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TITLE: A Single Arm Phase II Study of the Combination of DKN-01 and Nivolumab in Previously Treated Patients with Advanced Biliary Tract Cancer (BTC)

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Nivolumab, NSC # (if applicable), Bristol Myers Squibb

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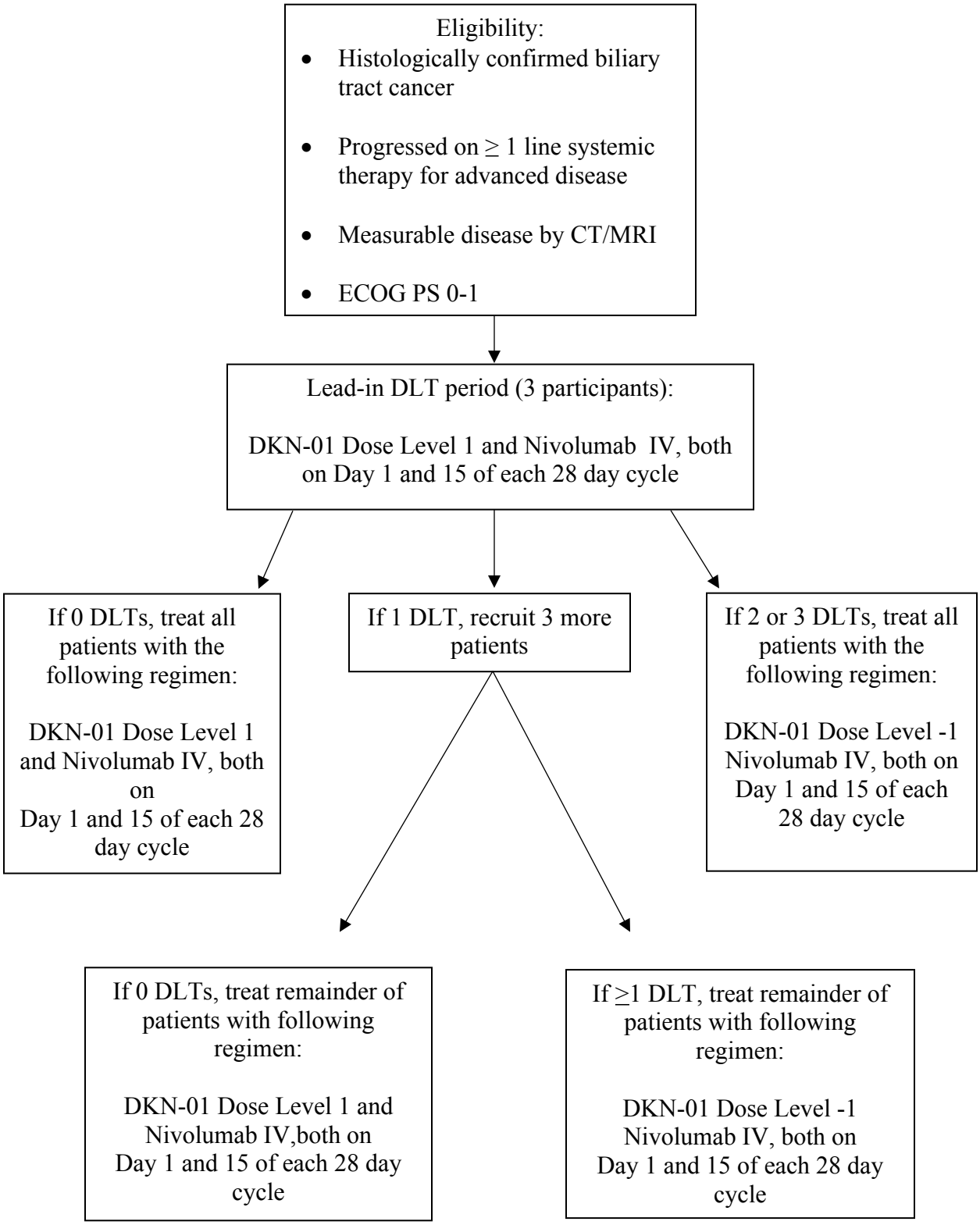
Amendments:
Amendment 1 – September 4, 2019
Amendment 2 – January 8, 2020
Amendment 3 – May 01, 2020
Amendment 4 – May 09, 2023

SCHEMA


Eligibility:
• Histologically confirmed biliary tract cancer
• Progressed on ≥ 1 line systemic therapy for advanced disease
• Measurable disease by CT/MRI
• ECOG PS 0-1
Lead-in DLT period (3 participants):
DKN-01 Dose Level 1 and Nivolumab IV, both on Day 1 and 15 of each 28 day cycle
If 0 DLTs, treat all patients with the following regimen:
DKN-01 Dose Level 1 and Nivolumab IV, both on Day 1 and 15 of each 28 day cycle
If 1 DLT, recruit 3 more patients
If 2 or 3 DLTs, treat all patients with the following regimen:
DKN-01 Dose Level -1 Nivolumab IV, both on Day 1 and 15 of each 28 day cycle
If 0 DLTs, treat remainder of patients with following regimen:
DKN-01 Dose Level 1 and Nivolumab IV,both on Day 1 and 15 of each 28 day cycle
If ≥1 DLT, treat remainder of patients with following regimen:
DKN-01 Dose Level -1 Nivolumab IV, both on Day 1 and 15 of each 28 day cycle

Contents

1. OBJECTIVES

1.1 Study Design

This study is a multicenter, open-label phase II trial evaluating DKN 01 and Nivolumab in previously treated patients with advanced biliary tract cancer (BTC). Patients will be enrolled in a single arm trial and treated with both DKN-01 and Nivolumab IV every 2 weeks, with a safety lead-in period. The first three patients on trial will be treated with DKN-01 at 600mg IV and Nivolumab 240mg IV every 2 weeks. If no DLTs are experienced, the remainder of patients on trial will be treated with DKN-01 at a dose of 600mg IV. If one patient meets criteria for a dose limiting toxicity (DLT), three additional patients will be enrolled on study. If no additional patients have a DLT, DKN-01 600mg IV will be used for remainder of patients on trial. If 2 of the first 3 patients or 2 of the total 6 patients have a DLT, the dose will be reduced to DKN-01 300mg IV for the remainder of the trial. Participants that are registered for the trial but do not begin treatment will be replaced. Tumor response will be assessed radiographically every 8 weeks by RECIST v1.1 criterion. Participants will receive treatment until disease progression, unacceptable toxicity, or death. Treatment beyond RECIST-defined progression will be permitted if the participant is deriving ongoing clinical benefit. No enrichment strategy will be used. However, we will do a pre-planned post-hoc subgroup analysis by tumor DKK1 and PD-L1 expression status on archival or fresh biopsy tissue. A Simon 2 stage design will be used, and if ≥ 1 of 13 patients respond in the first stage, the study will proceed to stage 2.

1.2 Primary Objectives

1.2.1 Evaluate the overall response rate (ORR) of the combination of DKN-01 and nivolumab in patients with advanced BTC after progression on ≥1 prior systemic therapy

1.3 Secondary Objectives

1.3.1 Evaluate the safety and tolerability of the combination of DKN-01 and nivolumab in patients with advanced BTC

1.3.2 Evaluate the median progression free survival (PFS) and median overall survival (OS) of the combination of DKN-01 and nivolumab in patients with advanced BTC

1.3.3 Evaluate the ORR, PFS and OS of subgroups stratified by tumor DKK1 and PD-L1 expression in a pre-planned post-hoc analysis

1.3.4 Explore molecular correlates of response, including circulating biomarkers, tumor tissue biomarkers, and stool-based gut microbiome biomarkers.

2. BACKGROUND

2.1 Biliary Tract Cancer

Biliary tract cancer (BTC) is a rare and fatal malignancy that can involve the intrahepatic bile ducts, extrahepatic bile ducts, or gallbladder. Tumors of the bile ducts are more specifically called cholangiocarcinoma (CCA), and the incidence of intrahepatic cholangiocarcinoma (ICC) is rising in the United States and globally[1, 2]. While surgery can be curative if the tumor is detected early, recurrences and detection in the advanced stages are common. The majority of patients present with unresectable disease, and the 5 year survival is 10-12%. BTCs are biologically, histologically, and anatomically heterogenous, and the one-size-fits all approach of standard chemotherapy has shown limited efficacy. The current standard systemic therapy for advanced biliary tract cancers (ABTCs) is the combination of Gemcitabine and Cisplatin, which showed an 11.7 month median overall survival compared to 8.1 months with Gemcitabine alone[3]. The field is wide open for new advancements, and targeted therapies offer a promising approach.

Currently, there is no approved second line treatment (CT2) for advanced BTC. Regimens that have been studied in the first or second line setting that are currently being used in the second line include infusional 5-FU-based, gemcitabine-based, or oxaliplatin-based combination regimens. Monotherapy with infusional 5-FU, capecitabine, or gemcitabine is also sometimes prescribed. None of these regimens have shown superiority as second line therapy in a randomized study, and none of them are a clear CT2 choice based on first line data.

2.2 DKN-01 and Nivolumab

A summary of the properties of DKN-01 and nivolumab and the clinical and nonclinical experience with the drug are contained in the Investigator's Brochure (IB) supplied by Leap Therapeutics and Bristol Myers Squibb, respectively. The IBs should be reviewed in conjunction with this study protocol.

2.2.1 **DKN-01**

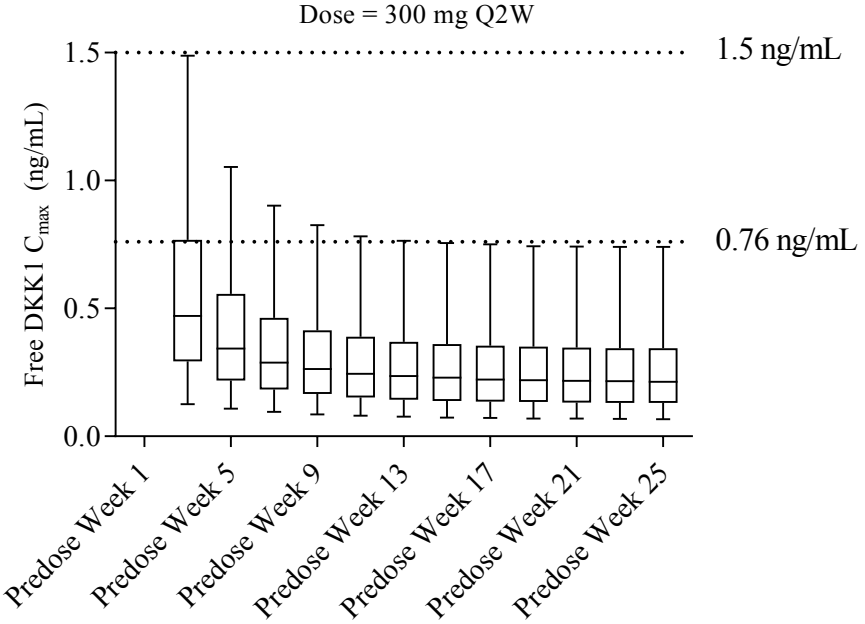
DKN-01 binds to and reduces the concentration of free DKK1 in serum. A xenograft study was conducted in rats to study the relationship between diminished levels of free DKK1 and antitumor activity. The study was carried out in athymic nude female rats (strain Hsd:RHFoxn1rnu) bearing implanted DKK1+ human A549 NSCLC cells. Animals were treated with either vehicle (buffered sterile diluent) or DKN-01 (0.05, 0.2, 1.0, or 5.0 mg/kg) administered IV once weekly for 4 weeks. DKN-01 and DKK1 serum concentrations were evaluated after the first and last weekly doses and at troughs between doses. The relationship between drug levels, DKK1 levels, and TGI was evaluated using a PK/pharmacodynamic model. At the end of the study, reduction in tumor growth was observed in all active treatment arms and reached statistical significance in the lowest and highest DKN-01 dose-treated groups. The greatest mean TGI observed was 44%. Moreover, two rats exhibited complete disappearance of

the tumor by the end of the study, one at 5 mg/kg DKN-01 and the other at the 0.05 mg/kg DKN-01 dose level.

A PK/pharmacodynamic model with target-mediated drug disposition adequately described the relationship between total DKN-01 concentration, free DKK1 concentration, and TGI in xenograft-bearing nude rats. Model-predicted free DKK1 concentrations were related quantitatively to TGI, with 50% maximal TGI achieved at approximately 50% reduction in free DKK1 serum levels.

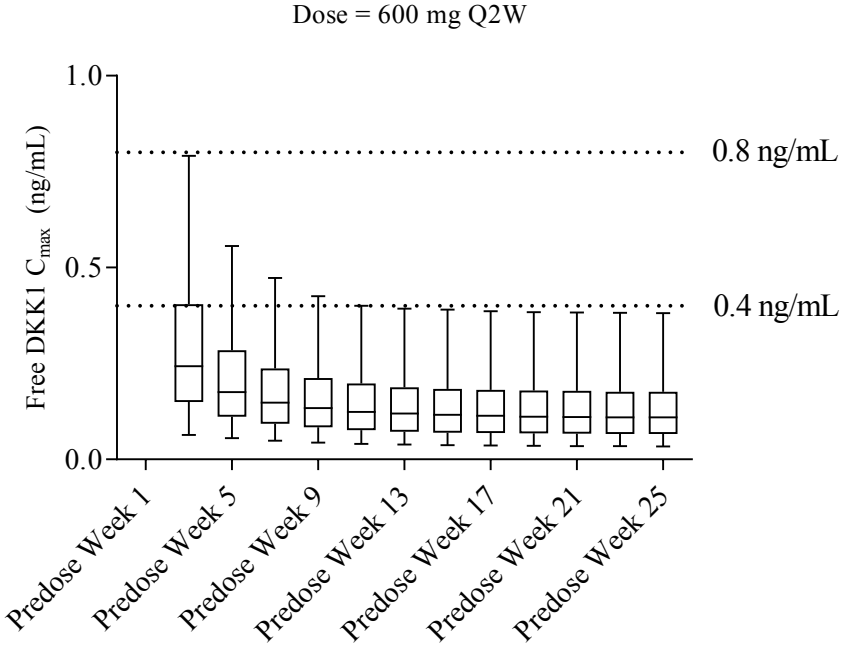
A similar PK/pharmacodynamic model was used to simulate human clinical DKN-01 concentrations and the resulting free DKK1 serum concentrations after IV drug administration once every 2 weeks (Q2W). These simulations focused on the maximal concentration for free DKK1 (DKK1 C_{max}) over the Q2W dosing interval. The initial baseline value of free DKK1 was approximately 5.4 ng/mL (data not shown). The graphs below demonstrate that DKK1 C_{max} is substantially reduced (relative to baseline) by the administration DKN-01. These plots illustrate that in the majority of patients (> 95%), DKK1 C_{max} concentrations could be suppressed to 1.5 ng/mL or less after one dose of 300 mg DKN-01 (Figure 1). In addition, after repeat 300 mg Q2W dosing, the highest free DKK1 C_{max} levels declined to approximately 0.76 ng/mL or less for 95% of patients (Figure 1). Simulations showed that when the DKN-01 dose was increased to 600 mg, the highest free DKK1 C_{max} was decreased to approximately 0.8 ng/mL after the first dose, and then to 0.4 ng/mL after repeat Q2W dosing to steady-state (**Error! Reference source not found.**).

Figure 1: Predicted Maximal Free Serum DKK1 (C_{max}) Concentrations after 300 mg DKN-01 IV Dosing Administered Once Every Two Weeks (Q2W)



*Plot whiskers represent an interval containing 80% of patients.

Figure 2: Predicted Maximal Free Serum DKK1 (C_{max}) Concentrations after 600 mg DKN-01 IV Dosing Administered Once Every Two Weeks (Q2W)*



*Plot whiskers represent an interval containing 90% of patients.

Several ongoing clinical studies have been conducted to determine the effect of DKN-01 on a variety of cancers. In Study DEK-DKK1-P102 (P102; NCT02013154), DKN-01 was dosed in combination with paclitaxel as well as in a separate monotherapy substudy. In this study, patients were administered DKN-01 at doses of 150 or 300 mg Q2W over a 28-day cycle. Patients in Study DEK-DKK1-P103 (P103; NCT02375880) were administered DKN-01 at doses of 150 or 300 mg in combination with gemcitabine and cisplatin on Days 1 and 8 of each 21-day cycle.

In both of these studies, PK/Pharmacodynamic modeling showed a dose-dependent decrease in free DKK1 concentrations. In P102 and P103 the median free DKK1 C_{max} was 0.39 ng/mL and 0.50 ng/mL (respectively), and the highest individual free DKK1 C_{max} was 0.86 and 0.77 ng/mL, respectively. In non-responders, the median C_{max} was 0.65 and 0.61 ng/mL (respectively) and the highest individual free C_{max} observed was 5.7 and 10.8 ng/mL, respectively. Thus, for patients showing a partial response, maximal free DKK1 C_{max} levels did not exceed 0.86 ng/mL and the median free C_{max} values were lower than those for the non-responders. Currently, it is not understood if the 0.86 ng/mL threshold is a meaningful target for efficacy, or if median free DKK1 C_{max} values (equal to or lower than 0.39-0.50 ng/mL) are important. However, it can be stated that there is an overall trend for lower free DKK1 levels in partial responders versus non-

responders. By extension, higher doses of DKN-01 (i.e., 600 mg) should lower free DKK1 levels sufficiently to see additional efficacy in patients.

Safety data for DKN-01 are available from 30 healthy subjects treated with a single dose of DKN-01 monotherapy and 229 patients with cancer treated with DKN-01, of whom 65 received DKN-01 monotherapy; 7 received DKN-01 in combination with len/dex; 69 received DKN-01 in combination with paclitaxel; 51 patients received DKN-01 in combination with gem/cis; and 37 received DKN-01 in combination with pembrolizumab.

Study DEK-DKK1-P100 was a 2-part (Parts A and B), Phase 1, multicenter, nonrandomized, open-label, multiple-dose, dose-escalation study of DKN-01 administered IV to patients 30 years of age or older. Study Part A (dose escalation) consisted of a standard 3 + 3 dose escalation designed to determine the MTD and DLTs of DKN-01 in patients with MM or advanced solid tumors. Study Part B (dose confirmation) was designed to administer DKN-01 at the MTD (or highest dose tested if the MTD is not reached) to further characterize safety and tolerability and to evaluate progression-free survival in patients with relapsed/refractory NSCLC.

A total of 32 patients were enrolled in the study and received study treatment. Thirteen patients were enrolled and treated in Part A and 19 patients in Part B. In Part A, DKN-01 doses of 75, 150, and 300 mg were administered IV QW and 600 mg was administered IV Q2W to different cohorts of patients. There were no DLTs observed in Part A; therefore, study Part B proceeded with enrolling a total of 19 patients who received 300 mg DKN-01 Q2W.

DKN-01 given IV at escalating doses from 75 mg QW to 600 mg Q2W in Part A and as a single IV agent at a dose of 300 mg Q2W in Part B was safe and well-tolerated.

Twenty-nine (90.6%) of 32 patients experienced at least one TEAE during the study. The incidence of TEAEs was similar across dose groups (100% in all dose groups in Part A and 84.2% in the 300 mg Q2W group in Part B). The most common types of TEAEs were gastrointestinal disorders (53.1%) and general disorders and administration site conditions (46.9%), primarily fatigue/asthenia. The most common TEAEs (those experienced by ≥10% of total patients) were nausea (31.3%), fatigue (28.1%), decreased appetite (25.0%), dyspnea and vomiting (21.9% each), constipation (18.8%), and asthenia, dysphagia, hypokalemia, pneumonia and productive cough (12.5% each).

Overall, 46.9% of patients experienced at least 1 treatment-related TEAE, with the most frequently reported treatment-related TEAEs being fatigue (25.0%), nausea (9.4%), and asthenia and dysgeusia (each 6.3%).

All treatment-related TEAEs were Grade 1 or 2 in severity. There were no related SAEs and no discontinuations due to a TEAE.

Study DEK-DKK1-P102 is an ongoing Phase 1 non-randomized, dose-escalating, open label, multi-center study conducted in multiple parts (Parts A through F). In addition, a separate

Monotherapy Substudy is being conducted concurrently with Parts B through F. A maximum of approximately 224 patients aged 18 years or older with histologically confirmed recurrent or refractory esophageal, gastro-esophageal junction or gastric cancer with progressive disease requiring therapy will be enrolled in the study.

As of 20 August 2018, 124 patients have been enrolled, including 9 patients in Part A; 20 in Part B; 25 in Part C, 3 in Part D; 2 in Part E; 37 in Part F; and 28 in the Monotherapy Substudy. Overall, 3 patients have received DKN-01 150 mg+paclitaxel; 56 have received DKN-01 300 mg+paclitaxel; 2 have received DKN-01 150 mg+pembrolizumab; 35 have received DKN-01 300 mg+pembrolizumab; and 28 patients have received DKN-01 300 mg as monotherapy in this study.

Among patients treated with DKN-01 as monotherapy (N=28) in the Monotherapy Substudy, the most common type of TEAEs were gastrointestinal disorders (18 patients; 64%) and the most common individual TEAEs were fatigue (13 patients; 46%), vomiting (8 patients; 29%), dehydration (7 patients; 25%), and nausea, constipation, and anemia (each 6 patients; 21%). At least 1 TEAE was considered by the Investigator to be DKN-01-related for 16 (57%) patients, with the most common such events being fatigue (7 patients; 25%), anemia and vomiting (each 4 patients; 14%), and nausea and decreased appetite (each 3 patients; 11%). All other DKN-01-related TEAEs were reported for ≤2 patients.

One (4%) patient treated with DKN-01 monotherapy experienced DKN-01-related Grade 3 TEAEs, hyponatremia and lymphopenia. All other DKN-01-related TEAEs were Grade 1 or 2 in intensity.

<u>DKN-01 combined with paclitaxel in esophagogastric cancers</u>
Among patients treated with DKN-01 300 mg+paclitaxel (N=56), the most common type of TEAEs were gastrointestinal disorders (41 patients; 73%) and the most common individual TEAEs were fatigue (29 patients; 52%), anemia (27 patients; 48%), alopecia and peripheral sensory neuropathy (each 19 patients; 34%), neutropenia (17 patients; 30%), cough (16 patients; 29%), and dyspnea (14 patients; 25%).

Among the 3 patients treated with the lower DKN-01 dose of 150 mg+paclitaxel, the TEAE profile was similar to that seen with the 300 mg dose, with the most common TEAEs being diarrhea and fatigue (each 3 patients; 100%) and arthralgia, headache, and toothache (each 2 patients; 67%). All other TEAEs were reported for 1 patient only at this dose level.
Thirty-two (57%) of 56 patients treated with DKN-01 300 mg +paclitaxel experienced a DKN-01-related TEAE, most commonly fatigue (13 patients; 23%), diarrhea (7 patients; 13%), nausea (6 patients; 11%), anemia and decreased appetite (each 5 patients; 9%), and constipation and neutropenia (each 4 patients; 7%).

Six (11%) patients experienced a DKN-01-related Grade 3 TEAE, including hypophosphatemia (2 patients; 4%), anemia, monocytosis, neutropenia, and peripheral neuropathy (1 patient; 2%). DKN-01-related TEAEs reported with DKN-01 150 mg + paclitaxel included single incidences of dysgeusia, fatigue, headache, peripheral sensory neuropathy, and stomatitis, with fatigue and peripheral sensory neuropathy being Grade 3 in intensity.

Among the 56 patients who received DKN-01 300 mg + paclitaxel, 22 (39%) experienced at least 1 SAE, with infections and infestations being the most common type (9 patients; 16%). Individual SAEs reported for >1 patient included pneumonia (4 patients; 7%), and aspiration, lung infection, and pulmonary embolism (each 2 patients; 4%). All SAEs were considered by the Investigator to be unrelated to DKN-01.

Three patients experienced a Grade 5 TEAE, including aspiration (2 patients) and pulmonary embolism and respiratory failure in 1 patient each. For all 3 patients, the TEAE resulting in death was considered by the Investigator to be unrelated to DKN-01.

DKN-01 combined with pembrolizumab in esophagogastric cancers
Among patients treated with DKN-01 300 mg+pembrolizumab (N=35), the most common type of TEAEs were gastrointestinal disorders (18 patients; 51%) and the most common individual TEAEs were fatigue (11 patients; 31%), and hyponatremia, blood alkaline phosphatase increased, and aspartate aminotransferase increased (each 8 patients; 23%).

Only 2 patients were treated with DKN-01 150 mg+pembrolizumab. (Two patients intended to be treated with DKN-01 150 mg were inadvertently treated with 300 mg DKN-01 due to site error; neither patient experienced a TEAE.) All TEAEs in this arm have occurred in 1 patient only; no individual TEAE occurred in both patients in this treatment arm.

Note that no immune system disorders or infusion-related reactions have been reported with DKN-01 in combination with pembrolizumab.

Fourteen (40%) patients receiving DKN-01 300 mg + pembrolizumab experienced at least 1 TEAE considered by the Investigator to be at least possibly related to DKN-01. With the 300 mg dose, the most common DKN-01-related TEAEs were AST increased (5 patients; 14%), fatigue (4 patients; 11%), and lymphopenia (3 patients; 9%); all other such events were reported for ≤2 patients.

One (50%) patient receiving DKN-01 150 mg + pembrolizumab experienced at least 1 TEAE considered by the Investigator to be at least possibly related to DKN-01, dysphonia and flushing. Three (9%) patients at the 300 mg dose level experienced a Grade 3 DKN-01-related TEAE, including anemia, diarrhea, and lymphopenia (each 1 patient; 3%).

Among patients who received DKN-01 300 mg + pembrolizumab, 9 (26%) experienced at least 1 SAE, with gastrointestinal disorders being the most common type (4 patients; 11%). The only SAE reported for >1 patient was decreased appetite (2 patients; 6%).

One patient experienced a Grade 5 (i.e., fatal) TEAE, gastrointestinal hemorrhage, with this event considered by the Investigator to be unrelated to study drug.

DKN-01 in combination with Gemcitabine/Cisplatin in biliary tract cancer
Study DEK-DKK1-P103 is a Phase 1 non-randomized, dose-escalating, open-label, multi-center study being conducted in 2 parts (Part A and Part B). In Part A, escalating doses of DKN-01

were administered to different cohorts of patients to evaluate safety and DLTs and to establish the MTD of DKN-01 when administered in combination with gemcitabine/cisplatin.

Overall, DKN-01 has been well tolerated and the MTD of DKN-01 in combination with gemcitabine and cisplatin was the highest dose tested, 300 mg, as there were no dose limiting toxicities in Part A of the study. However, two patients enrolled into Part B expansion and dosed at the MTD (300 mg) experienced DLT-equivalent toxicities including Grade 4 febrile E. Coli sepsis (Patient 104-013: Day 20) and Grade 3 hyperbilirubinemia (Patient 101-005: Day 106). All 51 (100%) patients experienced at least 1 TEAE. Overall, the most common TEAEs were thrombocytopenia (37 patients; 73%), neutropenia (36 patients; 71%), anemia (31 patients; 61%), leukopenia and fatigue (each 29 patients; 57%), and aspartate aminotransferase increased, blood alkaline phosphatase increased, and nausea (each 28 patients; 55%).

Overall, at least 1 TEAE was considered by the Investigator to be DKN-01 related for 44 (86%) of patients, with the most common such events including neutropenia (27 patients; 53%), fatigue and thrombocytopenia (each 25 patients; 49%), leukopenia and nausea (each 19 patients; 37%), AST increased (18 patients; 35%), and anemia (17 patients; 33%).

For 46 (90%) of 51 patients, at least 1 TEAE was considered by the Investigator to be ≥Grade 3 in intensity, with the most common type of such events being hematologic abnormalities within the blood and lymphatic system disorders SOC (38 patients; 75%), with the most common individual ≥Grade 3 TEAEs being neutropenia (30 patients; 59%), thrombocytopenia (17 patients; 33%), and leukopenia (13 patients; 26%).

For 32 (63%) patients, a ≥Grade 3 was considered by the Investigator to be DKN-01-related, most commonly neutropenia (24 patients; 47%), thrombocytopenia (11 patients; 22%), leukopenia (6 patients; 12%), anemia (5 patients; 10%), AST increased (4 patients; 8%), and lymphopenia and ALT increased (each 3 patients; 6%).

Twenty-five (49%) of 51 patients experienced at least 1 SAE in Study DEK-DKK1-P103, including 24 (51%) of 47 patients in the 300 mg combination group and 1 (25%) of 4 patients in the 150 mg combination group. SAEs reported for >1 patient included neutropenia and thrombocytopenia (each 4 patients; 8%), cholangitis and sepsis (each 3 patients; 6%), and bacteremia, upper gastrointestinal hemorrhage, acute kidney injury, and pyrexia (each 2 patients; 4%). All other SAEs were reported for 1 patient only. Six patients experienced SAEs that were considered at least possibly related to DKN-01, including thrombocytopenia and neutropenia (each 3 patients), Escherichia sepsis/sepsis (2 patients), and failure to thrive, leukopenia, and peritonitis bacterial (each 1 patient).

Four (8%) patients experienced a Grade 5 (i.e., fatal) TEAE, including acute kidney injury, nephrotic syndrome, sepsis, and shock, all of which were considered unrelated to all study drugs. Study DEK-DKK1-P204 is an ongoing Phase 2 basket study designed to evaluate DKN-01 activity either as monotherapy or, at the Investigator's discretion, in combination with paclitaxel in patients with recurrent EEC or recurrent platinum-resistant/refractory EOC who have received at least one prior systemic therapy for advanced disease. This basket study will enrich for activating β-catenin mutation and/or Wnt signaling alterations. A maximum of 94 evaluable

patients aged 18 years or older with histologically confirmed recurrent EEC or recurrent platinum-resistant/refractory EOC with PD requiring therapy will be enrolled in the study. Patients who are not eligible to receive paclitaxel will be enrolled in the monotherapy group by primary tumor.

For detailed information related to clinical safety, please refer to the Investigator's Brochure.

2.2.2 **Nivolumab**

In clinical trials, nivolumab has demonstrated an acceptable benefit-risk across multiple tumor types, including advanced melanoma, RCC, NSCLC, and some lymphomas. Overall, the safety profile is quite similar across multiple tumor types and is discussed further in the sections below, including overviews from studies CA209003 and CA20937.

CA209003 is a completed Phase 1 open label, multiple dose escalation study in 306 subjects with select previously treated advanced solid tumors, including melanoma, RCC, NSCLC, colorectal cancer, and hormone-refractory prostate cancer. Subjects received nivolumab at doses of 0.1, 0.3, 1, 3 or 10 mg/kg intravenously every 2 weeks, up to a maximum of 2 years of total therapy. A total of 306 subjects were treated with nivolumab in the dose range of 0.1 - 10 mg/kg.

No maximal tolerated dose was identified in CA209003. The incidence, severity and relationship of AEs were generally similar across dose levels and tumor types. Nivolumab related AEs of any grade occurred in 75.2% of subjects. Of the 306 treated subjects, 303 (99.0%) subjects have at least 1 reported AE regardless of causality. The most frequently reported AEs were fatigue (54.9%), decreased appetite (35.0%), diarrhea (34.3%), nausea (30.1%), and cough (29.4%). Treatment-related AEs were reported in 230 (75.2%) of the 306 subjects. The most frequently reported treatment-related AEs were fatigue (28.1%), rash (14.7%), diarrhea (13.4%), and pruritus (10.5%). Most treatment-related AEs were low grade. Treatment-related high grade (Grade 3 - 4) AEs were reported in 52 (17.0%) of subjects. The most common treatment-related high grade AEs were fatigue (2.3%) and diarrhea (1%). Drug-related SAEs occurred in 11.5% of subjects. Grade 3 - 4 drug-related SAEs reported in at least 2 subjects included: diarrhea (3 subjects, 1.0%), pneumonitis (3 subjects, 1.0%), pneumonia (2 subjects, 0.7%) and lipase increased (2 subjects, 0.7%).

Select AE categories (events with a potential inflammatory mechanism requiring more frequent monitoring and/or unique intervention such as immunosuppressants and/or endocrine replacement therapy) include: GI AEs, pulmonary AEs, renal AEs, hepatic AEs, skin AEs, and endocrinopathies. In addition, select AEs include a category for infusion reactions. Each category is composed of a discrete set of preferred terms, including those of greatest clinical relevance. These select AEs are considered events of interest based on the mechanism of action and were previously referred to as immune-related AEs or immune-mediated AEs. Most high grade events resolved following the treatment guidelines for the treatment of pulmonary events, GI events, hepatic events, renal events, and endocrine events, respectively. In conclusion, the safety profile at 3 mg/kg (n = 54) was similar to safety profile across the dose ranges from 0.1 mg/kg to 10 mg/kg (n = 306).

Treatment-related AEs leading to discontinuation were reported in 32 (10.5%) of the 306 treated subjects on CA209003. The most frequent of these were pneumonitis (8 subjects; 2.6%) and colitis (3 subjects; 1.0%). There were 3 (1%) drug related deaths; each occurred after development of pneumonitis.

CA209037 is an ongoing Phase 3, open-label study of nivolumab (3 mg/kg administered by intravenous [IV] infusion every 2 weeks [Q2W]) vs investigator's choice therapy in subjects with previously-treated advanced melanoma. As of 30-Apr-2014, 268 subjects have been treated with 3 mg/kg IV nivolumab in CA209037 with safety results as outlined below (source document interim CSR, DCN930081508)[4] that are consistent with the Phase 1 experience of CA209003.

In CA209037, nivolumab related AEs of any grade occurred in 67.5% of subjects. Of the 268 subjects treated with nivolumab, 255 (95.1%) subjects had at least 1 reported AE regardless of causality. The most frequently reported treatment-related AEs were fatigue (25.0%), pruritus (16.0%), diarrhea (11.2%), and nausea (9.3%). Most treatment-related AEs were low grade. Treatment-related high grade (Grade 3 - 4) AEs were reported in 24 (9.0%) of subjects. The most common treatment-related high grade AEs were fatigue (0.7%), anemia (0.7%), diarrhea (0.4%), and vomiting (0.4%). Drug-related SAEs occurred in 4.5% of subjects. Grade 3 - 4 drug-related SAEs reported in at least 2 subjects included diarrhea (2 subjects, 0.7%). In addition, drug-related SAE of hyperglycemia occurred in 0.7%.

Select AE categories (events with a potential inflammatory mechanism requiring more frequent monitoring and/or unique intervention such as immunosuppressants and/or endocrine replacement therapy) are:

- Skin (29.1%) including pruritus (16.0%) and rash (9.3%)
- GI (11.6%) including diarrhea (11.2%) and colitis (1.1%)
- Endocrine (7.8%) including hypothyroidism (7.8%) and hyperthyroidism (1.9%)
- Hepatic (4.5%) including AST increased (4.1%) and ALT increased (2.6%)
- Pulmonary (2.2%) including pneumonitis (1.9%)
- Hypersensitivity/infusion reaction (1.9%)
- Renal (1.5%) including increased creatinine (0.7%), increased urea (0.4%), and tubulointerstitial nephritis (0.4%).

In general, these select AEs were considered by the investigator to be related to study drug, except for AEs in the hepatic and renal select AE categories. There were few high-grade select adverse events (n = 20), and the majority of high-grade events (13 of 20) subsequently resolved, including those for which immunosuppressive therapy was not initiated.

Treatment-related AEs leading to discontinuation were reported in 6 (2.2%) of the 268 treated subjects in CA209037 including single events of colitis, pancreatitis, increased ALT, increased lipase, autoimmune neuropathy, and demyelination. There were no deaths due to drug-related toxicity in CA209037.

Taken together, these data from studies from CA209003 and CA209037 highlight the acceptable

safety profile with similar trends in AEs in the 574 subjects treated with nivolumab in these 2 studies. Additional details on the safety profile of nivolumab, including results from other clinical studies, are also available in the BMS-936558 (nivolumab) IB.

In addition to the clinical safety data outlined above, preliminary new non-clinical safety findings of adverse pregnancy outcomes and infant losses in the absence of overt maternal toxicity have been reported.[5] The findings of increased late stage pregnancy loss and early infant deaths/euthanasia in nivolumab exposed pregnant monkeys suggest a potential risk to human pregnancy if there is continued treatment with BMS-936558 (nivolumab) during pregnancy.

Summary of Nivolumab Clinical Activity

As of July 2015, > 8,600 subjects have received nivolumab in single- or multiple-dose Phase 1/2/3 studies or studies with nivolumab in combination with other therapeutics (ipilimumab, cytotoxic chemotherapy, anti-angiogenics, and targeted therapies).

In **CA209003**, the clinical activity of nivolumab was demonstrated in a variety of tumor types, including melanoma, RCC, and NSCLC. Clinical activity was noted across a range of doses (0.1 mg/kg, 0.3 mg/kg, 1 mg/kg, 3 mg/kg, and 10 mg/kg). In CA209003, a total of 306 subjects with melanoma, RCC, and NSCLC have been evaluated for clinical activity. A response of either CR or PR, as determined by investigator assessed tumor evaluations based on RECIST 1.1 criteria,[6] has been reported at all dose levels.

In NSCLC, the most active doses were 3 and 10 mg/kg. The overall ORR of 17% was reported with a 48-week progression-free survival rate (PFSR) of 22% (95% CI: 15 - 30%), and a 24-month overall survival rate of 24% (95% CI: 16 - 32%). Only a single response (1/33) was reported at 1 mg/kg. Durable responses were observed in both squamous and non-squamous subtypes. Historically, ORR of 5% to 10% and median PFS (mPFS) of 2 to 3 months has been reported with docetaxel treatment in previously-treated NSCLC subjects.

A complete response (CR) or partial response (PR) was reported in 31% (95% CI: 22% - 41%) of the 107 response-evaluable subjects with melanoma treated with nivolumab monotherapy Q2W at doses ranging from 0.1 to 10 mg/kg in CA209003. The responses were durable with a PFSR at 24 weeks of 38% (95% CI: 28 - 47%) and OS at 24 months of 48% (95% CI: 38 - 57%).

Of the 34 response evaluable RCC subjects in CA209003, responses were reported in both the 1 mg/kg (5 of 18 subjects, 27 %) and 10 mg/kg (5 of 16 subjects, 31%) treatment groups. PFSR at 24 weeks was 33% (95% CI: 14 - 55%) in the 1 mg/kg and 37% (95% CI: 14 - 61%) in the 10 mg/kg nivolumab treatment groups. OS at 24 months was 51% (95% CI: 30 - 64%) and 44% (95% CI: 20 - 66) in the 1 mg/kg and 10 mg/kg groups, respectively.

In **CA209037**, the ongoing Phase 3 study for subjects with previously-treated advanced melanoma of nivolumab (3 mg/kg administered by intravenous [IV] infusion every 2 weeks [Q2W]) vs investigator's choice therapy, as of 30-Apr-2014, 120 nivolumab-treated and 47 subjects in the investigator's choice arm are available for determination of the ORR using RECIST 1.1 criteria. As determined by imaging plus clinical review by a blinded independent

central review (BICR), the ORR for nivolumab vs the reference arm is 31.7% vs 10.6%, respectively. Four subjects in the nivolumab arm had a CR, whereas no subjects in the reference arm had a CR. Median progression-free survival was 4.7 months (95% CI: 2.3 - 6.5) vs 4.2 months (95% CI: 2.1 - 6.3) with a 6-month PFS rate of 48% (95% CI: 38 - 56) vs 34% (95% CI: 18 - 51) in the nivolumab (n = 122) vs reference arm (n = 60), respectively.

In addition, recent clinical trial data in MEL **(CA209066)**, and NSCLC **(CA209017 and CA209057)** have demonstrated OS benefit:

- In **CA209066**, a Phase 3 study of 418 previously untreated metastatic melanoma patients without a BRAF mutation, 1 year overall survival was 72.9% (95% CI: 65.5 to 78.9) in the nivolumab group as compared to 42.1% (95% CI: 33.0 to 50.9) in the dacarbazine group (P < 0.001). The median progression-free survival was 5.1 months in the nivolumab group versus 2.2 months in the dacarbazine group (hazard ratio for death or progression of disease, 0.43; 95% CI, 0.34 to 0.56; P < 0.001). The objective response rate was 40.0% (95% CI, 33.3 to 47.0) in the nivolumab group versus 13.9% (95% CI, 9.5 to 19.4) in the dacarbazine group (odds ratio, 4.06; P < 0.001). The survival benefit with nivolumab versus dacarbazine was observed across prespecified subgroups, including subgroups defined by status regarding the programmed death ligand 1 (PD-L1).[7] Additional supportive data for the clinical activity of nivolumab in advanced melanoma has been shown in a cohort of 107 advanced melanoma subjects in an outpatient setting who enrolled between 2008 and 2012. In 107 patients with advanced MEL treated with nivolumab**,** objective responses were observed in 31% of patients. Median OS (62% with two to five prior systemic therapies) was 16.8 months, and 1- and 2-year survival rates were 62% and 43%, respectively. Median progression-free survival was 3.7 months (95% CI, 1.9 to 9.1 months), with 1- and 2-year progression-free survival rates of 36% (95% CI, 27% to 46%) and 27% (95% CI, 17% to 36%), respectively.[8]
- In **CA209017**, a Phase 3 trial in 272 patients with metastatic squamous NSCLC with progression on or after platinum-based chemotherapy, subjects were randomized to nivolumab 3 mg/kg IV q2 week (n = 135) or docetaxel (n = 137) at 75 mg/m2 every 3 weeks. Median OS was 9.2 months in the nivolumab group (95% CI: 7.3 - 13.3) vs 6.0 months in the docetaxel group (95% CI: 5.1 - 7.3) (HR of 0.59, P = 0.00025).
- A Phase 3 clinical trial of nivolumab vs docetaxel in Non-squamous NSCLC (**CA209057**), nivolumab monotherapy demonstrated superior OS compared with docetaxel, with a clinically meaningful and statistically significant improvement observed (HR=0.73 [95.92% CI: 0.59, 0.89]; stratified log-rank test p-value = 0.0015). The median overall survival was 12.2 months (95% confidence interval [CI], 9.7 to 15.0) with nivolumab versus 9.4 months (95% CI, 8.1 to 10.7) with docetaxel. Results of secondary endpoints of ORR, DOR, and TTR further support the antitumor activity of nivolumab in this population. Pre-study (baseline) PD-L1 expression was predictive for benefit from nivolumab for all efficacy endpoints. Interaction p-values reported for PD-L1 expression subgroups by each of the pre-defined expression levels suggested a clinically important signal of a predictive association. In PD-L1 positive subjects, the nivolumab group showed improved efficacy vs docetaxel across all efficacy endpoints (OS, ORR, and PFS). In contrast, there were no meaningful

differences in efficacy between the treatment groups in the PD-L1 negative subgroups by any expression level.[9]

2.3 Rationale

Biliary tract cancer (BTC) is an aggressive malignancy with a poor prognosis, and there is no current standard for second or third line therapy for advanced BTC. Immunotherapy combination trials represent a promising strategy to address this unmet medical need given the critical role of the immune system in the pathogenesis of BTC. Chronic inflammation is a common underlying risk factor for BTC and can be due to a variety of etiologies including liver fluke infection, choledocholithiasis, sclerosing cholangitis, viral hepatitis, and alcohol. The success of immune checkpoint blockade depends on an endogenous antitumor immune response, a hallmark of which is the presence of tumor infiltrating lymphocytes (TILs). TILs were identified in approximately half of 375 BTC cases in one study[10] and 27/27 cases of CCA in another study[11]. Additionally, BTCs have been shown to suppress T cell responses in a variety of ways: myeloid-derived suppressor cells (MDSCs) have been identified in the blood of patients with BTC[12] and PD-L1 expression has been identified in BTC in 30-100% of samples across a variety of studies[11, 13, 14]. A strategy of reactivation of T cell responses is thus a seemingly rationale therapeutic approach in advanced BTC.

DKN-01 is a potent humanized IgG4 monoclonal antibody with neutralizing activity against dickkopf-1 (DKK1), a modulator of Wnt signaling pathway, which influences a number of important processes such as cell growth and differentiation and bone development and adult bone homeostasis[15-17]. In a study of 138 ICC samples, 38.4% of ICC tumor samples demonstrated DKK1 expression, and high DKK1 expression was associated with lymph node metastasis, advanced tumor stage, and lower 5 year survival rates than DKK1 negative patients[18]. In a phase Ib trial of DKN-01/Gemcitabine/Cisplatin in patients with advanced refractory BTC, the overall response rate (ORR) was 21.3% (10/47 evaluable patients), with all 10 patients experiencing a confirmed PR; all 10 of these patients were treated with DKN-01 300 mg. By tumor type, the ORR was 20.7% (6/29 patients) in intrahepatic CCA, 25.0% (1/4 patients) in extrahepatic CCA, and 20.0% (3/15 patients) in gallbladder cancer. Thirty-four patients experienced a best response of SD; thus, the disease control rate was 93.6% (44/47 patients). Among all 51 patients, median PFS was 35.7 weeks (95% CI, 23.3, 43.1); 25.5% (13/51) of patients were censored in the analysis, and among those with intra-hepatic biliary adenocarcinoma and gallbladder cancer, median PFS was 41.3 weeks (95% CI 19.9, 53.7) and 23.4 weeks (95% CI 8.4, 43.1), respectively. By DKN-01 dose, median PFS was 19.2 weeks (95% CI, 5.7, 48.1) and 37.7 weeks (95% CI, 23.4, 44.7) in the 150 mg and 300 mg groups, respectively. Among all 51 patients, median OS was 51.1 weeks (95% CI, 35.9, 64.9); at the time of the final analysis, 16 (31.4%) of 51 patients were alive at last follow-up. By DKN-01 dose, median OS was 30.9 weeks (95% CI, 15.1, 89.3) among patients who received DKN-01 150 mg + gem/cis (N=4) and 53.7 weeks (95% CI, 38.9, 69.9) among patients who received DKN-01 300 mg + gem/cis (N=47)[19]. Similarly, pembrolizumab has shown preliminary activity in biliary tract cancer (BTC) in the KEYNOTE 028 phase I trial with patients with treatment refractory advanced BTC with ≥ 1% PD-L1 positivity showed a 33% clinical benefit rate including a 17% ORR.[14]

Anti-PD-1 plus DKN-01 show additive effects in inhibiting tumor growth in a B16 syngeneic mouse model, suggesting this may be a viable clinical strategy. Studies with DKN-01

in this model also suggest that its locus of action is on innate immune cells (specifically natural killer cells) rather than on the tumor cells or the adaptive immune response.

Based on this rationale, the current phase II trial was designed to evaluate the activity of the combination of DKN-01 and nivolumab in patients with advanced BTC who have progressed on ≥1 line of systemic therapy.

2.4 Correlative Studies Background

Biliary tract cancers are a molecularly heterogeneous population of tumors, and identification of biomarkers that predict response to therapy is urgently needed to identify the subgroups most likely to benefit from therapies such as the combination of DKN-01 and nivolumab.

A) RNA and DNA analysis of Pre and post treatment Diagnostic Sample

Fresh frozen and archived formalin fixed paraffin embedded (FFPE) material from pre and post treatment biopsies will be analyzed for RNA and DNA markers to predict response to therapy.

We have developed a RNA-ISH assay applicable to FFPE sectioned slides for repeat non-coding RNAs that appear to be linked with immune infiltrates [20, 21]. We will evaluate repeat RNAs by RNA in situ hybridization (RNA-ISH) and immune cells by IHC (CD8, CD163, FOXP3)

Whole exome/whole genome DNA sequencing for recurrent genomic aberrations will be considered for analysis.

B) Blood Based Biomarkers for Disease Response Monitoring

Newer "liquid biopsy" technologies have emerged in recent years including circulating tumor (ctDNA) [22-24], circulating tumor cells (CTCs) [25-29], and exosomes [30, 31]. Each of these methods has different advantages and the ability to evaluate these technologies in selected patient cohorts for their diagnostic and predictive utility in cancer is essential. Our group has developed mature technologies for each of these methods to be fully evaluated in a clinical trial. Blood samples will be evaluated for these markers on study to determine if they correlate with response and potentially predict response based on the pretreatment blood sample.

Correlative studies showed that DKN-01 combined with chemotherapy was associated with transient increases in circulating immune cytokines such as plasma IFN-γ, IL-6, IL-8 and IL-10 but also with decreased TNF-α. A high plasma IL-6 and an increase (or no decrease) in TNF-α associated with worse outcomes. The combined regimen was also associated with changes in plasma biomarkers of angiogenesis at days 8 and 15, such as increased PlGF, VEGF-D, sTIE2 and sVEGFR1 but decreases VEGF and VEGF-C. The decreased in VEGF and increase in sVEGFR1 may indicate an inhibition of angiogenesis, however, plasma VEGF and VEGF-C levels were significantly increased after 3 weeks (1 cycle) of treatment suggesting pro-angiogeneic activity. High pretreatment VEGF-C, and decreased sVEGFR1 at day 8 and change in sTIE2 at day 15 associated with better survival outcomes. Plasma HGF at all time-points (but not changes after treatment) were associated with worse outcomes and with PK parameter changes after DKN-01 plus gemcitabine and cisplatin chemotherapy. Collectively, these data

indicate that HGF, sVEGFR1, IL-6, and TNF-α should be further evaluated as biomarkers of poor prognosis or treatment resistance.

C) Stool Based Gut Microbiome Biomarkers

As a critical modulator for host-tumor interactions, the gut microbiome may be a modifiable target for adjuvant therapy and a key marker for predicting immunotherapeutic efficacy.[32, 33] We propose to collect stool samples longitudinally throughout the study to understand microbial dynamics in response to treatment and identify those microbiota that may be used to predict disease-free progression (response), onset of treatment-related toxicities, or progression of disease/death.

3. PARTICIPANT SELECTION

3.1 Eligibility Criteria

3.1.1 Histologically confirmed intra- or extrahepatic cholangiocarcinoma or gallbladder cancer

3.1.2 Participants must have measurable disease by CT/MRI by RECIST version 1.1 criteria

3.1.3 Prior chemoembolization, radiofrequency ablation, or radiation to the liver is allowed as long as the patient has measurable disease outside of the treated area or measurable progression per RECIST v1.1 at the site of the treated area.

3.1.4 Documented progression after ≥1 line of systemic therapy for advanced BTC. Prior adjuvant chemotherapy qualifies as this 1 line if the last cycle of adjuvant therapy was completed within 6 months of radiological progression.

3.1.5 Age ≥ 18 years

3.1.6 ECOG performance status ≤1

3.1.7 Life expectancy of greater than 3 months

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

- Absolute neutrophil count ≥1,500/mcL
- Absolute lymphocyte count ≥0.4 x 10^9/L
- Platelets ≥75,000/mcL
- Hemoglobin ≥ 8.0 g/dL (prior transfusions are allowed if given ≥ 7 days before testing)
- Total bilirubin ≤ 2.0 x institutional upper limit of normal; except patients with Gilbert Syndrome who must have a total bilirubin level of < 3.0 x ULN
- AST(SGOT)/ALT(SGPT) ≤3 × institutional upper limit of normal; ≤ 5 x ULN in case of liver metastases
- Creatinine ≤ 2.0 x institutional upper limit of normal OR Creatinine clearance ≥30

mL/min/1.73 m^2 for participants with creatinine levels ≥ ULN
- International Normalized Ratio (INR) ≤ 1.5 x ULN unless patient is receiving anticoagulant therapy as long as INR is within therapeutic range of intended use of anticoagulants
- Serum albumin ≥ 2.5 g/dL

3.1.9 Subjects with hepatitis B or C are eligible to enroll if they have:
- Chronic HBV infection (evidenced by a positive HBV surface antigen or HBV DNA) as long as they have been on antiviral therapy for ≥ 4 weeks.
- Chronic or resolved HCV infection (evidenced by a detectable HCV RNA or antibody). Antiviral therapy is not required for chronic HCV.

3.1.10 Women of child-bearing potential and men must agree to use adequate contraception according to national guidelines (hormonal or barrier method of birth control; abstinence) prior to study entry, for the duration of study participation, and for 5 months for women and 7 months for men after completion of study drug administration.

3.1.11 Female subjects must be either of non-reproductive potential (i.e., post-menopausal by history: ≥ 50 years old and no menses for ≥1 year without an alternative medical cause; OR history of hysterectomy, OR history of bilateral tubal ligation, OR history of bilateral oophorectomy) or must have a negative serum pregnancy test upon study entry.

3.1.12 Ability to understand and the willingness to sign a written informed consent document.

3.2 Exclusion Criteria

3.2.1 Prior DKK1 inhibitor or anti-PD-1/PD-L1 treatment

3.2.2 Participants with Child-Pugh B or C cirrhosis

3.2.3 Participants with a diagnosis of ampullary cancer

3.2.4 Treatment with any of the following within the specified time frame prior to the first dose of DKN-01 and nivolumab:
- Any non-investigational or investigational anticancer therapy within 3 weeks or have not recovered from side effects of such therapy prior to treatment administration (mitomycin within prior 5 weeks). For targeted therapy, 5 half-lives are sufficient, even if <3 weeks. Concurrent participation in an observational study may be allowed after review by the Principal Investigator.
- Patients with locoregional therapy, e.g., transarterial chemoembolization (TACE), selective internal radiotherapy (SIRT), external beam radiation, or ablation within 4 weeks
- Palliative limited field radiotherapy (i.e. bone metastases) within 2 weeks
- Major surgery within the previous 4 weeks (the surgical incision should be fully healed prior to the first dose of treatment)

3.2.5 Any condition requiring systemic treatment with either corticosteroids (> 2mg daily dexamethasone equivalent) or other immunosuppressive medications within 14 days of starting the study medications. Premedication for hypersensitivity reactions (e.g. to contrast for CT or gadolinium for MRI) is allowed.

3.2.6 Subjects with autoimmune disease active within the last two years including but not limited to Crohn's disease, ulcerative colitis, myasthenia gravis, myositis, autoimmune hepatitis, systemic lupus erythematosus, rheumatoid arthritis, vascular thrombosis associated with antiphospholipid syndrome, Wegener's granulomatosis, Sjögren's syndrome, Guillain-Barré syndrome, multiple sclerosis, type I diabetes mellitus, vasculitis, or glomerulonephritis

- Patients with a history of autoimmune-related hypothyroidism on a stable dose of thyroid replacement hormone are eligible for this study.
- Patients with controlled Type I diabetes mellitus on a stable insulin regimen are eligible

3.2.7 History of idiopathic pulmonary fibrosis, organizing pneumonia (e.g., bronchiolitis obliterans), idiopathic pneumonitis, or evidence of active pneumonitis on screening chest CT scan. History of radiation pneumonitis/fibrosis in the radiation field is permitted.

3.2.8 History of osteonecrosis of the hip or evidence of structural bone abnormalities in the proximal femur on magnetic resonance imaging (MRI) scan that is symptomatic and clinically significant. Degenerative changes of the hip joint are not excluded.

3.2.9 Known osteoblastic bony metastasis. Screening of asymptomatic subjects without a history of metastatic bony lesions is not required

3.2.10 Fredericia's corrected QT interval (QTcF) ≥ 500 ms on ECG conducted during screening

3.2.11 History of allergic reactions attributed to compounds of similar chemical or biologic composition to DKN-01 or Nivolumab.

3.2.12 A serious illness or medical condition(s) including, but not limited to, the following:

- Known brain metastasis (not including primary brain tumors) unless patient is clinically stable for ≥ 1 month without systemic corticosteroids beyond physiologic replacement (>10 mg prednisone daily).
- Known acute systemic infection.
- Myocardial infarction, severe/unstable angina, symptomatic congestive heart failure
- New York Heart Association [NYHA] Class III or IV (see Appendix C, New York Heart Association [NYHA] Classification) within the previous 2 months; if >2 months, cardiac function must be within normal limits and the patient must be free of cardiac-related symptoms.
- Chronic nausea, vomiting, or diarrhea considered to be clinically significant in the opinion of the investigator.
- Congenital long QT syndrome, or any known history of torsade de pointes, or family history of unexplained sudden death.
- Other severe acute or chronic medical or psychiatric condition or laboratory abnormality that in the judgment of the investigator would make the patient inappropriate for entry into this study.

3.2.13 Patients with a history of another primary malignancy that is currently clinically significant, and has potential for metastases or currently requires active intervention (except for hormonal therapy for breast or prostate cancer).

3.2.14 Patients who received treatment with live vaccines within 30 days prior to the first dose of study medication. Examples of live vaccines include, but are not limited to, the following: measles, mumps, rubella, chicken pox, shingles, yellow fever, seasonal flu, H1N1 flu, rabies, BCG and typhoid vaccine.

3.2.15 .

3.2.16 Prior allogeneic stem cell or solid organ transplant.

3.2.17 Known or current evidence of HIV

3.2.18 Pregnant or lactating female.

3.3 Inclusion of Women and Minorities

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

4. REGISTRATION

4.1 General Guidelines for DF/HCC Institutions

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.

Following registration, participants may begin protocol-specific therapy and/or intervention. Issues that would cause treatment delays should be discussed with the Overall Principal Investigator (PI). 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-101) must be followed.

4.3 General Guidelines for Other Investigative Sites

Eligible participants will be entered on study centrally at the Massachusetts General Hospital Cancer Center by the Study Coordinator.

Following registration, participants should begin protocol therapy within 7 days. Issues that would cause treatment delays should be discussed with the Overall PI. 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

Not applicable.

5. TREATMENT PLAN

5.1 Treatment Regimen

DKN-01 and Nivolumab will be administered on day 1 and day 15 of each 28 day cycle. Treatment will be administered on an outpatient basis. Reported adverse events and potential risks are described in Section 7. Appropriate dose modifications are described in Section 6. No investigational or commercial agents or therapies other than those described below may be administered with the intent to treat the participant's malignancy.

Table 1:

Regimen Description					
Agent	**Premedications**	**Dose**	**Route**	**Schedule**	**Cycle Length**
DKN-01	None	600mg*	IV over 30 +/- 10 minutes	Days 1 and 15	28 days (4 weeks)
Nivolumab	None	240mg	IV over 30 +/- 10 min minutes, after completion of DKN-01	Days 1 and 15	

*Dose may be reduced to 300mg depending on results of DLT lead-in period

DKN-01
DKN-01 is to be administered as IV infusion over 30 minutes. The patient's vital signs (heart rate, respiratory rate, blood pressure, and temperature) should be determined within 60 minutes before, during (every 15 [±5] minutes), and 30 (±10) minutes and 2 hours (±15 minutes) after the infusion. No premedication is needed prior to administer DKN-01.

Nivolumab
Nivolumab is to be administered as IV infusion over 30 minutes. At the end of the infusion, flush the line with a sufficient quantity of normal saline or dextrose solution.

DKN-01 will be administered first followed by Nivolumab, with a minimum of 5 minutes between dosing.

5.2 Pre-Treatment Criteria

Treatment may be administered after review of hematology and chemistry laboratory values, but thyroid function tests can be pending at the time of administration.

5.2.1 Cycle 1, Day 1

- ANC $\geq 1500/mm^3$
- Platelet count $\geq 75\times10^9/L$
- AST and ALT at baseline grade and $\leq$ 5x ULN
- Total bilirubin $\leq$ 2x ULN

5.2.2 Subsequent Cycles

A new cycle may be initiated for patients meeting the following criteria:

- ANC $\geq 1000/mm^3$
- Platelet count $\geq 50\times10^9/L$
- Liver associated biochemical abnormalities (e.g., AST, ALT, bilirubin) $\leq$Grade 1 or resolved to baseline grade

- Any other drug-related AEs that may have occurred resolved to baseline or ≤ Grade 1 severity.

If these conditions are not met on Day 1 of a new cycle, the subject will be evaluated weekly and a new cycle of treatment will not be initiated until the toxicity has resolved as described above. The maximum treatment delay is 12 weeks for DKN-01 and 12 weeks for nivolumab. If, in the opinion of the investigator, a patient is receiving clinical benefit from the combination, patients may continue therapy after a discussion between the investigator and the Principle Investigator until developing documented progressive disease or otherwise meeting criteria for treatment discontinuation (Section 6.6).

If, in the opinion of the Investigator, a patient is receiving clinical benefit from treatment with the combination of DKN-01 plus nivolumab but has a compelling clinical reason after completion of Cycle 1 to discontinue one drug, the patient will be permitted to continue in the study with the other drug alone at the discretion of the treating clinician. Patients for whom the investigator decides it is in their best interest to stop DKN-01 and nivolumab treatment will be discontinued and end of treatment procedures completed.

5.3 Agent Administration

DKN-01
The vial is manufactured to deliver 20 mg of DKN-01 and must be reconstituted with 0.9% Sodium Chloride Solution. The drug product does not contain antimicrobial preservative and must be stored in refrigerated conditions (2ºC to 8ºC).

Reconstitution of the vial with 2.2 mL of 0.9% Sodium Chloride Solution gives a clear solution containing 10 mg/mL DKN-01. The reconstituted vials are stable for up to 8 hours at room temperature (up to 30°C) or refrigerated (2 to 8°C).The route of administration is IV infusion via syringe pump only (over a period of 30 minutes). Once the reconstituted DKN-01 has been removed from the vials into a syringe, it must be administered to the patient within 4 hours, while being stored at room temperature. Therefore, the maximum time limit from reconstitution to completion of administration is 12 hours. A list of infusion materials compatible with DKN-01 for Injection is provided in the study drug guidelines and in the Pharmacy Manual (Appendix C).

Study drug will be prepared and dispensed by qualified pharmacy staff under supervision of pharmacists.. The pharmacy must maintain an individual record for the patient.

The clinical trial centers will keep a trial specific authorization list which determines the persons responsible for handling of the investigational drugs. The responsible person regarding the following will keep accurate records in the clinical trial centers:

- receipt of IMP supply from sponsor (clinical trial center, principal investigator, identification of IMP, Batch No, Formulation, kind and size of packaging, date of expiry, number of study drugs per participant, number of reserve study drugs, number of study drugs in total, patient identification number, date and time of receipt); location of storage of IMP;

- dispensing of IMPs in the clinical trial center (date and time, number, batch no, patient identification number, volume of unused solution for injection when returning). IMPs will not be returned to the pharmacy, they will be immediately sent to destruction after use.

Nivolumab

Nivolumab 100 mg (10 mg/mL) will be packaged in an open-label fashion. Five or ten nivolumab 10 mL vials will be packaged within a carton.

Nivolumab is to be administered as an IV infusion through a 0.2-micron to 1.2-micron pore size, low-protein binding (polyethersulfone membrane) in-line filter at the protocol-specified doses. It is not to be administered as an IV push or bolus injection. Nivolumab injection can be infused undiluted (10 mg/mL) or diluted with 0.9% Sodium Chloride Injection, USP or 5% Dextrose Injection, USP to protein concentrations as low as 0.35 mg/mL. Instructions for dilution and infusion of nivolumab injection may be provided in the clinical protocol, pharmacy binder, or pharmacy reference sheet. Care must be taken to assure sterility of the prepared solution as the product does not contain any antimicrobial preservative or bacteriostatic agent. Nivolumab infusions are compatible with polyvinyl chloride (PVC) or polyolefin containers and infusion sets and glass bottles.

5.4 Definition of Dose-Limiting Toxicity (DLT)

Toxicities will be graded and documented according to the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE v 5.0 guidelines).

A DLT is defined as any clinically significant adverse event (AE) occurring within the first 28 days of starting the study treatment that is considered by the investigator to be possibly related or related to DKN-01 or nivolumab. These include the following events:

- Any death not clearly due to the underlying disease or extraneous causes
- Non- hematologic toxicity:
  - Grade 3 or higher
  - Hy's law
- Hematologic toxicity:
  - Grade 4 neutropenia or thrombocytopenia > 7 days
  - ≥Grade 3 thrombocytopenia with bleeding
  - Neutropenic fever
- Grade 3 hypersensitivity reaction to DKN-01 with premedication
- Grade 4 hypersensitivity reaction to DKN-01 with or without premedication
- Dosing delay greater than 28 days

The following will not be considered a DLT:

- Grade 3 nausea/vomiting or diarrhea < 72 hours with adequate antiemetic and other supportive care

- Grade 3 fatigue < 1 week
- ≥ Grade 3 electrolyte abnormality that lasts <24 to 72 hours, is not clinically complicated, and resolves spontaneously or responds to conventional medical interventions
- ≥ Grade 3 amylase or lipase that is not associated with symptoms or clinical manifestations of pancreatitis
- Alopecia
- Grade 3 leukopenia that resolves to ≤ grade 2 within 14 days
- Grade 4 leukopenia/lymphopenia that resolves to ≤ grade 2 within 21 days
- A drug-related fever ≤ Grade 3 will not be considered a DLT.
- Grade 3 hypersensitivity reaction to DKN-01 with premedication (Grade 3 hypersensitivity reaction to DKN-01 without premedication is not considered a DLT)
- Grade 4 hypersensitivity reaction to DKN-01 with or without premedication
- Dosing delay greater than 28 days

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

Dose escalation will proceed within each cohort according to the following scheme. Dose-limiting toxicity (DLT) is defined above.

Number of Participants with DLT at the Initial Dose Level in first 3 patients	**Escalation Decision Rule**
0 out of 3	Treat the remainder of participants at DKN-01 600mg.
1 out of 3	Three (3) additional participants will be entered at the 600mg dose level if only 3 participants were treated previously at that dose. If 0 DLTs are experienced, continue with 600mg dose level. If 1-3 DLTs are experienced, treat remainder of patients at 300mg dose level.
2-3 out of 3	Treat the remainder of participants at DKN-01 300mg.

5.5 General Concomitant Medication and Supportive Care Guidelines

Concomitant therapy includes any prescription medications or over the counter preparations used by a patient after 7 days preceding the screening evaluation and prior to the end of protocol treatment visit.

Allowed concomitant medications:

- Patients should receive full supportive care, including transfusions of blood and blood products, antibiotics, anti-emetics, etc., when appropriate.
- Hematopoetic growth factors support including erythroid (eg erythropoietin, darbepoetin), myeloid (eg granulocyte colony-stimulating factor, granulocyte-macrophage colony-stimulating factor), and thrombopoetic (eg romiplostim, eltrombopag) growth factors are allowed.
- Patients who experience infusion-associated symptoms may be treated symptomatically with acetaminophen, diphenhydramine, and/or ranitidine or another H2 receptor antagonist, as per standard practice. Serious infusion associated events manifested by dyspnea, hypotension, wheezing, bronchospasm, tachycardia, reduced oxygen saturation, or respiratory distress should be managed with supportive therapies as clinically indicated (e.g., supplemental oxygen and β2-adrenergic agonists).
- Systemic corticosteroids and TNF-α inhibitors may attenuate potential beneficial immunologic effects of treatment with nivolumab but may be administered at the discretion of the treating physician after consultation with the Principle Investigator. If feasible, alternatives to corticosteroids should be considered. Premedication may be administered for Cycles ≥ 2 at the discretion of the treating physician after consultation with the Principle Investigator. The use of inhaled and topical corticosteroids and mineralocorticoids (e.g., fludrocortisone) for patients with orthostatic hypotension or adrenocortical insufficiency is allowed. The use of steroids for prophylaxis for CT scans or MRIs is allowed.
- Patients who use hormonal therapy with GnRH agonists, oral contraceptives, hormone-replacement therapy, prophylactic or therapeutic anticoagulation therapy (such as low molecular weight heparin or warfarin at a stable dose level), or other allowed maintenance therapy should continue their use.
- Patients of reproductive potential should use highly effective means of contraception.

Allowed concomitant procedures:

- Biliary drainage procedures, including but not limited to endoscopic retrograde cholangiopancreatography (ERCP) and transhepatic percutaneous drains, are allowed during trial to address biliary obstruction and biliary stent exchanges at the discretion of the investigator.
- Paracenteses and thoracenteses
- Palliative extrahepatic or liver-directed radiation is allowed. Both drugs should be held for at least 1 day prior to the start of radiation, throughout radiation, and for at least 1 day after completion of radiation.
- Additional procedures such as ablation and chemoembolization may be allowed for oligometastatic progression after discussion with the Principal Investigator of the trial

Prohibited concomitant medications:

- Any concomitant therapy intended for the treatment of biliary tract cancer, whether health authority-approved or experimental, is prohibited. This includes but is not limited to the following: chemotherapy, hormonal therapy, immunotherapy, radiotherapy,

investigational agents, or herbal therapy.

- Patients must not receive live attenuated influenza vaccine within 4 weeks prior to Cycle 1, Day 1 or at any time during the study but may receive inactivated influenza vaccines.
- Medications with a known interaction with DKN-01 or nivolumab.
- Traditional herbal medicines should not be administered because the ingredients of many herbal medicines are not fully studied and their use may result in unanticipated drug-drug interactions that may cause or confound assessment of toxicity.
- Patients are not allowed to receive immunostimulatory agents, including but not limited to IFN-α, IFN-γ, or IL-2, during the entire study. These agents, in combination with nivolumab, could potentially increase the risk for autoimmune conditions.
- Patients should also not receive immunosuppressive medications, including but not limited to cyclophosphamide, azathioprine, methotrexate, and thalidomide. These agents could potentially alter the activity and the safety of nivolumab.

5.6 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:

- Disease progression
- Intercurrent illness that prevents further administration of treatment
- Unacceptable adverse event(s)
- Participant demonstrates an inability or unwillingness to comply with protocol requirements
- Participant decides to withdraw from the protocol therapy
- General or specific changes in the participant's condition render the participant unacceptable for further treatment in the judgment of the treating investigator
- Treatment delay of ≥ 12 weeks of either DKN-01 or Nivolumab or both

Participants will be removed from the protocol therapy when any of these criteria apply, and the relevant Off-Treatment/Off-Study information will be updated in OnCore. The reason for removal from protocol therapy, and the date the participant was removed, must be documented in the case report form (CRF). Alternative care options will be discussed with the participant.

Treatment beyond RECIST-defined progression may be permitted after discussion between the investigator and the Principle Investigator. The criteria to continue treatment includes: an absence of clinical symptoms or signs indicating clinically significant disease progression; no

decline in performance status; absence of rapid disease progression or threat to vital organs or critical anatomical sites [e.g., CNS metastasis, respiratory failure due to tumor compression, spinal cord compression] requiring urgent alternative medical intervention; no significant, unacceptable or irreversible toxicities related to study treatment; or if the participant is deriving ongoing clinical benefit.

In case of early study closure, all study participants may continue to receive study treatment as planned in the protocol provided that they benefit from it and all protocol-specified criteria for continuing study treatment are met.

5.7 Duration of Follow Up

For patients who stop treatment because of confirmed disease progression:
For 100 days after stopping protocol therapy
- Reporting of any AE/SAE

Follow-up visits will be conducted every 3 months ± 7days:
- Survival status
- Diagnosis of subsequent progression and / or new malignancy
- Subsequent anti-cancer therapy

For patients who stop treatment in the absence of disease progression:
For 100 days after stopping protocol therapy
- Reporting of any AE/SAE

Every 12 weeks/3 months ± 7days from start of treatment until 2 years after start of treatment relative to the date of treatment start and then every 12 weeks/3 months ± 7days until confirmed progression according to RECIST
- Tumor evaluation
    - Abdominal contrast enhanced (CE) CT or MRI staging and CE thoracic CT
    - CA 19-9
    - Survival status
    - Diagnosis of subsequent progression and / or new malignancy
    - Subsequent anti-cancer therapy

5.8 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
- Hospice

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-101.

6. DOSING DELAYS/DOSE MODIFICATIONS

6.1 General Guidelines for dose delays and 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 5.0can be downloaded from the CTEP website http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

Each cycle should be started with all study drugs unless one drug has been held/discontinued due toxicity.

If a subject is held on day 1 of a cycle, they can be re-evaluated and treated on day 8 ± 3 days if they meet the resumption criteria; if they are dosed on day 8 ± 3 days, the next dose will resume 2 weeks later. If they do not meet criteria for treatment on day 8 ± 3 days, then day 1 will be considered a missed dose, and they will continue with the next dose (day 15 dose) as scheduled.

If a subject is held on day 15 of a cycle, they can be re-evaluated and treated on day 22 ± 3 days if they meet the resumption criteria; if they are dosed on day 22 ± 3 days, the next dose will resume 2 weeks later. If they do not meet criteria for treatment on day 22 ± 3 days, then day 15 will be considered a missed dose, and they will continue with the next cycle as scheduled.

Interrupting dosing of the study drug(s)should depend on the attribution, as well as on the severity and seriousness of the toxicity to the patient subject, which is to be determined at the discretion of the Investigator. In other words, how likely the toxicity is due to a specific drug, as well as how severe and serious the toxicity is to the patient-subject, should inform among other considerations (including efficacy-safety analysis), which drug or combination of drugs should be held/discontinued.

Day 1 or Day 15 of any cycle may be delayed for a maximum of **12 weeks for DKN-01 or nivolumab** to allow patients to recover from toxicity or adverse events. A treatment delay due to reasons other the toxicity (ex. because of holidays, weekends, inclement weather, or other justifiable events) should be no more than 7 calendar days and not counted as a protocol violation. If a delay of therapy of more than the above mentioned days are necessary, this must discussed with the study principle investigator.

If a significant toxicity cannot be related to a specific drug of the combination, hold both drugs until resolution. Restart each drug according to its specific toxicity management guidelines.

Dose reductions are allowed for **DKN-01** but **not for nivolumab** (please refer to guidelines below).

6.2 Dose Levels of DKN-01 and Nivolumab

Table 2a:

Dose Levels for DKN-01 if 600mg is the phase II expansion dose	
Dose Level	**Dose**
Level 1 (Starting Dose)	DKN-01 600mg IV
Level -1	DKN-01 300mg IV
Level -2	DKN-01 150mg IV

Dose Levels for DKN-01 if 300mg is the phase II expansion dose	
Dose Level	**Dose**
Level 1 (Starting Dose)	DKN-01 300mg IV
Level -1	DKN-01 150mg IV
Level -2	DKN-01 75mg IV

Table 2b:

Dose Levels for Nivolumab	
Dose Level	Dose
Level 1 (Starting Dose)	Nivolumab 240mg IV
No dose reductions allowed for nivolumab	

6.3 Hematologic Toxicity Dose Adjustments

Table 3a: Hematologic Toxicity Dose Adjustments

Neutropenia	**DKN-01**	**Nivolumab**
≤ Grade 1 (ANC <LLN-1500/mm^3)	No change in dose	No change in dose
Grade 2 (ANC <1500-1000/mm^3)	No change in dose.	No change in dose.
Grade 3 (ANC <1000-500/mm^3) Or Grade 4 (ANC<500mm^3)	Hold until < Grade 2. Resume at one dose level lower, if indicated.**	Hold until < Grade 2. Resume at same dose level, if indicated.

Neutropenia	**DKN-01**	**Nivolumab**
Second occurrence of Grade 4 (ANC <500mm^3)	Off protocol therapy	Off protocol therapy

Table 3b: Hematologic Toxicity Dose Adjustments

Thrombocytopenia	**DKN-01**	**Nivolumab**
≤ Grade 1 (Plts <LLN-75,000/mm^3)	No change in dose	No change in dose
Grade 2 (Plts <75,000-50,000/mm^3)	No change in dose.	No change in dose.
Grade 3 (Plts <50,000-25,000/mm^3) Or Grade 4 (Plts <25,000/mm^3)	Hold until ≤ Grade 2. Resume at one dose level lower, if indicated.**	Hold until ≤ Grade 2. Resume at same dose level, if indicated.
Second occurrence of Grade 4 (Plts <25,000/mm^3)	Off protocol therapy	Off protocol therapy

6.4 DKN-01 Dose Adjustments for Non-hematologic toxicities

Table 4: DKN-01 Dose Adjustments for Non-hematologic toxicities

Toxicity Occurrence	DKN-01
First*	Restart at dose level 1 (starting dose) after toxicity resolves to ≤Grade 1 or baseline grade.
Second	Restart at reduced dose level -1 after toxicity resolves to ≤Grade 1 or baseline grade.
Third	Restart at reduced dose level -2 after toxicity resolves to ≤Grade 1 or baseline grade.
Fourth	Discontinue

*If the toxicity is judged to be significant in the opinion of the Investigator or the Investigator does not wish to dose at the same dosing level, he/she may contact the Principle Investigator to consider a one dose reduction level with the first occurrence of toxicity.
**If the toxicity is judged to be not drug-related in the opinion of the Investigator or the Investigator does not wish to reduce by one dose level, he/she may contact the Principle Investigator to consider proceeding at the same dose level for the first occurrence of toxicity.

Once a patient's DKN-01 dose has been reduced, no re-escalation to a previously received dose is allowed at any time during the study. Intra-patient dose escalation is not permitted at any time during the study.

- For patients who experience an infusion reaction to DKN-01, all attempts should be made to obtain blood samples for determination of anti-DKN-01 antibody (immunogenicity sample) and serum DKN-01 levels as close to the onset of the event as possible, at the resolution of the event, and 30 days following the event or just prior to the start of new therapy, whichever comes first. In addition, these samples may be used for determination

of pharmacodynamic markers.

6.5 DKN-01 Management for Non-hematologic toxicities

Table 5: Management of DKN-01 related toxicities

System	Severity	Management	Follow-up
Gastrointestinal			
	Grade 2 or 3 nausea, vomiting, or diarrhea	Withhold DKN-01	Symptoms Resolve to ≤Grade 1 or baseline Resume DKN-01 as per **Table 4** if symptoms have improved to mild severity or resolution.
	Grade 4 nausea, vomiting, or diarrhea	Permanently discontinue DKN-01	
Liver			
	Moderate: If baseline AST and ALT≤ grade 1, then AST or ALT >3.0 to <5.0 x ULN and/or Total bilirubin >2.0 to <3.0 x ULN	Withhold DKN-01 Increase frequency of liver function test monitoring until resolution	Symptoms Resolve to ≤Grade 1 or baseline Resume DKN- 01 as per **Table 4** if liver function tests are <3.0 x ULN or return to BL and bilirubin is <2.0 x ULN or returns to BL. If AST or ALT elevation continues to be >5 x ULN OR total bilirubin > 3 x ULN, see below.
	Severe or life-threatening: AST or ALT >5.0 x ULN and/or Total bilirubin >3.0 x ULN	Permanently discontinue DKN-01 Increase frequency of liver function test monitoring until resolution	
Skin			

	Moderate: Non-localized rash (diffuse, <50% of skin surface)	Withhold DKN-01 Administer topical corticosteroids if there is no improvement of symptoms within 1 week	Symptoms Resolve to ≤Grade 1 or baseline Resume DKN- 01 as per **Table 4** if dermatitis resolves or improves to mild (localized) symptoms If symptoms worsen, see below.
	Severe or life-threatening: Stevens-Johnson syndrome, toxic epidermal necrolysis or rash complicated by full thickness dermal ulceration, or necrotic, bullous or hemorrhagic manifestations	Permanently discontinue DKN-01 Administer systemic corticosteroid therapy	
Neurologic			
	Moderate: Moderate symptoms, clinically detectable with no impact on ADLs	Withhold DKN-01	Symptoms Resolve to ≤Grade 1 or baseline Resume DKN- 01 as per **Table 4** when symptoms resolve or return to BL If symptoms worsen, see below
	Severe or life-threatening: Severe symptoms (impact on ADLs) or life threatening	Permanently discontinue DKN-01	
Endocrine			
Fatigue Headache Mental status change Abdominal pain Unusual bowel habits Hypotension Abnormal thyroid function tests and/or serum chemistries	Moderate to life threatening: Document signs and/or symptoms of dysfunction Endocrinopathies requiring hormone replacement or medical intervention AEs requiring hospitalization, urgent medical intervention or interfering with ADLs	Withhold DKN-01 Evaluate endocrine function Consider radiographic pituitary gland imaging Continue to assess as indicated Initiate appropriate hormone-replacement therapy	Symptoms Resolve to ≤Grade 1 or baseline Resume DKN- 01 as per **Table 4** when patient is stable on hormone-replacement therapy (as indicated)

Table 6: Management of DKN-01 Infusion-related reactions

Grade	Management
Grade 1	Slow the infusion rate by 50%. Monitor the patient for worsening of condition. For subsequent infusions, premedicate with diphenhydramine hydrochloride 50 mg IV (or equivalent); additional premedication may be administered at the investigator's discretion.
Grade 2	Stop the infusion. Administer diphenhydramine hydrochloride 50 mg IV (or equivalent), acetaminophen 650 mg orally for fever, and oxygen. Resume the infusion at 50% of the prior rate once the infusion reaction has resolved or decreased to Grade 1. Monitor for worsening of condition. For subsequent infusions, premedicate with diphenhydramine hydrochloride 50 mg IV (or equivalent); additional premedication may be administered at the investigator's discretion.
Grade 3	Stop the infusion and disconnect the infusion tubing from the patient. Administer diphenhydramine hydrochloride 50 mg IV (or equivalent), dexamethasone 10 mg IV (or equivalent), bronchodilators for bronchospasm, and other medications/treatment as medically indicated.
Grade 4	Stop the infusion and disconnect the infusion tubing from the patient. Administer diphenhydramine hydrochloride 50 mg IV (or equivalent), dexamethasone 10 mg IV (or equivalent), and other medications/treatment as medically indicated. Give epinephrine or bronchodilators as indicated. Hospital admission for observation may be indicated. Patients who have a Grade 4 infusion reaction with or without premedication will not receive further DKN-01 treatment, but will continue to be followed on the protocol.

Nivolumab

6.6 Management of nivolumab specific adverse events

Toxicities associated or possibly associated with nivolumab treatment should be managed according to standard medical practice. Excessive activation of the immune system is a potential risk associated with nivolumab and has been observed when nivolumab is used in combination with other immunomodulating agents. Interrupt dosing of nivolumab depending on the attribution of the toxicity, at the discretion of the investigator. During this time, treatment may continue with the other non-attributable treatment agent(s). Additional tests, such as autoimmune serology or biopsies, should be used to evaluate for a possible immunogenic etiology. Although most immune-related adverse events observed with immunomodulatory agents have been mild and self-limiting, such events should be recognized early and treated promptly to avoid potential major complications. Discontinuation of nivolumab may not have an immediate therapeutic effect and in severe cases, immune-related toxicities may require acute

management with topical corticosteroids, systemic corticosteroids or other immunosuppressive agents.

The Investigator should consider the benefit-risk balance a given patient may be experiencing prior to further administration of nivolumab. In patients who have met the criteria for permanent discontinuation, resumption of nivolumab may be considered if the patient is deriving benefit and has fully recovered from the immune-related event. Patients can be rechallenged with nivolumab only after approval has been documented by both the investigator (or an appropriate delegate) and the Principle Investigator.

6.6.1 Gastrointestinal Toxicity

Immune-related colitis has been associated with the administration of nivolumab. Management guidelines for diarrhea or colitis are provided in Table 7.

Table 7: Management Guidelines for Gastrointestinal Events (Diarrhea or colitis)

Event	Management
Diarrhea or colitis, Grade 1	• Continue nivolumab. • Initiate symptomatic treatment. • Endoscopy is recommended if symptoms persist for > 7 days. • Monitor closely.
Diarrhea or colitis, Grade 2	• Withhold nivolumab for up to 12 weeks after event onset.[a] • Initiate symptomatic treatment. • Patient referral to GI specialist is recommended. • For recurrent events or events that persist > 5 days, initiate treatment with 1–2 mg/kg/day oral prednisone or equivalent. • If event resolves to Grade 1 or better, resume nivolumab.[b] • If event does not resolve to Grade 1 or better while withholding nivolumab, permanently discontinue nivolumab and contact Principle Investigator[c]
Diarrhea or colitis, Grade 3	• Withhold nivolumab for up to 12 weeks after event onset.[a] • Refer patient to gastrointestinal specialist for evaluation and confirmatory biopsy. • Initiate treatment with 1–2 mg/kg/day intravenous methylprednisolone or equivalent and convert to 1–2 mg/kg/day oral prednisone or equivalent upon improvement. • If event resolves to Grade 1 or better, resume nivolumab[b] • If event does not resolve to Grade 1 or better while withholding nivolumab, permanently discontinue nivolumab and contact Principle Investigator[c]
Diarrhea or colitis, Grade 4	• Permanently discontinue nivolumab and contact Principle Investigator.[c]

Event	Management
	• Refer patient to gastrointestinal specialist for evaluation and confirmation biopsy. • Initiate treatment with 1–2 mg/kg/day intravenous methylprednisolone or equivalent and convert to 1–2 mg/kg/day oral prednisone or equivalent upon improvement. • If event does not improve within 48 hours after initiating corticosteroids, consider adding an immunosuppressive agent. • If event resolves to Grade 1 or better, taper corticosteroids over ≥ 1 month.

[a]Nivolumab may be withheld for a longer period of time (i.e. > 12 weeks after event onset) to allow for corticosteroids (if initiated) to be reduced to ≤ 10 mg/day oral prednisone or equivalent. The acceptable length of the extended period of time must be agreed upon by the investigator and the Principle Investigator.

[b]If corticosteroids have been initiated, they must be tapered over ≥ 1 month to ≤ 10 mg/day oral prednisone or equivalent before nivolumab can be resumed.

[c]Resumption of nivolumab may be considered in patients who are deriving benefit and have fully recovered from the immune-related event. Patients can be rechallenged with nivolumab only after approval has been documented by both the investigator (or an appropriate delegate) and the Principle Investigator.

All events of diarrhea or colitis should be thoroughly evaluated for other more common etiologies. For events of significant duration or magnitude or associated with signs of systemic inflammation or acute phase reactants (e.g., increased CRP or platelet count or bandemia): Perform sigmoidoscopy (or colonoscopy, if appropriate) with colonic biopsy with three to five specimens for standard paraffin block to check for inflammation and lymphocytic infiltrates to confirm colitis diagnosis.

6.6.2 Hepatotoxicity

Immune-related hepatitis has been associated with the administration of nivolumab.
Eligible patients must have adequate liver function, as manifested by measurements of total bilirubin and hepatic transaminase, and liver function will be monitored throughout study treatment. Patients with right upper-quadrant abdominal pain and/or unexplained nausea or vomiting should have LFTs performed immediately and reviewed before administration of the next dose of study drug.

For patients with elevated LFTs concurrent medication, viral hepatitis, and toxic or neoplastic etiologies should be considered and addressed, as appropriate.
Management guidelines for hepatic events are provided in Table 8.

Table 8: Management Guidelines for Drug- Related Hepatic Events[a]

Event	Management	Follow up for all events
Hepatic event, Grade ≥2 (if baseline AST/ALT are WNL)	• Delay dosing of Nivolumab until re-treatment criteria are met • Increase frequency of monitoring to every 3-5 days	• Resume dosing when re-treatment criteria are met **If AST or ALT levels do not improve with a dose delay of 3–5 days or if levels worsen** • Initiate steroid therapy at 0.5–2 mg/kg/d methylprednisolone or oral equivalent **For ALT or AST levels >8× ULN** • Initiate steroid therapy promptly at 1–2 mg/kg/d methylprednisolone or oral equivalent **For all patients initiating steroids** • If AST or ALT levels do not improve within 3–5 days or levels worsen after the start of steroid therapy, discuss with Principal Investigator possibility of adding mycophenolate mofetil 1 g BID • Consult gastroenterologist • Taper steroids once AST or ALT levels have declined by 1 CTCAE grade • Taper steroids slowly over at least 1 month[b] • Resume immuno-oncology therapy when AST or ALT have returned to near baseline unless the criteria for permanent discontinuation are reached[c]
Hepatic event, Grade ≥3 (if baseline AST/ALT are Grade 1)	• Delay dosing of Nivolumab until re-treatment criteria are met • Increase frequency of monitoring to every 3-5 days	
Hepatic event, AST or ALT at 2x baseline or 8x ULN (whichever is lower) (if baseline AST/ALT are grade 2)	• Delay dosing of Nivolumab until re-treatment criteria are met • Increase frequency of monitoring to every 3-5 days	

LFT= liver function tests. WNL= within normal limits

[a]Nivolumab may be withheld for a longer period of time (>12 weeks after event onset) to allow for corticosteroids (if initiated) to be reduced to ≤ 10 mg/day oral prednisone or equivalent. The acceptable length of the extended period of time must be agreed upon by the investigator and the Principle Investigator.

[b]If corticosteroids have been initiated, they must be tapered over ≥ 1 month to ≤ 10 mg/day oral prednisone or equivalent before nivolumab can be resumed.

[c]Resumption of nivolumab may be considered in patients who are deriving benefit and have fully recovered from the immune-related event. Patients can be rechallenged with nivolumab only after approval has been documented by both the investigator (or an appropriate delegate) and the Principle Investigator.

6.6.3 Dermatologic toxicity

Treatment-emergent rash has been associated with nivolumab. The majority of cases of rash were mild in severity and self-limited, with or without pruritus.
A dermatologist should evaluate persistent and/or severe rash or pruritus. A biopsy should be

considered unless contraindicated. Management guidelines for dermatologic events are provided in Table 9.

Table 9: Management Guidelines for Dermatologic Events

Event	Management
Dermatologic event, Grade 1	• Continue nivolumab. • Consider treatment with topical corticosteroids and/or other symptomatic therapy (e.g., antihistamines).
Dermatologic event, Grade 2	• Continue nivolumab. • Consider patient referral to dermatologist. • Initiate treatment with topical corticosteroids. • Consider treatment with higher-potency topical corticosteroids if event does not improve
Dermatologic event, Grade 3	• Withhold nivolumab for up to 12 weeks after event onset.[a] • Refer patient to dermatologist. • Initiate treatment with 10 mg/day oral prednisone or equivalent, increasing dose to 1–2 mg/kg/day if event does not improve within 48–72 hours. • If event resolves to Grade 1 or better, resume nivolumab.[b] • If event does not resolve to Grade 1 or better, permanently discontinue nivolumab and contact Principle Investigator.[c]
Dermatologic event, Grade 4	• Permanently discontinue nivolumab and contact Principle Investigator.[c]

[a]Nivolumab may be withheld for a period of time beyond 12 weeks to allow for corticosteroids to be reduced to ≤ 10 mg/day oral prednisone or equivalent. The acceptable length of the extended period of time must be agreed upon by the investigator and the Principle Investigator.

[b]If corticosteroids have been initiated, they must be tapered over ≥ 1 month to ≤ 10 mg/day oral prednisone or equivalent before nivolumab can be resumed.

[c]Resumption of nivolumab may be considered in patients who are deriving benefit and have fully recovered from the immune-related event. Patients can be rechallenged with nivolumab only after approval has been documented by both the investigator (or an appropriate delegate) and the Principle Investigator.

6.6.4 Endocrine toxicity (including hypophysitis)

Thyroid disorders, adrenal insufficiency, diabetes mellitus and pituitary disorders have been associated with the administration of nivolumab. Patients with unexplained symptoms such as headache, fatigue, myalgias, constipation, or mental status changes, should be investigated for the presence of thyroid, pituitary, or adrenal endocrinopathies. The patient should be referred to an endocrinologist if an endocrinopathy is suspected. Thyroid-stimulating hormone (TSH) and free T3 and T4 levels should be measured to determine whether thyroid abnormalities are

present. Pituitary hormone levels and function tests (e.g. TSH, growth hormone, luteinizing hormone, follicle-stimulating hormone, testosterone, prolactin, adrenocorticotropic hormone [ACTH] levels and ACTH stimulation test) and magnetic resonance imaging (MRI) of the brain (with detailed pituitary sections) may help to differentiate primary pituitary insufficiency from primary adrenal insufficiency
Management guidelines for endocrine events are provided in Table 10.

Table 10: Management Guidelines for Endocrine Events

Event	Management
Asymptomatic hypothyroidism	• Continue nivolumab. • Initiate treatment with thyroid replacement hormone. • Monitor TSH monthly
Symptomatic hypothyroidism	• Withhold nivolumab. • Initiate treatment with thyroid replacement hormone. • Monitor TSH monthly • Consider patient referral to endocrinologist. • Resume nivolumab when symptoms are controlled and thyroid function is improving.
Asymptomatic hyperthyroidism	• TSH ≥ 0.1 mU/L and < 0.5 mU/L: • Continue nivolumab. • Monitor TSH every 4 weeks. • TSH < 0.1 mU/L: • Follow guidelines for symptomatic hyperthyroidism.
Symptomatic hyperthyroidism	• Withhold nivolumab. • Initiate treatment with anti-thyroid drug such as methimazole or carbimazole as needed. • Consider patient referral to endocrinologist. • Resume nivolumab when symptoms are controlled and thyroid function is improving. • Permanently discontinue nivolumab and contact Principle Investigator for life-threatening immune-related hyperthyroidism.[c]
Symptomatic adrenal insufficiency, Grade 2–4	• Withhold nivolumab for up to 12 weeks after event onset.[a] • Refer patient to endocrinologist. • Perform appropriate imaging. • Initiate treatment with 1–2 mg/kg/day intravenous methylprednisolone or equivalent and convert to 1–2 mg/kg/day oral prednisone or equivalent upon improvement. • If event resolves to Grade 1 or better and patient is stable on replacement therapy resume nivolumab.[b]

Event	Management
	• If event does not resolve to Grade 1 or better or patient is not stable on replacement therapy while withholding nivolumab, permanently discontinue nivolumab and contact Principle Investigator[c]
Hyperglycemia, Grade 1 or 2	• Continue nivolumab. • Initiate treatment with insulin if needed. • Monitor for glucose control.
Hyperglycemia, Grade 3 or 4	• Withhold nivolumab. • Initiate treatment with insulin. • Monitor for glucose control. • Resume nivolumab when symptoms resolve and glucose levels are stable.
Hypophysitis pan-hypopituitarism), Grade 2-3	• Withhold nivolumab for up to 12 weeks after event onset.[a] • Refer patient to endocrinologist. • Perform brain MRI (pituitary protocol). • Initiate treatment with 1–2 mg/kg/day IV methylprednisolone or equivalent and convert to 1–2 mg/kg/day oral prednisone or equivalent upon improvement.[a] • Initiate hormone replacement therapy if clinically indicated. • If event resolves to Grade 1 or better, resume nivolumab.[b] • If event does not resolve to Grade 1 or better while withholding nivolumab, permanently discontinue nivolumab and contact Principle Investigator.[c] • For recurrent hypophysitis, treat as a Grade 4 event.
Hypophysitis (pan-hypopituitarism), Grade 4	• Permanently discontinue nivolumab and contact Principle Investigator.[c] • Refer patient to endocrinologist. • Perform brain MRI (pituitary protocol). • Initiate treatment with 1–2 mg/kg/day IV methylprednisolone or equivalent and convert to 1–2 mg/kg/day oral prednisone or equivalent upon improvement.[a] • Initiate hormone replacement therapy if clinically indicated.

TSH = thyroid-stimulating hormone;

Event	Management

[a]Nivolumab may be withheld for a longer period of time (i.e., >12 weeks after event onset) to allow for corticosteroids (if initiated) to be reduced to ≤ 10 mg/day oral prednisone or equivalent. The acceptable length of the extended period of time must be agreed upon by the investigator and the Principle Investigator.

[b]If corticosteroids have been initiated, they must be tapered over ≥ 1 month to ≤ 10 mg/day oral prednisone or equivalent before nivolumab can be resumed.

[c]Resumption of nivolumab may be considered in patients who are deriving benefit and have fully recovered from the immune-related event. Patients can be rechallenged with nivolumab only after approval has been documented by both the investigator (or an appropriate delegate) and the Principle Investigator.

6.6.5 Pulmonary toxicity

Dyspnea, cough, fatigue, hypoxia, pneumonitis and pulmonary infiltrates have been associated with the administration of nivolumab. Patients will be assessed for pulmonary signs and symptoms throughout the study and will also have CT scan of the chest performed at every tumor assessment.

All pulmonary events should be thoroughly evaluated for other commonly reported etiologies such as pneumonia or other infection, lymphangitic carcinomatosis, pulmonary embolism, heart failure, chronic obstructive pulmonary disease, or pulmonary hypertension.

Management guidelines for pulmonary events are provided in Table 11.

Table 11: Management Guidelines for Pulmonary Events, Including Pneumonitis

Event	Management
Pulmonary event, Grade 1	• Continue nivolumab and monitor closely. • Re-evaluate on serial imaging. • Consider patient referral to pulmonary specialist.
Pulmonary event, Grade 2	• Withhold nivolumab for up to 12 weeks after event onset.[a] • Refer patient to pulmonary and infectious disease specialists and consider bronchoscopy or BAL. • Initiate treatment with 1–2 mg/kg/day oral prednisone or equivalent. • If event resolves to Grade 1 or better, resume nivolumab.[b] • if event does not resolve to Grade 1 or better while withholding nivolumab, permanently discontinue nivolumab and contact Principle Investigator.[c] • For recurrent events, treat as a Grade 3 or 4 event.
Pulmonary event, Grade 3 or 4	• Permanently discontinue nivolumab and contact Principle Investigator.[c] • Bronchoscopy or BAL is recommended. • Initiate treatment with 1–2 mg/kg/day oral prednisone or equivalent.

Event	Management
	• If event does not improve within 48 hours after initiating corticosteroids, consider adding an immunosuppressive agent. • If event resolves to Grade 1 or better, taper corticosteroids over ≥ 1 month.

BAL= bronchoscopic alveolar lavage; IVIG = intravenous immunoglobulin

[a]Nivolumab may be withheld for a longer period of time (i.e. >12 weeks after event onset) to allow for corticosteroids (if initiated) to be reduced to ≤ 10 mg/day oral prednisone or equivalent. The acceptable length of the extended period of time must be agreed upon by the investigator and the Principle Investigator.

[b]If corticosteroids have been initiated, they must be tapered over ≥ 1 month to ≤ 10 mg/day oral prednisone or equivalent before nivolumab can be resumed.

[c]Resumption of nivolumab may be considered in patients who are deriving benefit and have fully recovered from the immune-related event. Patients can be rechallenged with nivolumab only after approval has been documented by both the investigator (or an appropriate delegate) and the Principle Investigator.

6.6.6 Pancreatic Toxicity

Symptoms of abdominal pain associated with elevations of amylase and lipase, suggestive of pancreatitis, have been associated with the administration of nivolumab. The differential diagnosis of acute abdominal pain should include pancreatitis. Appropriate workup should include an evaluation for ductal obstruction, as well as serum amylase and lipase tests. Management guidelines for pancreatic events, including pancreatitis, are provided in Table 12.

Table 12: Management Guidelines for Pancreatic Events, Including Pancreatitis

Event	Management
Amylase and lipase elevation, Grade 2 or asymptomatic grade 3	• Continue nivolumab. • Monitor amylase and lipase weekly. • For prolonged elevation (e.g., > 3 weeks), consider treatment with 10 mg/day oral prednisone or equivalent.
Symptomatic grade 3 amylase and/or lipase elevation or any grade 4 amylase and/or lipase elevation	• Withhold nivolumab for up to 12 weeks after event onset.[a] • Refer patient to gastrointestinal specialist. • Monitor amylase and lipase every other day. • If no improvement, consider treatment with 1–2 mg/kg/day oral prednisone or equivalent. • If event resolves to Grade 1 or better, resume nivolumab.[b] • If event does not resolve to Grade 1 or better while withholding nivolumab, permanently discontinue nivolumab and contact Principle Investigator.[c] • For recurrent events, permanently discontinue nivolumab and contact Principle Investigator.[c]

Event	Management
Immune-related pancreatitis, Grade 2 or 3	• Withhold nivolumab for up to 12 weeks after event onset.[a] • Refer patient to gastrointestinal specialist. • Initiate treatment with 1–2 mg/kg/day intravenous methylprednisolone or equivalent and convert to 1–2 mg/kg/day oral prednisone or equivalent upon improvement. • If event resolves to Grade 1 or better, resume nivolumabb • If event does not resolve to Grade 1 or better while withholding nivolumab permanently discontinue nivolumab and contact Principle Investigator.[c] • For recurrent events, permanently discontinue nivolumab and contact Principle Investigator.[c]
Immune-related pancreatitis, Grade 4	• Permanently discontinue nivolumab and contact Principle Investigator.[c] • Refer patient to gastrointestinal specialist. • Initiate treatment with 1–2 mg/kg/day intravenous methylprednisolone or equivalent and convert to 1–2 mg/kg/day oral prednisone or equivalent upon improvement. • If event does not improve within 48 hours after initiating corticosteroids, consider adding an immunosuppressive agent. • If event resolves to Grade 1 or better, taper corticosteroids over ≥ 1 month.

[a]Nivolumab may be withheld for a longer period of time (i.e. > 12 weeks after event onset) to allow for corticosteroids (if initiated) to be reduced to ≤ 10 mg/day oral prednisone or equivalent. The acceptable length of the extended period of time must be agreed upon by the investigator and the Principle Investigator.

[b]If corticosteroids have been initiated, they must be tapered over ≥ 1 month to ≤ 10 mg/day oral prednisone or equivalent before nivolumab can be resumed.

[c]Resumption of nivolumab may be considered in patients who are deriving benefit and have fully recovered from the immune-related event. Patients can be rechallenged with nivolumab only after approval has been documented by both the investigator (or an appropriate delegate) and the Principle Investigator.

6.6.7 Ocular toxicity

An ophthalmologist should evaluate visual complaints (e.g., uveitis, retinal events). Management guidelines for ocular events are provided in Table 13.

Table 13: Management Guidelines for Ocular Events

Event	Management
Ocular event, Grade 1	• Continue nivolumab. • Patient referral to ophthalmologist is strongly recommended. • Initiate treatment with topical corticosteroid eye drops and topical immunosuppressive therapy.

Event	Management
	• If symptoms persist, treat as a Grade 2 event.
Ocular event, Grade 2	• Withhold nivolumab for up to 12 weeks after event onset.[a] • Patient referral to ophthalmologist is strongly recommended. • Initiate treatment with topical corticosteroid eye drops and topical immunosuppressive therapy. • If event resolves to Grade 1 or better, resume nivolumab.[b] • If event does not resolve to Grade 1 or better while withholding nivolumab permanently discontinue nivolumab and contact Principle Investigator [c]
Ocular event, Grade 3 or 4	• Permanently discontinue nivolumab and contact Principle Investigator.[c] • Refer patient to ophthalmologist. • Initiate treatment with 1–2 mg/kg/day oral prednisone or equivalent. • If event resolves to Grade 1 or better, taper corticosteroids over ≥ 1 month.

[a]Nivolumab may be withheld for a period of time (i.e. >12 weeks after event onset) to allow for corticosteroids (if initiated) to be reduced to ≤ 10 mg/day oral prednisone or equivalent. The acceptable length of the extended period of time must be agreed upon by the investigator and the Principle Investigator.

[b]If corticosteroids have been initiated, they must be tapered over ≥ 1 month to ≤ 10 mg/day oral prednisone or equivalent before nivolumab can be resumed.

[c]Resumption of nivolumab may be considered in patients who are deriving benefit and have fully recovered from the immune-related event. Patients can be rechallenged with nivolumab only after approval has been documented by both the investigator (or an appropriate delegate) and the Principle Investigator.

6.6.8 Immune-Related Myocarditis

Immune-related myocarditis has been associated with the administration of nivolumab. Immune-related myocarditis should be suspected in any patient presenting with signs or symptoms suggestive of myocarditis, including, but not limited to, dyspnea, chest pain, palpitations, fatigue, decreased exercise tolerance, or syncope. Immune-related myocarditis needs to be distinguished from myocarditis resulting from infection (commonly viral, e.g. in a patient who reports a recent history of gastrointestinal illness), ischemic events, underlying arrhythmias, exacerbation of pre-existing cardiac conditions, or progression of malignancy.

All patients with possible myocarditis should be urgently evaluated by performing cardiac enzyme assessment, an ECG, a chest X-ray, an echocardiogram, and a cardiac MRI as appropriate per institutional guidelines. A cardiologist should be consulted. An endomyocardial biopsy may be considered to enable a definitive diagnosis and appropriate treatment, if clinically indicated.

Patients with signs and symptoms of myocarditis, in the absence of an identified alternate etiology, should be treated according to the guidelines in Table 14.

Table 14: Management Guidelines for Immune-Related Myocarditis

Event	Management
Immune-related myocarditis, Grade 1	• Refer patient to cardiologist • Initiate treatment as per institutional guidelines.
Immune-related myocarditis, Grade 2	• Withhold nivolumab for up to 12 weeks after event onset a and contact EORTC Principle Investigator. • Refer patient to cardiologist • Initiate treatment as per institutional guidelines and consider antiarrhythmic drugs, temporary pacemaker, ECMO, or VAD as appropriate. • Consider treatment with 1–2 mg/kg/day IV methylprednisolone or equivalent and convert to 1–2 mg/kg/day oral prednisone or equivalent upon improvement.[a] • If event resolves to Grade 1 or better, resume nivolumab.[b] • If event does not resolve to Grade 1 or better while withholding nivolumab, permanently discontinue nivolumab and contact Principle Investigator.[c]
Immune-related myocarditis, Grade 3-4	• Permanently discontinue nivolumab and contact Principle Investigator.[c] • Refer patient to cardiologist • Initiate treatment as per institutional guidelines and consider antiarrhythmic drugs, temporary pacemaker, ECMO, or VAD as appropriate. • Initiate treatment with 1–2 mg/kg/day IV methylprednisolone or equivalent and convert to 1–2 mg/kg/day oral prednisone or equivalent upon improvement.[a,b] • If event does not improve within 48 hours after initiating corticosteroids, consider adding an immunosuppressive agent. • If event resolves to Grade 1 or better, taper corticosteroids over ≥ 1 month.

ECMO = extracorporeal membrane oxygenation; VAD = ventricular assist device;
IV = intravenous.

[a]Nivolumab may be withheld for a longer period of time (i.e., > 12 weeks after event onset) to allow for corticosteroids (if initiated) to be reduced to ≤ 10 mg/day oral prednisone or equivalent. The acceptable length of the extended period of time must be agreed upon by the investigator and the Principle Investigator.

[b]If corticosteroids have been initiated, they must be tapered over ≥ 1 month to ≤ 10 mg/day oral prednisone or equivalent before nivolumab can be resumed.

Event	Management

[c]Resumption of nivolumab may be considered in patients who are deriving benefit and have fully recovered from the immune-related event. Patients can be rechallenged with nivolumab only after approval has been documented by both the investigator (or an appropriate delegate) and the Principle Investigator.

6.6.9 Infusion-Related Reactions

No premedication is indicated for administration of Cycle 1 of nivolumab. However,patients who experience an infusion-related reaction with Cycle 1 of nivolumab may receive premedication with antihistamines or antipyretics/analgesics (e.g. acetaminophen) for subsequent infusions.
Note: Metamizole (dipyrone) is prohibited in treating nivolumab associated infusion-related reactions, due to its potential for causing agranulocytosis.
Guidelines for medical management of infusion-related reactions during Cycle 1 are provided in table 15. For subsequent cycles, infusion-related reactions should be managed according to institutional guidelines.

Table 15: Management Guidelines for Infusion-Related Reactions

Event	Management
IRR, Grade 1	• Reduce infusion rate to half the rate being given at the time of event onset. • After the event has resolved, the investigator should wait for 30 minutes while delivering the infusion at the reduced rate. • If the infusion is tolerated at the reduced rate for 30 minutes after symptoms have resolved, the infusion rate may be increased to the original rate.
IRR, Grade 2	• Interrupt nivolumab infusion. • Administer aggressive symptomatic treatment (e.g., oral or IV antihistamine, anti-pyretic, glucocorticoids, epinephrine, bronchodilators, oxygen). • After symptoms have resolved to baseline, resume infusion at half the rate being given at the time of event onset. • For subsequent infusions, consider administration of oral premedication with antihistamines, anti-pyretics and/or analgesics and monitor closely for IRRs.
IRR, Grade 3 or 4	• Stop infusion. • Administer aggressive symptomatic treatment (e.g., oral or IV antihistamine, anti-pyretic, glucocorticoids, epinephrine, bronchodilators, oxygen). • Permanently discontinue nivolumab and contact Principle Investigator.[a]

IRR = infusion-related reaction; IV = intravenous.

Event	Management

[a]Resumption of nivolumab may be considered in patients who are deriving benefit and have fully recovered from the immune-related event. Patients can be rechallenged with nivolumab only after approval has been documented by both the investigator (or an appropriate delegate) and the Principle Investigator.

6.6.10 Neurologic Disorders

Myasthenia gravis and Guillain-Barré syndrome have been observed with single-agent nivolumab. Patients may present with signs and symptoms of sensory and/or motor neuropathy. Diagnostic work-up is essential for an accurate characterization to differentiate between alternative etiologies. Management guidelines for neurologic disorders are provided in Table 16.

Table 16: Management Guidelines for Neurologic Disorders

Event	Management
Immune-related neuropathy, Grade 1	• Continue nivolumab. • Investigate etiology.
Immune-related neuropathy, Grade 2	• Withhold nivolumab for up to 12 weeks after event onset.[a] • Investigate etiology. • Initiate treatment as per institutional guidelines. • If event resolves to Grade 1 or better, resume nivolumab[b] • If event does not resolve to Grade 1 or better, permanently discontinue nivolumab and contact Principle Investigator[c]
Immune-related neuropathy, Grade 3 or 4	• Permanently discontinue nivolumab and contact Principle Investigator.[c] • Initiate treatment as per institutional guidelines.
Myasthenia gravis and Guillain-Barré syndrome (any grade)	• Permanently discontinue nivolumab and contact Principle Investigator.[c] • Refer patient to neurologist. • Initiate treatment as per institutional guidelines. • Consider initiation of 1–2 mg/kg/day oral or intravenous prednisone or equivalent.

[a]Nivolumab may be withheld for a longer period of time (i.e. >12 weeks after event onset) to allow for corticosteroids (if initated) to be reduced to ≤ 10 mg/day oral prednisone or equivalent. The acceptable length of the extended period of time must be agreed upon by the investigator and the Principle Investigator.

[b]If corticosteroids have been initiated, they must be tapered over ≥ 1 month to ≤ 10 mg/day oral prednisone or equivalent before nivolumab can be resumed.

[c]Resumption of nivolumab may be considered in patients who are deriving benefit and have fully recovered from the immune-related event. Patients can be rechallenged with nivolumab only after approval has been documented by both the investigator (or an appropriate delegate) and the Principle Investigator.

6.6.11 Immune-Related Meningoencephalitis

Immune-related meningoencephalitis is an identified risk associated with the administration of nivolumab. Immune-related meningoencephalitis should be suspected in any patient presenting with signs or symptoms suggestive of meningitis or encephalitis, including, but not limited to, headache, neck pain, confusion, seizure, motor or sensory dysfunction, and altered or depressed level of consciousness. Encephalopathy from metabolic or electrolyte imbalances needs to be distinguished from potential meningoencephalitis resulting from infection (bacterial, viral, or fungal) or progression of malignancy, or secondary to a paraneoplastic process.

All patients being considered for meningoencephalitis should be urgently evaluated with a CT scan and/or MRI scan of the brain to evaluate for metastasis, inflammation, or edema. If deemed safe by the treating physician, a lumbar puncture should be performed and a neurologist should be consulted.

Patients with signs and symptoms of meningoencephalitis, in the absence of an identified alternate etiology, should be treated according to the guidelines in Table 17.

Table 17: Management Guidelines for Immune-Related Meningoencephalitis

Event	Management
Immune-related meningoencephalitis, all grades	• Permanently discontinue nivolumab and contact Principle Investigator.[a] • Refer patient to neurologist. • Initiate treatment with 1–2 mg/kg/day IV methylprednisolone or equivalent and convert to 1–2 mg/kg/day oral prednisone or equivalent upon improvement. • If event does not improve within 48 hours after initiating corticosteroids, consider adding an immunosuppressive agent. • If event resolves to Grade 1 or better, taper corticosteroids over ≥ 1 month.

IV = intravenous.

[a]Resumption of nivolumab may be considered in patients who are deriving benefit and have fully recovered from the immune-related event. Patients can be rechallenged with nivolumab only after approval has been documented by both the investigator (or an appropriate delegate) and the Principle Investigator.

6.6.12 Immune-Related Nephritis

Immune-related nephritis is an identified risk associated with the administration of nivolumab. Immune-related nephritis has to be suspected if patient shows discolored urine and symptomatic elevation of creatinine.

Patients with signs and symptoms of nephritis, in the absence of an identified alternate etiology should be treated according to the guidelines in.

7. ADVERSE EVENTS: LIST AND REPORTING REQUIREMENTS

DEFINITIONS

ADVERSE EVENTS

An Adverse Event (AE) is defined as any new untoward medical occurrence or worsening of a preexisting medical condition in a clinical investigation participant administered study drug and that does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (such as an abnormal laboratory finding), symptom, or disease temporally associated with the use of investigational product, whether or not considered related to the investigational product.

A ***non-serious adverse event*** is an AE not classified as serious.

The protocol must include a definition for Serious Adverse Events (SAE)**.**

SERIOUS ADVERSE EVENTS

A **S*erious Adverse Event (SAE)*** is any untoward medical occurrence that at any dose:

- results in death
- is life-threatening (defined as an event in which the participant was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe)
- requires inpatient hospitalization or causes prolongation of existing hospitalization (see **NOTE** below)
- results in persistent or significant disability/incapacity
- is an important medical event (defined as a medical event(s) that may not be immediately life-threatening or result in death or hospitalization but, based upon appropriate medical and scientific judgment, may jeopardize the subject or may require intervention [eg, medical, surgical] to prevent one of the other serious outcomes listed in the definition above.) Examples of such events include, but are not limited to, intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalization.)
- Suspected transmission of an infectious agent (eg, pathogenic or nonpathogenic) via the study drug is an SAE.

Although pregnancy and potential drug-induced liver injury (DILI), are not always serious by regulatory definition, however, these events must be reported within the SAEs timeline.

Any component of a study endpoint that is considered related to study therapy should be reported as an SAE (eg, death is an endpoint, if death occurred due to anaphylaxis, anaphylaxis must be reported).

NOTE: (PI determines if this information regarding hospitalizations are considered SAEs and should be included in the protocol. This is supplemental information that is included in BMS-sponsored trials)

The following hospitalizations are not considered SAEs in BMS clinical studies:

- a visit to the emergency room or other hospital department < 24 hours, that does not result in admission (unless considered an important medical or life-threatening event)
- elective surgery, planned prior to signing consent
- admissions as per protocol for a planned medical/surgical procedure
- routine health assessment requiring admission for baseline/trending of health status (eg, routine colonoscopy)
- Medical/surgical admission other than to remedy ill health and planned prior to entry into the study. Appropriate documentation is required in these cases.

– Admission encountered for another life circumstance that carries no bearing on health status and requires no medical/surgical intervention (eg, lack of housing, economic inadequacy, caregiver respite, family circumstances, administrative reason).

Admission for administration of anticancer therapy in the absence of any other SAEs (applies to oncology protocols)

ADVERSE EVENT Collection and REPORTING INFORMATION:

- All Serious Adverse Events (SAEs) that occur following the subject's written consent to participate in the study through 100 days of discontinuation of dosing must be reported to BMS Worldwide Safety and Leap Therapeutics, whether related or not related to study drug. If applicable, SAEs must be collected that relate to any later protocol-specified procedure (eg, a follow-up skin biopsy).
    - ✓ **BMS Worldwide Safety Contact Information**
        - Worldwide.Safety@BMS.com
        - +1 609-818-3804
    - ✓ **Leap Therapeutics**
        - DKN_Safety@leaptx.com
        - Safety-Inbox@novellaclinical.com
- Following the subject's written consent to participate in the study, all SAEs, whether related or not related to study drug, are collected, including those thought to be associated with protocol-specified procedures. The investigator should report any SAE occurring after these aforementioned time periods, which is believed to be related to study drug or protocol-specified procedure.
- An SAE report should be completed for any event where doubt exists regarding its seriousness;
- If the investigator believes that an SAE is not related to study drug, but is potentially related to the conditions of the study (such as withdrawal of previous therapy or a complication of a study procedure), the relationship should be specified in the narrative section of the SAE Report Form.

An appropriate SAE form (e.g. ex-US = CIOMS form or USA = Medwatch form) should be used to report SAEs to BMS. If you prefer to use your own Institutional form, it must be reviewed by the BMS Protocol Manager prior to study initiation to ensure that at a minimum all of the data elements on the CIOMS form are present. Note: Please include the BMS Protocol number on the SAE form or on the cover sheet with the SAE form transmission.

- ✓ The CIOMS form is available at: http://www.cioms.ch/index.php/cioms-form-i
- ✓ The MedWatch form is available at: MedWatch 3500 Form

- The Sponsor will reconcile the clinical database AE cases (**case level only**) transmitted to BMS Global Pharmacovigilance (Worldwide.Safety@bms.com).
    - The Investigator will request from BMS GPV&E, aepbusinessprocess@bms.com the SAE reconciliation report and include the BMS protocol number every 3 months and prior to data base lock or final data summary
    - GPV&E will send the investigator the report to verify and confirm all SAEs have been transmitted to BMS GPV&E.
    - The data elements listed on the GPV&E reconciliation report will be used for case identification purposes. If the Investigator determines a case was not transmitted to BMS GPV&E, the case should be sent immediately to BMS (Worldwide.Safety@bms.com).

- In addition to the Sponsor Investigator's responsibility to report events to their local HA, suspected serious adverse reactions (whether expected or unexpected) shall be reported by BMS to the relevant competent health authorities in all concerned countries according to local regulations (either as expedited and/or in aggregate reports).
- In accordance with local regulations, BMS will notify sponsor investigators of all reported SAEs that are suspected (related to the investigational product) and unexpected (ie, not previously described in the IB). An event meeting these criteria is termed a Suspected, Unexpected Serious Adverse Reaction (SUSAR). Sponsor investigator notification of these events will be in the form of either a SUSAR Report or a Semi-Annual SUSAR Report.
    - ✓ Other important findings which may be reported by BMS as an Expedited Safety Report (ESR) include: increased frequency of a clinically significant expected SAE, an SAE considered associated with study procedures that could modify the conduct of the study, lack of efficacy that poses significant hazard to study subjects, clinically significant safety finding from a nonclinical (eg, animal) study, important safety recommendations from a study data monitoring committee, or sponsor or BMS decision to end or temporarily halt a clinical study for safety reasons.
    - ✓ Upon receiving an ESR from BMS, the investigator must review and retain the ESR with the IB. Where required by local regulations or when there is a central IRB/IEC for the study, the sponsor will submit the ESR to the appropriate IRB/IEC. The investigator and IRB/IEC will determine if the informed consent requires revision. The investigator should also comply with the IRB/IEC procedures for reporting any other safety information.

SAEs, whether related or not related to study drug, and pregnancies must be reported to BMS within 24 hours \ 1 Business Day of becoming aware of the event. SAEs must be recorded on either CIOMS, MedWatch, or approved site SAE form.

Pregnancies must be reported and submitted to BMS. BMS will perform due diligence follow-up using the BMS Pregnancy Form which the investigator must complete.

SAE Email Address: Worldwide.Safety@BMS.com

SAE Facsimile Number: +1 609-818-3804

If only limited information is initially available, follow-up reports are required. (Note: Follow-up SAE reports should include the same investigator term(s) initially reported.)

If an ongoing SAE changes in its intensity or relationship to study drug or if new information becomes available, a follow-up SAE report should be sent within 24 hours \ 1 Business Day to BMS using the same procedure used for transmitting the initial SAE report.

All SAEs should be followed to resolution or stabilization.

The causal relationship to study drug is determined by a physician and should be used to assess all adverse events (AE). The casual relationship can be one of the following:

Related: There is a reasonable causal relationship between study drug administration and the AE.

Not related: There is not a reasonable causal relationship between study drug administration and the AE.

The term "reasonable causal relationship" means there is evidence to suggest a causal relationship.

Adverse events can be spontaneously reported or elicited during open-ended questioning, examination, or evaluation of a subject. (In order to prevent reporting bias, subjects should not be questioned regarding the specific occurrence of one or more AEs.)

NONSERIOUS ADVERSE EVENT

- Non-serious Adverse Events (AE) are to be provided to BMS in aggregate via interim or final study reports as specified in the agreement or, if a regulatory requirement [eg, IND US trial] as part of an annual reporting requirement.
- Non-serious AE information should also be collected from following the subject's written consent to participate in the study.

Non-serious Adverse Event Collection and Reporting

The collection of non-serious AE information should begin following the subject's written consent to participate in the study. All non-serious adverse events (not only those deemed to be treatment-related) should be collected continuously during the treatment period and for a minimum of 100 days following the last dose of study treatment.

Non-serious AEs should be followed to resolution or stabilization, or reported as SAEs if they become serious. Follow-up is also required for non-serious AEs that cause interruption or discontinuation of study drug and for those present at the end of study treatment as appropriate.

Laboratory Test Abnormalities

All laboratory test results captured as part of the study should be recorded following institutional procedures. Test results that constitute SAEs should be documented and reported to BMS as such.

The following laboratory abnormalities should be documented and reported appropriately:

- any laboratory test result that is clinically significant or meets the definition of an SAE
- any laboratory abnormality that required the participant to have study drug discontinued or interrupted
- any laboratory abnormality that required the subject to receive specific corrective therapy.

It is expected that wherever possible, the clinical rather than laboratory term would be used by the reporting investigator (eg, anemia versus low hemoglobin value).

Drug Induced Liver Injury (DILI):

Wherever possible, timely confirmation of initial liver-related laboratory abnormalities should occur prior to the reporting of a potential DILI event. All occurrences of potential DILIs, meeting the defined criteria, must be reported as SAEs.

Potential drug induced liver injury is defined as:

1) AT (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 AT 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.

AEs of Special Interest (Product Specific Usually a regulatory requirement. Remove if not applicable)

Pregnancy

If, following initiation of the investigational product, it is subsequently discovered that a study participant is pregnant or may have been pregnant at the time of investigational product exposure, including during at least 5 half-lives after product administration, the investigational product will be permanently discontinued in an appropriate manner (eg, dose tapering if necessary for participant).

The investigator must immediately notify Worldwide.Safety@bms.com of this event and complete one of the following forms within 24 hours of awareness of the event via either the CIOMS, MedWatch or appropriate Pregnancy Surveillance Form in accordance with SAE reporting procedures.

Protocol-required procedures for study discontinuation and follow-up must be performed on the participant.

Follow-up information regarding the course of the pregnancy, including perinatal and neonatal outcome and, where applicable, offspring information must be reported on the CIOMS, MedWatch, BMS Pregnancy Surveillance Form, **or** approved site SAE form. A BMS Pregnancy Surveillance Form may be provided upon request.

Any pregnancy that occurs in a female partner of a male study participant should be reported to BMS. Information on this pregnancy will be collected on the Pregnancy Surveillance Form. In order for Sponsor or designee to collect any pregnancy surveillance information from the female partner, the female partner must sign an informed consent form for disclosure of this information.

Other Safety Considerations

Any significant worsening noted during interim or final physical examinations, electrocardiograms, X-rays, and any other potential safety assessments, whether or not these procedures are required by the protocol, should also be recorded as a non-serious or serious AE, as appropriate, and reported accordingly.

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 Expected Toxicities

See investigator's brochure for DKN-01 and Nivolumab for full and up-to-date list of expected toxicities.

7.1.1 Adverse Events Lists

7.1.1.1 Adverse Event List(s) for DKN-01

Risks associated with DKN-01 therapy. Events MAY BE SERIOUS (i.e. causing hospitalization, life-threatening, or where noted, may cause death) and include the following:

Very Common (>10%)
• Fatigue
• Nausea
Common (>1% and <10%)
• Vomiting
• Anemia (decrease in the amount of red blood cells in the blood)
• Decreased appetite
• Arthralgia (joint pain)
• Dysgeusia (changes in sense of taste)
• Hyponatremia* (decrease in blood sodium level)
• Lymphopenia* (decrease of lymphocyte concentration in blood)
• • Constipation
•
• Leukopenia (decreased white blood cell level)
• Muscle Spasms
• • Myalgia (muscle pain)
•
• • Oral pain
• Diarrhea (watery, loose or soft stools)
• Dizziness
• Abdominal distension (swelling)
• Abnormal liver function test (alanine aminotransferase and/or aspartate aminotransferase increased) which possibly can lead to liver damage
• Headache
• Low levels of phosphorous in the blood (hypophosphatemia)
• Muscle weakness
• Weakness (asthenia)
• Blood cholesterol increased
• Chills
• Indigestion (dyspepsia)
• Shortness of breath (dyspnea)
• Trouble sleeping (insomnia)
• Abnormal blood test that can indicate longer than normal blood clotting time (increased international normalized ratio)

• Excess protein in the urine (proteinuria) which may cause fluid retention
• Decreased number of platelets in the blood (thrombocytopenia). A low platelet count increases your risk of bleeding including: nosebleeds, bruising, stroke, and/or digestive system bleeding. If your platelet count it too low, you may need a platelet transfusion.

•
• Stomatitis (inflammation in the mouth)
• Weight decreased

* Severe

Risks associated with DKN-01 therapy when given in combination with a PD-1 inhibitor. Events MAY BE SERIOUS (i.e. causing hospitalization, life-threatening, or where noted, may cause death), and include the following:

Very Common (≥10%)

- Aspartate aminotransferase increased
- Fatigue
- Blood alkaline phosphatase increased (an enzyme from liver and bone), which may cause fatigue and yellowing of the skin and eyes

Common (≥1% and <10%)

- Alanine aminotransferase increased
- Lymphopenia* (low level of lymphocytes in blood)
- Anemia
- Hypocalcemia (low calcium concentration in the blood)
- Nausea
- Abdominal distension
- Anemia
- Arthralgia
- Diarrhea*
- Dysphonia (difficulty speaking)
- Dyspnea (Shortness of breath)
- Flushing
- Hypoalbuminemia
- Hyponatremia (low sodium level in blood)
- Hypophosphatemia (low level of phosphorous in blood)
- Leukopenia (decreased white blood cells)
- Neuropathy peripheral
- Night sweat
- Decreased appetite
- Blood creatinine increased which may indicate kidney injury
- Blood bilirubin increased (an abnormal liver test) which may cause yellowing of the skin and/or eyes

- Hypokalemia (low level of potassium in the blood)
- Myalgia (muscle pain)
- Generalized itching (pruritus)
- Fever (pyrexia)
- Generalized rash
- Vomiting
- Abdominal pain
- Weakness (asthenia)
- Dehydration
- Dizziness
- Dysgeusia (abnormal taste or sense of taste)
- Eczema
- Gamma-glutanyl transferase increased (abnormal liver test) which suggests possible liver damage
- Homer's Syndrome, which may cause decreased pupil size, a dropping eyelid and decreased sweating on the affected side of the face
- High blood sugar (hyperglycemia)
- High blood pressure (hypertension)
- Hypomagenseaemia (low levels of magnesium in blood)
- Influenza (Flu) like illness
- Pink or Purple Spots on the skin (Lichen planus)
- Muscle spasms
- Muscle spasticity
- Musculoskeletal pain
- Neutrophil count decreased (low white blood cell count) which can increase the risk of infection including fever, pain, redness, and/or difficult breathing. These symptoms may be serious or life threatening
- Painless detachment of the nail from the nail bed (onychoclasis)
- Low blood pressure after standing up (orthostatic hypotension)
- Excess fluid in the lungs (pleural effusion)
- Pneumonia
- High levels of protein in the urine (proteinuia) which may cause fluid retention
- Rash that is red and may be flat and/or have bumps (Rash maculopapular)
- Mouth sores or swelling (stomatitis)
- Fainting (syncope)
- Upper-airway cough syndrome
-

***** Severe

7.1.1.2 Adverse Event List for Nivolumab

Nivolumab may cause the side effects listed below, severity or duration of these side effects may vary for each participant. This information is based on data from

subjects in other clinical trials with nivolumab. In addition, there may be side effects that are not yet known that may occur.

Very common side effects of nivolumab are (≥ 10%)

- Diarrhea
- Fatigue
- Itching
- Rash

Common side effects of nivolumab include (≥1 to < 10%)

- Abdominal pain
- Alkaline phosphatase increased: lab test result associated with liver or bone abnormalities
- Allergic reaction/hypersensitivity
- ALT increased: lab test result associated with abnormal liver function
- Amylase increased: lab test result associated with pancreas inflammation
- AST increased: lab test result associated with abnormal liver function
- Bilirubin (liver function blood test) increased
- Chills
- Constipation
- Cough
- Creatinine increased: lab test result associated with decreased kidney Function
- Decreased appetite
- Dizziness or Vertigo (feeling off balance which can lead to dizziness)
- Dry mouth
- Dry skin
- Fever
- Headache
- High blood pressure
- Increased blood sugar
- Inflammation of the colon
- Inflammation of the mouth
- Infusion related reaction
- Joint pain or stiffness
- Lipase increased: lab test result associated with pancreas inflammation
- Loss of color (pigment) from areas of skin
- Lung inflammation (pneumonitis - see details below)
- Musculoskeletal pain
- Nausea
- Redness (of the skin)
- Shortness of breath
- Sodium levels in the blood low
- Swelling, including face, arms, and legs
- Thyroid gland function decreased (hypothyroidism). It is a condition where

- your thyroid gland doesn't produce enough hormones. The common signs
- of an underactive thyroid are tiredness, weight gain and feeling
- depressed.
- Thyroid gland function increased (hyperthryoidism). It is a condition in
- which the thyroid overproduces hormones. The common symptoms
- include weight loss, weakness, irregular heartbeat, and difficulty sleeping
- Tingling, burning, numbness or weakness, possibly in arms, legs, hands
- and feet
- Upper respiratory tract infection
- Vomiting

Uncommon side effects of nivolumab include (≥0.1 to < 1%)

- Adrenal gland function decreased
- Bronchitis
- Cranial nerve disorder
- Diabetes
- Dry eye
- Hair loss
- Heart rate increased
- Heart rhythm abnormal
- Hives
- Inflammation of the eye
- Inflammation of the kidney
- Inflammation of the pancreas
- Inflammation of the pituitary gland
- Inflammation of the stomach
- Inflammation of the thyroid gland
- Liver inflammation
- Low blood pressure
- Lung infiltrates, associated with infection or inflammation
- Pituitary gland function decreased
- Psoriasis: characterized by patches of abnormal, scaly skin
- Renal (kidney) failure or kidney injury
- Respiratory failure
- Vision blurred

Rare side effects of nivolumab include (≥0.01 to < 0.1%)

- Anaphylactic reaction (severe allergic reaction)
- Damage to the protective covering of the nerves in the brain and spinal
- cord
- Diabetes complications resulting in excess blood acids and diabetic coma
- Guillain-Barre syndrome, an autoimmune disorder associated with
- progressive muscle weakness or paralysis
- Inflammation of blood vessels

- Inflammation of the brain, potentially life-threatening or fatal
- Inflammation of the heart
- Muscle inflammation
- Myasthenic syndrome (neurologic syndrome characterized by muscle
- weakness) including myasthenia gravis, a nerve disease that may cause
- weakness of eye, face, breathing, and swallowing muscles.

- Pemphigoid: blistering of the skin or mouth caused by the immune system
- attacking healthy tissue
- Rhabdomyolysis: muscle fiber released into the blood stream which could
- damage your kidneys
- Rosacea: acne-like skin condition resulting in redness of face
- Sarcoidosis, a disease involving abnormal collections of inflammatory cells
- (granulomas) in organs such as lungs, skin, and lymph nodes
- Stevens Johnson syndrome: inflammatory disorder of skin and mucous
- membranes, resulting in blistering and shedding of skin
- Toxic epidermal necrolysis: a potentially fatal disease characterized by
- blistering and peeling of the top layer of skin resembling a severe burn
- Histiocytic necrotizing lymphadenitis or Kikuchi lymphadenitis: disorder of
- the lymph nodes which causes the lymph nodes to become enlarged,
- inflamed and painful, commonly affecting lymph nodes of the neck and
- possibly associated with fever or muscle and joint pains.
- Vogt Koyanagi Harada syndrome; a disease that affects the pigmented
- tissue; this may affect the eye leading to swelling, pain and/or blurred
- vision; the ear leading to hearing loss, ringing in the ears and/or skin
- leading to loss of skin color

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 sitehttps://ctep.cancer.gov/protocoldevelopment/electronic_applications/docs/CTCAE_v5_Quick_Reference_5x7.pdf

- **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.
  - Other AEs for the protocol that do not require expedited reporting are outlined in the

next section (Expedited Adverse Event Reporting) under the sub-heading of Protocol-Specific Expedited Adverse Event Reporting Exclusions.

- **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.

7.3 Adverse Event Reporting

7.3.1 In the event of an unanticipated problem or life-threatening complications treating investigators must immediately notify the Overall PI.

7.3.2 Investigators **must** report to the Overall PI any adverse event (AE) that occurs after the initial dose of study treatment, during treatment, or within 100 days of the last dose of treatment on the local institutional SAE form.

7.3.3 DF/HCC Adverse Event Reporting Guidelines

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

7.3.4 Protocol-Specific Adverse Event Reporting Exclusions

None

7.4 Reporting to the Food and Drug Administration (FDA)

The Overall PI, as study sponsor, will be responsible for all communications with the FDA. The Overall PI 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 Routine Adverse Event Reporting

All Adverse Events **must** be reported in routine study data submissions to the Overall PI on the toxicity case report forms. **AEs reported through expedited processes (e.g., reported to the IRB, FDA, etc.) must also be reported in routine study data submissions.**

8. PHARMACEUTICAL INFORMATION

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

DKN-01 will be administered first followed by Nivolumab, with a minimum of 5 minutes between dosing.

8.1 DKN-01

8.1.1 Description

DKN-01 is an IgG4 optimized for neutralizing activity against DKK1. The molecular formula is C6394 H9810 N1698 O2012 S42. Molecular weight is 144,013 Daltons (intact).

A summary of the PK of DKN-01 after IV administration is presented below.

Study DEK-DKK1-P100: Summary of DKN-01 Pharmacokinetic Parameters Following IV Administration

PK Parameter[a]	DKN-01 Dose				
	Part A				Part B
	75 mg QW	150 mg QW	300mg QW	600 mg Q2W	300 mg Q2W*
Cycle 1, Day 1					
C_{max} (µg/mL)	22.8	47.8	130	183	116
SD	(7.67)	(4.19)	(25.7)	(34.6)	(45.0)
N	3	3	3	3	19
C_{max}/Dose (µg/mL/mg)	0.303	0.319	0.434	0.305	0.388
SD	(0.102)	(0.0280)	(0.0857)	(0.0577)	(0.150)
N	3	3	3	3	19
AUC_{0-t} (µg·h/mL)[b]	1940	4380	10900	28400[c]	17400 c*
SD	(780)	(403)	(2320)	(2470)	(5430)
N	3	3	3	3	19
AUC_{0-t}/Dose[b](µg·h/mL/mg)	25.9	29.2	36.2	47.3	58.0
SD	(10.4)	(2.68)	(7.73)	(4.11)	(18.1)
N	3	3	3	3	19
Cycle 1, Day 22					
C_{max} (µg/mL)	43.2	105	224		
SD	(25.1)	(29.5)	(28.4)	NA	NA
N	3	3	3		

C_{max}/Dose (µg/mL/mg)	0.58576	0.701	0.748		
SD	(0.334)	(0.197)	(0.0948)	NA	NA
N	3	3	3		
AUC_{0-t} (µg·h/mL) [b]	5660	15800	25600		
SD	(3920)	(5610)	(4570)	NA	NA
N	3	3	3		
AUC_{0-t}/Dose (µg·h/mL/mg)[b]	75.5	105	85.5		
SD	(52.3)	(37.4)	(15.2)	NA	NA
N	3	3	3		

AUC_{0-t} = the area under the serum concentration versus time curve for the dosing interval; C_{max} = peak serum concentration; NC = not calculated; NA = not available; SD = standard deviation

* Note that the report excluded Subject 26 (N=18) possibly because there were issues with actual times for this subject. The TLFs did not exclude this subject. Differences were not notable. Data from the TLFs were reported here or were used to recalculate the appropriate AUC values.

[a] Values are reported as mean (standard deviation).

[b] Reported as AUC_{0-tau} in study report with tau equivalent to one week for QW dose groups vs. two weeks for Q2W dose groups.

[c] Data misreported in final TLFs, AUCs recalculated from individual values using nominal times.

8.1.2 **Form**

DKN-01 for Injection is supplied for clinical study use as a lyophilized formulation in a glass vial. The drug product is composed of DKN-01 and the inactive ingredients sucrose, polysorbate 80, sodium chloride, citric acid, and sodium citrate.

The vial is manufactured to deliver 20 mg of DKN-01 and must be reconstituted with 0.9% Sodium Chloride Injection, USP. The vials of lyophilized drug product do not contain antimicrobial preservative and must be stored in refrigerated conditions (2ºC to 8ºC).

Reconstitution of the vial with 2.2 mL of 0.9% Sodium Chloride Injection, USP gives a clear solution containing 10 mg/mL DKN-01. The reconstituted vials are stable for up to 8 hours at room temperature (up to 30°C) or refrigerated (2ºC to 8ºC). Once the reconstituted DKN-01 has been removed from the vial(s) into a syringe(s), it must be administered to the patient within 4 hours, while being stored at room temperature. Therefore, the maximum time limit from reconstitution to completion of administration is 12 hours.

The route of administration is IV infusion via syringe pump only (over a period of 30 minutes). For patients being treated at 600mg of DKN-01, DKN-01 will be administered in 2 equal dose volume of appropriate sized syringes via bifurcated tubing.

8.1.3 **Storage and Stability**

For use in clinical studies, DKN-01 for injection is supplied as a lyophilized formulation in a glass vial and should be stored in refrigerated conditions (2ºC to 8ºC).

8.1.4 **Compatibility**

Reconstitution of the vial with 2.2 mL of 0.9% Sodium Chloride Injection, USP gives a clear solution containing 10 mg/mL DKN-01. The reconstituted vials are stable for up to 8 hours at room temperature (up to 30°C) or refrigerated (2 to 8°C). Once the reconstituted DKN-01 has been removed from the vial(s) into a syringe, it must be administered to the patient within 4 hours, while being stored at room temperature. Therefore, the maximum time limit from reconstitution to completion of administration is 12 hours.

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 chemotherapeutic agent in a self-contained and protective environment.

8.1.6 **Availability**

Drug supplies and re-supplies will be provided free of charge by Leap Therapeutics.

DKN-01 for Injection is supplied for clinical study use as a lyophilized formulation in a glass vial.

For more information regarding the supply of DKN-01 please refer to the study drug guidelines and the Pharmacy Manual (Appendix C).

8.1.7 **Preparation**

Reconstitution of the vial with 2.2 mL of 0.9% Sodium Chloride Injection, USP gives a clear solution containing 10 mg/mL DKN-01. The reconstituted vials are stable for up to 8 hours at room temperature (up to 30°C) or refrigerated (2 to 8°C). Once the reconstituted DKN-01 has been removed from the vial(s) into a syringe, it must be administered to the patient within 4 hours, while being stored at room temperature. Therefore, the maximum time limit from reconstitution to completion of administration is 12 hours.

8.1.8 **Administration**

The route of administration is IV infusion via syringe pump only (over a period of 30 minutes). For patients being treated at 600mg of DKN-01, DKN-01 will be administered using a full 60ml syringe, or 2 x 30ml syringes.

8.1.9 **Ordering**

The investigator or designee will order DKN-01 from Leap Therapeutics according to

ordering instructions provide by the company.

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.)
The clinical trial centers will keep a trial specific authorization list which determines the persons responsible for handling of the investigational drugs. The responsible person regarding the following will keep accurate records in the clinical trial centers:

- receipt of IMP supply from sponsor (clinical trial center, principal investigator, identification of IMP, Batch No, Formulation, kind and size of packaging, date of expiry, number of study drugs per participant, number of reserve study drugs, number of study drugs in total, patient identification number, date and time of receipt);
- location of storage of IMP;
- dispensing and returning of IMPs in the clinical trial center (date and time, number, batch no, patient identification number, volume of unused solution for injection when returning).

Accountability of the investigational study drug(s) is under the responsibility of the investigator and can be delegated to an appropriately qualified person. Study drug accountability should be maintained by each site. Accountability records should include receipt date, batch numbers, expiry dates, patient SeqID, use by subject, dispensing dates, quantities (lowest unit) and stock balance.

8.1.11 **Destruction and Return**

In addition to internal accountability documentation on site, EORTC study-specific accountability and drug destruction forms will be supplied for this purpose, if site-specific forms are deemed not sufficiently detailed or do not provide enough information, according to EORTC Quality Assurance criteria.

The drug accountability and destruction forms will be verified during monitoring visits.
At the end of study, when all patients have stopped protocol treatment, complete drug reconciliation per batch should be available at the site for verification by EORTC in order to allow drug destruction or return procedure.

Both the unused and expired study medication must be destroyed, upon authorization of the sponsor, according to local regulations and procedures, and a copy of the destruction form must be returned to the EORTC Headquarters.

The medication provided for this trial is to be used only as indicated in this protocol and only for

the patients entered in this study.

8.2 Nivolumab

8.2.1 Description

Nivolumab, also referred to as BMS-936558-01 or BMS-936558, is a soluble protein consisting of 4 polypeptide chains, which include 2 identical heavy chains and 2 identical light chains. Nivolumab is produced from cell culture using a Chinese Hamster Ovary (CHO) cell line. The molecular formula is 6362 H9862 N1712 O1995 S42. The molecular weight is 146,221 daltons.

The PK of single-agent nivolumab was studied in patients over a dose range of 0.1 to 20 mg/kg administered as a single dose or as multiple doses of OPDIVO as a 60-minute intravenous infusion every 2 or 3 weeks. Nivolumab clearance (CL) decreases over time, with a mean maximal reduction (% coefficient of variation [CV%]) from baseline values of 24.5% (47.6%) resulting in a geometric mean steady-state clearance (CLss) (CV%) of 8.2 mL/h (53.9%) in patients with metastatic tumors; the decrease in CLss is not considered clinically relevant. Nivolumab clearance does not decrease over time in patients with completely resected melanoma, as the geometric mean population clearance is 24% lower in this patient population compared with patients with metastatic melanoma at steady state. The geometric mean volume of distribution at steady state (Vss) (CV%) is 6.8 L (27.3%), and geometric mean elimination half-life (t1/2) is 25 days (77.5%). Steady-state concentrations of nivolumab were reached by 12 weeks when administered at 3 mg/kg every 2 weeks, and systemic accumulation was 3.7-fold. The exposure to nivolumab increases dose proportionally over the dose range of 0.1 to 10 mg/kg administered every 2 weeks. The predicted exposure (Cavg and Cmax) of nivolumab after a 30-minute infusion is comparable to that observed with a 60-minute infusion.

Specific Populations: The population PK analysis suggested that the following factors had no clinically important effect on the clearance of nivolumab: age (29 to 87 years), weight (35 to 160 kg), gender, race, baseline LDH, PD-L1 expression, solid tumor type, tumor size, renal impairment, and mild hepatic impairment.

Renal Impairment: The effect of renal impairment on the clearance of nivolumab was evaluated by a population PK analysis in patients with mild (eGFR 60 to 89 mL/min/1.73 m2), moderate (eGFR 30 to 59 mL/min/1.73 m2), or severe (eGFR 15 to 29 mL/min/1.73 m2) renal impairment. No clinically important differences in the clearance of nivolumab were found between patients with renal impairment and patients with normal renal function.

Hepatic Impairment: The effect of hepatic impairment on the clearance of nivolumab was evaluated by population PK analyses in patients with HCC and in patients with other tumors with mild hepatic impairment (total bilirubin [TB] less than or equal to the ULN and AST greater than ULN or TB greater than 1 to 1.5 times ULN and any AST) and in

HCC patients with moderate hepatic impairment (TB greater than 1.5 to 3 times ULN and any AST). No clinically important differences in the clearance of nivolumab were found between patients with mild/moderate hepatic impairment.

Full details on the clinical pharmacology aspects of nivolumab can be found in the Investigator Brochure.

8.2.2 Form

Nivolumab is clear to opalescent, colorless to pale yellow liquid, light (few) particulates may be present. The drug products are sterile, non-pyrogenic, single-use, isotonic aqueous solutions for intravenous (IV) infusion. Nivolumab Injection (100 mg/Vial (10 mg/mL)) is also referred to as nivolumab injection.

8.2.3 Storage and Stability

Vials of nivolumab injection must be stored at 2°C to 8°C (36°F to 46°F) and protected from light and freezing. The unopened vials can be stored at room temperature (up to 25°C, 77°F) and room light for up to 48 hours.

8.2.4 Compatibility

Care must be taken to assure sterility of the prepared solution as the product does not contain any antimicrobial preservative or bacteriostatic agent. Nivolumab infusions are compatible with polyvinyl chloride (PVC) or polyolefin containers and infusion sets, and glass bottles.

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 chemotherapeutic agent in a self-contained and protective environment.

8.2.6 Availability

Investigational product documentation must be maintained that includes all processes required to ensure drug is accurately administered. This includes documentation of drug storage, administration and, as applicable, storage temperatures, reconstitution, and use of required processes (eg, required diluents, administration sets).

The sites are responsible for procuring IV bags, diluent, and in-line filters.

8.2.7 Preparation

DF/HCC sites will follow institutional standard practices and/or packet insert.

When the dose is fixed (eg, 240 mg, 360 mg, or 480 mg flat dose), nivolumab injection can be infused undiluted or diluted so as not to exceed a total infusion volume of 160 mL. For patients weighing less than 40 kilograms (kg), the total volume of infusion must not exceed 4 mL per kg of patient weight. During drug product preparation and handling, vigorous mixing or shaking is to be avoided.

The administration of nivolumab infusion must be completed within 24 hours of preparation. If not used immediately, the infusion solution may be stored under refrigeration conditions (2°C to 8°C, 36°F to 46°F) for up to 24 hours, and a maximum of 8 hours of the total 24 hours can be at room temperature (up to 25°C, 77°F) and room light. The maximum of 8 hours under room temperature and room light conditions includes the product administration period.

8.2.8 **Administration**

DF/HCC sites will follow institutional standard practices and/or packet insert.

For details on prepared drug storage and administration of nivolumab, please refer to the current Investigator Brochure.

Nivolumab is to be administered as a 30-minute IV infusion. At the end of the infusion, flush the line with a sufficient quantity of normal saline or dextrose solution.

8.2.9 **Ordering**

The investigator or designee will order Nivolumab from BMS according to ordering instructions provide by the company.

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.)

Study drug will be administered in the clinical facility. After administration of nivolumab, treatment compliance will be assessed by investigator report on the case report forms.

8.2.11 **Destruction and Return**

For this study, study drugs (those supplied by BMS or sourced by the investigator) such as partially used study drug containers, vials and syringes may be destroyed on site.

On-site destruction is allowed provided the following minimal standards are met:

- On-site disposal practices must not expose humans to risks from the drug.
- On-site disposal practices and procedures are in agreement with applicable laws and regulations, including any special requirements for controlled or hazardous substances.
- Written procedures for on-site disposal are available and followed. The procedures must be filed with the site's SOPs and a copy provided to BMS upon request.
- Records are maintained that allow for traceability of each container, including the date disposed of, quantity disposed, and identification of the person disposing the containers. The method of disposal, ie, incinerator, licensed sanitary landfill, or licensed waste disposal vendor must be documented.
- Accountability and disposal records are complete, up-to-date, and available for the Monitor to review throughout the clinical trial period.

It is the investigator's responsibility to arrange for disposal of all empty containers, provided that procedures for proper disposal have been established according to applicable federal, state, local, and institutional guidelines and procedures, and provided that appropriate records of disposal are kept.

9. BIOMARKER, CORRELATIVE, AND SPECIAL STUDIES

9.1 Biomarker Studies

We propose baseline circulating biomarkers on all 30 patients and paired tumor biopsies in 15 patients. Biopsies will be done at baseline and cycle 1 day 21 ± 4 days to assess early predictive markers of response and non-response. In responders, biopsies will be done at baseline and progression to assess mechanisms of resistance. The analyses will be performed in the labs of Dr. Dan Duda, Dr. David Ting and Dr. Nir Hacohen at the Massachusetts General Hospital Cancer Center. We also propose collecting baseline and serial stools samples in patients that consent to this, and the analyses will be performed in Dr. Andrew Chan's lab.

9.1.1 Circulating biomarkers

We will further validate blood-based immune and angiogenesis biomarkers, such as circulating CD4+ and CD8+ lymphocytic and myeloid-suppressor (CD14+, PD-L1+) as well as plasma markers, that are candidates for pharmacodynamic biomarkers or biomarkers of response to DKN-01 (see Section 2.4B).

Circulating tumor cells (CTCs) isolated from serial blood samples using the CTC-iChip (an antigen-agnostic microfluidic device) and circulating exosomal RNA will be analyzed in parallel by RNA-seq of the coding and non-coding transcriptomes to derive novel biomarkers. Additionally, ctDNA will be isolated from samples and undergo whole exome sequencing.

9.1.2 Tumor Tissue biomarkers:

We will evaluate baseline tumor tissue expression of the targets (DKK1 and PD-L1) to assess for predictors of response as well as TIL presence, tissue distribution and activation (Granzyme B, proliferation markers) post-therapy in any available biopsy tissue.

We will perform single-cell RNA-sequencing and T-cell receptor (TCR) sequencing of fresh pre- and post-treatment biopsies to characterize the tumor microenvironment at baseline and the changes observed due to treatment. This data will be leveraged to identify novel predictive gene expression signatures, pathways and immune cell subsets relevant for response. Samples will be processed and analyzed by the Nir Hacohen lab at MGH at the Center for Translational Immunology. In addition, the Hacohen lab will preserve core biopsies to perform spatial analysis by spatial transcriptomics and multiplex immunohistochemistry (IHC) to validate key findings by single-cell RNA-sequencing, and for RNA in situ hybridization (RNA-ISH) to detect repeat RNAs, which have been linked with changes in the immune microenvironment. The Hacohen lab has significant experience and well-validated pipelines for processing fresh tumor samples for single-cell RNA-sequencing and multiplex IHC, and has established pipelines for computational analysis of these samples.

9.1.3 Stool Microbiome biomarkers:

We propose to collect stool samples longitudinally throughout the study from all participants to understand microbial dynamics in response to treatment and identify those microbiota that may be used to predict disease-free progression (response), onset of treatment-related toxicities, or progression of disease/death. 16s and whole shotgun metagenomic/transcriptomic sequencing will be used to identify the changes in gut microbiota taxonomy, genomic diversity, and/or microbial pathways associated with drug intervention and whether there are specific associations that predict response, toxicity, or progression. The Chan laboratory has extensive experience in the handling, processing, sequencing, and data analysis required to execute these proposed correlative studies.

9.2 Laboratory Correlative Studies

9.2.1 **Circulating Biomarkers**

Blood will be processed for CTC purification, plasma collection, and mononuclear cell isolation. Approximately 10 mL of blood will be processed through the CTC-iChip platform for CTC isolation. We will divide the CTC product into three different analyses including 1) Standard IF staining 2) RNA-ISH staining and 3) bulk frozen CTC product for future qRT-PCR or sequencing analysis. The bulk frozen CTC product will be preserved using RNAlater stabilization solution (Qiagen Cat No. 76106). RNAlater has been shown to significantly increase RNA recovery from CTC products and still permits the isolation and analysis of DNA and proteins from CTC product. One tube of RNAlater stabilized CTC product will be purified

for RNA using the RNeasy Micro Kit (Qiagen Cat No. 74004). The other tube of RNAlater stabilized CTC product will be flash frozen and stored at -80°C for future exploratory research.

The other 10 mL of blood will have plasma and mononuclear cells separated by Ficoll based density centrifugation followed by immediate flash freezing and storage at -80°C. A 1-2 mL aliquot of plasma will be thawed and processed for exosomes capture on the $^{HB\text{-}Exo}$Chip and/or ultracentrifugation based methods. RNA will be isolated from exosomes using TRIzol (Thermo Fisher Cat No. 10296028) based purification. This RNA will be either used immediately for analysis or stored at -80°C.

9.2.1.1 Collection of Specimen(s)

All patients will have blood drawn into four 10 mL EDTA purple top tubes for analysis before treatment and then every two months on treatment for the duration of the study.

9.2.1.2 Handling of Specimens(s)
Specimens will be stored at room temperature and sent within 1 hour to the CTC laboratory for processing at the MGH Cancer Center, Building 149, Charlestown, MA.

9.2.1.3 Shipping of Specimen(s)

Blood will be transported to MGH Cancer Center, Building 149, Charlestown, MA via Partners Healthcare approved vendor US Ground.

9.2.1.4 Site(s) Performing Correlative Study:

MGH Cancer Center – Charlestown Navy Yard Building 149

9.2.2 **Tumor tissue biomarkers**

Pre- and post-treatment biopsies will be collected for 1) fresh tissue, 2) fresh frozen in liquid nitrogen or dry ice bath, and 3) formalin for standard formalin fixed paraffin embedding (FFPE).

We will evaluate baseline tumor tissue expression of the targets (DKK1 and PD-L1) as well as TIL presence, tissue distribution and activation (Granzyme B, CD69, proliferation markers) post-therapy in any available biopsy tissue using existing antibodies against PD-L1, DKK1, Granzyme B, CD69 and Ki-67. The antibody used for PD-L1 will be anti-human CD274 Ab (clone: 29E.2A3). DKK1 will be evaluated by RNAScope.

Fresh tissue biopsies will under single-cell RNA-sequencing using standard pipelines (10X genomics). Briefly, scRNAseq samples will undergo dissociation into single-cell suspension, then be loaded onto a Chromium Single Cell A Chip (10X Genomics) and processed through the Chromium Controller to generate Gel Beads in Emulsion (GEMs). RNA-seq libraries will be prepared using the Chromium 5’ Library and Gel Bead kit (10X Genomics) following the manufacturer’s protocol. Libraries will be subsequently sequenced on an Illumina HiSeqX with paired end reads. Read count quantification for each sample and downstream analysis will be performed according to established

computational pipelines and initially include unsupervised clustering and cell annotation to identify cell subsets, differential gene expression between selected patient and cell subsets, analysis of cellular states and cell-trajectories and reconstruction of TCRs to investigate T cell clonality. All analysis will be implemented in Seurat (v3.0) in the R programming environment.

Fresh frozen tumors will be processed for RNA and DNA extraction with the AllPrep spin column kit (Qiagen 80204). RNA will be analyzed by RNA-sequencing using the Illumina Total RNA-seq library construction and sequencing on the NextSeq 2500 platform. Excess RNA and DNA will be archived and stored at -80 degrees C. DNA may be analyzed by whole genome,whole exome, or targeted gene sequencing. A single fresh frozen core will be stored for potential future spatial analysis with either SlideSeq or multiplex IHC.

FFPE slides will be stained using the ACD RNAscope platform on the automated Leica Bond RX platform. Sequential slides will be stained for HSATII, LINE-1, TERRA, HERV-H, PPIB (RNA control) and other RNA markers identified by RNA-seq.

9.2.2.1 Collection of Specimen(s)

Between one (1) and six (6) fresh, fresh frozen and FFPE biopsies will be obtained for research biopsies performed by interventional gastroenterology or interventional radiology.

9.2.2.2 Handling of Specimens(s)

Fresh frozen biopsies will be frozen immediately with liquid nitrogen or dry ice freezing solution (dry ice combined with ethanol). FFPE samples will be placed in 10% formalin. All samples will be transported immediately to the MGH Center for Translational Immunology.

9.2.2.3 Shipping of Specimen(s)

Biopsies will be transported to the MGH Center for Translational Immunology at MGH Jackson building on the 9th floor.

9.2.2.4 Site(s) Performing Correlative Study

MGH Center for Translational Immunology, Jackson building 9th floor.

9.2.3 **Stool microbiome biomarkers**

We will obtain self-collected stool specimens from participants at pre-treatment/baseline and at the end of each treatment cycle (returned at the time of radiologic assessment). We may additionally ask participants to provide a sample surrounding any treatment-related toxicities and a final sample at the time of progression or when the participant is taken off-treatment.

Participation in all stool collections is optional and their decision to participate in any given collection time point or not will not affect the participant's participation in other aspects of the

trial. Therefore, we anticipate that participants may provide as few as 1 stool sample or up to 10 stool samples. Given the individual treatment plans and the optional nature of the collection, not all participants will provide the same number of samples. These non-invasive stool collections pose no additional known risk to the participants.

We will perform RNA and DNA purification from stool aliquots and sequencing according to protocols optimized in our previous validation study[34] in which we have collected over 932 unique specimens and failed to recover high quality RNA and DNA from only 4 samples. Libraries for metagenome and metatranscriptome will be constructed using the Illumina TruSeq method with ~180nt inserts and sequenced on the Illumina HiSeq 2000 platform targeting a minimum of ~2 Gnt/sample with 100nt paired-end reads. Quality control and removal of host 'contaminant' sequences will be conducted using the KneadData pipeline. Taxonomic profiles will be determined by MetaPhlAn2 (Metagenomic Phylogenetic Analysis), which identifies taxa to the species level and quantifies their relative abundances. Meta'omic data will then be piped into HUMAnN2 (HMP Unified Metabolic Analysis Network tools 2) to generate functional genomic profiles, the detailed species-specific reconstructions of microbial metabolic pathways, and their complement of gene orthologs, taxonomic distributions, and abundances. Within HUMAnN2, taxonomic specific profiles of UniRef orthologous gene families, MetaCyc, Unipathway, and KEGG pathways are generated to comprehensively determine the metabolic potential of each sample. MaAsLin, a sparse regression multivariate generalized linear model, will be used to test associations between clinical metadata, including trial outcomes and microbial community abundance and function. As this is a rapidly evolving field of study, the exact tools used may change, but any replacement will perform the same range of function.

9.2.3.1 Collection of Specimen(s)

Participants will be provided with an at-home stool collection kit with necessary instructions and will return specimens (stored in a room temperature stabilization buffer) at each clinical visit. Participants will be provided with a subsequent replacement kit for their next collection at each of these visits.

9.2.3.2 Handling of Specimens(s)

Stool samples will be stored in a room temperature stabilization buffer provided in the at-home collection kits. Participants will bring samples with them to a clinical visit where study staff will coordinate pick-up by or delivery to a member of the Chan Laboratory. Stool samples sent to the Chan laboratory will be homogenized using a spatula and separated into aliquots of 100 mg for storage at -80°C prior to DNA and RNA extraction. All samples will be processed and frozen within 3 hours of receipt in the laboratory.

9.2.3.3 Shipping of Specimen(s)

Stool samples can also be shipped at room temperature (within 5 days of collection) to or coordinated with the laboratory team to be picked up from trial coordinators in the clinical

setting:

Chan Laboratory
c/o David A. Drew, Ph.D.
Massachusetts General Hospital
55 Fruit St., GRJ-815
Boston, MA 02114

Deidentified extracted DNA/RNA or stool samples may be sent to:

Broad Institute
Attn: BSP platform
301 Binney Street Lab 5076
Cambridge, MA 02142
617-714-8952

for sequencing. However, we may choose to use other comparable sequencing services according to resources at the time.

9.2.3.4 Site(s) Performing Correlative Study

Chan Laboratory and Broad Institute.

STUDY CALENDAR

Baseline evaluations are to be conducted within 1 week prior to start of protocol therapy and laboratory parameters do not need to re-meet eligibility on c1d1 if the treating investigator feels it is safe and in the best interest of the patient to proceed. Scans and x-rays must be done ≤3 weeks prior to the start of therapy.

Assessments must be performed prior to administration of any study agent. Study assessments and agents should be administered within ± 3 days of the protocol-specified date, unless otherwise noted.

	Screening	On Treatment Period						Off Treatment
		Cycle 1 (28 days)				Cycles 2 and beyond (28 days)		
	Pre-Study (-14 days)	Day 1	Day 8	Day 15	Day 22	Day 1	Day 15	
DKN-01		X		X		X	X	
Nivolumab		X		X		X	X	
Informed consent[b]	X							
Demographics	X							
Medical history	X							
Concurrent meds	X	X----------X						
Physical exam	X	X	X	X	X	X	X	X
Vital signs[c]	X	X	X	X	X	X	X	X
Height	X							
Weight	X	X	X	X	X	X	X	X
ECOG Performance status	X	X	X	X	X	X	X	X
Mismatch repair protein status	X							
CBC w/diff	X	X	X	X	X	X	X	X
Serum chemistry[d]	X	X	X	X	X	X	X	X
Viral tests at Screening (HBV/HCV)[e]	X							
Thyroid function tests[f]	X	X				X		X
Creatine phosphokinase (CPK)[g]	X	X				X		X
Blood coagulation	X	X	X	X	X	X	X	

test (INR)								
Amylase and lipase	X	X	X	X	X	X	X	
CA19-9	X	X				X		
EKG	X	(as indicated)						
Adverse event evaluation		X----------X						X
Tumor measurements	X[h]	Tumor measurements are repeated every 8 weeks.[i]						X
Radiologic evaluation	X[h]	Radiologic measurements should be performed every 8 weeks.[i]						X
B-HCG	X[j]	X				X		
Circulating biomarkers (Duda Lab)	X	X		X		X[k]		X[l]
Circulating biomarkers (Ting Lab)		X						X[l]
Tumor biopsy[m]	X				X			
Return at-home Stool Collection	X	X[n]				X[n]		X

a: Off-study evaluation at the time of discontinuation of treatment or up to 28 days afterwards
b: Informed consent can be signed up to 28 days prior to Cycle 1 Day 1.
c: On treatment days, the patient's vital signs (heart rate, respiratory rate, blood pressure, and temperature) should be determined within 60 minutes before and 2 hours (±15 minutes) after the DKN-01 infusion. During infusion the vital signs should be collected as clinically indicated.
d: Albumin, alkaline phosphatase, total bilirubin, bicarbonate, BUN, calcium, chloride, creatinine, glucose, LDH, phosphorus, potassium, total protein, SGOT [AST], SGPT [ALT], sodium.
e: Testing will be performed at Screening and will include HBV/HCV serology (HBsAg, HBsAb, HBcAb, and HCV antibody) and viral load assessment (HBV DNA and HCV RNA). Patients who have detectable HBV DNA or HCV RNA at Screening will perform the respective viral load test every 2 cycles (8 weeks).
f: TSH and free T4 will be assessed at Screening, Baseline, and every 2 cycles thereafter (8 weeks). T3 or free T3 levels will be assessed at Screening and Baseline. Patient can receive dose prior to receiving the results for thyroid labs.
g: Patient can receive dose prior to receiving the results for CPK.
h: Radiologic evaluation within 21 days of c1d1
i: The window for tumor measurements and radiologic evaluation is +/- 7 days
j: Serum pregnancy test (women of childbearing potential) at baseline and every 4 weeks. For day 1 of each cycle (including c1d1), serum pregnancy test needs to be within 24 hours of nivolumab dosing.
k: Circulating biomarkers will be collected on c2d1 but not on day 1 of subsequent cycles. See Biomarker Cheat Sheet on Alert page, section 9, and Appendix B for full details.
l: Circulating biomarkers should be collected at progression

m: Fifteen patients will have paired tumor biopsies while on treatment. Biopsies will be done at baseline (within 28 days of c1d1) and at c1d22 (+/- 4 days) to assess early predictive markers of response and non-response. In 4 responders, biopsies will be done at baseline and progression to assess mechanisms of resistance. If the treating investigator feels the biopsy is unsafe or not feasible for the patient, they may reach out to the Principle Investigator for discussion of omission of biopsy.
n: Participants who consent to optional stool collection will collect samples at home prior to the start of each treatment cycle but return these on the first day of each treatment cycle. Patients will ideally provide a stool sample every cycle, but the number of samples they provide over time is voluntary
o:

10. MEASUREMENT OF EFFECT

Objective tumor response and time to progression will be measured in this study using the revised international criteria (1.1) proposed by the RECIST (Response Evaluation Criteria in Solid Tumors) committee.

The complete RECIST criteria are included in the published RECIST document available at http://www.eortc.org/RECIST.

10.1 Antitumor Effect – Solid Tumors

For the purposes of this study, participants should be re-evaluated for response every *8* weeks. In addition to a baseline scan, confirmatory scans should also be obtained usually at the next schedule scan vist but not less than 4 weeks following initial documentation of objective response.

Response and progression will be evaluated in this study using the new international criteria proposed by the Response Evaluation Criteria in Solid Tumors (RECIST) guideline (version 1.1) [*Eur J Ca* 45:228-247, 2009]. 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.

10.1.1 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.

10.1.2 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 or might not be considered measurable.

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, leptomeningeal disease, 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.

10.1.3 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.

10.1.4 Response Criteria

10.1.4.1 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.

10.1.4.2 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).

10.1.4.3 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.

10.1.4.4 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.

Response (partial or complete) according to RECIST 1.1 with confirmation requires a confirmatory scan preferably at the next regularly scheduled imaging visit and no

earlier than 4 weeks after the prior assessment of response.

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.
** 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

10.1.5 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.

10.1.6 Progression-Free 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.

10.1.7 Response Review

Radiological images will be independently reviewed by experts independent of the study in our Tumor Imaging Metrics Core (TIMC).

11. 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).

11.1 Data Reporting

11.1.1 Method

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

11.1.2 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 (ODQ) in accordance with DF/HCC policies.

11.2 Data Safety Monitoring

The DF/HCC Data and Safety Monitoring Committee (DSMC) will review and monitor toxicity and accrual data from this study. The committee is composed of medical oncologists, research nurses, pharmacists and biostatisticians with direct experience in cancer clinical research. Information that raises any questions about participant safety will be addressed with the Overall PI and study team.

The DSMC will review each protocol up to four times a year with the frequency determined by the outcome of previous reviews. Information to be provided to the committee may include: up-to-date participant accrual; current dose level information; DLT information; all grade 2 or higher unexpected adverse events that have been reported; summary of all deaths occurring within 30 days of intervention for Phase I or II protocols; for gene therapy protocols, summary of all deaths while being treated and during active follow-up; any response information; audit results, and a summary provided by the study team. Other information (e.g. scans, laboratory values) will be provided upon request.

11.3 Collaborative Agreements Language N/A

This is an investigatory initiated study sponsored by Leap Therapeutics and BMS. Data will be shared between the participating institution and the two companies

12. STATISTICAL CONSIDERATIONS

12.1 Study Design/Endpoints

The primary endpoint is to investigate the overall response rate (ORR) of the combination of DKN-01 and nivolumab.

We hypothesize that the combination of DKN-01 and nivolumab will have a true ORR 20%, while the null hypothesis would be < 5%. The ORR of 5% was selected on the basis of studies of systemic therapy beyond the first line in unselected patients with refractory advanced biliary tract cancer showing an ORR in the single digits[35].

To detect a difference of 15% (20% vs 5%) with 80% power and using a one-sided binomial test with 5% type 1 error, we require a total of 27 patients. We recommend enrolling 30 patients to allow for 10% attrition. The anticipated follow-up is 12 months.

We will use a Simon's two-stage design. After testing the treatment on 13 patients in the first stage, the trial will be terminated if 0 respond. If the trial goes on to the second stage, a total of 27 patients will be studied. If the total number responding is less than or equal to 3, the treatment is rejected. If the chosen dose is 300mg, the patients treated at 600mg will not be included in the two-stage design. The patients treated at 600mg will not be replaced.

If the treatment is actually not effective, there is a 0.042 probability of concluding that it is. If the treatment is actually effective, there is a 0.199 probability of concluding that it is not.

All patients with measurable disease who receive at least one dose of both DKN-01 and nivolumab will be considered evaluable.

Secondary Endpoints:
1) Efficacy Endpoints:

- Progression Free Survival (PFS) – PFS is defined using the RECIST criteria for progressive disease. Patients discontinued from study for clinical progression will also be evaluable for this endpoint.
- Median Overall Survival (OS) – Median OS is defined as the length of time from trial registration that half of the enrolled patients in the study are still alive. Kaplan-Meier estimates of OS rates will be calculated along with their corresponding 95% confidence intervals. Cox proportional hazards regression modeling of OS will be used to assess the effect of the combination of DKN-01 and nivolumab on OS while controlling for other confounders.
- ORR, PFS and OS of subgroups stratified by tumor DKK1 and PD-L1 expression status in a pre-planned post-hoc analysis will also be assessed.

2) Biologic correlative studies of target inhibition – Categorical measures related to each marker will be correlated with response to the combination of DKN-01 and nivolumab using Fisher's exact tests. Kaplan-Meier survival curves and Cox proportional hazard models will be used to explore the relationship between the biomarker categories and PFS and OS as described in detail in section 9.4. These correlative endpoints are exploratory in nature.
3) Tolerability and safety – All enrolled patients who receive at least one dose of DKN-01 or nivolumab will be assessed for adverse events by the Common Toxicity Criteria for Adverse Events, v4.03, and the relationship of each AE to the study drugs will be documented.

12.2 Sample Size, Accrual Rate and Study Duration

A total of 30 evaluable participants will be accrued within 24 months. The anticipated follow-up is 12 months. The total planned study duration is 36 months.

Accrual Targets					
Ethnic Category	**Sex/Gender**				
	Females		**Males**		**Total**
Hispanic or Latino	2	+	1	=	3

Not Hispanic or Latino	15	+	12	=	27
Ethnic Category: Total of all subjects	17	+	13	=	30
Racial Category					
American Indian or Alaskan Native	0	+	0	=	0
Asian	2	+	2	=	4
Black or African American	1	+	1	=	2
Native Hawaiian or other Pacific Islander	0	+	0	=	0
White	14	+	10	=	30
Racial Category: Total of all subjects	17	+	13	=	30

12.3 Stratification Factors

The patients will be stratified in a pre-planned post-hoc analysis by PD-L1status and DKK1 status.

12.4 Interim Monitoring Plan

An early stopping rule is planned, which states that if the ORR is 0% after 13 patients are treated, we will terminate the study.

12.5 Analysis of Primary Endpoints

See Section 12.1.

12.6 Analysis of Secondary Endpoints

See Section 12.1.

12.7 Reporting and Exclusions

12.7.1 Evaluation of Toxicity

All patients who receive at least 1 dose of at least one study drug will be considered evaluable for toxicity.

12.7.2 Evaluation of the Primary Efficacy Endpoint

Total accrual may be adjusted to account for patients inevaluable for response, ie those patients not receiving at least one dose of the study drugs. Participants that are registered for the trial but do not begin treatment will be replaced and not included in the analysis of the primary endpoint.

13. PUBLICATION PLAN

The results should be made public within 24 months 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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APPENDIX A PERFORMANCE STATUS CRITERIA

ECOG Performance Status Scale	
Grade	Descriptions
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.
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).
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.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.
5	Dead.

APPENDIX B BIOASSAY TEMPLATES

Duda lab

Test and Control Articles

Material	Source
Human ProInflammatory Panel 1 V-PLEX™ Plus (Cat #K15049G-2)	Meso-Scale Discovery
Human Angiogenesis Panel 1 V-PLEX Kit (Cat #K15190D-2):	Meso-Scale Discovery
Human CXCL12/SDF1alpha Quantikine ELISA Kit (Cat #DSA00)	R&D Systems
Human HGF Quantikine ELISA Kit (Cat #DHG00)	R&D Systems

Plasma and peripheral blood mononuclear cells will be collected from all patients with advanced carcinoma enrolled in this clinical trial. We will measure potential biomarkers at baseline (pre-treatment) and then weekly for the first cycle of treatment (Days 8, 15), pre-treatment on Cycle 2 Day 1 and at the end of treatment (or early termination).

Experimental Methods

Eight cc of blood were collected in purple top (plasma EDTA) vacutainers, with a minimum of 5cc required. All samples were shipped on dry ice to the Steele Laboratories at MGH. Blood was separated by centrifugation in refrigerated centrifuge for 20 min at 2000 rpm into plasma and a cellular phase. The plasma was prepared in standard fashion and stored at –78 degrees Celsius until analysis. Plasma analysis was carried out for a panel of circulating inflammatory and angiogenic molecules previously identified as potential biomarkers. All measurements were performed in duplicate in a CLIA-Certified Core.

1. Human ProInflammatory Panel: IFN-γ, IL-1β, IL-10, IL-12 p70, IL-13, IL-2, IL-4, IL-6, IL-8, TNF-α
2. Human Angiogenesis Panel: bFGF, sFLT-1/sVEGFR1, PlGF, sTIE-2, VEGF, VEGF-C, VEGF-D
3. Human SDF1alpha and HGF

Each participant will have 2 vials of blood (~8 mL each) collected at the time points listed in Section 2.1. The blood must be processed and stored within 30-45 minutes after collection.

Materials and Labeling of Blood Tubes and Cryovials

Labels will be provided for the vacutainer blood tubes and the cryovials. All labels will have space for sites to provide the following information:

- Participant study number;
- Participant initials and date of birth

- Time point when sample was collected;
- For the blood tubes, a "BL" and for the plasma cryovials, a "PL" to distinguish which label should be used for each tube.

The site will be responsible for obtaining the appropriate tubes for the blood and plasma specimens. The list of supply options are as follows:
For Blood Draw Tubes (3 Options)
1. SARSTEDT Monovette® EDTA KE (9 mL), Part # 02.1333.001; or
2. Becton-Dickinson VacutainerTM K2E (10 mL), Part # 367525; or
3. Greiner Bio-One Vacuette® K3E EDTA K3 (9 mL), Part # 455036; and
For Plasma Cryovials (1 Option)
4. Nunc, Internally Threaded Cryotube, (3.6 mL vials), Fischer Scientific, Part # 12-565-162N.

Blood Draw and Centrifugation

- Prior to the blood draw, the blood vacutainer should be pre-cooled by placing tube in a bucket with wet ice.
- Directly from the participant's IV line, draw the 2 vials of blood using 2 pre-labeled blood vacutainer tubes.
- Record time of blood draw on Blood Processing Form (BPF).
- Immediately invert the blood tube several times to ensure proper mixing with the preservative.
- Place the blood tube in a bucket filled with wet ice.
- Centrifuge the blood tube at 700g (2000rpm) for 20 minutes at 4°C with no break at the end of centrifugation for plasma extraction.
- Using a sterile pipette, pipette the top clear layer (careful not to disturb the bottom red layer) and aliquot equally into 3 pre-labeled 1.8 mL Nunc cryovials.
- Visually check the plasma for hemolysis.
- If the plasma appears to be yellow and clear, please proceed with processing the plasma and record the observation on the BPF form.
- If the plasma appears to be dark red, please discard the plasma and sign and date the BPF.
- After the plasma has been extracted, check the red blood cells by sticking two wooden applicator sticks in the tube and observe the sticks for clots. Record if there was clotting observed, check yes or no on the BPF.
- Record numbers of vials on BPF.
- Immediately store the cryovials into a –80°C freezer*.
- Record time of freeze, location of the vials (i.e., freezer number, shelf, box number, and room #, as applicable) on the BPF.

* If a –80°C freezer is not available on site, the plasma specimens should be shipped to the core facility on dry ice the same day as processing. See Section 4.0 for shipping procedures.

Labeling and Storing Specimens
For tracking purposes, each specimen and its associated forms should be labeled with the site

number and case number. All cryovials should be stored in a monitored –80° C freezer. A separate freezer box should be set a side for the storage of all the plasma vials. They will be shipped in bulk to the testing core facility at completion of study.

SHIPPING SPECIMENS TO CORE FACILITY

When blood collection has been completed for all participants and after the participants go off trial the plasma samples should be shipped to the Steele Laboratory at Massachusetts General Hospital on dry ice in a Styrofoam box. The Styrofoam container should be packed with at least 7 pounds of dry ice, and make sure the top is completely covered with dry ice. Seal the Styrofoam container within a tight-fitting cardboard shipping box. A copy of the Plasma Shipping (PS) Form for each set of samples should be placed within a separate zip-lock plastic bag and placed on top of the Styrofoam container lid before the external shipping box is sealed.

If the site does not have –80°C storage capability, the plasma samples should be sent to the Steele Laboratory at Massachusetts General Hospital after the blood has been processed. The Styrofoam container should be packed with at least 2 inches of dry ice on the bottom, and completely covered on the top with an additional 2 inches or more of dry ice. Seal the Styrofoam container within a tight-fitting cardboard shipping box. A copy of the Plasma Shipping (PS) Form for each set of samples should be placed within a separate zip-lock plastic bag and placed on top of the Styrofoam container lid before the external shipping box is sealed.

If the site does not have facilities and trained personnel for plasma separation, the blood samples should be shipped to the Steele Laboratory at Massachusetts General Hospital with cold packs in a Styrofoam box within 24 hours (DO NOT FREEZE). Seal the Styrofoam container within a tight-fitting cardboard shipping box. A copy of the Plasma Shipping (PS) Form for each set of samples should be placed within a separate zip-lock plastic bag and placed on top of the Styrofoam container lid before the external shipping box is sealed.

Specimens should be shipped Monday to Wednesday only by overnight FedEx to the Testing Core Facility. On the day of shipment, the study coordinator will notify the Testing Core Facility via e-mail at annak@steele.mgh.harvard.edu or FAX (617-724-5841, ATTN: A. Khachatryan or J. Kahn) so they know to expect the upcoming shipment. Include the estimated date of arrival and the FedEx tracking number.

NOTE: The subject line of the e-mail/FAX should include the following so that the Testing Core Facility staff can distinguish plasma specimens sent by the sites.
Plasma Specimen Shipment--Site Name
Upon receipt of specimens, the Testing Core Facility will reconcile the materials and notify the site study coordinator of missing specimens, damaged specimens, or any concerns to be addressed.

Shipping Materials and Process

The appropriate shipping materials for specimens are the following:

- Storage boxes for plasma tubes (Fisherbrand, 5 3/4” x 5 3/4” x 4 7/8”, Part # 03-395-01, and dividers, Part # 03-395-11);
- Large size zip-locked bags;

- Multi-purpose insulated bio-shippers (Thermosafe Bio-Shippers; dimensions 14" x 10" x 14");
- Biohazard bags;
- M3 carton sealing tape;
- Shipping labels to indicate: "Notice: Keep Frozen" use only for Dry Ice shipments, Upright arrows, "Diagnostic Specimens – Not restricted, Packed in Compliance with IATA Packing Instruction 650", and "Class 9 – Dry Ice" label; and "Keep Refrigerated" use only for Cold Pack shipments.
- Dry ice or Cold Packs

The packing process for dry ice shipments includes the following:

- Place all plasma tubes in storage freezer boxes, tape the sides of the boxes;
- Place one freezer box, each separately, in a large zip-locked bag;
- Pack the Styrofoam container with at least 7 pounds of dry ice;
- Place the bagged freezer boxes in the middle of the bio-shipper (you can fit as many as the box allows);
- Pack the Styrofoam container with an additional dry-ice on the top of the boxes to cover the top;
- Place a copy of the Plasma Shipping Form (PS) for a single participant inside one biohazard bag, in the form slot (use as many forms/bags as necessary to cover the contents of the box to be shipped);
- Close the lid, place all bagged shipping forms on top of the lid, and seal the shipping container with tape.
- Maintain a copy of the transmittal log at the site.
- The packing process for cold pack shipments includes the following:
- Place all blood tubes in storage freezer boxes, tape the sides of the boxes
- Place one freezer box, each separately, in a large zip-locked bag.
- Place the bagged freezer boxes in the middle of the bio-shipper (you can fit as many as the box allows)
- Pack the Styrofoam container with 4-6 cold packs surrounding the boxes with the blood tubes.
- Place a copy of the Plasma Shipping Form (PS) for a single participant inside one biohazard bag, in the form slot (use as many forms/bags as necessary to cover the contents of the box to be shipped).
- Close the lid, place all bagged shipping forms on top of the lid, and seal the shipping container with tape.
- Maintain a copy of the transmittal log at the site.

Labeling Shipping Containers

Label each shipping container with the FedEx shipping label to include the following:

1. The study coordinator's return address.
2. The Testing Core Facility address:

ATTN: Mrs. Anna Khachatryan / Mrs. Julia Kahn

MGH, Cox-734
100 Blossom St.
Boston, MA 02114, USA
Phone: (617) 726-4088 or (617) 726-8143 or (617) 724-1353
Fax: (617) 724-5841
Pager: 14082
Email: annak@steele.mgh.harvard.edu, julia@steele.mgh.harvard.edu

3. "Notice: Keep Frozen", "Class 9 – Dry Ice" stickers or "Keep Refrigerated", Upright arrows, and "Diagnostic Specimens – Not restricted, Packed in Compliance with IATA Packing Instruction 650".

Summary Shipping Task List
The following summarizes the tasks to be completed by the site for a scheduled shipment:

- Prepare transmittal paperwork and retain copies at the site.
- Send a notification e-mail or FAX to the Testing Core Facility listing the items being shipped, including: the FedEx tracking number, total number of plasma tubes in the shipment, and the expected date of arrival. Please note "Specimen Shipment—Site Name." in the e-mail or FAX 'Subject' line.
- Pack the plasma specimens according to instructions above.
- Label each shipping container with the FedEx shipping label and affix all appropriate shipping labels.
- Maintain a copy of the transmittal log at the site.

Cellular Biomarkers
Circulating mononuclear cells will be collected for analysis of lymphocytic and myeloid populations.

Collection and Handling Procedures for Cellular biomarkers for Dr. Duda's lab (Steele Laboratories)

- Collect blood in an 8.0 ml Sodium Citrate tube (Blue Tiger Top Tube, see http://www.bd.com/vacutainer/products/molecular/citrate/)
- Invert several times to ensure mixing with the anticoagulant.
- Place a label on this blue tiger top vial of blood and cover the label with freezer tape.
- Place the labeled vial on wet ice and send to lab for further processing.

Processing of Specimen

- Within two hours of collection of the blue tiger top tube, the lab will centrifuge the blue tiger top tube at 4ºC temperature in a horizontal rotator for 25 minutes at 1600 g.
- After centrifugation, the mononuclear cells will be visible in a whitish layer just under the plasma. Carefully remove (and discard) the top plasma layer. Transfer the mononuclear layer (500-900 microliters) in the CPT tube to a labeled cyrovial. (Nunc. Product #377267)

- Add equal volume of freezing media (RPMI-1640 media with 20% dimethyl sulfoxide is then added. The cryotubes should be labeled and immediately be placed in a -80ºC degree freezer.

Sample Labels for Lab Samples
Complete the labels with patient identifiers printing each label with Study-No., patient ID, initials and day/time of sample collection (24-hour clock format, i.e., 6:30 pm = 18:30). A label example is provided below:

Study-No.: Investigator:
Patient-ID: Patient Initials: Date of birth:
Date of sampling: (mm/dd/yy) Time of Sampling: (hh:mm) (24-hr)
Sample Type: (Cells or Plasma)

APPENDIX C NEW YORK HEART ASSOCIATION [NYHA] CLASSIFICATION

Class	Patient Symptoms
I	No limitation of physical activity. Ordinary physical activity does not cause undue fatigue, palpitation, dyspnea (shortness of breath).
II	Slight limitation of physical activity. Comfortable at rest. Ordinary physical activity results in fatigue, palpitation, dyspnea (shortness of breath).
III	Marked limitation of physical activity. Comfortable at rest. Less than ordinary activity causes fatigue, palpitation, or dyspnea.
IV	Unable to carry on any physical activity without discomfort. Symptoms of heart failure at rest. If any physical activity is undertaken, discomfort increases.