TITLE: A Phase 2 Study of the Safety, Efficacy, and Immune Response of CRS-207, Pembrolizumab, Ipilimumab, and Tadalafil in Patients with Previously Treated Metastatic Pancreatic Adenocarcinoma

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Commercial Agents:
KEYTRUDA® (Pembrolizumab, MK-3475)
YERVOY® (Ipilimumab, BMS-734016)
Tadalafil (generic)

Johns Hopkins University Supplied Agent: CRS-207 (*Lm* Δ*actA*/Δ*inlB*/hMesothelin)

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1. OBJECTIVES

1.1 Primary Objective

To determine the objective response rate (ORR) using Response Evaluation Criteria for Solid Tumors (RECIST 1.1) in subjects with previously treated metastatic pancreatic cancer treated with tadalafil, pembrolizumab, ipilimumab, and CRS-207.

1.2 Secondary Objective

1.2.1 To assess safety and characterize toxicities when combining tadalafil, pembrolizumab, ipilimumab, and CRS-207 in subjects with metastatic pancreatic adenocarcinoma.

1.3 Exploratory Objectives

1.3.1 To assess the overall survival (OS).

1.3.2 To assess progression free survival (PFS), disease control rate (DCR), duration of response (DOR), and time to objective response (TOR) by RECIST 1.1.

1.3.3 To assess ORR, PFS, DCR, DOR, and TOR by immune Response Evaluation Criteria for Solid Tumors (iRECIST).

1.3.4 To measure tumor marker kinetics (CA 19-9) in subjects receiving treatment and correlate with OS, PFS, and best overall response.

1.3.5 To collect peripheral blood mononuclear cells (PBMC), plasma, and serum to identify potential therapeutic targets, biomarkers and predictors of response (OS, PFS and best overall response) and autoimmune toxicity.

- Measure pre- and post-treatment changes in PBMCs including effector, helper, and regulatory T cells, NK cells, monocytes, and macrophages through cell phenotyping analysis and gene expression profiling.

- Correlate induction of *Listeria monocytogenes* (*Lm*)- and mesothelin antigen-specific T cell responses and changes to the T cell epitope repertoire with OS, PFS, and best overall response.

- Correlate telomere length of lymphocytes to help predict response (OS, PFS, and best overall response).

- Correlate the induction of anti-thyroglobulin and anti-galectin-3 antibody responses with response (OS, PFS, and best overall response).

- Proteomic approaches will be used on pre- and post-treatment sera to identify targets and biomarkers of response (OS, PFS, and best overall response) or toxicity.

1.3.6 To collect archived tissue and pre- and post-treatment biopsies to test for predictors of response (OS, PFS, and best overall response) and future targets for combinatorial therapy.

- Immunohistochemistry (IHC) and/or gene expression profiling will be used to compare the nature of tumors and immune infiltrates for responders versus non-responders.
- Next-generation sequencing of T cell receptor (TCR) genes may be used to compare the tumor infiltrating T cell repertoire in responders and non-responders
- Up-regulation of immune inhibitory molecules (such as programmed death-ligand 1 [PD-L1]) will be evaluated in the pre- and post-treatment samples.
- Proteomic approaches to quantify protein expression and activation of specific signaling pathways in tumors from responders versus non-responders.

1.4 Study Design

This is an open-label, phase 2 study to evaluate the safety and clinical activity of tadalafil, pembrolizumab, ipilimumab, and CRS-207 in subjects with metastatic pancreatic adenocarcinoma who have progressed after at least 1 prior chemotherapy regimen.

The primary endpoint of this study is objective response rate (ORR) using RECIST. The treatment will be considered inactive and of no interest for further evaluation if the ORR is 5% or less and considered active if the ORR is 30% or greater.

A total of 17 patients will be enrolled on the study. If a total of 3 or more responses are observed, we will conclude that the ORR is higher than 5% and that the regimen warrants further study. This design has 92% power to reject the null hypothesis of an ORR of 5% in favor of the alternative hypothesis of 30%, with one-sided type 1 error 0.0502 (target type I error of 0.1). Additional patients may be enrolled as needed until at least 10 paired biopsies are obtained, but will not be included in the primary analysis of efficacy endpoint.

The study will consist of a screening period (within 28 days of first dose), a treatment period per the table below, and a follow-up period.

Subjects will receive treatment every 3 weeks for 6 cycles of treatment within a course. A course of treatment will be 18 weeks and courses can be repeated. The treatment schedule can be found in **Table 1**. Subjects will come to the clinic for dosing and/or assessments on Days 1 and 2 of each cycle and additional days for safety and immune monitoring follow-up per the study schedules in **Section 9**.

Dose reductions are only allowed for tadalafil per **Section 5.1**. If the investigator assesses a drug-related toxicity (that requires discontinuation) to be related to an individual component of the treatment schedule, dosing for that study drug alone may be discontinued while dosing is delayed

until the subject meets criteria to resume treatment of the other study drugs. The relationship to the discontinued study drug should be well documented in the source documents and permission from the Principal Investigator and Co-Principal Investigator needs to be obtained prior to continuation with the other study drugs. As of protocol version 5.0, enrollment to this study has been completed, and any patients that remain on study after February 22, 2024 will discontinue CRS-207 (no further drug supply) and may remain on study to continue to receive pembrolizumab, ipilimumab, and tadalafil.

Enrollment for the first three patients will be staggered by three weeks to evaluate patients for adverse events before the next patient is enrolled (the fourth patient may be enrolled 3 weeks after the third patient). The proportion of treated subjects with unacceptable toxicity will be monitored routinely. Complete unacceptable toxicity criteria can be found in **Section 4.7**.

At the investigator's discretion, subjects may receive additional courses of the assigned treatment regimen if they are clinically stable and meet dosing eligibility criteria. All subjects may continue in the treatment period up to a maximum of 2 years, or until discontinuation due to unacceptable toxicity, lack of clinical benefit as determined by the investigator, subject withdrawal, or termination of the study by IND Sponsor. Subjects that begin a new course prior to the 2 year cut-off may complete that course prior to coming off study. Subjects may continue on treatment with radiographic disease progression if subject is clinically stable and investigator believes the treatment is providing benefit. Criteria for removal from treatment are found in **Section 4.10**. Subjects will return to the study site 28 (±7) days after the final administration of study treatment for an end-of-treatment (EOT) evaluation. To eliminate any potentially residual CRS-207, subjects will initiate a 7-day course of antibiotics 7 days after the subject's last dose of CRS-207 and prior to receiving any subsequent cancer-related therapy (or if the patient will be having a semi-permanent indwelling device placed while on study) per **Section 4.5**. Subjects who are still receiving treatment at the time of study close may complete the current treatment course and the EOT evaluation prior to transitioning to long-term follow-up. Subjects will be considered in the treatment period until 28 days after the last dose of study drug. Blood cultures through a peripheral vein and also through a central line (if applicable) will be collected to monitor for the presence of CRS-207 per **Section 4.13**.

After completion of treatment and EOT assessments, all subjects, including those who did not receive treatment, will continue to be followed every three months (+/- 2 weeks) by telephone, e-mail, or optional clinic visit until death, withdrawal of consent, or closure of study. Subjects will also be contacted at 90 days (+14 day reporting window) from the last dose of pembrolizumab/ ipilimumab or 28 days (+7 day reporting window) from the last dose of tadalafil or CRS-207 if the subject is no longer receiving pembrolizumab/ipilimumab due to toxicity, whichever reporting period is longer. Information on survival and new cancer therapies will be collected. In addition, all subjects that received at least one dose of CRS-207 will be monitored for CRS-207 infection for one year per **Section 4.13**.

All subjects who discontinue study treatment should continue to be monitored for disease status by radiologic imaging every approximately every two months per standard of care until: 1) the start of a new antineoplastic therapy (information of the new cancer therapy will be collected), 2) disease progression, 3) death, 4) withdrawal of consent, or 5) the close of the study, whichever occurs first.

All subjects will be followed after their last dose of study drug for the development of adverse events (AEs) and serious adverse events (SAEs) as described in **Section 6.5.1**.

The primary analysis will be conducted when all treated subjects have documented response or progression by RECIST, or have discontinued the study. Information on survival may continue to be gathered for supplementary analyses after the completion of the primary analysis. At the conclusion of the study, all remaining subjects will be offered enrollment in a long-term follow-up study and continue to be followed for survival.

2. BACKGROUND

2.1 Study Disease

Despite decades of basic and clinical research, effective therapy for the treatment of patients with pancreatic ductal adenocarcinoma (PDA) remains one of the greatest unmet clinical needs in oncology today. Currently, PDA accounts for approximately 8% of all cancer-related mortality and has the lowest 5-year survival rate among all cancer types in the United States. PDA is currently the 4th leading cause of death from cancer in the U.S. with estimates in 2021 for 60,430 people diagnosed and about 48,220 dying from the disease[1]. Worldwide it will claim more than 300,000 lives this year[2]. It is projected that by 2030, pancreatic cancer will become the second leading cause of cancer-related death in the US[3].

Most patients are initially diagnosed with advanced disease that is inoperable with median survival of less than 1 year. Patients with advanced disease are usually treated with chemotherapy, with the intent of prolonging survival and palliating symptoms (pain, weight loss and decrease in performance status). From 1997, gemcitabine was the standard chemotherapy for advanced pancreatic cancer after demonstrating a significant improvement in survival compared to 5-fluorouracil (5-FU)[4]. Median survival was 5.65 months for gemcitabine-treated patients and 4.41 months for 5-FU treated patients, while overall tumor response rates were 5.4% and 0%, respectively.

Until recently, only erlotinib, an oral epidermal growth factor (EGF) inhibitor, was shown in a Phase 3 study to modestly improve median OS in combination with gemcitabine over gemcitabine alone (6.24 months for the doublet versus 5.91 months for gemcitabine alone) without a significant difference in ORR between the treatments[5]. In 2011, a Phase 2/3 trial conducted by a French consortium study group demonstrated FOLFIRINOX, a combined regimen of oxaliplatin, irinotecan, fluorouracil, and leucovorin significantly increased survival in patients with pancreatic cancer over gemcitabine alone. Median OS was 11.1 months versus 6.8 months for each treatment, respectively (hazard ratio [HR] for death, 0.57; 95% confidence interval [CI], 0.45 to 0.73; $p < 0.001$). The ORR was also increased to 31.6% from 9.4% ($p < 0.001$). Adverse events were increased in the FOLFIRINOX group and 5.4% of patients in this group experienced febrile neutropenia[6]. Although FOLFIRINOX represents an efficacious regimen in pancreatic cancer, there are still concerns about its potential toxicity and it is being reserved for the most fit patients.

In the MPACT (**M**etastatic **P**ancreatic **A**denocarcinoma **C**linical **T**rial) study, *nab*-paclitaxel combined with gemcitabine demonstrated a statistically significant and clinically meaningful median OS of 8.5 versus 6.7 months (HR 0.72, $p < 0.0001$ including a 59% increase in one-year survival (35% versus 22%, $p=0.0002$) and demonstrated double the rate of survival at two years

(9% versus 4%, p=0.02) as compared to gemcitabine alone in previously untreated patients with metastatic pancreatic cancer. *nab*-/gemcitabine also demonstrated a statistically significant improvement in key secondary endpoints compared to gemcitabine alone, including a 31% reduction in the risk of progression or death with a median PFS of 5.5 versus 3.7 months (HR 0.69, $p < 0.0001$) and an ORR of 23% compared to 7% (response rate ratio of 3.19, $p < 0.0001$)[7]. The MPACT study and regimen formed the basis for full FDA approval in September 2013 for the first-line treatment of metastatic adenocarcinoma of the pancreas.

Nanoliposomal irinotecan in combination with 5-FU was recently approved for patients with previously treated pancreatic cancer. However, it is unknown if this combination is better than 5-FU and irinotecan (FOLFIRI) as 5-FU was the comparator and there was no benefit in patients who previously received irinotecan so the value of this drug is questionable in FOLFIRINOX treated patients[8]. Therapies for patients with metastatic pancreatic cancer are urgently needed.

2.2 Rationale

Therapeutic benefit of immunotherapy in pancreatic cancer remains to be seen. The poor antigenicity of neoplastic cells and uniquely immunosuppressive stromal compartment are likely significant contributors to resistance, by inhibiting T cell priming and infiltration into the tumor microenvironment (TME). Single agent therapy using vaccines to induce antigen-specific T cells or targeting immune checkpoints such as programmed death-1 (PD-1) or cytotoxic T-lymphocyte associated protein-4 (CTLA-4) does not address these complex immunosuppressive mechanisms at play both at the systemic level and in the TME.

Current research evaluating responsiveness and resistance to immunotherapy has focused on tumor intrinsic factors and in particular, genetic sequencing data. However, these studies have had few revelations since PDA is typically genetically bland. Emerging data suggests that tumor extrinsic factors, especially the unique PDA desmoplastic stroma, orchestrates immunotherapy resistance. In particular, myeloid cells including tumor associated macrophages (TAMs) and myeloid derived suppressor cells (MDSCs) infiltrate PDA stroma and support neoplasia initiation, progression and metastasis. Multifunctional pathways such as CXCR4, CCR2/CCR5, and ARG1/NOS2 have been implicated in myeloid and stromal driven immune suppression. But it remains poorly understood how (i) PDA-targeted vaccine strategies with or without ICIs modulate these cells, and (ii) whether therapeutic targeting of these cells will enhance the activity of immunotherapy against PDA.

Effective immunotherapy in pancreatic cancer may require combinations of 3 or more agents to overcome this tolerance.

Dual Blockade with Pembrolizumab and Ipilimumab

The PD-1 receptor-ligand interaction is a major pathway hijacked by tumors to suppress immune control[9]. The normal function of PD-1, expressed on the cell surface of activated T cells under healthy conditions, is to down-modulate unwanted or excessive immune responses, including autoimmune reactions. The ligands for PD-1 (PD-L1 and PD-L2) are constitutively expressed or can be induced in various tumors[10-13]. Binding of either PD-1 ligand to PD-1 inhibits T cell activation triggered through the T cell receptor. The observed correlation of clinical prognosis with PD-L1 expression in multiple cancers suggests that the PD-1/PD-L1 pathway plays a critical

role in tumor immune evasion and should be considered as an attractive target for therapeutic intervention.

CTLA-4, an activation-induced T-cell surface molecule, is a member of the CD28:B7 immunoglobulin superfamily that competes with CD28 for B7. CTLA-4 mediated signals are inhibitory and turn off T cell-dependent immune responses[14]. The proposed mechanism of action for ipilimumab is interference of the interaction of CTLA-4 with B7 molecules on APCs, with subsequent blockade of the inhibitory modulation of T-cell activation promoted by the CTLA 4/B7 interaction.

While OPDIVO® (nivolumab), in combination with YERVOY® (ipilimumab), is currently FDA approved for the treatment of patients across multiple types of cancers, the combination of KEYTRUDA® (pembrolizumab) with ipilimumab is still under investigation. In a study evaluating low-dose ipilimumab (1 mg/kg) plus pembrolizumab (200 mg) following progression on anti-PD-1 immunotherapy in advanced melanoma, significant antitumor activity with durable long-term responses were observed[15]. In addition, the combination with low-dose ipilimumab was more tolerable with a grade 3-4 adverse event rate of 27%[15] in comparison to the 59% reported with higher doses of ipilimumab in combination with nivolumab[16]. Another study in previously treated advanced non-small-cell lung cancer (NSCLC) patients also demonstrated some anti-tumor activity but with higher toxicity rates given the higher doses of pembrolizumab and ipilimumab[17].

Immunotherapies in Pancreatic Cancer

CRS-207 is a live, attenuated, double-deleted *Lm* engineered to secrete mesothelin, a tumor-associated antigen which is overexpressed in most pancreatic cancers. An *Lm* vaccine construct permits access to both major histocompatibility complex (MHC) class I and II antigen processing pathways and has the capacity to stimulate both adaptive and innate immunity. A microorganism-based construct can naturally stimulate innate immunity via "danger signals" and toll-like receptors, serving as a natural vaccine adjuvant. Listeria also induces IFNβ expression through a stimulator of interferon genes (STING)-dependent pathway. Initial studies using CRS-207 in metastatic PDA (mPDA) were used in combination with GVAX pancreas vaccine (allogeneic pancreatic cancer cells modified to express GM-CSF) as a heterologous prime boost strategy using CY/GVAX Pancreas as a prime and CRS 207 as a boost vaccine. A survival benefit was observed compared to CY/GVAX Pancreas alone in an initial phase 2 study[18]. In a follow up study, ECLIPSE, with 3 treatment arms (A: CY/GVAX Pancreas + CRS-207, B: CRS-207, C: single agent chemotherapy), the study did not meet its primary endpoint and Arm A did not extend survival compared to Arm C. There was however, a partial response in Arm A. Although the study was not designed to compare Arm B to Arm C, these curves overlapped. The median survivals were 3.8, 5.4, and 4.6 months, respectively[19]. This study suggests that vaccines will likely need to be combined with immune checkpoint inhibition, but also supports studying CRS-207 alone as an interesting vaccine platform.

Based on the observation that PDAs upregulate immune checkpoint expression following vaccine treatment, we have undertaken several clinical trials in mPDA combining vaccines with one or more immune checkpoint inhibitors (several of which are ongoing: NCT03190265 and NCT03006302). In a completed study combining CY/GVAX Pancreas/CRS-207 with or without nivolumab, three patients had objective responses, and changes in the TME, including increase in $CD8^+$ T cells and a decrease in $CD68^+$ myeloid cells, were observed in long-term survivors of the

arm receiving nivolumab[20]. In addition, T cell receptor (TCR) repertoire analyses on PBL from patients treated with either ipilimumab +/- GVAX and GVAX+CRS-207 +/- nivolumab revealed that a net diversification of the peripheral TCR repertoire with ipilimumab and an increase in T cell clonality with nivolumab that correlated with improved survival[21]. These data suggested that ipilimumab increases the number of T cells expanded and available for vaccine priming and nivolumab expands the tumor specific T cells that have been primed by the vaccine, further supporting their use in combination.

So far, we have focused on inducing and expanding cancer recognizing T cells. Our reported studies and preliminary data demonstrate the ability to induce T cell responses that in some patients results in T cell infiltration and durable tumor regressions. However, these modest therapeutic effects suggest that there are other immunological partners to be targeted. Multiplexed immunohistochemistry (mIHC) imaging of PDAs treated with neoadjuvant GVAX demonstrated higher PD-L1 expression in myeloid cells in the TME. Also, analysis of PDA from our prime boost vaccine with and without nivolumab in mPDA patients, showed that a higher myeloid inflamed status in pretreatment PDAs predicted shorter survival, whereas pre-treatment lymphoid cell populations did not predict response (**Fig. 1**). Moreover, preliminary analysis of the peripheral blood samples by mass cytometry (Cytof) demonstrated several features of circulating immune cells associated with better clinical outcomes, notably lower abundance of myeloid cells at baseline, and trends toward lower CXCR4, CXCR2 and CCR2 expression in myeloid cells, and higher HLADR. These results suggest the critical importance of understanding the crosstalk between the myeloid and T cell compartments as well as the potential utility of targeting the myeloid-stromal signaling to enhance antitumor activity[20].

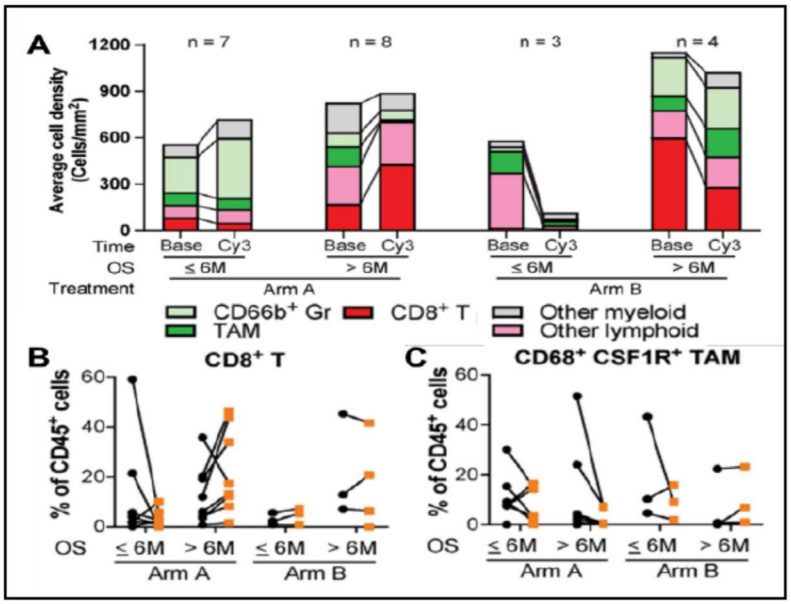


Figure 1: Changes in immune cell composition are associated with survival in patients treated with vaccine + nivo. FFPE tissue sections derived from biopsies obtained at baseline and after GVAX/CRS207 with (Arm A) or without nivo (Arm B) were subjected to immune-detection by multiplexed imaging. (A) Immune cell densities (cell number per mm2) of CD8+ T cell, other lymphoid lineages cells, CD68+CSF1R+ tumor associated macrophages (TAMs), CD66b+ granulocytes, and other myeloid cell lineages are shown, comparing baseline and post Cy3 status among short and long OS groups. (B, C) Frequency of CD8+ T cells (B) and CD68+ myeloid cells (C) are exhibited as cell percentages of total CD45+ immune cells, comparing baseline and post Cy3 status among short and long OS groups. Cy=cycle; In B, black circles =pre-treatment and orange squares are Cy3. Arm A: vaccine+nivo; Arm B: vaccine only.

We have also evaluated the combination of GVAX and ipilimumab in both treatment refractory advanced PDA and as maintenance after induction chemotherapy for newly diagnosed mPDA. While GVAX + ipilimumab did not improve survival over continuation of chemotherapy in the maintenance treatment of non-biomarker selected mPDA, clear biologic effects on peripheral and

intratumoral immune cells were observed[22].

[redacted]

Phosphodiesterase 5 inhibitors (PDE5i)

In addition to pro-apoptotic tumor effects, PDE5i have been found to have immunomodulatory properties including decreasing MDSCs and Tregs and increasing effector T cells in preclinical models. The widely available PDE5i tadalafil has also been shown to downregulate the expression and activity of ARG1 and NOS2, key MDSC-mediated immunosuppressive effector pathways, in intratumoral MDSCs in the CT26 mouse model[23]. Studies of tadalafil in melanoma and head and neck squamous cell cancer (HNSCC) patients have shown biologic activity, with increased T cell

activation and infiltration and decreased MDSCs[24-27]. A preliminary report from a study of nivo +/- tadalafil for neoadjuvant treatment of HNSCC showed that tadalafil enhanced T cell and myeloid cell infiltration in 47 randomized patients with a biologic effect in the TME[27]. [redacted]

[redacted]

Tadalafil has been evaluated extensively for safety and is currently FDA approved for use in erectile dysfunction (ED) and benign prostatic hyperplasia (BPH). The recommended dosing for ED/BPH is 5 mg for daily dosing and up to 20 mg on demand dosing for ED based on individual efficacy and tolerability. For our study, we have chosen a dose of tadalafil based on data from a randomized, prospective, double blinded, placebo controlled, phase II clinical trial in HNSCC which demonstrated reversal of immunosuppression at a dose of 20 mg/day[24]. This included reduction of MDSC numbers in tadalafil-treated patients, as well as increase in T-cell activation and expansion. Our study will allow dose reductions based on tolerability.

A better understanding of predictors of response as well as the mechanisms of resistance to immunotherapy remains a critical unmet need, as it will allow better patient selection and identification of additional targets for including in combination therapy approaches. Our overarching hypothesis will test whether repolarization or depletion of myeloid and stromal cells in the PDA TME, will augment activity of immunotherapy combinations and recruit the highest quality T cells to the antitumor response. In this study, we will be combining the CRS-207 vaccine with the myeloid-stromal targeting phosphodiesterase 5 inhibitor tadalafil and the immune checkpoint inhibitors nivolumab and ipilimumab.

3. PATIENT SELECTION

3.1 Inclusion Criteria

3.1.1 Age ≥18 years.

3.1.2 Have histologically- or cytologically-proven ductal adenocarcinoma of the pancreas. Patients with mixed histology (>30% non-adenocarcinoma component) will be excluded.

3.1.3 Have metastatic disease.

3.1.4 Have documented radiographic disease progression at the time of study enrollment, after previous systemic chemotherapy given in a neoadjuvant, adjuvant, locally advanced or metastatic setting.

3.1.5 Presence of at least one lesion with measurable disease as defined by 10 mm in longest diameter for a soft tissue lesions or 15 mm in short axis for a lymph node by RECIST 1.1.

3.1.6 Patient's acceptance to have a tumor biopsy of an accessible lesion at baseline and on treatment if the lesion can be biopsied with acceptable clinical risk (per **Section 8.1** as judged by the investigator**).**

3.1.7 ECOG performance status 0 or 1 (**Appendix A**).

3.1.8 Adequate organ and marrow function as defined below:

- Leukocytes ≥ 3,000/mcL
- Absolute neutrophil count ≥ 1,500/mcL
- Lymphocyte count ≥ 800/mcL
- Platelets ≥ 100 × 10^3/uL
- Hemoglobin ≥ 8.0 g/dL
- Total bilirubin ≤ upper limit of normal (ULN) except subjects with Gilbert Syndrome, who can have total bilirubin < 3.0 mg/dL
- AST(SGOT) and ALT(SGPT) ≤2.0 × ULN
- Alkaline phosphatase ≤5.0 × ULN
- Creatinine Creatinine clearance (CrCl) ≥ 50 mL/min (if using the modified Cockcroft-Gault formula below):

$$\textit{Female}\ \text{CrCl} = \frac{(140 - \text{age in years}) \times \text{weight in kg} \times 0.85}{72 \times \text{serum creatinine in mg/dL}}$$

$$\textit{Male}\ \text{CrCl} = \frac{(140 - \text{age in years}) \times \text{weight in kg} \times 1.00}{72 \times \text{serum creatinine in mg/dL}}$$

- Albumin ≥3.0 g/dL

3.1.9 Women of childbearing potential (WOCBP) must have a negative serum pregnancy test (minimum sensitivity 25 IU/L or equivalent units of human chorionic gonadotropin [HCG]). WOCBP is defined in **Section 4.8.** Patients with a positive HCG due to tumor secretion may be permitted to enroll if lack of pregnancy can be documented (e.g. transvaginal ultrasound or serial HCG) and with approval by the Principal Investigator.

- WOCBP must agree to follow instructions for method(s) of contraception from the time of enrollment for the duration of treatment with study drug(s) plus 5 half-lives of study drug(s) plus 4 weeks (duration of ovulatory cycle) for a total of 5 months post treatment completion.

- Men who are sexually active with WOCBP must agree to follow instructions for method(s) of contraception for the duration of treatment with study drug(s) plus 5 half-lives of study drug(s) plus 90 days (duration of sperm turnover) for a total of 7 months post-treatment completion.

- At least one barrier method of contraception must be employed by all sexually active patients (male and female), regardless of other methods, to prevent the transfer of body fluids.

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

3.2 Exclusion Criteria

3.2.1 Patient has a known history or evidence of brain metastases.

3.2.2 Patient who has had chemotherapy, radiation, or biological cancer therapy within 14 days prior to the first dose of study drug.

3.2.3 Patient has received an investigational agent or used an investigational device within 28 days of the first dose of study drug.

3.2.4 Patient is expected to require any other form of systemic or localized antineoplastic therapy while on study.

3.2.5 Patients who have had surgery within 28 days of dosing of investigational agent, excluding minor procedures (dental work, skin biopsy, etc.), celiac plexus block, and biliary stent placement.

3.2.6 Patients who have received any prophylactic vaccine within 14 days of first dose of study drug (7 days for the COVID vaccine) or received a live vaccine within 30 days of planned start of study therapy.

3.2.7 Have used any systemic steroids within 14 days of study treatment.

3.2.8 Use more than 4 g/day of acetaminophen.

3.2.9 Use of organic nitrates. Note: Medicines called nitrates include nitroglycerin that is found in tablets, sprays, ointments, pastes, or patches. Nitrates can also be found in other medicines such as isosorbide dinitrate or isosorbide mononitrate. Some recreational drugs called "poppers" also contain nitrates, such as amyl nitrite and butyl nitrite.

3.2.10 Use of alpha blocking antihypertensives (i.e. prazosin, doxazosin, terazosin).

3.2.11 Use of guanylate cyclase (GC) stimulators such as riociguat

3.2.12 Consumption of substantial amounts of alcohol (≥5 units/day)

3.2.13 Use of strong or moderate cytochrome P450 3A4 (CYP3A4) inhibitor or inducer. Potential interactions with patient medications should be checked in a clinical database such as EPIC or Lexicomp (https://www.uptodate.com/drug-interactions/?&redirect=true#di-druglist)

3.2.14 Patients on immunosuppressive agents (e.g., TNF pathway inhibitors, PI3 kinase inhibitors) within 7 days of study treatment.

3.2.15 Patient has a known allergy to both penicillin and sulfa.

3.2.16 History of severe hypersensitivity reaction to any monoclonal antibody.

3.2.17 History of severe hypersensitivity to tadalafil or any of the excipients of this product

3.2.18 Have current or prior history of infection or clinically significant adverse events (AEs) associated with an exogenous implant(s) or device(s) that has not and cannot be easily removed.

3.2.19 Subjects who have implanted medical devices that pose high risks for colonization and cannot be easily removed (e.g., artificial heart valves, pacemakers, prosthetic joints, orthopedic screw(s), metal plate(s)) if infection occurs. Other common devices such as venous access devices (e.g., Port-a-Cath or Mediport) may be permitted as well as arterial and venous stents and dental and breast implants.

3.2.20 Evidence of clinical ascites. Trace or small amounts of radiographic ascites may be approved by the Principal Investigator.

3.2.21 Have clinically significant and/or malignant pleural effusion (pleural effusions that are not clinically significant are allowed, defined as no more than 25% fluid level of the corresponding hemithorax).

3.2.22 Uncontrolled intercurrent illness including, but not limited to, ongoing or active infection, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situations that would limit compliance with study requirements.

3.2.23 Subjects with active, known or suspected autoimmune disease. Subjects with Graves or Hashimoto's disease, vitiligo, type I diabetes mellitus, psoriasis not requiring systemic treatment, or conditions not expected to recur in the absence of an external trigger are permitted to enroll.

3.2.24 Presence of any tissue or organ allograft, regardless of need for immunosuppression, including corneal allograft. Instances where loss of the graft is not a clinical concern (such as dental bone grafts or skin grafts placed only to promote skin growth) can be approved by the Principal Investigator. Patients with a history of allogeneic hematopoietic stem cell transplant will be excluded.

3.2.25 All toxicities attributed to prior anti-cancer therapy other than alopecia and fatigue must have resolved to grade 1 (National Cancer Institute Common Terminology Criteria for Adverse Events [CTCAE], version 5) or baseline before administration of study drug. Subjects with toxicities attributed to prior anti-cancer therapy which are not expected to resolve and result in long-lasting sequelae, such as neuropathy after chemotherapy, are permitted to enroll.

3.2.26 Have received a diagnosis of human immunodeficiency virus (HIV), hepatitis B or hepatitis C (patients who are hepatitis C antibody positive may be enrolled if they are confirmed with negative viral load at screening)

3.2.27 Patient has a pulse oximetry of <92% on room air.

3.2.28 Patient is on supplemental home oxygen.

3.2.29 Patient has an unhealed surgical wound or ulcer, or a bone fracture considered non-healing.

3.2.30 Patient has clinically significant heart disease.

- Angina requiring treatment with long-acting nitrates
- Positive cardiac stress test without documented evidence of subsequent, effective cardiac intervention
- Uncontrolled arrhythmia
- Within 90 days of start of treatment:
    - Angina requiring treatment with short-acting nitrates
    - Unstable angina
    - Myocardial Infarction
    - Coronary artery bypass graft surgery
    - Percutaneous coronary intervention (for example, angioplasty or stent placement)
- Any evidence of heart disease (NYHA≥Class III) within 6 months of planned tadalafil administration
- Left ventricular outflow obstructions, such as aortic stenosis and idiopathic hypertrophic subaortic stenosis

3.2.31 Patient has valvular heart disease that requires antibiotic prophylaxis for prevention of endocarditis.

3.2.32 Prior history of non-arterial ischemic optic retinopathy.

3.2.33 History of significant hypotensive episode requiring hospitalization within 6 months.

3.2.34 History of hypotension and/or blindness and/or sensorineural hearing loss during prior treatment with tadalafil or other PDE-5 inhibitors

3.2.35 History of known hereditary degenerative retinal disorders, including retinitis pigmentosa

3.2.36 History of stroke within prior 6 months.

3.2.37 Have insufficient peripheral venous access to permit completion of the study dosing and compliance with study phlebotomy regimen

3.2.38 Patient is, at the time of signing informed consent, a regular user (including "recreational use") of any illicit drugs (does not include marijuana or its derivatives) or other substance abuse (including alcohol) that could potentially interfere with adherence to study procedures or requirements.

3.2.39 Patient is unwilling or unable to follow the study schedule for any reason.

3.2.40 Patient is pregnant or breastfeeding.

3.2.41 Have rapidly progressing disease, as judged by the investigator (e.g., rapid progression through prior treatment[s]).

3.3 Inclusion of Women and Minorities

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

4. TREATMENT PLAN

4.1 Agent Administration

Treatment will be administered on an outpatient basis. Enrollment for the first three patients will be staggered by three weeks to evaluate patients for adverse events before the next patient is enrolled (the fourth patient may be enrolled 3 weeks after the third patient). Dosing delays are described in **Section 5**. No investigational or commercial agents or therapies other than those described below in **Table 2** may be administered with the intent to treat the subject's malignancy.

Table 1: Treatment Schedule

TREATMENT SCHEDULE			
Tadalafil	**Pembrolizumab**	**Ipilimumab**	**CRS-207**
Days 3-21* Cycles 1-6	Day 1, Cycles 1-6	Day 1, Cycles 1, 3, 5	Day 2, Cycles 1-6

* Daily starting on Day 1 after discontinuation of CRS-207

Table 2: Regimen Description

REGIMEN DESCRIPTION				
Agent	***Premedications; Precautions***	***Dose***	***Route***	***Course Length***
Tadalafil	None	20mg daily	Oral	18 weeks (6 cycles with 21 day cycles)
CRS-207	650 mg acetaminophen; NS pre- and post-infusion to total 1500ml (suggested: 500ml pre and 1000ml post). Subjects may also be pre-medicated with anti-emetics (suggested: IV ondansetron and IV fosaprepitant).	1×10^9 CFU in 100ml NS	IV infusion over 1 hour**	
Pembrolizumab	No prophylactic pre-medication will be given unless indicated by previous experience in an individual subject per **Section 4.2.3**.	200 mg	IV infusion over 30 min*	
Ipilimumab	No prophylactic pre-medication will be given unless indicated by previous experience in an individual subject per **Section 4.2.3**.	50 mg	IV infusion over 30 min*	

*Infusion times are approximate (-10/+15 min) and may need to be adjusted based on subject tolerability.
**Infusion times are approximate (± 15 min) and may need to be adjusted based on subject tolerability.

Please see **Section 5.2** for guidance regarding dosing delays. If the investigator assesses a drug-related toxicity (that requires discontinuation) to be related to an individual component of the treatment schedule, dosing for that study drug alone may be discontinued while dosing is delayed until the subject meets criteria to resume treatment of the other study drugs. The relationship to the discontinued study drug should be well documented in the source documents and permission from the Principal Investigator needs to be obtained prior to continuation with the other study drugs.

As of protocol version 5.0, any patients that remain on study after February 22, 2024 will

discontinue CRS-207 (no further drug supply) and may remain on study to continue to receive pembrolizumab, ipilimumab, and tadalafil.

4.1.1 Tadalafil

Tadalafil is primarily metabolized in the liver. Per the package insert, it is not recommended to administer tadalafil to patients with severe hepatic impairment. Therefore, Tadalafil administration will begin on Day 3 after review of hepatic laboratories and blood pressure for patients receiving CRS-207. Tadalafil administration will begin on Day 1 for patients who have discontinued CRS-207.

Subjects will self-administer their dose of tadalafil once a day without regard to food. The dose of tadalafil should be taken as close to the regularly scheduled 24-hour dosing interval as possible. If a dose is missed, missed doses may be taken on the same day (but not more than one dose per day). If a dose is vomited, it will be skipped.

4.1.2 CRS-207

4.1.4 Pembrolizumab

Pembrolizumab is to be administered as a 30 minute IV infusion of 200 mg. It is not to be administered as an IV push or bolus injection. At the end of the infusion, flush the line with a sufficient quantity of normal saline. Subjects should be observed for a minimum of 30 minutes before administration of ipilimumab.

Antiemetic medications should not be routinely administered prior to dosing of drugs. See **Section 4.2.3** for subsequent premedication recommendations following a pembrolizumab-related infusion reaction.

4.1.5 Ipilimumab

Ipilimumab is to be administered as a 30 minute IV infusion of 50 mg. It is not to be administered as an IV push or bolus injection. At the end of the infusion, flush the line with a sufficient quantity of normal saline.

Antiemetic medications should not be routinely administered prior to dosing of drugs. See **Section 4.2.3** for subsequent premedication recommendations following an ipilimumab-related infusion reaction.

4.2 General Concomitant Medication and Supportive Care Guidelines

4.2.1 Tadalafil

Acute reactions will be managed using standard therapy for acute drug reactions as per institutional guidelines.

4.2.2 CRS-207

Guidance on treatment of the common infusion reactions related to CRS-207 dosing is as follows:

- **Fevers:** Despite the acetaminophen premedication, subjects can spike fevers up to 40°C starting at the end of the CRS-207 infusion generally through the next 24 hours. Oral ibuprofen (400 to 800 mg) and acetaminophen (650 to 1000 mg) may be used in alternate sequence every 4 hours.

- **Rigors**: Rigors (generally once or twice per infusion) have been observed to start during or at the end of a CRS-207 infusion through 24 hours. IV narcotics such as morphine or meperidine may be administered per institutional policy. Oral morphine or non-steroidal anti-inflammatory drugs (NSAIDs) (e.g., aspirin, ibuprofen, naproxen) may be used as home treatment.

- **Blood pressure**: Decreases in blood pressure have been observed necessitating additional IV fluids during the 4 hour observation period (up to 1 or 2 liters). Reasons for this include the development of fever, compartmental shifts of fluid

resulting from the CRS-207 infusion and the use of narcotics. Some subjects have also been slightly hypotensive at 24 hours upon arrival to the clinic after CRS-207 administration. Subjects are encouraged to hydrate themselves liberally at home with oral fluids.

- **Nausea and vomiting**: Nausea and vomiting have been reported and observed within 24 hours after CRS-207 infusion. Subjects may be given anti-emetics as needed.

Blood draws for clinical hematology and serum chemistry will be done the day after the CRS-207 infusion. Any unexpected grade 3 or greater laboratory abnormalities should be repeated within 24-72 hours. Grade 3 or greater creatinine, AST, ALT, and bilirubin should be repeated within 24-72 hours as well.

4.2.3 Pembrolizumab and Ipilimumab

Pembrolizumab and ipilimumab are fully human monoclonal immunoglobulin (Ig) G4 antibodies. Subjects should be closely monitored for potential AEs during antibody infusion and potential AEs throughout the study.

4.2.3.1 Infusion Reactions

Since pembrolizumab and ipilimumab contain only human immunoglobulin protein sequences, it is unlikely to be immunogenic and induce an infusion or hypersensitivity reaction. However, if such a reaction were to occur, it might manifest with fever, chills, rigors, headache, rash, pruritis, arthralgias, hypo- or hypertension, bronchospasm, or other symptoms. All grade 3 or 4 infusion reactions should be reported within 24 hours to the Principal Investigator and reported as an SAE if criteria are met. Infusion reactions should be graded according to CTCAE (version 5) guidelines. Treatment recommendations are provided below and may be modified based on local treatment standards and guidelines as appropriate:

For grade 1 symptoms (mild reaction; infusion interruption not indicated; intervention not indicated):

Remain at bedside and monitor subject until recovery from symptoms. The following prophylactic premedications are recommended for future infusions: diphenhydramine 50 mg (or equivalent) and/or acetaminophen 325 to 1000 mg at least 30 minutes before additional pembrolizumab or ipilimumab administrations.

For grade 2 symptoms (moderate reaction requires therapy or infusion interruption but responds promptly to symptomatic treatment [e.g., antihistamines, non-steroidal anti-inflammatory drugs, narcotics, corticosteroids, bronchodilators, IV fluids]; prophylactic medications indicated for 24 hours):

Stop the pembrolizumab or ipilimumab infusion, begin an IV infusion of normal saline, and treat the subject with diphenhydramine 50 mg IV (or equivalent) and/or acetaminophen 325 to 1000 mg; remain at bedside and monitor subject until

resolution of symptoms. Corticosteroid or bronchodilator therapy may also be administered as appropriate. If the infusion is interrupted, restart the infusion at 50% of the original infusion rate when symptoms resolve; if no further complications ensue after 30 minutes, the rate may be increased to 100% of the original infusion rate. Monitor subject closely. If symptoms recur then no further pembrolizumab or ipilimumab will be administered at that visit. Administer diphenhydramine 50 mg IV, and remain at bedside and monitor the subject until resolution of symptoms. The amount of study drug infused must be recorded on the case report form (CRF). The following prophylactic premedications are recommended for future infusions: diphenhydramine 50 mg (or equivalent) and/or acetaminophen 325 to 1000 mg should be administered at least 30 minutes before additional pembrolizumab or ipilimumab administrations.

For grade 3 or grade 4 symptoms (severe reaction, grade 3: prolonged [i.e., not rapidly responsive to symptomatic medication and/or brief interruption of infusion]; recurrence of symptoms following initial improvement; hospitalization indicated for other clinical sequelae [e.g., renal impairment, pulmonary infiltrates]; grade 4: (life threatening; pressor or ventilator support indicated):

Immediately discontinue infusion of pembrolizumab or ipilimumab. Begin an IV infusion of normal saline, and treat the subject as follows. Recommend bronchodilators, epinephrine 0.2 to 1 mg of a 1:1,000 solution for subcutaneous administration or 0.1 to 0.25 mg of a 1:10,000 solution injected slowly for IV administration, and/or diphenhydramine 50 mg IV with methylprednisolone 100 mg IV (or equivalent), as needed. Subject should be monitored until the investigator is comfortable that the symptoms will not recur. Pembrolizumab and ipilimumab will be permanently discontinued. Investigators should follow their institutional guidelines for the treatment of anaphylaxis. Remain at bedside and monitor subject until recovery from symptoms. In the case of late-occurring hypersensitivity symptoms (e.g., appearance of a localized or generalized pruritis within 1 week after treatment), symptomatic treatment may be given (e.g., oral antihistamine, or corticosteroids).

Please refer to **Section 5.2** for guidelines regarding ipilimumab, CRS-207, and tadalafil treatment delays following a pembrolizumab or ipilimumab infusion-related reaction.

4.2.3.2 Pembrolizumab and Ipilimumab-Related Adverse Events

Blocking PD-1 or CTLA-4 function may permit the emergence of auto-reactive T cells and resultant clinical autoimmunity. Rash/pruritus, diarrhea/colitis, pneumonitis, hepatitis, and hypothyroidism were drug-related, presumptive autoimmune events noted in previous pembrolizumab and ipilimumab studies.

For the purposes of this study, a pembrolizumab or ipilimumab-related AE is defined as an AE of unknown etiology, associated with drug exposure and is consistent with an immune phenomenon. Efforts should be made to rule out neoplastic, infectious, metabolic, toxin or other etiologic causes. Serological, immunological, and

histological (biopsy) data should be used to support the diagnosis of an immune-mediated toxicity. Suspected pembrolizumab or ipilimumab-related AEs must be documented on an AE or SAE CRF. Identification and treatment of pembrolizumab and ipilimumab-related AEs can be found in the **NCCN's guidelines for the management of immunotherapy-related toxicities**. Additional guidance can be found in the pembrolizumab and ipilimumab package inserts. Antibiotics will also be administered to subjects who have not yet received antibiotics for CRS-207 and the subject requires steroids for a suspected pembrolizumab or ipilimumab-related AE (**Section 4.5**).

Subjects who experience a grade 2 or higher pembrolizumab or ipilimumab-related AE should be discussed with the Principal Investigator and IND sponsor immediately.

4.3 Prohibited and/or Restricted Medications and Devices

The following therapies or devices are not permitted during the treatment period (if administered, the subject may be removed from the study):

- Any non-study anticancer chemotherapy or immunotherapy (approved or investigational)
- Any major surgery or surgical procedure; if required must be discussed with the Principal Investigator to determine if it is appropriate for the subject to continue study treatment
- TNF pathway inhibitors or PI3 kinase inhibitors
- Another investigational agent
- Live vaccines (examples of live vaccines include, but are not limited to: measles, mumps, rubella, chicken pox, yellow fever, rabies, BCG, and typhoid [oral] vaccine). Seasonal influenza vaccines for injection are generally killed virus vaccines and are allowed. However, intranasal influenza vaccines (e.g. Flu-Mist®) are live attenuated vaccines, and are not allowed.
- Where possible, subjects should not receive any dose of the COVID-19 vaccine within 7 days of pembrolizumab, ipilimumab, and CRS-207.
- The use of anticoagulants is known to increase the risk of gastrointestinal hemorrhage. Since gastrointestinal hemorrhage is an adverse reaction with pembrolizumab and ipilimumab, subjects who require concomitant anticoagulant therapy should be monitored closely.
- Implanted medical devices that pose high risks for colonization and cannot be easily removed (e.g., artificial heart valves, pacemakers, prosthetic joints, orthopedic screw(s), metal plate(s)) if infection occurs are prohibited. Other common devices such as venous access devices (e.g., Port-a-Cath or Mediport), arterial and venous stents, and dental and breast implants may be permitted if approved by the Principal Investigator.
- Palliative (limited-field) radiation therapy is permitted, but only for pain control and with approval by the Principal Investigator or IND Sponsor.
- Systemically active steroids can be used but should be reported to the Principal Investigator and/or IND Sponsor. Steroid treatment should be completed at least 14 days prior to resuming study-related treatments. Patients requiring adrenal replacement steroid doses $\leq$ 10 mg daily prednisone equivalent (in the absence of active autoimmune disease) may resume treatment if approved by the Principal Investigator or the IND Sponsor.

Patients requiring replacement doses of steroids are required to discontinue treatment with CRS-207.

- If steroids or immunosuppressive agents are required during treatment, prophylactic antibiotics will be administered as outlined in **Section 4.5.**
- Use of organic nitrates. Note: Medicines called nitrates include nitroglycerin that is found in tablets, sprays, ointments, pastes, or patches. Nitrates can also be found in other medicines such as isosorbide dinitrate or isosorbide mononitrate. Some recreational drugs called "poppers" also contain nitrates, such as amyl nitrite and butyl nitrite.
- Use of guanylate cyclase (GC) stimulators such as riociguat
- Substantial amounts of alcohol (≥5 units/day) are not recommended while taking tadalafil because tadalafil can potentiate the hypotensive effects
- Alpha-blocking antihypertensives (such as terazosin, doxazosin mesylate, prazosin, alfuzosin) are prohibited. Alpha blockers for the treatment of benign prostatic hyperplasia should be used with caution.
- Use of anti-hypertensives should be used with caution
- Use of strong or moderate CYP3A4 inducers (rifampicin) are not recommended because they can decrease tadalafil exposure
- Use of strong or moderate CYP3A4 inhibitors are not recommended because they can increase tadalafil exposure. Patient medications should be checked in a clinical database such as EPIC or Lexicomp (https://www.uptodate.com/drug-interactions/?&redirect=true#di-druglist)

In addition, the following therapies should not be administered during the treatment period unless medically necessary and approval must be obtained from the Principal Investigator for a subject to continue dosing if therapy is given concurrently with study participation:

- General anesthesia
- Aspirin >325 mg/day (chronic daily use of aspirin ≤325 mg/day and heparin flushes for central lines are allowed except during CRS-207 infusions through 4 days after each CRS-207 infusion.)
- More than 4 g/day of acetaminophen
- Systemic antibiotics

4.4 Other Restrictions and Precautions

Palliative (limited-field) radiation therapy is permitted, but only for pain control to sites present at baseline and with approval by the Principal Investigator or IND Sponsor.

If subjects receive immunosuppressive medications on or after study, prophylactic antibiotics to prevent CRS-207 infection are strongly recommended for the duration of the treatment with the immunosuppressant (recommended oral 80 mg trimethoprim / 400 mg sulfamethoxazole once daily or 160 mg trimethoprim / 800 mg sulfamethoxazole (DS) three days a week).

4.5 Antibiotic Administration

4.5.1 Antibiotic Regimen for Semi-Permanent Indwelling Device Placement

An antibiotic regimen should be initiated for any patient that is having a semi-permanent

indwelling device placed such as biliary stents/drains, vascular devices, and pleuryx catheters (excluding patients who discontinued CRS-207). If possible, it is recommended that patients receive a dose of Ampicillin/Sulbactam (Unasyn, 3g) with Gentamicin (3 mg/kg) pre-operatively. This should be followed by 8-hour intervals of 1g Amoxicillin (160 mg trimethoprim/800 mg sulfamethoxazole at 12-hour intervals for allergic patients) for 3 days post-operatively.

4.5.2 EOT: 7 Days after Last CRS-207 Dose in Each Treatment Course, or Suspected CRS-207 Infection, or Steroid Administration

An antibiotic course will be initiated for each subject 7 days after the subject's last dose of CRS-207 for each treatment course, within 7 days of the decision to discontinue treatment if treatment is discontinued midcourse to ensure clearance of CRS-207 before additional courses or subsequent cancer-related therapy, or 7 days after the subject's last dose of CRS-207 if CRS-207 is discontinued and the patient remains on study to receive the other study drugs. For subjects who do not have a central line, a 7-day course of oral amoxicillin (1G at 8-hour intervals) or trimethoprim/sulfamethoxazole in penicillin-allergic subjects (160 mg trimethoprim/800 mg sulfamethoxazole at 12-hour intervals) will be initiated for each subject. If the patients do not tolerate the 1G dose, the antibiotics can be dose reduced to 500mg. Subjects with a central line will receive 2 doses [2g ampicillin 6 hours apart or 3-5 mg/kg trimethoprim/ sulfamethoxazole 8 hours apart (in penicillin-allergic subjects)] of IV antibiotics through the port, followed by 6 days of oral antibiotics [1G at 8-hour intervals amoxicillin or 160 mg trimethoprim/800 mg sulfamethoxazole at 12-hour intervals (in penicillin-allergic subjects)] started within 6 hours of completing the IV ampicillin (or within 8 hours from IV trimethoprim/sulfamethoxazole). If the subject is withdrawn from the study more than 7 days after administration of CRS-207, antibiotics will be administered as soon as possible after study withdrawal.

Should a patient require emergent implant of a prohibited device (as described in **Section 4.3**) while on therapy, the patient will receive a 14-day IV antibiotic regimen appropriate for the coverage of wild-type listeriosis.

Antibiotics will also be administered to subjects who have not yet received antibiotics for CRS-207 and the subject requires steroids for a suspected pembrolizumab/ipilimumab-related AE or non-related AE. After completion of 7-day course of antibiotics for listeria clearance, antibiotic prophylaxis should be given for the duration of the treatment with the steroid (recommended oral 80 mg trimethoprim / 400 mg sulfamethoxazole once daily or 160 mg trimethoprim / 800 mg sulfamethoxazole (DS) three days a week).

During or after study, subjects who are confirmed to have a blood culture positive for CRS-207 after more than 7 days post-infusion, will receive a minimum 14-day course of IV antibiotics (see below). In addition, IV ampicillin (or trimethoprim/sulfamethoxazole in penicillin-allergic subjects) plus gentamicin should be initiated earlier for possible infectious complications of CRS-207 for subjects who are suspected of having CRS-207 infection and meet the specified criteria (see below).

Subjects with clinical or laboratory signs or symptoms of persistent infection who require initiation of antibiotics other than specified by protocol should have a clinically-relevant evaluation, including appropriate bacterial cultures. Culture of cerebrospinal fluid should be obtained for subjects with suspected central nervous system infection. In such instances, analysis of

cerebrospinal fluid should also include cell count, protein, glucose and Gram stain. IV ampicillin (or trimethoprim/sulfamethoxazole in penicillin-allergic subjects) plus gentamicin should be initiated for possible infectious complications of CRS-207 for subjects who are suspected of having persistent CRS-207 infection and meet the criteria listed below:

- Flu-like symptoms Grade 3 or greater lasting for ≥12 hours
- Fever Grade 4 or higher (>40.0ºC for >24 hours)
- Persistent fever >39ºC lasting for ≥48 hours
- Infection Grade 3 or higher (infection with interventional radiology or operative intervention indicated)
- Evidence of abscess
- Clinical signs or symptoms (e.g., neurologic signs or symptoms), which, in the judgment of the investigator, necessitate starting antibiotics

The preferred antibiotic regimen if persistent CRS-207 infection is suspected or confirmed is IV administration of ampicillin (or trimethoprim/sulfamethoxazole in penicillin-allergic subjects) plus gentamicin. For this purpose, initial doses of ampicillin should be approximately 12 g/day (divided doses every 3 to 4 hours), with gentamicin 3 mg/kg daily in divided doses every 8 hours (with adjustments according to renal function and serum levels of gentamicin). In penicillin-allergic subjects, initial IV doses of trimethoprim/sulfamethoxazole should be 15 to 20 mg/kg/day (based on trimethoprim component) divided four times per day, with gentamicin 3 mg/kg/day in divided doses every 8 hours (with adjustments according to renal function and serum levels of gentamicin). For those individuals who receive IV antibiotics, the course of therapy is anticipated to be greater than or equal to 14 days, depending on clinical course. Antibiotic treatment may be completed with use of oral antibiotics, if clinically indicated.

If during the course of treatment the subject develops a reaction to antibiotics such that neither penicillin derivatives nor trimethoprim/sulfamethoxazole can be safely administered, alternative antibiotics such as tetracyclines should be given in all of the above situations. The choice of alternative should be made in discussion with the Principal Investigator, and consultation with infectious disease should be considered.

Suspected or confirmed infection with CRS-207 and/or Listeria is considered an adverse event of special interest (AESI) and should be reported following SAE reporting procedures (**Section 6.1.3**) irrespective of temporal relationship to study drug administration. This includes scheduled blood cultures during surveillance monitoring that are positive for CRS-207 or if a subject presents with symptoms suspicious for a Listeria-like infection and/or is tested positive for Listeria at a local hospital/clinic.

4.6 Definition of an Overdose for this Protocol

Overdose of pembrolizumab or ipilimumab are defined as:
An overdose is defined as the accidental or intentional administration of any dose of a product that is considered both excessive and medically important. All occurrences of overdose must be reported as SAEs (see **Section 6.5.1** for reporting details). Appropriate supportive treatment should be provided if clinically indicated.

All reports of overdose with and without an AE must be reported within 24 hours to the Principal Investigator and IND Sponsor (Dr. Elizabeth Jaffee).

4.7 Unacceptable Toxicity

Unacceptable toxicities are defined as any pembrolizumab, ipilimumab, CRS-207, or tadalafil-related toxicities that require treatment discontinuation per **Sections 4.10.2, 4.10.3, or 4.10.4**.

Exception: While grade 3 and above pembrolizumab and ipilimumab infusion reactions require treatment discontinuation of that drug per **Section 4.10.2**, they will not be considered unacceptable toxicities.

Unexpected Grade 3 or greater laboratory abnormalities should be repeated within 24-72 hours if clinically indicated and monitored as necessary to determine if event meets toxicity criteria. Grade 3 or greater creatinine, AST, ALT, and bilirubin should be repeated within 24-72 hours as well.

If the investigator assesses a drug-related toxicity (that requires discontinuation) to be related to an individual component of the treatment schedule, dosing for that study drug alone may be discontinued while dosing is delayed until the subject meets criteria to resume treatment of the other study drugs. The relationship to the discontinued study drug should be well documented in the source documents and permission from the Principal Investigator and co-Principal Investigator needs to be obtained prior to continuation with the other study drugs.

Starting from the 6th patient, if more than 33% of patients (that is, 2/6, 3/9, 4/12, 5/15, 6/17) are observed to experience an unacceptable toxicity within the first cycle of treatment, enrollment will be paused and the safety data will be reviewed by the study team and IND Sponsor before the study enrolls additional patients. In addition, enrollment will be paused if a patient's death is assessed as possibly related to any of the study drugs while the data is reviewed by the study team and IND sponsor.

4.8 WOCBP, Contraception, Use in Pregnancy, Use in Nursing

A WOCBP is defined as any female who has experienced menarche and who has not undergone surgical sterilization (hysterectomy or bilateral oophorectomy) and is not postmenopausal. Menopause is defined clinically as 12 months of amenorrhea in a woman over age 45 years in the absence of other biological or physiological causes. In addition, women under the age of 62 years must have a documented serum follicle stimulating hormone (FSH) level > 40mIU/mL to confirm menopause.

Women treated with hormone replacement therapy (HRT) are likely to have artificially suppressed FSH levels and may require a washout period in order to obtain a physiologic FSH level. The duration of the washout period is a function of the type of HRT used. The duration of the washout period below are suggested guidelines and the investigators should use their judgment in checking serum FSH levels. If the serum FSH level is >40 mIU/ml at any time during the washout period, the woman can be considered postmenopausal:

- 1 week minimum for vaginal hormonal products (rings, creams, gels)
- 4 week minimum for transdermal products

- 8 week minimum for oral products

4.8.1 Contraception

The investigational agents used in this protocol may have adverse effects on a fetus *in utero*. Furthermore, it is not known if the investigational agents have transient adverse effects on the composition of sperm. Non-pregnant, non-breast-feeding women may be enrolled if they are considered highly unlikely to conceive. Highly unlikely to conceive is defined as 1) surgically sterilized, or 2) postmenopausal (defined as a woman who is > 45 years of age and has not had menses for greater than 12 months and women under the age of 62 must have a documented serum follicle stimulating hormone (FSH) level less than 40mlU/mL will be considered postmenopausal). or 3) amenorrheaic for < 2 years without a hysterectomy and oophorectomy and with a documented FSH value in the postmenopausal range, or) not heterosexually active for the duration of the study, or 5) heterosexually active and willing to use 2 methods of birth control (which is also required for the female partners of male subjects). The 2 birth control methods can be 2 barrier methods *or* a barrier method plus a hormonal method to prevent pregnancy, used throughout the study starting with Visit 1 through 5 months after the last dose of study drug. Male subjects enrolled in this study must also agree to use an adequate method of contraception starting with Visit 1 through 7 months after the last dose of study drug.

Investigators shall counsel WOCBP and male subjects who are sexually active with WOCBP on the importance of pregnancy prevention and the implications of an unexpected pregnancy Investigators shall advise WOCBP and male subjects who are sexually active with WOCBP on the use of highly effective methods of contraception. Highly effective methods of contraception have a failure rate of < 1% per year when used consistently and correctly.

HIGHLY EFFECTIVE METHODS OF CONTRACEPTION

- Male condoms with spermicide
- Hormonal methods of contraception including combined oral contraceptive pills, vaginal ring, injectables, implants, and intrauterine devices (IUDs) such as Mirena by WOCBP subject or male subject's WOCBP partner.
- Nonhormonal IUDs, such as ParaGard
- Tubal ligation
- Vasectomy
- Complete abstinence*

*Complete abstinence is defined as complete avoidance of heterosexual intercourse and is an acceptable form of contraception for all study drugs. Abstinence is only acceptable when this is in line with the preferred and usual lifestyle of the subject. Periodic abstinence (e.g., calendar, ovulation, symptothermal, profession of abstinence for entry into a clinical trial, post-ovulation methods) and withdrawal are not acceptable methods of contraception. Subjects who choose complete abstinence are not required to use a second method of contraception, but female subjects must continue to have pregnancy tests. Acceptable alternate methods of highly effective contraception must be discussed in the event that the subject chooses to forego complete abstinence.

LESS EFFECTIVE METHODS OF CONTRACEPTION

- Diaphragm with spermicide
- Cervical cap with spermicide
- Vaginal sponge
- Male condom without spermicide*
- Progestin only pills by WOCBP subject or male subject's WOCBP partner
- Female condom*

*A male and female condom must not be used together

Subjects should be informed that taking the study drug may involve unknown risks to the fetus (unborn baby) if pregnancy were to occur during the study. In order to participate in the study they must adhere to the contraception requirement (described above) for the duration of the study. If there is any question that a subject will not reliably comply with the requirements for contraception, that subject should not be entered into the study.

4.8.2 Use in Pregnancy

The investigational agents used in this protocol may have adverse effects on a fetus; therefore, women with a positive pregnancy test at screening will not be eligible for enrollment. If a subject inadvertently becomes pregnant while on treatment, the subject will immediately be removed from the study. The study team will contact the subject at least monthly and document the subject's status until the pregnancy has been completed or terminated.

Pregnancy in female subjects throughout the study or within 5 months of completing treatment as well as any pregnancy in partners of male subjects throughout the study or within 7 months of completing the study should be reported initially as a serious adverse event (see SAE reporting procedures in **Section 6.5.1** and **6.5.5**) by the investigator within 24 hours of learning of its occurrence. Pregnancy information must be reported on the Pregnancy Form.

Protocol required procedures for study discontinuation and follow-up must be performed on the subject unless contraindicated by pregnancy (e.g., x-ray studies). Other appropriate pregnancy follow-up procedures should be considered if indicated.

Follow-up information regarding the course of the pregnancy, including any voluntary or spontaneous termination, perinatal and neonatal outcome and where applicable, offspring information must be reported on the Pregnancy Follow-up Form. Pregnancy outcomes must also be collected for the female partners of any males in this trial. Consent to report information regarding these pregnancy outcomes should be obtained from the female partner.

4.8.3 Use in Nursing Women

Since many drugs are excreted in human milk, and because of the potential for serious adverse reactions in the nursing infant, subjects who are breast-feeding are not eligible for enrollment.

4.8.4 All Subjects (Male and Female)

All sexually active patients must use at least a barrier method (i.e., condom) to prevent transmission of body fluids.

4.9 Duration of Therapy

Subjects will receive treatment every 3 weeks for 6 cycles of treatment within a course (18 weeks total). At the investigator's discretion, subjects who are clinically stable and meet dosing requirements (per **Section 5.2**) at the end of the first course may receive additional courses of their assigned treatment for up to a maximum of 2 years. Subjects that begin a new course prior to the 2 year cut-off may complete that course prior to coming off study. The additional course(s) may start as early as 3 weeks (+7 days) from last dose of previous course and all assessments will be followed per the study schedule in **Section 9**, with the first dose of the additional course corresponding to Day 1, Cycle 1 of the study schedule. The following assessments are not required during additional courses:

- HLA-typing
- Tumor biopsies

4.10 Criteria for Removal from Treatment

The reason for study removal and the date the subject was removed will be documented in the CRF. A subject will be discontinued from the trial for any of the following reasons:

- The subject or legal representative (such as a parent or legal guardian) withdraws consent for participation in the study.

A subject must be discontinued from treatment (but may continue to be monitored in the post-treatment follow-up portion of the trial) for any of the following reasons:

- The subject or legal representative (such as a parent or legal guardian) withdraws consent for treatment
- Intercurrent illness that prevents further administration of treatment
- Unacceptable toxicities that do not resolve to allow for treatment continuation with the remainder of the study regimen (see **Section 4.7**). If the study treatment has provided clinical benefit (as defined in **Section 4.10.1**), the IND sponsor may approve trial continuation for patients experiencing toxicities that are not life threatening or of major clinical concern (such as transient hypotension).
- Disease progression as defined in **Section 4.10.1**
- Need for >2 dose delays due to the same drug-related toxicity as per the dose delay guidelines (see **Section 5.2**)
- If, in the opinion of the Investigator, a change or temporal or permanent discontinuation of therapy would be in the best interest of the subject,
- Noncompliance with trial treatment or procedure requirements,

- Subject is lost to follow-up
- Subject becomes pregnant

4.10.1 Disease Progression

CRS-207, pembrolizumab, and ipilimumab are expected to trigger immune-mediated responses, which require activation of the immune system prior to the observation of clinical responses. Such immune activation may take weeks to months to be evident. Some subjects may have objective volume increase of tumor lesions or other disease parameters within weeks following the start of immunotherapy. Such subjects may not have had sufficient time to develop the required immune activation or, in some subjects, tumor volume or other disease parameter increases may represent infiltration of lymphocytes into the original tumor. In conventional studies, such tumor volume or relevant laboratory parameter increases during the first 2-4 months of the study would constitute disease progression and lead to discontinuation of imaging to detect response, thus disregarding the potential for subsequent immune-mediated clinical response. This phenomenon was observed in approximately 10% of subjects in the Phase 1 study of nivolumab and has also been reported for ipilimumab monotherapy[28].

Subjects will be permitted to continue with treatment beyond RECIST 1.1 defined PD as long as they meet the following criteria:
- Investigator-assessed clinical benefit, and
- Subject is tolerating study drug.

The assessment of clinical benefit should take into account whether the subject is clinically deteriorating and unlikely to receive further benefit from continued treatment. The following criteria need to be taken into consideration:
- No decline in ECOG performance status.
- Absence of rapid progression of disease or of progressive tumor at critical anatomical sites (e.g., cord compression) requiring urgent alternative medical intervention.

All decisions to continue treatment beyond PD must be discussed with the Principal Investigator or Co-Principal Investigator and documented in the study records.

Tumor assessments will be made using RECIST 1.1 (**Appendix B**) and iRECIST (**Appendix C**).

4.10.2 Pembrolizumab and Ipilimumab-Related Adverse Events

Permanent discontinuation of pembrolizumab and ipilimumab should be considered for any of the following:

1. Severe or life-threatening related AEs, including, but not limited to, any of the following (the IND Sponsor must be notified in the event of these AEs):
    - Any grade 2 treatment-related uveitis, eye pain, or blurred vision that does not respond to topical therapy and does not improve to Grade 1 severity within 2 weeks of starting therapy OR requires systemic treatment

- Any grade 3 non-skin, drug-related AE lasting > 7 days, with the following exceptions:

  - Grade 3 treatment-related uveitis, pneumonitis, bronchospasm, colitis, neurologic toxicity, hypersensitivity reaction, or infusion reaction (applies to pembrolizumab and ipilimumab only) of any duration requires discontinuation
  - Diarrhea, nausea, or vomiting that resolves to < grade 3 within 24 hours of intervention
  - Grade 3 fatigue does not require discontinuation
  - Grade 3 treatment-related endocrinopathies adequately controlled with only physiologic hormone replacement do not require discontinuation
  - Grade 3 treatment-related laboratory abnormalities do not require treatment discontinuation except:

    - Grade 3 treatment-related thrombocytopenia > 7 days OR that is associated with bleeding requires discontinuation

    - Any treatment-related liver function test (LFT) abnormality that meets the following criteria require discontinuation:
      - Total bilirubin > 5 × ULN
      - Concurrent AST or ALT > 3 × ULN **and** total bilirubin > 2 × ULN

- Any grade 4 treatment-related AE or laboratory abnormality, except for the following events which do not require discontinuation:

  - Grade 4 amylase or lipase abnormalities that are not associated with symptoms or clinical manifestations, or radiographic signs of pancreatitis. It is recommended to consult with the Principal Investigator for grade 4 amylase or lipase abnormalities.

  - Isolated grade 4 electrolyte imbalances/abnormalities that are not associated with clinical sequelae and are corrected with supplementation/appropriate management within 72 hours of their onset.

  - Transient, self-correcting grade 4 AST or ALT that occur after the CRS-207 infusion and resolves within 2 weeks.

  - Grade 4 lymphopenia and leukopenia.

  - Grade 4 treatment-related endocrinopathy adverse events, such as adrenal insufficiency, ACTH deficiency, hyper- or hypothyroidism, or glucose intolerance, which resolve or are adequately controlled with physiologic hormone replacement (corticosteroids, thyroid hormones) or glucose-controlling agents, respectively, may not require discontinuation after discussion with and approval from the Principal Investigator.

- Any dosing interruption lasting > 6 weeks with the following exceptions:

  - Dosing interruptions to allow for prolonged steroid tapers to manage drug-related adverse events are allowed. Prior to re-initiating treatment in a subject with a dosing interruption lasting > 6 weeks, the Principal Investigator must be consulted. Tumor assessments should continue as per protocol even if dosing is interrupted.

  - Dosing interruptions > 6 weeks that occur for non-drug-related reasons may be allowed if approved by the Principal Investigator. Prior to re-initiating treatment in a subject with a dosing interruption lasting > 6 weeks, the Principal Investigator must be consulted. Tumor assessments should continue as per protocol even if dosing is interrupted.

Any AE, laboratory abnormality, or intercurrent illness which, in the judgment of the Investigator, presents a substantial clinical risk to the subject with continued pembrolizumab or pembrolizumab/ipilimumab dosing.

In order to standardize the management of irAEs for all subjects, treatment management algorithms can be found in the **NCCN's guidelines for the management of immunotherapy-related toxicities**. Additional AE treatment management guidance included in the package inserts might be considered for individual cases.

Subjects that are required to stop treatment with pembrolizumab/ipilimumab due to toxicity may stay on study and receive tadalafil and CRS-207 (assessment schedule per **Section 9.2**) once the pembrolizumab/ipilimumab-related toxicity(s) has resolved to a grade 1.

4.10.2 CRS-207-Related Adverse Events

Permanent discontinuation of CRS-207 should be considered for any of the following:

- Any treatment-related ≥ grade 3 AEs. Exceptions include: fever, chills, rigors, hypertension, hypotension, syncope, or hypoxia occurring within 12 hours of CRS-207 administration
- A fever of >40°C that lasts for greater than 24 hours and does not respond to antipyretics.
- Clinically significant hypotension unresponsive to IV fluids (e.g., systolic blood pressure [BP] <90 mm Hg or mean arterial pressure <55 mm Hg as measured on two separate occasions at least 10 minutes apart).
- Initiation of antibiotic therapy, coincident with simultaneous isolation of CRS-207 from a normally sterile body site, other than blood (e.g., cerebrospinal fluid, joint fluid).

Subjects that are required to stop treatment with CRS-207 due to toxicity may stay on study and receive pembrolizumab, ipilimumab, and tadalafil (assessment schedule per **Section 9.2**) once the pembrolizumab/ipilimumab-related toxicity(s) has resolved to a grade 1.

4.10.4 Tadalafil-Related Adverse Events

Permanent discontinuation of tadalafil should be considered for any of the following:

- Severe or life-threatening tadalafil-related AEs, including, but not limited to, any of the following (the IND Sponsor must be notified in the event of these AEs):

    1. Any grade 2 drug-related retinal vascular disorder, eye pain, or blurred vision/visual field defect that does not respond to topical therapy and does not improve to Grade 1 severity within 2 weeks of starting therapy OR requires systemic treatment

    2. Any grade 3 or above clinically significant drug-related AEs that recurs after 2 dose reductions or the toxicity does not resolve to < grade 3 within 24 hours of intervention

Subjects that are required to stop treatment with tadalafil due to toxicity may stay on study and receive pembrolizumab, ipilimumab, and CRS-207 (assessment schedule per **Section 9.2**) once the pembrolizumab/ipilimumab-related toxicity(s) has resolved to a grade 1.

4.11 End of Treatment (EOT) Visit

All subjects will return to the study site 28 days (± 7 days) after the final study treatment (i.e., completion of the final course or upon early discontinuation) for an EOT evaluation. Procedures and assessments performed at these visits and beyond should follow the respective guidelines described in **Sections 4.12 and 9.0** as appropriate.

If the EOT visit occurs early (e.g., 1 week prior to the expected visit as protocol allows) or if the patient cannot return due to disease progression, an assessment for AEs should be made by telephone or email on day 28 (± 1 day) after last dose of study drug and documented.

To eliminate any potentially residual CRS-207, subjects will be administered a 7-day course of antibiotics per **Section 4.5**. Blood cultures for surveillance of CRS-207 will also be collected per **Section 4.13**.

4.12 Duration of Follow-Up

Treated subjects will begin the follow-up period after they complete the EOT visit. Subjects will be contacted every three months (+/- 1 month) to monitor overall survival until death, withdrawal of consent, or study closure. Information of other cancer therapies after discontinuation from the study treatment will be collected. Subjects will also be contacted at 90 days (+14 day reporting window) from the last dose of pembrolizumab/ipilimumab if the subject was still receiving pembrolizumab/ipilimumab at the time of treatment discontinuation to monitor drug toxicity. In addition, all subjects that received at least one dose of CRS-207 will be monitored for CRS-207 infection for one year per **Section 4.13**.

Subjects who discontinued study treatment without documented disease progression should continue to be monitored for disease status by radiologic imaging. Disease monitoring should continue to be assessed approximately every two months per standard of care until: 1) start of a

new antineoplastic therapy (information of the new cancer therapy will be collected), 2) disease progression, 3) death, 4) withdrawal of consent, or 5) study closure, whichever occurs first.

Subjects who are discontinued from the study treatment due to an unacceptable drug-related AE will be monitored for safety until the resolution of the AE to ≤ grade 1 or stabilization or until initiation of a new therapy for their cancer, whichever occurs first.

All subjects will be followed after their last dose of study drug for the development of AEs and SAEs as described in **Section 6.5.1**

At the conclusion of the study, all remaining subjects who have received at least one dose of study treatment will be offered enrollment in a long-term follow-up study and continue to be evaluated for survival. Subjects who are still receiving treatment at the time of study close may complete the current treatment course (up to 6 cycles) and EOT visit prior to transitioning to participation in the separate long-term follow-up study.

4.13 Blood Cultures for CRS-207 Surveillance

Patients who stop treatment will have blood samples from their peripheral vein drawn at the EOT visit to assess clearance of CRS-207. Patients who discontinue CRS-207 but remain on study to receive other agents should have blood cultures drawn within 4 weeks of the last dose of CRS-207. After the first culture, blood will continue to be collected for CRS-207 culture at 3, 6, 9, and 12 months (+/- 1 week window for each collection) for up to 1 year to monitor for the presence of CRS-207. For subjects with a central line, a blood sample will also be taken through the central line at time points indicated for CRS-207 testing. Subjects with samples positive for the presence of Listeria will initiate IV antibiotics per **Section 4.5** and be re-tested until negative cultures are confirmed.

4.13.1 Confirmed Listeria Infection

In the event a subject has a positive Listeria culture at any time during or after study participation (except within 7 days after a CRS-207 infusion), the IND Sponsor should be notified within 24 hours of the adverse event of special interest (AESI) per **Section 6.1.3**.

If Listeria has been confirmed at the clinical site or an external laboratory, all efforts should be made to obtain a sample of the bacterial isolate from the original positive culture and submit to the IND Sponsor or designee for strain confirmation; records on all samples cultured during this period must be obtained and provided to the Sponsor. Refer to the Central Laboratory Manual for sample collection and shipping instructions.

4.13.2 Suspected Infection with CRS-207 or Listeria

In the case of a suspected persistent CRS-207 or Listeria infection that has not been confirmed by culture, collection of blood (peripheral and port for those with indwelling ports), urine and stool samples in duplicate is recommended. One set of samples should be cultured locally for Listeria per institutional guidelines. Culture of cerebrospinal fluid should be obtained for subjects with suspected central nervous system infection. In such instances, analysis of cerebrospinal fluid should also include cell count, protein, glucose,

and Gram stain. If samples are positive for Listeria, the IND Sponsor must be notified immediately.

5. DOSING DELAYS/DOSE MODIFICATIONS

5.1 Dose Modifications

Dose reduction or dose increase of CRS-207, pembrolizumab, and ipilimumab will not be permitted.

Up to two dose reductions for tadalafil are allowed. Patients who experience grade 3 or 4 tadalafil-related toxicities that are eligible for retreatment will be dose reduced. If the grade 3 or 4 toxicity resolves to grades 0-2, then a dose modification of tadalafil is allowed to 10mg/day dosage. Dose reduction for persistent grade <3 toxicity may also be considered at the discretion of the investigator. A second dose reduction to 5mg/day is allowed. If three dose reductions are required, further treatment with tadalafil will be discontinued. Patients may continue to receive other drugs if tadalafil is discontinued.

Patients with a creatinine clearance of 30-50 mL/min will be dose reduced to 10mg/day. A second dose reduction to 5mg/day is allowed.

5.2 Dosing Delays

Dosing of study therapy will be delayed for the following laboratory criteria:

Day 1 of each cycle:

- AST/ALT >3 × ULN
- Total bilirubin > 1.5 x ULN or direct bilirubin > 2.0 × ULN for subjects with Gilbert's disease
- Creatinine clearance (CrCl) < 30 mL/min
- Hemoglobin < 7.5 g/dL
- ANC < 1000/uL
- Platelets < 80 × 10^3/uL

Day 3 of each cycle (N/A for patients no longer receiving CRS-207):

- AST/ALT >5 × ULN
- Total bilirubin > 1.5 x ULN or direct bilirubin > 2.0 × ULN for subjects with Gilbert's disease
- Creatinine clearance (CrCl) < 30 mL/min

If a subject's Day 3 laboratory results require a dosing delay of tadalafil, tadalafil may be dispensed to the patient but the patient will be instructed to delay the start of tadalafil until the laboratory values come back within dosing range.

All scheduled cycles within a course are to be given approximately 3 weeks apart. If necessary, a scheduled cycle may be delayed for up to 1 week. In this case, subsequent cycles should continue

so that a subject can still receive all 6 cycles given that the cycles are a minimum of 3 weeks apart and they have not experienced an AE necessitating discontinuation. If delayed more than 1 week, the Principal Investigator must be contacted for further instructions on continued treatment. Additional delays or modifications to the treatment schedule must be approved by the Principal Investigator or IND Sponsor.

If a delay occurs between Day 1 and 2 in a cycle:

- Pembrolizumab and ipilimumab-related infusion reactions must resolve to baseline prior to administration of ipilimumab, tadalafil or CRS-207.
- If a pembrolizumab-related infusion reaction prevents subsequent infusion of ipilimumab on the same day, the dose of ipilimumab should be replaced within 72 hours. In such instances, at least 18 days must elapse between the replacement dose of ipilimumab and the administration of the next dose of pembrolizumab combined with ipilimumab. If the dose of ipilimumab cannot be replaced within 72 hours, resume ipilimumab at the next scheduled dose per **Section 9**.
- Resume Day 2 treatment schedule (CRS-207) and assessments without repeating Day 1 study treatments (pembrolizumab/ipilimumab) if the delay is within 72 hours.
- If the delay is longer than 72 hours, repeat Day 1 and Day 2 (if applicable) study treatments/assessments with a minimum of 2 weeks from the previous Day 1 treatment. This includes steroid treatment requiring at least a 14 day washout prior to resuming study-related treatments.

Administration of study drug should be delayed for the following:

- Dosing criteria are not met
- Any grade ≥ 2 non-skin, treatment-related AE, with the following exceptions:
    - Grade 2 drug-related fatigue or laboratory abnormalities do not require a treatment delay
    - Grade 2 hypothyroidism or thyroiditis
- Any grade ≥3 skin treatment-related AE
- Any ≥ grade 3 treatment-related laboratory abnormality, with the following exceptions for asymptomatic amylase or lipase:
    - Grade 3 or 4 amylase or lipase abnormalities that are not associated with symptoms or clinical manifestations, or radiographic signs of pancreatitis do not require a dose delay. It is recommended to consult with the Principal Investigator for grade 3 amylase or lipase abnormalities.
    - Isolated grade 3 or 4 electrolyte imbalances/abnormalities that are not associated with clinical sequelae and are corrected with supplementation/appropriate management
- Any AE, laboratory abnormality, or intercurrent illness which, in the judgment of the investigator, warrants delaying the dose of study drug.

Guidance for Investigators is provided in the current pembrolizumab and ipilimumab package inserts. Additionally, management algorithms have been developed to assist Investigators with select toxicities and can be found in the **NCCN's guidelines for the management of immunotherapy-related toxicities.**

Subjects may resume treatment with pembrolizumab and ipilimumab when the treatment-related AE(s) resolve to grade ≤1 or baseline value, with the following exceptions:

- Subjects may resume treatment in the presence of grade 2 fatigue.
- Subjects who have not experienced a Grade 3 drug-related skin AE may resume treatment in the presence of Grade 2 skin adverse event
- Treatment-related pulmonary toxicity, diarrhea, or colitis must have resolved to baseline before treatment is resumed.
- Treatment-related endocrinopathies adequately controlled with only physiologic hormone replacement may resume treatment, which include grade 2 hyperglycemia, hypothyroidism and thyroiditis.

6. ADVERSE EVENTS: LIST AND REPORTING REQUIREMENTS

This study will use the descriptions and grading scales found in the revised CTCAE version 5 for AE reporting.

Information about all AEs, whether volunteered by the subject, discovered by investigator questioning, or detected through physical examination, laboratory test or other means, will be collected, recorded, and followed as appropriate.

6.1 Definitions

6.1.1 Adverse Event

An AE is defined as any undesirable sign, symptom or medical condition occurring after starting the study drug (or therapy) even if the event is not considered to be related to the study. An undesirable medical condition can be symptoms (e.g., nausea, chest pain), signs (e.g., tachycardia, enlarged liver) or the abnormal results of an investigation (e.g., laboratory findings, electrocardiogram). Medical conditions/diseases present before starting the study treatment are only considered AEs if they worsen after starting the study treatment (any procedures specified in the protocol). New medical conditions / diseases occurring before starting the study treatment but after signing the informed consent form will not be recorded as AEs. Additionally, expected progression of the disease being studied will not be recorded as an adverse event.

Laboratory abnormalities: Laboratory abnormalities present at the screening visit will be recorded as pre-treatment signs and symptoms. After study treatment administration, all grade 3 and 4 clinical laboratory results that represent an increase in severity from baseline will be reported as AEs. A grade 1 or 2 clinical laboratory abnormality should be reported as an AE only if it is considered clinically significant by the investigator (induce clinical signs or symptoms or require therapy).

6.1.2 Serious Adverse Event

A SAE is an undesirable sign, symptom or medical condition which:

- Results in death

- Is life threatening (defined as an event in which the subject 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 for exceptions) for >24 hours
- Results in persistent or significant disability/incapacity
- Is a congenital anomaly/birth defect (note: reports of congenital anomalies/birth defects must also be reported on the Pregnancy Form)
- 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 [e.g., 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.)
- Potential drug induced liver injury (DILI) is also considered an important medical event.
- Hemophagocytic lymphohistiocytosis is also considered an important medical event.
- Suspected transmission of an infectious agent (eg, pathogenic or nonpathogenic) via the study drug is an SAE.
- Is a new cancer (that is not a condition of the study)
- Is associated with an overdose
- Is a pregnancy or pregnancy outcome of spontaneous abortion, missed abortion, benign hydatidiform mole, blighted ovum, fetal death, intrauterine death, miscarriage, or stillbirth.

Events **not** considered to be SAEs are hospitalizations for:

- Admissions as per protocol for a planned medical/surgical procedure or to facilitate a procedure
- Routine health assessment requiring admission for baseline/trending of health status (e.g., routine colonoscopy)
- Medical/surgical admission for purpose other than remedying ill health state and was 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 (e.g., lack of housing, economic inadequacy, care-giver respite, family circumstances, administrative).
- Admissions for monitoring of treatment-related infusion reactions that do not otherwise meet the criteria for a SAE.

6.1.3 Adverse Events of Special Interest (AESI)

Suspected infection with CRS-207 and/or Listeria are considered adverse events of special interest (AESI) and should be reported following SAE reporting procedures in **Section 6.5** irrespective of temporal relationship to study drug administration.

In the event a subject has a positive Listeria culture at any time during or after study participation, the event should be reported to the IND Sponsor within 24 hours of the event.

All AESIs must be reported for the duration of the study regardless of causality.

6.2 Assessment of Causality

The relationship of an AE to the administration of the study drug is to be assessed by the investigator according to the following definitions:

- No (unrelated, not related, no relation): The time course between the administration of study drug and the occurrence or worsening of the adverse event rules out a causal relationship and another cause (concomitant drugs, therapies, complications, etc.) is suspected.

- Yes (related): The time course between the administration of study drug and the occurrence or worsening of the adverse event is consistent with a causal relationship and no other cause (concomitant drugs, therapies, complications, etc.) can be identified.

The following factors should also be considered:

- The temporal sequence from study drug administration - The event should occur after the study drug is given. The length of time from study drug exposure to event should be evaluated in the clinical context of the event.
- Underlying, concomitant, intercurrent diseases - Each report should be evaluated in the context of the natural history and course of the disease being treated and any other disease the subject may have.
- Concomitant medication - The other medications the subject is taking or the treatment the subject receives should be examined to determine whether any of them might be recognized to cause the event in question.
- Known response pattern for this class of study drug - Clinical and/or preclinical data may indicate whether a particular response is likely to be a class effect.
- Exposure to physical and/or mental stresses - The exposure to stress might induce adverse changes in the recipient and provide a logical and better explanation for the event.
- The pharmacology and pharmacokinetics of the study drug - The known pharmacologic properties (absorption, distribution, metabolism, and excretion) of the study drug should be considered.

<u>Assessment of Grade:</u>

The investigator will make an assessment of grade for each AE and SAE reported during the study, which will be recorded in the CRF. The assessment will be based on the National Cancer Institute's CTCAE (Version 5) and graded as shown below:

- Grade 1: Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated
- Grade 2: Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental activities of daily living

- Grade 3: Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care activities of daily living
- Grade 4: Life-threatening consequences; urgent intervention indicated
- Grade 5: Death related to AE

Any AE that changes in grade during its course will be recorded in the CRF at the highest level experience by the subject.

6.3 Expectedness

Unexpected AE: An AE, which varies in nature, intensity or frequency from information on the investigational drug/agent provided in the product IB, package insert or safety reports. Any AE that is not included in the IB, package insert, safety reports or informed consent is considered "unexpected".

Expected (known) AE: An AE, which has been reported in the IB, package insert or safety reports. An AE is considered "expected", only if it is included in the IB document as a risk.

6.4 Handling of Expedited Safety Reports

In accordance with local regulations, the IND Sponsor or designee will notify investigators of all SAEs that are unexpected (i.e., not previously described in the IB), and related to tadalafil, CRS-207, pembrolizumab, or ipilimumab. This notification will be in the form of an expedited safety report (ESR) that is to be faxed to the investigators and the study coordinators. Upon receiving such notices, the investigator must review and retain the notice with the IB and where required by local regulations, the investigator will submit the ESR to the appropriate IRB. The investigator and IRB will determine if the informed consent requires revision. The investigator should also comply with the IRB procedures for reporting any other safety information.

6.5 Reporting

6.5.1 Adverse Events and Serious Adverse Events

All AEs (both related and unrelated) will be captured on the appropriate study-specific CRFs. All AEs experienced by subjects will be collected and reported from the first dose of the investigational agent, throughout the study, and will be followed for 28 days after last dose of study drug unless related to the investigational agent.

Subjects who experience a grade 2 or higher pembrolizumab or ipilimumab-related AE should be discussed with the Principal Investigator.

Report AEs to the IND Sponsor within 24 hours once identified as an unacceptable toxicity (defined in Section 4.7).

Elizabeth Jaffee: [redacted]

Report all AESI to the IND Sponsor within 24 hours once identified (defined in Section 6.1.3):

Elizabeth Jaffee: [redacted]

All SAEs (including deaths) occurring from the first dose of the study drug through 90 days (+ 14 day reporting window) after the last dose of pembrolizumab or ipilimumab or within 7 days prior to initiation of a new antineoplastic treatment (whichever comes first) will be collected and reported. If the subject never received or is no longer receiving pembrolizumab or ipilimumab due to toxicity, all SAEs (including deaths) occurring from the first dose of the study drug through 28 days from the last dose of tadalafil CRS-207 (whichever reporting period is longer). All SAEs (including deaths) that the investigator considers related to study drug occurring after the follow-up periods must be reported.

Subjects who have an ongoing AE/SAE related to the study procedures and/or medication(s) may continue to be periodically contacted by a member of the study staff until the event is resolved or determined to be irreversible by the investigator.

SAEs will be reported promptly to the IND Sponsor within 24 hours of initial notification of the SAE. If this falls on a weekend or holiday, an email notification is acceptable but must be followed by an SAE reporting form on the next business day.

SAE reports and any other relevant safety information are to be sent to:

Elizabeth Jaffee: [redacted]

6.5.2 Follow-up of Adverse Events and Serious Adverse Events

After the initial AE or SAE report, the investigator is required to proactively follow each subject and provide further information to the safety department in regards to the subject's condition.

All AE(s) and SAE(s) will be followed until:

- Resolution
- The condition stabilizes
- The event is otherwise explained
- The subject is lost to follow-up
- Death

As soon as relevant information is available, a follow-up SAE report will be submitted to the IND Sponsor.

6.5.3 Overdose

An overdose is defined as the accidental or intentional administration of any dose of a product that is considered both excessive and medically important. All occurrences of overdose must be reported as SAEs.

6.5.4 Potential 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 under the seriousness category checked as 'other medically important event'. Potential drug induced liver injury is defined as:

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

6.5.5 Pregnancy Reporting

Although pregnancy and lactation are not always serious by regulatory definition, it is the responsibility of investigators or their designees to report any pregnancy or lactation in a subject (spontaneously reported to them) that occurs during the trial or within 5 months of completing the trial as an SAE. This also includes the pregnancy of a male subject's female partner who has provided written consent to provide information regarding pregnancy, which occurs during the trial or within 7 months of completing the trial.

If a subject or partner of a subject participating in the study becomes pregnant, the investigator must report the pregnancy within 24 hours of discovery or knowledge of the event. To report a pregnancy, complete the SAE form with the seriousness category checked as 'other important medical event'. When the form is completed, the IND Sponsor will be notified of the event.

All subjects or partners of subjects who become pregnant must be followed to the completion/termination of the pregnancy. If the pregnancy ends for any reason before the anticipated date, the investigator should notify the IND Sponsor. At the completion of the pregnancy, the investigator will document the outcome of the pregnancy. Pregnancy outcomes of spontaneous abortion, missed abortion, benign hydatidiform mole, blighted ovum, fetal death, intrauterine death, miscarriage and stillbirth must also be reported as SAEs (Important Medical Events). If the pregnancy continues to term, the outcome (health of infant) must also be reported to the IND Sponsor.

6.5.6 Institutional Review Board (IRB) and Institutional Biosafety Committee (IBC)

Serious adverse events will be reported to the IRB and IBC per institutional standards. Upon receipt, follow-up information will be given to the IRB and IBC (as soon as relevant information is available) per institutional standards.

6.5.7 Food and Drug Administration (FDA)

All reporting to the FDA will be completed by the IND Sponsor.

6.5.7.1 Expedited IND Safety Reports

7 Calendar-Day Telephone or Fax Report:
The IND Sponsor is required to notify the FDA of any fatal or life-threatening adverse event that is unexpected and assessed by the investigator to be possibly related to the investigational agent. Such reports are to be telephoned or faxed (301-827-9796) to the FDA within 7 calendar days of first learning of the event. Follow-up information will be submitted to the FDA as soon as relevant information is available.

15 Calendar-Day Written Report:
The IND Sponsor is required to notify the FDA of any SAE that is unexpected and related to the investigational agent in a written IND Safety Report.

Written IND Safety Reports should include an Analysis of Similar Events in accordance with regulation 21 CFR § 312.32. All safety reports previously filed with the IND concerning similar events should be analyzed. The new report should contain comments on the significance of the new event in light of the previous, similar reports.

Written IND safety reports with Analysis of Similar Events are to be submitted to the FDA within 15 calendar days of first learning of the event. Follow-up information will be submitted to the FDA as soon as relevant information is available.

6.5.7.2 IND Annual Reports

In accordance with the regulation 21 CFR § 312.33, the IND Sponsor shall within 60 days of the anniversary date that the IND went into effect submit a brief report of the adverse events and progress of the investigation. Please refer to Code of Federal Regulations, 21 CFR § 312.33 for a list of the elements required for the annual report. All IND annual reports will be submitted to the FDA by the IND Sponsor.

7. PHARMACEUTICAL INFORMATION

7.1 Tadalafil

7.1.1 Agent Accountability

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

7.1.2 Mode of Action

Tadalafil is a selective inhibitor of cyclic guanosine monophosphate (cGMP)-specific phosphodiesterase type 5 (PDE5). PDE5 is found in the smooth muscle of the corpus

cavernosum, prostate, and bladder as well as in vascular and visceral smooth muscle, skeletal muscle, urethra, platelets, kidney, lung, cerebellum, heart, liver, testis, seminal vesicle, and pancreas. In addition to pro-apoptotic tumor effects, PDE5 inhibitors have been found to have immunomodulatory properties including decreasing MDSCs and Tregs and increasing effector T cells in preclinical models.

7.1.3 Description

Tadalafil is available as almond-shaped tablets. Tablets are available in different sizes and different shades of yellow. Each tablet contains 2.5, 5, 10, or 20 mg of tadalafil and the following inactive ingredients: croscarmellose sodium, hydroxypropyl cellulose, hypromellose, iron oxide, lactose monohydrate, magnesium stearate, microcrystalline cellulose, sodium lauryl sulfate, talc, titanium dioxide, and triacetin.

7.1.4 Packaging and Labeling Information

Supplies will be labeled in accordance with regulatory requirements.

7.1.5 Storage

Clinical supplies must be stored in a secure, limited-access location under the storage conditions specified on the label. Receipt and dispensing of trial medication must be recorded by an authorized person at the trial site. Refer to the package insert for storage conditions.

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

7.1.6 Stability

Refer to the package insert for stability information. Follow expiration dates listed on clinical supply.

7.1.7 Route of Administration

Oral administration

7.1.8 Subject Care Implications

Tadalafil should be taken at about the same time of day each day. Tadalafil may be taken with or without meals. If a dose is missed, missed doses may be taken on the same day (but not more than one dose per day). If a dose is vomited, it will be skipped.

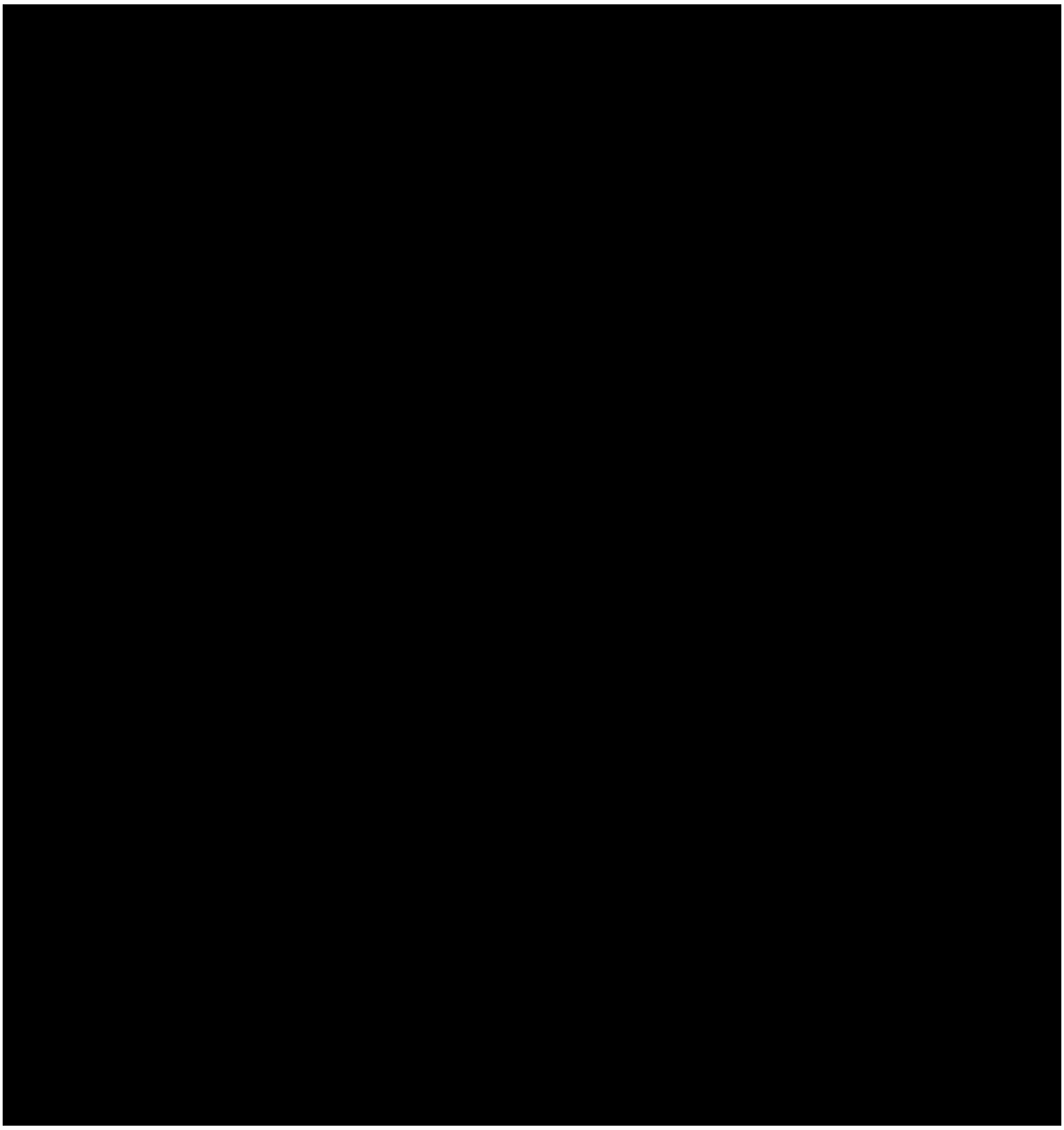
Tadalafil should not be taken with increased intake of alcohol (for example, 5 glasses of wine) as this may result in increased headaches, dizziness, increased heart rate, and hypotension.

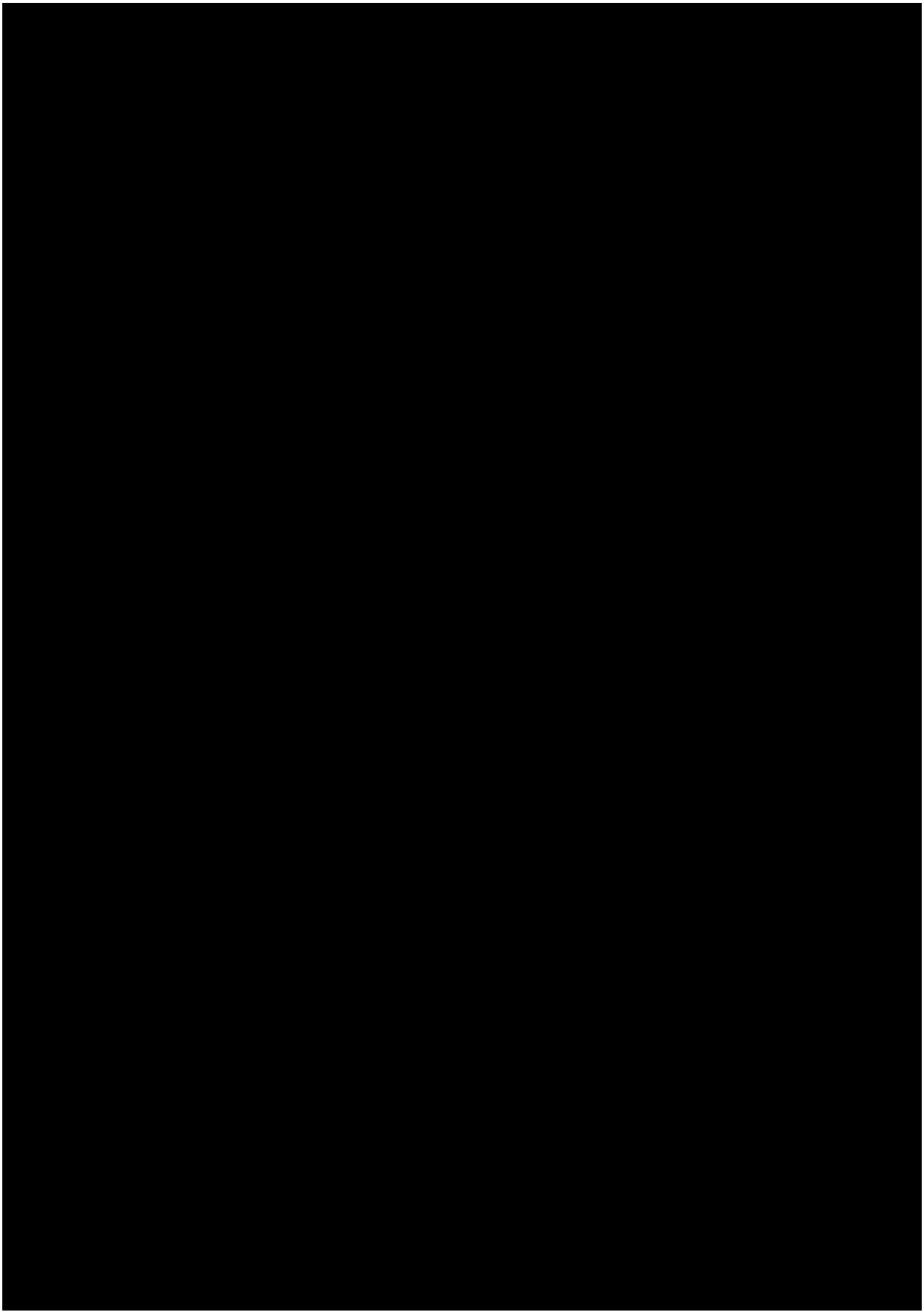
7.1.9 Returns and Reconciliation

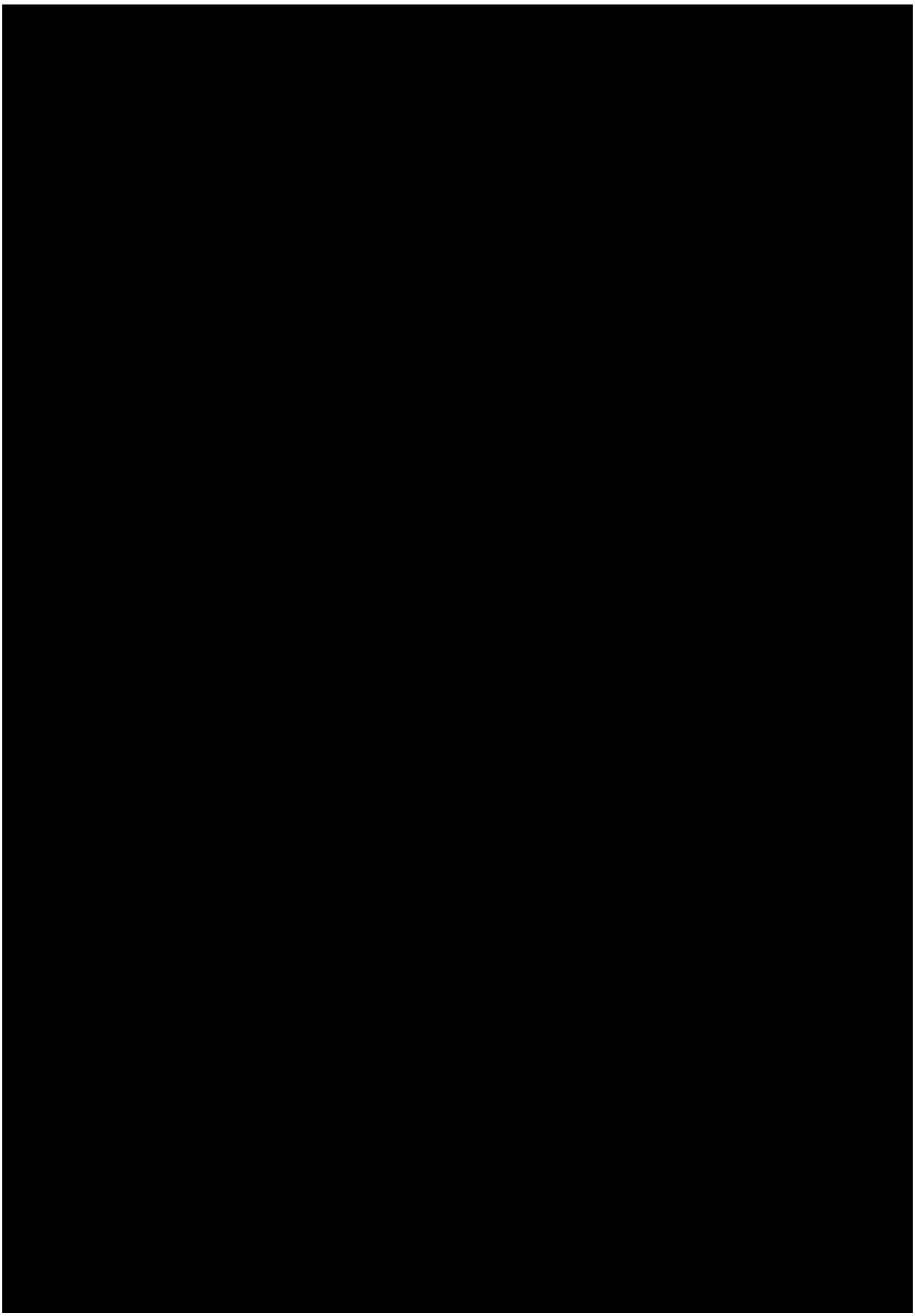
The investigator is responsible for keeping accurate records of the clinical supplies and the amount remaining at the conclusion of the trial.

Upon completion or termination of the study, all unused and/or partially used investigational product will be destroyed at the site per institutional policy. 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.

7.2 CRS-207







7.3 Pembrolizumab (KEYTRUDA®)

7.3.1 Agent Accountability

The IND sponsor or the IND Sponsor's representative shall take responsibility for and shall take all steps to maintain appropriate records and ensure appropriate supply, storage, handling, distribution and usage of investigational product in accordance with the protocol and any applicable laws and regulations.

7.3.2 Mode of Action

Pembrolizumab is a fully human monoclonal immunoglobulin (Ig) G4 antibody that binds to the PD-1 cell surface membrane receptor, a negative regulatory molecule expressed by activated T and B lymphocytes. Inhibition of the interaction between PD-1 and its ligands (PD-L1 and 2) promotes immune responses and antigen-specific T cell responses to both foreign antigens as well as self-antigens.

7.3.3 Description

KEYTRUDA (pembrolizumab) injection is a sterile, preservative-free, clear to slightly opalescent, colorless to slightly yellow solution for intravenous use. Each vial contains 100 mg of pembrolizumab in 4 mL of solution. Each 1 mL of solution contains 25 mg of pembrolizumab and is formulated in: L-histidine (1.55 mg), polysorbate 80 (0.2 mg), sucrose (70 mg), and Water for Injection, USP.

7.3.4 Packaging and Labeling Information

Supplies will be labeled in accordance with regulatory requirements.

7.3.5 Preparation

Refer to the package insert for preparation instructions.

7.3.6 Storage

Clinical supplies must be stored in a secure, limited-access location under the storage conditions specified on the label. Receipt and dispensing of trial medication must be recorded by an authorized person at the trial site. Refer to the package insert for storage conditions.

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

7.3.7 Stability

Refer to the package insert for stability information. Follow expiration dates listed on clinical supply.

7.3.8 Route of Administration

Pembrolizumab is to be administered as a 30 minute IV infusion line containing a sterile, non-pyrogenic, low-protein binding 0.2 micron to 5 micron in-line or add-on filter. At the end of the infusion, flush the line with a sufficient quantity of normal saline (approximately 30-50mL).

7.3.9 Subject Care Implications

The most common side effects include fatigue, musculoskeletal pain, decreased appetite, pruritus, diarrhea, nausea, rash, pyrexia, cough, dyspnea, constipation, pain, and abdominal pain.

KEYTRUDA is a monoclonal antibody that belongs to a class of drugs that bind to either the programmed death-receptor 1 (PD-1) or the PD-ligand 1 (PD-L1), blocking the PD-1/PD-L1 pathway, thereby removing inhibition of the immune response, potentially breaking peripheral tolerance and inducing immune-mediated adverse reactions. Important immune-mediated adverse reactions listed under the package insert may not include all possible severe and fatal immune-mediated adverse reactions.
Immune-mediated adverse reactions, which may be severe or fatal, can occur in any organ system or tissue and can affect more than one body system simultaneously. Immune-mediated adverse reactions can occur at any time after starting treatment with a PD-1/PD-L1 blocking antibody. While immune-mediated adverse reactions usually manifest during treatment with PD-1/PD-L1 blocking antibodies, immune-mediated adverse reactions can also manifest after discontinuation of PD-1/PD-L1 blocking antibodies.

Early identification and management of immune-mediated adverse reactions are essential to ensure safe use of PD-1/PD-L1 blocking antibodies. Monitor patients closely for symptoms and signs that may be clinical manifestations of underlying immune-mediated adverse reactions. Evaluate liver enzymes, creatinine, and thyroid function at baseline and periodically during treatment. In cases of suspected immune-mediated adverse reactions, initiate appropriate workup to exclude alternative etiologies, including infection. Institute medical management promptly, including specialty consultation as appropriate.

Withhold or permanently discontinue KEYTRUDA depending on severity (**NCCN's guidelines for the management of immunotherapy-related toxicities**). Additional guidance can be found in the package insert. In general, if KEYTRUDA requires interruption or discontinuation, administer systemic corticosteroid therapy (1 to 2 mg/kg/day prednisone or equivalent) until improvement to Grade 1 or less. Upon improvement to Grade 1 or less, initiate corticosteroid taper and continue to taper over at least 1 month. Consider administration of other systemic immunosuppressants in patients whose immune-mediated adverse reactions are not controlled with corticosteroid therapy

7.3.10 Returns and Reconciliation

The investigator is responsible for keeping accurate records of the clinical supplies and the amount remaining at the conclusion of the trial.

Upon completion or termination of the study, all unused and/or partially used investigational product will be destroyed at the site per institutional policy. 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.

7.4 Ipilimumab (YERVOY®)

7.4.1 Agent Accountability

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

7.4.2 Mode of Action

CTLA-4 is a negative regulator of T-cell activity. Ipilimumab is a monoclonal antibody that binds to CTLA-4 and blocks the interaction of CTLA-4 with its ligands, CD80/CD86. Blockade of CTLA-4 has been shown to augment T-cell activation and proliferation, including the activation and proliferation of tumor infiltrating T-effector cells. Inhibition of CTLA-4 signaling can also reduce T-regulatory cell function, which may contribute to a general increase in T cell responsiveness, including the anti-tumor immune response.

7.4.3 Description

YERVOY (ipilimumab) injection, for intravenous use is a sterile, preservative-free, clear to slightly opalescent, colorless to pale-yellow solution, which may contain a small amount of visible translucent-to-white, amorphous ipilimumab particulates. It is supplied in single-dose vials of 50 mg/10 mL or 200 mg/40 mL. Each milliliter contains 5 mg of ipilimumab and the following inactive ingredients: diethylene triamine pentaacetic acid (DTPA) (0.04 mg), mannitol (10 mg), polysorbate 80 (vegetable origin) (0.1 mg), sodium chloride (5.85 mg), tris hydrochloride (3.15 mg), and Water for Injection, USP at a pH of 7.

7.4.4 Packaging and Labeling Information

Supplies will be labeled in accordance with regulatory requirements.

7.4.5 Preparation

Refer to the package insert for preparation instructions.

7.4.6 Storage

Clinical supplies must be stored in a secure, limited-access location under the storage conditions specified on the label. Receipt and dispensing of trial medication must be

recorded by an authorized person at the trial site. Refer to the package insert for storage conditions.

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

7.4.7 Stability

Refer to the package insert for stability information. Follow expiration dates listed on clinical supply.

7.4.8 Route of Administration

Ipilimumab is to be administered as a 30 minute IV through an intravenous line containing a sterile, non-pyrogenic, low-protein-binding in-line filter. At the end of the infusion, flush the line with a sufficient quantity of normal saline (approximately 30-50mL).

Pembrolizumab is to be administered as a 30 minute IV infusion line containing a sterile, non-pyrogenic, low-protein binding 0.2 micron to 5 micron in-line or add-on filter. At the end of the infusion, flush the line with a sufficient quantity of normal saline (approximately 30-50mL).

7.4.9 Subject Care Implications

Most common adverse reactions with YERVOY are fatigue, diarrhea, pruritus, rash, and colitis.

YERVOY is a fully human monoclonal antibody that blocks T-cell inhibitory signals induced by the CTLA-4 pathway, thereby removing inhibition of the immune response with the potential for induction of immune-mediated adverse reactions. Immune-mediated adverse reactions listed herein may not be inclusive of all possible severe and fatal immune-mediated reactions.

Immune-mediated adverse reactions, which may be severe or fatal, can occur in any organ system or tissue. Immune-mediated adverse reactions can occur at any time after starting YERVOY. While immune-mediated adverse reactions usually manifest during treatment, immune-mediated adverse reactions can also manifest after discontinuation of YERVOY. Early identification and management are essential to ensure safe use of YERVOY. Monitor for signs and symptoms that may be clinical manifestations of underlying immune-mediated adverse reactions. Evaluate clinical chemistries including liver enzymes, creatinine, adrenocorticotropic hormone (ACTH) level, and thyroid function at baseline and before each dose. Institute medical management promptly, including specialty consultation as appropriate.

Withhold or permanently discontinue YERVOY depending on severity (**NCCN's guidelines for the management of immunotherapy-related toxicities**). Additional guidance can be found in the package insert. In general, if YERVOY requires interruption or discontinuation, administer systemic corticosteroid therapy (1 to 2 mg/kg/day prednisone or equivalent) until improvement to Grade 1 or less. Upon improvement to

Grade 1 or less, initiate corticosteroid taper and continue to taper over at least 1 month. Consider administration of other systemic immunosuppressants in patients whose immune-mediated adverse reactions are not controlled with corticosteroid therapy.

7.4.10 Returns and Reconciliation

The investigator is responsible for keeping accurate records of the clinical supplies and the amount remaining at the conclusion of the trial.

Upon completion or termination of the study, all unused and/or partially used investigational product will be destroyed at the site per institutional policy. 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.

8. CORRELATIVE/SPECIAL STUDIES

Research samples will be collected at the discretion of the PI based on availability of supplies and safety of patient and staff. Sample collection, processing, storage, and shipment instructions will be provided in the Laboratory Manual.

8.1 Tumor Tissue Studies

Tumor biopsies will be collected (if a subject's tumor is thought to be reasonably safe and easy to biopsy) at baseline and at Cycle 3 (4-6 cores per time point). Lesions will be considered acceptable for biopsy if palpable or accessible by imaging (CT or US) guidance when necessary, and if not in close proximity to a vital cardiovascular structure. If a biopsy was done within 21 days before first dose, archived tissue from this biopsy may be used as baseline sample. Fine needle aspiration will not be acceptable. Additional optional biopsies may be obtained later in the course of study treatment.

Attempts will be made to obtain archived tissue samples from all subjects. Archived FNA biopsy samples do not contain sufficient tissue and will not be collected.

Detailed instructions for tissue collection, processing, storage, and shipment will be provided in the Laboratory Manual.

To explore the association of OS, PD-L1 positivity, and tumor-infiltrating lymphocyte characteristics with clinical responses, archived tumor tissue and tumor tissue obtained at baseline and during treatment (Cycle 3) will be compared. PD-L1 expression may predict response to anti-PD-1[29,30]. However, PD-L1 is also upregulated in response to IFN-γ released by infiltrating T cells and could potentially be a predictor of response to any active immunotherapy. Pre- and post-treatment tumor biopsies will also be analyzed for PD-1 expression as well as infiltration of immune cells (effector T cells, Tregs, B cells, dendritic cells, etc). Characterization of immune checkpoint expression as well as immune infiltrates may be predictive of response to therapy and may also give insight into next generation combinatorial approaches. Preliminary data from a pancreatic cancer immunotherapy study suggests that induction of a $T_h1$and T_h17 phenotype at the

tumor itself predicts response. Furthermore, upregulation of other inhibitory molecules such as IL-10 and TGF-β may identify other targets for combinatorial strategies. Dependent on availability of paired tissue samples, additional analysis including determination of gene signatures of tumor and immune cells will be performed to look for patterns of response associated with immune activation and changes in the tumor.

Attempts will be made to obtain archived tissue samples from all subjects.

8.2 Peripheral Blood Mononuclear Cells (PBMCs)

Whole blood for isolation of PBMCs will be collected prior to dosing on Day 1 of Cycles 1, 2, 4, and 6 only during Course 1. Pre- and post-treatment changes in PBMCs including effector, helper, and regulatory T cells, NK cells, and macrophages through cell phenotyping analysis and gene expression profiling will be measured. In addition, induction of Listeria and mesothelin antigen-specific T cell responses and changes to the T cell epitope repertoire will also be evaluated.

The cellular immune responses directed against *Lm* and mesothelin will be evaluated by using enzyme-linked immunosorbent spot (ELISPOT) and intracellular cytokine staining. Post-treatment expression of PD-1 and other lymphocyte activation markers will be measured as well. These responses will be correlated with OS. PBMCs are isolated and stored frozen (liquid nitrogen) until use. Detailed instructions for collection, processing, storage, and shipment are provided in the Laboratory Manual.

8.3 Serum and Plasma Marker Studies

Sera will be collected prior to dosing on Day 1 of Cycles 1-6 only during Course 1. Additionally, subjects will have whole blood for serum drawn 20-26 hours post-CRS-207 infusion only during Course 1. Plasma will be collected prior to dosing on Day 1 of Cycles 1, 2, 4, and 6 only during Course 1. Humoral immune responses, including anti-Lm, anti-mesothelin, anti-thyroglobulin and anti-galectin 3 antibodies will be evaluated by using enzyme-linked immunosorbent assay (ELISA). In addition, potential therapeutic targets, biomarkers, and predictors of response and autoimmune toxicity will be evaluated. Sera and plasma are isolated and stored frozen (-80ºC) until use. Detailed instructions for collection, processing, storage, and shipping are provided in the Laboratory Manual.

8.4 Diagnostic Tissue Samples

Tissue, fluid, or blood may be collected from standard of care procedures used to treat or diagnose immune-related toxicities.

8.5 Genomic Analysis

Genomic sequencing library construction, whole genome/exome sequencing, whole transcriptome sequencing, microbial sequencing, neoepitope prediction, mutation burden, and bioinformatic analysis will be performed either at an on-campus laboratory or at an off-campus sequencing service. All the samples will be de-identified before sending to any laboratory for sequencing. The FASTQ files, BAM files and VCF files will be generated and analyzed.

Genomic sequencing data will be stored and computations conducted using a JH IT managed

subscription of Azure.

Clinical analysis. Several CLIA-certified laboratories now offer molecular profiling of cancer specimens in commercial and noncommercial settings and provide these results to patients and their physicians (e.g. Foundation Medicine, PGDx, Michigan Center for Translational Pathology, or JHU CLIA Laboratories). It is possible, therefore, that some of our research analyses will be conducted in these CLIA-certified environments. If tissue or cells are evaluated with next generation sequencing strategies to provide a molecular profile of individual cancer specimens in a CLIA-certified facility, these results will be made available to the patient and their physician. Patient confidentiality will be maintained, and the patient's identity will not be publicly linked to any study results. Researchers may use the data set generated in the CLIA assay setting to study genetic alterations across a large number of genes important in cancer. Germline mutations are only identified in punitive cancer genes. Researchers will use the data set for exploratory research to study cancer cell heterogeneity. Some of the sequencing data obtained from the NGS strategies will be uploaded to government sponsored databases, such as GEO and dbGAP. The results of the research studies may be published but subjects will not be identified in any publication.

If a germline alteration of clinical importance (as judged by the Investigator) to the subject and his or her family members is identified by a CLIA-certified test in the course of this analysis, attempts will be made in writing to contact the subject and/or family members for genetic counseling referral.

9. STUDY CALENDAR

Study Procedures	Pre-Study	Cycle 1[24]				Cycle 2				Cycle 3				Cycle 4				Cycle 5				Cycle 6				EOT[27]
		D1	D2[29]	D3[29]	D9[29]	D1	D2[29]	D3[29]	D9[29]	D1	D2[29]	D3[29]	D9[29]	D1	D2[29]	D3[29]	D9[29]	D1	D2[29]	D3[29]	D9[29]	D1	D2[29]	D3[29]	D9[29]	
Visit Windows (days)[1]	-28 to D1	-	-	-	±1	-2/ +7	-	-	±1	-2/ +7	-	-	±1	-2/ +7	-	-	±1	-2/ +7	-	-	±1	-2/ +7	-	-	±1	+/- 7
Tadalafil[2]		X[2]																								
Pembrolizumab[3]		X				X				X				X				X				X				
Ipilimumab[3]		X								X								X								
CRS-207			X				X				X				X				X				X			
Hydration			X	X			X	X			X	X			X	X			X	X			X	X		
Informed consent	X																									
Inclusion/ exclusion criteria	X																									
Demographics	X																									
Medical, Cancer, & Con Med History[4]	X																									
Con Meds, Adverse Events		X	X	X	X[25]	X	X	X	X[25]	X	X	X	X[25]	X	X	X	X[25]	X	X	X	X[25]	X	X	X	X[25]	X
Physical Exam, ECOG PS[5]	X	X				X				X				X				X				X				X
Vitals, Weight, & Height[6]	X	X	X	X		X	X	X		X	X	X		X	X	X		X	X	X		X	X	X		X
Hematology, Chemistry[7, 13]	X	X		X	X	X		X	X	X		X	X	X		X	X	X		X	X	X		X	X	X
Endocrine[8, 13]		X				X				X				X				X				X				X
Urinalysis[9]	X																									
Virology[10]	X																									
Coagulation panel[11]	X																									
Pregnancy Test[12, 13]	X	X[26]				X				X				X				X				X				
CA19-9 [13]	X	X				X				X				X				X				X				
ECG[14]	X																									
Scans, RECIST/iRECIST[15]	X													X												X

Study Procedures	Pre-Study	Cycle 1[24]				Cycle 2				Cycle 3				Cycle 4				Cycle 5				Cycle 6				EOT[27]
		D1	D2[29]	D3[29]	D9[29]	D1	D2[29]	D3[29]	D9[29]	D1	D2[29]	D3[29]	D9[29]	D1	D2[29]	D3[29]	D9[29]	D1	D2[29]	D3[29]	D9[29]	D1	D2[29]	D3[29]	D9[29]	
Whole blood for PBMC[16, 20]		X				X								X								X				X
Whole blood for plasma[16, 20]		X				X								X								X				X
Serum[16, 20]		X		X		X		X		X		X		X		X		X		X		X		X		X
HLA[17, 20]		X																								
Archival Tissue[18, 20]	X																									
Tumor Biopsies[19, 20]	X									X																
Antibiotics[21,22]																									X[28]	X[28]
Blood sample for CRS-207 testing[23]																										X

In order to minimize the need for research-only in-person visits, telemedicine visits may be substituted for in person clinical trial visits or portions of clinical trial visits where determined to be appropriate and where determined by the investigator not to increase the participants risks. Prior to initiating telemedicine for study visits the study team will explain to the participant, what a telemedicine visit entails and confirm that the study participant is in agreement and able to proceed with this method. Telemedicine acknowledgement will be obtained in accordance with the Guidance for Use of Telemedicine in Research. In the event telemedicine is not deemed feasible, the study visit will proceed as an in-person visit. Telemedicine visits will be conducted using HIPAA compliant method approved by the Health System and within licensing restrictions.

1: If necessary, a scheduled cycle may be delayed for up to 1 week. Longer delays to be approved by the IND Sponsor and/or Principal Investigator.
2: Tadalafil administration occurs on Days 3-21 (daily starting on Day 1 for patients no longer receiving CRS-207) after review of hepatic laboratories and blood pressure on Day 3 (or Day 1 for patients no longer receiving CRS-207).
3: Order of administration is Pembrolizumab followed by Ipilimumab. Subjects should be observed for a minimum of 30 minutes between each infusion.
4: Cancer history includes: primary site of cancer, gross location of primary tumor, secondary sites of cancer, histology, histologic grade, date of initial diagnosis, date of metastatic diagnosis, prior cancer therapy regimens.
5: Complete physical examination and assessment of ECOG PS will be completed at baseline; focused physical examinations and assessment of ECOG PS will be conducted thereafter. Day 1 Physical examination and ECOG status may be done up to 1 day prior to dosing.
6: Blood pressure, pulse, and temperature are required as indicated. Weight and pulse oximetry will be obtained at baseline and prior to each cycle. Height will be taken at or prior to screening only. Only weight and blood pressure are required on Day 3 of each cycle. Pembrolizumab: vitals will be collected prior to the infusion. Ipilimumab: vitals will be collected prior to and at the end of the infusion (-5/+ 15 minutes). CRS-207: vital signs will be obtained prior to and then every 30 minutes (± 15 minutes) during infusion and every hour (-5/+ 15 minutes) during post-infusion follow-up. Subjects will be

observed for at least 4 hours after each CRS-207 infusion. Subjects who are not stable enough to be released at 4 hours after infusion should continue to be monitored until stable. Presence of fever alone does not indicate subject is not clinically stable.

7: Clinical hematology: CBC with differential ANC, ALC, AEC, and platelet count; serum chemistry: sodium, potassium, chloride, bicarbonate, glucose, BUN, creatinine, ALT, AST, alkaline phosphatase, total bilirubin, direct bilirubin, amylase, total protein, albumin, calcium, magnesium, and phosphate. Required labs on Day 3 only after CRS-207 dosing: Any unexpected Grade 3 or greater laboratory abnormalities should be repeated within 24-72 hours. Grade 3 or greater creatinine, AST, ALT, and bilirubin should be repeated within 24-72 hours as well.

8: TSH (Total T3 and free T4 if TSH abnormal and clinically indicated).

9: Bilirubin, blood, glucose, ketones, leukocytes, nitrite, pH, color, protein, RBC and WBC count, and specific gravity.

10: Virology screen: HIV antibody, hepatitis B surface antigen and hepatitis C antibody; additional virology may also be evaluated. Subjects who are hepatitis C antibody positive and confirmed negative viral load at screening will be allowed to enroll.

11: Coagulation panel: D-dimer, fibrinogen, international normalized ratio of prothrombin time, APTT

12: Pregnancy tests will be administered to WOCBP: serum pregnancy test is required at screening; urine pregnancy tests are required before doses on Day 1 of dosing weeks.

13: Labs may be collected within a window of up to 3 days prior to dosing. Blood draws must not be collected from a central line for at least 4 days after infusion of CRS-207.

14: ECG should be performed at baseline.

15: Spiral CT of thorax, abdomen and pelvis (and other imaging studies as clinically indicated to evaluate suspected sites of metastatic disease). If a subject cannot have a CT scan (e.g., allergy to contrast dye), an MRI should be performed. 3-D CT scans and RECIST reads will not be used to determine eligibility at baseline. On study radiologic evaluations and tumor measurements (RECIST and iRECIST per **Appendix B** and **Appendix C**) will be performed every 10 weeks (± 1 week; starting from the date of first treatment) including the EOT evaluation (± 4 weeks). If the EOT visit occurs early, scans do not need to be repeated if one has been done within the past 6 weeks. Weeks are in reference to calendar week and should not be adjusted due to dosing delays.

16: Up to 120 mL of whole blood for PBMC isolation may be drawn up to 72 hours prior to dosing and must be processed by sponsor-qualified operators within 6 hours of collection and stored in liquid nitrogen. Approximately 20 mL of whole blood for plasma collection will be drawn as indicated. Approximately 5.0 mL of blood for serum for immune monitoring will be drawn as indicated. Day 3 blood draws should be taken between 20 and 26 hours after start of dosing. Collection of whole blood for isolation of PBMCs, plasma, and serum will only be drawn during Course 1. EOT samples are optional (collection at the discretion of the PI).

17: HLA-typing to include HLA class I type A and B, low resolution. HLA typing is only done during the first course of study treatment. If HLA is missed during Cycle 1, it may be collected during subsequent cycles.

18: Attempts to obtain surgical or biopsy archival tumor samples will be made for every subject until the sample is obtained or documentation that the sample cannot be obtained. Detailed instructions for tissue collection, processing and shipment are provided in the Laboratory Manual.

19: Tumor biopsies to be taken (if a subject's tumor is thought to be reasonably safe and easy to biopsy) at baseline and at Cycle 3 (4-6 cores per timepoint). If a biopsy was done within 21 days before first dose, archived tissue from this biopsy may be used as baseline sample. The Cycle 3 biopsy has a ± 1 week window. Additional optional biopsies may be obtained later in the course of study treatment. Fine needle aspiration will not be acceptable. Biopsies will only be collected during the first course of study treatment. Detailed instructions for tissue collection, processing and shipment are provided in the Laboratory Manual.

20: Research samples will be collected at the discretion of the PI based on availability of supplies and safety of patient and staff.

21: A 7-day course of antibiotics will be administered 7 days after final dose of CRS-207 in each course (or after final dose if discontinued early or 7 days after the subject's last dose of CRS-207 if CRS-207 is discontinued and the patient remains on study to receive the other study drugs) per **Section 4.5**. Antibiotic regimens must be completed prior to initiation of any other cancer-related therapy. Antibiotics should also be administered to subjects who have not received antibiotics after CRS-207 treatment and the subject is administered steroids for the treatment of suspected pembrolizumab or ipilimumab-related AE.

22: Refer to **Section 4.5** for the recommended antibiotic regimen for patients having a semi-permanent indwelling device placed while on study.

23: Blood for CRS-207 culture will be collected at EOT (or within 4 weeks of the last dose of antibiotics if patients do not complete 6 cycles of treatment or if patients discontinue CRS-207 but remain on study to receive other agents) to assess clearance of CRS-207. After the first culture, blood will continue to be collected for CRS-207 culture at 3, 6, 9, and 12 months from last dose of CRS-207 (+/- 1 week window for each collection). For subjects with a central line, blood samples should be collected from **both peripheral and central lines**.

24: Cycle 1 Day 1 evaluations do not need to be repeated if they were conducted within 3 days of the pre-study evaluations. The additional course(s) may start as early as 3 weeks (+7 days) from last dose of previous course.

25: Day 9 (± 1 day) adverse event assessments may be conducted by telephone or email.

26: Course 2 and beyond only

27: Subjects will return to the study site for an EOT evaluation. EOT follow-up will occur 28 (±7) days after the final dose. NOTE: CT scan assessment at EOT will occur 28 days (± 4 weeks) after the final dose. If the EOT visit occurs early and the patient cannot return due to disease progression, an assessment for AEs should be made by telephone or email on day 28 (±1) after last study dose in place of the PE/PS/vitals (clinical laboratory values should still be collected). Subjects who discontinue from treatment should be contacted every three months (+/- 1 month) to monitor overall survival. Information of other cancer therapies after discontinuation from the study treatment will be collected as well. Subjects should be contacted by telephone or email at 90 days (+ 14 day reporting window) to assess for treatment related toxicities that occur in the follow-up period.

28: Site personnel will contact the subject by telephone (prior to and within 3 days after completion) to facilitate compliance with antibiotic treatment and document in source.

29: Not required if patient discontinues CRS-207

10. STUDY ENDPOINTS

10.1 Primary Endpoint

The primary endpoint is ORR, which is defined as the proportion of subjects with PR or CR according to RECIST. Subjects who discontinue due to toxicity or clinical progression prior to post-baseline tumor assessments will be considered as non-responders. Subjects who discontinue for other reasons prior to their first post-baseline tumor assessment will be replaced and not included in the primary efficacy analysis.

10.2 Secondary Endpoint

The secondary endpoint is as follows:

- Safety assessed by the following measures:
  - Number of patients who have grade 3 or higher drug-related toxicities
  - Frequency of drug-related toxicity by grade
  - Frequency of pembrolizumab-related infusion reactions
  - Frequency of ipilimumab-related infusion reactions
  - Frequency of CRS-207-related infusion reactions
  - Frequency of immune-related AEs
  - Frequency of unacceptable toxicities
  - Frequency of treatment-emergent changes from normal to abnormal values in key laboratory parameters:
    - Vital signs: BP, pulse, temperature
    - Physical examination
    - Changes in ECG readings
    - Clinical hematology: complete blood count (CBC) with differential ANC, ALC, AEC, and platelet count
    - Clinical serum chemistry: sodium, potassium, chloride, bicarbonate, glucose, blood urea nitrogen (BUN), creatinine, ALT, AST, alkaline phosphatase, amylase, bilirubin (total), total protein, albumin, calcium, magnesium and phosphate

10.3 Exploratory Endpoints

Exploratory endpoints are as follows:

- Overall survival (OS), progression-free survival (PFS), disease control rate (DCR), duration of response (DOR), and time to objective response (TOR) assessed using RECIST 1.1.
  - OS is defined as the number of months from the date of first dose until death or end of follow-up (OS will be censored on the date the subject was last known to be alive for subjects without documentation of death at the time of analysis).
  - PFS is defined as the number of months from the date of first dose to disease progression (PD or relapse from CR as assessed using RECIST 1.1 criteria) or death due to any cause. PFS will be censored at the date of the last scan for subjects without documentation of disease progression at the time of analysis.
  - DCR is defined as the proportion of subjects with SD, PR, or CR according to RECIST 1.1.

  - DOR is defined as the number of months from the first documentation of a response to date of disease progression.
  - TOR is defined as the number of months from the date of first dose to the date of first response (PR or CR as assessed using RECIST 1.1 criteria).
- Tumor marker kinetics measured by change in serum CA19-9 concentrations from baseline
- ORR, PFS, DCR, DOR, and TOR assessed using iRECIST
- Humoral and cellular immune responses directed against *Lm* and mesothelin assessed by using the following measures:
  - ELISPOT or intracellular cytokine staining assays of PBMC
  - Induction of proinflammatory cytokines and chemokines in the serum
  - ELISA detection of mesothelin- and *Lm*-specific antibodies in the serum
- Immune subset analyses by IHC and gene expression profiling of tumor tissue
- Immune subset analyses in PBMCs including effector, helper, and regulatory T cells, NK cells, and macrophages
- Telomere length of lymphocytes
- Thyroglobulin and galectin-3 antibody responses
- Peripheral blood specimens, intratumoral core biopsy specimens, and resection specimens will be studied using a variety of laboratory techniques including but not limited to: immunohistochemistry (IHC), flow cytometry, CITE-Seq, RNA-Seq, whole exome sequencing, whole genome sequencing, T cell receptor and B cell receptor sequencing, ChIP-seq, ATAC-seq, and MBD-seq.

11. DATA REPORTING / REGULATORY REQUIREMENTS

AE guidelines and instructions for AE reporting can be found in **Section 6.0 (Adverse Events: List and Reporting Requirements)**.

Dr. Elizabeth Jaffee will be holding the IND for this study. She will comply with all regulated reporting requirements to the FDA.

11.1 Data Collection and Processing

All information will be collected on study-specific CRFs by study staff. These data will be reviewed for completeness and accuracy by the Principal Investigator.

CRFs will be used to capture study results and data. The study coordinator or other authorized study personnel will transcribe data from source documents onto paper or eCRFs. Before or between visits, the Principal Investigator, IND Sponsor, or designee may request copies of the CRFs for preliminary medical review. Once the CRFs are complete and source-verified, the investigator must sign and date all required pages, verifying the accuracy of all data contained within the CRF.

11.2 Safety Meetings

Scheduled meetings will take place weekly and will include the principal investigator, study coordinator(s), nurse(s), sub-investigators (as appropriate), collaborators (as appropriate), and biostatisticians (as appropriate) involved with the conduct of the protocol. During these meetings

matters related to the following will be discussed: safety of protocol participants, validity and integrity of the data, enrollment rate relative to expectation, characteristics of participants, retention of participants, adherence to protocol (potential or real protocol violations), data completeness, and progress of data for objectives.

11.3 Monitoring

The SKCCC Compliance Monitoring Program will provide external monitoring for JHU-affiliated sites in accordance with SKCCC DSMP (Version 6.0, 02/21/2019). The SMC Subcommittee will determine the level of patient safety risk and level/frequency of monitoring. The PI shall internally monitor the progress of the trial, including review and confirmation of all safety/treatment-related outcomes, response assessments, safety reports and/or any related source documentation. The protocol will be monitored externally by the SKCCC CRO QA Office. Additional data and safety monitoring oversight will also be performed by the SKCCC Safety Monitoring Committee (SMC - as defined in the DSMP).

11.4 Study Documentation

11.4.1 Informed Consent and Authorization for use and Disclosure of Protected Health Information

Written informed consent and authorization of use and disclosure of protected health information (PHI) must be obtained from each subject (or the subject's legally authorized representative) before performing any study-specific screening/baseline period evaluations. The ICF and authorization for use and disclosure of PHI, which is prepared by the investigator or the site, must be reviewed and approved by the IND Sponsor, the study monitor (if applicable), and the site's IRB before the initiation of the study.

11.4.2 Investigator Study Files

Documentation about the investigator and study staff, the IRB and the institution, is required before study site initiation. A list of required documents will be provided by the IND Sponsor or designee to each participating investigator. Copies of these documents as well as supplemental information, such as the investigator's obligations, IB, clinical study protocol and amendments, safety information, investigational agent information, biological samples and laboratory procedures, SRM, study logs and IND Sponsor/investigator/study monitor correspondence will be kept on-site in study site-specific binders.

The IND Sponsor or designee will be responsible for maintaining original and backup of all CRF data. The investigator is responsible for maintaining backup of all electronic data systems used for primary documentation or source documentation. Backup of electronic data will be performed periodically as described in the site-specific SOPs. Backup records must be stored at a secure location on site and backup and recovery logs must be maintained to facilitate data recovery. If an electronic medical records system that is not supported by the IND Sponsor or designee (or is discontinued or decommissioned) is used, the investigator must maintain a system to retrieve these records or arrange for the transfer of these records to an alternate electronic format or to paper.

Changes to any electronic records require an audit trail, in accordance with 21 CFR 11.10(e), and should include who made the changes and when and why the changes were made. An audit trail is defined as a secure, computer-generated, time-stamped electronic record that will allow reconstruction of the course of events relating to the creation, modification and deletion of an electronic record. Audit trails must be created incrementally, in chronological order and in a manner that does not allow new audit trail information to overwrite existing data. Audit trails should be in a readable format and readily available at the study site and any other location where electronic study records are maintained.

Audit trails are generated automatically for eCRFs. The investigator is responsible for maintaining audit trails of all electronic data systems used for primary documentation or source documentation.

11.4.3 Case Report Forms and Source Documentation

The investigator must make study data accessible to the site monitor, to other authorized representatives of the IND Sponsor (or designee) and to the appropriate regulatory authority inspectors. The original CRF for each subject will be checked against source documents at the study site by the site monitor.

11.4.4 Retention of Study Documents

According to ICH E6, Section 4.9, all CRFs, as well as supporting paper and electronic documentation and administrative records, must be retained for at least 2 years after the last approval of a marketing application and until there are no pending or contemplated marketing applications, or at least 2 years have elapsed since the formal discontinuation of clinical development of an individual product. Longer retention periods may apply. The IND Sponsor will notify investigators as to when documents no longer need to be retained. No study documents will be destroyed or moved to a new location without prior written approval from the IND Sponsor. If the investigator relocates, retires or withdraws from the clinical study for any reason, all records required to be maintained for the study should be transferred to an agreed-upon designee, such as another investigator at the institution where the study was conducted.

Audit trails for electronic documents must be retained for a period at least as long as that required for the subject electronic records to which they pertain. The investigator must retain either the original or a certified copy of audit trails.

11.4.5 Data Confidentiality and Subject Anonymity

All information about the nature of the proposed investigation provided by the IND Sponsor or their representative to the investigator (with the exception of information required by law or regulations to be disclosed to the IRB, the subject or the appropriate regulatory authority) must be kept in confidence by the investigator.

The anonymity of participating subjects must be maintained. Subjects will be identified by their initials and an assigned subject number on CRFs and other documents retrieved from

the site or sent to the IND Sponsor, regulatory agencies, or central laboratories/reviewers. Documents that identify the subject (e.g., the signed ICF) must be maintained in strict confidence by the investigator, except to the extent necessary to allow auditing by the appropriate regulatory authority, the study monitor, IND Sponsor or their representative.

12. STATISTICAL CONSIDERATIONS

12.1 Study Design/Endpoints

Sample Size

This is an open-label, phase 2 study to evaluate the safety and clinical activity of tadalafil, pembrolizumab, ipilimumab, and CRS-207 in subjects with metastatic pancreatic adenocarcinoma who have progressed after at least 1 prior chemotherapy regimen.

The primary endpoint of this study is objective response rate (ORR) using RECIST. The treatment will be considered inactive and of no interest for further evaluation if the ORR is 5% or less and considered active if the ORR is 30% or greater.

A total of 17 evaluable patients will be enrolled for ORR. Patients will be considered evaluable for the primary endpoint if they receive at least one dose of study drug. If 3 or more responses are observed, we will conclude that the ORR is higher than 5% and that the regimen warrants further study. This design has 92% power to reject the null hypothesis of an ORR of 5% in favor of the alternative hypothesis of 30%, with one-sided target type 1 error of 0.1 (actual type error is 0.0502). Additional patients may be enrolled as needed until at least 10 paired biopsies are obtained, but will not be included in the primary analysis of efficacy endpoint.

Statistical Analyses

The primary endpoint is objective response, defined as complete response (CR) or partial response (PR) according to RECIST criteria. Objective response rate (ORR) will be estimated as the proportion of subjects whose best overall response is either a CR or PR per RECIST with corresponding 95% exact CI. The primary population for the analysis is all subjects who receive at least one dose of study drug, and have at least one post-baseline tumor assessments or discontinue due to toxicity or clinical progression prior to post-baseline tumor assessments. Subjects who discontinue due to toxicity or clinical progression prior to post-baseline tumor assessments will be considered as non-responders. Subjects who discontinue for other reasons prior to their first post-baseline tumor assessments will be replaced and not included in the primary efficacy analysis.

The secondary and exploratory endpoints are listed in **Section 10.2** and **10.3**.

PFS, OS, TOR, and DOR will be summarized using Kaplan-Meier methods. DCR and their associated 95% exact CI will be calculated.

All biomarker measures, T-cell response, immune phenotype and inflammatory response evaluation will be listed, tabulated, and where appropriate plotted. Tumor tissue IHC, functional analysis and genomic analysis will be listed, tabulated and where appropriate plotted. For the

continuous data, summary statistics and the corresponding changes (or percent changes) from baseline will be provided at each time-point of assessment as well as for the changes from baseline to the peak value. Categorical data will be summarized using frequency table. As this is an open-label study without a control treatment, statistical analyses will be done to aid in the understanding of the results. The associations of biomarkers with objective response, or time-to-event endpoints may be assessed using logistic regression and Cox regression, respectively. Correlation of baseline expression levels or changes in expression levels with response or time-to-event endpoints will identify potential responsive (or resistant) subgroups.

12.2 Safety Analysis

The safety analysis will be performed in all subjects who receive at least one dose of study treatment. AE data will be listed individually and incidence of AEs summarized by system organ class and preferred terms within a system organ class. When calculating the incidence of AEs, each AE (based on preferred terminology defined by CTCAE version 5.0) will be counted only once for a given subject. In analyses of grade and causality, if the same AE occurs on multiple occasions, the highest grade and strongest relationship to study drug will be assumed. If 2 or more AEs are reported as a unit, the individual terms will be reported as separate experiences.

Changes in vital signs, hematology and clinical chemistry parameters from baseline to the end of the study will be examined. Toxicity will be tabulated by type and grade. Treatment-emergent changes from normal to abnormal values in key laboratory parameters will be identified.

Toxicity will be monitored throughout the trial. Starting from the 6^{th} patient, if more than 33% of patients (that is, 2/6, 3/9, 4/12, 5/15, 6/17) are observed to experience an unacceptable toxicity within the first cycle of treatment, enrollment will be paused and the safety data will be reviewed by the study team and IND Sponsor before we enroll additional patients. Complete unacceptable toxicity criteria can be found in **Section 4.7**. In addition, enrollment will be paused if a patient's death is assessed as possibly related to any of the study drugs while the data is reviewed by the study team and IND sponsor.

REFERENCES

1 Siegel, R. L., Miller, K. D., Fuchs, H. E. & Jemal, A. Cancer Statistics, 2021. *CA: A Cancer Journal for Clinicians* **71**, 7-33, doi:https://doi.org/10.3322/caac.21654 (2021).

2 Torre, L. A. *et al.* Global cancer statistics, 2012. *CA: a cancer journal for clinicians* **65**, 87-108, doi:10.3322/caac.21262 (2015).

3 Rahib, L. *et al.* Projecting cancer incidence and deaths to 2030: the unexpected burden of thyroid, liver, and pancreas cancers in the United States. *Cancer Res* **74**, 2913-2921, doi:10.1158/0008-5472.CAN-14-0155 (2014).

4 Burris, H. A., 3rd *et al.* Improvements in survival and clinical benefit with gemcitabine as first-line therapy for patients with advanced pancreas cancer: a randomized trial. *J Clin Oncol* **15**, 2403-2413 (1997).

5 Moore, M. J. *et al.* Erlotinib plus gemcitabine compared with gemcitabine alone in patients with advanced pancreatic cancer: a phase III trial of the National Cancer Institute of Canada Clinical Trials Group. *J Clin Oncol* **25**, 1960-1966, doi:10.1200/JCO.2006.07.9525 (2007).

6 Conroy, T. *et al.* FOLFIRINOX versus gemcitabine for metastatic pancreatic cancer. *N Engl J Med* **364**, 1817-1825, doi:10.1056/NEJMoa1011923 (2011).

7 Von Hoff, D. D. *et al.* Increased survival in pancreatic cancer with nab-paclitaxel plus gemcitabine. *N Engl J Med* **369**, 1691-1703, doi:10.1056/NEJMoa1304369 (2013).

8 Wang-Gillam, A. *et al.* Nanoliposomal irinotecan with fluorouracil and folinic acid in metastatic pancreatic cancer after previous gemcitabine-based therapy (NAPOLI-1): a global, randomised, open-label, phase 3 trial. *Lancet* **387**, 545-557, doi:10.1016/S0140-6736(15)00986-1 (2016).

9 Disis, M. L. Immune regulation of cancer. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* **28**, 4531-4538, doi:10.1200/JCO.2009.27.2146 (2010).

10 Dong, H. *et al.* Tumor-associated B7-H1 promotes T-cell apoptosis: a potential mechanism of immune evasion. *Nature medicine* **8**, 793-800, doi:10.1038/nm730 (2002).

11 Sharpe, A. H. & Freeman, G. J. The B7-CD28 superfamily. *Nature reviews. Immunology* **2**, 116-126, doi:10.1038/nri727 (2002).

12 Brown, J. A. *et al.* Blockade of programmed death-1 ligands on dendritic cells enhances T cell activation and cytokine production. *J Immunol* **170**, 1257-1266 (2003).

13 Francisco, L. M., Sage, P. T. & Sharpe, A. H. The PD-1 pathway in tolerance and autoimmunity. *Immunological reviews* **236**, 219-242, doi:10.1111/j.1600-065X.2010.00923.x (2010).

14 Postow, M. A., Harding, J. & Wolchok, J. D. Targeting immune checkpoints: releasing the restraints on anti-tumor immunity for patients with melanoma. *Cancer J* **18**, 153-159 (2012).

15 Olson, D. J. *et al.* Pembrolizumab Plus Ipilimumab Following Anti-PD-1/L1 Failure in Melanoma. *J Clin Oncol*, JCO2100079, doi:10.1200/JCO.21.00079 (2021).

16 Hodi, F. S. *et al.* Nivolumab plus ipilimumab or nivolumab alone versus ipilimumab alone in advanced melanoma (CheckMate 067): 4-year outcomes of a multicentre, randomised, phase 3 trial. *Lancet Oncol* **19**, 1480-1492, doi:10.1016/S1470-2045(18)30700-9 (2018).

17 Gubens, M. A. *et al.* Pembrolizumab in combination with ipilimumab as second-line or later therapy for advanced non-small-cell lung cancer: KEYNOTE-021 cohorts D and H. *Lung Cancer* **130**, 59-66, doi:10.1016/j.lungcan.2018.12.015 (2019).

18 Le, D. T. *et al.* Safety and survival with GVAX pancreas prime and Listeria Monocytogenes-expressing mesothelin (CRS-207) boost vaccines for metastatic

pancreatic cancer. *J Clin Oncol* **33**, 1325-1333, doi:10.1200/JCO.2014.57.4244 (2015).
19 Le, D. T. *et al.* Results from a Phase IIb, Randomized, Multicenter Study of GVAX Pancreas and CRS-207 Compared with Chemotherapy in Adults with Previously Treated Metastatic Pancreatic Adenocarcinoma (ECLIPSE Study). *Clin Cancer Res* **25**, 5493-5502, doi:10.1158/1078-0432.CCR-18-2992 (2019).
20 Tsujikawa, T. *et al.* Evaluation of Cyclophosphamide/GVAX Pancreas Followed by Listeria-mesothelin (CRS-207) With or Without Nivolumab in Patients with Pancreatic Cancer. *Clin Cancer Res*, doi:10.1158/1078-0432.CCR-19-3978 (2020).
21 Hopkins, A. C. *et al.* T cell receptor repertoire features associated with survival in immunotherapy-treated pancreatic ductal adenocarcinoma. *JCI Insight* **3**, doi:10.1172/jci.insight.122092 (2018).
22 Wu, A. A. *et al.* A Phase II Study of Allogeneic GM-CSF-Transfected Pancreatic Tumor Vaccine (GVAX) with Ipilimumab as Maintenance Treatment for Metastatic Pancreatic Cancer. *Clin Cancer Res* **26**, 5129-5139, doi:10.1158/1078-0432.CCR-20-1025 (2020).
23 Serafini, P. *et al.* Phosphodiesterase-5 inhibition augments endogenous antitumor immunity by reducing myeloid-derived suppressor cell function. *The Journal of experimental medicine* **203**, 2691-2702, doi:10.1084/jem.20061104 (2006).
24 Califano, J. A. *et al.* Tadalafil augments tumor specific immunity in patients with head and neck squamous cell carcinoma. *Clin Cancer Res* **21**, 30-38, doi:10.1158/1078-0432.CCR-14-1716 (2015).
25 Hassel, J. C. *et al.* Tadalafil has biologic activity in human melanoma. Results of a pilot trial with Tadalafil in patients with metastatic Melanoma (TaMe). *Oncoimmunology* **6**, e1326440, doi:10.1080/2162402X.2017.1326440 (2017).
26 Weed, D. T. *et al.* The Reversal of Immune Exclusion Mediated by Tadalafil and an Anti-tumor Vaccine Also Induces PDL1 Upregulation in Recurrent Head and Neck Squamous Cell Carcinoma: Interim Analysis of a Phase I Clinical Trial. *Front Immunol* **10**, 1206, doi:10.3389/fimmu.2019.01206 (2019).
27 Luginbuhl, A. J. *et al.* 1116PD - A window of opportunity trial of preoperative nivolumab with or without tadalafil in squamous cell carcinoma of the head and neck (SCCHN): Safety, clinical, and correlative outcomes. *Annals of Oncology* **30**, v453, doi:https://doi.org/10.1093/annonc/mdz252.008 (2019).
28 Wolchok, J. D. *et al.* Guidelines for the evaluation of immune therapy activity in solid tumors: immune-related response criteria. *Clin Cancer Res* **15**, 7412-7420, doi:10.1158/1078-0432.CCR-09-1624 (2009).
29 Topalian, S. L. *et al.* Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. *The New England journal of medicine* **366**, 2443-2454, doi:10.1056/NEJMoa1200690 (2012).
30 Brahmer, J. R. *et al.* Phase I study of single-agent anti-programmed death-1 (MDX-1106) in refractory solid tumors: safety, clinical activity, pharmacodynamics, and immunologic correlates. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* **28**, 3167-3175, doi:10.1200/JCO.2009.26.7609 (2010).
31 Seymour, L. *et al.* iRECIST: guidelines for response criteria for use in trials testing immunotherapeutics. *Lancet Oncol* **18**, e143-e152, doi:10.1016/S1470-2045(17)30074-8 (2017).

APPENDIX A: Performance Status Criteria

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

APPENDIX B: Response Evaluation Criteria in Solid Tumors (RECIST) 1.1 Criteria for Evaluating Response in Solid Tumors

RECIST version 1.1 will be used in this study for assessment of tumor response. While either CT or MRI may be used utilized, as per RECIST 1.1, CT is the preferred imaging technique in this study.

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, as ≥10 mm with CT scan, or ≥10 mm with 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 unless there is evidence of progression in the irradiated site. 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, and abdominal masses (not followed by CT or MRI), are considered as 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 subject, 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.

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.

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 subject 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).

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 subject's best response assignment will depend on the achievement of both measurement and confirmation criteria.

For Subjects with Measurable Disease (i.e., Target Disease)

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

* See RECIST 1.1 manuscript for further details on what is evidence of a new lesion.

** Only for non-randomized trials with response as primary endpoint.

*** In exceptional circumstances, unequivocal progression in non-target lesions may be accepted as disease progression.

Note: Subjects 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.

Reference

E.A. Eisenhauer, P. Therasse, J. Bogaerts, L.H. Schwartz, D. Sargent, R. Ford, J. Dancey, S. Arbuck, S. Gwyther, M. Mooney, L. Rubinstein, L. Shankar, L. Dodd, R. Kaplan, D. Lacombe, J. Verweij. New response evaluation criteria in solid tumours: Revised RECIST guideline (version 1.1). Eur J Cancer. 2009 Jan;45(2):228-47.

APPENDIX C: Description of the iRECIST Process For Assessment of Disease Progression

Assessment at Screening and Prior to RECIST 1.1 Progression

Until radiographic progression based on RECIST 1.1, there is no distinct iRECIST assessment.

Assessment and Decision at RECIST 1.1 Progression

In participants who show evidence of radiological PD by RECIST 1.1 the Investigator will decide whether to continue a participant on study treatment until repeat imaging is obtained using the criteria outlined in **Section 4.10.1**.

If the Investigator decides to continue treatment, the participant may continue to receive study treatment and the tumor assessment should be repeated 4 to 8 weeks later to confirm PD by iRECIST, per Investigator assessment.

Tumor flare may manifest as any factor causing radiographic progression per RECIST 1.1, including:

- Increase in the sum of diameters of target lesion(s) identified at baseline to ≥ 20% and ≥ 5 mm from nadir
    - Please note: the iRECIST publication uses the terminology "sum of measurements", but "sum of diameters" will be used in this protocol, consistent with the original RECIST 1.1 terminology.
- Unequivocal progression of non-target lesion(s) identified at baseline
- Development of new lesion(s)

iRECIST defines new response categories, including **iUPD** (unconfirmed progressive disease) and **iCPD** (confirmed progressive disease). For purposes of iRECIST assessment, the first visit showing progression according to RECIST 1.1 will be assigned a visit (overall) response of iUPD, regardless of which factors caused the progression.

At this visit, target and non-target lesions identified at baseline by RECIST 1.1 will be assessed as usual.

New lesions will be classified as measurable or non-measurable, using the same size thresholds and rules as for baseline lesion assessment in RECIST 1.1. From measurable new lesions, up to 5 lesions total (up to 2 per organ), may be selected as New Lesions – Target. The sum of diameters of these lesions will be calculated, and kept distinct from the sum of diameters for target lesions at baseline. All other new lesions will be followed qualitatively as New Lesions – Non-target.

Assessment at the Confirmatory Imaging

On the confirmatory imaging, the participant will be classified as progression confirmed (with an overall response of iCPD), or as showing persistent unconfirmed progression (with an overall response of iUPD), or as showing disease stability or response (iSD/iPR/iCR).

Confirmation of Progression

Progression is considered confirmed, and the overall response will be iCPD, if ANY of the following occurs:

- Any of the factors that were the basis for the initial iUPD show worsening
  - For target lesions, worsening is a further increase in the sum of diameters of ≥ 5 mm, compared to any prior iUPD time point
  - For non-target lesions, worsening is any significant growth in lesions overall, compared to a prior iUPD time point; this does not have to meet the "unequivocal" standard of RECIST 1.1
  - For new lesions, worsening is any of these:
    - An increase in the new lesion sum of diameters by ≥ 5 mm from a prior iUPD time point
    - Visible growth of new non-target lesions
    - The appearance of additional new lesions
- Any new factor appears that would have triggered PD by RECIST 1.1

Persistent iUPD

Progression is considered not confirmed, and the overall response remains iUPD, if:

- None of the progression-confirming factors identified above occurs AND
- The target lesion sum of diameters (initial target lesions) remains above the initial PD threshold (by RECIST 1.1)

Additional imaging for confirmation should be scheduled 4 to 8 weeks from the scan on which iUPD is seen. This may correspond to the next visit in the original visit schedule. The assessment of the subsequent confirmation scan proceeds in an identical manner, with possible outcomes of iCPD, iUPD, and iSD/iPR/iCR.

Resolution of iUPD

Progression is considered not confirmed, and the overall response becomes iSD/iPR/iCR, if:

- None of the progression-confirming factors identified above occurs, AND
- The target lesion sum of diameters (initial target lesions) is not above the initial PD threshold.

The response is classified as iSD or iPR (depending on the sum of diameters of the target lesions), or iCR if all lesions resolve.

In this case, the initial iUPD is considered to be pseudo-progression, and the level of suspicion for progression is "reset". This means that the next visit that shows radiographic progression, whenever it occurs, is again classified as iUPD by iRECIST, and the confirmation process is repeated before a response of iCPD can be assigned.

Detection of Progression at Visits After Pseudo-progression Resolves

After resolution of pseudo-progression (i.e., achievement of iSD/iPR/iCR), iUPD is indicated by any of the following events:

- Target lesions
  - Sum of diameters reaches the PD threshold (≥ 20% and ≥ 5 mm increase from nadir) either for the first time, or after resolution of previous pseudo-progression. The nadir is always the smallest sum of diameters seen during the entire trial, either before or after an instance of pseudo-progression.
- Non-target lesions
  - If non-target lesions have never shown unequivocal progression, their doing so for the first time results in iUPD.
  - If non-target lesions had shown previous unequivocal progression, and this progression has not resolved, iUPD results from any significant further growth of non-target lesions, taken as a whole.
- New lesions
  - New lesions appear for the first time
  - Additional new lesions appear
  - Previously identified new target lesions show an increase of ≥ 5 mm in the new lesion sum of diameters, from the nadir value of that sum
  - Previously identified non-target lesions show any significant growth

If any of the events above occur, the overall response for that visit is iUPD, and the iUPD evaluation process (see Assessment at the Confirmatory Imaging above) is repeated. Progression must be confirmed before iCPD can occur.

The decision process is identical to the iUPD confirmation process for the initial PD, except in one respect. If new lesions occurred at a prior instance of iUPD, and at the confirmatory scan the burden of new lesions has increased from its smallest value (for new target lesions, their sum of diameters is ≥5 mm increased from its nadir), then iUPD cannot resolve to iSD or iPR. It will remain iUPD until either a decrease in the new lesion burden allows resolution to iSD or iPR, or until a confirmatory factor causes iCPD.

Additional details about iRECIST are provided in the iRECIST publication [31].

Table 2: Comparison between RECIST 1.1 and iRECIST

	RECIST 1.1	iRECIST
Definitions of disease: numbers, sites and target or non	Measurable are diameters greater than 10 mm (15 for nodes maximum of 5 (2 per organ)	No change from RECIST 1.1
CR, PR or SD	Cannot have met criteria for progression	Can have had iUPD (more than once) but not iCPD before iCR, iPR or iSD
Confirmation of CR or PR	Only in non-randomized studies	As per RECIST 1.1
Confirmation of SD	Not required	As per RECIST 1.1
New lesions	Progression: recorded but not measured	iUPD but only becomes iCPD if on the next scan there are new lesions or the size increases by greater than 5 mm
Confirmation of progression	Not required	Required
Consideration of clinical status	Not required	Clinical stability considered at iUPD to decide treatment continuation

Table 3: Trajectory of progression in iRECIST

Target Lesions: iCR, Non-target: iCR, no new lesions	iCR	iCR
Target lesions: iCR, Non-target: non iCR/non iUPD, no new lesions	iPR	iPR
Target Lesions: iPR, Non-target: non iCR/non iUPD, no new lesions	iPR	iPR
Target lesions: iSD, Non-target: non iCR/non iUPD, no new lesions	iSD	iSD
Target lesions: iUPD with no change or with a decrease from the last time point, Non-target: iUPD with no change or decrease from last time point, new lesions	NA	New lesions confirm iCPD if new lesions previously identified and increased in size (≥ 5 mm in sum of measures for new lesions or any increase for new lesion non-target) or increase in number. If no change is seen in new lesions assignment remains iUPD
Target lesions: iSD, iPR, iCR, non-target: iUPD, no	iUPD	Remains iUPD unless iCPD is confirmed by increase in

new lesions		the size of non-targets (does not need to meet RECIST 1.1 criteria)
Target lesions: iUPD, non-target: non iCR/non iUPD, no new lesions	iUPD	Remains iUPD unless iCPD confirmed on the basis of further increase ≥ 5 mm; otherwise stays as iUPD
Target lesions: iUPD, non-target: iUPD, no new lesions	iUPD	Remains iUPD unless iCPD confirmed on previously identified targets iUPD ≥ 5 mm or non-target iUPD
Target lesions: iUPD, non-targets: iUPD, new lesions	iUPD	Remains iUPD unless iCPD confirmed by increase of ≥ 5 mm previously identified target, or non-target or an increase in size or number of new lesions
Target lesions non iUPD or progression, non-targets: non iUPD or progression, new lesions	iUPD	Remains iUPD unless iCPD confirmed by increase in size or number of new lesions previously identified.

Target lesions, non-target lesions and new lesions are defined according to RECIST 1.1 criteria: if no pseudoprogression occurs, RECIST 1.1 and iRECIST categories for CR, PR and SD are the same. * Previously identified in the assessment prior to this time point. 'I' indicates immune responses assigned using iRECIST