institution have developed IHC staining on paraffin embedded tissues for PD-L1, PD-L2, TIM-3, and LAG-3 through our center for Immuno-Oncology Pathology Core (Scott Rodig MD, PhD, Core Director). PD-L1 IHC has recently been established in a CLIA approved laboratory and the remaining assays for CLIA laboratory conduct are being finalized.

  These investigators have published the methods, protocols, and data establishing the sensitivity and specificity of IHC staining assays using the monoclonal antibodies recognizing PD-L1 (CD274, B7-H1, antibody clone 7G11, generated in the lab of Gordon Freeman, DFCI) and PD-L2 (CD273, B7-DC, clone 9E5, generated in the laboratory of Gordon Freeman, DFCI).[89,90]

  Tumor and immune cell expression of PD-L1 will be evaluated at different cut-off points (i.e. 1%, 5%, 10%, 50%). All IHC stained slides will be evaluated by a pathologist and H-scores will be reported. A subset of slides will be reviewed by a second pathologist to ensure concordance of interpretation.

  The semi-quantitative scoring for this study is in accordance with those published previously and as described above, will include scores for both the neoplastic and non-neoplastic cells within the tumor microenvironment. Data derived from pathologist visual scoring (semi-quantitative, but with increased specificity for delineating neoplastic and non-neoplastic cells) and pathologist-assisted, automated scoring (quantitative, but without accurately delineating neoplastic and non-neoplastic cells) for each marker of interest will be assessed for its clinical value.

- FFPE tumor slides will also be assessed by IHC and/or IF assays for other markers, including MHC class I and II markers (HLA-ABC, HLA-DR) and dendritic cell markers (CD11c, CD1c, CD123), as deemed appropriate and informative based on the literature at

the time of correlative science performance.

Assay 3: Bulk and Single-Nucleus RNA Sequencing

RNA analysis may be performed, and tissue for RNA analysis will be stored, in the DFCI-T-Hub. Messenger RNA (mRNA) expression within tumor biopsy specimens may be assessed using the most current and informative methodologies at the time that correlative science is performed on all specimens. NanoString signatures and/or whole transcriptome sequencing may be used. Potential genes of interest, based on prior immune profiling of breast tumors include CXCL9, CCL5, CD8ACD80, CXCL13, IGKC, CD21, IDO1, PD-1, PD-L1, PD-L2, CTLA4, and FOXP3.[91]

Perou and others have previously shown that within TNBC, exists all of the intrinsic subtypes of breast cancer. Thus, we propose to use the PAM50 algorithm[92] and the Claudin-low predictor[93] in order to determine the intrinsic subtype of each sample. Specifically, using the RNA-seq data, a global normalization step will be performed to adjust the TNBC RNA-seq data obtained in this study relative to the TNBC subset of patients in the TCGA RNA-seq study. Once this normalization is complete, the PAM50 algorithm will be run as described in Parker et al. 2009, and then the Claudin-low predictor will be run as described in Prat et al., thus classifying samples into 1 of 6 intrinsic subtypes. The intrinsic subtype classifications will be tested for associations with disease response to treatment using univariate and multivariate testing in models that include the standard clinical parameters and with models including other genomic predictors.

For a subset of patients who are enrolled at DFCI, single-nucleus RNA sequencing will be performed on biopsies at baseline, on treatment, and, when available, on progression. These studies will determine whether changes in RNA expression signatures specific to tumor and immune cells correlate with treatment response and resistance. These analyses will also investigate whether particular immune cell subsets are enriched in responders compared to non-responders, as suggested by studies in other tumors treated with checkpoint inhibitors. Additional single-cell techniques may be added to the single-cell RNA sequencing, such as cellular indexing of transcriptomes and epitopes by sequencing (CITE-seq), a method that quantifies cell-surface proteins at a single-cell level.[94]

Assay 4: Whole Exome, Whole Genome Sequencing, DNA Methylation

All DNA sequencing will be performed at the Broad Institute; this may include SNP-arrays for germline genomic analyses, as well as exome and/or whole genome sequencing. Tumor biopsy specimens may be assessed using the most current and informative methodologies at the time that correlative science is performed. Potential assays that may be performed on frozen or FFPE metastatic samples and/or FFPE primary samples include:

- Whole Genome sequencing depth of $\geq$ 40X coverage, or Whole Exome sequencing depth of $\geq$ 500X coverage.
- AFFY SNP 6.0, or Illumina SNP arrays for genotypes and DNA copy number changes may be performed.
- DNA methylation: Illumina Infinium 450K methylation arrays.

Previously performed, CLIA-approved NGS panels (i.e., DFCI's Oncopanel) on participants (either from the pre-screening population or the enrolled population) may be requested and utilized in these analyses.

9.3.4 Sites Performing Correlative Studies and/or Data Analysis

De-identified biospecimens and data may be shared with the following entities for the purposes described above:

DFCI Breast Translational Hub-Smith (T-Hub)DFCI Center for Immuno-Oncology
DFCI Core Blood and Tissue Bank
The Broad Institute of MIT
Nanostring Technologies, Inc
Brigham and Women's Hospital (including Center for Advanced Molecular Diagnostics)
Harvard Medical School
Yale Cancer Center
Adaptive Biotechnologies
Foundation Medicine, Inc.
Discovery Life Sciences/Qualtek
Reveal Genomics
BostonGene
[redacted]
Ventana

9.4 Blood Collection

Research blood collection is mandatory for all patients for flow cytometry of PBMCs and potential DNA isolation. The samples will be banked in the DFCI Breast Translational Hub-Smith (T-Hub)for these and future research purposes. These specimens will become the property of the DF/HCC.

The following research blood samples are required:

Cycle 1 Day 1:

- 2 lavender top tubes (10 mL) for whole blood
- 2 Streck tubes (10 mL) for whole blood
- 2 CPT tubes (8 mL each) for whole blood

With Restaging (Every 9 weeks, coinciding with Day 1 of that cycle):

- 2 Streck tubes (10 mL) for whole blood
- 2 CPT tubes (8 mL each) for whole blood

Off Treatment (with or without progressive disease):

- 2 Streck tubes (10 mL) for whole blood
- 2 CPT tubes (8 mL each) for whole blood

The following research blood samples are optional for patients who come off treatment for a reason other than progressive disease until progressive disease, start of another anti-cancer therapy, or death:

Follow Up (every 9-12 weeks):

- 2 Streck tubes (10 mL) for whole blood
- 2 CPT tubes (8 mL each) for whole blood

If sample collection is missed at baseline or at the time of progression, then the sample should be drawn at a future appointment before starting another anti-cancer therapy if possible.

For retreatment patients the following research blood samples are required:

With Restaging (Every 9 weeks, coinciding with Day 1 of that cycle):

- 2 Streck tubes (10 mL) for whole blood
- 2 CPT tubes (8 mL each) for whole blood

End of Treatment (with or without progressive disease):

- 2 Streck tubes (10 mL) for whole blood
- 2 CPT tubes (8 mL each) for whole blood

9.4.1 Shipping

Blood samples will be prepared and shipped to the DFCI T-Hub per the instructions in the laboratory manual.

9.5 Blood Samples – Potential Testing

9.5.1 Immune Markers

Blood will be collected at baseline, with restaging (every 9 weeks prior to the infusion of study drugs), and at time of progression for evaluation of immune markers. The banked samples will be used to analyze DNA, RNA, and protein in future studies.

9.5.1.1 Collection

Two aliquots of 8 ml of whole blood will be collected in CPT tubes. Collection with other CPT tube sizes (for the same total volume) may be acceptable after discussion with the PI and Coordinating Center (i.e., four 4ml CPTs). The blood sample will be collected and processed at baseline, with restaging (every 9 weeks) prior to the infusion of study drugs, and at time of progression for evaluation of immune markers.

9.5.1.2 Potential Testing

9.5.1.2.1 Flow Cytometry

PBMCs will be generated as described in the Lab Manual and used to assess immune cell populations. Surface staining with a panel of antibodies and flow cytometry on PBMCs will then be performed. A selection of the following antibodies may be used, and/or additional antibodies, as deemed appropriate and informative based on the state of the immune profiling literature at the time of correlative science is performed: CD8, PD-1, PD-L1, PD-L2, CD4, FOXP3, CD127.

9.5.2 Cell-free DNA (cfDNA) analysis

Blood will be collected at baseline, every 9 weeks prior to infusion of study drugs, and at time of progression for evaluation of cell-free DNA (cfDNA). The banked samples will be used to analyze DNA, RNA and protein in future studies.

9.5.2.1 Collection of cfDNA specimen(s)

Two 10 ml aliquots of whole blood will be collected in Streck Tubes. The blood sample will be collected and processed at baseline, with restaging (every 9 weeks prior to infusion of study drugs), and at time of progression.

9.5.3 Germline Mutational Status

Mutations identified in target genes will be assessed using germline DNA from the baseline blood sample. We will assess germline mutational status of clinically actionable genes related to familial risks of breast, colon, ovarian, endometrial, and pancreatic cancers, as well as melanoma. Genes will be analyzed both for single-base changes and large rearrangements by next-generation sequencing. Germline mutational profiling will be included as a secondary correlative analysis in this trial to explore the activity of sacituzumab govitecan and pembrolizumab, compared to sacituzumab govitecan alone, in patients with HR+ / HER2- MBC and germline mismatch repair and other DNA repair pathway alterations.

9.5.3.1 Collection of germline specimen(s)

An additional two 10 ml of whole blood will be collected in lavender top tubes at baseline. The blood sample will be collected and processed at baseline. Fill the lavender top tube completely and immediately mix by gentle inversion 8 to 10 times. Inadequate or delayed mixing may result in inaccurate results. The banked samples will be used to analyze DNA, RNA, and protein in future studies. In addition, one of these tubes will be used to determine the uridine diphosphate-glucuronosyl transferase 1A1 (*UGT1A1*) genotype to associate this with the incidence of adverse events, particularly neutropenia, as this genotype has been linked to increased toxicity of irinotecan in other studies.[32] Exploratory analysis will also be done for efficacy.

9.5.4 Sites Performing Correlatives

De-identified biospecimens and data may be shared with the following entities for the purposes described above:

DFCI Core Blood and Tissue Bank
DFCI Center for Immuno-Oncology

DFCI Breast Translational Hub-Smith (T-Hub)
The Broad Institute of MIT
Brigham and Women's Hospital
Nanostring Technologies, Inc.
Adaptive Biotechnologies
Foundation Medicine, Inc.
Reveal Genomics
BostonGene

9.6 Stool Collection

9.6.1 Collection

All stool samples will be collected by each patient at home using a home-based, self-collection and mail method for stool that has been proven to provide nearly equivalent metagenomic and metatranscriptomic data as the state-of-the-art, fresh-frozen sample collection protocol.[95]

Samples will be collected at the below timepoints:

- Baseline
- After 3-6 weeks of treatment (any time from C2D1-C3D1 is allowed)
- At time of progression for those patients who achieved response (partial or complete) to treatment

Retreatment patients will only collect a sample at the below timepoint:

- At the time of progression after retreatment, if response was again observed

A patient questionnaire is included in the kits. The questionnaire should be completed at the time of stool collection and mailed back along with the sample. Stool samples and questionnaires that are not collected at the protocol-specified collection time points will not be protocol violations. Retreatment stool samples are optional.

9.6.2 Handling and shipping of stool specimens

All kits will be provided to the patients at a clinic visit. Patients will also be provided with a mailer in which to return the sample. All samples will be shipped within 24 hours of collection at ambient temperature to the DFCI Breast Translational Hub-Smith (T-Hub), where they will be processed and stored (see Lab Manual). Samples will be stored until shipped out to an external lab vendor, such as Microbiome Dx, who will perform the analysis of the samples.

9.6.3 Analysis

We will quantify microbiome features from amplicon, metagenome, and metatranscriptome using established pipelines to identify strain-level taxonomic, functional, transcriptional, and microbially-mediated metabolite profiles associated with response to pembrolizumab (see Section 13.7.2). This work will determine the association of these microbiome features with response to

the combination immunotherapy-containing regimen versus the monotherapy regimen without immunotherapy.

9.6.4 Sites performing correlative analysis

De-identified biospecimens and data may be shared with the following entities for the purposes described above:

BWH/Harvard Cohorts Biorepository
Microbiome Dx – External Lab Vendor
DFCI Breast Translational Hub-Smith (T-Hub)
Brigham and Women's Hospital

9.6.5 Diet and Physical Activity Assessments

Dietary composition will be assessed through the Block Fat/Sugar/Fruit/Vegetable Screener, supplemented with 3 additional questions about fiber intake from the Block Vegetable/Fruit/Fiber Screener. The dietary assessment contains 58 total questions and takes about 10-12 minutes to complete. Analysis produces estimates of saturated fat, trans fat, total sugars, "added sugars" (in sweetened cereals, soft drinks, and sweets), fruit and fruit juice, vegetable intake, glycemic load, glycemic index and fiber intake.

Physical activity patterns will be assessed with the Leisure Score Index of Godin Leisure-Time Exercise Questionnaire (LSI). The LSI is a short instrument which asks participants to quantify the number of minutes spent in the last week engaging in vigorous, moderate, and light/mild physical activity. The LSI has undergone extensive reliability and validity testing in a number of cancer and general populations[96]. We will be using a modified form of the instrument which includes not only frequency of activity but also exercise duration. Weekly minutes of moderate and vigorous physical activity will be calculated at each time point using standard scoring algorithms.

Please refer to Appendices B and C for the Diet and Physical Activity Assessments.

Assessments will be conducted at the below timepoints (in conjunction with collection of stool samples):

- Baseline
- After 3-6 weeks of treatment (any time from C2D1-C3D1 is allowed)
- At time of progression for those patients who achieved response (partial or complete) to treatment

Retreatment patients will only collect complete an assessment at the below timepoint:

- At the time of progression after retreatment, if response was again observed

The assessments should be completed at the time of stool collection and mailed back along with the sample and stool questionnaire. Diet and physical activity assessments that are not collected at the protocol-specified collection time points will not be protocol violations. Retreatment

assessments are optional.

9.7 Additional Translational Analyses

The above-mentioned analyses may be altered based on novel developments in the field of cancer immune profiling at the time of correlative science. Additional markers or alternative technologies (based on scientific developments and/or novel technologies) may also be used, to explore potential prognostic or predictive candidate markers/panels or markers related to treatment benefit and/or safety, to improve diagnostic tests, or to understand breast cancer biology.

9.8 Future Use of Specimens and Data

All biospecimens and data will be banked for future research and may be made available to other researchers for proposed research projects. These projects will be approved by the PI, Breast Users Committee, and/or the IRB, as appropriate.

9.9 Patient Reported Outcomes

We plan to assess patient reported outcomes utilizing the EORTC QLQ-C30 (Appendix E) and EuroQOL EQ5D-5L (Appendix F) instruments, as well as specific questions from the NCI PRO-CTCAE (Appendix G) library. The questions have been selected carefully to capture the most common adverse events associated with sacituzumab govitecan and pembrolizumab.

The QLQ-C30 measure comprises 9 multiple-item scales and 6 single items. Multiple-item scales of QLQ-C30 consisted of 6 functional scales (physical, role, emotional, cognitive, social and global QOL) and 3 symptom scales (fatigue, nausea and vomiting, pain). Six single-item scales of QLQ-C30 involve dyspnea, sleep disturbance, appetite loss, constipation, diarrhea and financial impact. All of the derived scales range in score from 0 to 100. For the overall HRQoL and functioning scales, a higher score is correlated with better HRQoL, whereas a higher score represents worse HRQoL for symptom scales.

The EQ-5D-5L generic QOL questionnaire is comprised of 5 dimensions: mobility, self-care, usual activities, pain or discomfort, and anxiety or depression. Each dimension has 3 levels: (1) no problem, (2) some problem, or (3) extreme problem. Thus, the final scoring consists of 243 possible combinations or health states. The utility value for each state is assigned on the basis of a set of preference weights (tariffs) elicited from the general population and is specific for each country.

PRO-CTCAE is a PRO measure developed to evaluate symptomatic toxicity in patients on cancer clinical trials. Treatment-related symptoms were selected from PRO-CTCAE for collection in this protocol based on the previously reported adverse event profile for sacituzumab govitecan and pembrolizumab. These symptoms are decreased appetite, nausea, vomiting, constipation, diarrhea, abdominal pain, shortness of breath, hair loss, and fatigue. PRO-CTCAE responses are scored from 0 to 4 with no standardized scoring rules.

Assessments will be conducted at the below timepoints:

- Baseline
- Every 9 weeks at the day 1 visit following restaging visits
- At the end of treatment visit

Retreatment patients will complete assessments at the below timepoint:

- Every 9 weeks at the day 1 visit following restaging visits
- At the end of treatment visit

The assessments should be completed in clinic at study visits at the above timepoints. Quality of life assessments that are not collected at the protocol-specified collection time points will not be protocol violations. Retreatment assessments are optional.

10 STUDY CALENDAR

Screening evaluations are to be conducted within 28 days prior to study treatment initiation unless otherwise specified. Screening assessments occurring within 3 days prior to initiating study treatment do not need to be repeated on Cycle 1 Day 1.

As detailed in the Study Calendar, a negative pregnancy test in women of child-bearing potential must be documented within 2 weeks before the first dose of study medication.

In the event that the participant's condition is deteriorating, laboratory evaluations should be repeated within 48 hours prior to initiation of the next cycle of therapy.

Assessments must be performed prior to administration of any study agent. Study assessments and agents should be administered within $\pm$ 2 days of the protocol-specified date for Day 1 and Day 8, unless otherwise noted; There should be a minimum of 14 days ($\pm$ 2 days) between Day 8 sacituzumab govitecan dosing and Day 1 sacituzumab govitecan dosing of the next cycle.

Dose delays of both drugs are allowed per protocol. If Day 1 dosing is delayed, all subsequent assessments will be adjusted accordingly. For patients randomized to the combination arm, if drugs are administered on different days in the same 21-day cycle, every effort should be made to administer both drugs on the same day in subsequent cycles (all assessments being adjusted accordingly), provided that criteria are met per protocol and that 14 days ($\pm$ 2 days) have elapsed since the administration of the previous dose of sacituzumab govitecan and 21 $\pm$ 4 days since the administration of the previous dose of pembrolizumab. If Day 8 sacituzumab govitecan dosing is delayed, causing adjustment to Day 1 of the following cycle, it is recommended to administer pembrolizumab on the same day as Day 1 of the next cycle of sacituzumab govitecan.

For retreatment patients, any baseline assessments (including tumor imaging and research samples) should be completed within 14 days of Day 1 of second course treatment, unless stated otherwise.

	Screening /Baseline (-28 days to C1D1)	Cycle 1 Day 1	Cycle 1 Day 8	Cycle 2 Day 1	Cycle 2 Day 8	Cycle 3 Day 1	Cycle 3 Day 8	Subsequent Cycles Day 1	Subsequent Cycles Day 8[r]	End of Treatment[m]	Follow-Up (every 6 months)	EDC Timepoints *CRF builder-use only*
Archival Tumor Retrieval (See Section 9.1)	X											N/A
Demographics	X											Baseline
Medical History[a]	X											Baseline
Physical exam[b], performance status	X	X		X		X		X		X		Baseline, Day 1 all cycles, EOT
Concurrent medications (See Section 5.6)	X	X	X	X	X	X	X	X	X	X		N/A
Adverse Event assessment		X	X	X	X	X	X	X	X	X	X[p]	All visits after baseline
Vital signs[c]	X	X	X	X	X	X	X	X	X	X		N/A
Weight[d]	X	X	X	X	X	X	X	X	X	X		Baseline, Day 1 all cycles, EOT
Height	X											Baseline
Hematology panel[e]	X	X	X	X	X	X	X	X	X	X		Baseline, Day 1 & 8 all cycles, EOT
Chemistry panel[f]	X	X	X	X	X	X	X	X	X	X		Baseline, Day 1 & 8 all cycles, EOT
TSH/fT4[g]	X	X		X		X		X		X		Baseline, Day 1 all cycles, EOT
Coagulation panel (INR/PT/aPTT)	X											N/A
Pregnancy test[h]	X											N/A
Tumor Assessment[i]	X							X[i]		X[n]	X[n]	Baseline, Q9 wks, EOT, F/U
Brain MRI[j]	X							X		X		Baseline, Q9 wks, EOT, F/U
Research Blood (See Section 9.3)		X						X[k]		X	X[o]	N/A
Research Biopsy (See Section 9.2)	X			X						X		Baseline, C2/3, EOT
Research Stool Sample and Diet/Activity Questionnaires (See Section 9.5 & 9.6)	X[l]			X						X[l]		Baseline, C2/3, EOT

Patient Reported Outcomes (See Section 9.9)	X[q]							X[q]		X[q]		Baseline, Q9 wks, EOT
Survival Assessment (See Section 5.8)											X	Follow up (FU)

a. Medical history includes clinically significant diseases, recent infections (including administration of any antibiotics within the last 28 days), surgeries, and cancer history (including prior cancer therapies and procedures).
b. A complete physical examination will be performed at baseline. A limited physical exam will be completed prior to therapy on Day 1 of every cycle beginning with Cycle 1.
c. Vital signs should be collected pre-dose on dosing days and are to include measurements of heart rate, respiratory rate, systolic and diastolic blood pressure while the patient is in a seated position, and oral or tympanic temperature.
d. Weight adjustments for sacituzumab govitecan will be done according to institutional standards. See Section 8.2.7.
e. Hematology includes hemoglobin, hematocrit, platelet count, RBC count, WBC count, and percent and absolute differential count. Results must be available prior to the administration of study drug.
f. Chemistry testing includes sodium, potassium, chloride, bicarbonate, glucose, BUN, creatinine, calcium, phosphorus, magnesium, total bilirubin, total protein, albumin, ALT, AST, alkaline phosphatase, and LDH. Results must be available prior to the administration of study drug.
g. TSH/fT4 will be drawn on day 1 of the first 4 cycles and then every 9 weeks at the day 1 following restaging visits
h. In female subjects of child-bearing potential as defined in the eligibility criteria, pregnancy test (serum or urine) must be performed within **2 weeks** before the first dose of study medication. If the urine test is positive or cannot be confirmed as negative, then a serum test is required.
i. Tumor assessments should consist of 1) CT and/or MRI of the chest/abdomen/pelvis, 2) any other imaging studies as clinically indicated by the treating physician. Tumor assessments will be performed at baseline, every 3 cycles/9 weeks with a window of -7 days to + 3 days (e.g., between Cycle 3 Day 15 and Cycle 4 Day 4). Additional scans are permitted as clinically indicated. All known sites of disease documented at screening should be re-assessed at each subsequent tumor evaluation. See Section 11.1.3 Participants who have their treatment held or delayed will continue to have restaging scans that align with cycles of treatment received, rather than weeks on study.
j. Brain MRIs are not mandatory at baseline in patients without history of brain metastases and in whom there are no concerning signs or symptoms. If participants have a history of brain metastases, brain imaging (MRI preferred; CT if necessary) is required at baseline and with restaging scans. If participant received radiation therapy within 6 weeks of registration, then baseline brain imaging is not needed and the most recent brain imaging pre-radiation treatment may be used; the next brain imaging can occur with the first restaging. Brain MRI should be done with and without contrast. If a participant is unable to have an MRI, a CT of the brain with contrast is acceptable. If CT with contrast is contraindicated, a CT without contrast is acceptable.
k. Research blood will be drawn with restaging (every 9 weeks, coinciding with day 1 of the cycle). If research blood tubes are drawn on a day where treatment is held and delayed, they do not need to be re-drawn at the subsequent visit where treatment is administered.
l. Stool sample may be collected within 4 weeks of starting treatment and 4 weeks from progression, as long as no intervening therapy (otherwise, within 2 weeks).
m. End of treatment (EOT) visit is to occur within 45 days of final administration of study treatment, at the time of the subsequent visit that follows progression/other off treatment reason. If the EOT visit occurs within 30 days of final study treatment, an additional AE assessment is performed 30-45 days from final administration of study treatment; this may be conducted in person or by phone by the investigator or suitably trained and qualified delegate (e.g. MD, RN, NP, etc.).
n. For those stopping treatment for reasons other than progressive disease (i.e., complete response), tumor measurements should continue to be repeated every 9-12 weeks until initiation of retreatment on this study, another anti-cancer therapy, progression, or death, whichever occurs first.
o. Samples during follow up should be drawn every 9-12 weeks, alongside tumor assessments, for patients who come off treatment for reasons other than progressive disease until the initiation of another anti-cancer therapy, progression, or death, whichever occurs first.

p. An adverse event assessment is performed 90 days (-15/+30 days) after the last dose of pembrolizumab (Arm A patients only). This may be conducted in person or by phone by the investigator or suitably trained and qualified delegate (e.g., MD, RN, NP, etc.).
q. Patient reported outcomes per Section 9.9 should be performed during screening or on C1D1 prior to treatment (baseline), at the clinic visit following a restaging scan, and at end of treatment/progressive disease.
r. For subjects on Arm A, if sacituzumab govitecan is discontinued and pembrolizumab is continued, Day 8 assessments (AE assessment, labs, vitals, etc.) are not needed.

11 MEASUREMENT OF EFFECT

For the purposes of this study, participants should be re-evaluated for response every 9 weeks. If treatment is delayed, scans will also be delayed to align with cycles of treatment received, rather than weeks on study.

11.1 Antitumor Effect – Solid tumors

Response and progression in sites of metastases will be evaluated in this study using the international criteria proposed by the RECIST 1.1 criteria[97]. Changes in the largest diameter (unidimensional measurement) of the tumor lesions and the shortest diameter in the case of malignant lymph nodes are used in the RECIST criteria.

For any subject who shows first radiologic evidence of progressive disease (PD) by RECIST 1.1 and is deemed clinically stable, it is at the discretion of the investigator to continue treating the subject until progression is confirmed at the next scheduled restaging. These cases should be discussed with the Principal Investigator.

- If progression is not confirmed on the subsequent scan, the subject should continue to receive treatment if the treating investigator feels that the participant is clinically stable, demonstrates improved condition, or is clearly continuing to benefit from the treatment. In this case, radiographic scans should continue to be performed according to the study calendar (i.e., every 9 weeks).

- If radiologic progression is confirmed on the subsequent scan, then the subject should be discontinued from all study treatment.

In all participants, the date of progression for the primary endpoint will be documented as the first date progression was observed.

11.1.1 RECIST 1.1 Definitions

Evaluable for Target Disease response. Only those participants who have measurable disease present at baseline, have received at least one cycle of therapy, and have had their disease re-evaluated will be considered evaluable for target disease response. These participants will have their response classified according to the definitions stated below. (Note: Participants who exhibit objective disease progression prior to the end of cycle 1 will also be considered evaluable.)

Evaluable Non-Target Disease Response. Participants who have lesions present at baseline that are evaluable but do not meet the definitions of measurable disease, have received at least one cycle of therapy, and have had their disease re-evaluated will be considered evaluable for non-target disease. The response assessment is based on the presence, absence, or unequivocal progression of the lesions.

11.1.2 Disease Parameters

Measurable disease. Measurable lesions are defined as those that can be accurately measured in at least one dimension (longest diameter to be recorded) as ≥ 20 mm by chest x-ray or ≥ 10 mm with CT scan, MRI, or calipers by clinical exam. All tumor measurements must be recorded in millimeters (or decimal fractions of centimeters).

Note: Tumor lesions that are situated in a previously irradiated area might be considered measurable if the investigator thinks it appropriate to include them.

Malignant lymph nodes. To be considered pathologically enlarged and measurable, a lymph node must be ≥15 mm in short axis when assessed by CT scan (CT scan slice thickness recommended to be no greater than 5 mm). At baseline and in follow-up, only the short axis will be measured and followed.

Non-measurable disease. All other lesions (or sites of disease), including small lesions (longest diameter <10 mm or pathological lymph nodes with ≥10 to <15 mm short axis), are considered non-measurable disease. Bone lesions, ascites, pleural/pericardial effusions, lymphangitis cutis/pulmonitis, inflammatory breast disease, abdominal masses (not followed by CT or MRI), and cystic lesions are all considered non-measurable.

Note: Cystic lesions that meet the criteria for radiographically defined simple cysts should not be considered as malignant lesions (neither measurable nor non-measurable) since they are, by definition, simple cysts.

'Cystic lesions' thought to represent cystic metastases can be considered as measurable lesions, if they meet the definition of measurability described above. However, if non-cystic lesions are present in the same participant, these are preferred for selection as target lesions.

Target lesions. All measurable lesions up to a maximum of 2 lesions per organ and 5 lesions in total, representative of all involved organs, should be identified as **target lesions** and recorded and measured at baseline. Target lesions should be selected on the basis of their size (lesions with the longest diameter), be representative of all involved organs, but in addition should be those that lend themselves to reproducible repeated measurements. It may be the case that, on occasion, the largest lesion does not lend itself to reproducible measurement in which circumstance the next largest lesion which can be measured reproducibly should be selected. A sum of the diameters (longest for non-nodal lesions, short axis for nodal lesions) for all target lesions will be calculated and reported as the baseline sum diameters. If lymph nodes are to be included in the sum, then only the short axis is added into the sum. The baseline sum diameters will be used as reference to further characterize any objective tumor regression in the measurable dimension of the disease.

Non-target lesions. All other lesions (or sites of disease) including any measurable lesions over and above the 5 target lesions should be identified as **non-target lesions** and should also be recorded at baseline. Measurements of these lesions are not required, but the

presence, absence, or in rare cases unequivocal progression of each should be noted throughout follow up.

11.1.3 Methods for Evaluation of Disease

All measurements should be taken and recorded in metric notation using a ruler, calipers, or a digital measurement tool. All baseline evaluations should be performed as closely as possible to the beginning of treatment and never more than 4 weeks before the beginning of the treatment.

The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during follow-up. Imaging-based evaluation is preferred to evaluation by clinical examination unless the lesion(s) being followed cannot be imaged but are assessable by clinical exam.

Clinical lesions. Clinical lesions will only be considered measurable when they are superficial (*e.g.*, skin nodules and palpable lymph nodes) and ≥10 mm in diameter as assessed using calipers (*e.g.*, skin nodules). In the case of skin lesions, documentation by color photography, including a ruler to estimate the size of the lesion, is recommended.

Chest x-ray. Lesions on chest x-ray are acceptable as measurable lesions when they are clearly defined and surrounded by aerated lung; however, CT is preferable.

Conventional CT and MRI. This guideline has defined measurability of lesions on CT scan based on the assumption that CT thickness is 5mm or less. If CT scans have slice thickness greater than 5 mm, the minimum size of a measurable lesion should be twice the slice thickness. MRI is also acceptable in certain situations (e.g., for body scans).

Use of MRI remains a complex issue. MRI has excellent contrast, spatial, and temporal resolution; however, there are many image acquisition variables involved in MRI, which greatly impact image quality, lesion conspicuity, and measurement. Furthermore, the availability of MRI is variable globally. As with CT, if an MRI is performed, the technical specifications of the scanning sequences used should be optimized for the evaluation of the type and site of disease. Furthermore, as with CT, the modality used at follow-up should be the same as was used at baseline and the lesions should be measured/assessed on the same pulse sequence. It is beyond the scope of the RECIST guidelines to prescribe specific MRI pulse sequence parameters for all scanners, body parts, and diseases. Ideally, the same type of scanner should be used, and the image acquisition protocol should be followed as closely as possible to prior scans. Body scans should be performed with breath-hold scanning techniques, if possible.

FDG-PET. While FDG-PET response assessments need additional study, it is sometimes reasonable to incorporate the use of FDG-PET scanning to complement CT scanning in assessment of progression (particularly possible 'new' disease). New lesions on the basis of FDG-PET imaging can be identified according to the following algorithm:

(a) Negative FDG-PET at baseline, with a positive FDG-PET at follow-up is a sign of PD based on a new lesion.
(b) No FDG-PET at baseline and a positive FDG-PET at follow-up: If the positive FDG-PET at follow-up corresponds to a new site of disease confirmed by CT, this is PD. If the positive FDG-PET at follow-up is not confirmed as a new site of disease on CT, additional follow-up CT scans are needed to determine if there is truly progression occurring at that site (if so, the date of PD will be the date of the initial abnormal FDG-PET scan). If the positive FDG-PET at follow-up corresponds to a pre-existing site of disease on CT that is not progressing on the basis of the anatomic images, this is not PD.
(c) FDG-PET may be used to upgrade a response to a CR in a manner similar to a biopsy in cases where a residual radiographic abnormality is thought to represent fibrosis or scarring. The use of FDG-PET in this circumstance should be prospectively described in the protocol and supported by disease-specific medical literature for the indication. However, it must be acknowledged that both approaches may lead to false positive CR due to limitations of FDG-PET and biopsy resolution/sensitivity.

Note: A 'positive' FDG-PET scan lesion means one which is FDG avid with an uptake greater than twice that of the surrounding tissue on the attenuation corrected image.

PET-CT. At present, the low dose or attenuation correction CT portion of a combined PET-CT is not always of optimal diagnostic CT quality for use with RECIST measurements. However, if the site can document that the CT performed as part of a PET-CT is of identical diagnostic quality to a diagnostic CT (with IV and oral contrast), then the CT portion of the PET-CT can be used for RECIST measurements and can be used interchangeably with conventional CT in accurately measuring cancer lesions over time. Note, however, that the PET portion of the CT introduces additional data which may bias an investigator if it is not routinely or serially performed.

MIBG (meta-iodobenzylguanidine). The following is recommended, to assure high quality images are obtained.

Patient preparation: Iodides, usually SSKI (saturated solution of potassium iodide), are administered to reduce thyroidal accumulation of free radioiodine, preferably beginning the day prior to injection and continuing for 3 additional days (4 days total). For infants and children, one drop t.i.d. is sufficient, for adolescents 2 drops t.i.d., and for adults 3 drops t.i.d. Participants and/or parents are asked about exposure to potential interfering agents. If none is noted, an indwelling intravenous line is established. The dose of MIBG is administered by slow intravenous injection over 90 seconds.

Images from the head to the distal lower extremities should be obtained.

I-123 MIBG scintigraphy is performed to obtain both planar and tomographic images.

Planar: Anterior and posterior views from the top of the head to the proximal lower extremities are obtained for 10 minutes at 24 hours and occasionally at 48 hours

following injection of 10 mCi/1.7 square meters of body surface area (~150 µCi/kg, maximum 10 mCi). Anterior views of the distal lower extremities are adequate. A large field of view dual head gamma camera with low energy collimators is preferred.

SPECT: Most participants receiving I-123 MIBG also undergo SPECT at 24 hours, using a single or multi-headed camera with a low energy collimator. The camera is rotated through 360 degrees, 120 projections at 25 seconds per stop. Data are reconstructed using filtered back projections with a Butterworth filter and a cut off frequency of 0.2-0.5. SPECT/CT may be performed at institutions with this capacity.

Ultrasound. Ultrasound is not useful in assessment of lesion size and should not be used as a method of measurement. Ultrasound examinations cannot be reproduced in their entirety for independent review at a later data and, because they are operator dependent, it cannot be guaranteed that the same technique and measurements will be taken from one assessment to the next. If new lesions are identified by ultrasound in the course of the study, confirmation by CT or MRI is advised. If there is concern about radiation exposure from CT, MRI may be used instead of CT in selected instances.

Endoscopy, Laparoscopy. The utilization of these techniques for objective tumor evaluation is not advised. However, such techniques may be useful to confirm complete pathological response when biopsies are obtained or to determine relapse in trials where recurrence following complete response (CR) or surgical resection is an endpoint.

Tumor markers. Tumor markers alone cannot be used to assess response. If markers are initially above the upper normal limit, they must normalize for a participant to be considered in complete clinical response. Specific guidelines for both CA-125 response (in recurrent ovarian cancer) and PSA response (in recurrent prostate cancer) have been published [*JNCI* 96:487-488, 2004; *J Clin Oncol* 17, 3461-3467, 1999; *J Clin Oncol* 26:1148-1159, 2008]. In addition, the Gynecologic Cancer Intergroup has developed CA-125 progression criteria which are to be integrated with objective tumor assessment for use in first-line trials in ovarian cancer [*JNCI* 92:1534-1535, 2000].

Cytology, Histology. These techniques can be used to differentiate between partial responses (PR) and complete responses (CR) in rare cases (*e.g.*, residual lesions in tumor types, such as germ cell tumors, where known residual benign tumors can remain).

The cytological confirmation of the neoplastic origin of any effusion that appears or worsens during treatment when the measurable tumor has met criteria for response or stable disease is mandatory to differentiate between response or stable disease (an effusion may be a side effect of the treatment) and progressive disease.

11.1.3.1 Evaluation of Target Lesions

Complete Response (CR): Disappearance of all target lesions. Any pathological lymph nodes (whether target or non-target) must have reduction in short axis to <10 mm.

Partial Response (PR): At least a 30% decrease in the sum of the diameters of target lesions, taking as reference the baseline sum diameters.

Progressive Disease (PD): At least a 20% increase in the sum of the diameters of target lesions, taking as reference the smallest sum on study (this includes the baseline sum if that is the smallest on study). In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5 mm. (Note: the appearance of one or more new lesions is also considered progressions).

Stable Disease (SD): Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum diameters while on study.

11.1.3.2 Evaluation of Non-Target Lesions

Complete Response (CR): Disappearance of all non-target lesions and normalization of tumor marker level. All lymph nodes must be non-pathological in size (<10 mm short axis).

Note: If tumor markers are initially above the upper normal limit, they must normalize for a patient to be considered in complete clinical response.

Non-CR/Non-PD: Persistence of one or more non-target lesion(s) and/or maintenance of tumor marker level above the normal limits.

Progressive Disease (PD): Appearance of one or more new lesions and/or *unequivocal progression* of existing non-target lesions. *Unequivocal progression* should not normally trump target lesion status. It must be representative of overall disease status change, not a single lesion increase.

Although a clear progression of "non-target" lesions only is exceptional, the opinion of the treating physician should prevail in such circumstances, and the progression status should be confirmed at a later time by the review panel (or Principal Investigator).

11.1.3.3 Evaluation of New Lesions

The finding of a new lesion should be unequivocal (i.e., not due to difference in scanning technique, imaging modality, or findings thought to represent something other than tumor (for example, some 'new' bone lesions may be simply healing or flare of pre-existing lesions). However, a lesion identified on a follow-up scan in an anatomical location that was not scanned at baseline is considered new and will indicate PD. If a new lesion is equivocal (because of small size etc.), follow-up evaluation will clarify if it truly represents new disease and if PD is confirmed, progression should be declared using the date of the initial scan on which the lesion was discovered.

11.1.3.4 Evaluation of Best Overall Response

The best overall response is the best response recorded from the start of the treatment until disease progression/recurrence (taking as reference for progressive disease the smallest measurements recorded since the treatment started). The patient's best response assignment will depend on the achievement of both measurement and confirmation criteria.

For Participants with Measurable Disease (*i.e.*, Target Disease)

Target Lesions	Non-Target Lesions	New Lesions	Overall Response	Best Overall Response when Confirmation is Required*
CR	CR	No	CR	≥ 4 wks Confirmation**
CR	Non-CR/Non-PD	No	PR	≥ 4 wks Confirmation**
CR	Not evaluated	No	PR	
PR	Non-CR/Non-PD/not evaluated	No	PR	
SD	Non-CR/Non-PD/not evaluated	No	SD	Documented at least once ≥ 4 wks from baseline**
PD	Any	Yes or No	PD	No prior SD, PR or CR
Any	PD***	Yes or No	PD	
Any	Any	Yes	PD	

* See RECIST 1.1 manuscript for further details on what is evidence of a new lesion.
** Only for non-randomized trials with response as primary endpoint.
*** In exceptional circumstances, unequivocal progression in non-target lesions may be accepted as disease progression.

Note: Participants with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be reported as "*symptomatic deterioration.*" Every effort should be made to document the objective progression even after discontinuation of treatment.

For Participants with Non-Measurable Disease (*i.e.*, Non-Target Disease)

Non-Target Lesions	New Lesions	Overall Response
CR	No	CR
Non-CR/non-PD	No	Non-CR/non-PD*
Not all evaluated	No	Not evaluated
Unequivocal PD	Yes or No	PD
Any	Yes	PD

* 'Non-CR/non-PD' is preferred over 'stable disease' for non-target disease since SD is increasingly used as an endpoint for assessment of efficacy in some trials so to assign this category when no lesions can be measured is not advised

11.1.4 Duration of Response

Duration of overall response: The duration of overall response is measured from the time measurement criteria are met for CR or PR (whichever is first recorded) until the first date that recurrent or progressive disease is objectively documented (taking as reference for progressive disease the smallest measurements recorded since the treatment started, or death due to any cause. Participants without events reported are censored at the last disease evaluation).

Duration of overall complete response: The duration of overall CR is measured from the time measurement criteria are first met for CR until the first date that progressive disease is objectively documented, or death due to any cause. Participants without events reported are censored at the last disease evaluation.

Duration of stable disease: Stable disease is measured from the start of the treatment until the criteria for progression are met, taking as reference the smallest measurements recorded since the treatment started, including the baseline measurements.

11.1.5 Time to Response

Time to objective response: The time to objective response is defined as the time from randomization to the date of the first documented CR or PR (whichever is first recorded).

11.1.6 Clinical Benefit Rate

Clinical Benefit Rate: Clinical Benefit Rate (CBR) is defined as CR, PR and stable disease (SD) for $\geq$ 24 weeks.

11.1.7 Survival

Overall Survival: Overall Survival (OS) is defined as the time from randomization (or registration) to death due to any cause, or censored at date last known alive.

Progression-Free Survival: Progression-Free Survival (PFS) is defined as the time from randomization (or registration) to the earlier of progression or death due to any cause. Participants alive without disease progression are censored at date of last disease evaluation.

Time to Progression: Time to Progression (TTP) is defined as the time from randomization (or registration) to progression, or censored at date of last disease evaluation for those without progression reported.

11.1.8 Response Review

Central Review will be conducted by the DF/HCC Tumor Imaging Metric Core for DF/HCC Institutions.

11.2 Other Response Parameters

N/A

12 DATA REPORTING / REGULATORY REQUIREMENTS

Adverse event lists, guidelines, and instructions for AE reporting can be found in Section 7.0 (Adverse Events: List and Reporting Requirements).

12.1 Data Reporting

12.1.1 Method

The Office of Data Quality (ODQ) will collect, manage, and perform quality checks on the data for this study.

12.1.2 Responsibility for Data Submission

Investigative sites within DF/HCC or DF/PCC are responsible for submitting data and/or data forms to the Office of Data Quality in accordance with DF/HCC SOPs.

12.2 Data Safety Monitoring

The DF/HCC Data and Safety Monitoring Board (DSMB) will review and monitor study progress, toxicity, safety and other data from this study. The board is chaired by a medical oncologist from outside of DF/HCC and has external and internal representation. Information that raises any questions about participant safety or protocol performance will be addressed by the Sponsor-Investigator, statistician and study team. Should any major concerns arise, the DSMB will offer recommendations regarding whether or not to suspend the study.

The DSMB will meet twice a year to review accrual, toxicity, response and reporting information. Information to be provided to the DSMB may include participant accrual; treatment regimen information; adverse events and serious adverse events reported by category; summary of any deaths on study; audit results; and a summary provided by the study team. Other information (e.g., scans, laboratory values) will be provided upon request.

12.3 Multicenter Guidelines

This protocol will adhere to DF/HCC Policy MULTI-100 and the requirements of the DF/HCC Multi-Center Data and Safety Monitoring Plan. The specific responsibilities of the Sponsor-Investigator, Coordinating Center, and Participating Institutions and the procedures for auditing are presented in Appendix H.

12.4 Collaborative Research and Future Use of Data and Samples

Tissue, blood, stool, bodily fluids, and other materials derived from these will be collected in this study to analyze genes, DNA, RNA, proteins and cells for the study's correlative endpoints and potential future research, utilizing new types of biomarker testing as it becomes available.

These samples and any data generated as a part of these clinical trials may be used for future research studies and may be provided to collaborating investigators both within and outside of the DF/HCC for either correlative endpoints or secondary use. Samples and data may be shared with outside non-profit academic investigators, as well as with for-profit pharmaceutical investigators or commercial entities, with whom we collaborate. When samples or data are sent to collaborators and when any research is performed on them, all information will be identified with a code, and will not contain any PHI, such as name, birthday, or MRNs.

In order to allow the greatest amount of research to be performed on the specimens and information generated as a part of this trial, researchers in this study may share results of genetic sequencing with other scientists. De-identified specimen or genetic data may be placed into one of more publicly accessible scientific databases, such as the National Institutes of Health's Database for Genotypes and Phenotypes (dbGaP). The results from the correlative research on this study will be shared with these public databases. Through such databases, researchers from around the world will have access to de-identified samples or data for future research. More detailed information, beyond the public database, may only be accessed by scientists at other research centers who have received special permission to review de-identified data.

13 STATISTICAL CONSIDERATIONS

13.1 Study Design/Endpoints

This is a randomized phase II open label study of sacituzumab govitecan +/- pembrolizumab for patients with HR+ / HER2- MBC. Sacituzumab govitecan will be administered at a dose of 10 mg/kg IV on days 1 and 8 of each 21-day cycle, and pembrolizumab will be administered at a dose of 200 mg IV on day 1 of each 21-day cycle. The target enrollment is 110 patients; 55 per arm of treatment.

The primary endpoint is PFS defined as the time from study randomization to disease progression, per RECIST 1.1 or medical judgment, the latter based on established clinical parameters, such as rising tumor markers and physical exam evidence of progression, i.e., worsening chest wall disease, or death due to any cause, whichever occurred first. Patients alive without disease progression are censored at the date of last disease evaluation. The primary objective is to compare in the intent-to-treat (ITT) population (as defined in Section 13.5) the PFS in patients randomized to receive sacituzumab govitecan in combination with pembrolizumab (Arm A) versus those randomized to receive sacituzumab govitecan monotherapy (Arm B).

<u>Secondary endpoints include:</u>

- PFS according to RECIST 1.1 in the PD-L1-positive population (defined as CPS ≥1)
- Overall response rate (ORR) according to RECIST 1.1 in the ITT and PD-L1+ population
- Overall survival (OS) in the ITT and PD-L1+ population
- Clinical benefit rate (CBR) according to RECIST 1.1, defined as CR, PR or stable disease for ≥ 24 weeks in the ITT and PD-L1+ population
- Time to progression (TTP) in the ITT and PD-L1+ population
- Duration of response (DOR) and time to objective response (TTOR) in the ITT and PD-L1+ population
- Toxicity graded according to NCI CTCAE, Version 5.0

13.2 Sample Size, Accrual Rate and Study Duration

Based on data of recent trials in a similar population of patients with metastatic breast cancer treated with sacituzumab govitecan, a median PFS of 5.5 months would be expected with sacituzumab govitecan monotherapy.[32] Currently, there are no published data about the PFS of patients with HR+ / HER2- MBC treated with sacituzumab govitecan in combination with anti-PD-1 therapy. We anticipate that achieving a PFS of at least 8.5 months for combination therapy (Arm A) in the present study would make the regimen worthy of further investigation. Considering a one-sided 0.1 type-I error and 80% power to detect a 3-month absolute difference in median PFS at the time of final analysis (hazard ratio = 0.65), 110 patients would need to be enrolled on the trial.

The final analysis will occur when 100 PFS events are observed, or 2 months after the last patient is randomized, whichever occurs first. An interim analysis for futility will occur when ~50% of the information is obtained (50 PFS events). The study would stop early if the Z-statistic from a log-rank test is less than 0. It is estimated that the interim analysis will occur approximately 18 months after the first patient is randomized.

The expected accrual rate is 4-5 patients per month, with accrual of 110 patients anticipated to be completed at 24 months. The expected study duration to primary analysis of PFS is 2.5 to 3 years.

13.2.1 Change in timing of the final analysis

At the time of the October 2023 DSMB reporting, it became clear that 100 PFS events would not be reached in this study (due to higher rates of treatment discontinuation for reasons other than progression than expected) and so the final PFS analysis would only occur 9 months after the last patient was randomized. This would result in a longer study timeline than expected (45 months since accrual was completed at 36 months). Changing the final analysis from 9 months after the last patient is randomized to 2 months after the last patient is randomized (i.e., 38-month study duration to primary PFS analysis) would result in approximately 5% decrease in study power. Given the treatment landscape and emerging data for TROP2 antibody drug conjugates for metastatic HR-positive/HER2-negative breast cancer, this relatively small loss of power was considered acceptable against the alternative of waiting to report the results and, hence, the final analysis will now be conducted 2 months after the last patient is randomized.

13.3 Stratification Factors

There are no planned stratification factors for this trial.

13.4 Interim Monitoring Plan

We will perform a safety run-in analysis in the first 12 patients randomized to the combination therapy arm. If there are 4 or more dose-limiting toxicity (DLTs) in the first 12 patients included, enrollment will be halted to discuss whether the study will be amended, with re-evaluation of the appropriate dosing schedule and study design or closed. The probability of observing 4 or more DLTs in the first 12 if the true DLT rate is 15% or lower is less than 10%. The probability of observing 4 or more DLTs in the first 12 if the true DLT rate is 30% or higher is greater than 50%. Section 5.4 describes the DLT definitions used in this current study, which will be assessed in the first cycle (21 days). In the case that enrollment continues, these first 12 patients will be included in the efficacy analysis.

An additional interim analysis for futility will occur when ~50% of the information is obtained (after 50 PFS events have occurred). The study would stop early if the Z-statistic from a log-rank test is less than 0.

13.5 Analysis of Primary Endpoints

The primary objective is to compare the median PFS achieved with the experimental combination of sacituzumab govitecan plus pembrolizumab versus sacituzumab govitecan alone, and this will be assessed among all patients who initiated protocol therapy according to the randomized treatment assignment.

PFS is defined as the time from study randomization to disease progression, according to RECIST 1.1 or medical judgment, the latter based on established clinical parameters, such as rising tumor markers and physical exam evidence of progression, i.e., worsening chest wall disease, or death due to any cause, whichever occurred first. Patients alive without disease progression are censored at the date of last disease evaluation. Definitive analysis of the efficacy data will be based on the modified intent-to-treat principle where all patients who initiate protocol therapy will be analyzed as randomized.

The Kaplan-Meier estimates will also be calculated separately for patients allocated to each treatment regimen. Multivariable Cox proportional hazards models will be used to estimate treatment efficacy in PFS with adjusting for potential confounders such as age at study entry, stage (locally advanced or metastatic), visceral metastases (yes or no), level of ER expression (1-9% vs. 10% or greater), and rapid recurrence after completion of adjuvant therapy < 6 months (yes or no). Potential interactions between treatment and clinical factors will be explored in the Cox models. Model diagnostics will be performed to check whether the proportionality assumption is valid in those models. Should it fail, appropriate analysis, such as models with time-dependent effects, will be explored. An exploratory subset analysis in the eligible-only population will also be performed, and results of this analysis will be compared with those from the intent-to-treat population in the primary analysis. Any substantial difference will be carefully examined.

13.6 Analysis of Secondary Endpoints

Efficacy Endpoints

- Among patients who initiated protocol therapy and whose tumor was centrally confirmed to be PD-L1-positive (as defined by CPS ≥1 using the PharmDx 22C3 assay), the efficacy, as assessed by PFS, of sacituzumab govitecan plus pembrolizumab will be compared to sacituzumab govitecan alone. PD-L1 status will be defined by central confirmation of PD-L1 testing on the baseline research biopsy, if available; if not available, then the central PD-L1 testing results from the archival sample will be used. The comparison of PFS between treatment arms in the PD-L1-positive subgroup will be exploratory.

- In the modified ITT and PD-L1-positive population, all patients who initiated protocol therapy will also be evaluated for ORR (according to RECIST 1.1), CBR, DOR, TTOR and OS.

  Radiographic response will be assessed using RECIST 1.1 criteria, as defined in section 11. Objective response will require confirmatory scans or exam as indicated. The ORR (CR + PR), according to RECIST 1.1, will be reported with 95% confidence intervals. Clinical benefit is defined as CR, PR or SD ≥ 24 weeks according to RECIST 1.1 and will be reported with 95% confidence intervals. Median DOR and TTOR will be reported with ranges. Overall survival, defined as the time from randomization (or registration) to death due to any cause with censoring at date last known alive, will be reported with Kaplan Meier estimates.

  For OS, the log-rank test will be one-sided. Treatment efficacy in these secondary endpoints will be assessed by Cox models adjusting for clinical factors such as age at study entry, stage (locally advanced or metastatic), visceral metastases (yes or no), level of ER expression (1-9% vs. 10% or greater), and rapid recurrence after completion of adjuvant therapy < 6 months (yes or no). Dichotomous endpoints of response will be compared using Cochran-Maentel-Haenszel chi-squared tests.

Safety and tolerability

All patients will be evaluable for toxicity from the time of their first treatment with any study agent. Toxicity will be graded according to NCI CTCAE, Version 5.0. Toxicities will be summarized by maximum grade and by treatment arm. Incidence rate of each toxicity will be reported with 95% confidence intervals. The incidence rates of any grade 3 or higher toxicity will be compared across arms using a two-sided Fisher's exact test.

13.7 Correlative Endpoints

13.7.1 Tumor biopsies and TIL assessment

Previous studies have demonstrated that, in addition to direct cytotoxic effects, chemotherapy-induced cell death can be immunogenic, causing T cell tumor infiltration and sensitizing tumors to immune checkpoint blockade.[98] Cytotoxic CD8+ T cell infiltration has been reported to increase after treatment with chemotherapy across different tumor types.[99-101] Recently, Herbst et al.

demonstrated that patients who had an increase of at least 5% in expression of PD-L1 in the tumor microenvironment were more likely to respond to the PD-L1 inhibitor atezolizumab.[58] Modifications in molecular signatures of the tumor microenvironment also correlated with response to therapy.

Tumor-infiltrating lymphocytes (TILs) by histological assessment will be quantitative and evaluated as a percentage of cells stained (0-100%). Data from Loi and colleagues suggest that the mean number of TILs in first-line HR+ / HER2- MBC is likely similar to that observed in first-line metastatic HER2-positive samples from the CLEOPATRA study.[102] The median TIL value was 10% with a mean number of TILs of 17% (standard deviation as a continuous variable of 21%). TILs will be analyzed as a continuous variable using descriptive statistics to summarize the distribution observed among study participants.

A) To assess the relationship between baseline TILs and progression-free survival, we plan to perform a mandatory research biopsy of a safely accessible tumor lesion at baseline. The association of TILs and PFS will be evaluated in a proportional hazards regression model with treatment effect and study stratification factors as covariates.[103] Linear and non-linear components of the model will be assessed using Wald-type tests. If a non-linear association is detected, the point estimate and 95% prediction bands will be reported for the log relative hazard over the entire distribution of TILs. If no significant non-linear effects are observed, the step-down model will evaluate the linear multivariate proportional hazards regression model and report the adjusted hazard ratio and 95% confidence interval for a linear increase in TILs. In addition, modification of the treatment effect will be explored as an interaction test, and if non-significant (two-sided alpha = 0.05), the step-down model with main effects will be reported.

B) To assess the absolute change in the percentage of TILs before and after starting treatment, a mandatory biopsy at 3-6 weeks post-treatment (any time between C2D1 and C3D1) is required, if tumor is safely accessible. This on-treatment biopsy will be performed in, at least, the first 27 patients with accessible disease in each arm. Assuming the drop-out/non-evaluable rate will be 10%, 24 paired samples will be available.

Descriptive statistics will be used to summarize the distribution of TILs observed at baseline and after 3-6 weeks of therapy (e.g., mean, standard deviation, median, and inter-quartile range). The evaluation of change in TILs within each arm will be based on a paired t-test (absolute difference) using a one-sided alpha = 0.05 for each arm. Based on the variability of TILs reported by Loi and colleagues in paired specimens before and after treatment with chemotherapy for TNBC, with 24 evaluable paired specimens anticipated per arm and a standard deviation of eight, there will be 82% power to detect an increase in mean TILs from 22.3% to 26.5%, under an assumption of an additive shift model with a Gaussian common density function.

If paired biopsies at baseline and at 3-6 weeks after treatment are obtained in 24 paired samples in each arm (assuming a drop-out/non-evaluable rate of 10% in the entire study population), there will be statistical power to detect the following change in TILs between both study arms. Assuming that the mean difference in TILs from pre-treatment to 3-6 weeks after starting protocol therapy is 10.4% in Arm A (sacituzumab govitecan + pembrolizumab) and 4.2% in Arm B (sacituzumab govitecan alone), with a standard deviation of 8, using a Wilcoxon-Mann-Whitney test with one-

sided alpha of 0.05, the study has 80% power to detect the difference of change in TILs between Arm A (sacituzumab govitecan + pembrolizumab) and Arm B (sacituzumab govitecan alone).

Optional repeat biopsies will be performed at time of progression to explore changes in TILs and other tissue markers. Descriptive statistics will be used to summarize the change in TILs, and inferences on treatment-effects will use non-parametric tests (e.g., Wilcoxon rank sum) with two-sided alpha 0.05. The association of changes in TILs with clinical outcomes of PFS and OS will be exploratory and hypothesis generating in the subset of patients with evaluable repeat biopsies. Analysis plans will consider using Cox models with time-varying covariates to account for the post-baseline assessments.

C) For secondary correlative endpoints to characterize the expression of tumor markers by immunohistochemistry (IHC) and/or immunofluorescence (IF), descriptive statistics and agglomerative hierarchical clustering techniques will be used to summarize the distribution and patterns of profiles observed in baseline samples. Tests of association with clinical outcomes (PFS, OS, ORR) will be exploratory using Cox proportional hazard and logistic regression models, and inferences will use the Benjamini-Hochberg method to control the false discovery rate. Any positive findings will require validation in future studies with independent samples for unbiased estimates of the association with treatment effects and clinical outcomes.

Efficacy endpoints will be analyzed according to Trop-2 expression, which will be evaluated as percent of positive cells, intensity, a composite score, or another method. Analyses of Trop-2 expression are descriptive and exploratory in nature and intended to examine the distribution of Trop-2 expression levels at different biopsy sites and assess associations between Trop-2 expression and efficacy outcomes, such as PFS, OS and ORR, for which separate analyses will be performed according to Trop-2 expression (above and below median) and treatment group.

D) Single-cell RNA sequencing will be performed on biopsies at baseline, on treatment, and, when available, at progression for a subset of patients enrolled at DFCI to assess the relationship of timepoint-specific tumor and immune cell subpopulations with clinical outcomes in exploratory analyses. In addition, changes in single-cell RNA expression signatures across these timepoints will be correlated with response. Standard preprocessing and quality control algorithms will be performed to filter out low quality cells and to normalize the expression data. Differentially expressed genes will be identified using the Benjamini-Hochberg procedure to control the false discovery rate for multiple comparisons. Descriptive statistics and clustering techniques, including principal component analysis and t-distributed stochastic neighbor embedding, will be employed to summarize the distributions of the expression data and identify tumor and immune cell subpopulations. Changes in cell subpopulations between baseline, on-treatment, and at progression biopsies will be assessed with Wilcoxon signed-rank tests, while differences in cell subpopulations between responders and non-responders will be compared with Mann-Whitney tests. Preliminary associations of tumor and immune cell subpopulations with progression-free and overall survival will be explored using Cox proportional hazard models.

13.7.2 Immunogenomic analysis

Tumor and matched germline blood sequencing data will be processed with standard quality control methods, for instance, excluding samples with poor sequencing coverage and low estimated purity as previously described.[104] We will use established whole exome sequencing (WES) pipelines to determine single nucleotide variants, small insertions/deletions, and larger structural variants with computational filtering of artifacts introduced by FFPE-based DNA extraction and DNA oxidation during sequencing.[104,105] We will limit our search to highly deleterious variants, specifically known hotspot or putative truncating mutations, such as frameshift insertions/deletions, nonsense mutations, and splice-site alterations. Additional standard analysis methods will be used to assess tumor purity and ploidy, copy number alterations, and mutational clonality, the latter to distinguish genomic alterations more likely to be founder mutations from those more likely to have been acquired with evolution or recurrence.[104,106] Established analysis procedures will likewise be applied to RNA sequencing (RNAseq) data to estimate transcript abundances, differential gene expression, and significantly enriched pathways.[107] The association of response with genomic alterations, both individual genes and genes combined in pathways, will be reported by contingency tables and tested for significance using Fisher's exact test. The false discovery rate will be controlled with the Benjamini-Hochberg method.

We will compare genomic and transcriptomic markers between responders and non-responders in both an unbiased analysis and a directed assessment of immune mechanisms, including antigen presentation, interferon-γ signaling, T-cell effector pathways, and mutational signatures.[107-109] In addition, we will investigate antigen presentation genes, such as MHC class I genes (*HLA-A*, *HLA-B*, *HLA-C*, *B2M*), MHC class II genes (*HLA-DMA/B HLA-DOA/B*, *HLA-DPA1/B1*, *HLA-DQA1/2, HLA-DQB1/2, HLA-DRA, HLA-DRB/1/3/4/5*), master regulators of these two families of genes (*NLRC5* and *CIITA*, respectively), genes implicated in peptide processing and loading (*TAP1*, *TAP2*, *TAPBP*), and genes related to interferon-γ, which is known to upregulate MHC class I, including *JAK1/2*, interferon-γ receptor 1/2 (*IFNGR1/2*), interferon regulatory factor 1 (*IRF1*), suppressor of cytokine signaling 1 (*SOCS1*), and protein inhibitor of activated STAT4 (*PIAS4*).[59,110]

WES and RNAseq analyses will also investigate the association between immune pathways and response. WES data will be analyzed to calculate tumor mutation burden, which is associated with response to immunotherapy in other tumors.[108] HLA genotypes will be assessed with OptiType, an integer linear programming algorithm that predicts HLA genotype with an overall accuracy of 97%.[111] Neoantigen prediction will be accomplished with NetMHCpan, a method that utilizes both binding affinity and antigen processing data obtained from mass spectrometry.[111]

Similarly, RNAseq data will be analyzed to estimate immune cell subpopulations and overall immune cell infiltrate with the digital cytometry method CIBERSORTx.[112] The cytolytic activity of the local immune cell infiltrate will be calculated as the geometric mean of *PRF1* and *GZMA* expression,[113] and gene set enrichment analysis will be performed using canonical molecular signatures,[114] such as the Hallmark gene sets,[115] as well as a carefully curated compendium of immune signatures.[116] Existing transcriptional signatures reported to be associated with response to immunotherapy, such as interferon-γ and T cell effector signatures,[110,117] will be assessed in the subset of patients treated with pembrolizumab. As bulk RNAseq only detects T cell receptors

(TCR) on the most abundant T cells at a rate close to 1 TCR per 10 million reads,[118] we will consider target specific TCR sequencing methods, which capture greater TCR diversity.[119]

13.7.3 Analysis of intestinal microbiome

Microbial DNA is extracted using the Mag-Bind Universal Pathogen DNA Kit (Omega Bio-Tek). Briefly, 250 mg of the specimen is transferred to a deep-well plate for bead beating followed by DNA precipitation and purification following the manufacturer's instructions. Finally, DNA is eluted in 100 uls of Elution Buffer and stored at -80°C until further use. 16S sequencing libraries are generated by amplifying the v3-v4 hypervariable regions of the 16S gene in a polymerase chain reaction using primers F341and R785. Resulting amplicons are tagged with unique molecular barcodes that are later used to demultiplex sequencing reads into individual sample buckets. Libraries are loaded on a MiSeq flow cell and sequenced following Illumina's loading instructions. Sequence data are retrieved from the instrument by converting base call format files into fastq files for data processing purposes.

MicrobiomeDX uses BacPro™, a proprietary algorithm, to inspect and validate sequencing files by employing demultiplexing, trimming, merging, and quality filtering steps. Paired sequencing reads are merged using an overlap of 25 bp allowing for 10 base mismatches. Merged sequences are dereplicated and clustered in a de-novo fashion using VSEARCH, while filtering out sequence chimeras and singletons. Representative sequences from each cluster are mapped against the SILVA database at 99% sequence identity to obtain accurate taxonomic classifications and relative abundances. In parallel, feature tables are constructed to derive alpha diversity indices, and distance matrices are built to derive beta diversity indices. The BacPro™ pipeline generates a comprehensive report that includes alpha diversity scores describing community richness and evenness, taxonomic composition with relative abundances, and beta diversity metrics to determine the in-between sample differences based on the bacterial communities identified.

13.7.4 Patient Reported Outcomes

Summary statistics of the scores for the derived functional and symptom quality of life scales according to the scoring manuals and global health status scores will be summarized and compared by treatment arm at each time point.

13.8 Reporting and Exclusions

13.8.1 Evaluation of Efficacy

For this Phase II trial, the efficacy evaluable population is a modified intent-to-treat (ITT) population. The modified ITT population consists of all patients who initiate protocol therapy, even if there are major protocol therapy deviations.

13.8.2 Evaluation of Safety

The safety population will be used in the safety data summaries. The safety population consists of all patients who took at least one dose of sacituzumab govitecan or pembrolizumab on study and who have at least one post-baseline safety assessment. Note that a patient who had no adverse events constitutes a safety assessment. Patients who have received at least one dose of study drug but have no post-treatment safety data of any kind will be excluded. Evaluation of safety analyses will use treatments as treated rather than as planned.

14 PUBLICATION PLAN

The results should be made public within 1 year of reaching the end of the study. The end of the study is the time point at which the last data items are to be reported, or after the outcome data are sufficiently mature for analysis, as defined in the section on Sample Size, Accrual Rate and Study Duration. If a report is planned to be published in a peer-reviewed journal, then that initial release may be an abstract that meets the requirements of the International Committee of Medical Journal Editors. A full report of the outcomes should be made public no later than three (3) years after the end of the study.

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121. Sacituzumab Govitecan Package Insert

APPENDIX A PERFORMANCE STATUS CRITERIA

ECOG Performance Status Scale		**Karnofsky Performance Scale**	
Grade	Descriptions	Percent	Description
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.	100	Normal, no complaints, no evidence of disease.
		90	Able to carry on normal activity; minor signs or symptoms of disease.
1	Symptoms, but ambulatory. 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).	80	Normal activity with effort; some signs or symptoms of disease.
		70	Cares for self, unable to carry on normal activity or to do active work.
2	In bed <50% of the time. Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours.	60	Requires occasional assistance but is able to care for most of his/her needs.
		50	Requires considerable assistance and frequent medical care.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.	40	Disabled, requires special care and assistance.
		30	Severely disabled, hospitalization indicated. Death not imminent.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.	20	Very sick, hospitalization indicated. Death not imminent.
		10	Moribund, fatal processes progressing rapidly.
5	Dead.	0	Dead.

APPENDIX B 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 for the duration of study treatment and 3 months after the last dose of study treatment:

- 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 9 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 consistently and correctly as described in Table 1 starting with the first dose of study medication through 180 days (6 months) after the last dose of study medication.

Table 1 Highly Effective Contraception Methods

Highly Effective Methods That Have Low User Dependency *Failure rate of <1% per year when used consistently and correctly.*
• 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.)
Notes: Use should be consistent with local regulations regarding the use of contraceptive methods for participants of clinical studies.

Pregnancy Testing

WOCBP should only be included after a negative highly sensitive urine or serum pregnancy test.

Following initiation of treatment, pregnancy testing will be performed whenever an expected menstrual cycle is missed or when pregnancy is otherwise suspected; at the time points specified in the Schedule of Activities, and as required locally.

APPENDIX C DIET AND PHYSICAL ACTIVITY ASSESSMENTS

Please note: the below is a copy of the **Block Fat/Sugar/Fruit/Vegetable Screener** for reference. *Please use the official Scantron version of the Screener rather than the appendix.*

FOOD QUESTIONNAIRE

RESPONDENT ID #

TODAY'S DATE

Jan Feb Mar Apr May Jun Jul Aug Sep Oct Nov Dec

DAY

YEAR 2008 2009 2010 2011 2012 2013 2014 2015 2016 2017 2018 2019 2020 2021 2022 2023

ABOUT YOU

SEX
- Male
- Female

If female, are you pregnant or breast feeding?
- No
- Yes
- Not female

AGE

WEIGHT pounds

HEIGHT ft. in.

ABOUT THIS SURVEY

- This form is about the foods you usually eat. It will take about 10 - 15 minutes to complete.
- Please answer each question as best you can. Estimate if you aren't sure.
- USE ONLY A NO. 2 PENCIL.
- Fill in the ovals completely, and erase completely if you make any changes.

INSTRUCTIONS

Think about your eating habits over the past year or so. Remember breakfast, lunch, dinner, snacks and eating out.

There are two kinds of questions for each food:

- **"How Many Days per Week"**, on average, do you usually eat the food
- **"How Much"** do you usually eat of the food.

	HOW MANY DAYS PER WEEK? None or less than 1	1 day	2 days	3-4 days	5-6 days	Every day	HOW MUCH ON THOSE DAYS?		
1. Glasses of milk, not counting on cereal or coffee (any kind).	○	○	○	○	○	○	1 8 oz glass	2	3+
2. Real 100% fruit juice, like orange juice, apple juice, or fruit smoothies. Don't count fruit flavored soft drinks or drinks like Sunny Delight.	○	○	○	○	○	○	Small 6 oz glass	1 cup	2+ cups
3. Vegetable juice, like tomato juice, V8, carrot.	○	○	○	○	○	○	Small 6 oz glass	1 cup	2+ cups
4. Snapple, Koolaid, instant lemonade, instant iced tea, cordial - regular or sugar-free.	○	○	○	○	○	○	1 glass	2	3+
5. Drinks with some juice, like Hawaiian Punch, Sunny Delight, Knudsen, Hi-C, cranberry juice.	○	○	○	○	○	○	1 glass	2	3+
6. Any kind of soft drink, soda or pop, like Coke, cola, Sprite, Gingerale, Crush, Fanta, regular or sugar-free.	○	○	○	○	○	○	1 glass/can	2	3+
7. Beer	○	○	○	○	○	○	1 glass/can	2	3+

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	HOW MANY DAYS PER WEEK?						HOW MUCH ON THOSE DAYS?		
	NONE OR LESS THAN 1	1 DAY	2 DAYS	3-4 DAYS	5-6 DAYS	EVERY DAY			
8. Eggs, or breakfast sandwiches with eggs, like Egg McMuffins (McDonalds).	○	○	○	○	○	○	○ 1 egg	○ 2	○ 3+
9. Cold cereal, any kind.	○	○	○	○	○	○	○ 1 small bowl	○ 1 medium bowl	○ 1 large bowl
10. Hot Cereal, cooked cereal like oatmeal or porridge, grits, or cream of wheat.	○	○	○	○	○	○	○ 1 small bowl	○ 1 medium bowl	○ 1 large bowl
11. Real sugar or honey in coffee or tea or on cereal.	○	○	○	○	○	○	○ 1 tsp	○ 2	○ 3+
12. Cheese, sliced cheese or cheese spread, including on sandwiches.	○	○	○	○	○	○	○ 1 slice	○ 2	○ 3+
13. Lunch meats like bologna, salami, sliced ham, turkey lunch meat, or any other cold meat cuts.	○	○	○	○	○	○	○ 1 slice	○ 2	○ 3+
14. Hamburgers, cheeseburgers, meat balls or meat loaf.	○	○	○	○	○	○	○ 1 small/3 oz	○ 1 large	○ 2 large
15. Hot dogs, or sausage like Polish, Italian or chorizo.	○	○	○	○	○	○	○ 1 hotdog	○ 2	○ 3+
16. Other beef or pork, such as steak, roast beef, ribs, or in sandwiches, tacos, burritos.	○	○	○	○	○	○	○ 3 oz small	○ 4-6 oz medium	○ 7+ oz large
17. Fried chicken, including chicken nuggets, wings, chicken patty.	○	○	○	○	○	○	○ 1 medium piece	○ 2 medium pieces or 6 nuggets	○ 3 medium pieces
18. Fish, any kind.	○	○	○	○	○	○	○ 2 oz	○ 4 oz	○ 6 oz
19. Pizza.	○	○	○	○	○	○	○ 1 slice	○ 2	○ 3+
20. Spaghetti, lasagna, other pasta, or noodles.	○	○	○	○	○	○	○ 1 cup	○ 2	○ 3+
21. Rice, or dishes made with rice.	○	○	○	○	○	○	○ 1 cup rice	○ 2	○ 3+
22. Green salad and vegetables you put in green salad.	○	○	○	○	○	○	○ 1 cup	○ 2	○ 3+
23. Any kind of fruit, fresh or canned (not counting juice).	○	○	○	○	○	○	○ 1 fruit or 1/2 cup	○ 2 fruits or 1 cup	○ 3 fruits or 2 cups
24. French fries, home fries, hash browns.	○	○	○	○	○	○	○ small (McD)	○ medium	○ large
25. Potatoes not fried, like baked, mashed.	○	○	○	○	○	○	○ 1/2 cup or 1/2 potato	○ 1 cup	○ 2+ cups
26. Vegetable soup, or stew with vegetables.	○	○	○	○	○	○	○ 1 cup	○ 1 1/2 cups	○ 2+ cups

	HOW MANY DAYS PER WEEK?						HOW MUCH ON THOSE DAYS?		
	NONE OR LESS THAN 1	1 DAY	2 DAYS	3-4 DAYS	5-6 DAYS	EVERY DAY			
27. ALL other vegetables you eat, as a side dish or in any kind of dish, not counting salad or potatoes.	○	○	○	○	○	○	○ 1/2 cup altogether	○ 1 cup	○ 2 cups
28. Bread, rolls, bagels.	○	○	○	○	○	○	○ 1 slice	○ 2	○ 3+
29. Biscuits, muffins, croissants.	○	○	○	○	○	○	○ 1	○ 2	○ 3+
30. Snack chips like potato chips, tortilla, corn chips, Fritos, Doritos, popcorn (not pretzels).	○	○	○	○	○	○	○ 1 small handful	○ 1 oz bag 1 cup	○ Big bag 2 cups
31. Crackers, like Ritz, soda-crackers, Cheez-Its, or any other snack cracker.	○	○	○	○	○	○	○ 3-4 small crackers	○ 5-10 crackers	○ a lot
32. Ice cream, ice cream bars.	○	○	○	○	○	○	○ 1/2 cup	○ 1 cup	○ 2+ cups
33. Doughnuts.	○	○	○	○	○	○	○ 1	○ 2	○ 3+
34. Cake, cookies, or snack cakes like cupcakes, Twinkies or any other pastry.	○	○	○	○	○	○	○ 1 small piece	○ 1 medium	○ 2+
35. Pie including fast food pies or snack pies.	○	○	○	○	○	○	○ 1 small piece	○ 1 medium	○ 2+
36. Chocolate candy like chocolate bars, M&Ms, Mars Bars, Reeses.	○	○	○	○	○	○	○ 1 mini	○ 1 medium	○ 1 large
37. Any other candy (not chocolate) like hard candy, Lifesavers, Skittles, Starburst.	○	○	○	○	○	○	○ 1-2 pieces	○ 1/2 package	○ 1 package
38. Margarine (not butter) on bread or on vegetables.	○	○	○	○	○	○	○ 1 teaspoon	○ 2 teaspoons	○ 3 teaspoons
39. Butter (not margarine) on bread or on vegetables.	○	○	○	○	○	○	○ 1 teaspoon	○ 2 teaspoons	○ 3 teaspoons
40. Fat or oil in cooking.	○	○	○	○	○	○			

For each of the questions below, please fill in the oval that best describes your usual eating habits.

41. What kind of milk do you usually drink?	○ Whole milk ○ Reduced-fat 2% milk ○ Low-fat 1% milk	○ Skim milk ○ Soy milk ○ Rice milk	○ I don't drink milk or soy milk
42. If you drink soft drinks or pop, is it usually:	○ Diet or sugar free soft drinks	○ Regular ○ I don't drink soft drinks	
43. If you drink Snapple, KoolAid, instant iced tea, or instant lemonade, is it usually:	○ Sugar-free ○ Regular	○ I don't drink these	
44. If you eat hot dogs, are they usually:	○ Low Fat or turkey hot dogs	○ Regular hot dogs ○ I don't eat hot dogs	
45. If you eat lunch meats, are they usually:	○ Low Fat or turkey	○ Regular	○ I don't eat lunch meats
46. If you eat snacks like chips, are they usually:	○ Trans-fat free	○ Regular	○ I don't know ○ I don't eat them

For each of the questions below, please fill in the oval that best describes your usual eating habits.

47. If you eat crackers, are they usually:
- Trans-fat free
- Triscuits, Graham crackers, Ry-Vita
- Saltines or other snack crackers
- I don't eat them

48. If you eat ice cream, is it usually:
- Low carb, low sugar
- Low fat or ice milk
- Regular
- Premium
- I don't eat it

49. If you eat cake, snack cakes, cookies and other pastries, are they usually:
- Low carb, low sugar
- Low fat
- Regular
- I don't eat it

50. If you eat chocolate candy, is it usually:
- Low carb, low sugar
- Low fat
- Regular
- I don't eat it

51. If you eat other candy, not chocolate, is it usually:
- Sugar-free
- Regular
- I don't eat it

52. When you use margarine, is it usually:
- Stick margarine
- Soft tub margarine
- Low-fat margarine
- Butter-margarine blend
- Non-hydrogenated and trans-fat free
- I don't eat it

53. What kind of fat or oil do you usually use in cooking? MARK ONLY 1 or 2.
- Spray oil (i.e. Pam), or no oil
- Butter
- Butter/margarine blend
- Stick margarine
- Soft tub margarine
- Low-fat margarine
- Corn oil, vegetable oil
- Olive oil, canola oil
- Lard, fatback, bacon fat
- Crisco
- Trans-fat free brand
- I don't know, or don't cook

54. What kind of cold cereal do you usually eat?
Choose 1 or 2 that you eat most often.
(If you usually eat just one kind, mark one.)
- Low-carb cereals like Atkins, Low-Carb Special K
- Cheerios (plain), Shredded Wheat, Wheat Chex, Wheaties
- Sweetened cereals like Frosted Flakes, Honey Nut Cheerios, Fruit Loops, Cap'n Crunch, granola, instant sweetened oatmeal
- Other cold cereals, like Corn Flakes, Rice Krispies, Bran Flakes
- I don't eat cereal.

55. What kind of bread do you usually eat?
- Italian, French or local bakery
- Regular sliced white bread
- Dark bread like rye, cracked wheat
- 100% whole wheat
- I don't know or I don't eat bread

SOME LAST QUESTIONS ABOUT YOU

Are you
- Hispanic or Latino
- Not Hispanic or Latino

What race do you consider yourself to be? (MARK ALL THAT APPLY)
- White
- Black or African American
- Asian
- American Indian or Alaska Native
- Native Hawaiian or Other Pacific Islander
- Do not wish to provide this information

Thank you very much for filling out this questionnaire.
Please take a minute to go back and fill in anything you may have skipped.

PLEASE DO NOT WRITE IN THIS AREA

SERIAL #

APPENDIX D DIET AND PHYSICAL ACTIVITY ASSESSMENTS

Below is a copy of the **Gordin Leisure-Time Exercise Questionnaire (LSI)** with three additional questions about fiber intake from the **Block Vegetable/Fruit/Fiber Screener**.

Godin Leisure-Time Questionnaire

When answering these questions please:

- only count exercise sessions that lasted 10 minutes or longer in duration.
- only count exercise that was done during free time (i.e., not occupation or housework).
- note that the main difference between the first three categories is the intensity of the endurance (aerobic) exercise.
- please write the average frequency on the first line and the average duration on the second.
- if you did not do any exercise in one of the categories, please write in "0".

Considering a typical week (7 days) how many times on the average did you do the following kinds of exercise in the last month?

	Times Per Week	Average Duration (min.)
a. VIGOROUS/STRENUOUS EXERCISE (HEART BEATS RAPIDLY, SWEATING) (e.g., running, aerobics classes, cross country skiing, vigorous swimming, vigorous bicycling).	_____	_____
b. MODERATE EXERCISE (NOT EXHAUSTING, LIGHT PERSPIRATION) (e.g., fast walking, tennis, easy bicycling, pilates, easy swimming, popular and folk dancing).	_____	_____
c. LIGHT/MILD EXERCISE (MINIMAL EFFORT, NO PERSPIRATION) (e.g., easy walking, yoga, golfing with a cart, and bowling).	_____	_____
d. STRENGTH TRAINING (eg. Dumbbells or Nautilus machines)	_____	_____

In addition to the above, please complete the below questions from the Dietary Fruit-Vegetable-Fiber Screener ©

Think about your eating habits over the past month or so. About how often do you eat each of the following foods? Remember breakfast, lunch, dinner, snacks and eating out. Mark one box for each food.

Fiber	Less than 1/Week	Once a Week	2-3 times a Week	4-6 times a Week	Once a Day	2+ a Day
Fiber cereals like Raisin Bran, Shredded Wheat or Fruit-n-Fiber	☐	☐	☐	☐	☐	☐
Beans such as baked beans, pinto, kidney, or lentils (not green beans)	☐	☐	☐	☐	☐	☐
Dark bread such as whole wheat or rye	☐	☐	☐	☐	☐	☐

BLOCK DIETARY DATA SYSTEMS
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(510) 704-8514

APPENDIX E EORTC QLQ-C30

We are interested in some things about you and your health. Please answer all of the questions yourself by circling the number that best applies to you. There are no "right" or "wrong" answers. The information that you provide will remain strictly confidential.

Please fill in your initials: ☐☐☐☐
Your birthdate (Day, Month, Year): ☐☐☐☐☐☐☐☐
Today's date (Day, Month, Year): ☐☐☐☐☐☐☐☐

	Not at All	A Little	Quite a Bit	Very Much
1. Do you have any trouble doing strenuous activities, like carrying a heavy shopping bag or a suitcase?	1	2	3	4
2. Do you have any trouble taking a long walk?	1	2	3	4
3. Do you have any trouble taking a short walk outside of the house?	1	2	3	4
4. Do you need to stay in bed or a chair during the day?	1	2	3	4
5. Do you need help with eating, dressing, washing yourself or using the toilet?	1	2	3	4
During the past week:				
6. Were you limited in doing either your work or other daily activities?	1	2	3	4
7. Were you limited in pursuing your hobbies or other leisure time activities?	1	2	3	4
8. Were you short of breath?	1	2	3	4
9. Have you had pain?	1	2	3	4
10. Did you need to rest?	1	2	3	4
11. Have you had trouble sleeping?	1	2	3	4
12. Have you felt weak?	1	2	3	4
13. Have you lacked appetite?	1	2	3	4
14. Have you felt nauseated?	1	2	3	4

15. Have you vomited?	1	2	3	4
16. Have you been constipated?	1	2	3	4
17. Have you had diarrhea?	1	2	3	4
18. Were you tired?	1	2	3	4
19. Did pain interfere with your daily activities?	1	2	3	4
20. Have you had difficulty in concentrating on things, like reading a newspaper or watching television?	1	2	3	4
21. Did you feel tense?	1	2	3	4
22. Did you worry?	1	2	3	4
23. Did you feel irritable?	1	2	3	4
24. Did you feel depressed?	1	2	3	4
25. Have you had difficulty remembering things?	1	2	3	4
26. Has your physical condition or medical treatment interfered with your <u>family</u> life?	1	2	3	4
27. Has your physical condition or medical treatment interfered with your <u>social</u> activities?	1	2	3	4
28. Has your physical condition or medical treatment caused you financial difficulties?	1	2	3	4

For the following questions please circle the number between 1 and 7 that best applies to you

29. How would you rate your overall <u>health</u> during the past week?

1	2	3	4	5	6	7
Very poor						Excellent

30. How would you rate your overall <u>quality of life</u> during the past week?

1	2	3	4	5	6	7

APPENDIX F EUROQOL EQ-5D-5L

Under each heading, please tick the ONE box that best describes your health TODAY.

MOBILITY

I have no problems in walking about ☐
I have slight problems in walking about ☐
I have moderate problems in walking about ☐
I have severe problems in walking about ☐
I am unable to walk about ☐

SELF-CARE

I have no problems washing or dressing myself ☐
I have slight problems washing or dressing myself ☐
I am unable to wash or dress myself ☐

USUAL ACTIVITIES *(e.g., work, study, housework, family or leisure activities)*

I have no problems doing my usual activities ☐
I have slight problems doing my usual activities ☐
I have moderate problems doing my usual activities ☐
I have severe problems doing my usual activities ☐
I am unable to do my usual activities ☐

PAIN / DISCOMFORT

I have no pain or discomfort ☐
I have slight pain or discomfort ☐
I have moderate pain or discomfort ☐
I have severe pain or discomfort ☐
I have extreme pain or discomfort ☐

ANXIETY / DEPRESSION

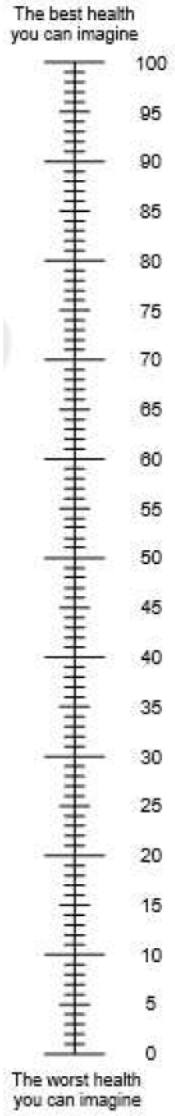
I am not anxious or depressed ☐
I am slightly anxious or depressed ☐
I am moderately anxious or depressed ☐

I am severely anxious or depressed ☐

I am extremely anxious or depressed ☐

- We would like to know how good or bad your health is TODAY.
- This scale is numbered from 0 to 100.
- 100 means the best health you can imagine. 0 means the worst health you can imagine.
- Mark an X on the scale to indicate how your health is TODAY.
- Now, please write the number you marked on the scale in the box below.

YOUR HEALTH TODAY = ☐



APPENDIX G NCI PRO-CTCAE™ QUESTIONS FOR ALL PATIENTS

As individuals go through treatment for their cancer they sometimes experience different symptoms and side effects. For each question, please check or mark an ☒ in the one box that best describes your experiences over the past 7 days…

1.	In the last 7 days, what was the SEVERITY of your DECREASED APPETITE at its WORST?				
	○ None	○ Mild	○ Moderate	○ Severe	○ Very severe
	In the last 7 days, how much did DECREASED APPETITE INTERFERE with your usual or daily activities?				
	○ Not at all	○ A little bit	○ Somewhat	○ Quite a bit	○ Very much

2.	In the last 7 days, how OFTEN did you have NAUSEA?				
	○ Never	○ Rarely	○ Occasionally	○ Frequently	○ Almost constantly
	In the last 7 days, what was the SEVERITY of your NAUSEA at its WORST?				
	○ None	○ Mild	○ Moderate	○ Severe	○ Very severe

3.	In the last 7 days, how OFTEN did you have VOMITING?				
	○ Never	○ Rarely	○ Occasionally	○ Frequently	○ Almost constantly
	In the last 7 days, what was the SEVERITY of your VOMITING at its WORST?				
	○ None	○ Mild	○ Moderate	○ Severe	○ Very severe

4.	In the last 7 days, what was the SEVERITY of your CONSTIPATION at its WORST?				
	○ None	○ Mild	○ Moderate	○ Severe	○ Very severe

5.	In the last 7 days, how OFTEN did you have LOOSE OR WATERY STOOLS (DIARRHEA/DIARRHOEA)?				
	○ Never	○ Rarely	○ Occasionally	○ Frequently	○ Almost constantly

6.	In the last 7 days, how OFTEN did you have PAIN IN THE ABDOMEN (BELLY AREA)?				
	○ Never	○ Rarely	○ Occasionally	○ Frequently	○ Almost constantly
	In the last 7 days, what was the SEVERITY of your PAIN IN THE ABDOMEN (BELLY AREA) at its WORST?				
	○ None	○ Mild	○ Moderate	○ Severe	○ Very severe
	In the last 7 days, how much did PAIN IN THE ABDOMEN (BELLY AREA) INTERFERE with your usual or daily activities?				
	○ Not at all	○ A little bit	○ Somewhat	○ Quite a bit	○ Very much

7.	In the last 7 days, what was the SEVERITY of your SHORTNESS OF BREATH at its WORST?				
	○ None	○ Mild	○ Moderate	○ Severe	○ Very severe
	In the last 7 days, how much did your SHORTNESS OF BREATH INTERFERE with your usual or daily activities?				
	○ Not at all	○ A little bit	○ Somewhat	○ Quite a bit	○ Very much

8.	In the last 7 days, what was the SEVERITY of your COUGH at its WORST?				
	○ None	○ Mild	○ Moderate	○ Severe	○ Very severe
	In the last 7 days, how much did COUGH INTERFERE with your usual or daily activities?				
	○ Not at all	○ A little bit	○ Somewhat	○ Quite a bit	○ Very much

9.	In the last 7 days, what was the SEVERITY of your WHEEZING (WHISTLING NOISE IN THE CHEST WITH BREATHING) at its WORST?				
	○ None	○ Mild	○ Moderate	○ Severe	○ Very severe

10.	In the last 7 days, did you have any RASH?	
	○ Yes	○ No

11.	In the last 7 days, did you have any HAIR LOSS?				
	○ Not at all	○ A little bit	○ Somewhat	○ Quite a bit	○ Very much

12.	In the last 7 days, what was the SEVERITY of your ITCHY SKIN at its WORST?				
	○ None	○ Mild	○ Moderate	○ Severe	○ Very severe

13.	In the last 7 days, did you have any HIVES (ITCHY RED BUMPS ON THE SKIN)?	
	○ Yes	○ No

14.	In the last 7 days, how OFTEN did you have ACHING MUSCLES?				
	○ Never	○ Rarely	○ Occasionally	○ Frequently	○ Almost constantly
	In the last 7 days, what was the SEVERITY of your ACHING MUSCLES at their WORST?				
	○ None	○ Mild	○ Moderate	○ Severe	○ Very severe
	In the last 7 days, how much did ACHING MUSCLES INTERFERE with your usual or daily activities?				
	○ Not at all	○ A little bit	○ Somewhat	○ Quite a bit	○ Very much

15.	In the last 7 days, how OFTEN did you have ACHING JOINTS (SUCH AS ELBOWS, KNEES, SHOULDERS)?				
	○ Never	○ Rarely	○ Occasionally	○ Frequently	○ Almost constantly
	In the last 7 days, what was the SEVERITY of your ACHING JOINTS (SUCH AS ELBOWS, KNEES, SHOULDERS) at their WORST?				
	○ None	○ Mild	○ Moderate	○ Severe	○ Very severe
	In the last 7 days, how much did ACHING JOINTS (SUCH AS ELBOWS, KNEES, SHOULDERS) INTERFERE with your usual or daily activities?				
	○ Not at all	○ A little bit	○ Somewhat	○ Quite a bit	○ Very much

16.	In the last 7 days, what was the SEVERITY of your FATIGUE, TIREDNESS, OR LACK OF ENERGY at its WORST?				
	○ None	○ Mild	○ Moderate	○ Severe	○ Very severe
	In the last 7 days, how much did FATIGUE, TIREDNESS, OR LACK OF ENERGY INTERFERE with your usual or daily activities?				
	○ Not at all	○ A little bit	○ Somewhat	○ Quite a bit	○ Very much

Do you have any other symptoms that you wish to report?	
○ Yes	○ No

Please list any other symptoms:

1.	In the last 7 days, what was the SEVERITY of this symptom at its WORST?				
	○ None	○ Mild	○ Moderate	○ Severe	○ Very severe
2.	In the last 7 days, what was the SEVERITY of this symptom at its WORST?				
	○ None	○ Mild	○ Moderate	○ Severe	○ Very severe
3.	In the last 7 days, what was the SEVERITY of this symptom at its WORST?				
	○ None	○ Mild	○ Moderate	○ Severe	○ Very severe

APPENDIX H DF/HCC MULTICENTER DATA & SAFETY MONITORING PLAN

DFCI IRB Protocol #: 20-153

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1. INTRODUCTION

The Dana-Farber/Harvard Cancer Center Multi-Center Data and Safety Monitoring Plan (DF/HCC DSMP) outlines the procedures for conducting a DF/HCC Multi-Center research protocol. The DF/HCC DSMP serves as a reference for any sites external to DF/HCC that are participating in a DF/HCC clinical trial.

1.1 Purpose

To establish standards that will ensure that a Dana-Farber/Harvard Cancer Center Multi-Center protocol will comply with Federal Regulations, Health Insurance Portability and Accountability Act (HIPAA) requirements and applicable DF/HCC Standard Operating Procedures.

2 GENERAL ROLES AND RESPONSIBILITIES

For DF/HCC Multi-Center Protocols, the following general responsibilities apply, in addition to those outlined in DF/HCC Policies for Sponsor-Investigators:

2.1 Coordinating Center

The Coordinating Center is the entity that provides administrative support to the DF/HCC Sponsor in order that he/she may fulfill the responsibilities outlined in the protocol document and DSMP, and as specified in applicable regulatory guidelines (i.e., CTEP Multi-Center Guidelines).

The general responsibilities of the Coordinating Center may include but are not limited to:

- Assist in protocol development.
- Maintain FDA correspondence as applicable.
- Review registration materials for eligibility and register participants from Participating Institutions in the DF/HCC clinical trial management system (CTMS).
- Distribute protocol and informed consent document updates to External Sites as needed.
- Oversee the data collection process from External Sites.
- Maintain documentation of Serious Adverse Event (SAE) reports and deviations/violation submitted by External Sites and provide to the DF/HCC Sponsor for timely review and submission to the DFCI IRB, as necessary.
- Distribute serious adverse events reported to the DF/HCC Sponsor that fall under the DFCI IRB Adverse Event Reporting Policy to all External Sites.
- Provide External Sites with information regarding DF/HCC requirements that they will be expected to comply with.
- Carry out plan to monitor External Sites either by on-site or remote monitoring.
- Maintain Regulatory documents of all External Sites which includes but is not limited to the following: local IRB approvals/notifications from all Participating Institutions, confirmation of Federalwide Assurances (FWAs) for all sites, all SAE submissions, Screening Logs for all sites, IRB approved consents for all sites
- Conduct regular communications with all External Sites (conference calls, emails, etc.) and maintain documentation all relevant communications.

2.2 External Site

An External Site is an institution that is outside the DF/HCC and DF/PCC consortium that is collaborating with DF/HCC on a protocol where the sponsor is a DF/HCC investigator. The External Site acknowledges the DF/HCC Sponsor as having the ultimate authority and responsibility for the overall conduct of the study.

Each External Site is expected to comply with all applicable DF/HCC requirements stated within this Data and Safety Monitoring Plan and/or the protocol document.

The general responsibilities for each External Site may include but are not limited to:

- Document the delegation of research specific activities to study personnel.
- Commit to the accrual of participants to the protocol.
- Submit protocol and/or amendments to their local IRB.
- Maintain regulatory files as per sponsor requirements.
- Provide the Coordinating Center with regulatory documents or source documents as requested.
- Participate in protocol training prior to enrolling participants and throughout the trial as required (i.e. teleconferences).
- Update Coordinating Center with research staff changes on a timely basis.
- Register participants through the Coordinating Center prior to beginning research related activities.
- Submit Serious Adverse Event (SAE) reports to local IRB per institutional requirements and to the Coordinating Center, in accordance with DF/HCC requirements.
- Submit protocol deviations and violations to local IRB per institutional requirements and to the DF/HCC Sponsor in accordance with DF/HCC requirements.
- Order, store and dispense investigational agents and/or other protocol mandated drugs per federal guidelines and protocol requirements.
- Have office space, office equipment, and internet access that meet HIPAA standards.
- Participate in any quality assurance activities and meet with monitors or auditors at the conclusion of a visit to review findings.
- Promptly provide follow-up and/or corrective action plans for any monitoring queries or audit findings.
- Notify the sponsor immediately of any regulatory authority inspection of this protocol at the External Site.

3 DF/HCC REQUIREMENTS FOR MULTI-CENTER PROTOCOLS

Certain DF/HCC Policy requirements apply to External Sites participating in DF/HCC research. The following section will clarify DF/HCC requirements and further detail the expectations for participating in a DF/HCC Multi-Center protocol.

3.1 Protocol Revisions and Closures

The External Sites will receive notification of protocol revisions and closures from the Coordinating Center. When under a separate IRB, it is the individual External Site's responsibility to notify its IRB of these revisions.

- **Protocol revisions:** External Sites will receive written notification of protocol revisions from the Coordinating Center. All protocol revisions should be IRB approved and implemented within a timely manner from receipt of the notification.

- **Protocol closures and temporary holds:** External Sites will receive notification of protocol closures and temporary holds from the Coordinating Center. Closures and holds will be effective immediately. In addition, the Coordinating Center, will update the External Sites on an ongoing basis about protocol accrual data so that they will be aware of imminent protocol closures.

3.2 Informed Consent Requirements

The DF/HCC approved informed consent document will serve as a template for the informed consent for External Sites. The External Site consent form must follow the consent template as closely as possible and should adhere to specifications outlined in the DF/HCC Guidance Document on Model Consent Language for Investigator-Sponsored Multi-Center Trials. This document will be provided separately to each External Site upon request.

External Sites must send their version of the informed consent document to the Coordinating Center for sponsor review and approval. If the HIPAA authorization is a separate document, please submit to the sponsor for the study record. Once sponsor approval is obtained, the External site may submit to their IRB of record, as applicable. In these cases, the approved consent form must also be submitted to the Coordinating Center after approval by the local IRB for all consent versions.

The Principal Investigator (PI) at each External Site will identify the appropriate members of the study team who will be obtaining consent and signing the consent form for protocols. External Sites must follow the DF/HCC requirement that for all interventional drug, biologic, or device research, only attending physicians may obtain initial informed consent and any re-consent that requires a full revised consent form.

3.3 IRB Re-Approval

Verification of IRB re-approval from the External Sites is required in order to continue research activities. There is no grace period for continuing approvals.

The Coordinating Center will not register participants if a re-approval letter is not received from the External Site on or before the anniversary of the previous approval date.

3.4 DF/HCC Multi-Center Protocol Confidentiality

All documents, investigative reports, or information relating to the participant are strictly confidential. Whenever reasonably feasible, any participant specific reports (i.e., Pathology Reports, MRI Reports, Operative Reports, etc.) submitted to the Coordinating Center should be de-identified. It is recommended that the assigned protocol case number (as described below) be used for all participant specific documents. Participant initials may be included or retained for cross verification of identification.

3.5 Participant Registration and Randomization

Please refer to Protocol Section 4.3 and 4.4 for participant registration information. Treatment cannot begin until site has received confirmation that participant has been registered with DF/HCC CTMS.

3.5.1 Initiation of Therapy

Participants must be registered with the DF/HCC CTMS before the initiation of treatment or other protocol-specific interventions. Treatment and other protocol-specific interventions may not be initiated until the External Site receives confirmation of the participant's registration from the Coordinating Center. The DF/HCC Sponsor and DFCI IRB must be notified of any violations to this policy.

3.5.2 Eligibility Exceptions

No exceptions to the eligibility requirements for a protocol without DFCI IRB approval will be permitted. All External Sites are required to fully comply with this requirement. The process for requesting an eligibility exception is defined below.

3.6 Data Management

DF/HCC develops case report forms (CRF/eCRFs), for use with the protocol. These forms are designed to collect data for each study. DF/HCC provides a web-based training for all eCRF users.

3.6.1 Data Forms Review

Data submissions are monitored for timeliness and completeness of submission. If study forms are received with missing or questionable data, the submitting institution will receive a written or electronic query from the DF/HCC Office of Data Quality, Coordinating Center, or designee.

Responses to all queries should be completed and submitted within 14 calendar days.

If study forms are not submitted on schedule, the External Sites will periodically receive a Missing Form Report from the Coordinating Center noting the missing forms.

3.7 Protocol Reporting Requirements

3.7.1 Protocol Deviations, Exceptions and Violations

Federal Regulations require an IRB to review proposed changes in a research activity to ensure that researchers do not initiate changes in approved research without IRB review and approval, except when necessary to eliminate apparent immediate hazards to the participant. DF/HCC requires all departures from the defined procedures set forth in the IRB approved protocol to be reported to the DF/HCC Sponsor and to the IRB of record.

3.7.2 Reporting Procedures

Requests to deviate from the protocol require approval from the IRB of record and the sponsor.

All protocol violations must be sent to the Coordinating Center in a timely manner. The Coordinating Center will provide training for the requirements for the reporting of violations.

3.7.3 Guidelines for Processing IND Safety Reports

The DF/HCC Sponsor will review all IND Safety Reports per DF/HCC requirements and ensure that all IND Safety Reports are distributed to the External Sites as required by DF/HCC Policy. External Sites will review/submit to the IRB according to their institutional policies and procedures.

4 MONITORING: QUALITY CONTROL

The Coordinating Center, with the aid of the DF/HCC Office of Data Quality, provides quality control oversight for the protocol.

4.1 Ongoing Monitoring of Protocol Compliance

The External Sites may be required to submit participant source documents to the Coordinating Center for monitoring. External Sites may also be subject to on-site monitoring conducted by the Coordinating Center.

The Coordinating Center will implement ongoing monitoring activities to ensure that External Sites are complying with regulatory and protocol requirements, data quality, and participant safety. Monitoring practices may include but are not limited to source data verification, and review and analysis of eligibility requirements, informed consent procedures, adverse events and all associated documentation, review of study drug administration/treatment, regulatory files, protocol departures reporting, pharmacy records, response assessments, and data management.

Additionally, a plan will be formulated to provide regular and ongoing communication to

External Sites about study related information which will include participation in regular teleconferences initiated by the Coordinating Center. Teleconferences will occur monthly and will continue regularly until completion of protocol therapy.
Upon completion of protocol therapy, teleconferences will occur every 3 months until study completion. Additional communication may be distributed via "Newsletter" or email as deemed appropriate by DF/HCC Sponsor.

On-Site Monitoring: On-site monitoring will occur on an as-needed basis. External Sites will be required to provide access to participants' complete medical record and source documents for source documentation verification during the visit. In addition, External Sites should provide access to regulatory documents, pharmacy records, local policies related to the conduct of research, and any other trial-related documentation maintained by the External Site. On-site monitoring visits can be substituted with remote (virtual) monitoring visits at the discretion of the Principal Investigator.

Remote Monitoring: Remote monitoring will be performed on an as-needed basis by the Clinical Trial Monitor. Sites will be asked to provide source documentation via email or mail as specified by the Clinical Trial Monitor for virtual monitoring.

4.2 Monitoring Reports

The DF/HCC Sponsor will review all monitoring reports to ensure protocol compliance. The DF/HCC Sponsor may increase the monitoring activities at External Sites that are unable to comply with the protocol, DF/HCC Sponsor requirements or federal and local regulations.

4.3 Accrual Monitoring

Prior to extending a protocol to an external site, the DF/HCC Sponsor will establish accrual requirements for each External Site. Accrual will be monitored for each participating institution by the DF/HCC Sponsor or designee. Sites that are not meeting their accrual expectations may be subject to termination. Sites are expected to accrue at least 3 patients per year.

5 AUDITING: QUALITY ASSURANCE

5.1 DF/HCC Internal Audits

All External Sites are subject to audit by the DF/HCC Office of Data Quality (ODQ). Typically, approximately 3-4 participants would be audited at the site over a 2-day period. If violations which impact participant safety or the integrity of the study are found, more participant records may be audited.

5.2 Audit Notifications

It is the External Site's responsibility to notify the Coordinating Center of all external audits or inspections (e.g., FDA, EMA, NCI) that involve this protocol. All institutions will forward a

copy of final audit and/or re-audit reports and corrective action plans (if applicable) to the Coordinating Center, within 12 weeks after the audit date.

5.3 Audit Reports

The DF/HCC Sponsor will review all final audit reports and corrective action plans, if applicable. The Coordinating Center must forward any reports to the DF/HCC ODQ per DF/HCC policy for review by the DF/HCC Audit Committee. For unacceptable audits, the DF/HCC Audit Committee would forward the final audit report and corrective action plan to the DFCI IRB as applicable.

5.4 External Site Performance

The DF/HCC Sponsor and the DFCI IRB are charged with considering the totality of an institution's performance in considering institutional participation in the protocol.

External Sites that fail to meet the performance goals of accrual, submission of timely and accurate data, adherence to protocol requirements, and compliance with state and federal regulations, may be put on hold or closed.