Abbreviated Title: Nivo, tadalafil, vancomycin
NIH Protocol: 19C0033
Version Date: 01/19/2022
NCT: NCT03785210

Title: Phase II Study of Nivolumab (anti-PD1), Tadalafil and Oral Vancomycin in Patients with Refractory Primary Hepatocellular Carcinoma or Liver Dominant Metastatic Cancer from Colorectal or Pancreatic Cancers

NCI Principal Investigator: Tim F. Greten, MD
Thoracic & GI Malignancies Branch
National Cancer Institute
Building 10, Room 2B38B
9000 Rockville Pike
Bethesda, MD 20892
240-760-6114
gretentf@mail.nih.gov

Investigational Agents:

Drug Name:	Nivolumab	Tadalafil	Vancomycin
IND Number:	133973	133973	133973
Sponsor:	NCI CCR	NCI CCR	NCI CCR
Manufacturer:	Bristol Myers Squibb	Generic	Generic
Supplier:	Bristol Myers Squibb	NIH Clinical Center Pharmacy	NIH Clinical Center Pharmacy

PRÉCIS

Background:

- Current treatment options for patients with liver cancers, including hepatocellular carcinoma (HCC) and advanced liver cancers are limited and take no account of the known biological and genetic heterogeneity in these diseases. Median survival for advanced disease remains poor at approximately 1 year.
- Nivolumab is a fully human monoclonal immunoglobulin G4 (IgG4) antibody that is specific for human programmed death-1 (PD-1, cluster of differentiation 279 [CD279]) cell surface membrane receptor. Nivolumab has been approved by FDA for the treatment of HCC and other solid tumors.
- Tadalafil is a phosphodiesterase type 5 (PDE5) inhibitor which have been approved by the FDA for the treatment of pulmonary arterial hypertension, benign prostatic hyperplasia and erectile dysfunction, with a relative safe clinical profile. PDE5 inhibitors have been examined in multiple malignancies and cancer cell lines for their direct anticancer activities, for their efficacy as chemo-sensitizers and for cancer chemoprevention.
- Oral vancomycin is antibiotic that has effect on altering gut commensal bacteria subsequently inducing a liver-selective anti-tumor effect.
- The aim of the study is to evaluate whether the immunomodulatory effect induced by PDE5 inhibitor and oral vancomycin can be enhanced by immune checkpoint inhibition in advanced liver cancer.

Objective:

- To determine the Best Overall Response (BOR) according to Response Evaluation Criteria (RECIST 1.1) to combined treatment of nivolumab, oral vancomycin and tadalafil in patients with refractory primary HCC or liver dominant metastatic cancer from colorectal cancer (CRC) or pancreatic adenocarcinoma (PDAC).

Eligibility:

- Histologically confirmed, hepatocellular carcinoma (HCC)

  Or
- Histologically confirmed carcinoma highly suggestive of a diagnosis of HCC

  Or
- Histologically confirmed advanced colorectal or pancreatic malignancy with liver involvement as dominant site of metastasis
- Measurable lesion, accessible for biopsy.
- Age $\geq$ 18 years
- ECOG $\leq$ 1
- Acceptable renal, bone marrow and liver function.
- Willingness to undergo two mandatory tumor biopsies.

Design:

- The proposed study is a phase II study of combined nivolumab, oral vancomycin and tadalafil treatment in patients with HCC or liver dominant metastatic cancer from colorectal or pancreatic cancers.

- Treatment will be delivered in cycles consisting of 4 weeks (+/- 3 days) until progression or unacceptable toxicity.
- Patients will be seen in Clinical Center on monthly basis with disease status evaluation every 8 (+/-1) weeks after start of study therapy.

TABLE OF CONTENTS

STATEMENT OF COMPLIANCE

The trial will be carried out in accordance with International Conference on Harmonisation Good Clinical Practice (ICH GCP) and the following:

- United States (US) Code of Federal Regulations (CFR) applicable to clinical studies (45 CFR Part 46, 21 CFR Part 50, 21 CFR Part 56, 21 CFR Part 312, and/or 21 CFR Part 812)

National Institutes of Health (NIH)-funded investigators and clinical trial site staff who are responsible for the conduct, management, or oversight of NIH-funded clinical trials have completed Human Subjects Protection and ICH GCP Training.

The protocol, informed consent form(s), recruitment materials, and all participant materials will be submitted to the Institutional Review Board (IRB) for review and approval. Approval of both the protocol and the consent form must be obtained before any participant is enrolled. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented to the study. In addition, all changes to the consent form will be IRB-approved; an IRB determination will be made regarding whether a new consent needs to be obtained from participants who provided consent, using a previously approved consent form

1 INTRODUCTION

1.1 Study Objectives

1.1.1 Primary Objective:

- To determine the Best Overall Response (BOR) according to Response Evaluation Criteria (RECIST 1.1) to combined treatment of nivolumab, oral vancomycin and tadalafil in patients with refractory primary HCC or liver dominant metastatic cancer from colorectal cancer (CRC) or pancreatic adenocarcinoma (PDAC).

1.1.2 Secondary Objectives:

- To assess the safety and tolerability of nivolumab in combination with oral vancomycin and tadalafil in patients with refractory primary HCC or liver dominant metastatic cancer from CRC or PDAC.
- To assess overall survival (OS) of nivolumab combined with oral vancomycin and tadalafil in patients with refractory HCC or liver dominant metastatic cancer from CRC or PDAC.

1.1.3 Exploratory Objectives:

- To measure changes in immune cell population in the peripheral blood and tumors of patients treated on this study, including CD4+, CD8+, MDSCs, MAITS NKT cells, IFNgamma signaling pathway, perforin and granzyme.
- To measure secreted cytokines in the peripheral blood to monitor the relative level change with the treatment
- To measure changes in bile acid in the peripheral blood of patients treated in this study.
- To evaluate tadalafil and oral vancomycin pharmacokinetic level to monitor compliance and association between the level and response rate or immune parameters

- To evaluate TCR beta sequencing to analyze the T cell response from PBMC and tumor samples
- To assess gene and protein expression and metabolite profile from tumor to evaluate the association of the change immune cell signature, signaling pathways and metabolite with clinical outcome
- To measure changes in gut microbiome in the stool of patients treated on this study.
- To evaluate the incidence of vancomycin-resistant enterococci (VRE) development during the trial.
- To evaluate the relationship between the response rate, overall survival and immune parameters, gut microbiome, and bile acid component in patients treated on this study.

1.2 Background and Rationale

1.2.1 HCC And Immune Checkpoint Inhibitors (ICIs)

Hepatocellular carcinoma (HCC) is the fifth most common cancer worldwide and the second leading cause of cancer associated mortality with an average life expectancy of 6-9 months[1]. In the United States, there were 39,230 new cases and 27,170 deaths related to liver cancer in 2016[2]. By 2030, these numbers are expected to rise, primarily due to increase rates of hepatitis C virus (HCV) related cirrhosis[3]. In early stage disease, treatment has traditionally comprised surgery (partial resection or transplantation) or locoregional therapies like ablation or chemoembolization. Survival rates are varied between the different modalities. However, most patients relapse with recurrent and often, metastatic disease.

In advanced disease, systemic therapy with sorafenib or lenvatinib is the standard first line treatment. Sorafenib is a potent oral multikinase inhibitor that prevents tumor cell growth and angiogenesis. It is approved for inoperable or metastatic HCC based on two randomized phase III clinical trials. In the SHARP trial, sorafenib improved median overall survival (OS) by 3 months compared to best supportive care (BSC); 10.7 months in the sorafenib arm and 7.9 months in the BSC arm (HR 0.69;95% CI 0.55-0.87, p<0.001)[4]. In a similarly designed study by Cheng, et al, Asian patients were enrolled to receive sorafenib versus placebo. Median OS improved by approximately 2 months, 6.5 months in the sorafenib arm vs 4.2 months with placebo (HR 0.68, 95% CI 0.50-0.93, p=0.014)[5]. Recent FDA-approved lenvatinib for advanced HCC based on REFLECT trial showed non-inferior but not statistically superior to sorafenib for OS (HR 0.92; 95% CI: 0.79, 1.06). Median OS in the lenvatinib arm was 13.6 months and 12.3 months in the sorafenib arm. REFLECT also demonstrated a statistically significant improvement in progression-free survival (PFS) with lenvatinib as compared to sorafenib. Median PFS was 7.3 months in the lenvatinib arm and 3.6 months in the sorafenib arm (HR 0.64; 95% CI: 0.55, 0.75; p<0.001). The overall response rate was higher for the lenvatinib arm as compared to sorafenib (19% vs. 7% per RECIST 1.1).[6]

For patients who progress following first line treatment, regorafenib and recently nivolumab, are approved as second line agents. Regorafenib is a multikinase inhibitor targeting tumor growth and angiogenesis. In a study comparing regorafenib versus placebo in patients with advanced HCC who progressed through sorafenib, regorafenib improved OS by approximately 4 months (HR 0.63, 95% CI 0.50-0.79, p<0.001), and progression free survival (HR 0.46, 95% CI 0.37-0.56, p<0.001) [7].

To date, there have been 4 published immunotherapy trials in HCC (**Table 1**) [8-11]. The first was a single-arm phase II trial evaluating anti-CTLA-4 tremelimumab in 21 patients with HCC in the setting of second line treatment[10]. Tremelimumab showed promising antitumor activity and an acceptable safety profile. The partial response rate was 17.6% (3/17), and the disease control rate was 76% (13/17). Duration of response was 3.6, 9.2, and 15.7 months in the 3 responders, respectively. More recently, tremelimumab in combination with loco-reginal therapy has demonstrated activity and feasibility [8]. In this pilot study, 32 patients with HCC received tremelimumab at 2 dose levels (3.5 mg/kg in 6 patients and 10 mg/kg in 26 patients) every 4 weeks for 6 doses, then every 3 months. In conjunction with this, 7 patients with Barcelona Clinic Liver Cancer (BCLC) stage B disease underwent Transarterial chemoembolization, 10 patients with BCLC stage C disease underwent radiofrequency ablation, and 11 patients with BCLC stage C disease underwent cryoablation. Rapid disease progression was observed in 4 patients who were then excluded. Response assessment was performed in the 19 patients with evaluable lesions outside of the ablation zone. The partial response rate was 26% (5/19) and the disease control rate was 84% (16/19). CheckMate-040 was the largest study to evaluate immunotherapy in HCC thus far[9]. This was a multicenter phase I/II dose escalation and expansion study investigating nivolumab in patients with advanced HCC. A total of 214 patients with advanced HCC and Child-Pugh score of 6 or less received nivolumab 3 mg/kg every 2 weeks on the dose expansion phase. The study included patients with and without chronic viral hepatitis, including active HBC (31%) and HCV (21%) but not those with active co-infection with HBV and HCV or with hepatitis D virus infection. Most patients had advanced progressive disease, with 68% having extrahepatic spread and 76% having progressed despite prior systemic therapy. Partial responses were observed in 15% and 20% of patients in the dose-escalation and dose-expansion phases, respectively. Disease control rates were 64% and 58%, respectively. Responses were durable, averaging 17 months and 10 months, respectively. On the basis of CheckMate-040, FDA granted nivolumab accelerated approval in September 2017 for patients with advanced HCC previously treated with sorafenib. Following the aforementioned strong objective activity of nivolumab in the second-line scenario, phase III trials are testing the effect of nivolumab against sorafenib for patients naive to systemic therapy (NCT02576509). Recently, pembrolizumab (another PD-1–blocking agent) showed clinical activity for sorafenib-exposed patients (NCT02702401, KEYNOTE-240)[11]. In this study, 104 eligible patients were enrolled and treated, and objective response was 17% (18/104). The best overall responses were one (1%) complete and 17 (16%) partial responses; meanwhile, 46 (44%) patients had stable disease, 34 (33%) had progressive disease. Overall, the ORR is still suboptimal from these strategies.

Table 1: Key immunotherapy trials in Hepatocellular Carcinoma (HCC)					
Drug(s)	**Target**	**Population**	**No.**	**RR**	**Reference**
Tremelimumab	CTLA-4	Advanced HCC	21	17.6%	Sangro et al.[10]
Tremelimumab + Ablation	CTLA-4	Advanced HCC	32	26.3%	Duffy et al. [8]
Nivolumab	PD-1	Advanced HCC (naïve to Sorafenib)	80	22.5%	EI-Khoueiry et al. [9]
		Advanced HCC (Intolerant or progressed to Sorafenib)	182	18.6%	
Pembrolizumab	PD-1	Advanced HCC (Intolerant or progressed to Sorafenib)	104	17.3%	Zhu et al. [11]

1.2.2 Liver Metastases and Current Treatment Options

Tumors metastatic to the liver are more common than primary tumors. The most common sites of primary tumor are breast, lung, and colorectal cancer. Some authors have reported hepatic metastases in as many as 40 to 50% of adult patients with extrahepatic primary tumors[12]. Due to the central role in the portal circulation, the liver is a frequent site for metastatic tumor, especially for tumors of the digestive tract such as colon and pancreas [13-16]. The incidence of liver metastases is different among the primary tumor types. Accordingly, liver metastasis affects more than 50% of colorectal cancer (CRC) patients during their lifespan[17] and 60% metastatic pancreatic adenocarcinoma (PDAC) patients[13]. Liver metastases also occur at high frequencies in other solid tumors but are usually detected in the late stage of tumor progression. About 8% of prostate cancer patients develop clinically evident liver metastases during their treatment period [18] and a much higher percentage (25–45%) of prostate cancer patients show liver involvement at autopsy[19, 20]. A similar pattern is also observed in breast cancer patients where less than 5% of the patients have clinically evident liver metastases at the time of diagnosis [21, 22].

Liver metastases are associated with a poor prognosis and remain a major cause of morbidity and mortality in patients with malignant tumors of different origins. The presence of liver metastases still remains a clinically significant and therapeutically frustrating problem in clinical oncology secondary to the sanctuary effect of the liver in relation to systemic chemotherapy. For example, 15 to 25% of patients with CRC present with liver metastases, and another 25 to 50% develop liver metastasis within 3 years following resection of the primary tumor [23, 24].

Table 2: Key immunotherapy trials in CRC						
Drug(s)	**Target**	**Population**	**No.**	**Liver metastases**	**RR**	**Reference**
dMMR CRC						
Pembrolizumab	PD-1	Refractory CRC	11	55%	40%	Le et al. [25]
Nivolumab	PD-1	Refractory CRC	74	N/A	32%	Overman

Table 2: Key immunotherapy trials in CRC

Drug(s)	Target	Population	No.	Liver metastases	RR	Reference
						et al. [26]
Nivolumab + Ipilimumab	PD-1 + CTLA-4	Refractory CRC	119	N/A	55%	Overman et al. [27]
pMMR CRC						
Pembrolizumab	PD-1	Refractory CRC	21	52%	0%	Le et al. [25]
Unselected CRC						
Tremelimumab	CTLA-4	Refractory CRC	47	N/A	2%	Chung et al. [28]
Nivolumab	PD-1	Refractory CRC	19	N/A	0%	Topalian et al. [29] [30]
BMS-936559	PD-L1	Refractory CRC	18	N/A	0%	Brahmer et al. [30]
Atezolizumab + Bevacizumab	PD-L1	Refractory CRC	14	N/A	7%	Bendell et al. [31]
Atezolizumab + FOLFOX/bev		Metastatic CRC (70% first line)	30	N/A	40%	
Atezolizumab + Cobimetinib	PD-L1 + MEK	Refractory CRC	23	N/A	17%	Bendell et al. [32]

Conventional treatment of liver metastases include surgery (hepatic resection), intervention (embolization and transcatheter arterial chemoembolization), systemic chemotherapy, and radiofrequency ablation. Among them, only hepatic resection for metastatic disease has gained general acceptance as a potentially curative option. The potentially curative treatment of liver metastasis of CRC with surgical resection has been reported for many years, with 5-year survival rates now of 50%-60% [33, 34]. Moreover, if patients are well selected, up to 20% can achieve long-term disease-free survival after metastectomy [33-35]. The role of surgery for metastases from neuroendocrine tumors on long-term outcome is also well-documented [36, 37]. Surgical resection of hepatic metastases from gastric adenocarcinoma was associated with a significantly improved overall survival [38]. It is also noted that surgical resection of hepatic metastases was associated with a significantly improved overall 1-year and 3-year survival with median survival of 9.9 months for all PDAC patients with liver metastases [39]. Surgical resection allows for the maximum response rates, but unfortunately, the number of patients that are eligible for resection and/or ablation based on a finite number of liver metastasis and liver-only disease is small. Only 10%-20% of liver metastases from CRC are amenable to resection. Nevertheless, recurrences

occur in two-thirds of patients after resection for liver metastases within the first 2 years. One multi-center study of 1669 patients found that more than half developed recurrences, with median disease-free survival duration of 16.3 months[40].

Table 3: Key immunotherapy trials in Pancreatic adenocarcinoma (PDAC)

Drug(s)	Target	Population	No.	Liver metastases	RR	Reference
Ipilimumab	CTLA-4	Advanced/Metastatic PDAC	27	N/A	3.7%	Royal et al. [41]
Ipilimumab + Gemcitabine	CTLA-4	Advanced/Metastatic PDAC	13	N/A	15%	Anjali et al. [42]
Ipilimumab + GVAX	CTLA-4	Advanced/Metastatic PDAC	30	N/A	0%	Le et al. [43]
BMS-936559	PD-L1	Advanced/Metastatic PDAC	14	N/A	0%	Brahmer et al.[30]
Pembrolizumab + Gem/Nab	PD-1	Advanced/Metastatic PDAC	11	N/A	18%	Weiss et al. [44]
Pembrolizumab + Gem/Nab	PD-1	Advanced/Metastatic PDAC	15	N/A	20%	Weiss et al. [45]
MEDI4736	PD-L1	Advanced/Metastatic PDAC	10-20	N/A	?%	Segal et al. [46]
CP-870,893	CD40	Advanced/Metastatic PDAC	22	82%	18%	Beatty et al. [47]

Despite the positive and encouraging results in HCC, clinical results appear different in patients with liver metastases. No single trial specifically targets patients with liver metastatic disease. However, the majority of trials testing ICIs included patients with liver metastasis from CRC (**Table 2**) and PDAC (**Table 3**).

1.2.3 Tumor Microenvironment (TME)

It is critical to understand the mechanism of immune evasion during tumorigenesis in order to develop innovative and effective therapeutics based on cancer immunology. There is emerging data suggesting that the tumor microenvironment (TME) controls anti-tumor immunity by accumulation of regulatory T cells (Tregs), natural killer T cells (NKT), tumor associated macrophages (TAMs), indoleamine 2,3-dioxygenase (IDO) and myeloid derived suppressor cells (MDSCs) [48]. MDSCs form a critical link between the innate and adaptive immune system through their ability to influence the fate of Tregs in antigen specific T cell tolerance through diverse mechanisms [49]. MDSCs are a heterogeneous population of immature and immunosuppressive myeloid cells, which display a variety of pro-tumoral effects. They promote tumor angiogenesis through VEGF production[50] and subvert both innate and adaptive antitumor immunity[51]. MDSCs impair CD4+ and CD8+ T-cell responses via increased arginase activity,

leading to arginine depletion[52], and through the production of reactive oxygen and nitrogen species that disrupt TCR signaling[53]. MDSCs abrogate hepatic NK-cell activity via membrane-bound TGF-β[54] and facilitate the expansion of Tregs cells and the induction of Tregs cells through IL-10 and TGF-β production. Increased numbers of CD14+ HLA-DR$^{-/low}$ MDSCs have been found in both tumor tissue and peripheral blood from patients with HCC, and elevated cell counts were related to tumor progression[50, 52]. Nevertheless, MDSCs affect T-cell function, survival and trafficking. MDSCs express galectin-9 that binds to TIM-3 on T cells, inducing T cell apoptosis [55]. MDSCs also express ADAM17 that down-regulates L-selectin (CD62L) levels on T cells, limiting their homing to lymph nodes and tumors[56]. Additionally, MDSCs interact with liver macrophages and causes their up-regulation of B7-H1, further strengthening the immunosuppressive phenotype[57]. MDSCs can also impair NK cell function. In human HCC, MDSCs (CD14^{+}HLA-DR$^{-/low}$) inhibit NK cell cytotoxicity and cytokine release, which is mediated by the NKp30 receptor[52]. Tumor-derived IL-1β induces Ly6C negative MDSCs which also inhibit NK cell development and function[58]. Together, MDSCs contribute to immunosuppressive network through multiple mechanisms and are potential immunotherapy targets for liver cancer. Therefore, the abundance of these immunosuppressive factors in the HCC or metastatic liver cancer TME calls for a multi-targeted approach based on solid rationale for synergy.

NKT cells represent another important cell type in the liver cancer TME. The liver has the largest number of NKT cells, which play critical roles in the pathogenesis of liver diseases and liver cancer [59]. NKT cells are a heterogeneous group of nonconventional T lymphocytes expressing both NK and T cell markers that share phenotypic and functional characteristics with NK cells. NKT cells not only recognize malignant cells directly and protect against tumor progression in a CD1d-dependent manner[60], but also kill CD1d-expressing tumor-associated macrophages to suppress tumor growth[61]. We recently described that NKT cell activity is regulated by the ratio of primary bile acid to secondary bile acid, which can be modified by the gut microbiome[62]. Nevertheless, clinical evidence has showed high intratumoral NKT cells, high intratumoral IFN-g or combination of both high was associated with prolonged overall and recurrence-free survival in patients with HCC after curative resection[63]. Overall, it is generally believed that NKT cells play anti-tumor roles through the production of IFN-g promoting NK cell activation and killing tumor cells or tumor-associated macrophages.

Therefore, we hypothesize that antitumor effect of ICIs will be boosted by augmenting NKT cells and suppressing MDSCs would be a promising synergistic strategy to treat liver cancer (**Figure 1**).

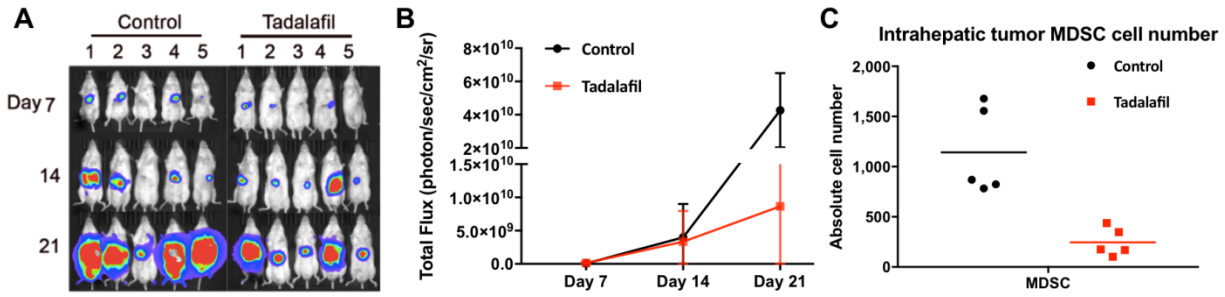


Figure 1: Tadalafil suppressed tumor growth and reduced tumor MDSC number. (A) RIL-175 hepatoma cells ($5x10^5$/ 20µL) were orthotopically implanted into the livers of male B6(Cg)-Tyr^{c-2J}/J mice. The establishment and growth of tumors were blindly monitored by BLI with the Xenogen IVIS. BLI represents proliferation rate through luciferase total flux signals. They were followed up for 21 days. Tadalafil (2mg/kg), a PDE5 inhibitor, was daily administered intraperitoneally in tumor bearing mice. (B) Analysis of BLI images was carried out by Living Image 2.50 software (PerkinElmer, Waltham, MA, USA). Calculated ROIs are graphed. (C) Accumulation of intrahepatic tumoral MDSCs was determined by flow cytometry.

1.2.4 PDE5 Inhibitors Augment Immunotherapy by Targeting on TME

Phosphodiesterase type 5 (PDE5) inhibitor, e.g. sildenafil and tadalafil, have been approved by the FDA for the treatment of pulmonary arterial hypertension, benign prostatic hyperplasia and erectile dysfunction, with a relative safe clinical profile. In the last decade, a significant number of studies have reported an increased expression of PDE5 in several human cancers compared to normal or surrounding non-neoplastic tissues[64-67]. Concomitantly, PDE5 inhibitors have been examined in multiple malignancies and cancer cell lines for their direct anticancer activities, for their efficacy as chemo-sensitizers and for cancer chemoprevention [68, 69]. Interestingly, the suppression of MDSCs by PDE5 inhibitor was well documented recently. In preclinical mouse models, it has been demonstrated that PDE5 inhibitor, sildenafil, and tadalafil significantly inhibited the MDSC functions by the downregulation of iNOS and ARG1 activities, leading to the activation of antitumor immunity and the prolongation of survival of tumor-bearing mice [70-72]. Recently, we found the treatment with a PDE5 inhibitor, tadalafil, caused suppression of tumor growth by reversing MDSC suppressor function (**Figure 1**). In 2015, two randomized, prospective, double blinded, placebo controlled, phase II clinical trials to determine the activity of PDE5 inhibitors on immune function in head and neck squamous cell carcinoma (HNSCC) patients were conducted[70, 73] (**Figure 2**). Results showed that tadalafil can reverse tumor-specific immune suppression in these patients by lowering MDSCs and Tregs and increasing tumor-specific $CD8^+$ T cells in a dose-dependent fashion, and augments general and tumor-specific immunity in HNSCC patients. In an open-labeled pilot study, the biologic effects, safety and efficacy of palliative treatment with tadalafil was assessed in patients with advanced metastatic melanoma [74]. Stable disease was achieved in 3/12 patients (25%). Median progression-free survival was 4.6 months, median overall survival 8.5 months. Stable patients displayed significantly higher numbers of $CD8^+$ tumor infiltrating lymphocytes (TILs) in the center of metastases before treatment as compared with progressive patients. Upon therapy, they showed increased expression of ζ-chain (used as a marker of T cell activation) in $CD8^+$and $CD4^+$TILs and $CD8^+$T cells in the peripheral blood as compared with baseline. Tadalafil improved clinical outcome of advanced melanoma patients by enhancing antitumor immunity. It was also demonstrated that the addition of tadalafil in a patient with end-stage relapsed/refractory multiple myeloma demonstrated reduced MDSC function and generated a dramatic and durable anti-myeloma immune and clinical response[75]. All of these clinical studies elaborate the decreased amounts of MDSC by PDE5 inhibitor and their immunosuppressive pattern correlated with an increased T cell reactivity and improved clinical outcome of advanced cancer patients. Currently, there are two clinical trials ongoing to evaluate the efficacy of combination of PDE5 inhibitor with regorafenib on advanced solid tumor (NCT02466802) and with vaccine on HNSCC (NCT02544880), respectively. No clinical trial is evaluating the therapeutic effects of PDE5 inhibitor on liver cancer or liver metastases with ICIs.

Based on the available pre-clinical and clinical trial data, it is highly suggested PDE5 inhibitor likely has synergistic antitumor efficacy when combined with ICIs (**Figure 1**).

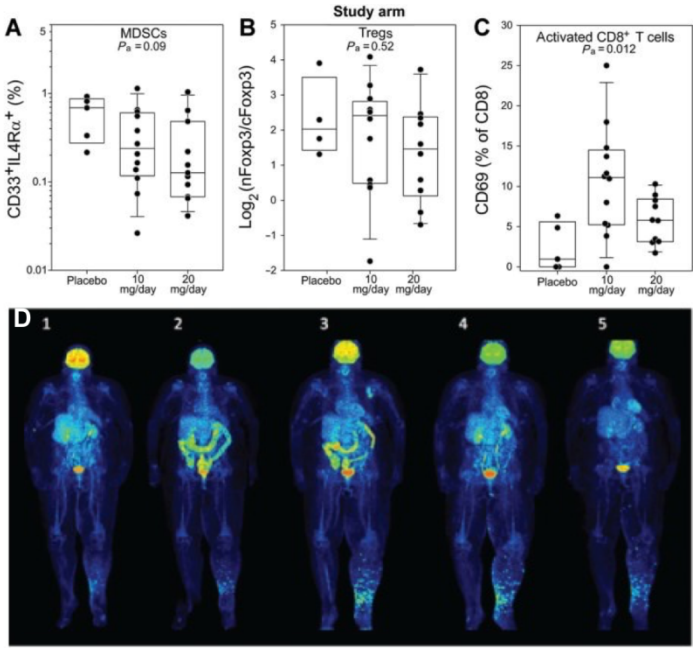


Figure 2: Tadalafil suppressed tumor growth by modulating tumor microenvironment. (A-C): tadalafil modulates tumor microenvironment. CD33/IL4Rα (A), CD4/FoxP3 (B), or CD8/CD69 (C) intratumoral concentration was evaluated by immune-fluorescence microscopy in HNSCC. D. Representative image of PET-CT scans from metastatic melanoma patient prior (1), after 4 cycles of ipilimumab (2), and 6 weeks later to exclude pseudo-progression (3); here progressive disease was confirmed, and the patient included into the TaMe trial; after 8 weeks of treatment with tadalafil 5 mg daily PO (4) and after 5 months (5). The scans show progressive disease under ipilimumab (1–3) and stable disease under tadalafil (3–5) – with a good regression of cutaneous metastases in the leg and a progressing left inguinal lymph node.

1.2.4.1 Tadalafil toxicity

Because clinical trials are conducted under widely varying conditions, adverse reaction rates observed in the clinical trials of a drug cannot be directly compared to rates in the clinical trials of another drug and may not reflect the rates observed in practice. Tadalafil was administered to over 9000 men during clinical trials worldwide. In trials of tadalafil for once daily use, a total of 1434, 905, and 115 were treated for at least 6 months, 1 year, and 2 years, respectively. For tadalafil for use as needed, over 1300 and 1000 subjects were treated for at least 6 months and 1 year, respectively. In addition, as discussed above, tadalafil has been administrated to study the efficacy to improve antitumor immunity with daily dose for short period times.

1.2.4.1.1 Tadalafil for Use as Needed for Erectile Dysfunction (ED)

In eight primary placebo-controlled clinical studies of 12 weeks duration, mean age was 59 years (range 22 to 88) and the discontinuation rate due to adverse events in patients treated with tadalafil 10 or 20 mg was 3.1%, compared to 1.4% in placebo treated patients.

When taken as recommended in the placebo-controlled clinical trials, the following adverse reactions were reported (**Table 4**) for tadalafil for use as needed:

Table 4: Treatment-Emergent Adverse Reactions Reported by ≥2% of Patients Treated with Tadalafil (10 or 20 mg) and More Frequent on Drug than Placebo in the Eight Primary Placebo-Controlled Clinical Studies (Including a Study in Patients with Diabetes) for tadalafil for Use as Needed for ED

Adverse Reaction	Placebo (N=476)	Tadalafil 5 mg (N=151)	Tadalafil 10 mg (N=394)	Tadalafil 20 mg (N=635)
Headache	5%	11%	11%	15%
Dyspepsia	1%	4%	8%	10%
Back pain	3%	3%	5%	6%
Myalgia	1%	1%	4%	3%
Nasal congestion	1%	2%	3%	3%
Flushing [a]	1%	2%	3%	3%
Pain in limb	1%	1%	3%	3%

a The term flushing includes: facial flushing and flushing

1.2.4.1.2 Tadalafil for Once Daily Use for ED

In three placebo-controlled clinical trials of 12 or 24 weeks duration, mean age was 58 years (range 21 to 82) and the discontinuation rate due to adverse events in patients treated with tadalafil was 4.1%, compared to 2.8% in placebo-treated patients.

The following adverse reactions were reported (**Table 5**) in clinical trials of 12 weeks duration:

Table 5: Treatment-Emergent Adverse Reactions Reported by ≥2% of Patients Treated with tadalafil for Once Daily Use (2.5 or 5 mg) and More Frequent on Drug than Placebo in the Three Primary Placebo-Controlled Phase 3 Studies of 12 weeks Treatment Duration (Including a Study in Patients with Diabetes) for tadalafil for Once Daily Use for ED

Adverse Reaction	Placebo (N=248)	Tadalafil 2.5 mg (N=196)	Tadalafil 5 mg (N=304)
Headache	5%	3%	6%
Dyspepsia	2%	4%	5%
Nasopharyngitis	4%	4%	3%
Back pain	1%	3%	3%
Upper respiratory tract infection	1%	3%	3%
Flushing	1%	1%	3%
Myalgia	1%	2%	2%
Cough	0%	4%	2%
Diarrhea	0%	1%	2%
Nasal congestion	0%	2%	2%
Pain in extremity	0%	1%	2%
Urinary tract infection	0%	2%	0%
Gastroesophageal reflux disease	0%	2%	1%
Abdominal pain	0%	2%	1%

The following adverse reactions were reported (**Table 6**) over 24 weeks treatment duration in one placebo-controlled clinical study:

Table 6: Treatment-Emergent Adverse Reactions Reported by ≥2% of Patients Treated with tadalafil for Once Daily Use (2.5 or 5 mg) and More Frequent on Drug than Placebo in One Placebo-Controlled Clinical Study of 24 Weeks Treatment Duration for tadalafil for Once Daily Use for ED			
Adverse Reaction	**Placebo (N=94)**	**Tadalafil 2.5 mg (N=96)**	**Tadalafil 5 mg (N=97)**
Nasopharyngitis	5%	6%	6%
Gastroenteritis	2%	3%	5%
Back pain	3%	5%	2%
Upper respiratory tract infection	0%	3%	4%
Dyspepsia	1%	4%	1%
Gastroesophageal reflux disease	0%	3%	2%
Myalgia	2%	4%	1%
Hypertension	0%	1%	3%
Nasal congestion	0%	0%	4%

1.2.4.1.3 Tadalafil for Once Daily Use for Benign Prostate Hyperplasia (BPH) and for ED and BPH

In three placebo-controlled clinical trials of 12 weeks duration, two in patients with BPH and one in patients with ED and BPH, the mean age was 63 years (range 44 to 93) and the discontinuation rate due to adverse events in patients treated with tadalafil was 3.6% compared to 1.6% in placebo-treated patients. Adverse reactions leading to discontinuation reported by at least 2 patients treated with tadalafil included headache, upper abdominal pain, and myalgia. The following adverse reactions were reported (**Table 7**).

Table 7: Treatment-Emergent Adverse Reactions Reported by ≥1% of Patients Treated with tadalafil for Once Daily Use (5 mg) and More Frequent on Drug than Placebo in Three Placebo-Controlled Clinical Studies of 12 Weeks Treatment Duration, including Two Studies for Tadalafil for Once Daily Use for BPH and One Study for ED and BPH		
Adverse Reaction	**Placebo (N=576)**	**Tadalafil 5 mg (N=581)**
Headache	2.3%	4.1%
Dyspepsia	0.2%	2.4%
Back pain	1.4%	2.4%
Nasopharyngitis	1.6%	2.1%
Diarrhea	1.0%	1.4%
Pain in extremity	0.0%	1.4%

Table 7: Treatment-Emergent Adverse Reactions Reported by ≥1% of Patients Treated with tadalafil for Once Daily Use (5 mg) and More Frequent on Drug than Placebo in Three Placebo-Controlled Clinical Studies of 12 Weeks Treatment Duration, including Two Studies for Tadalafil for Once Daily Use for BPH and One Study for ED and BPH		
Adverse Reaction	**Placebo (N=576)**	**Tadalafil 5 mg (N=581)**
Myalgia	0.3%	1.2%
Dizziness	0.5%	1.0%

1.2.4.1.4 Tadalafil for Once Daily Use for HNSCC

In a study of HNSCC with tadalafil to modify antitumor immunity, the following AEs were reported (**Table 8**) [73].

Table 8: **Treatment-Emergent Adverse Reactions Reported with Tadalafil for Once Daily for at least 20 days**							
		Possible			**Probable**		
	Symptoms	**Placebo**	**10**	**20**	**Placebo**	**10**	**20**
Grade 1	Back pain			2			
	Constipation						1
	Cough			1			
	Diarrhea		1	1			
	Ear, nose and throat examination abnormal		1				
	Headache	1		2	1		
	Neck pain	1		2			
	Pain in extremity						1
	Pulmonary			1	1		
	Sexual	1					
	TOTAL AE grade 1	*3*	*2*	*9*	*2*	*0*	*2*
	Total patients with AE grade 1	**2**	**2**	**4**	**1**	**0**	**2**
Grade 2	Back pain					1	
	Headache					1	
	Neck pain					1	
	Pain in extremity			1			1
	Total AE grade 2			*1*		*3*	*1*

Table 8: **Treatment-Emergent Adverse Reactions Reported with Tadalafil for Once Daily for at least 20 days**							
		Possible			**Probable**		
	Symptoms	**Placebo**	**10**	**20**	**Placebo**	**10**	**20**
	Total patients with AE grade 2			**1**		**1**	**1**
Grade 3	Back pain					1	1
	Myalgia						1
	Total AE grade 3					*1*	*2*
	Total patients with AE grade 3					**1**	**2**
Note: placebo (n=5); 10 mg arm (n=14); 10 mg arm (n=15). AE= Adverse Events							

Additional, less frequent adverse reactions (<1%) reported in the controlled clinical trials of tadalafil for BPH or ED and BPH included: gastroesophageal reflux disease, upper abdominal pain, nausea, vomiting, arthralgia, and muscle spasm.

Back pain or myalgia was reported at incidence rates described in **Table 4, Table 5, Table 6, Table 7, Table 8**. In tadalafil clinical pharmacology trials, back pain or myalgia generally occurred 12 to 24 hours after dosing and typically resolved within 48 hours. The back pain/myalgia associated with tadalafil treatment was characterized by diffuse bilateral lower lumbar, gluteal, thigh, or thoracolumbar muscular discomfort and was exacerbated by recumbency. In general, pain was reported as mild or moderate in severity and resolved without medical treatment, but severe back pain was reported with a low frequency (<5% of all reports). When medical treatment was necessary, acetaminophen or non-steroidal anti-inflammatory drugs were generally effective; however, in a small percentage of subjects who required treatment, a mild narcotic (e.g., codeine) was used. Overall, approximately 0.5% of all subjects treated with tadalafil for on demand use discontinued treatment as a consequence of back pain/myalgia. In the 1-year open label extension study, back pain and myalgia were reported in 5.5% and 1.3% of patients, respectively. Diagnostic testing, including measures for inflammation, muscle injury, or renal damage revealed no evidence of medically significant underlying pathology. In studies of tadalafil for once daily use, adverse reactions of back pain and myalgia were generally mild or moderate with a discontinuation rate of <1% across all indications.

Across all studies with any tadalafil dose, reports of changes in color vision were rare (<0.1% of patients).

The following adverse reactions have been identified during post approval use of tadalafil. Because these reactions are reported voluntarily from a population of uncertain size, it is not always possible to reliably estimate their frequency or establish a causal relationship to drug exposure. These events have been chosen for inclusion either due to their seriousness, reporting frequency, lack of clear alternative causation, or a combination of these factors.

Cardiovascular and Cerebrovascular — Serious cardiovascular events, including myocardial infarction, sudden cardiac death, stroke, chest pain, palpitations, and tachycardia, have been reported post marketing in temporal association with the use of tadalafil. Most, but not all, of these patients had preexisting cardiovascular risk factors. Many of these events were reported to occur

during or shortly after sexual activity, and a few were reported to occur shortly after the use of tadalafil without sexual activity. Others were reported to have occurred hours to days after the use of tadalafil and sexual activity. It is not possible to determine whether these events are related directly to tadalafil, to sexual activity, to the patient's underlying cardiovascular disease, to a combination of these factors, or to other factors.

Body as a Whole — hypersensitivity reactions including urticaria, Stevens-Johnson syndrome, and exfoliative dermatitis ***Nervous*** — migraine, seizure and seizure recurrence, transient global amnesia

Ophthalmologic — visual field defect, retinal vein occlusion, retinal artery occlusion Non-arteritic anterior ischemic optic neuropathy (NAION), a cause of decreased vision including permanent loss of vision, has been reported rarely post marketing in temporal association with the use of phosphodiesterase type 5 (PDE5) inhibitors, including tadalafil. Most, but not all, of these patients had underlying anatomic or vascular risk factors for development of NAION, including but not necessarily limited to: low cup to disc ratio ("crowded disc"), age over 50, diabetes, hypertension, coronary artery disease, hyperlipidemia, and smoking. It is not possible to determine whether these events are related directly to the use of PDE5 inhibitors, to the patient's underlying vascular risk factors or anatomical defects, to a combination of these factors, or to other factors.

Otologic — Cases of sudden decrease or loss of hearing have been reported post marketing in temporal association with the use of PDE5 inhibitors, including tadalafil. In some of the cases, medical conditions and other factors were reported that may have also played a role in the otologic adverse events. In many cases, medical follow-up information was limited. It is not possible to determine whether these reported events are related directly to the use of tadalafil, to the patient's underlying risk factors for hearing loss, a combination of these factors, or to other factors

Urogenital — priapism

1.2.5 Gut Microbiome and Liver Cancer Control

Various bacteria populating in mammal gastrointestinal tract are an indispensable part in intestine ecosystem and play a pivotal role in gut barrier[76]. Commensal bacteria have been showed to regulate host immune system through the crosstalk with host intestinal epithelial cells and lymphatic cells[77]. In addition, the bacterial metabolism is another factor influencing the host

immune homeostasis[77]. It is well known that oral antibiotics results in short- and long-term changes of the intestinal microbiome in humans[78, 79]. For instance, oral vancomycin is noted to reduce fecal microbial diversity with a decrease of gram-positive bacteria (mainly *Firmicutes*) and a compensatory increase in gram-negative bacteria (mainly *Proteobacteria*). Concurrently, vancomycin increases plasma primary bile acids with decreased fecal secondary bile acids that is predominantly associated with altered *Firmicutes*[80]. In line, this change might have implication on host immunity.

The gut microbiome has emerged as a critical factor regulating anti-tumor immunity controlling the efficacy of chemo- and immunotherapies[81-85]. Most recently, it was noted that there were significant differences in the diversity and composition of the patient gut microbiome of responders versus non-responders to anti-PD-1 treatment in melanoma [84]. This study further found patient fecal microbiome samples (n = 43, 30 responders, 13 non-responders) significantly higher alpha diversity and relative abundance of bacteria of the *Ruminococcaceae* family in responding patients. The intestinal microbiome change by oral antibiotics may change the clinical

outcome to ICIs. It was found that antibiotics inhibited the clinical benefit of anti-PD-1 or anti-PD-L1 in patients with advanced cancer. Fecal microbiota transplantation from responder into germ-free or antibiotic-treated mice ameliorated the antitumor effects of PD-1 blockade, whereas fecal microbiota transplantation from non-responder failed to do so. Surprisingly, analysis of patient stool samples at diagnosis revealed correlations between clinical responses to PD-1 blockade and the relative abundance of *Akkermansia muciniphila*. Oral supplementation with *A. muciniphila* after Fecal microbiota transplantation with no responder feces restored the efficacy of PD-1 blockade in an interleukin-12–dependent manner by increasing the recruitment of CCR9+CXCR3+CD4+ T lymphocytes into mouse tumor beds[85]. These data have important implications for the treatment of cancer patients with ICIs.

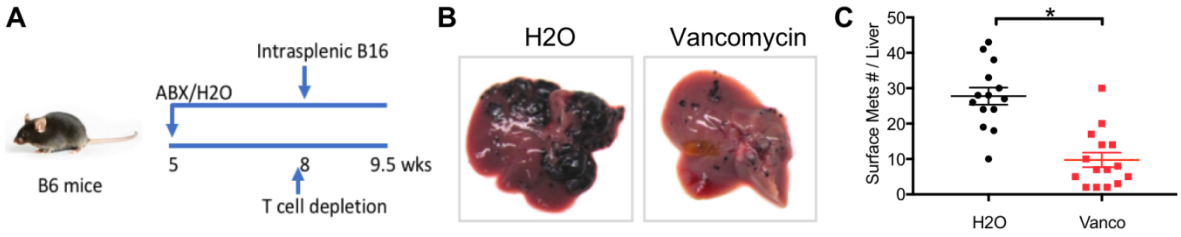


Figure 3: Wildtype mice, were treated with ABX or H2O for 3 weeks (A). Then mice were given intra splenic B16 tumor cell injection. Liver metastases were measured. Representative images and liver surface metastatic tumor counts are shown. Liver metastasis was measured. Representative images, cumulative liver metastasis counts are shown. N=16 for H2O, 14 for ABX. $p<0.05$, one-way ANOVA**.**

Profound effects of the gut microbiome on HCC development have been described[86, 87]. Recently, we demonstrated that altering gut commensal bacteria by oral vancomycin induced a liver-selective anti-tumor effect[62] (**Figure 3**). An increase of hepatic $CXCR6^+$ NKT cells was observed, independent of mouse strain, gender or presence of liver tumor. Hepatic NKT cells showed an activated phenotype and produced more IFNg upon antigen stimulation. In *vivo* functional studies confirmed that NKT cells mediated the liver-selective tumor inhibition. The data also showed that NKT cell accumulation was regulated by CXCL16 expression of liver sinusoidal endothelial cells, which was controlled by gut microbiome-mediated primary-to-secondary bile acid conversion. Interestingly, previous study on healthy volunteer, treated with oral vancomycin, showed decreased fecal secondary bile acids with a simultaneous postprandial increase in primary bile acids in plasma (**Figure 4**)[80]. This finding indicates there are certain similarity between human and mice regarding the metabolic change of bile acid to oral vancomycin. Feeding secondary bile acids or colonization of bile acid-metabolizing bacteria reversed both NKT cell accumulation and inhibition of liver tumor growth in mice with altered gut commensal bacteria. This study suggests a new link between gut bacteria-controlled bile acid metabolism and liver anti-tumor immune-surveillance.

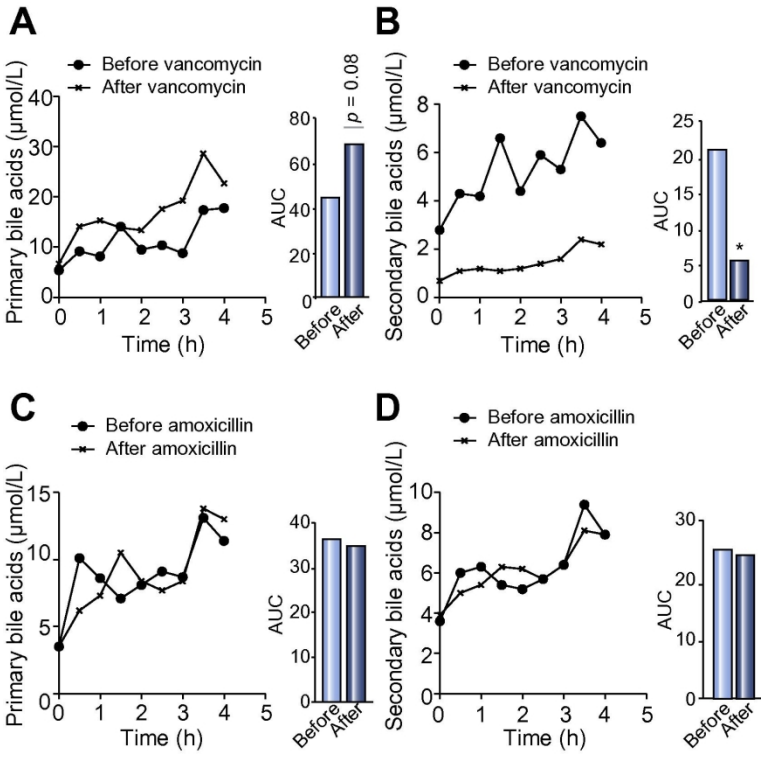


Figure 4: Effect of antibiotics on plasma bile acid concentration. After vancomycin treatment the postprandial rise in plasma concentrations of primary bile acids is increased (A), whereas the concentration of secondary bile acids is decreased (B). Amoxicillin treatment did not have an effect on either postprandial primary (C) or secondary (D) plasma bile acids. Data are expressed as medians (n = 10 per group), ∗p <0.05.

1.2.6 Nivolumab

PD-1 is a negative regulatory molecule that is expressed transiently following T-cell activation and on chronically stimulated T cells characterized by an "exhausted" phenotype. Subsequently, PD-1 positive T cells lose function and proliferative capacity while enhancing a suppressive tumor microenvironment. PD-1 may act together with other T-cell modulating molecules, including CTLA-4, TIM-3, lymphocyte-activation gene 3 (LAG-3) as well as indoleamine-pyrrole 2,3-dioxygenase 1 (IDO-1), cytokines, and transforming growth factor beta. Two ligands specific for PD-1 have been identified: PD-ligand 1 (PD-L1, also known as B7-H1 or CD274, expressed on tumor, antigen-presenting cells [APCs], and dendritic cells [DCs]) and PD-L2 (also known as B7-DC or CD273, expressed on endothelial cells). The interaction of PD- 1 with PD-L1 and PD-L2 results in negative regulatory stimuli that down-modulate the activated T-cell immune response through SHP-1 phosphatase. PD-L1 expression is found on a number of tumors, and is associated with poor prognoses based on OS in many tumors[88].

Nivolumab (OPDIVO®, BMS-936558, MDX-1106, and ONO-4538) is a fully human monoclonal immunoglobulin G4 (IgG4) antibody (HuMAb) that is specific for human programmed death-1 (PD-1, cluster of differentiation 279 [CD279]) cell surface membrane receptor[88]. Nivolumab binds to cynomolgus monkey PD-1 but not mouse, rat, or rabbit molecules. Clinical activity of

nivolumab has been evidenced by the recent FDA approval in patients with melanoma, non-small cell lung cancer (NSCLC), SCLC, renal cell carcinoma (RCC), HCC, dMMR CRC, HNSCC and urothelial cancer. Specifically, on the basis of CheckMate-040, FDA granted nivolumab accelerated approval in September 2017 for patients with advanced HCC previously treated with sorafenib.

1.2.6.1 Pharmacokinetics

Pharmacokinetics (PK) of nivolumab was linear in the range of 0.3 to 10 mg/kg, with dose-proportional increases in maximum serum concentration (C_{max}) and area under the concentration-time curve from time zero to infinity ($AUC_{0-\infty}$), with low to moderate inter-subject variability observed at each dose level[88]. Clearance of nivolumab is independent of dose in the dose range (0.1 to 10 mg/kg) and tumor types studied. Body weight normalized dosing showed approximately constant trough concentrations over a wide range of body weights. The mean terminal elimination half-life of BMS-936558 is 17 to 25 days consistent with the half-life of endogenous IgG4.

1.2.6.2 Efficacy

In the multicenter phase I/II dose escalation and expansion study, CheckMate-040, total 214 patients with advanced HCC and Child-Pugh score of 6 or less received nivolumab 3 mg/kg every 2 weeks on the dose expansion phase[9]. Partial responses were observed in 15% and 20% of patients in the dose-escalation and dose-expansion phases, respectively. Disease control rates were 64% and 58%, respectively. Responses were durable, averaging 17 months and 10 months, respectively.

In the monotherapy cohort of CheckMate-142, nivolumab provided durable responses with 31% investigator-assessed objective response rate (ORR) (median duration of response not yet reached with median follow-up of 12.0 months), 69% sustained disease control, 54% (9 months) and 50% (12 months) PFS, and 78% (9 months) and 73% (12 months) OS in previously treated patients with dMMR/MSI-H mCRC [26]. In the combination therapy cohort of CheckMate-142, at median follow-up of 13.4 months, nivolumab plus ipilimumab demonstrated 55% investigator-assessed ORR (95% CI, 45.2 to 63.8), and 80% disease control rate in the patients with dMMR/MSI-H mCRC. Median duration of response was not reached and most responses (94%) were ongoing at data cutoff. PFS rates were 76% (9 months) and 71% (12 months); respective OS rates were 87% and 85%. Statistically significant and clinically meaningful improvements were observed in patient-reported outcomes, including functioning, symptoms, and quality of life [27].

1.2.6.3 Toxicology

A maximum tolerated dose (MTD) of nivolumab was not defined[29]. Serious adverse events (SAEs) occurred in 32 of 296 patients (11%) similar to the immune-related inflammatory events seen with ipilimumab: pneumonitis, vitiligo, colitis, hepatitis, hypophysitis, and thyroiditis (with noted pulmonary toxicity resulting in 3 deaths. Renal failure, symptomatic pancreatic and DM, neurologic events, vasculitis, insomnia and hematologic toxicity have also been reported). In combination with ipilimumab in the concurrent-regimen, grade 3 to 4 treatment-related adverse events (AEs) occurred in 32% of patients and were manageable. Patients (13%) who discontinued treatment because of study drug-related AEs had an ORR (63%) consistent with that of the overall population[27].

1.2.6.4 Pharmacodynamics/Biomarkers

Tumor-cell expression (melanoma) of PD-L1 was characterized in combination with ipilimumab with the use of IHC staining and pharmacodynamics changes in the peripheral-blood absolute lymphocyte count[89]. With PD-L1 positivity defined as expression in at least 5% of tumor cells, biopsy specimens from 21 of 56 patients (38%) were PD-L1–positive. Among patients treated with the concurrent regimen of nivolumab and ipilimumab, ORs were observed in patients with either PD-L1–positive tumor samples (6 of 13 patients) or PD-L1–negative tumor samples (9 of 22). In the sequenced regimen cohorts, a higher number of overall responses was seen among patients with PD-L1–positive tumor samples (4 of 8 patients) than among patients with PD-L1– negative tumor samples (1 of 13) suggesting the possibility that these tumors have higher response rates to the combination. The relationship between PDL-1 expression and responses may not be present in patients treated with the combination. Tissue expression of PDL-2, interferon-γ (IFN- γ), IDO, and T cell $CD8^+$ are of current interest. Until more reliable data based on standardized procedures for tissue collection and assays are available, PD-L1 status cannot be used to select patients for treatment at this time.

1.2.7 PDE5 Inhibitor and Oral Vancomycin in Combination with Nivolumab

Because the mechanisms of action of PDE5 inhibitor, oral vancomycin and PD-1 are non-redundant targeting at MDSCs, NKT cells and PD-1 pathways as abovementioned, the combination may have additive or synergistic activity (**Figure 5**). There has no such treatment strategy developing or developed.

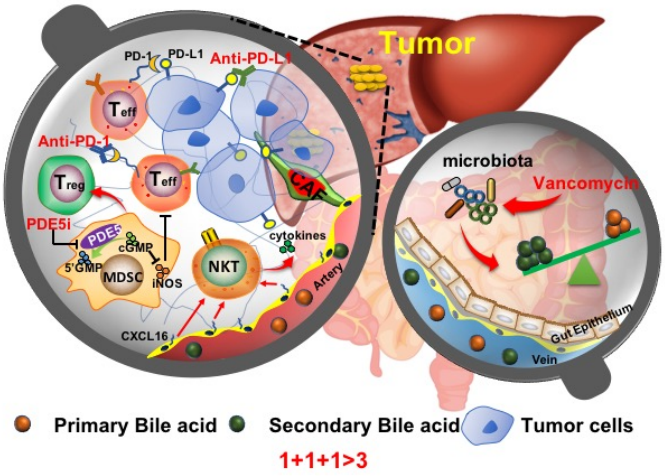


Figure 5: Concept of the protocol.

1.2.7.1 PDE5 inhibitor and oral vancomycin in combination with nivolumab regimen dose justification

Per the FDA approval for HCC treatment with nivolumab, patients can be treated with 240 mg every 2 weeks or 480 mg every 4 weeks for HCC.

As discussed above, patients with HNSCC received tadalafil 20 mg once a day (20 mg daily) for at least 10 days demonstrated augmentation of general and tumor-specific immunity[70]. However,

in the other study, it seemed that tadalafil immunomodulatory activity was maximized at an intermediate dose (10 mg daily) but not at higher doses (20 mg daily)[73]. The potential mechanism is a possible off-target effect on PDE11 at high dosages that, by increasing intracellular cAMP, may negatively affect antitumor immunity.

Vancomycin is poorly absorbed after oral administration. During multiple dosing of 250 mg every 8 hours for 7 doses, fecal concentrations of vancomycin in volunteers exceeded 100 mg/kg in the majority of samples. No blood concentrations were detected, and urinary recovery did not exceed 0.76%. Oral vancomycin remains the mainstay of therapy for severe infections produced by Clostridium difficile, the most prevalent cause of healthcare-associated infectious diarrhea in developed countries. Its short- and long-term effects on the human intestinal microbiota remain largely unknown, however, 125 mg 4 times daily for 10 days has been used as initial standard dose for non-severe Clostridium difficile infection.

Since there are no previous reports regarding this combination therapy in cancer patients, we will choose the FDA approved standard dose and previous reported single agent dose as initial one. The dose may need titrate for the future study after the complicate analysis after this trial.

2 ELIBILITY ASSESSMENT AND ENROLLMENT

2.1 Eligibility Criteria

2.1.1 Inclusion Criteria

- Patients must have
  - histopathological confirmation of HCC (Cohort 1)

    OR

  - histopathological confirmation of carcinoma in the setting of clinical and radiological characteristics which, together with the pathology, are highly suggestive of a diagnosis of HCC (Cohort 1)

    OR

  - histopathological confirmation of advanced colorectal or pancreatic malignancy with liver involvement as dominant site of metastasis (Per multidiscipline tumor board review and approval) (Cohort 2).
- Patients must have disease that is not amenable to potentially curative resection, transplantation or ablation.
- Patients must have progressed on, been intolerant to, or refused prior sorafenib/lenvatinib and/or atezolizumab/bevacizumab therapy (Cohort 1 only).
- Subjects must have progressed on or after standard systemic chemotherapy (at least one line of chemotherapy for patients with liver metastasis from PDAC, at least two lines of chemotherapy for patients with liver metastasis from CRC) (Cohort 2 only).
- Patients must have evaluable or measurable disease per RECIST 1.1 (See Section **6.3.1**).
- Patients must have lesion accessible for biopsy and be willing to undergo pre- and post-treatment biopsies.

- ECOG performance status of 0 to 1 (**Appendix A**)
- If liver cirrhosis is present, patient must have a Child-Pugh score ≤7 (**Appendix F**)
- Active chronic HBV infected subjects must be on antivirals and have HBV DNA <100IU/mL. HCV infected subjects can be enrolled with close HCV RNA level monitoring.
- Age ≥ 18 years. Because no dosing or adverse event data are currently available on the use of nivolumab in combination with tadalafil and vancomycin in patients <18 years of age, children are excluded from this study, but will be eligible for future pediatric trials
- Adequate hematological function defined by:
  - white blood cell (WBC) count ≥ 3×10^9/L
  - absolute neutrophil count (ANC) ≥ 1.5×10^9/L,
  - lymphocyte count ≥ 0.5×10^9/L,
  - platelet count ≥ 60×10^9/L, and
  - Hgb ≥9 g/ dL (more than 48 hours post-completion of blood transfusion))
- Adequate hepatic function defined by:
  - a total bilirubin level ≤ 1.5 × ULN,
  - an AST level <5×ULN,
  - an ALT level <5 ×ULN.
- Adequate renal function defined by:
  - Creatinine clearance (CrCl) ≥50 mL/min/1.73 m^2 by 24 hours urine collection or as predicted by the Cockcroft-Gault formula:

$$\text{CrCl} = \frac{(140 - \text{age (y)}) \times (\text{weight in kg}) \times (0.85, \text{if female}) \times 1.73\ m^2}{72 \times \text{Serum Creatinine (mg/dL)} \times \text{pt.'s BSA } (m^2)}$$

- The effects of nivolumab on the developing human fetus are unknown. For this reason, women of child-bearing potential and men must agree to use adequate contraception (hormonal or barrier method of birth control; abstinence) prior to study entry and for the duration of study participation and up to 5 months (women) and 7 months (men) after the last dose of the drug. Should a woman become pregnant or suspect she is pregnant while she or her partner is participating in this study, she should inform her treating physician immediately.
- Patients with a history of cardiovascular disease may be enrolled per cardiology consultation and approval with echocardiogram and troponin level in normal range at the time of enrollment.
- Patient must be able to understand and willing to sign a written informed consent document.

2.1.2 Exclusion Criteria

- Patients who have had standard-of-care anti-cancer therapy or therapy with investigational agents (e.g. chemotherapy, immunotherapy, endocrine therapy, targeted therapy, biologic therapy, tumor embolization, monoclonal antibodies or other investigation agents), large field radiotherapy, or major surgery within 4 weeks prior to enrollment.
- Therapy with antibiotics within 30 days prior to enrollment.
- Therapy with nitrates, alpha-blockers, or cytochrome P450 (CYP3A4) inhibitors within 7-days prior to enrollment and for whom stopping is unsafe and/or a safe substitute is not medically recommended. Use of PDE5 inhibitors such as vardenafil (Levitra®), tadalafil (Cialis®), and sildenafil citrate (Viagra®) ≤15-days prior to enrollment.
- The patient must not be currently on a corticosteroid dose greater than physiologic replacement dosing defined as 10 mg of cortisone per day or its equivalent.
- For PDAC patients with liver metastases, primary PDAC has not been resected (unless the primary is in the tail of the pancreas).
- Patients with known brain metastases will be excluded from this clinical trial because of their poor prognosis and because they often develop progressive neurologic dysfunction that would confound the evaluation of neurologic and other adverse events.
- Have signs of liver failure, e.g. clinically significant ascites, encephalopathy, or variceal bleeding within 6 months prior to enrollment.
- Prior major liver resection: remnant liver <50% of the initial liver volume. Patients with a biliary stent can be included.
- Patients with active autoimmune disease or history of autoimmune disease that might recur, which may affect vital organ function or require immune suppressive treatment including systemic corticosteroids. These include but are not limited to patients with a history of immune related neurologic disease, multiple sclerosis, autoimmune (demyelinating) neuropathy, Guillain-Barre syndrome or CIDP, myasthenia gravis; systemic autoimmune disease such as SLE, connective tissue diseases, scleroderma, inflammatory bowel disease (IBD), Crohn's, ulcerative colitis, hepatitis; and patients with a history of toxic epidermal necrolysis (TEN), Stevens-Johnson syndrome, or phospholipid syndrome. Such diseases should be excluded because of the risk of recurrence or exacerbation of disease.

  Of note, patients with vitiligo, endocrine deficiencies including thyroiditis managed with replacement hormones including physiologic corticosteroids are eligible. Patients with rheumatoid arthritis and other arthropathies, Sjogren's syndrome and psoriasis controlled with topical medication and patients with positive serology, such as antinuclear antibodies (ANA), anti-thyroid antibodies should be evaluated for the presence of target organ involvement and potential need for systemic treatment but should otherwise be eligible.
- Have history of idiopathic pulmonary fibrosis (including bronchiolitis obliterans with organizing pneumonia) or evidence of active pneumonitis on screening chest CT scan.

- 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.
- Patients with myocardial infarction or myocarditis within 12 months prior to enrollment.
- History of severe or unstable cerebrovascular disease.
- Sustained hypotension (<90/50 mmHg) or uncontrolled hypertension (>160/100 mmHg)
- Stroke within 6 months prior to enrollment.
- HIV-positive patients are excluded because HIV causes complicated immune deficiency and study treatment can possess more risks for these patients.
- Have had prior transplant of any kind.
- Have ascites.
- History of allergic reactions attributed to compounds of similar chemical or biologic composition to nivolumab, tadalafil or vancomycin.
- History of severe hypersensitivity reaction to any monoclonal antibody.
- Prior invasive malignancy (except non-melanomatous skin cancer) unless disease free for a minimum of 3 years prior to enrollment.
- Pregnant women are excluded from this study because nivolumab's potential for teratogenic or abortifacient effects is unknown. Because there is an unknown but potential risk for adverse events in nursing infants secondary to treatment of the mother with nivolumab, breastfeeding should be discontinued if the mother is treated with nivolumab

2.1.3 Recruitment Strategies

The study will be posted on the CCR website and on clinicaltrials.gov. NIH social media platforms may also be used to notify potential subjects of the study. Outside providers and colleagues may directly refer patients for screening into this study.

2.2 Screening Evaluation

2.2.1 Screening activities performed after a consent for screening has been signed

The following activities will be performed only after the subject has signed the consent for study #01-C-0129 on which screening activities will be performed. Assessments performed at outside facilities or on another NIH protocol within the timeframes below may also be used to determine eligibility once a patient has signed the consent.

Studies should be done within 28 days prior to enrollment unless otherwise noted below.

- Complete Medical History and Physical Evaluation (including height, weight, vital signs, and ECOG performance status).
- Laboratory Evaluation
    - Hematological Profile: CBC with differential and platelet count.

- Biochemical Profile: electrolytes, BUN, creatinine, AST, ALT, total and direct bilirubin, calcium, phosphorus, albumin, magnesium.
- PT, INR, PTT, fibrinogen (if clinically indicated)
- 24-hour urine collection (if creatinine clearance is tested this way)
- ACTH
- morning cortisol
- Thyroid tests TSH, T3, T4
- Uric acid, amylase and lipase
- Tumor marker CEA, AFP, CA19-9
- Serum or urine pregnancy test for female participants of childbearing potential (within 2 weeks prior to enrollment).
- HIV, Hepatitis B and C serology and/or viral load
- TB testing (if clinically indicated)
- Troponin I (if clinically indicated)
- Urinalysis

- Cardiology Consultation (if clinically indicated)
- Echocardiogram (if clinically indicated)
- EKG
- CT scan of chest, abdomen and pelvis (or MRI of abdomen if clinically indicated)
- Documentation of histologic cancer confirmation (at any time point prior to enrollment; required from each participant to confirm diagnosis). If there is no available documentation, biopsy will be performed to confirm the diagnosis.

2.3 Participant Registration and Status Update Procedures

Registration and status updates (e.g. when a participant is taken off protocol therapy and when a participant is taken off-study) will take place per CCR SOP ADCR-2, CCR Participant Registration & Status Updates found here.

2.3.1 Treatment Assignment Procedures (for registration purposes only)

Cohorts

Number	Name	Description
1	Cohort 1	Patients with HCC or patients with carcinoma highly suggestive of a diagnosis of HCC
2	Cohort 2	Patients with liver metastases from CRC or PDAC

Arms

Number	Name	Description
1	Arm 1	Nivolumab, tadalafil and oral vancomycin

Treatment Assignment

Patients in Cohort 1 and 2 will be directly assigned to treatment in Arm 1

2.4 Baseline Evaluation

Tests performed during screening do not need to be repeated if done in designated time frame prior to start of study treatment.

Within 28 days prior to first dose:

- CT scan of chest, abdomen and pelvis (or MRI of abdomen if clinically indicated)
- HLA subtype- HLA-A, B, C and sequenced based HLA-A-02* (any time prior to treatment)
- Baseline research biopsy (does not need to be repeated if biopsy was done during screening)

Within 3 days prior to first dose:

- Concomitant medications
- Baseline signs and symptoms evaluation
- Stool collection or rectal swab for bile acid
- Research blood for mixed meal test for bile acid and for whole exome panel

3 STUDY IMPLEMENTATION

3.1 Study Design

The proposed study is an open label, single-arm study of nivolumab, oral vancomycin and tadalafil in patients with refractory primary HCC or liver dominant metastatic cancer from CRC or PDAC, see **Table 9** and **Schema 1**.

Treatment will be delivered in cycles consisting of 4 weeks (+/- 3 days).

Treatment will continue until off treatment criteria are met (see Section **3.6.1**).

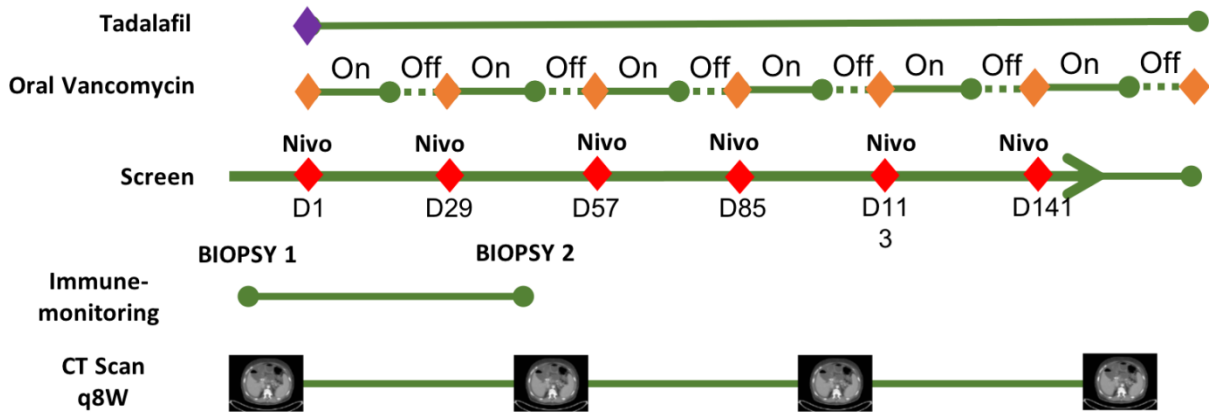
If one or two study drugs permanently discontinued because of toxicity, treatment with the other drug/s will continue.

Patients will be monitored every 8 (+/- 1) weeks with imaging per **Study Calendar**.

Patients will have two mandatory research biopsies: at baseline and anytime during week 3 of Cycle 2 while patient is still on oral vancomycin. (If patient's disease progresses before week 3 of cycle 2, the post-treatment biopsy may be performed per PI discretion at the time of progression).

Table 9: Treatment regimen		
Nivolumab	**Tadalafil**	**Oral Vancomycin**

Table 9: Treatment regimen		
480 mg intravenously every 4 weeks	10 mg PO daily	125 mg every 6-hour PO from week 1 to week 3, week 4 off, 500 mg total daily



Schema 1: Treatment and monitoring schedule

3.2 Drug Administration

3.2.1 Nivolumab Administration

Nivolumab will be given on Day 1 of every cycle at a flat dose of 480 mg. Nivolumab will be administered over approximately 30 to 60-minutes via IV infusion (See section **14.1.5**).

3.2.2 Tadalafil Administration

Tadalafil will be given orally at 10 mg dose daily.

Patients will begin tadalafil dosing on the Day 1 of Cycle 1 and will continue to receive tadalafil on every day of every cycle.

In case of missing dose or vomiting after taking tadalafil, patients will be instructed not to increase next day dosage and not to repeat taking tadalafil on this day.

Patients will complete and return Patient's Diary (**Appendix B).**

Missing of 20% of the doses for any given cycle is allowed.

3.2.3 Vancomycin Administration

Vancomycin will be given orally 125 mg every 6 hours at 500 mg total daily dose.

Patients will begin vancomycin dosing on the Day 1 of Cycle 1 and will continue to receive vancomycin from week 1 to week 3 with week 4 off treatment on every cycle.

If vancomycin administered earlier than scheduled or missed, dose can be taken in the time period of +/- 2 hours of scheduled time. In case of vomiting after taking vancomycin, next administration of vancomycin should proceed as scheduled.

Patients will complete and return Patient's Diary (**Appendix B**).

Missing of 20% of the doses for any given cycle is allowed.

3.2.4 Sequence and Monitoring of Dose Administration

On day 1 of every cycle tadalafil and vancomycin can be administered at any time prior to or after nivolumab injection.

Vital signs will be collected within 1 hour before nivolumab infusions, at least once during the infusions, and within 30 minutes after the completion of the infusion.

In the event of a ≤Grade 2 infusion-related reaction, the infusion rate of study drug may be decreased by 50% or interrupted until resolution of the event and re-initiated at 50% of the initial rate until completion of the infusion. For patients with a ≤Grade 2 infusion related reaction, subsequent infusions must be administered at 50% of the initial rate. Acetaminophen and/or an antihistamine (e.g. diphenhydramine) or equivalent medications per institutional standard may be administered at the discretion of the investigator. If the infusion related reaction is ≥Grade 3 or higher in severity, study drug will be discontinued. See also **Appendix D**

As with any antibody, allergic reactions to dose administration are possible. Therefore, appropriate drugs and medical equipment to treat acute anaphylactic reactions must be immediately available, and study personnel must be trained to recognize and treat anaphylaxis, as per local institutional guidelines.

3.3 Dose Delay or Modifications

3.3.1 General Guidance

When, at the beginning of a treatment cycle, treatment delay related to either tadalafil or vancomycin treatment alone is indicated, treatment with nivolumab may not be delayed. If treatment delay is due to nivolumab only, tadalafil and vancomycin treatment may be continued without delay.

If, in the opinion of the investigator, a toxicity is considered to be due solely to one drug (e.g. flushing by tadalafil, dysgeusia by oral vancomycin), the dose of the other drugs does not require modification.

3.3.2 Nivolumab

Dose modifications of nivolumab may be required in the event of treatment-related toxicity. General guidelines regarding dose modification are provided in **Appendix C**.

3.3.2.1 Adverse Events of Special Interest for Nivolumab

An adverse event of special interest (AESI) is one of scientific and medical interest specific to understanding of the Investigational Product and may require close monitoring. An AESI may be serious or non-serious.

AESIs for nivolumab include but are not limited to events with a potential inflammatory or immune-mediated mechanism and which may require more frequent monitoring and/or interventions such as steroids, immunosuppressants and/or hormone replacement therapy. These AESIs are being closely monitored in clinical studies with nivolumab. An immune-mediate adverse event (imAE) is defined as an adverse event that is associated with drug exposure and is consistent with an immune-mediated mechanism of action and where there is no clear alternate

aetiology. Serologic, immunologic, and histologic (biopsy) data, as appropriate, should be used to support an imAE diagnosis. Appropriate efforts should be made to rule out neoplastic, infectious, metabolic, toxin, or other etiologic causes of the imAE.

AESIs observed with nivolumab include:

- Diarrhea / Colitis
- Pneumonitis / ILD
- ALT/AST increases / hepatitis / hepatotoxicity
- Neuropathy / neuromuscular toxicity (e.g. Guillain-Barré, and myasthenia gravis)
- Endocrinopathies (i.e. events of hypophysitis, hypopituitarism adrenal insufficiency, diabetes insipidus, hyper- and hypothyroidism and type I diabetes mellitus)
- Rash / Dermatitis
- Nephritis / Blood creatinine increases
- Pancreatitis (or labs suggestive of pancreatitis - increased serum lipase, increased serum amylase)
- Other inflammatory responses that are rare with a potential immune-mediated etiology include, but are not limited to, myocarditis, pericarditis, and uveitis.

In addition, infusion-related reactions and hypersensitivity/anaphylactic reactions with a different underlying pharmacological etiology are also considered AESIs.

More specific guidelines for AESIs evaluation and treatment are described in detail in the Dosing Modification and Toxicity Management Guidelines (see **Appendix C**).

If new or worsening pulmonary symptoms (e.g. dyspnea) or radiological abnormality suggestive of pneumonitis/interstitial lung disease is observed, toxicity management as described in detail in the Dosing Modification and Toxicity Management Guidelines (see **Appendix C**) will be applied. It is strongly recommended to perform a full diagnostic workup, to exclude alternative causes such as lymphangitic carcinomatosis, infection, allergy, cardiogenic edema, or pulmonary hemorrhage. In the presence of confirmatory HRCT scans where other causes of respiratory symptoms have been excluded, a diagnosis of pneumonitis (ILD) should be considered and the Dosing Modification and Toxicity Management Guidelines should be followed.

For nivolumab, AESIs will comprise the following:

- Pneumonitis: AEs of pneumonitis are also of interest, as pneumonitis has been observed with use of anti-PD-1 mAbs (but not with anti-PD-L1 mAbs). Initial work-up should include a high-resolution CT scan, ruling out infection, and pulse oximetry. Pulmonary consultation is highly recommended. Guidelines for the management of patients with immune-mediated AEs (imAEs) including pneumonitis are provided in **Appendix C**.
- Pneumonitis (ILD): The following assessments, and additional assessments if required, will be performed to enhance the investigation and diagnosis of potential cases of pneumonitis. The results of the assessment will be collected.

- Physical examination: Signs and symptoms (cough, shortness of breath and pyrexia, etc.) including auscultation for lung field will be assessed.
- SpO2: Saturation of peripheral oxygen (SpO2)
- Other items: When pneumonitis (ILD) is suspected during study treatment, the following markers should be measured where possible:
    - ILD Markers (KL-6, SP-D) and β-D-glucan

- Tumor markers: Particular tumor markers which are related to disease progression.
- Additional Clinical chemistry: CRP, LDH
- Infusion reactions: AEs of infusion reactions (also termed infusion-related reactions) are of special interest to BMS and are defined, for the purpose of this protocol, as all AEs occurring from the start of nivolumab infusion up to 48 hours after the infusion start time. For all infusion reactions, SAEs should be reported to BMS Patient safety.
- Hypersensitivity reactions: Hypersensitivity reactions as well as infusion-related reactions have been reported with anti PD-L1 and anti-PD-1 therapy [90]. As with the administration of any foreign protein and/or other biologic agents, reactions following the infusion of mAbs can be caused by various mechanisms, including acute anaphylactic (IgE-mediated) and anaphylactoid reactions against the mAbs and serum sickness. Acute allergic reactions may occur, may be severe, and may result in death. Acute allergic reactions may include hypotension, dyspnea, cyanosis, respiratory failure, urticaria, pruritus, angioedema, hypotonia, arthralgia, bronchospasm, wheeze, cough, dizziness, fatigue, headache, hypertension, myalgia, vomiting, and unresponsiveness. Guidelines for the management of patients with hypersensitivity (including anaphylactic reaction) and infusion-related reactions are provided in **Appendix D**.
- Hepatic function abnormalities (hepatotoxicity): nivolumab induced liver injury is defined as concurrent ALT ≥10 × ULN AND total bilirubin ≥2 × ULN or baseline value (if elevated total bilirubin at study entry), AND no other immediately apparent possible causes of ALT elevation and hyperbilirubinemia, including, but not limited to, tumor progression, acute viral hepatitis, cholestasis, pre-existing hepatic disease or the administration of other drug(s), herbal medications and substance known to be hepatotoxic. Guidelines for management of patients with hepatic function abnormality are provided in **Appendix C**.
- Gastrointestinal disorders: Diarrhea/colitis is one of the most commonly observed treatment emergent SAE when nivolumab is used. In rare cases, colon perforation may occur that requires surgery (colectomy) or can lead to a fatal outcome if not properly managed. Guidelines on management of diarrhea and colitis in patients receiving nivolumab are provided in **Appendix C**.
- Endocrine disorders: Immune-mediated endocrinopathies include hypophysitis, adrenal insufficiency, and hyper- and hypothyroidism. Guidelines for the management of patients with immune-mediated endocrine events are provided in **Appendix C**.
- Pancreatic disorders: Immune-mediated pancreatitis includes autoimmune pancreatitis, and lipase and amylase elevation. Guidelines for the management of patients with immune-mediated pancreatic disorders are provided in **Appendix C**.

- Neurotoxicity: Immune-mediated nervous system events include encephalitis, peripheral motor and sensory neuropathies, Guillain-Barré, and myasthenia gravis. Guidelines for the management of patients with immune-mediated neurotoxic events are provided in **Appendix C**.
- Nephritis: Consult with Nephrologist. Monitor for signs and symptoms that may be related to changes in renal function (e.g. routine urinalysis, elevated serum BUN and creatinine, decreased creatinine clearance, electrolyte imbalance, decrease in urine output, proteinuria, etc.)
- Patients should be thoroughly evaluated to rule out any alternative etiology (e.g. disease progression, infections etc.)
- Steroids should be considered in the absence of clear alternative etiology even for low grade events (Grade 2), in order to prevent potential progression to higher grade event. Guidelines for the management of patients with immune-mediated neurotoxic events are provided in **Appendix C**.

3.3.2.2 Nivolumab Dose Delay Criteria

Dose delay criteria apply for all nivolumab-related adverse events. Treatment delay up to 8 weeks from the last dose is allowed. If treatment is not resumed during 8 weeks, patients will be taken off nivolumab treatment.

After nivolumab discontinuation, the patient can continue with tadalafil and vancomycin treatment.

Nivolumab administration should be delayed for the following:

- Any grade ≥ 2 non-skin, drug-related adverse event, with the following exceptions: Grade 2 drug-related fatigue or laboratory abnormalities do not require a treatment delay
- Any grade 3 skin, drug-related adverse event, any grade 3 drug-related laboratory abnormality, with the following exceptions for asymptomatic amylase or lipase, ALT, or AST:
    - Grade 3 amylase or lipase abnormalities that are not associated with symptoms or clinical manifestations of pancreatitis do not require a dose delay.
    - If a subject has a baseline AST or ALT that is within normal limits, delay dosing for drug-related Grade ≥ 2 toxicity (2-grade shift).
    - If a subject has baseline AST or ALT within the grade 1 toxicity range, delay dosing for drug-related Grade ≥ 3 toxicity (2-grade shift).
    - If a subject has baseline AST or ALT within the Grade 2 toxicity range, delay dosing for drug-related increase in AST or ALT at 2x baseline value or when AST or ALT is 8x ULN (whichever is lower).
    - Any adverse event, laboratory abnormality, or intercurrent illness which, in the judgment of the investigator, warrants interrupting the dose of study medication. subjects who require delay of nivolumab should be re-evaluated weekly or more frequently if clinically indicated. It is recommended to monitor elevations in AST or ALT approximately every 3 days till levels peak or begin to decline. Nivolumab

dosing can be resumed when re-treatment criteria are met. Tumor assessments for all subjects should continue as per protocol even if study drug dosing is delayed.

Resumption after delay is discussed in section **3.3.2.4** below.

3.3.2.3 Nivolumab Dose Discontinuation Criteria

Nivolumab administration should be permanently discontinued if at least one of the following drug-related adverse event(s) occurs:

- Any ≥ Grade 2 drug-related uveitis, eye pain, or reduction of visual acuity 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 adverse event lasting > 7 days, with the following exceptions:
  - Grade 3 drug-related uveitis, pneumonitis, bronchospasm, neurological toxicity, hypersensitivity reaction, or infusion reaction of any duration requires discontinuation.
  - Grade 3 drug-related endocrinopathies adequately controlled with only physiologic hormone replacement do not require discontinuation.
  - Grade 3 drug-related laboratory abnormalities do not require treatment discontinuation except:
  - Grade 3 drug-related thrombocytopenia > 7 days or associated with bleeding requires discontinuation
  - Any drug-related liver function test (LFT) abnormality that meets the following criteria require discontinuation:
    - AST or ALT > 10 x ULN for > 2 weeks,
    - AST or ALT > 15 x ULN irrespective of duration,
    - T. bilirubin > 8 x ULN irrespective of duration for subjects with elevated bilirubin at study entry or > 5 x ULN for those with normal T bilirubin at entry,
    - Concurrent AST or ALT > 3 x ULN and T. bilirubin > 5 x ULN for subjects entering treatment with a normal bilirubin and up to 8 x ULN for subjects with elevated bilirubin
- Any drug-related Grade 4 drug-related adverse event or laboratory abnormality, except for the following events which do not require discontinuation:
  - Grade 4 neutropenia < 7 days
  - Grade 4 lymphopenia or leukopenia
  - Grade 4 amylase or lipase abnormalities that are not associated with symptoms or clinical manifestations of pancreatitis.

- 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
- For Grade 4 endocrinopathy adverse events such as hyper- or hypothyroidisms, or glucose intolerance, which resolve or are adequately controlled with physiologic hormone replacement (steroids, thyroid hormones) or glucose controlling agents, respectively

- Dosing delays to allow for prolonged steroid tapers to manage drug-related adverse events are allowed. Tumor assessments should continue as per protocol even if dosing is delayed. Periodic study visits to assess safety and laboratory studies should also continue every 6 weeks or more frequently if clinically indicated during such dosing delays.
- Dosing delays lasting > 6 weeks from the previous dose that occur for non-drug-related reasons may be allowed. Tumor assessments should continue as per protocol even if dosing is delayed. Periodic study visits to assess safety and laboratory studies should also continue every 6 weeks or more frequently if clinically indicated during such dosing delays.
- Any adverse event, laboratory abnormality or intercurrent illness which, in the judgment of the Investigator, presents a substantial clinical risk to the subject with continued nivolumab dosing.

3.3.2.4 Nivolumab Dose Resumption Criteria

Subjects may resume treatment with study drug when the drug-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 toxicity.
- Subjects with baseline Grade 1 AST, ALT, or total bilirubin who require dose delays for reasons other than a drug-related hepatic event may resume treatment in the presence of Grade 2 AST, ALT, or total bilirubin.
- Subjects who require dose delays for drug-related increased AST, ALT, or bilirubin may resume treatment when hepatic parameters are at baseline or Grade 1.
- Subjects with AST, ALT or bilirubin values meeting discontinuation parameters (Section **3.3.2.3**) should have treatment permanently discontinued.
- Drug-related pulmonary toxicity, diarrhea, or colitis must have resolved to baseline before treatment is resumed.
- Drug-related endocrinopathies adequately controlled with only physiologic hormone replacement may resume treatment.
- Subjects who delay study treatment due to any Grade ≥ 3 amylase or lipase abnormality that is not associated with symptoms or clinical manifestations of pancreatitis, and that is assessed by the investigator not to be related to nivolumab, may resume nivolumab when the amylase or lipase abnormality has resolved to Grade < 3.

Resuming treatment after a delay of up to 8 weeks from the previous dose is permitted with the following exceptions:

- Dosing delays to allow for prolonged steroid tapers to manage drug-related adverse events are allowed. Tumor imaging assessments should continue as per protocol even if dosing is interrupted.
- Dosing delays > 8 weeks from the last dose that occur for non-drug-related reasons may be allowed.

3.3.3 Tadalafil

If patient's CrCl is reduced to 30 - 50 mL/minute (as measured by the Cockcroft-Gault formula), the dose of tadalafil will be reduced to 5 mg daily.

If patient's CrCl is reduced less than 30 mL/minute, tadalafil will be held until the renal function improved:

- to 30 - 50 mL/minute, tadalafil will resume at 5 mg daily;
- to > 50 mL/minute, tadalafil will resume at 10 mg daily;

If renal function is not improved after 4 weeks, tadalafil will be permanently discontinued.

If patient experiences priapism, hearing loss, vision change, or hypotension that is associated with the use of tadalafil, tadalafil will be permanently discontinued.

If tadalafil is permanently discontinued, treatment with nivolumab and vancomycin will continue.

3.3.4 Oral Vancomycin

Vancomycin will be held temporally for adverse events ≥ grade 1 confirmed by PI as associated with oral vancomycin:

- nephrotoxicity
- ototoxicity
- uncontrolled nausea, diarrhea or abdominal pain.

Vancomycin can be resumed at full dose after toxicity resolves to baseline. If toxicity is not resolved for 4 weeks, vancomycin will be permanently discontinued.

Vancomycin will be discontinued if patient develops anaphylactoid reactions that is confirmed to be associated with oral vancomycin.

If vancomycin is permanently discontinued, treatment with nivolumab and tadalafil will continue.

3.4 Study Calendar

	Screening[1]	Baseline[1]	All Cycles Day 1 [1]	28 Days Safety FU[11,13]	Long Term FU[12, 13]
Nivolumab[2]			X		
Tadalafil[3]			X		
Oral vancomycin[4]			X		
Medical History	X				
Height	X				
Cardiology consult [5]	X				
Echocardiogram [5]	X				
Histologic confirmation of disease	X				
TB testing [5]	X				
24-hour urine (if creatinine clearance is tested this way)	X				
Troponin I [5]	X				
HIV, Hepatitis B and C serology and/or viral load [6]	X		X		
EKG	X		X		
Physical exam, weight and ECOG	X		X	X	X
Vital Signs	X		X[7]	X	
CBC w/differential, Platelets	X		X	X	X
Biochemical profile[8]	X		X	X	X
Radiologic Evaluation[9]	X	X	X		
HLA		X			
Urinalysis	X				
Thyroid tests TSH, T3, T4	X		X	X	
Uric acid, amylase and lipase	X		X	X	
ACTH and morning cortisol	X				
PT, INR, PTT, fibrinogen	X[5]		X	X	
Serum or urine pregnancy test	X		X		

	Screening[1]	**Baseline[1]**	**All Cycles Day 1 [1]**	**28 Days Safety FU[11,13]**	**Long Term FU[12, 13]**
Tumor marker CEA, AFP, CA19-9	X		X	X	
Concomitant medications	X	X	X		
Adverse event evaluation			X	X	X
Baseline signs and symptoms		X			
Tumor biopsy[10]		X	X		
Research blood for Immune monitoring[14]			X		
Research blood for cytokines [14]			X		
Research blood for tadalafil PK[15]			X		
Research blood for mixed meal test for bile acid[16]		X	X		
Research blood for vancomycin level [17]			X		
Research blood for TCR beta sequencing			X		
Research blood for whole exome panel		X			
Stool collection or rectal swab for bile acid[19]		X	X		
Stool collection or rectal swab for Vancomycin-resistant enterococci (VRE) development [18]			X		
Stool collection or rectal swab for microbial DNA sequences [18]			X		
Nutrition consult and completion of food diary (**Appendix H**) [19]		X	X		
Phone call or e-mail for survival every 6 month					X

[1] Baseline and C1D1 evaluations do not need to be repeated if performed at screening or baseline in designated time frame. All evaluations will be done within 72 hours before treatment initiation on Day 1 of every cycle. If treatment does not start within 28 days after enrollment, screening evaluations will be repeated.

[2] 480 mg of nivolumab via IV infusion on Day 1 of each cycle.

[3] 10 mg of tadalafil PO on every day of every cycle.

[4] 125 mg of vancomycin every 6 hours (500 mg daily dose) from Day 1 to day 21 of every cycle.

[5] if clinically indicated.

[6] If screening serology results indicate potential HBV or HCV infection, viral load will be tested before every nivolumab infusion

[7] For vital signs see Section **3.2.**

[8] Biochemical Profile: electrolytes, BUN, creatinine, AST, ALT, total and direct bilirubin, calcium, phosphorus, albumin, magnesium.

[9] CT scan of chest, abdomen and pelvis (or MRI of abdomen if clinically indicated) every 8 (+/- 1) weeks after start of study therapy.

[10] Mandatory tumor biopsies will be performed at baseline and any day during week 3 of cycle 2 while patient is taking oral vancomycin. If patient's disease progresses before C3D1, the post-treatment biopsy may be performed per PI discretion at the time of progression.

[11] +/- 1 week

[12] Follow up visits are planned to be performed at 60 (+/- 14 days) and 90 (+/- 14 days) days after treatment discontinuation to evaluate patient's safety. After this visit, subjects will be followed every 6 months (± 1 month) for survival by phone call or e-mail.

[13] If subjects are not willing to come to NIH for FU visits, they will be contacted by phone call or e-mail for survival and adverse events.

[14] On Day 1 of cycles 1-4, then every 12 weeks until PD.

[15] Before patient takes tadalafil on day 1 of every cycle.

[16] At baseline and C1D10, an IV line will be placed and a mixed meal test (Boost Plus Vanilla) will be performed with collection of blood samples at 0 (+/- 10), 30 (+/- 10), 60 (+/- 15), 90 (+/- 20), 120 (+/- 20), 150 (+/- 20), and 180 (+/- 30) min for bile acid analysis. T=0 is the time patient ingests mixed meal. If patient is still on the protocol treatment after 6 months, patient will repeat the mixed meal test on day 10 of whatever cycle the patient is on.

[17] Before patient takes vancomycin on day 1 of every cycle.

[18] On day 1 of every cycle.

[19] At baseline and C1D10. If patient is still on the protocol treatment after 6 months, this will be performed on day 10 of whatever cycle patient is on.

3.5 Costs and Compensation

3.5.1 Costs

NIH does not bill health insurance companies or participants for any research or related clinical care that participants receive at the NIH Clinical Center. If some tests and procedures performed outside the NIH Clinical Center, participants may have to pay for these costs if they are not covered by insurance company. Medicines that are not part of the study treatment will not be provided or paid for by the NIH Clinical Center.

3.5.2 Compensation

No compensation is offered on this study.

3.5.3 Reimbursement

The NCI will cover the costs of some expenses associated with protocol participation. Some of these costs may be paid directly by the NIH and some may be reimbursed to the participant/guardian as appropriate. The amount and form of these payments are determined by the NCI Travel and Lodging Reimbursement Policy.

3.6 Criteria for Removal from Protocol Therapy and Off Study Criteria

Prior to removal from study, efforts must be made to have all subjects complete a safety visit approximately 90 days following the last dose of study therapy

3.6.1 Criteria for Removal from Protocol Therapy

- Participant requests to be withdrawn from active therapy
- Unacceptable Toxicity as defined in the Section **3.3** and **Appendix C**
- Positive pregnancy test or intent to become pregnant
- Investigator discretion
- Initiation of therapy that prevents further administration of study treatment (See Section **4**)
- Progressive Disease. NOTE: While RECIST PD will be noted and recorded the immune-related RECIST criteria (**Appendix E**) will be applied to determine discontinuation of study treatment.
- Intercurrent illness that prevents further administration of treatment

3.6.2 Off Study Criteria

- Lost to follow-up
- Death
- Participant requests to be withdrawn from study
- Investigator discretion
- PI decision to end the study

3.6.3 Lost to Follow-up

A participant will be considered lost to follow-up if he or she fails to return for the 60 or 90 day scheduled safety visits post-treatment or is unable to be contacted by the study site staff.
The following actions must be taken if a participant fails to return to the clinic for a required study visit:

- The site will attempt to contact the participant and reschedule the missed visit within 2 weeks and counsel the participant on the importance of maintaining the assigned visit schedule and ascertain if the participant wishes to and/or should continue in the study.
- Before a participant is deemed lost to follow-up, the investigator or designee will make every effort to regain contact with the participant (where possible, 3 telephone calls and, if necessary, an IRB approved certified letter to the participant's last known mailing address or local equivalent methods). These contact attempts should be documented in the participant's medical record or study file.
- Should the participant continue to be unreachable, he or she will be considered to have withdrawn from the study with a primary reason of lost to follow-up.

4 CONCOMITANT MEDICATIONS/MEASURES

All routine and appropriate supportive care (including blood products) will be provided during this study, as clinically indicated, and in accordance with the standard of care practices. Clinical judgment should be utilized in the treatment of any AE experienced by the patient.

Drugs with laxative properties and herbal or natural remedies for constipation should be used with caution through to 90 days after the last dose of immune checkpoint inhibitors.

Drugs with significant ototoxicity should be used with caution

4.1 Excluded Concomitant Medications

Table 10: Prohibited concomitant medications

Prohibited medication/class of drug:	**Usage:**
Any investigational anticancer therapy other than those under investigation in this study	Should not be given concomitantly whilst the patient is on study treatment
mAbs against CTLA-4, PD-1, or PD-L1 other than those under investigation in this study	Should not be given concomitantly whilst the patient is on study treatment
Any concurrent chemotherapy, radiotherapy, immunotherapy, or biologic or hormonal therapy for cancer treatment other than those under investigation in this study	Should not be given concomitantly whilst the patient is on study treatment. (Concurrent use of hormones for non-cancer-related conditions [e.g. insulin for diabetes and hormone replacement therapy] is acceptable. Local treatment of isolated lesions, excluding target lesions, for palliative intent is acceptable [e.g. by local surgery or radiotherapy])

Table 10: Prohibited concomitant medications	
Prohibited medication/class of drug:	**Usage:**
Immunosuppressive medications including, but not limited to, systemic corticosteroids at doses exceeding 10 mg/day of prednisone or equivalent, methotrexate, azathioprine, and tumor necrosis factor-α blockers	Should not be given concomitantly. (Use of immunosuppressive medications for the management of IP-related AEs, premedication for patients who had infusion reaction, or in patients with contrast allergies is acceptable). In addition, use of inhaled, topical, and intranasal corticosteroids is permitted.
Viagra	Should not be given concomitantly whilst the patient is on study treatment
Microbiome changing drugs, including but not limited to probiotics	Should not be given concomitantly whilst the patient is on study treatment

4.2 Methods of Contraception

4.2.1 Female Patient of Child-bearing Potential

Females of childbearing potential who are sexually active with a non-sterilized male partner must use effective method of contraception (**Table 11**) from the time of screening and must agree to continue using such precautions for 5 months after the last dose of nivolumab + oral vancomycin + tadalafil combination therapy. Non-sterilized male partners of a female patient must use male condom plus spermicide throughout this period. Cessation of birth control after this point should be discussed with a responsible physician. Not engaging in sexual activity for the total duration of the drug treatment and the drug washout period is an acceptable practice; however, periodic abstinence, the rhythm method, and the withdrawal method are not acceptable methods of birth control. Female patients should also refrain from breastfeeding throughout this period.

4.2.2 Male Patients with a Female Partner of Childbearing Potential

Non-sterilized males who are sexually active with a female partner of childbearing potential must use a male condom plus spermicide from screening through 7 months after the last dose of nivolumab + oral vancomycin + tadalafil combination therapy. Engaging in sexual activity is an acceptable practice; however, occasional abstinence, the rhythm method, and the withdrawal method are not acceptable methods of contraception. Male patients should refrain from sperm donation throughout this period.

Female partners (of childbearing potential) of male patients must also use a highly effective method of contraception throughout this period (**Table 11**).

4.2.3 Highly Effective Methods of Contraception.

Highly effective methods of contraception, defined as one that results in a low failure rate (i.e. less than 1% per year) when used consistently and correctly are described in **Table 11**. Note that some contraception methods are not considered highly effective (e.g. male or female condom with or without spermicide; female cap, diaphragm, or sponge with or without spermicide; non-copper containing intrauterine device; progestogen-only oral hormonal contraceptive pills where

inhibition of ovulation is not the primary mode of action [excluding Cerazette/desogestrel which is considered highly effective]; and triphasic combined oral contraceptive pills).

Table 11: Highly Effective methods of contraception (<1% failure rate)

Barrier/Intrauterine methods	Hormonal Methods
• Copper T intrauterine device • Levonorgesterel-releasing intrauterine system (e.g. Mirena®)[a]	• Etonogestrel implants: e.g. Implanon or Norplant • Intravaginal device: e.g. ethinylestradiol and etonogestrel • Medroxyprogesterone injection: e.g. Depo-Provera • Normal and low dose combined oral contraceptive pill • Norelgestromin/ethinylestradiol transdermal system • Cerazette (desogestrel)

[a] This is also considered a hormonal method

4.3 Blood Donation

Subjects should not donate blood while participating in this study, and for 5 months (women) and 7 months (men) after the last dose of nivolumab + oral vancomycin + tadalafil combination therapy.

5 CORRELATIVE STUDIES FOR RESEARCH

5.1 Biospecimen Collection

A description of correlative studies including a brief statement of rationale and processing information is made below.

Test/assay	Sample volume (approx.)	Type of tube[a] / tissue sample	Collection point	Location of specimen analysis[1]
Immune-monitoring by FACS	Blood, 120 mL	EDTA (Purple top tubes)	See Study Calendar **3.4**	Blood Processing Core (BPC)
Serum cytokines by ELISA	2mL	2 green top tube (GTT) or CPT tubes with sodium citrate	See Study Calendar **3.4**	Blood Processing Core (BPC)
Tadalafil PK	4 mL	Sodium heparin green top tube (GTT)	See Study Calendar **3.4**	Blood Processing Core (BPC)

Test/assay	Sample volume (approx.)	Type of tube[a] / tissue sample	Collection point	Location of specimen analysis[1]
Vancomycin Pharmacokinetic Analysis	4 mL	Sodium heparin green top tube (GTT)	See Study Calendar **3.4**	Blood Processing Core (BPC)
Mixed meal test for bile acid levels in blood	5 mL	Sodium heparin green top tube (GTT)	See Study Calendar **3.4**	Blood Processing Core (BPC)
IHC for immune cell infiltration	Tumor sample		At time of biopsies	Laboratory of Pathology
Proteomics	Tumor sample		At time of biopsies	Greten Lab
Metabolomics	Tumor sample		At time of biopsies	Greten Lab
TCRbeta sequencing	10 mL	EDTA (Purple top tube)	See Study Calendar **3.4**	Blood Processing Core (BPC)
TCRbeta sequencing	Tumor sample		At time of biopsies	Greten Lab
RNA seq	Tumor sample		At time of biopsies	Greten Lab
Whole exome/panel	Tumor sample		At time of biopsies	Greten Lab
Whole exome/panel	Blood, 2.5 mL	1 PAX gene DNA tube	See Study Calendar **3.4**	Greten Lab
Fecal bile acid composition	Stool sample or rectal swab	Feccontainer tubes or rectal swabs	See Study Calendar **3.4**	Greten Lab
Vancomycin-resistant enterococci (VRE) development	Stool sample or rectal swab	Feccontainer tubes or rectal swabs	See Study Calendar **3.4**	NIH Department of Laboratory Medicine
DNA sequencing of the microbiome	Stool sample or rectal swab	Feccontainer tubes or rectal	See Study Calendar **3.4**	Greten Lab

a. Please note that tubes and media may be substituted based on availability with the permission of the PI or laboratory investigator.

5.2 Correlative Studies of Blood Samples

Blood samples will be collected at time points indicated in the Study Calendar **3.4**. Blood samples will be initially sent to the Blood Processing Core (BPC) for barcoding and storage. On certain occasions, the blood may also be brought to the Greten lab for processing and analysis

5.2.1 Immune Monitoring

We will analyze PBMC for quantitative and functional changes of effector cells by FACS. The effect on (i) CD4 T cell number and activity, (ii) CD8 T cell number and activity, (iii) NK cell number and activity, (iv) Treg number, (v) MDSC: frequency + functional assay, and (vi) the detection of tumor-associated antigens using tetramer assay. These experiments will be done in Blood Processing Core (BPC).

5.2.2 Serum Cytokines

We will analyze cytokine profile from sera for cytokines and chemokines by ELISA, that are associated with treatment efficacy and outcome. These experiments will be done in Blood Processing Core (BPC).

5.2.3 Tadalafil Pharmacokinetic Analysis

Blood samples for the determination of tadalafil plasma trough levels will be obtained from participating patients at time points indicated in Study Calendar **3.4**. Bioanalytical measurements will be conducted on an ultra HPLC-MS/MS system by the Clinical Pharmacology Program (CPP)

5.2.4 Vancomycin Pharmacokinetic Analysis

Blood samples for the determination of vancomycin plasma trough levels will be obtained from participating patients at time points indicated in Study Calendar **3.4**. Bioanalytical measurements will be conducted on an ultra HPLC-MS/MS system by the Clinical Pharmacology Program (CPP)

5.2.5 Bile Acid Analysis

Fasting blood samples will be drawn. At T = 0 min the participants ingest a liquid meal containing 25% of total daily energy expenditure (Boost Plus Vanilla; 1.5 kcal/mL; 49% carbohydrates, 35% fat and 16% protein). Participants are asked to drink the liquid meal within 10 min. Blood samples for determination of plasma bile acid levels will be drawn at time points indicated in Study Calendar **3.4 and Appendix I**. The blood samples will be collected on baseline and ten days after oral vancomycin is taken during the first treatment cycle. Bile acid profile is measured using liquid chromatography tandem mass spectrometry (LC-MS/MS) as described[91]. Moreover, fecal bile acid composition was determined from rectal swab at baseline and 10 days after oral vancomycin treatment, using gas chromatography (GC). The total amount of primary and secondary bile salts is calculated as the sum of the individually quantified bile salts. AUC will be calculated. These experiments will be done in Dr. Figg lab.

5.2.6 Food Diary for Mixed Meal Study

It has been well demonstrated the diet has great impact on gut microbiota. Therefore, a food diary for a mixed meal study (**Appendix H**) will be used in this study to assess the dietary composition, in addition to nutrition consult. A specialist will evaluate the diary in detail. The completion of this diary will take 15-30 minutes with the time of consultation included.

Patients will have nutrition consult and complete the diary on the same days as bile acid analysis (See Study Calendar **3.4**.)

Non-English-speaking subjects will complete this instrument with help of an interpreter.

5.3 Tumor Studies (Non-genetic Analysis)

Mandatory tumor biopsies will be performed at baseline and anytime during week 3 of Cycle 2 while patient is still on oral vancomycin. If patient disease progresses before week 3 of Cycle 2, post-treatment biopsy may be performed per PI discretion at the time of progression. It is preferred that at **least two core biopsies ≥18 gauge in diameter and ≥1 cm** in length will be obtained.

The use of imaging to facilitate biopsies will be decided by members of the Interventional Radiology team and may include ultrasound, CT scan, PET scan or MRI.

Tumor biopsies are mandatory on this study, however, biopsies and local anesthesia will be administered only if they are of minimal risk to the participant, as determined by the investigators and Interventional Radiology. If these procedures are not considered to be of minimal risk to the participant, they will not be done. Patient will continue treatment.

5.3.1 Tumor Tissue Analysis

5.3.1.1 IHC

IHC will be performed on tumor tissue for assessment of immune cell infiltration (e.g. CD3+ CD4/8 cells, PDL1 etc.). Immunohistochemistry will be performed in the Laboratory of Pathology under the direction of Dr. David Kleiner.

5.3.1.2 Omics Study

Tumor samples will be collected for proteomics and metabolomics studies to investigate the differential expression of protein and metabolite that might be associated with the treatment outcome or efficacy. These experiments will be done in Dr. Greten lab.

5.3.2 Stool Collection and Processing

Stool samples or rectal swabs will be collected by the patients at time points indicated in the Study Calendar **3.4**.

Specimens will be frozen and kept at -20°C until analysis, which will be done in batches.

Samples will be collected for bile acid composition (Section **5.2.5**), Vancomycin-resistant enterococci (VRE) development DNA and sequencing of the microbiome (Section **5.5.3**).

5.3.2.1 Vancomycin-resistant Enterococci (VRE) Development

Stool specimen will be collected to evaluate VRE development given prolonged vancomycin exposure increase the odds of VRE. The protocol will evaluate VRE incidence as one of safety monitor.

5.4 Sample Storage, Tracking and Disposition

Samples will be ordered in CRIS and tracked through a Clinical Trial Data Management system. Should a CRIS screen not be available, the CRIS downtime procedures will be followed. All samples will be sent to Blood Processing Core (BPC) for processing and storage until they are

distributed to the designated place of analysis as described in the protocol. Samples will not be sent outside NIH without appropriate approvals and/or agreements, if required.

All samples will be barcoded, with data entered and stored in the secure databases. These databases create a unique barcode ID for every sample and sample box, which cannot be traced back to patients without database access. The data recorded for each sample includes the patient ID, name, trial name/protocol number, time drawn, cycle time point, dose, material type, as well as box and freezer location. Patient demographics associated with the clinical center patient number are provided in the system. For each sample, there are notes associated with the processing method (delay in sample processing, storage conditions on the ward, etc.).
Barcoded samples are stored in barcoded boxes in a locked freezer at either -20 or -80^{0}C according to stability requirements.
Access to stored clinical samples is restricted. Samples will be stored until requested by a researcher named on the protocol. All requests are monitored and tracked in database. All researchers are required to sign a form stating that the samples are only to be used for research purposes associated with this trial (as per the IRB approved protocol) and that any unused samples must be returned. It is the responsibility of the NCI Principal Investigator to ensure that the samples requested are being used in a manner consistent with IRB approval.

5.4.1 Samples Managed by Dr. Figg's Blood Processing Core (BPC)

5.4.1.1 BPC processing/contact Information

Please e-mail NCIBloodcore@mail.nih.gov at least 24 hours before transporting samples (the Friday before is preferred).

For sample pickup, page 102-11964.

For immediate help, call 240-760-6180 (main blood processing core number) or, if no answer, 240-760-6190 (main clinical pharmacology lab number).

For questions regarding sample processing, contact NCIBloodcore@mail.nih.gov.

5.4.2 Samples Managed by the Laboratory of Dr. Tim Greten

Contact information:

Sophie Wang

Building 10 Rm 3B44

Phone: 240-858-3218

E-mail: sophie.wang@nih.gov

5.4.3 Protocol Completion/Sample Destruction

All specimens obtained in the protocol are used as defined in the protocol. Any specimens that are remaining at the completion of the protocol will be stored in the conditions described in sections above. The study will remain open so long as sample or data analysis continues. Samples from consenting subjects will be stored until they are no longer of scientific value or if a subject withdraws consent for their continued use, at which time they will be destroyed.

If the patient withdraws consent the participant's data will be excluded from future distributions, but data that have already been distributed for approved research use will not be able to be retrieved.

The PI will record any loss or unanticipated destruction of samples as a deviation. Reporting will be per the requirements of section **7.2**.

5.5 Samples for Genetic/Genomic Analysis

5.5.1 TCR Beta Sequencing

PBMC and tumor samples will be collected for TCR beta sequencing performed in Blood Processing Core (BPC) and Dr. Greten' lab to analyze the T cell response.

5.5.2 Whole exome/Panel (tumor/normal) and RNAseq (tumor)

Whole exome will be done on tumor and blood sample. RNAseq will be done on tumor for expressed somatic mutations and neoantigen discovery. Tumor material for these analyses will be sent to the laboratory of Dr Greten. Specifically, the expression of different signaling pathway, including interferon gamma signaling, immune cell functional molecular, e.g. perforin and granzyme, will be investigated

5.5.3 Microbial DNA Sequence

Samples sequenced to study microbial communities will contain human DNA sequences. However, bioinformatic analyses of the sequences will remove sequences containing significant alignment to publicly available human DNA sequences. These data will be coded and stored at the NIH Intramural Sequencing Center (NISC) per standard protocols for intramural sequencing. The resulting sequences will be analyzed. Publicly available human DNA sequences likely do not include all possible DNA sequences and could result in fragments of human DNA sequences being incorporated into the microbial DNA analysis. Microbial DNA sequence from this study will be published and posted on the web. Coded information obtained from sequencing samples will be placed into an open access (public) scientific database such as Genbank: http://www.ncbi.nlm.nih.gov/Genbank/. For example, we will deposit 16S rRNA gene sequences in Genbank. Microbial sequencing is also deposited in the *Short Read Trace Archive* that is a component of Genbank. All of the data will be coded to minimize any risk of identifying an individual. Samples will be reported without any unique identifiable features of the subject.

5.5.4 Management of Results

Subjects will be contacted if a clinically actionable gene variant is discovered. Clinically actionable findings for the purpose of this study are defined as disorders appearing in the American College of Medical Genetics and Genomics recommendations for the return of incidental findings that is current at the time of primary analysis. (A list of current guidelines is maintained on the CCR intranet: https://ccrod.cancer.gov/confluence/display/CCRCRO/Incidental+Findings+Lists. Subjects will be contacted at that time with a request to provide a blood sample to be sent to a CLIA certified laboratory**.**

5.5.5 Genetic Counseling

If the research findings are verified in the CLIA certified lab, the subject will be offered the opportunity to come to NIH (at our expense) to have genetic education and counseling with the NCI Genetics Branch to explain this result. If the subject does not want to come to NIH, a referral to a local genetic healthcare provider will be provided (at their expense). This is the only time during the course of the study that incidental findings will be returned. No interrogations regarding clinically actionable findings will be made after the primary analysis.

6 DATA COLLECTION AND EVALUATION

6.1 Data Collection

The PI will be responsible for overseeing entry of data into a 21 CFR Part 11-compliant data capture system provided by the NCI CCR and ensuring data accuracy, consistency and timeliness. The principal investigator, associate investigators/research nurses and/or a contracted data manager will assist with the data management efforts. Primary and final analyzed data will have identifiers so that research data can be attributed to an individual human subject participant.

All adverse events, including clinically significant abnormal findings on laboratory evaluations, regardless of severity, will be followed until return to baseline or stabilization of event.

Document AEs from the first study intervention, Study Day 1 of cycle 1 through 90 days after the study agent (s) was/were administered. Beyond 90 days after the last intervention, only adverse events which are serious and related to the study intervention need to be recorded.

An abnormal laboratory value will be recorded in the database as an AE only if the laboratory abnormality is characterized by any of the following:

- Results in discontinuation from the study
- Is associated with clinical signs or symptoms
- Requires treatment or any other therapeutic intervention
- Is associated with death or another serious adverse event, including hospitalization.
- Is judged by the Investigator to be of significant clinical impact

If any abnormal laboratory result is considered clinically significant, the investigator will provide details about the action taken with respect to the test drug and about the patient's outcome.

Adverse Events of grade 1 will not be collected.

The results of the full diagnostic workup, blood and sputum culture, hematological parameters etc. will be captured in the eCRF.

Information on all concomitant medications, administered blood products, as well as interventions occurring during the study must be recorded on the patient's eCRF in C3D.

End of study procedures: Data will be stored according to HHS, FDA regulations and NIH Intramural Records Retention Schedule as applicable.

Loss or destruction of data: Should we become aware that a major breech in our plan to protect subject confidentiality and trial data has occurred, this will be reported expeditiously per requirements in section **7.2.1**.

6.2 Data Sharing Plans

6.2.1 Human Data Sharing Plan

The PI will share coded linked human data generated in this research for future research

- in a NIH-funded or approved public repository clinicaltrials.gov, dbGaP and GenBank
- in BTRIS

- in publication and/or public presentations

at the time of publication or shortly thereafter.

6.2.2 Genomic Data Sharing Plan

Unlinked genomic data will be deposited in public genomic databases such as dbGaP in compliance with the NIH Genomic Data Sharing Policy.

6.3 Response Criteria

For the purposes of this study, patients should be re-evaluated for response every 8 weeks (+/- 1 week). Whilst immune-related RECIST criteria (**Appendix E**) will be taken into consideration regarding continuation of therapy in the event of growth (with a requirement for confirmation of PD), standard RECIST criteria will be the primary method used for evaluation of the primary endpoint.

Response and progression will be evaluated in this study using the new international criteria proposed by the revised Response Evaluation Criteria in Solid Tumors (RECIST) guideline (version 1.1) [92, 93] and Modified Immune-related response criteria (Section **6.3.3.5**). Changes in the largest diameter (unidimensional measurement) of the tumor lesions and the shortest diameter in the case of malignant lymph nodes are used in the RECIST criteria.

6.3.1 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:

- By chest x-ray: >20 mm;
- By CT scan:
  - Scan slice thickness 5 mm or under as >10 mm with CT scan
  - Scan slice thickness >5 mm: double the slice thickness
- With calipers on clinical exam: >10 mm.

All tumor measurements must be recorded in millimeters (or decimal fractions of centimeters).

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/pneumonitis, 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 patient, 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.

6.3.2 Methods for Evaluation of Measurable Disease

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

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

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

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

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

Use of MRI remains a complex issue. MRI has excellent contrast, spatial, and temporal resolution; however, there are many image acquisition variables involved in MRI, which greatly impact image quality, lesion conspicuity, and measurement. Furthermore, the availability of MRI is variable globally. As with CT, if an MRI is performed, the technical specifications of the scanning sequences used should be optimized for the evaluation of the type and site of disease. Furthermore, as with CT, the modality used at follow-up should be the same as was used at baseline and the

lesions should be measured/assessed on the same pulse sequence. It is beyond the scope of the RECIST guidelines to prescribe specific MRI pulse sequence parameters for all scanners, body parts, and diseases. Ideally, the same type of scanner should be used, and the image acquisition protocol should be followed as closely as possible to prior scans. Body scans should be performed with breath-hold scanning techniques, if possible.

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

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

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

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

6.3.3 Response Criteria

6.3.3.1 Evaluation of Target Lesions

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

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

6.3.3.2 Evaluation of Non-Target Lesions

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

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

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

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

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

6.3.3.3 Evaluation of Best Overall Response

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

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

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

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

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

6.3.3.4 Duration of Response

Duration of overall response: The duration of overall response is measured from the time measurement criteria are met for CR or PR (whichever is first recorded) until the first date that recurrent or progressive disease is objectively documented (taking as reference for progressive disease the smallest measurements recorded since the treatment started).

The duration of overall CR is measured from the time measurement criteria are first met for CR until the first date that progressive disease is objectively documented.

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

6.3.3.5 Modified Immune-mediated response criteria (imRC)

Modified immune-mediated response criteria (imRC) will also be employed in this study. This new classification is based on the recent learning from clinical studies with cancer immunotherapies that even if some new lesions appear at the beginning of a treatment or if the total tumor burden does not increase substantially, tumor regressions or stabilizations might still occur later. The irRC were created using bi-dimensional measurements (as previously widely used in the World Health Organization criteria). For this trial, the concepts of the irRC are combined with RECIST 1.1 to come up with the modified imRC. Please refer to **Appendix E** for further details.

6.4 Toxicity Criteria

The following adverse event management guidelines are intended to ensure the safety of each patient while on the study. The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 5.0. A copy of the CTCAE version 5.0 can be downloaded from the CTEP web site (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm#ctc_50).

7 NIH REPORTING REQUIREMENTS/DATA AND SAFETY MONITORING PLAN

7.1 Definitions

Please refer to definitions provided in Policy 801: Reporting Research Events found here.

7.2 OHSRP Office of Compliance and Training / IRB Reporting

7.2.1 Expedited Reporting

Please refer to the reporting requirements in Policy 801: Reporting Research Events and Policy 802 Non-Compliance Human Subjects Research found here. Note: Only IND Safety Reports that meet the definition of an unanticipated problem will need to be reported per these policies.

7.2.2 IRB Requirements for PI Reporting at Continuing Review

Please refer to the reporting requirements in Policy 801: Reporting Research Events found here.

7.3 NCI Clinical Director Reporting

Problems expeditiously reported to the OHSRP in iRIS will also be reported to the NCI Clinical Director. A separate submission is not necessary as reports in iRIS will be available to the Clinical Director.

In addition to those reports, all deaths that occur within 30 days after receiving a research intervention **s**hould be reported via email to the Clinical Director unless they are due to progressive disease.

To report these deaths, please send an email describing the circumstances of the death to Dr. Dahut at NCICCRQA@mail.nih.gov within one business day of learning of the death.

7.4 NIH Required Data and Safety Monitoring Plan

The clinical research team will meet weekly when patients are being actively treated on the trial to discuss each patient.

All data will be collected in a timely manner and reviewed by the principal investigator or a lead associate investigator. Events meeting requirements for expedited reporting as described in section **7.2.1** will be submitted within the appropriate timelines.

The principal investigator will review adverse event and response data on each patient to ensure safety and data accuracy. The principal investigator will personally conduct or supervise the investigation and provide appropriate delegation of responsibilities to other members of the research staff.

8 SPONSOR PROTOCOL/SAFETY REPORTING

8.1 Definitions

8.1.1 Adverse Event

Any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment. An adverse event (AE) can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not related to the medicinal (investigational) product (ICH E6 (R2))

8.1.2 Serious Adverse Event (SAE)

An adverse event or suspected adverse reaction is considered serious if in the view of the investigator or the sponsor, it results in any of the following:

- Death,
- A life-threatening adverse event (see section **8.1.3**)
- Inpatient hospitalization or prolongation of existing hospitalization
    - A hospitalization/admission that is pre-planned (i.e., elective or scheduled surgery arranged prior to the start of the study), a planned hospitalization for pre-existing

condition, or a procedure required by the protocol, without a serious deterioration in health, is not considered a serious adverse event.

- A hospitalization/admission that is solely driven by non-medical reasons (e.g., hospitalization for patient or subject convenience) is not considered a serious adverse event.
- Emergency room visits or stays in observation units that do not result in admission to the hospital would not be considered a serious adverse event. The reason for seeking medical care should be evaluated for meeting one of the other serious criteria.

- Persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- A congenital anomaly/birth defect
- Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse drug experience when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

8.1.3 Life-threatening

An adverse event or suspected adverse reaction is considered "life-threatening" if, in the view of either the investigator or sponsor, its occurrence places the patient or subject at immediate risk of death. It does not include an adverse event or suspected adverse reaction that, had it occurred in a more severe form, might have caused death. (21CFR312.32)

8.1.4 Severity

The severity of each Adverse Event will be assessed utilizing the CTCAE version 5.0.

8.1.5 Relationship to Study Product

All AEs will have their relationship to study product assessed using the terms: related or not related.

- Related – There is a reasonable possibility that the study product caused the adverse event. Reasonable possibility means that there is evidence to suggest a causal relationship between the study product and the adverse event.
- Not Related – There is not a reasonable possibility that the administration of the study product caused the event.

8.2 Assessment of Safety Events

AE information collected will include event description, date of onset, assessment of severity and relationship to study product and alternate etiology (if not related to study product), date of resolution of the event, seriousness and outcome. The assessment of severity and relationship to the study product will be done only by those with the training and authority to make a diagnosis and listed on the Form FDA 1572 as the site principal investigator or sub-investigator. AEs occurring during the collection and reporting period will be documented appropriately regardless of relationship. AEs will be followed through resolution.

SAEs will be:

- Assessed for severity and relationship to study product and alternate etiology (if not related to study product) by a licensed study physician listed on the Form FDA 1572 as the site principal investigator or sub-investigator.
- Recorded on the appropriate SAE report form, the medical record and captured in the clinical database.
- Followed through resolution by a licensed study physician listed on the Form FDA 1572 as the site principal investigator or sub-investigator.

For timeframe of recording adverse events, please refer to section **6.1**. All serious adverse events recorded from the time of first investigational product administration must be reported to the sponsor with the exception of any listed in section **8.4**.

8.3 Reporting of Serious Adverse Events

Any AE that meets protocol-defined serious criteria or meets the definition of Adverse Event of Special Interest that require expedited reporting must be submitted immediately (within 24 hours of awareness) to OSRO Safety using the CCR SAE report form. Any exceptions to the expedited reporting requirements are found in section **8.4**.

All SAE reporting must include the elements described in section **8.2**.

SAE reports will be submitted to the Center for Cancer Research (CCR) at: OSROSafety@mail.nih.gov and to the CCR PI and study coordinator. CCR SAE report form and instructions can be found at: https://ccrod.cancer.gov/confluence/display/CCRCRO/Forms+and+Instructions.

Following the assessment of the SAE by OSRO, other supporting documentation of the event may be requested by the OSRO Safety and should be provided as soon as possible.

8.4 Waiver of expedited reporting to CCR

As death due to disease progression is part of the study objectives (OS), and captured as an endpoint in this study, death due to disease progression will not be reported in expedited manner to the sponsor. However, if there is evidence suggesting a causal relationship between the study drug and the event, report the event in an expedited manner according to section **8.3**.

Hospitalization that is deemed to be due to disease progression, and not attributable to the intervention will not be reported as an SAE. The event, and the assessment that it was caused by disease progression will be documented in the medical records. The causality assessment of hospitalization will be re-evaluated any time when new information is received. If the causality assessment changes from disease progression to related to the study intervention, SAE report will be sent to the Sponsor in an expedited manner according to section **8.3**. If there is any uncertainty whether the intervention is a contributing factor to the event, the event should be reported as AE or SAE as appropriate.

8.5 Safety Reporting to the Manufacturer, Bristol Myers Squibb

All events listed below must be reported in the defined timeline to CCRsafety@mail.nih.gov. The CCR Office of Regulatory Affairs will send all reports to the manufacturer as described below.

8.5.1 Death Reporting

All deaths that occur during the study, or within 90-day post-last dose of nivolumab/oral vancomycin/tadalafil safety follow-up period must be reported to BMS as follows:

- Death that is clearly the result of disease progression should be documented but should not be reported as an SAE.
- Where death is not due (or not clearly due) to progression of the disease under study, the AE causing the death must be reported to BMS as a SAE within 24 hours of Sponsor awareness. The report should contain a comment regarding the co-involvement of progression of disease, if appropriate, and should assign main and contributory causes of death.
- Deaths with an unknown cause should always be reported as a SAE.

8.5.2 Reporting of Serious Adverse Events

All SAEs will be reported, whether considered causally related to the investigational product, or to the study procedure(s). The reporting period for SAEs is the period immediately following the time that written informed consent is obtained through 90 days after the last dose of nivolumab/oral vancomycin/tadalafil or until the initiation of alternative anticancer therapy.

Sponsor must inform the FDA, via a MedWatch, of any serious or unexpected adverse events. SAEs and pregnancies must be reported to BMS within one business day of Sponsor becoming aware of the event. SAEs must be recorded on MedWatch or equivalent form. Pregnancies must be reported and submitted to BMS. BMS will perform due diligence follow-up using the BMS pregnancy Form (**Appendix G**) which the investigator must complete. It is the responsibility of the sponsor to compile all necessary information and ensure that the FDA receives a report according to the FDA reporting requirement timelines and to ensure that these reports are also submitted to BMS at the above time points.

* A cover page should accompany the MedWatch form indicating the following:

- "Notification from an Investigator Sponsored Study"
- The investigator IND number assigned by the FDA
- The investigator's name and address
- The trial name/title and BMS ISS reference number (CA209-8R3) (BMS only)

* Sponsor must also indicate, either in the SAE report or the cover page, the causality of events in relation to all study medications and if the SAE is related to disease progression, as determined by the principal investigator.

* Send SAE report and accompanying cover page by way of email to BMS's designated mailbox: Worldwide.Safety@BMS.com. SAE FAX Number: +1 609-818-3804

If a non-serious AE becomes serious, this and another relevant follow-up information must also be provided to BMS, and the FDA.

For all infusion reactions, SAEs should be reported to BMS Patient safety.

8.5.3 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 occurrence of potential DILIs, meeting

the defined criteria, must be reported as SAEs (See Section **8.3** for reporting details). The following definition takes into account anticipated baseline compromise of liver function in patients.

Potential drug induced liver injury is defined as:

1) Concurrent ALT ≥ 10 times upper limit of normal (ULN)

AND

2) Total bilirubin ≥ 2 times ULN or baseline value (if elevated bilirubin at study entry),

AND

3) No other immediately apparent possible causes of AT elevation and hyperbilirubinemia, including, but not limited to, viral hepatitis, pre-existing chronic or acute liver disease, or the administration of other drug(s) known to be hepatotoxic.

Drug induced liver injury (DILI) in a study subject, with or without associated clinical manifestations, is required to be reported as "hepatic function abnormal" within 24 hours of Sponsor knowledge of the event to the BMS Patient Safety using the designated Safety e-mailbox Worldwide.Safety@BMS.com unless a definitive underlying diagnosis for the abnormality (e.g., cholelithiasis or bile duct obstruction) that is unrelated to investigational product has been confirmed.

If the definitive underlying diagnosis for the abnormality has been established and is unrelated to investigational product, the decision to continue dosing of the study subject will be based on the clinical judgment of the investigator.

If no definitive underlying diagnosis for the abnormality is established, dosing of the study subject must be interrupted immediately. Follow-up investigations and inquiries must be initiated by the investigational site without delay.

Each reported event of hepatic function abnormality will be followed by the investigator and evaluated by the sponsor and BMS.

8.6 Reporting Pregnancy

All required pregnancy reports/follow-up to OSRO will be submitted to: OSROSafety@mail.nih.gov and to the CCR PI and study coordinator. Forms and instructions can be found here: https://ccrod.cancer.gov/confluence/display/CCRCRO/Forms+and+Instructions

8.6.1 Maternal exposure

If a patient becomes pregnant during the course of the study, the study treatment should be discontinued immediately, and the pregnancy reported to the Sponsor no later than 24 hours of when the Investigator becomes aware of it. The Investigator should notify the Sponsor no later than 24 hours of when the outcome of the Pregnancy becomes known,

Pregnancy itself is not regarded as an SAE. However, congenital abnormalities or birth defects and spontaneous miscarriages that meet serious criteria (section **8.1.2**) should be reported as SAEs.

The outcome of all pregnancies (spontaneous miscarriage, elective termination, ectopic pregnancy, normal birth, or congenital abnormality) should be followed up and documented.

8.6.2 Paternal exposure

Male patients should refrain from fathering a child or donating sperm during the study and for 7 months after the last dose of study drug (s).

Pregnancy of the patient's partner is not considered to be an AE. However, the outcome of all pregnancies (spontaneous miscarriage, elective termination, ectopic pregnancy, normal birth, or congenital abnormality) occurring from the date of the first dose until 7 months after the last dose should, if possible, be followed up and documented.

8.7 Regulatory Reporting for Studies Conducted Under CCR-Sponsored IND

Following notification from the investigator, CCR, the IND sponsor, will report any suspected adverse reaction that is both serious and unexpected. CCR will report an AE as a suspected adverse reaction only if there is evidence to suggest a causal relationship between the study product and the adverse event. CCR will notify FDA and all participating investigators (i.e., all investigators to whom the sponsor is providing drug under its INDs or under any investigator's IND) in an IND safety report of potential serious risks from clinical trials or any other source, as soon as possible, in accordance to 21 CFR Part 312.32.

All serious events will be reported to the FDA at least annually in a summary format.

8.8 Sponsor Protocol Non-Adherence Reporting

Protocol non-adherence is defined as any noncompliance with the clinical trial protocol, GCP, or protocol-specific procedural requirements on the part of the participant, the Investigator, or the study site staff inclusive of site personnel performing procedures or providing services in support of the clinical trial.

It is the responsibility of the study Staff to document any protocol non-adherence identified by the Staff or the site Monitor on the OSRO Site Protocol Non-Adherence Log. The protocol-specific, cumulative non-adherence log should be maintained in the site essential documents file and provided to OSRO as required over the duration of the study. In addition, any non-adherence to the protocol should be documented in the participant's source records and reported to the local IRB per their guidelines. OSRO required protocol non-adherence reporting is consistent with E6(R2) GCP: Integrated Addendum to ICH E6(R1): 4.5 Compliance with Protocol; 5.18.3 (a), and 5.20 Noncompliance; and ICH E3 16.2.2 Protocol deviations.

9 CLINICAL MONITORING

As a sponsor for clinical trials, FDA regulations require the CCR to maintain a monitoring program. The CCR's program allows for confirmation of: study data, specifically data that could affect the interpretation of primary and secondary study endpoints; adherence to the protocol, regulations, ICH E6, and SOPs; and human subjects protection. This is done through independent verification of study data with source documentation focusing on:

- Informed consent process
- Eligibility confirmation
- Drug administration and accountability
- Adverse events monitoring

- Response assessment.

The monitoring program also extends to multi-site research when the CCR is the coordinating center.

This trial will be monitored by personnel employed by a CCR contractor. Monitors are qualified by training and experience to monitor the progress of clinical trials. Personnel monitoring this study will not be affiliated in any way with the trial conduct.

Clinical site monitoring is conducted to ensure that the rights of the participants are protected, that the study is implemented per the approved protocol, Good Clinical Practice and standard operating procedures, and that the quality and integrity of study data and data collection methods are maintained. Monitoring for this study will be performed by NCI CCR Office of Sponsor and Regulatory Oversight (OSRO) Monitoring based on OSRO standards, FDA Guidance E6(R2) Good Clinical Practice: Integrated Addendum to ICH E6(R1) March 2018, and applicable regulatory requirements.

Details of clinical site monitoring will be documented in a Clinical Monitoring Plan (CMP) developed by OSRO. CMPs will be protocol-specific, risk-based and tailored to address human subject protections and integrity of the study data. The intensity and frequency of monitoring will be based on several factors, including study type, phase, risk, complexity, expected enrollment rate, and any unique attributes of the study and the site. OSRO Monitoring visits and related activities will be conducted throughout the life cycle of each protocol, with the first activity being before study start to conduct a Site Assessment Visit (SAV) (as warranted), followed by a Site Initiation Visit (SIV), Interim Monitoring Visit(s) (IMVs), and a study Close-Out Visit (COV).

Some monitoring activities may be performed remotely, while others will take place at the study site(s). Monitoring visit reports will describe visit activities, observations, findings of protocol non-adherence and associated action items or follow-up required for resolution of findings. Monitoring reports will be distributed to the study PI, NCI CCR QA, coordinating center (if applicable) and the OSRO regulatory file.

If protocol non-adherence is identified by the Monitor (i.e., any noncompliance with the clinical trial protocol, GCP, or protocol-specific procedural requirements on the part of the participant, the Investigator, or the site Staff) the Monitor will note the observation, review with site Staff and if unresolved, request that the Staff document the non-adherence on the protocol-specific OSRO Site Protocol Non-Adherence Log (see Section **8.8**).

10 STATISTICAL CONSIDERATIONS

10.1 Study Objective

10.1.1 Primary Objective

- The primary objective of this pilot trial is to preliminarily determine the best overall response according to RECIST 1.1 to combined treatment of nivolumab, oral vancomycin and tadalafil in the treatment of patients with HCC or non-HCC liver dominant metastases from CRC and PDAC.

10.1.2 Secondary Objectives

- To assess the safety and tolerability of nivolumab in combination with oral vancomycin and tadalafil in patients with refractory primary HCC or liver dominant metastatic cancer from CRC or PDAC.
- To assess overall survival (OS) of nivolumab combined with oral vancomycin and tadalafil in patients with refractory HCC or liver dominant metastatic cancer from CRC or PDAC.

10.2 Sample Size Determination

Separately in HCC and Non-HCC patients, a small one-stage phase II design will be utilized:

In HCC cohort 12 and in Non-HCC cohort 13 evaluable patients will be enrolled. With 12/13 patients, an exact binomial test with a one-sided 0.10 significance level will have 84% power to detect the difference between an unacceptably low 4% response rate (PR + CR) and a desirable, targeted response rate (PR + CR) of 25%. In practice, if there are at least 2 responses among the 12/13 patients, then the lower one-sided 90% confidence bound is at least 4.5%. Simultaneously, the upper one-sided 90% confidence bound is at least 38.6%. Thus, attaining 2 or more responses would be sufficient to demonstrate ruling out a 4% response rate and also be consistent with a desirable 25% rate or greater.

If the response rate (PR + CR) in a cohort is identified as being adequate, consideration will be given to enrolling replicate cohorts as appropriate by amendment to evaluate additional patients independently from the initial 12/13 patients.

It is expected that up to 5-6 HCC patients and 5-6 non-HCC can be enrolled within one year; thus, 2-3 years may be required to accrue 12 evaluable HCC patients and 13 evaluable non-HCC patients (25 total evaluable patients). To allow for a small number of inevaluable patients, the accrual ceiling will be set at 27 patients.

<u>As of Amendment version date 12/22/2021:</u>

The trial will no longer enroll any patients with HCC but will enroll only additional patients without HCC. There were 5 evaluable patients with HCC enrolled prior to December 2021, and up to 20 evaluable non-HCC patients will be enrolled based on the following:

With 20 patients, an exact binomial test with a one-sided 0.10 significance level will have 82.3% power to detect the difference between an unacceptably low 5% response rate (PR + CR) and a desirable, targeted response rate (PR + CR) of 21%. In practice if there are at least 3 responses among the 20 patients, then the lower two-sided 90% exact mid-p Clopper-Pearson confidence bound is at least 5.2% (and the lower one sided 90% confidence bound is at least 6.9%). Simultaneously, the upper two-sided 90% confidence bound is at least 32.0% (and the upper one-sided 90% confidence bound is 28.0%). Thus, attaining 3 or more responses would be sufficient to demonstrate ruling out a clinically not interesting 5% (that is, will not be worse than 5%) response rate and be consistent with a desirable 21% rate or greater.

As of December 2021, there are 12 non-HCC patients enrolled. It is expected that the remaining 5 non-HCC patients can be enrolled in less than one year. With 5 HCC patients and 20 non-HCC patients, the total evaluable patients will be 25. To allow for a small number of inevaluable patients, the accrual ceiling will remain at 27.

10.3 Populations for Analyses

All patients received treatments, regardless of drug types included in this study and had at least one re-staging will be evaluated for response (PR + CR).

10.4 Statistical Analyses

10.4.1 General Approach

The fraction of patients who experience a response will be reported along with confidence intervals.

10.4.2 Analysis of the Primary Endpoints

In each cohort, the fraction of patients who experience a response (PR + CR) will be reported along with 80% and 95% two-sided confidence intervals.

10.4.3 Analysis of the Secondary Endpoint

The safety of the treatment will be monitored, and any toxicities identified will be reported by type and grade.

Overall survival will be calculated from the on-study date using the Kaplan-Meier method.

10.4.4 Safety Analyses

Patients will be assessed for toxicity by reporting the grades of toxicity and the type of toxicity observed for all patients.

10.4.5 Baseline Descriptive Statistics

Baseline demographic characteristics will be reported.

10.4.6 Planned Interim Analyses

There are no planned interim analyses for efficacy.

10.4.7 Exploratory Analyses

The following objectives will result in descriptive or comparative analyses when adequate data exist to perform them:

- To measure changes in immune cell population in the peripheral blood and tumors of patients treated on this study, including CD4+, CD8+, MDSCs, MAITS NKT cells, IFNgamma signaling pathway, perforin and granzyme.
- To measure secreted cytokines in the peripheral blood to monitor the relative level change with the treatment
- To measure changes in bile acid in the peripheral blood of patients treated in this study.
- To evaluate tadalafil and oral vancomycin pharmacokinetic level to monitor compliance and association between the level and response rate or immune parameters
- To evaluate TCR beta sequencing to analyze the T cell response from PBMC and tumor samples
- To assess gene and protein expression and metabolite profile from tumor to evaluate the association of the change immune cell signature, signaling pathways and metabolite with clinical outcome

- To measure changes in gut microbiome in the stool of patients treated on this study.
- To evaluate the incidence of vancomycin-resistant enterococci (VRE) development during the trial.
- To evaluate the relationship between the response rate, overall survival and immune parameters, gut microbiome, and bile acid component in patients treated on this study.

Any exploratory evaluations which generate quantitative measures will be done using descriptive statistics including confidence intervals when appropriate. Any statistical tests performed for evaluation of exploratory objectives will be done without formal adjustment for multiple comparisons, but in the context of the number of tests performed.

11 COLLABORATIVE AGREEMENT

11.1 Cooperative Research and Development Agreement (CRADA)

The CRADA for this protocol (03259) has been executed between Thoracic & GI Malignancies Branch, NCI, NIH and Bristol-Myers Squibb, the manufacturer of nivolumab.

12 HUMAN SUBJECTS PROTECTIONS

12.1 Rationale for Subject Selection

This study was designed to include women and minorities but was not designed to measure differences of intervention effects. Males and females will be recruited with no preference to gender. No exclusion to this study will be based on race. Minorities will actively be recruited to participate.

12.2 Participation of Children

Children (younger than 18 years) will not be included in this protocol due to the limited data on nivolumab in children and the different biology of childhood malignancy.

12.3 Participation of Subjects Unable to Give Consent

Adults unable to consent are excluded from enrolling in the protocol. However, it is possible that subjects enrolled in the protocol may permanently lose the capacity to consent for themselves during the course of this study. If a subject loses the capacity to consent during the course of the study for reasons unrelated to protocol participation and is receiving direct benefit from the study therapy in the opinion of the PI, the subject may be retained on the study therapy with the permission of the alternate decision maker. If the permanent loss of capacity is due to toxicity from the study therapy or disease progression, the participant will be removed from study therapy as required per protocol, but may be retained on study to be followed for safety reasons and/or overall survival as described in the Study Calendar **3.4** The follow-up procedures involve non-invasive, low risk studies. The benefit of a safety follow up would outweigh the risks of such procedures.

All subjects will be offered the opportunity to fill in their wishes for research and care, and assign a substitute decision maker on the "NIH Advance Directive for Health Care and Medical Research Participation" form so that another person can make decisions about their medical care in the event that they become incapacitated or cognitively impaired during the course of the study. Note: The PI or AI will contact the NIH Ability to Consent Assessment Team (ACAT) for evaluation to assess ongoing capacity of the subjects and to identify a LAR, as needed.

Please see section **12.6.1** for consent procedure.

12.4 Evaluation of Benefits and Risks/Discomforts for All Participants

12.4.1 Benefits

The study drug may help to control the disease. The results may help the investigators learn more about the disease and develop new treatments for patients with this disease.

12.4.2 Risks

The primary risk to patients participating in this research study is from the toxicity of nivolumab, oral vancomycin and tadalafil.

All care will be taken to minimize study treatment side effects, but they can be unpredictable in nature and severity. Patients will be examined and evaluated prior to enrollment. All evaluations to monitor the treatment of patients will be recorded in the patient chart. If patients suffer any physical injury as a result of the participation in this study, immediate medical treatment is available at the Clinical Center, National Cancer Institute, Bethesda, Maryland.

Although no compensation is available, any injury will be evaluated and treated in keeping with the benefits or care to which patients are entitled under applicable regulations. In all publications and presentations resulting from this trial, patients' anonymity will be protected to the maximum extent possible. Authorized personnel from the National Cancer Institute (NCI) and Food and Drug Administration (FDA) or other regulatory authorities may have access to research files in order to verify that patients' rights have been safeguarded. In addition, patient names will be given to the Central Registration to register and verify patients' eligibility.

12.4.2.1 Risk of Biopsy

All care will be taken to minimize risks that may be incurred by tumor sampling. However, there are procedure-related risks (such as bleeding, infection and visceral injury) that will be explained fully during informed consent.

12.4.2.2 Risks of Sedation

Biopsies will be done under sedation. Potential side effects of sedation include headache, nausea and drowsiness. These side effects usually go away quickly.

12.4.2.3 Risks of exposure to Ionizing Radiation

The study will involve radiation from the following sources:

- Up to 6 CT scans per year for disease assessment
- 2 CT scans for the collection of 2 mandatory biopsies

Subjects in this study may have up to 6 CT scans and 2 CT guided biopsies and be exposed to approximately 8.2 rem per year. This amount is more than would be expected from everyday background radiation. Being exposed to excess radiation can increase the risk of cancer. The risk of getting cancer from the radiation exposure in this study is 0.8 out of 100 (0.8%) and of getting a fatal cancer is 0.4 out of 100 (0.4%).

12.4.2.4 Risks of CT Scans

In addition to the radiation risks discussed above, CT scans may include the risks of an allergic reaction to the contrast. Participants might experience hives, itching, headache, difficulty breathing, increased heartrate and swelling.

12.4.2.5 Blood Collection Risks

Risks of blood draws include pain and bruising in the area where the needle is placed, lightheadedness, and rarely, fainting. When large amounts of blood are collected, low red blood cell count (anemia) can develop.

12.4.2.6 Risks of EKG

Risks include some minor skin irritation from the electrodes

12.4.2.7 Stool Collection or Rectal Swabs

There is no physical risk involved with stool or rectal swab collection.

12.4.2.8 Questionnaires Risk

Questionnaires may contain questions that are sensitive in nature. The participants are asked to only answer questions they are comfortable with

12.4.2.9 Other Risks

Risks include the possible occurrence of any of a range of side effects which are listed in the Consent Document or this protocol document. Frequent monitoring for adverse effects will help to minimize the risks associated with administration of the study agents.

12.4.2.10 Risk of Losing Data

This includes the risk that data obtained during this study, including data related to genotype, DNA sequencing or risk for disease tendency or trait can be released to members of the public, insurers, employers, or law enforcement agencies. Although there are no plans to release results to the patients, family members or health care providers, this risk will be included in the informed consent document

12.4.2.11 Non-Physical Risks of Genetic Research

Risk of receiving unwanted information
Anxiety and stress may arise as a result of the anticipation that unwanted information regarding disease related DNA sequencing or disease tendencies, or misattributed paternity. Patients will be clearly informed that the data related to DNA sequencing and genetic analysis is coded, investigational and will not be shared with patients, family members or health care providers.

Risk related to possibility that information may be released
This includes the risk that data related to genotype, DNA sequencing or risk for disease tendency or trait can be released to members of the public, insurers, employers, or law enforcement agencies. Although there are no plans to release results to the patients, family members or health care providers, this risk will be included in the informed consent document

12.5 Risks/Benefits Analysis for All Participants

It is possible that treatment on this protocol may reduce tumor burden or lessen symptoms caused by the cancer. While treatment on this protocol may not individually benefit subjects, the knowledge gained from this study may help others in the future who have gastric cancer. Potential risks include the possible occurrence of any of a range of side effects listed. The risks and benefits of participation for adults who become unable to consent, are no different than those described for patients who are less vulnerable

12.6 Consent Process and Documentation

The informed consent document will be provided as a physical or electronic document to the participant or consent designee(s) as applicable for review prior to consenting. A designated study investigator will carefully explain the procedures and tests involved in this study, and the associated risks, discomforts and benefits. In order to minimize potential coercion, as much time as is needed to review the document will be given, including an opportunity to discuss it with friends, family members and/or other advisors, and to ask questions of any designated study investigator. A signed informed consent document will be obtained prior to entry onto the study.

The initial consent process as well as re-consent, when required, may take place in person or remotely (e.g., via telephone or other NIH approved remote platforms used in compliance with policy, including HRPP Policy 303) per discretion of the designated study investigator and with the agreement of the participant. Whether in person or remote, the privacy of the subject will be maintained. Consenting investigators (and participant, when in person) will be located in a private area (e.g., clinic consult room). When consent is conducted remotely, the participant will be informed of the private nature of the discussion and will be encouraged to relocate to a more private setting if needed.

Consent will be documented with required signatures on the physical document (which includes the printout of an electronic document sent to participant) or as described below, with a manual (non-electronic) signature on the electronic document. When required, witness signature will be obtained similarly as described for the investigator and participant.

Manual (non-electronic) signature on electronic document:

When a manual signature on an electronic document is used for the documentation of consent at the NIH Clinical Center, this study will use the following to obtain the required signatures:

- Adobe platform (which is not 21 CFR Part 11 compliant); or,
- iMedConsent platform (which is 21 CFR Part 11 compliant)

During the consent process, participants and investigators will view individual copies of the approved consent document on screens at their respective locations (if remote consent); the same screen may be used when in the same location, but is not required.
Both the investigator and the participant will sign the document using a finger, stylus or mouse.
Note: Refer to the CCR SOP PM-2, Obtaining and Documenting the Informed Consent Process for additional information (e.g., verification of participant identity when obtaining consent remotely) found here.

12.6.1 Consent Process for Adults Who Lack Capacity to Consent to Research Participation

For participants addressed in section **12.3**, an LAR will be identified consistent with Policy 403 and informed consent obtained from the LAR, as described in Section **12.6.**

13 REGULATORY AND OPERATIONAL CONSIDERATIONS

13.1 Study Discontinuation and Closure

This study may be temporarily suspended or prematurely terminated if there is sufficient reasonable cause. Written notification, documenting the reason for study suspension or termination,

will be provided by the suspending or terminating party to study participants. If the study is prematurely terminated or suspended, the Principal Investigator (PI) will promptly inform study participants, the Institutional Review Board (IRB), and sponsor and will provide the reason(s) for the termination or suspension. Study participants will be contacted, as applicable, and be informed of changes to study visit schedule.

Circumstances that may warrant termination or suspension include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to participants
- Demonstration of efficacy that would warrant stopping
- Insufficient compliance to protocol requirements
- Data that are not sufficiently complete and/or evaluable
- Determination that the primary endpoint has been met
- Determination of futility

Study may resume once concerns about safety, protocol compliance, and data quality are addressed, and satisfy the sponsor, IRB and as applicable, Food and Drug Administration (FDA).

13.2 Quality Assurance and Quality Control

The clinical site will perform internal quality management of study conduct, data and biological specimen collection, documentation and completion. An individualized quality management plan will be developed to describe a site's quality management.

Quality control (QC) procedures will be implemented beginning with the data entry system and data QC checks that will be run on the database will be generated. Any missing data or data anomalies will be communicated to the site(s) for clarification/resolution.

Following written Standard Operating Procedures (SOPs), the monitors will verify that the clinical trial is conducted and data are generated and biological specimens are collected, documented (recorded), and reported in compliance with the protocol, International Conference on Harmonisation Good Clinical Practice (ICH GCP), and applicable regulatory requirements (e.g., Good Laboratory Practices (GLP), Good Manufacturing Practices (GMP)).

The investigational site will provide direct access to all trial related sites, source data/documents, and reports for the purpose of monitoring and auditing by the sponsor, and inspection by local and regulatory authorities.

13.3 Conflict of Interest Policy

The independence of this study from any actual or perceived influence, such as by the pharmaceutical industry, is critical. Therefore, any actual conflict of interest of persons who have a role in the design, conduct, analysis, publication, or any aspect of this trial will be disclosed and managed. Furthermore, persons who have a perceived conflict of interest will be required to have such conflicts managed in a way that is appropriate to their participation in the design and conduct of this trial. The study leadership in conjunction with the NCI has established policies and procedures for all study group members to disclose all conflicts of interest and will establish a mechanism for the management of all reported dualities of interest.

13.4 Confidentiality and Privacy

Participant confidentiality and privacy is strictly held in trust by the participating investigators, their staff, and the sponsor. This confidentiality is extended to cover testing of biological samples and genetic tests in addition to the clinical information relating to participants. Therefore, the study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the sponsor.

All research activities will be conducted in as private a setting as possible.

The study monitor, other authorized representatives of the sponsor, representatives of the Institutional Review Board (IRB), and/or regulatory agencies may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the participants in this study. The clinical study site will permit access to such records.

The study participant's contact information will be securely stored at each clinical site for internal use during the study. At the end of the study, all records will continue to be kept in a secure location for as long a period as dictated by the reviewing IRB, Institutional policies, or sponsor requirements.

Study participant research data, which is for purposes of statistical analysis and scientific reporting, will be transmitted to and stored at the NCI CCR. This will not include the participant's contact or identifying information. Rather, individual participants and their research data will be identified by a unique study identification number. The study data entry and study management systems used by the clinical site(s) and by NCI CCR research staff will be secured and password protected. At the end of the study, all study databases will be archived at the NIH.

To further protect the privacy of study participants, a Certificate of Confidentiality has been issued by the National Institutes of Health (NIH). This certificate protects identifiable research information from forced disclosure. It allows the investigator and others who have access to research records to refuse to disclose identifying information on research participation in any civil, criminal, administrative, legislative, or other proceeding, whether at the federal, state, or local level. By protecting researchers and institutions from being compelled to disclose information that would identify research participants, Certificates of Confidentiality help achieve the research objectives and promote participation in studies by helping assure confidentiality and privacy to participants.

14 PHARMACEUTICAL INFORMATION

14.1 Nivolumab (133973)

14.1.1 Source:

Bristol Myers Squibb will supply investigational nivolumab. Only 100mg, 10mg/mL nivolumab vials will be provided for this study. There are 5 vials per carton.

14.1.2 Toxicity:

Nivolumab is most commonly associated with immune-related adverse reactions. Most of these, including severe reactions, resolved following initiation of appropriate medical therapy or withdrawal of nivolumab.

Refer to investigator brochure for detailed toxicity information.

14.1.3 Formulation and Preparation

Nivolumab Injection is a clear to opalescent, colorless to pale yellow liquid; light (few) particulates may be present. The drug product is a sterile, nonpyrogenic, single-use, isotonic aqueous solution formulated in sodium citrate, sodium chloride, mannitol, diethylenetriamine pentacetic acid (pentetic acid) and polysorbate 80 (Tween® 80), pH 6.0.

Nivolumab injection can be infused undiluted (10 mg/mL) or diluted with 0.9% Sodium Chloride Injection, USP or 5% Dextrose, USP to concentrations no less than 1 mg/mL.

14.1.4 Stability and Storage

Store intact vials at 2°C to 8°C (36°F to 46°F); do not freeze. Protect from light. Do not shake. Shelf-life surveillance of the intact vials is ongoing.

Store the prepared infusion solution at room temperature or refrigerated. Do not freeze solutions prepared for infusion. Refer to investigator brochure for detailed stability information.

14.1.5 Administration Procedures

Intravenous infusion 30 to 60 minutes. Do not administer as an IV push or bolus injection.

Administer through a 0.2 micron to 1.2-micron pore size, low-protein binding in-line filter.

14.2 Tadalafil

14.2.1 Source

Tadalafil tablets will be provided by the NIH Clinical Center Pharmacy according to standard pharmacy procedures.

14.2.2 Description

Tadalafil is a selective inhibitor of cyclic guanosine monophosphate (cGMP)-specific phosphodiesterase type 5 (PDE5). Tadalafil has the empirical formula C22H19N3O4 representing a molecular weight of 389.41. The chemical designation is pyrazino [1′,2′:1,6] pyrido[3,4-b] indole-1,4-dione, 6-(1,3-benzodioxol-5-yl)-2,3,6,7,12,12a hexahydro-2-methyl-, (6R,12aR)-. It is a crystalline solid that is practically insoluble in water and very slightly soluble in ethanol.

14.2.3 How Supplied

Tadalafil tablets are available in 2.5 mg, 5 mg, 10 mg and 20 mg. Excipient information may vary depending on manufacturer (particularly for generics). Consult specific product labeling for detailed information.

Appearance of the generic drug product may differ depending on the manufacturer and patients will be notified of this difference.

14.2.4 Toxicity

Very common (in 100 people receiving tadalafil, 11 may have):

- headaches

Common risks (in 100 people receiving tadalafil, more than 2 and up to 10 may have):

- stomach upset,
- pain in belly,

- diarrhea,
- nausea,
- reflux disease,
- muscle or back pain, pain in arms and legs,
- flushing (redness or warmth of the face, neck, or chest),
- flu-like symptoms (such as stuffy nose, sneezing, or sore throat),
- infection,
- cough,

Rare risks (in 100 people receiving tadalafil, 1 or fewer may have):

- fatigue,
- face swelling,
- chest pain,
- heart problems,
- low blood pressure,
- elevated liver enzyme in the blood
- dry mouth,
- hemorrhoids,
- dizziness,
- luck of sleep,
- rash,
- blurred vision, changes in color vision, eye pain, swelling around eyes
- abnormal ejaculation
- a painful or prolonged erection lasting 4 or more hours;
- sudden decreased vision (including permanent blindness, in one or both eyes);
- a sudden decrease or loss of hearing, sometimes with ringing in the ears and dizziness

14.2.5 Administration:

Please see section **3.2.2**.

14.3 Oral Vancomycin

14.3.1 Source

Vancomycin capsules will be provided by the NIH Clinical Center Pharmacy according to standard pharmacy procedures

14.3.2 Mode of Action

Vancomycin is an antibacterial drug that inhibits bacterial cell wall synthesis by blocking glycopeptide polymerization through binding tightly to D-alanyl-D-alanine portion of cell wall precursor.

14.3.3 Description

Vancomycin CAPSULES for oral administration contain chromatographically purified vancomycin hydrochloride, a tricyclic glycopeptide antibiotic derived from *Amycolatopsis orientalis* (formerly *Nocardia orientalis*), which has the chemical formula $C_{66}H_{75}Cl_2N_9O_{24}$•HCl. The molecular weight of vancomycin hydrochloride is 1485.73; 500 mg of the base is equivalent to 0.34 mmol. The capsules contain vancomycin hydrochloride equivalent to 125 mg (0.08 mmol) or 250 mg (0.17 mmol) vancomycin. The capsules also contain F D & C Blue No. 2, gelatin, iron oxide, polyethylene glycol, titanium dioxide, and other inactive ingredients.

14.3.4 How Supplied

Vancomycin capsules are available in 125 mg and 250 mg strength. Excipient information may vary depending on the manufacturer (particularly for generics). Consult specific product labeling for detailed information.

Appearance of the generic drug product may differ depending on the manufacturer and patients will be notified of this difference.

14.3.5 Administration:

Please see section **3.2.3**.

14.3.6 Storage

Store at controlled room temperature, 59° to 86°F (15° to 30°C).

15 REFERENCES

1. Llovet, J.M., et al., *Hepatocellular carcinoma.* Nat Rev Dis Primers, 2016. **2**: p. 16018.
2. Siegel, R.L., K.D. Miller, and A. Jemal, *Cancer statistics, 2016.* CA Cancer J Clin, 2016. **66**(1): p. 7-30.
3. Rein, D.B., et al., *Forecasting the morbidity and mortality associated with prevalent cases of pre-cirrhotic chronic hepatitis C in the United States.* Dig Liver Dis, 2011. **43**(1): p. 66-72.
4. Llovet, J.M., et al., *Sorafenib in advanced hepatocellular carcinoma.* N Engl J Med, 2008. **359**(4): p. 378-90.
5. Cheng, A.L., et al., *Efficacy and safety of sorafenib in patients in the Asia-Pacific region with advanced hepatocellular carcinoma: a phase III randomised, double-blind, placebo-controlled trial.* Lancet Oncol, 2009. **10**(1): p. 25-34.
6. Kudo, M., et al., *Lenvatinib versus sorafenib in first-line treatment of patients with unresectable hepatocellular carcinoma: a randomised phase 3 non-inferiority trial.* Lancet, 2018. **391**(10126): p. 1163-1173.
7. Bruix, J., et al., *Regorafenib for patients with hepatocellular carcinoma who progressed on sorafenib treatment (RESORCE): a randomised, double-blind, placebo-controlled, phase 3 trial.* Lancet, 2017. **389**(10064): p. 56-66.
8. Duffy, A.G., et al., *Tremelimumab in combination with ablation in patients with advanced hepatocellular carcinoma.* J Hepatol, 2017. **66**(3): p. 545-551.
9. El-Khoueiry, A.B., et al., *Nivolumab in patients with advanced hepatocellular carcinoma (CheckMate 040): an open-label, non-comparative, phase 1/2 dose escalation and expansion trial.* Lancet, 2017. **389**(10088): p. 2492-2502.
10. Sangro, B., et al., *A clinical trial of CTLA-4 blockade with tremelimumab in patients with hepatocellular carcinoma and chronic hepatitis C.* J Hepatol, 2013. **59**(1): p. 81-8.
11. Zhu, A.X., et al., *Pembrolizumab in patients with advanced hepatocellular carcinoma previously treated with sorafenib (KEYNOTE-224): a non-randomised, open-label phase 2 trial.* Lancet Oncol, 2018.
12. Ananthakrishnan, A., V. Gogineni, and K. Saeian, *Epidemiology of primary and secondary liver cancers.* Semin Intervent Radiol, 2006. **23**(1): p. 47-63.
13. Griffin, J.F., et al., *Patterns of failure after curative resection of pancreatic carcinoma.* Cancer, 1990. **66**(1): p. 56-61.
14. Holch, J.W., et al., *Pattern and Dynamics of Distant Metastases in Metastatic Colorectal Cancer.* Visc Med, 2017. **33**(1): p. 70-75.
15. Ryan, D.P., T.S. Hong, and N. Bardeesy, *Pancreatic adenocarcinoma.* N Engl J Med, 2014. **371**(22): p. 2140-1.
16. Turdean, S., et al., *Current data in clinicopathological characteristics of primary hepatic tumors.* Rom J Morphol Embryol, 2012. **53**(3 Suppl): p. 719-24.

17. Misiakos, E.P., N.P. Karidis, and G. Kouraklis, *Current treatment for colorectal liver metastases.* World J Gastroenterol, 2011. **17**(36): p. 4067-75.

18. Pouessel, D., et al., *Liver metastases in prostate carcinoma: clinical characteristics and outcome.* BJU Int, 2007. **99**(4): p. 807-11.

19. Bubendorf, L., et al., *Metastatic patterns of prostate cancer: an autopsy study of 1,589 patients.* Hum Pathol, 2000. **31**(5): p. 578-83.

20. de la Monte, S.M., G.W. Moore, and G.M. Hutchins, *Metastatic behavior of prostate cancer. Cluster analysis of patterns with respect to estrogen treatment.* Cancer, 1986. **58**(4): p. 985-93.

21. Tabariès, S. and P. Siegel, *Liver Metastasis: Biology and Clinical Management* Breast cancer liver metastasis. , ed. P. Brodt. 2011: Springer Science.

22. Lee, Y.T., *Patterns of metastasis and natural courses of breast carcinoma.* Cancer Metastasis Rev, 1985. **4**(2): p. 153-72.

23. Khatri, V.P., K.G. Chee, and N.J. Petrelli, *Modern multimodality approach to hepatic colorectal metastases: solutions and controversies.* Surg Oncol, 2007. **16**(1): p. 71-83.

24. Power, D.G., B.R. Healey-Bird, and N.E. Kemeny, *Regional chemotherapy for liver-limited metastatic colorectal cancer.* Clin Colorectal Cancer, 2008. **7**(4): p. 247-59.

25. Ambrosetti, M.C., G.A. Zamboni, and R.P. Mucelli, *Distribution of liver metastases based on the site of primary pancreatic carcinoma.* Eur Radiol, 2016. **26**(2): p. 306-10.

26. Overman, M.J., et al., *Nivolumab in patients with metastatic DNA mismatch repair-deficient or microsatellite instability-high colorectal cancer (CheckMate 142): an open-label, multicentre, phase 2 study.* Lancet Oncol, 2017. **18**(9): p. 1182-1191.

27. Overman, M.J., et al., *Durable Clinical Benefit With Nivolumab Plus Ipilimumab in DNA Mismatch Repair-Deficient/Microsatellite Instability-High Metastatic Colorectal Cancer.* J Clin Oncol, 2018. **36**(8): p. 773-779.

28. Chung, K.Y., et al., *Phase II study of the anti-cytotoxic T-lymphocyte-associated antigen 4 monoclonal antibody, tremelimumab, in patients with refractory metastatic colorectal cancer.* J Clin Oncol, 2010. **28**(21): p. 3485-90.

29. Topalian, S.L., et al., *Safety, activity, and immune correlates of anti-PD-1 antibody in cancer.* N Engl J Med, 2012. **366**(26): p. 2443-54.

30. Brahmer, J.R., et al., *Safety and activity of anti-PD-L1 antibody in patients with advanced cancer.* N Engl J Med, 2012. **366**(26): p. 2455-65.

31. Bendell, J.C., et al., *Safety and efficacy of MPDL3280A (anti-PDL1) in combination with bevacizumab (bev) and/or FOLFOX in patients (pts) with metastatic colorectal cancer (mCRC).* Journal of Clinical Oncology 2015. **33**(3 (Suppl)): p. 1.

32. Bendell, J.C., et al., *Clinical activity and safety of cobimetinib (cobi) and atezolizumab in colorectal cancer (CRC).* Journal of Clinical Oncology, 2016. **34**(15 (Suppl)): p. 1.

33. Smith, J.J. and M.I. D'Angelica, *Surgical management of hepatic metastases of colorectal cancer.* Hematol Oncol Clin North Am, 2015. **29**(1): p. 61-84.

34. Nordlinger, B., et al., *Perioperative FOLFOX4 chemotherapy and surgery versus surgery alone for resectable liver metastases from colorectal cancer (EORTC 40983): long-term results of a randomised, controlled, phase 3 trial.* Lancet Oncol, 2013. **14**(12): p. 1208-15.

35. Tomlinson, J.S., et al., *Actual 10-year survival after resection of colorectal liver metastases defines cure.* J Clin Oncol, 2007. **25**(29): p. 4575-80.

36. Yuan, C.H., et al., *Meta-analysis of Liver Resection Versus Nonsurgical Treatments for Pancreatic Neuroendocrine Tumors with Liver Metastases.* Ann Surg Oncol, 2016. **23**(1): p. 244-9.

37. Uggeri, F., et al., *Metastatic liver disease from non-colorectal, non-neuroendocrine, non-sarcoma cancers: a systematic review.* World J Surg Oncol, 2015. **13**: p. 191.

38. Markar, S.R., et al., *Influence of Surgical Resection of Hepatic Metastases From Gastric Adenocarcinoma on Long-term Survival: Systematic Review and Pooled Analysis.* Ann Surg, 2016. **263**(6): p. 1092-101.

39. Yu, X., et al., *Dose surgical resection of hepatic metastases bring benefits to pancreatic ductal adenocarcinoma? A systematic review and meta-analysis.* Int J Surg, 2017. **48**: p. 149-154.

40. de Jong, M.C., et al., *Rates and patterns of recurrence following curative intent surgery for colorectal liver metastasis: an international multi-institutional analysis of 1669 patients.* Ann Surg, 2009. **250**(3): p. 440-8.

41. Royal, R.E., et al., *Phase 2 trial of single agent Ipilimumab (anti-CTLA-4) for locally advanced or metastatic pancreatic adenocarcinoma.* J Immunother, 2010. **33**(8): p. 828-33.

42. Mohindra, N.A., et al., *Results of the phase Ib study of ipilimumab and gemcitabine for advanced pancreas cancer.* Journal of Clinical Oncology, 2015. **33**(15 (suppl.)): p. e15281.

43. Le, D.T., et al., *Evaluation of ipilimumab in combination with allogeneic pancreatic tumor cells transfected with a GM-CSF gene in previously treated pancreatic cancer.* J Immunother, 2013. **36**(7): p. 382-9.

44. Weiss, G.J., et al., *A phase Ib study of pembrolizumab plus chemotherapy in patients with advanced cancer (PembroPlus).* Br J Cancer, 2017. **117**(1): p. 33-40.

45. Weiss, G.J., et al., *Phase Ib/II study of gemcitabine, nab-paclitaxel, and pembrolizumab in metastatic pancreatic adenocarcinoma.* Invest New Drugs, 2018. **36**(1): p. 96-102.

46. Segal, N.H., et al., *A PHASE I MULTI-ARM DOSE-EXPANSION STUDY OF THE ANTI-PROGRAMMED CELL DEATH-LIGAND-1 (PD-L1) ANTIBODY MEDI4736: PRELIMINARY DATA.* Annals of Oncology, 2014. **25**(suppl_4): p. iv365.

47. Beatty, G.L., et al., *A phase I study of an agonist CD40 monoclonal antibody (CP-870,893) in combination with gemcitabine in patients with advanced pancreatic ductal adenocarcinoma.* Clin Cancer Res, 2013. **19**(22): p. 6286-95.

48. Rabinovich, G.A., D. Gabrilovich, and E.M. Sotomayor, *Immunosuppressive strategies that are mediated by tumor cells.* Annu Rev Immunol, 2007. **25**: p. 267-96.

49. Serafini, P., et al., *Myeloid-derived suppressor cells promote cross-tolerance in B-cell lymphoma by expanding regulatory T cells.* Cancer Res, 2008. **68**(13): p. 5439-49.

50. Arihara, F., et al., *Increase in CD14+HLA-DR -/low myeloid-derived suppressor cells in hepatocellular carcinoma patients and its impact on prognosis.* Cancer Immunol Immunother, 2013. **62**(8): p. 1421-30.

51. Ostrand-Rosenberg, S., et al., *Cross-talk between myeloid-derived suppressor cells (MDSC), macrophages, and dendritic cells enhances tumor-induced immune suppression.* Semin Cancer Biol, 2012. **22**(4): p. 275-81.

52. Hoechst, B., et al., *A new population of myeloid-derived suppressor cells in hepatocellular carcinoma patients induces CD4(+)CD25(+)Foxp3(+) T cells.* Gastroenterology, 2008. **135**(1): p. 234-43.

53. Nagaraj, S., et al., *Altered recognition of antigen is a mechanism of CD8+ T cell tolerance in cancer.* Nat Med, 2007. **13**(7): p. 828-35.

54. Li, H., et al., *Cancer-expanded myeloid-derived suppressor cells induce anergy of NK cells through membrane-bound TGF-beta 1.* J Immunol, 2009. **182**(1): p. 240-9.

55. Sakuishi, K., et al., *Emerging Tim-3 functions in antimicrobial and tumor immunity.* Trends Immunol, 2011. **32**(8): p. 345-9.

56. Hanson, E.M., et al., *Myeloid-derived suppressor cells down-regulate L-selectin expression on CD4+ and CD8+ T cells.* J Immunol, 2009. **183**(2): p. 937-44.

57. Ilkovitch, D. and D.M. Lopez, *The liver is a site for tumor-induced myeloid-derived suppressor cell accumulation and immunosuppression.* Cancer Res, 2009. **69**(13): p. 5514-21.

58. Elkabets, M., et al., *IL-1beta regulates a novel myeloid-derived suppressor cell subset that impairs NK cell development and function.* Eur J Immunol, 2010. **40**(12): p. 3347-57.

59. Gao, B. and S. Radaeva, *Natural killer and natural killer T cells in liver fibrosis.* Biochim Biophys Acta, 2013. **1832**(7): p. 1061-9.

60. Metelitsa, L.S., et al., *Expression of CD1d by myelomonocytic leukemias provides a target for cytotoxic NKT cells.* Leukemia, 2003. **17**(6): p. 1068-77.

61. Song, L., et al., *Valpha24-invariant NKT cells mediate antitumor activity via killing of tumor-associated macrophages.* J Clin Invest, 2009. **119**(6): p. 1524-36.

62. Ma, C., et al., *Gut microbiome-mediated bile acid metabolism regulates liver cancer via NKT cells.* Science, 2018. **360**(6391).

63. Xiao, Y.S., et al., *Combination of intratumoral invariant natural killer T cells and interferon-gamma is associated with prognosis of hepatocellular carcinoma after curative resection.* PLoS One, 2013. **8**(8): p. e70345.

64. Catalano, S., et al., *Expression and Function of Phosphodiesterase Type 5 in Human Breast Cancer Cell Lines and Tissues: Implications for Targeted Therapy.* Clin Cancer Res, 2016. **22**(9): p. 2271-82.

65. Piazza, G.A., et al., *Exisulind, a novel proapoptotic drug, inhibits rat urinary bladder tumorigenesis.* Cancer Res, 2001. **61**(10): p. 3961-8.

66. Pusztai, L., et al., *Phase I and II study of exisulind in combination with capecitabine in patients with metastatic breast cancer.* J Clin Oncol, 2003. **21**(18): p. 3454-61.

67. Whitehead, C.M., et al., *Exisulind-induced apoptosis in a non-small cell lung cancer orthotopic lung tumor model augments docetaxel treatment and contributes to increased survival.* Mol Cancer Ther, 2003. **2**(5): p. 479-88.

68. Barone, I., et al., *Phosphodiesterase type 5 and cancers: progress and challenges.* Oncotarget, 2017. **8**(58): p. 99179-99202.

69. Savai, R., et al., *Targeting cancer with phosphodiesterase inhibitors.* Expert Opin Investig Drugs, 2010. **19**(1): p. 117-31.

70. Califano, J.A., et al., *Tadalafil augments tumor specific immunity in patients with head and neck squamous cell carcinoma.* Clin Cancer Res, 2015. **21**(1): p. 30-8.

71. Lin, S., et al., *Phosphodiesterase-5 inhibition suppresses colonic inflammation-induced tumorigenesis via blocking the recruitment of MDSC.* Am J Cancer Res, 2017. **7**(1): p. 41-52.

72. Serafini, P., et al., *Phosphodiesterase-5 inhibition augments endogenous antitumor immunity by reducing myeloid-derived suppressor cell function.* J Exp Med, 2006. **203**(12): p. 2691-702.

73. Weed, D.T., et al., *Tadalafil reduces myeloid-derived suppressor cells and regulatory T cells and promotes tumor immunity in patients with head and neck squamous cell carcinoma.* Clin Cancer Res, 2015. **21**(1): p. 39-48.

74. 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, 2017. **6**(9): p. e1326440.

75. Noonan, K.A., et al., *Targeting immune suppression with PDE5 inhibition in end-stage multiple myeloma.* Cancer Immunol Res, 2014. **2**(8): p. 725-31.

76. Brestoff, J.R. and D. Artis, *Commensal bacteria at the interface of host metabolism and the immune system.* Nat Immunol, 2013. **14**(7): p. 676-84.

77. Filyk, H.A. and L.C. Osborne, *The Multibiome: The Intestinal Ecosystem's Influence on Immune Homeostasis, Health, and Disease.* EBioMedicine, 2016. **13**: p. 46-54.

78. Dethlefsen, L., et al., *The pervasive effects of an antibiotic on the human gut microbiota, as revealed by deep 16S rRNA sequencing.* PLoS Biol, 2008. **6**(11): p. e280.

79. Dethlefsen, L. and D.A. Relman, *Incomplete recovery and individualized responses of the human distal gut microbiota to repeated antibiotic perturbation.* Proc Natl Acad Sci U S A, 2011. **108 Suppl 1**: p. 4554-61.

80. Vrieze, A., et al., *Impact of oral vancomycin on gut microbiota, bile acid metabolism, and insulin sensitivity.* J Hepatol, 2014. **60**(4): p. 824-31.

81. Iida, N., et al., *Commensal bacteria control cancer response to therapy by modulating the tumor microenvironment.* Science, 2013. **342**(6161): p. 967-70.

82. Vetizou, M., et al., *Anticancer immunotherapy by CTLA-4 blockade relies on the gut microbiota.* Science, 2015. **350**(6264): p. 1079-84.

83. Viaud, S., et al., *The intestinal microbiota modulates the anticancer immune effects of cyclophosphamide.* Science, 2013. **342**(6161): p. 971-6.

84. Gopalakrishnan, V., et al., *Gut microbiome modulates response to anti-PD-1 immunotherapy in melanoma patients.* Science, 2018. **359**(6371): p. 97-103.

85. Routy, B., et al., *Gut microbiome influences efficacy of PD-1-based immunotherapy against epithelial tumors.* Science, 2018. **359**(6371): p. 91-97.

86. Dapito, D.H., et al., *Promotion of hepatocellular carcinoma by the intestinal microbiota and TLR4.* Cancer Cell, 2012. **21**(4): p. 504-16.

87. Yoshimoto, S., et al., *Obesity-induced gut microbial metabolite promotes liver cancer through senescence secretome.* Nature, 2013. **499**(7456): p. 97-101.

88. *Nivolumab Investigator Brochure, BMS-936558, MDX1106.* . 2013.

89. Wolchok, J.D., et al., *Nivolumab plus ipilimumab in advanced melanoma.* N Engl J Med, 2013. **369**(2): p. 122-33.

90. Brahmer, J.R., *PD-1-targeted immunotherapy: recent clinical findings.* Clin Adv Hematol Oncol, 2012. **10**(10): p. 674-5.

91. Alnouti, Y., I.L. Csanaky, and C.D. Klaassen, *Quantitative-profiling of bile acids and their conjugates in mouse liver, bile, plasma, and urine using LC-MS/MS.* J Chromatogr B Analyt Technol Biomed Life Sci, 2008. **873**(2): p. 209-17.

92. Eisenhauer, E.A., et al., *New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1).* Eur J Cancer, 2009. **45**(2): p. 228-47.

93. Wolchok, J.D., et al., *Guidelines for the evaluation of immune therapy activity in solid tumors: immune-related response criteria.* Clin Cancer Res, 2009. **15**(23): p. 7412-20.

16 APPENDICES

16.1 Appendix A: Performance Status Criteria

ECOG Performance Status Scale	
Grade	Descriptions
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.
1	Symptoms, but ambulatory. Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (e.g., light housework, office work).
2	In bed <50% of the time. Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.
5	Dead.

16.2 Appendix B: Patient's Medication Diary _____

Cycle_____ Patient's ID _____

INSTRUCTIONS TO THE PATIENT:

1. Complete one form for each cycle of treatment
2. You will take vancomycin 125 mg every 6 hours a day for 3 weeks on and 1 week off
3. You will take tadalafil 10 mg tadalafil daily.
4. Record the date, the number of tablets that you took, and when you took them.
5. If you have any comments or notice any side effects, please record them in the comments column.
6. Please bring this form and your bottles (even it is empty) when you come for your clinic visits.

Day	**Date**	**Tadalafil, time**	**Vancomycin, time**				**Comments**
1							
2							
3							
4							
5							
6							
7							
8							
9							
10							

Day	**Date**	**Tadalafil, time**	**Vancomycin, time**				**Comments**
11							
12							
13							
14							
15							
16							
17							
18							
19							
20							
21							
22							
23							
24							
25							
26							

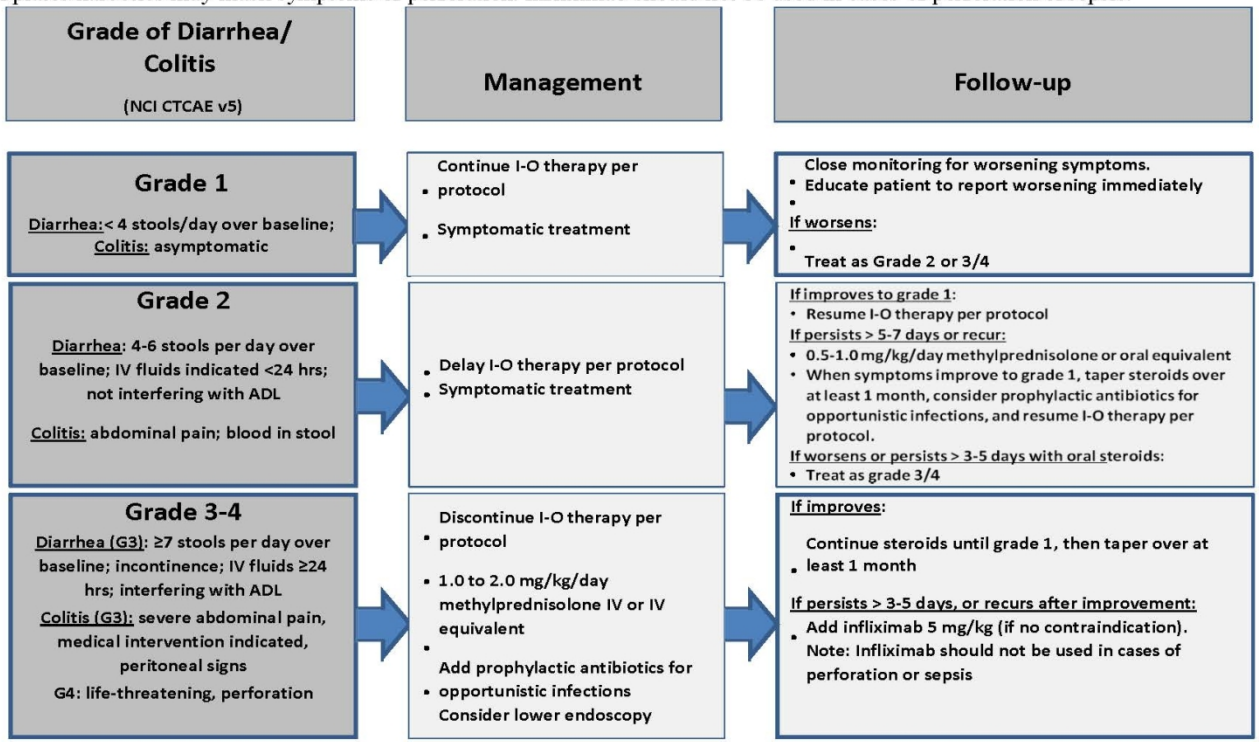
Day	**Date**	**Tadalafil, time**	**Vancomycin, time**				**Comments**
27							
28							

Patient's signature: _____

16.3 Appendix C: Management Algorithms For Endocrinopathy, Gastrointestinal, Hepatic, Neurological, Pulmonary, Renal, And Skin Adverse Events

GI Adverse Event Management Algorithm

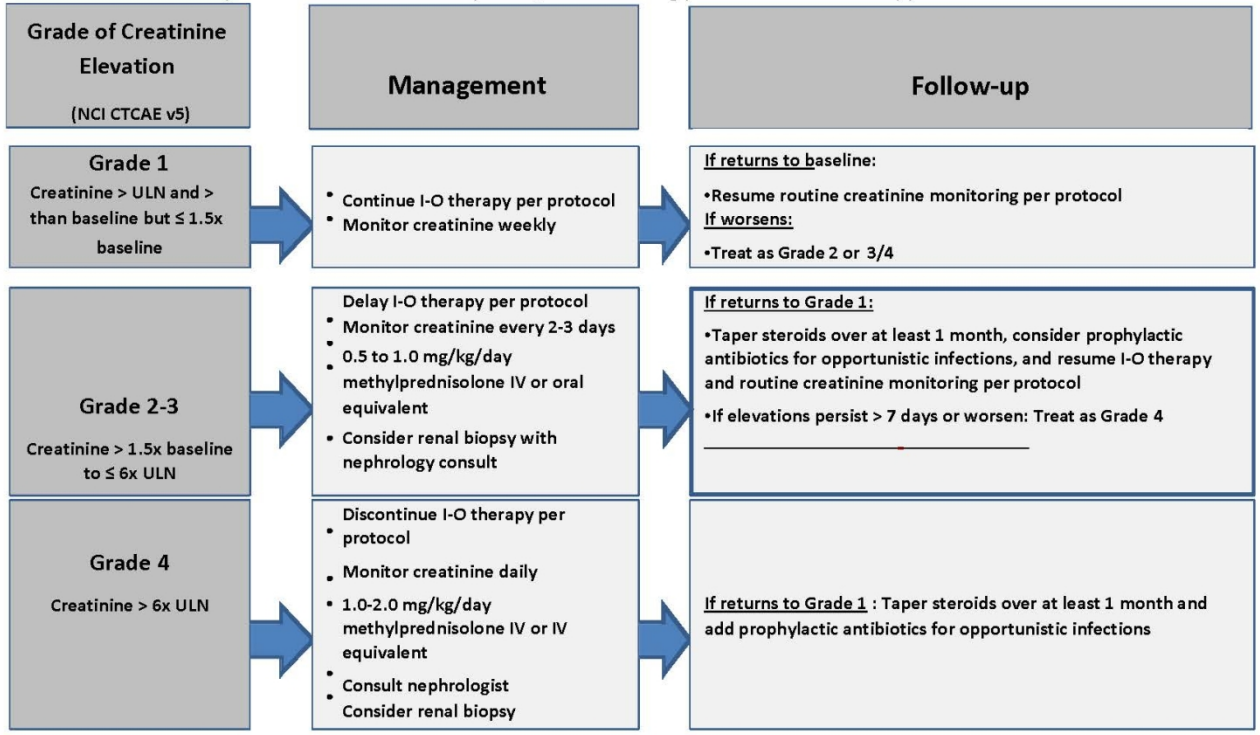
Rule out non-inflammatory causes. If non-inflammatory cause is identified, treat accordingly and continue I-O therapy.
Opiates/narcotics may mask symptoms of perforation. Infliximab should not be used in cases of perforation or sepsis.



Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (e.g. prednisone) at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of oral corticosteroids.

Renal Adverse Event Management Algorithm

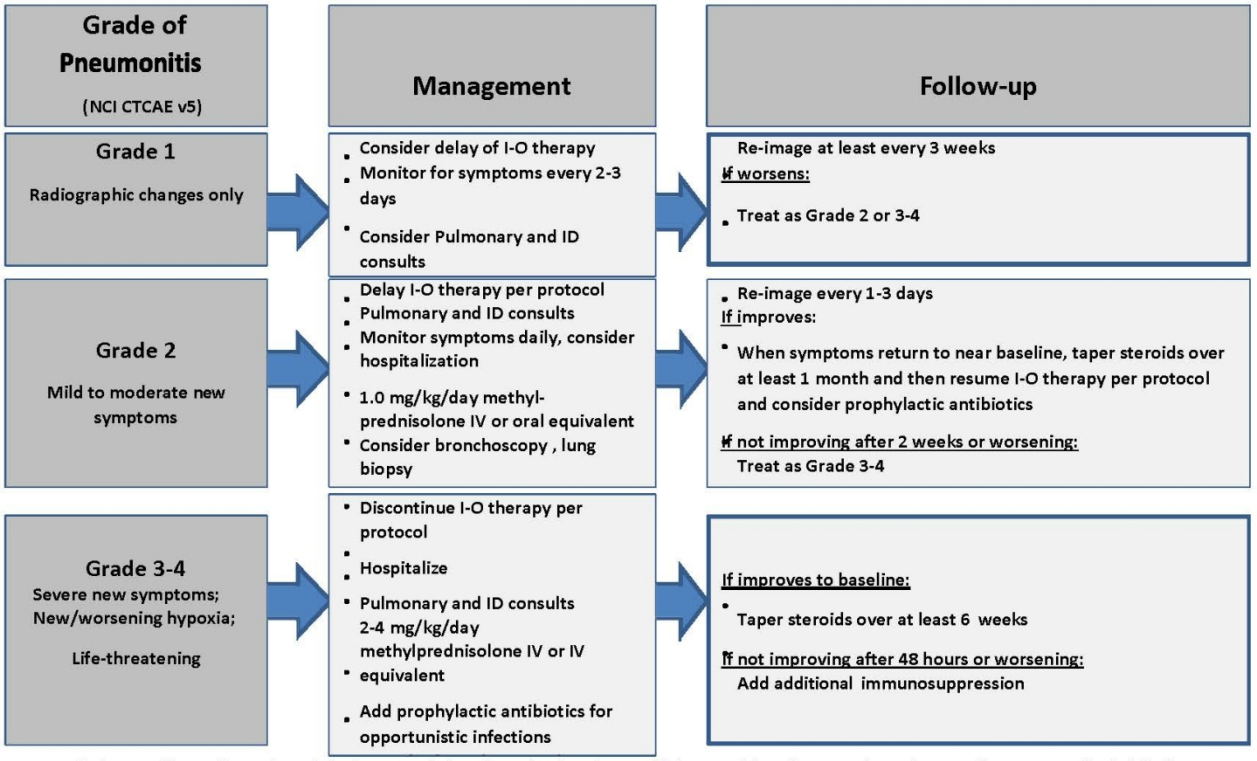
Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue I-O therapy.



Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (e.g. prednisone) at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of oral corticosteroids.

Pulmonary Adverse Event Management Algorithm

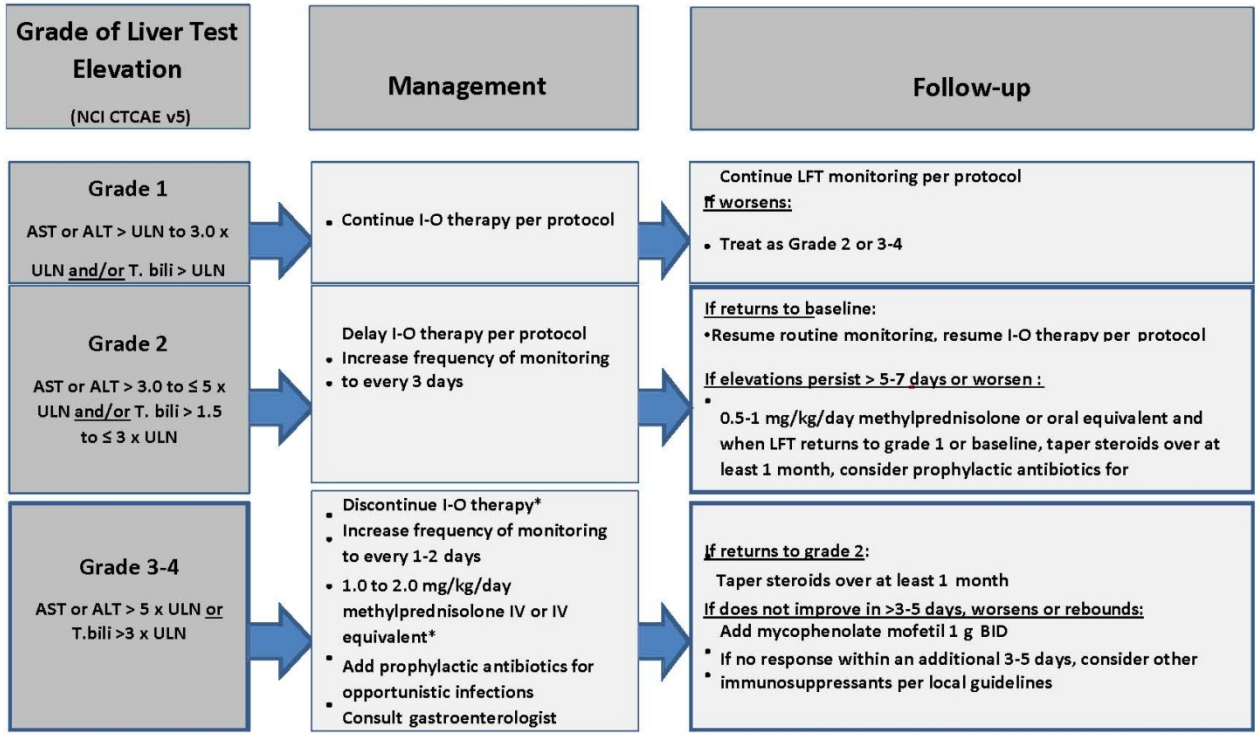
Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue I-O therapy. Evaluate with imaging and pulmonary consultation.



Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (e.g. prednisone) at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of oral corticosteroids

Hepatic Adverse Event Management Algorithm

Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue I-O therapy. Consider imaging for obstruction.

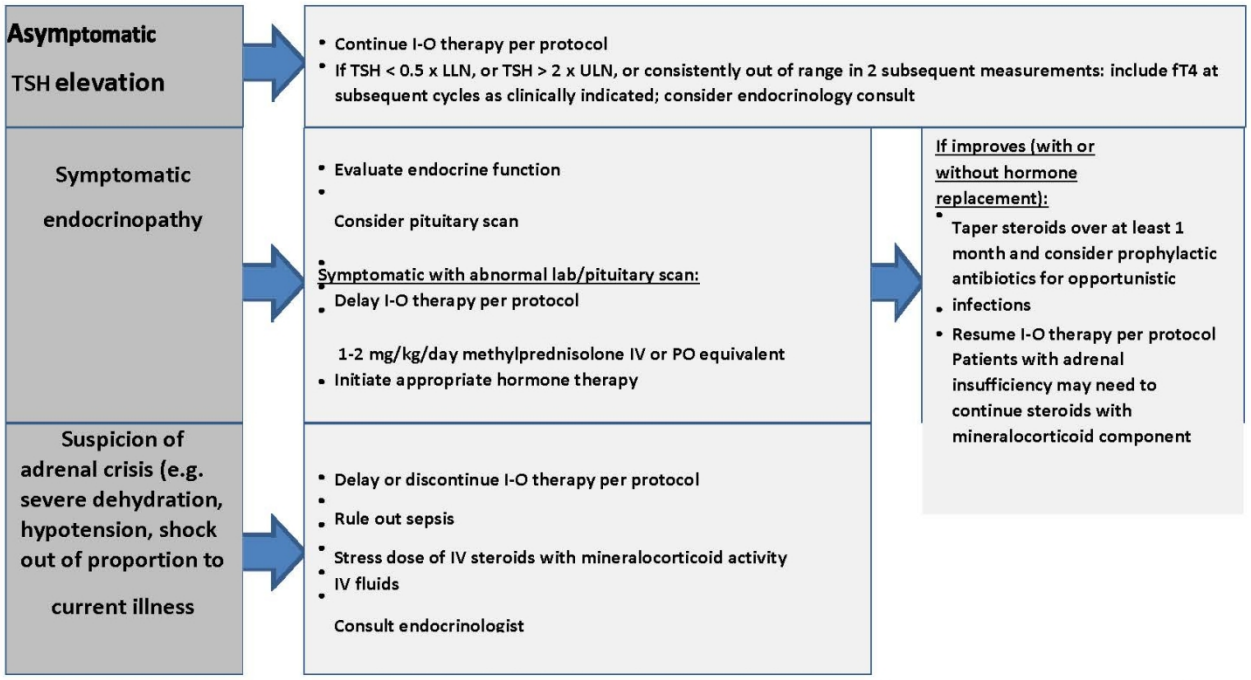


Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (e.g. prednisone) at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of oral corticosteroids.

*The recommended starting dose for grade 4 hepatitis is 2 mg/kg/day methylprednisolone IV.

Endocrinopathy Adverse Event Management Algorithm

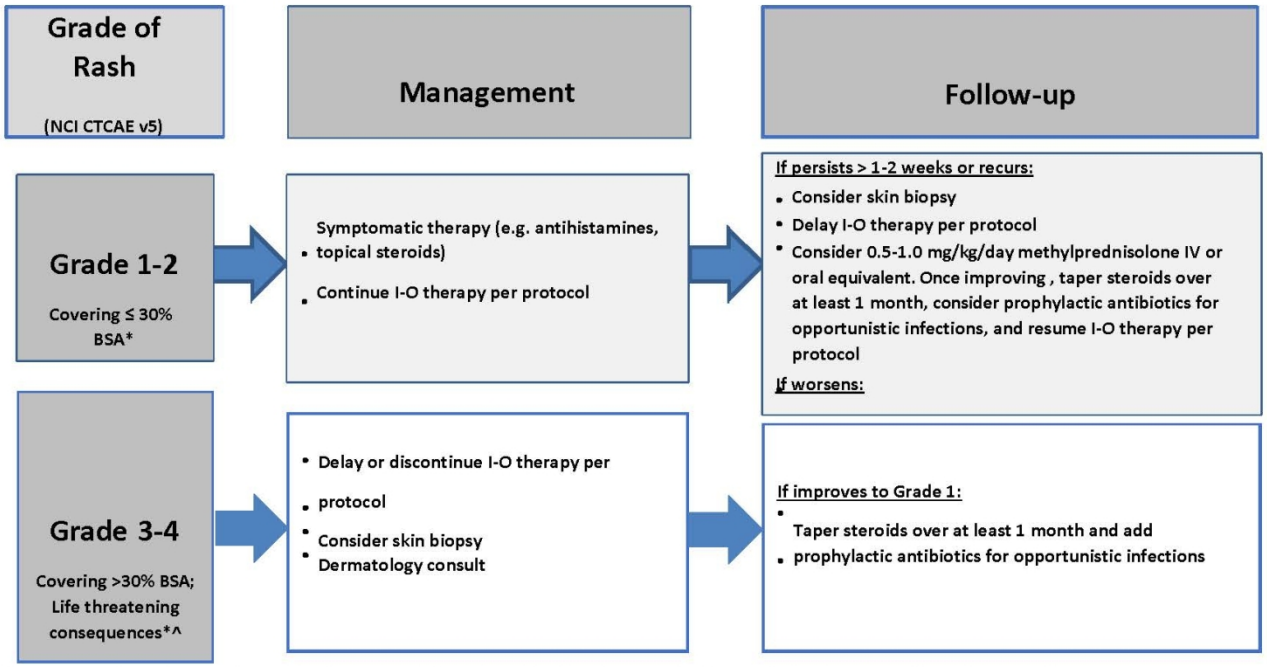
Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue I-O therapy. Consider visual field testing, endocrinology consultation, and imaging.



Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (e.g. prednisone) at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of oral corticosteroids.

Skin Adverse Event Management Algorithm

Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue I-O therapy.



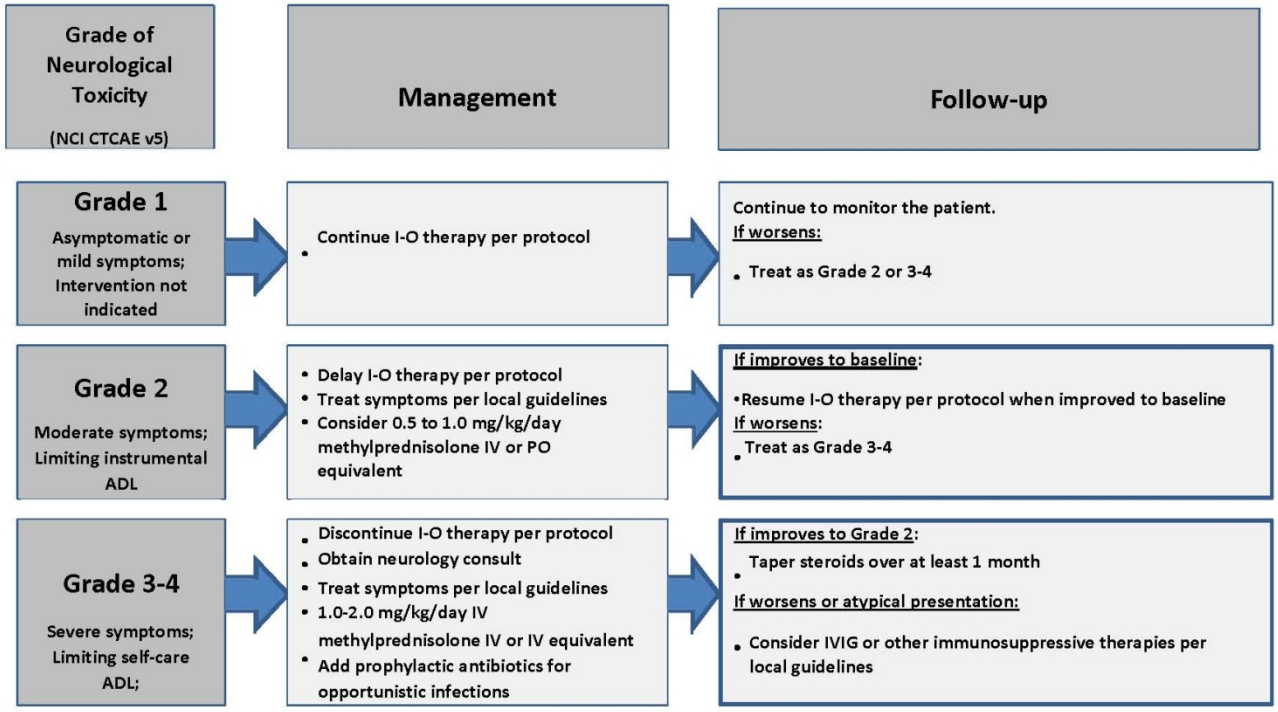
Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (e.g. prednisone) at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of oral corticosteroids.

*Refer to NCI CTCAE v5 for term-specific grading criteria.

^If SJS/TEN is suspected, withhold I-O therapy and refer patient for specialized care for assessment and treatment. If SJS or TEN is diagnosed, permanently discontinue I-O therapy.

Neurological Adverse Event Management Algorithm

Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue I-O therapy.



Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (e.g. prednisone) at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of oral corticosteroids.

16.4 Appendix D: Infusion-related Reaction Management Algorithm

Infusion-related Reactions		
Severity Grade of the Event (CTCAE 5.0	**Dose Modifications**	**Toxicity Management**
Any Grade	General Guidance	**For Any Grade:** − Manage per institutional standard at the discretion of investigator. − Monitor patients for signs and symptoms of infusion-related reactions (e.g., fever and/or shaking chills, flushing and/or itching, alterations in heart rate and blood pressure, dyspnoea or chest discomfort, or skin rashes) and anaphylaxis (e.g., generalized urticaria, angioedema, wheezing, hypotension, or tachycardia).
Grade 1 or 2	**For Grade 1:** • The infusion rate of study drug/study regimen may be decreased by 50% or temporarily interrupted until resolution of the event. **For Grade 2:** • The infusion rate of study drug/study regimen may be decreased 50% or temporarily interrupted until resolution of the event. • Subsequent infusions must be given at 50% of the initial infusion rate.	**For Grade 1 or 2:** − Acetaminophen and/or antihistamines may be administered per institutional standard at the discretion of the investigator. − Consider premedication per institutional standard prior to subsequent doses. − Steroids should not be used for routine premedication of Grade ≤2 infusion reactions.
Grade 3 or 4	**For Grade 3 or 4:** • Permanently discontinue study drug/study regimen.	**For Grade 3 or 4:** − Manage severe infusion-related reactions per institutional standards (e.g., IM epinephrine, followed by IV diphenhydramine and ranitidine, and IV glucocorticoid).

CTCAE - Common Terminology Criteria for Adverse Events; IM - Intramuscular; IV Intravenous; NCI - National Cancer Institute.

Non-immune-mediated Reactions		
Severity Grade of the Event (CTCAE 5.0)	**Dose Modifications**	**Toxicity Management**
Any Grade	• Note: Dose modifications are not required for AEs not deemed to be related to study treatment (i.e., events due to underlying disease) or for laboratory abnormalities not deemed to be clinically significant.	Treat accordingly, as per institutional standard.
Grade 1	• No dose modifications.	
Grade 2	• Hold study drug/study regimen until resolution to ≤Grade 1 or baseline.	
Grade 3	• Hold study drug/study regimen until resolution to ≤Grade 1 or baseline. • For AEs that downgrade to ≤Grade 2 within 7 days or resolve to ≤Grade 1 or baseline within 14 days, resume study drug/study regimen administration. Otherwise, discontinue study drug/study regimen.	
Grade 4	• Discontinue study drug/study regimen (Note: For Grade 4 labs, decision to discontinue should be based on accompanying clinical signs/symptoms, the Investigator's clinical judgment, and consultation with the Sponsor.).	

AE - Adverse event; CTCAE - Common Terminology Criteria for Adverse Events; NCI - National Cancer Institute.

16.5 Appendix E: Modified immune-related response criteria (irRC)

This new classification is based on the recent learning from clinical studies with cancer immunotherapies that even if some new lesions appear at the beginning of a treatment or if the total tumor burden does not increase substantially, tumor regressions or stabilizations might still occur later. The irRC were created using bi-dimensional measurements (as previously widely used in the World Health Organization criteria). For this trial, the concepts of the irRC are combined with RECIST 1.1 to come up with the modified irRC.

For modified irRC, only target and measurable lesions are taken into account. In contrast to the RECIST 1.1 criteria, the modified irRC criteria (a) require confirmation of both progression and response by imaging at 6 weeks after initial imaging and (b) do not necessarily score the appearance of new lesions as progressive disease if the sum of lesion diameters of target lesions (minimum of 10 mm per lesion, maximum of 5 target lesions, maximum of 2 per organ) and measurable new lesions does not increase by ≥ 20%.

The same method of assessment and the same technique should be used to characterize each identified and reported target lesion(s) at baseline, during the trial, and at the end of trial visit. All measurements should be recorded in metric notation. The modified irRC based on RECIST 1.1 are displayed below.

Modified immune-related response criteria are defined as follows:

New measurable lesions: Incorporated into tumor burden.

New non-measurable lesions: Do not define progression but precludes (irCR).

Overall irCR:	Complete disappearance of all lesions (whether measurable or not) and no new lesions. All measurable lymph nodes also must have a reduction in short axis to 10 mm.
Overall irPR:	Sum of the longest diameters of target and new measurable lesions decreases ≥ 30%.
Overall irSD:	Sum of the longest diameters of target and new measurable lesions neither irCR, irPR, (compared to baseline) or irPD (compared to nadir).
Overall irPD:	Sum of the longest diameters of target and new measurable lesions increases ≥ 20% (compared to nadir), confirmed by a repeat, consecutive observations at least 4 weeks (normally it should be done at 6 weeks) from the date first documented.

Overall Responses Derived from Changes in Index, Non-Index, and New Lesions

Measurable Response	Non-Measurable Response		Overall Response Using Modified irRC
Index and New, Measurable Lesions (Tumor Burden)[1]	**Non-Index Lesions**	**New, Non-Measurable Lesions**	
Decrease 100%	Absent	Absent	irCR[2]
Decrease 100%	Stable	Any	irPR[2]
Decrease 100%	Unequivocal progression	Any	irPR[2]
Decrease ≥ 30%	Absent / Stable	Any	irPR[2]
Decrease ≥ 30%	Unequivocal progression	Any	irPR[2]
Decrease < 30% to increase < 20%	Absent / Stable	Any	irSD
Decrease < 30% to increase < 20%	Unequivocal progression	Any	irSD
Increase ≥ 20%	Any	Any	irPD

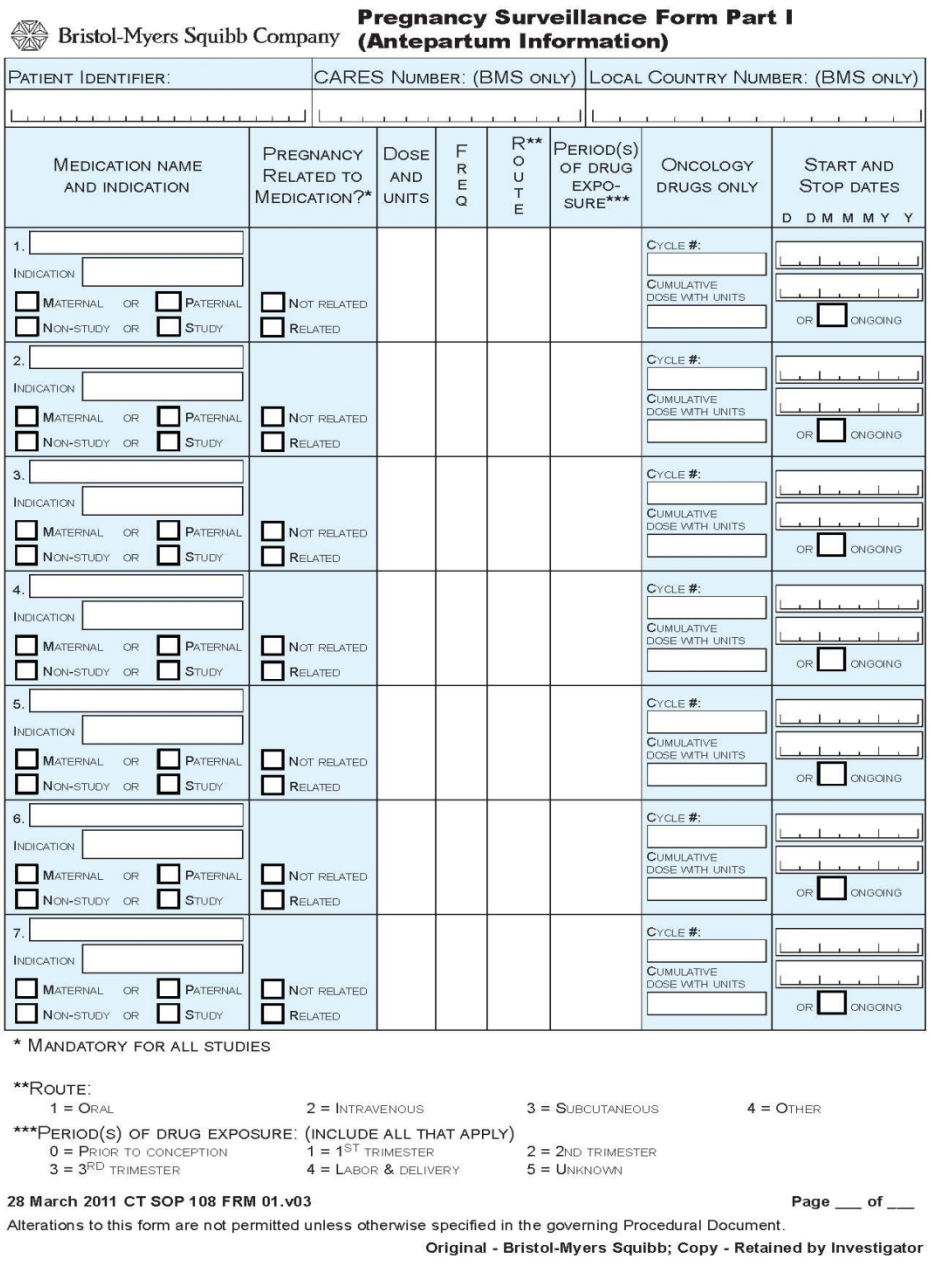
[1] Decreases assessed relative to baseline
[2] Assuming that the response (irCR and irPR) and progression (irPD) are confirmed by a second, consecutive assessment at least 4 weeks apart (normally it should be done 6 weeks apart).

16.6 Appendix F: Child-Pugh Classification System

Parameter	Points assigned		
	1	2	3
Ascites	Absent	Slight	Moderate
Bilirubin	<2 mg/dL (<34.2 micromol/liter)	2-3 mg/dL (34.2 to 51.3 micromol/liter)	>3 mg/dL (>51.3 micromol/liter)
Albumin	>3.5 g/dL (35 g/liter)	2.8-3.5 g/dL (28 to 35 g/liter)	<2.8 g/dL (<28 g/liter)
Prothrombin time			
Seconds over control	<4	4-6	>6
INR	<1.7	1.7-2.3	>2.3
Encephalopathy	None	Grade 1-2	Grade 3-4

Modified Child-Pugh classification of the severity of liver disease according to the degree of ascites, the plasma concentrations of bilirubin and albumin, the prothrombin time, and the degree of encephalopathy. A total score of 5-6 is considered grade A (well-compensated disease); 7-9 is grade B (significant functional compromise); and 10-15 is grade C (decompensated disease). These grades correlate with one- and two-year patient survival: grade A - 100 and 85 percent; grade B - 80 and 60 percent; and grade C - 45 and 35 percent.

16.7 Appendix G: Pregnancy Form



Bristol-Myers Squibb Company

Pregnancy Surveillance Form Part I (Antepartum Information)

PATIENT IDENTIFIER:	CARES NUMBER: (BMS ONLY)	LOCAL COUNTRY NUMBER: (BMS ONLY)

MEDICATION NAME AND INDICATION	PREGNANCY RELATED TO MEDICATION?*	DOSE AND UNITS	FREQ	ROUTE**	PERIOD(S) OF DRUG EXPOSURE***	ONCOLOGY DRUGS ONLY	START AND STOP DATES (D DM M M Y Y)
1. INDICATION ☐ MATERNAL OR ☐ PATERNAL ☐ NON-STUDY OR ☐ STUDY	☐ NOT RELATED ☐ RELATED					CYCLE #: CUMULATIVE DOSE WITH UNITS	OR ☐ ONGOING
2. INDICATION ☐ MATERNAL OR ☐ PATERNAL ☐ NON-STUDY OR ☐ STUDY	☐ NOT RELATED ☐ RELATED					CYCLE #: CUMULATIVE DOSE WITH UNITS	OR ☐ ONGOING
3. INDICATION ☐ MATERNAL OR ☐ PATERNAL ☐ NON-STUDY OR ☐ STUDY	☐ NOT RELATED ☐ RELATED					CYCLE #: CUMULATIVE DOSE WITH UNITS	OR ☐ ONGOING
4. INDICATION ☐ MATERNAL OR ☐ PATERNAL ☐ NON-STUDY OR ☐ STUDY	☐ NOT RELATED ☐ RELATED					CYCLE #: CUMULATIVE DOSE WITH UNITS	OR ☐ ONGOING
5. INDICATION ☐ MATERNAL OR ☐ PATERNAL ☐ NON-STUDY OR ☐ STUDY	☐ NOT RELATED ☐ RELATED					CYCLE #: CUMULATIVE DOSE WITH UNITS	OR ☐ ONGOING
6. INDICATION ☐ MATERNAL OR ☐ PATERNAL ☐ NON-STUDY OR ☐ STUDY	☐ NOT RELATED ☐ RELATED					CYCLE #: CUMULATIVE DOSE WITH UNITS	OR ☐ ONGOING
7. INDICATION ☐ MATERNAL OR ☐ PATERNAL ☐ NON-STUDY OR ☐ STUDY	☐ NOT RELATED ☐ RELATED					CYCLE #: CUMULATIVE DOSE WITH UNITS	OR ☐ ONGOING

* MANDATORY FOR ALL STUDIES

**ROUTE:
1 = ORAL 2 = INTRAVENOUS 3 = SUBCUTANEOUS 4 = OTHER

***PERIOD(S) OF DRUG EXPOSURE: (INCLUDE ALL THAT APPLY)
0 = PRIOR TO CONCEPTION 1 = 1ST TRIMESTER 2 = 2ND TRIMESTER
3 = 3RD TRIMESTER 4 = LABOR & DELIVERY 5 = UNKNOWN

28 March 2011 CT SOP 108 FRM 01.v03 **Page ___ of ___**

Alterations to this form are not permitted unless otherwise specified in the governing Procedural Document.

Original - Bristol-Myers Squibb; Copy - Retained by Investigator

Bristol-Myers Squibb Company

Pregnancy Surveillance Form Part I
(Antepartum Information)

PATIENT IDENTIFIER:	CARES NUMBER: (BMS ONLY)	LOCAL COUNTRY NUMBER: (BMS ONLY)

PRENATAL DIAGNOSTIC TESTING	BASE-LINE	DATE D D M M M Y Y	TEST RESULTS UNITS	NORMAL RANGE LOW	NORMAL RANGE HIGH
	☐				
	☐				
	☐				
	☐				
	☐				
	☐				
	☐				

DESCRIBE RESULTS IN DETAIL, IF APPLICABLE:

REPORTER INFORMATION: ☐ BMS STUDY INVESTIGATOR ☐ NON-BMS STUDY SPONSOR ☐ OTHER*

*QUALIFICATION: (COMPLETE ONLY IF "OTHER" IS CHECKED)

☐ PHYSICIAN ☐ PHARMACIST ☐ NURSE/NURSE PRACTITIONER ☐ OTHER HEALTH PROFESSIONAL
☐ CONSUMER ☐ ATTORNEY ☐ OTHER NON-HEALTH PROFESSIONAL

PERSON COMPLETING THE FORM (IF DIFFERENT FROM INVESTIGATOR/SPONSOR) :

PRINTED NAME

SIGNATURE

DATE: D D M M M Y Y

INSTITUTION/ORGANIZATION:

STREET ADDRESS: | CITY: | STATE/PROVINCE:

POST CODE: | COUNTRY: | PHONE NUMBER:

INVESTIGATOR/SPONSOR/OTHER:

LAST NAME

FIRST NAME | MIDDLE INITIAL

SIGNATURE:

DATE: D D M M M Y Y

Bristol-Myers Squibb Company

Pregnancy Surveillance Form Part II (Pregnancy Outcome)

PATIENT IDENTIFIER:	CARES NUMBER: (BMS ONLY)	LOCAL COUNTRY NUMBER: (BMS ONLY)

PREGNANCY OUTCOME:

MODE OF DELIVERY :

LABOR/DELIVERY COMPLICATIONS ☐ NO ☐ YES*

IF YES, SPECIFY

☐ SINGLE GESTATION ☐ MULTIPLE GESTATION (# ☐ of ☐)
COMPLETE AN OUTCOME FORM FOR EACH FETUS/INFANT

DATE PREGNANCY ENDED: D D M M M Y Y

GESTATIONAL AGE AT OUTCOME ☐☐ WEEKS ☐ UNKNOWN

ASSESSED BY: ☐ OBSTETRICAL DATES ☐ FETUS/INFANT PHYSICAL EXAM

DID OBSTETRICAL COMPLICATIONS OR MATERNAL/PATERNAL MEDICAL CONDITIONS OCCUR DURING THIS PREGNANCY ?
☐ NO ☐ YES*
☐ UNKNOWN IF YES, SPECIFY:

*FOR ANY COMPLICATIONS NOTED ABOVE, REPORT THE ADVERSE EVENT APPROPRIATELY (FOR STUDIES, REFER TO STUDY-SPECIFIC INSTRUCTIONS)

GENDER:	BIRTH WEIGHT:	BIRTH LENGTH:	HEAD CIRCUMFERENCE:	APGAR SCORE:
☐ MALE ☐ FEMALE ☐ UNKNOWN	☐☐ / ☐☐ lbs/oz ☐☐☐☐ grams	☐ inches ☐ cm	☐ inches ☐ cm	1 MIN. ☐☐ 5 MIN. ☐☐

☐ LIVE BIRTH NORMAL (PROCEED TO PART III)

☐ LIVE BIRTH ABNORMAL ☐ FETAL DEATH ☐ NEONATAL DEATH (IF ANY ARE CHECKED, COMPLETE SECTIONS BELOW)

☐ PRE-TERM ☐ TERM ☐ POST TERM
☐ SMALL FOR GESTATIONAL AGE
☐ INTRAUTERINE GROWTH RETARDATION
☐ DRUG WITHDRAWAL SYNDROME IN THE NEONATE
☐ MALFORMATION (SPECIFY BELOW)
☐ POST-NATAL/NEONATAL COMPLICATIONS (E.G. PERINATAL ASPHYXIA, INFECTION, RESPIRATORY DISTRESS) (SPECIFY):

FETAL DEATH
☐ ECTOPIC ☐ MISCARRIAGE/SPONTANEOUS ABORTION ☐ STILLBIRTH
☐ INDUCED ABORTION/ELECTIVE TERMINATION
AUTOPSY/PATHOLOGY REPORT ☐ NO ☐ YES ☐ UNKNOWN

NEONATAL DEATH:
CAUSE : DATE: D D M M M Y Y

PLACENTAL ABNORMALITIES ☐ NO ☐ YES ☐ UNKNOWN
IF YES, SPECIFY:
PATHOLOGY REPORT AVAILABLE ☐ NO ☐ YES ☐ UNKNOWN

FAMILY HISTORY OF CONGENITAL ABNORMALITIES/BIRTH DEFECTS:
☐ NO ☐ YES ☐ UNKNOWN
IF YES, SPECIFY:

PRIOR PREGNANCIES WITH CONGENITAL ABNORMALITIES/BIRTH DEFECTS: ☐ NO ☐ YES
IF YES, SPECIFY #/TYPE :

PRIOR STILLBIRTHS: ☐ NO ☐ YES
IF YES, SPECIFY # :

PRIOR SPONTANEOUS ABORTIONS: ☐ NO ☐ YES
IF YES, SPECIFY #:

SPECIFY ANY PRIOR PREGNANCY COMPLICATIONS:

HISTORY OF FERTILITY TREATMENTS (E.G. IVF):
☐ NO ☐ YES IF YES, SPECIFY:

DESCRIBE ANY CONGENITAL MALFORMATIONS/ABNORMALITIES, STRUCTURAL DEFECTS AND OTHER FETAL/NEONATAL COMPLICATIONS:

CAUSALITY (MANDATORY FOR STUDIES)

IN THE INVESTIGATOR'S OPINION, WAS THE DEFECT/MEDICAL PROBLEM RELATED TO MEDICATION UNDER STUDY ? : ☐ NOT RELATED ☐ RELATED
IF RELATED, PLEASE COMMENT ON SPECIFIC EVENT(S) AND MEDICATION(S) BELOW:
IF NOT RELATED, INDICATE WHAT THE DEFECT/MEDICAL PROBLEM WAS ATTRIBUTED TO:

Bristol-Myers Squibb Company

Pregnancy Surveillance Form Part III (Infant Follow-up)

PATIENT IDENTIFIER:	CARES NUMBER: (BMS ONLY)	LOCAL COUNTRY NUMBER: (BMS ONLY)

CURRENT INFANT AGE: ☐ AGE UNITS: ☐ DAYS ☐ WEEKS ☐ MONTHS

☐ NO PROBLEMS ☐ MEDICAL PROBLEMS NOTED (SPECIFY AND DESCRIBE FINDINGS AND/OR PLANNED EVALUATIONS; E.G. DIAGNOSTIC TESTING, CONSULTATIONS, ETC)

CAUSALITY (MANDATORY FOR ALL STUDIES): IN THE INVESTIGATOR'S OPINION WERE ANY PROBLEMS NOTED ABOVE RELATED TO THE MEDICATION UNDER STUDY ? ☐ NOT RELATED ☐ RELATED (PLEASE SPECIFY):

MATERNAL BREASTFEEDING: ☐ NO ☐ YES HOW LONG:

MATERNAL DRUGS TAKEN WHILE BREASTFEEDING: ☐ NO ☐ YES (IF YES, SPECIFY)

REPORTER INFORMATION: ☐ BMS STUDY INVESTIGATOR ☐ NON-BMS STUDY SPONSOR ☐ OTHER*

*QUALIFICATION: (COMPLETE ONLY IF "OTHER" IS CHECKED)

☐ PHYSICIAN ☐ PHARMACIST ☐ NURSE/NURSE PRACTITIONER ☐ OTHER HEALTH PROFESSIONAL
☐ CONSUMER ☐ ATTORNEY ☐ OTHER NON-HEALTH PROFESSIONAL

PERSON COMPLETING THE FORM (IF DIFFERENT FROM INVESTIGATOR/SPONSOR) :

PRINTED NAME

SIGNATURE

DATE: D D M M M Y Y

INSTITUTION/ORGANIZATION:

STREET ADDRESS:	CITY:	STATE/PROVINCE:

POST CODE:	COUNTRY:	PHONE NUMBER:

INVESTIGATOR/SPONSOR/OTHER:

LAST NAME

FIRST NAME MIDDLE INITIAL

SIGNATURE:

DATE: D D M M M Y Y

16.8 Appendix H: Food Diary for Mixed Meal Study

19C0033	PLACE	TIME	FOODS AND BEVERAGES	AMOUNT	COMPLETE DESCRIPTION	REVIEWER'S COMMENTS
NAME:						
Day of Week:						
Date: / /						
Is this a typical day? __yes __no If no, give reason:						
Daytime phone: ( )						
Birthdate of patient: / /						
Age of patient: ___ years Male Female						
Study ID number: __ __ __ __ __ __ __						
C1D0						
C1D10						

RD/HT reviewer: _____

16.9 Appendix I : Mixed Meal PK Sheet

19C0033 Nivolumab Tadalifil & Vancomycin

Drink: Boost Vanilla Plus

Amount Consumed: _____

Mixed meal test for bile acids in blood

(Time 0 is prior to the drink, all other time points are after completion of the drink)

Date: _____ Clock used: _____

Baseline: _____ C1D10: _____

Time Interval (Minutes)	Schedule Time (Military)	Actual Time (Military)	Comments	Signature	Reason if missed
0					
30					
60					
90					
120					
150					
180					

5 ml Sodium Heparin green top tube (GTT) at every time interval

Page Dr. Figg's lab at 102-11964 for specimen pickup