Phase II Trial of TG4010 plus Nivolumab in Previously Treated Patients with Metastatic Non-Small Cell Lung Cancer (NSCLC)

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PROTOCOL SIGNATURE PAGE

Protocol Number: UCDCC#263

Protocol Title: Phase II Trial of TG4010 plus Nivolumab in Previously Treated Patients with Metastatic Non-Small Cell Lung Cancer (NSCLC)

I have read this protocol and agree that it contains all necessary details for carrying out this study. I will conduct the study as outlined herein and will complete the study within the time designated, in accordance with all stipulations of the protocol and in accordance with Good Clinical Practices, local regulatory requirements, and the Declaration of Helsinki.

I will provide copies of the protocol and all pertinent information to all individuals responsible to me who assist in the conduct of this study. I will discuss this material with them to ensure that they are fully informed regarding the study agent(s) and the conduct of the study.

_____
Investigator Name (print)

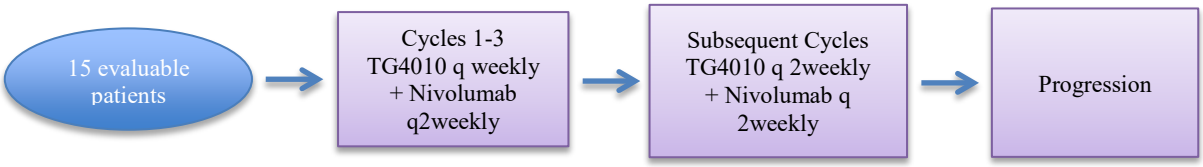
_____
Investigator Signature

_____
Institution Name

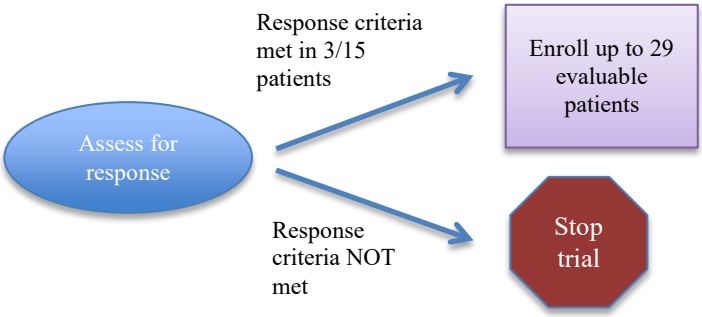
_____
Date

OVERALL SCHEMA

Summary of treatment schedule



Interim and final analysis



Patients will be enrolled in an initial cohort (Interim analysis, n=15 evaluable patients). Patients will be treated with TG4010 weekly (D1) and nivolumab q 2weekly (D1), for a total of 3 cycles, each cycle is 14 days. In subsequent cycles, patients will be treated with TG4010 q 2weekly (D1) and nivolumab q 2weekly (D1). All patients will be treated until progression, intolerable toxicity, or unwillingness to continue therapy. This initial cohort will be assessed for an objective response.

If 3 responses occur, the study will continue and a total of 33 patients will be accrued to the trial with plan for 29 evaluable patients. If < 3 responses are observed the trial will be stopped.

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List of Abbreviations

ADCC	Antibody-dependent cell mediated cytotoxicity
ADL	Activities of daily living
AE	Adverse event
ALK	Anaplastic lymphoma kinase
ALT	Alanine aminotransferase/serum glutamic pyruvic transaminase/SGPT
AST	Aspartate aminotransferase/serum glutamic oxaloacetic transaminase/SGOT
CNS	Central nervous system
CTCAE	Common Terminology Criteria for Adverse Events
CRF	Case Report/Record Form
CRP	C-reactive protein
DCR	Disease control rate
EBV	Epstein Barr virus
ECG	Electrocardiogram
EGFR	Epidermal growth factor receptor
FACS	Fluorescence-activated cell sorting
FDA	U.S. Food and Drug Administration
FFPE	Formalin fixed paraffin embedded
FNA	Fine needle aspiration
GMO	Genetically Modified Organism
Hr	Hour
HBsAg	Hepatitis B surface antigen
HBV	Hepatitis B virus
HCV	Hepatitis C virus
i.v.	Intravenous(ly)
ICH	International Conference on Harmonization
IEC	Independent Ethics Committee
IHC	Immunohistochemical
IND	Investigational New Drug
IRB	Institutional Review Board
irRC	Immune related Response Criteria
LVEF	left ventricular ejection fraction
LFTs	Liver function tests
MDRD	Modification of Diet in Renal Disease
MDSC	Myeloid derived suppressor cells
MRI	Magnetic resonance imaging
NSCLC	Non small cell lung cancer
ORR	Objective response rate
OS	Overall survival
PBMC	Peripheral blood mononuclear cell
PD-1	Programmed death-1
PD-L1	Programmed death-ligand 1
PFS	Progressive-free survival
qPCR	Quantitative polymerase chain reaction
REB	Research Ethics Board

RECIST	Response Evaluation Criteria in Solid Tumors
SAE	Serious Adverse Event
SD	Stable disease
SPD	Sum of the products of the two largest perpendicular diameters
TAA	Tumor associated antigens
TNBK	T, B, and natural killer
TNF	Tumor necrosis factor
TNM	Tumor-node-metastasis
TrPAL	Triple positive activated lymphocytes
TSH	Thyroid-stimulating hormone
TTP	Time to progression
SOP	Standard Operating Procedure
ULN	Upper limit of normal

1.0 Background

1.1 Advanced Stage Non-Small Cell Lung Cancer

Lung cancer is the leading cause of cancer deaths worldwide due to the majority of patients presenting with incurable, metastatic disease. In recent years, our increased understanding of the molecular biology of lung cancer has led to the development of more efficacious therapies for patients with advanced stage non-small cell lung cancer (NSCLC). As a result, overall survival has modestly increased by several months with a median survival of 1 year and small subsets of patients with an EGFR mutation or ALK gene fusion tumor enjoy prolonged survival beyond two years with targeted tyrosine kinase inhibitors (1). Nonetheless, there remains a great need to discover and develop novel agents that will improve survival for our patients.

Immunotherapy, particularly blockade of checkpoint molecules such as CTLA-4, PD-1 and PD-L1, is rapidly transforming cancer care demonstrating antitumor activity across a spectrum of solid tumors and hematological malignancies. However the number of responders is relatively low as discussed below approximately 20%. One possible explanation for this finding is the potent immunosuppressive effects of established tumors. Furthermore, it has been repeatedly demonstrated that poorly antigenic tumors or tumors with a poor T cell infiltrate are less likely to respond to checkpoint inhibitors (2).Thus, combinations of immune therapies that target distinct steps of tumor immunity may result in stronger and more durable responses (3). The purpose of vaccines in the setting of cancer is to induce tumor-specific antigen responses in non-tumor environments, where the immune system is more likely to be functional. This, in turn, induces innate and adaptive immunity specific to an antigen or epitope, leading to the development of tumor-infiltrating lymphocytes. The presence of tumor infiltrating lymphocytes, increased by such vaccines, could enhance the response of checkpoint inhibitors. There are several steps in the cancer immunity cycle that can be exploited to increase an anticancer immune response (4). This study seeks to focus on two steps: the facilitation of neoantigen presentation to dendritic cells for subsequent T cell activation and the activity of the T-cells inside the tumor by removing local suppression.

1.2 Nivolumab

Nivolumab is a fully human IgG4-blocking monoclonal antibody directed against PD-1 (programmed death receptor-1). This immune checkpoint inhibitor antibody binds PD-1 on activated immune cells to disrupt the interaction between PD-1 and PD-L1, PD-L2 ligands. Preclinical data has demonstrated that inhibition of the interaction between PD-1 and PD-L1 can enhance T-cell responses and mediate antitumor activity in vitro (5, 6).

A Phase I trial of nivolumab evaluated dosing, schedule, and safety in patients with advanced melanoma, non-small cell lung cancer, castration-resistant prostate cancer, renal cell cancer or colorectal cancer (7). Of the 296 patients enrolled into the trial, 122 had non-small cell lung cancer. In the entire cohort studied, the most common treatment-related adverse events were fatigue, rash, diarrhea, pruritus, decreased appetite, and nausea. In 14% of patients, Grade 3 or 4 treatment-related adverse events were observed. Of particular note, drug-related adverse events associated with immune suppression were observed, and these included pneumonitis, vitiligo,

colitis, hepatitis, hypophysitis, and thyroiditis. Across histologic subtypes of NSCLC and dose levels there were 22 objective responses (18%). These objective responses (OR) spanned squamous and non-squamous histologic subtypes. An additional 13 patients (10%) had stable disease (SD) for $\geq$ 24 weeks. The progression-free survival (PFS) was 2.3 months (8). The median survival was subsequently reported to be 9.9 months with a 1 and 2 year overall survival (OS) rate 42% and 24%, respectively (8).

The promising results of this trial led to Checkmate 063, a Phase II, single-arm, multi-center study evaluating the activity and safety of nivolumab studied patients with advanced, refractory squamous NSCLC. A total of 117 patients were enrolled in the trial, and received nivolumab 3 mg/kg IV every 2 weeks until progression or intolerable adverse effects. Of these, 17 patients (14.5%) had an objective response with median time to response of 3.3 months, and the median duration of response was not reached. In addition, 30 (26%) patients had stable disease with a median duration of 6.0 months. In this study, Grade 3-4 treatment-related adverse events were reported in 20 (17%) patients. Fatigue (4%), pneumonitis (3%) and diarrhea (3%) comprised these Grade 3-4 toxicities. Two treatment-associated deaths were also reported, caused by pneumonia and ischemic stroke (9).

Most recently, nivolumab has been FDA approved for the treatment of patients with metastatic squamous non-small cell lung cancer with progression on or after a platinum-based chemotherapy regimen. This approval was based on results of a Phase III trial, Checkmate-017, in which nivolumab (3 mg/kg IV every 2 weeks) was compared to docetaxel (75 mg/m2 IV every 3 weeks) (10). A total of 272 patients were randomized to the study. A 41% reduction in risk of death (HR: 0.59, 95% CI: 0.44-0.79) was observed with nivolumab, with the median overall survival in the nivolumab arm was 9.2 months (95% confidence interval [CI], 7.3 to 13.3) compared to 6 months (95% CI, 5.1 to 7.3) in the docetaxel arm. The confirmed objective response rate was significantly higher with nivolumab than with docetaxel (20% [95% CI, 14 to 28] vs 9% [95% CI, 5 to 15]; p=0.008). The median duration of response was not reached in the nivolumab arm. Median progression free survival was 3.5 months (95% CI, 2.1 to 4.9) in the nivolumab group and 2.8 months (95% CI, 2.1 to 3.5) in the docetaxel group (HR for death or disease progression of 0.62; 95% CI 0.47 to 0.81; p<0.001). The most frequently reported treatment-related adverse events with nivolumab were fatigue (16% of patients), decreased appetite (11% of patients), asthenia (10%). Adverse events of special interest were hypothyroidism (4% with nivolumab vs 0% with docetaxel), diarrhea (8% vs 20%), pneumonitis (5% vs 0%), increased blood creatinine level (3% vs 2%) and rash (4% vs 6%). Three treatment-related select Grade 3 adverse events were reported in the nivolumab group- one case each of tubulointerstitial nephritis, colitis, and pneumonitis (11).

In nonsquamous histology the Phase III open-label, randomized CheckMate-057 trial evaluating previously treated patients was stopped early as the study met its endpoint of overall survival favoring nivolumab. Median overall survival was 12.2 months in the nivolumab arm vs 9.4 months in the docetaxel arm (HR= 0.73 [96% CI: 0.59-0.89], p = 0.0015). There was not a statistically significant difference in progression-free survival in the two arms. However, an overall response rate of 19% (95% CI 15-24) was noted in the nivolumab arm (N=292) vs 12% (95% CI 9-17) in the docetaxel arm (N=290). Median duration of response in this trial was 17.2 months (range of 1.8 months to >22.6 months) in the nivolumab arm vs 5.6 months (>1.2 months

to >15.2 months). In the nivolumab arm, 7% had treatment-related SAEs with 5% Grade 3-4 AEs, while in the docetaxel arm 20% treatment-related SAEs were reported with 18% Grade 3-4 AEs. In the nivolumab arm, treatment-related AEs led to discontinuation in 5% of patients, while in the docetaxel arm 15% of treatment-related AEs of any grade led to discontinuation from the trial. Regarding treatment-related select AEs, hypothyroidism occurred in 7% of patients in the nivolumab, though there were no Grade 3-4 endocrinopathies. Diarrhea was observed in 8% of patients with nivolumab, with 1 Grade 3/4 toxicity; diarrhea was noted at 23% in the docetaxel arm with 1 Grade 3/4 toxicity. Transaminitis was noted in 3% of patients in the nivolumab arm v 1% in the docetaxel arm. Pneumonitis was observed in 3% of nivolumab-treated patients, with 1% Grade 3/4 toxicity; <1% of patients in the docetaxel arm experienced pneumonitis. Skin rashes were observed with a rash in 9% of nivolumab-treated patients, pruritus in 8%, and erythema in 1%. In docetaxel-treated patients, rash was reported in 3% of patients, pruritus in 1%, erythema in 4%. Infusion-related reactions were observed in 3% of patients in both the docetaxel and nivolumab arms (12). Based on these results, nivolumab has demonstrated antitumor activity with an acceptable toxicity profile in NSCLC patients with nonsquamous histology (11, 12).

1.3 TG4010

TG4010 is a modified, Vaccinia virus Ankara (MVA), a non-propagative significantly attenuated Vaccinia virus strain, that contains sequences coding for the human MUC1 (human mucin 1) antigen and interleukin 2 (IL-2) (13). MVA is a highly attenuated strain of vaccinia virus that does not integrate or propagate in most mammalian cells. It has an extensive safety history. MUC1 was selected as the tumor antigen target because it is overexpressed in many cancers. Importantly MUC1 covers most of the tumor cell surface. MUC1 is aberrantly glycosylated in cancer cells compared to normal cells. This hypoglycosylation makes MUC1 more vulnerable to processing by the proteasome in APCs (antigen presenting cells) and tumor cells. As a result MUC1 peptides (epitopes) are presented on the tumor cell surface by MHC class 1 molecules. In lung cancer MUC1 expression by immunohistochemistry is 80% (14, 15). In other solid tumors MUC1 is expressed on 90% of breast and liver cancers, 80% in stomach cancer, 70% in colorectal cancer, and 60% in prostate cancer examined (14,15). IL-2 is a potent stimulator of T and NK cells and is included in this vector to solicit a local effect. The mechanism of action of TG4010 is to induce MUC1 antigen expression in a non-tumor environment with a functional immune system to 1) induce MUC1 specific immunity via MUC1 tumor antigen presentation to T cells through MHC class I and II molecules and 2) to induce a nonspecific activation of the immune system via the vaccine virus and IL-2.

Over 500 patients have been enrolled in clinical studies of TG4010 (16). In the Phase I dose escalation studies, 13 patients received repeated IM (intramuscular) injections of TG4010 at doses of 5.0×10^6, 5.0×10^7, or 1.0×10^8 PFU (plaque forming units). TG4010 was well tolerated up to the highest dose level. The adverse reactions were typical of a vaccine therapy with local injection site reaction being most common. Three trials have been conducted in patients with NSCLC. The first trial in untreated patients with advanced lung cancer and MUC1 expressing tumors randomized patients to receive TG4010 1.0×10^8 PFU subcutaneously (SC) weekly over 6 weeks then once every 3 weeks or to TG4010 plus navelbine (25 mg/m^2, D1 and 8) and cisplatin (100 mg/m^2, D1) every 21 days for 6 cycles or until disease progression (16).

Patients in the monotherapy arm were allowed to add navelbine and cisplatin upon progression. In the TG4010 alone arm no responses were observed in 21 patients. Two patients (10%) had stable disease up to 211 days. Fourteen patients proceeded to the combination phase. Two patients achieved a response (14%). Forty-four patients were enrolled in the immediate combination arm. 13/37 evaluable patients (35.1%) achieved an ORR and 25/37 (67%) patients had disease control for > 3 months. The median time to progression (TTP) was 4.8 months and OS (overall survival) was 12.7 months. The combination arm exceeded its primary endpoint of an ORR in at least 11 in 33 evaluable patients suggesting that this combination may improve outcome for patients with advanced NSCLC.

A randomized Phase IIB trial was launched comparing gemcitabine and cisplatin for 6 cycles plus TG4010 until progression or gemcitabine and cisplatin (17). Patients were required to have advanced disease, no previous therapy and a MUC1 expressing tumor. The primary endpoint was a 6 month progression free survival (PFS) rate of greater than 40%. A total of 148 patients were enrolled. The patients were predominantly male (72%) and had adenocarcinoma (65%). Table 1 shows the combination met its primary endpoint and demonstrated a favorable ORR. TTP and OS were numerical higher in the combination arm.

Table 1. Efficacy of chemotherapy ± TG4010

Efficacy Parameter	TG4010 + Chemotherapy (N=74)	Chemotherapy (N=74)
Response rate	42%	28%
PFS at 6 months	43%	35%
Median TTP	5.9 months	5.2 months
Median OS	10.7 months	10.3 months

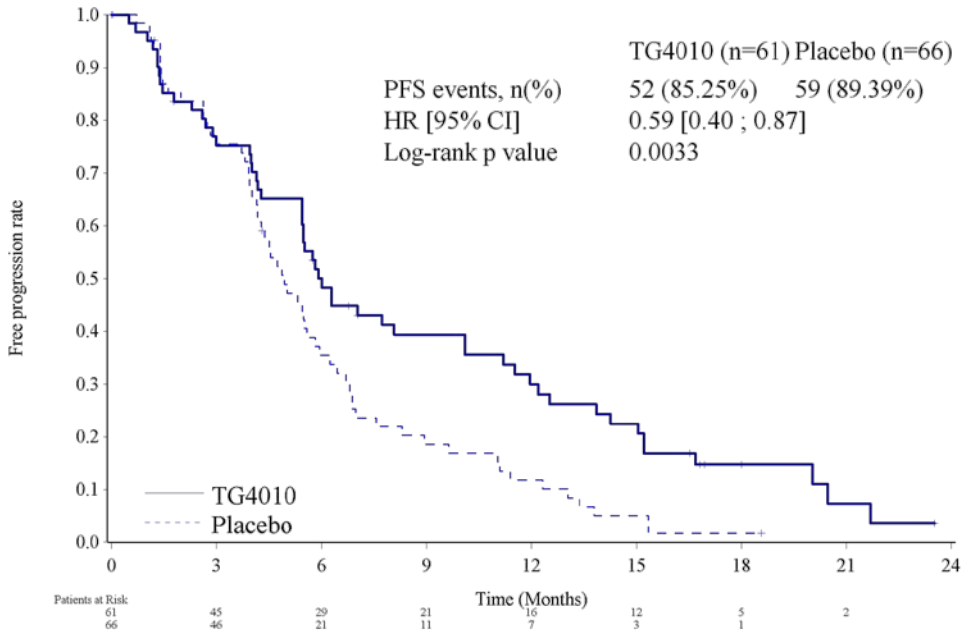
1.4 Correlative Studies

An analysis of the immunological data identified the pretreatment level of CD16+CD56+CD69+ lymphocytes (called TrPAL for Triple Positive Activated Lymphocytes) as a potential predictive biomarker of TG4010 safety and efficacy. Patients with normal baseline levels of TrPAL had a significant improvement in RR, PFS, TTP and OS when treated with TG4010 plus chemotherapy at 54%, 56%, 6.3 mos and 17.1 mos compared to 28%, 38%, 4.7 mos and 11.3 mos with chemotherapy alone. In contrast, patients with a high level of TrPAL had a significant decrease in RR, shorter PFS, TTP, and OS with the TG4010 regimen versus chemotherapy alone. These poorer outcomes were associated with an increase in the number of serious adverse events. These results may seem counterintuitive in that one would have predicted a higher level of TrPAL to be beneficial. However, evidence suggests that beyond a given threshold of immune activity, activated NK and NKT cells can limit the magnitude of the adaptive immune response by secreting IL-10 and killing dendritic cells, macrophages, and effector lymphocytes through direct cytotoxicity (18). Hence the immune response is decreased and tumors grow.

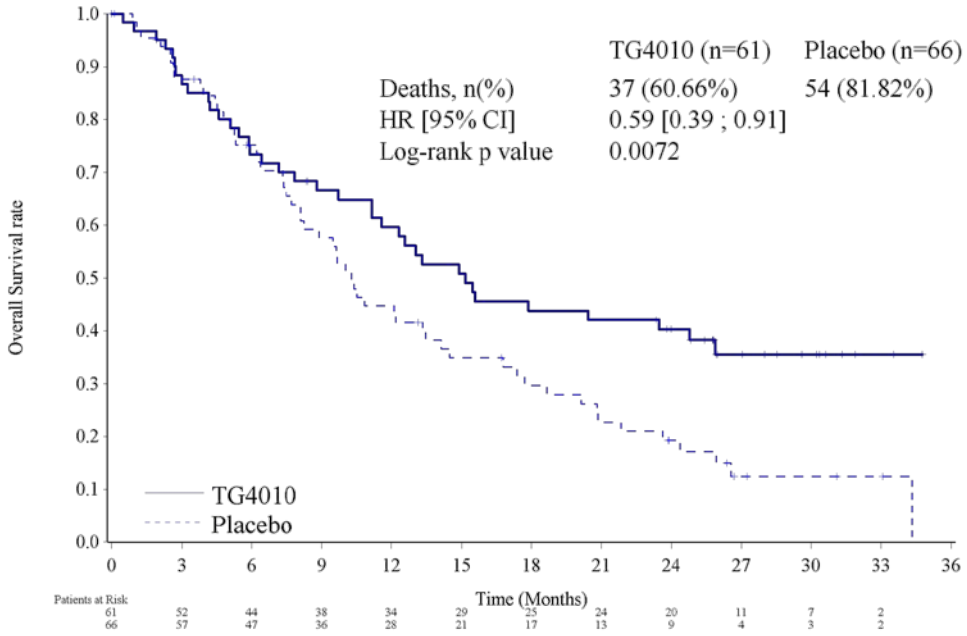
The promising results from this trial and the identification of a potential predictive biomarker of activity led to a randomized, placebo-controlled Phase IIB/III trial (TIME trial) of patients with previously untreated Stage IV NSCLC. The primary objective of the phase IIB part was to

validate the biomarker using PFS with a Bayesian probability that the Hazard Ratio (HR) of < 1 is > 95%. Patients had their TrPAL status determined to allow their classification in the normal or above normal TrPAL cohort. Within each cohort (normal TrPAL cohort 170 patients and above normal TrPAL cohort 52 patients, respectively) patients were then randomized 1:1using a dynamic allocation to TG4010 + chemotherapy or placebo + chemotherapy. In patients with normal TrPAL levels, the PFS HR was 0.75 (95% CI 0.54-1.03) with a posterior probability (HR < 1) of 98.4% in patients treated with TG4010 compared with placebo. In the above normal TrPAL group, the HR for PFS was 0.77 (CI 0.42-1.40) with a posterior probability (HR < 1) of 68.7% and posterior probability (HR > 1) of 31.3%. In preplanned subgroup analyses there was a statistical significant PFS advantage for the TG4010 regimen in patients with nonsquamous histology in the overall population (N=196, HR=0.69; 95% CI 0.51-0.94; p=0.0093) and in nonsquamous patients with lowTrPAL (N=127, HR=0.59; 95% CI 0.40-0.87, p=0.0033). Overall survival also favors the TG4010 arm: patients with non-squamous histology had a median overall survival of 14.6 months (95% CI 11.1-20.4) vs 10.8 months (95% CI 9.5-14.5) with placebo with a HR of 0.73 (95% CI 0.52 – 1.01 p=0.030). In the low TrPAL group, median OS was 15.1 months (95% CI: 11.1 – 25.9) vs 10.3 months (95% CI: 8.1 – 14.1) in the placebo group, with a HR of 0.59 (95% CI: 0.39 - 0.91), p=0.0.0072. In the high TrPAL group, no difference was seen in PFS or OS in patients with non-squamous histology. The occurrence of adverse events, severe AEs and Serious AEs were similar in both arms of the study except there were more injection site reactions in the TG4010 arm at 32.7% as compared to 3.7% in the placebo arm. No grade 3 or 4 injection site reactions were observed. The phase III portion of the trial is planned (19).

PFS for Patients with Low TrPAL, Non-Squamous Tumors



OS for Patients with Low TrPAL, Non-Squamous Tumors



1.5 Risks of TG4010 treatment

TG4010 has been reported to be well tolerated in all clinical trials completed to date. Overall, 380 patients have been treated with TG4010. In 270 patients (excluding the TIME study) treated injection site reactions were the most common adverse event (26%). No grade 3 or 4 injection site reactions have occurred. Other mild to moderate adverse events occurring in> 5% of patients included fatigue (20.7%), injection site erythema (12.2%) pyrexia (9.3%), injection site pain (7.8%), injection site induration (6.3%), influenza like illness (5.9%) and myalgias (5.5%). Specific risks associated with each component of TG4010 are described below. Further information about risks is available in the IB (15).

1.5.1 Risks associated with MVA

Immunization with MVA

For patients who have been immunized previously against smallpox with a vaccinia virus preparation (i.e., Elstree or Wyeth), the use of a cancer vaccine based on the MVA strain may pose even less of an issue, since previous vaccination provides a further element of safety related to pre-immunization }. For those patients who have not been vaccinated against smallpox, the use of this MVA construct should carry at most the same risk-benefit profile as smallpox vaccination, particularly considering the enhanced safety profile of MVA compared with other strains of vaccinia virus. Live vaccinia virus such as Dryvax and ACAM2000 used to vaccinate preventively against smallpox in the frame of a military program in the US (for bioterrorism

preparedness) has shown rare cardiac side effects such as myo-or pericarditis. There is today no such observation related to MVA, however a cardiac monitoring is implemented in this study.

Possible adverse effects related to MVA
According to a field study carried out on over 7,000 primary vaccines receiving an Intra-dermal (ID) MVA strain (vaccines included children under and over the age of 3 years), the most frequent adverse events (AEs) observed were local reaction (redness), fever (2% of vaccines), and 'flu-like' symptoms (4% of vaccines). Contrary to what had been observed with other vaccinia strains, fewer adverse reactions were reported during the vaccination campaign with MVA. Two more recent studies also showed that MVA has an acceptable safety profile in vaccinia-naïve and vaccinia-immune volunteers.

1.5.2 Risks associated with immunization against MUC1

Potential autoimmune toxicity from TG4010 related to cross reactivity with natural MUC1 protein cannot be ruled out. However, no evidence of autoimmune phenomena against MUC1-expressing tissues/organs was reported in the animal toxicity studies performed with TG4010 as well as in the clinical trials performed to date. During the clinical development of TG4010, autoimmunity parameters, *e.g.,* anti-DNA antibodies, anti-thyroid stimulating hormone, and anti-Thyroid Peroxydase (TPO) antibodies, were measured in patients with no clinically significant change.

1.5.3 Risks associated with induction of IL2 expression

IL2 protein, administered systemically, has been associated with a variety of toxicities. These toxicities have severely limited the therapeutic utility of systemic IL2. However, IL2 protein expressed by TG4010 from the human IL2 coding sequence appears to be localized at the site of injection and expressed at a low level, and there has been no evidence of systemic IL2 toxicity associated with TG4010 during toxicology studies and clinical trials.

1.5.4 Risks associated with viral dissemination of TG4010

Similar to the MVA wild type virus, TG4010 is a non-propagative and highly attenuated recombinant virus, poorly replicative in most mammalian cells. These properties support the non-spreading character of this recombinant virus which was confirmed in patients injected with TG4010 and monitored for viral shedding in blood and urine.

Indeed, viral dissemination was monitored by Polymerase Chain Reaction (PCR) using blood and urine samples from the 13 patients treated with TG4010 in Phase I studies. The limit of detection was around 90 plaque forming units (PFU)/mL in blood samples and around 400 PFU/mL in urine samples. No presence of the viral genome was detected by PCR in the blood or urine samples analyzed.

In Phase II studies, 81 patients (41 patients with NSCLC [TG4010.05 study] and 40 patients with prostate cancer [TG4010.03 study]) were monitored for viral dissemination. Using more sensitive assays, the urine and blood samples were all negative for the presence of viral DNA measured by PCR or nested-PCR. The limit of quantification was 5 PFU/300 µL in blood and 25 PFU/250 µL in the urine for TG4010.05 and 25 PFU/500 µL in blood and 100 PFU/280 µL in the urine for TG4010.03. However, as shown from the biodistribution data obtained in the rat, TG4010 viral DNA was present at the injection site 48 hours after the first injection in all animals tested, whereas it was present only in a very few samples outside the injection site (2 out of 210 samples were positive outside the injection site). These data confirm the low capacity for viral dissemination of this MVA vector which remains localized to the injection site up to the death of the infected cells.

Based on this observation, the injection site can be considered as a potential route by which TG4010 might be envisaged to come into contact with human beings other than the intended patient or enter the environment. Further evaluations were performed in the Phase IIb part of TG4010.14 study. Shedding data obtained by swabbing at the injection site were collected in a subset of patients. Viral DNA was quantifiable 6 hours after TG4010 injection but in very low concentration as compared to the injected dose, representing a very low risk of dissemination in the environment.

Overall these data confirm that spreading of the vector is confined to the injection site. This is consistent with MVA characteristics of being a non-propagative, non-integrative and poorly replicative virus.

In summary TG4010 has demonstrated consistent efficacy with a mild toxicity profile in patients with advanced NSCLC.

1.6 Combination therapy (TG4010 and immune checkpoint inhibitors)

We hypothesize that due to the different immune mechanisms of action of TG4010 and nivolumab, the combination will enhance the immune cellular response and lead to an increase in antitumor activity over nivolumab alone. Preclinical data have also recently been reported which support this hypothesis. CT26-MUC1 cell lines have been developed that have been demonstrated to result in MUC1+ subcutaneous and lung tumors. BALB/c mice were injected subcutaneously with CT26-MUC1 cells. On Days 2 and 9, mice were treated subcutaneously with TG4010 or an empty MVA vector. On Days 10, 13, 15, and 17, mice received anti-PD-1 (IgG2a, BioXCell). At Day 33, 60% of mice treated with the combination of TG4010 + anti-PD-1 therapy were tumor-free in this model compared to 20% in those treated with single agent TG4010 and 40% in the arm treated with single agent anti-PD-1 therapy (20).

In the most recent results communicated on the Phase IIB portion of the TIME trial, PD-L1 expression was measured in 160 of the 222 patients randomized to the trial. 138 of these patients had non-squamous cancer of the lung, and 70% of these patients had <5% PD-L1 expression. In these patients with low PD-L1 expression, median progression-free survival was 5.7 months (95% CI 4.4 – 8.3) in the TG4010 arm vs 4.2 months (95% CI 3.9 – 5.5), which was statistically significant (HR=0.64 [95% CI 0.41-0.99]; p = 0.024). In these same patients median OS was

15.6 months versus 10.8 months (HR = 0.65 [0.41-1.05]; p=0.039). The finding that TG4010 does have evidence of activity in patients with low PD-L1 expression supports the hypothesis that it may enhance activity in combination with immune checkpoint inhibitors (19).

This Phase II trial is designed to evaluate the safety and efficacy of this combination in previously treated patients. If promising results were seen, a randomized trial of the combination versus nivolumab would be pursued. Patients will not be selected based on TrPAL levels but TrPAL will be evaluated along with a host of immune assays.

2.0 Study Objectives and Endpoints

Primary Objectives

To evaluate the efficacy of nivolumab plus TG4010 in previously treated patients with Stage IV non squamous NSCLC with respect to Objective Response Rate (ORR) by RECIST 1.1.

Secondary Objectives

- Define the safety and toxicity profile of nivolumab plus TG4010 by CTCAE v4.
- Determine progression-free survival by RECIST 1.1.
- Determine overall survival
- Determine the duration of response and the occurrence of responses over time
- Determine the rate and duration of stable disease
- Determine the disease control rate

Exploratory Objectives

Imaging

- Determine ORR, DOR and PFS by irRC.

Tissue

a) IHC to assess tumor infiltrating immune cells (CD8, CD4, FoxP3) and expression of other markers with potential prognostic and/or predictive value on efficacy outcomes including MUC-1 and PD-L1 as well as new biomarkers.
b) qRT-PCR evaluation of gene signatures in the tumor microenvironment including: cytokines, T-cell activation markers, immunosuppressive enzymes and molecules (IDO, arginase, CTLA4, PD-1/PD-L1), macrophage polarization, etc.
c) RNAseq for identification of tumor neo-antigens
d) Flow Cytometry quantification, immunophenotyping, and activation / functional assessment of tumor infiltrating immune cells including myeloid-derived suppressor cells (MDSC), regulatory T (Treg) cells, T/B/NK cell immunophenotyping and activated T cells

Blood

a) Flow cytometry assessment of Natural Killer (NK) cells and Triple Positive Activated Lymphocytes (TrPAL) levels in order to analyze their value as a predictive biomarker of TG4010 activity in patients who received prior chemotherapy
b) Flow Cytometry quantification, immunophenotyping, and activation / functional assessment of tumor infiltrating immune cells including myeloid-derived suppressor cells (MDSC), regulatory T (Treg) cells, T/B/NK cell immunophenotyping and activated T cells
c) Evaluation of MUC-1, MVA, known Tumor Associated Antigens (TAA) and neo-antigens specific T-cell responses using a HLA-A*02:01 restricted tetramers
d) Evaluation of MUC-1 and MVA specific humoral responses
e) qRT-PCR evaluation of gene signatures in circulating cells including: cytokines, T-cell activation markers, immunosuppressive enzymes and molecules (IDO, arginase, CTLA4, PD-1/PD-L1), macrophage polarization, etc.
f) Peripheral blood cytokine / chemokine profiling

Table 2. Study Endpoints

Endpoints	Methods
Primary Endpoint:	
Objective Response Rate	RECIST 1.1
Secondary Endpoints:	
Safety and Toxicity	CTCAE v4.0
Progression Free Survival	RECIST 1.1
Overall Survival	Survival Follow-up
Duration of Response	RECIST 1.1
Stable Disease Rate	RECIST 1.1
Disease Control Rate	RECIST 1.1
Exploratory Endpoints:	
Imaging	
ORR, DOR and PFS	irRC
Tissue	
Tumor evaluation for MUC-1, PD-L1, CD8, CD4 and FOXP3 expression	IHC
Gene expression signatures in the tumor micro-environment	qRT-PCR
Identification of tumor neo-antigens	RNAseq
Quantification, immunophenotyping, and activation/functional assessment of tumor infiltrating cells including myeloid-derived suppressor cells (MDSC), regulatory T (Treg) cells, T/B/NK cell immunophenotyping and activated T cells	Flow cytometry
Blood (serum or PBMCs)	
Blood levels of NK cells and TrPAL	Flow cytometry
Quantification, immunophenotyping, and activation / functional assessment of circulating immune cells including myeloid-derived suppressor cells (MDSC), regulatory T (Treg) cells, T/B/NK cell immunophenotyping and activated T cells	Flow cytometry
Evaluation of MUC-1, MVA, known TAA and neo-antigen specific T-cell responses	Tetramers by Flow cytometry
MUC-1 and MVA specific humoral responses	ELISA
Gene expression signatures of circulating cells	qRT-PCR
Immune-related soluble factors and cytokines profiling	Multiplex assay

3.0 Overview of Study Design

This is a phase II, multi-center, single arm, open-label study evaluating the combination of TG4010 plus nivolumab in previously treated patients with metastatic NSCLC.

3.1 Treatment Schedule

Agent	Dose	Route	Schedule
Nivolumab	240 mg	IV	Every 2 weeks
TG4010	10^8 PFU	SC	Weekly x 6 then every 2 weeks

Please see section 5.0 for treatment administration details.

3.2 Treatment Duration

Patients will continue on therapy until one of the following criteria applies:

- Disease progression (unless potential pseudo-progression as described below)
- Intercurrent illness that prevents further administration of treatment
- Unacceptable adverse events
- Patient decides to withdraw from the study
- General or specific changes in the patient's condition render the patient unacceptable for further treatment in the judgment of the investigator
- Pregnancy
- Delay in treatment > 42 days due to toxicity
- Failure of patient to adhere to study requirements

In case, TG4010 or Nivolumab is stopped due to toxicity, the other drug will continue as planned per protocol.

Patients who show evidence of clinical benefit will be permitted to continue nivolumab and TG4010 after RECIST v1.1 criteria for progressive disease are met if they meet all of the following criteria:

- Evidence of clinical benefit as assessed by the investigator
- Absence of symptoms and signs (including worsening of laboratory values, e.g., new or
- worsening hypercalcemia) indicating unequivocal progression of disease
- No decline in ECOG performance status that can be attributed to disease progression
- Absence of tumor progression at critical anatomical sites (e.g., leptomeningeal disease) that cannot be managed by protocol-allowed medical interventions
- Patients must sign a new consent form.

For all treated patients, the maximum treatment duration will be 2 years.

3.3 Overall Study Plan

The overall study plan will consist of a screening period, a study treatment phase, a PFS follow up phase (if applicable), a safety follow-up and a survival follow-up. For safety, all patients will be followed for 100 days after the last dose of study treatment or until all treatment related clinical significant toxicities resolve to ≤ grade 1. Survival status (date of death or date of last contact) will be recorded.

3.4 Duration of Follow-Up

Patients will be followed up to 2 years for efficacy after removal from study or death, whichever occurs first. Patients removed from the study for unacceptable adverse event(s) will be followed for safety until resolution or stabilization of the adverse event, and in any case as for any adverse event for a minimal duration of 100 days after the last dose of study treatment.

4.0 Subject Selection

Patients will be recruited in the medical oncology clinics of the University of California Davis Medical Center and participating centers. The minimum number of patients enrolled on this phase II study is 15 patients. Assuming 10% loss to follow up or withdrawal of consent, a total of 33 patients will be included. The best overall response at interim analysis will be assessed in 15 evaluable patients (17 patients included). If 3 or more patients have an objective response, accrual will continue up to 33 patients to have 29 evaluable patients.

4.1 Inclusion Criteria

Patients must satisfy all of the following criteria for entry into the protocol:

1. Male or female patients, age ≥ 18 years old
2. Histologically confirmed non-squamous NSCLC. Patients with adenocarcinoma must have had EGFR and ALK mutational testing. Those with an actionable mutations/rearrangements are excluded.
3. Stage IIIB or IV patients must have progressed after a platinum based chemotherapy; a maximum of 3 previous systemic regimens are allowed (one regimen can be a tyrosine kinase inhibitor). Patients with Stage I-IIIB NSCLC who have progressed within 6 months of a full dose platinum based regimen as adjuvant therapy or with radiotherapy are eligible. Patients who received weekly low dose chemotherapy with radiation only are not eligible.
4. At least one measurable lesion by CT scan or MRI based on RECIST version 1.1
5. PS 0 or 1 on the ECOG scale (see Appendix).
6. Minimum life expectancy of 3 months.

7. Adequate hematological, hepatic, and renal function:
   - Hemoglobin $\geq$ 10.0 g/dL
   - White blood cells (WBC) $\geq$ 3.0 x 10^9/L including:
     - Neutrophils $\geq$ 1.5 x 10^9/L
     - Total lymphocyte count $\geq$ 0.5 x 10^9/L
   - Platelet counts $\geq$ 100 x 10^9/L
   - Serum alkaline phosphatase $\leq$ 3 x ULN in absence of liver or bone metastases and $\leq$ 5 x ULN in patients with documented bone or liver metastases
   - Total bilirubin $\leq$ 1.5 x ULN
   - Serum transaminases (alanine aminotransferase [ALT] and aspartate aminotransferase [AST]) $\leq$ 2.5 x ULN in the absence of liver metastases and $\leq$ 5 x ULN in case of liver metastases
   - Glomerular Filtration Rate $\geq$ 60 mL/min (according to MDRD formula or Cockroft & Gault formula)
   - Serum albumin $\geq$ 30 g/L
8. Effective contraception during the study period and for 5 months after the last study treatment administration (male and female patient)
9. Subjects with Type I diabetes mellitus, hypothyroidism only requiring hormone replacement, skin disorders (such as vitiligo, psoriasis, alopecia) not requiring systemic treatment, or conditions not expected to recur in the absence of an external trigger are permitted to enroll
11. Ability to understand and the willingness to sign a written informed consent

4.2 Exclusion Criteria

Patients will be excluded from the study for any of the following reasons:

1. Patients having active Central Nervous System (CNS) metastases. Patients adequately treated and neurologically returned to baseline (except for residual signs of symptoms related to the CNS treated) for at least 2 weeks prior to enrolment are allowed. In addition, patients must be either off corticosteroids or on a stable or decreasing dose of < 10 mg daily prednisone or equivalent.
2. Prior exposure to cancer immunotherapy including any immune checkpoint inhibitor and/or cancer vaccines
3. Prior history of other malignancy except:
   - Basal cell carcinoma of skin
   - Cervical intra-epithelial neoplasia
   - Other cancer curatively treated with no evidence of disease for at least 2 years

4. Patients under chronic treatment with systemic corticosteroids or other immunosuppressive drugs (eg cyclosporine) for a period of at least 4 weeks and whose treatment was not stopped 1 week prior to start of the study treatment (D1 of Cycle 1)
5. Positive serology for Human Immunodeficiency Virus (HIV) or Hepatitis C Virus (HCV); presence in the serum of the antigen HBs
6. Patient with any underlying medical condition that the treating physician considers might be aggravated by treatment or which is not controlled (e.g. elevated troponin or creatinine, uncontrolled diabetes)
7. Patients with major surgery or radiotherapy within 4 weeks prior to the start of the study treatment (ie D1 of Cycle 1). However, prior surgery or radiation therapy aimed at local palliation or attempted local disease control (except in case of thoracic radiotherapy) is permitted but has to be completed one week before treatment start.
8. Pregnant or nursing (lactating) women, where pregnancy is defined as the state of a female after conception and until the termination of gestation, confirmed by a positive hCG laboratory test (> 10 mIU/mL). Pregnancy is ruled out by a beta hCG test completed if necessary with an ultrasound as false positive results can be seen due to paraneoplastic syndrome.
9. Women of child-bearing potential, defined as all women physiologically capable of becoming pregnant, UNLESS they are:
    - Women whose sexual orientation precludes intercourse with a male partner
    - Women whose partners have been sterilized by vasectomy or other means
    - Using a highly effective method of birth control (ie one that results in a less than 1% per year failure rate when used consistently and correctly, such as implants, injectables, combined oral contraceptives, and some intrauterine devices [IUDs]; periodic abstinence (eg calendar, ovulation, symptothermal, post-ovulation methods) is not acceptable)
10. Patient with an organ allograft
11. Known allergy to eggs, gentamicin, or platinum containing compounds
12. Hypersensitivity to the active substance or to any of the excipients
13. Participation in a clinical study with an investigational product within 4 weeks prior to the start of the study treatment (ie D1 of Cycle 1)
14. Patient unable or unwilling to comply with the protocol requirements
15. Subject has active, known or suspected autoimmune disease, including systemic lupus erythematodes, Hashimoto thyroiditis, scleroderma, polyarteritis nodosa, or autoimmune hepatitis.
16. Subject has any peripheral neuropathy $\geq$ NCI CTCAE Grade 2 at enrollment
17. Subject has a history of interstitial lung disease, history of slowly progressive dyspnea and unproductive cough, sarcoidosis, silicosis, idiopathic pulmonary fibrosis, pulmonary hypersensitivity pneumonitis or multiple allergies. Any lung disease that may interfere with the detection or management of suspected drug-related pulmonary toxicity.

18. History of any of the following cardiovascular conditions within 12 months of enrollment: cardiac angioplasty or stenting, myocardial infarction, unstable angina, coronary artery by-pass graft surgery, symptomatic peripheral vascular disease, class III or IV congestive heart failure, as defined by the New York Heart Association.
19. Left ventricular ejection fraction (LVEF) less than the LLN as assessed by echocardiography.

5.0 Study Treatment

Treatment will be administered on an outpatient basis.

5.1 Nivolumab

5.1.1 Formulation, Packaging, and Handling

Nivolumab (also referred to as BMS-936558 or MDX-1106) 40 and/or 100 mg vials (10 mg/mL) will be supplied by Bristol-Myers Squibb in its commercial packaging with brand name "OPDIVO 10 mg/mL concentrate for solution for infusion" (pack size of one 10 mL vial (Type I glass) with a stopper (coated butyl rubber) and a flip-off seal (aluminium)), and labeled appropriately as investigational material for this study. Nivolumab is a programmed death receptor-1 (PD-1) blocking antibody. Nivolumab vials must be stored in the refrigerator at 2-8^{o}C, protected from light and freezing. If stored in a glass front refrigerator, vials should be stored in the carton. The product does not contain a preservative, and as such after preparation, nivolumab should be used immediately. If not used immediately, nivolumab infusions should be stored at room temperature (20-25°C) and room light for no more than 4 hours from time of preparation. This includes room temperature storage of infusion in IV container and time for administration of infusion. Alternatively, nivolumab infusion can be stored under refrigeration at 2 to 8^{o}C (36-46^{o}F) for no more than 24 hours from the time of infusion preparation and protected from light. The infusion should not be frozen.

Recommended safety measures for preparation and handling of nivolumab include laboratory coats and gloves.

For complete details on drug preparation, administration, storage conditions, clinical pharmacology, pharmacokinetics, and known precautions and adverse reactions please see the Nivolumab IB and /or Summary of Product Characteristics (SmPC).

5.1.2 Administration

The drug product solution should be visually inspected for particulate matter and discoloration prior to administration. Nivolumab is a clear to opalescent, colorless to pale-yellow solution that may contain few light particles. Discard the vial if the solution is cloud, is discolored, or contains extraneous particulate matter other than a few translucent-to-white, proteinaceous particles. Do not shake the vial.

Preparation of nivolumab should be performed by trained personnel in accordance with Summary of Product Characteristics and good practices rules, especially with respect to asepsis. The required volume of nivolumab should be withdrawn and transferred into an intravenous container. Nivolumab should be then be diluted with either 9 mg/mL (0.9%) sodium chloride solution for injection, USP or 50 mg/mL (5%) glucose solution for injection, USP, to prepare an infusion with a final concentration ranging from 1 mg/mL to 10 mg/mL. Diluted solution should be mixed by gentle inversion, but should not be shaken. Partially used or empty vials of nivolumab should be discarded.

Nivolumab infusion must not be administered as an intravenous push or bolus injection. Use an infusion set and an in-line, sterile, non-pyrogenic, low protein binding filter (pore size of 0.2 µm to 1.2 µm). Nivolumab infusion is compatible with PVC and polyolefin containers, glass bottles, PVC infusion sets and in-line filters with polyethersulfone membranes with pore sizes of 0.2 µm to 1.2 µm.

Patients should be administered nivolumab 240 mg as an in intravenous infusion over 30 minutes every 2 weeks (+/- 1 day for cycles 1 to 3 and +/- 3 days for subsequent cycles). For the first infusion, the patient's vital signs (heart rate, respiratory rate, blood pressures, and temperature) should be determined within 60 minutes before, during (every 15 [±5] minutes), and 30 (±10) minutes after the infusion. For subsequent infusions, vital signs will be collected within 60 minutes before infusion. Patients will be informed about the possibility of delayed post-infusion symptoms and instructed to contact their study physician if they develop acute symptoms.

NIVOLUMAB IS TO BE ADMINISTERED AFTER TG4010.

5.1.3 Disposal and Destruction

Do not store any unused portion of the infusion solution for reuse. Any unused medicinal product or waste material will be disposed of in accordance with local requirements.

Alternatively, unused medicinal product may be returned to BMS with the appropriate documentation. The site's method of drug supply destruction must be agreed upon by BMS.

Accurate records of all investigational product received at, dispensed from, returned to, and disposed of by the study site should be recorded on the Drug Log.

5.1.4 Supplier

Nivolumab will be supplied by Bristol-Myers Squibb for this study at no cost to the study participant.

5.2 TG4010

5.2.1 Formulation, Packaging, Handling

TG4010 is a suspension of recombinant Modified Vaccinia virus Ankara (MVA), a significantly attenuated strain of Vaccinia virus, carrying coding sequences for the human MUC1 antigen and human Interleukin-2 (IL2).

TG4010 is supplied in individual 4-mL glass vials (Type I glass). Each vial is intended for single use (i.e., 1 injection to 1 patient). The recoverable volume of TG4010 in each vial is 0.5 mL with an infectious titer of 1 x 10^8 PFU. TG4010 is a colorless to whitish clear or slightly turbid liquid, with possible presence of product particles or filaments (for further information, see TG4010 Investigator's Brochure and Technical Sheet).

The primary labels on the vials as well as the secondary labels (secondary packaging) are in the language of countries where the study is to be performed. Labels are compliant with local regulatory requirements for investigational material.

Packaging for shipment of TG4010 is compliant with the IATA (International Air Transport Association) and ADR (International Carriage of Dangerous Goods by Road) regulations for air and road transport of infectious substances (UN 3373 regulations). TG4010 is shipped on dry-ice with the official transport designation "Biological Substance, Category B".

The TG4010 must be stored at -20°C +/- 5°C in a freezer under the supervision of the study Pharmacist / Investigator (or his/her delegate). The vials will be dispensed only with the written authorization of the Investigator to staff that have been specifically designated and trained for this study.

TG4010 is a Genetically Modified Organism (GMO). As such, and whenever relevant, it must be handled according to national regulatory requirements. The Investigator's Brochure, as well as a Technical Sheet and a Preparation Procedure that are provided by Transgene detail instructions for TG4010 handling (biosafety requirements, preparation, administration, destruction) and incident management.

During all TG4010 handlings, lab coat, goggles, gloves and mask must be worn. All transport of TG4010 (vial or syringe containing the dose to be injected) must be done using a leakproof container/bag.

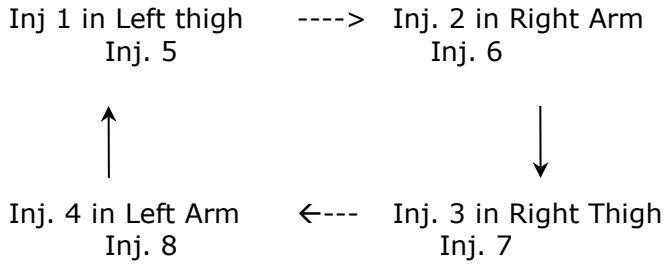
5.2.2 Administration

Prior to the administration, TG4010 must be prepared under aseptic conditions in compliance with requirements for every human injectable preparation. Aseptic conditions mean the standard hospital conditions for injectable preparations that ensure the sterility of TG4010 solutions.

TG4010 doses will be prepared and handled according to the instructions provided in the Preparation Procedure provided by Transgene.

Patients will receive SC injections of TG4010 at the dose of 1 x 10^8 PFU weekly for 6 weeks and then once every 2 weeks.

Four injection sites will be used (left and right arm, left and right thigh) according to a rotation schedule. It is preferable that the first injection is administered in either the right or left thigh. The second injection is to be given in the opposite side arm, the third in the same side thigh, and the fourth in the opposite side arm. For example, if the left thigh is the site of the first vaccination, the second vaccination would be in the right arm, the third in the right thigh, and the fourth in the left arm. This pattern is recommended and will be repeated for subsequent injections.



If the pattern cannot be followed exactly, it is more important to alternate between the arms and thighs than side to side.

TG4010 IS TO BE ADMINISTERED BEFORE NIVOLUMAB

5.2.3 Disposal and Destruction

Disposal instructions of the waste generated during TG4010 preparation and administration are available in the Technical Sheet.

In case of incident while handling TG4010, the actions recommended are described in the Technical Sheet. All incidents must be documented by a written report immediately sent to Transgene Medical Affairs or its designee:

Emergency 24-hour telephone number (Europe and USA): (33) (0)3 68 33 28 80

In Europe: Fax: (33) (0)3 88 27 91 41

In US: Fax: + (1) (617) 679 80 50

5.2.4 Supplier

TG4010 will be supplied by Transgene for this study at no cost to the study participant.

5.3 Associated Therapies

The use of the following drugs will not be restricted during the course of the study:

- Antiemetics
- Steroids (no more than 5 consecutive days, chronic exposure to be excluded)
- Bisphosphonates

Radiotherapy for pain relief (eg bone metastases) is permitted. Effusions will be drained if necessary. These supportive interventions must be reported.

6.0 Dose Modification and Dose Omissions

6.1 Dose Modifications

There will be no dose reductions for nivolumab and TG4010 in this study. If an AE is related to only one agent and dose omission is required, the other agent should continue as planned.

6.1.1 Dose Omissions: Nivolumab

Because of the potential for clinically meaningful nivolumab-related AEs requiring early recognition and prompt intervention, management algorithms have been developed for suspected AEs of selected categories.

Dose delay criteria apply for all nivolumab-related adverse events. Nivolumab must be delayed until treatment can resume.

Nivolumab administration should be delayed for the following:

Any Grade ≥ 2 non-skin, drug-related AE, with the following exceptions:

- Grade 2 drug-related fatigue or laboratory abnormalities do not require a treatment delay
- Any Grade 3 skin, drug-related AE

Any Grade 3 drug-related laboratory abnormality, with the following exceptions for lymphopenia, leukopenia, AST, ALT, total bilirubin, or asymptomatic amylase or lipase:

- Grade 3 lymphopenia or leukopenia does not require dose delay.
- If a subject has a baseline AST, ALT, or total bilirubin that is within normal limits, delay dosing for drug-related Grade ≥ 2 toxicity.
- If a subject has baseline AST, ALT, or total bilirubin within the Grade 1 toxicity range, delay dosing for drug-related Grade ≥ 3 toxicity.

- Any Grade ≥ 3 drug-related amylase or lipase abnormality that is not associated with symptoms or clinical manifestations of pancreatitis does not require dose delay. The Investigator should be consulted for such Grade ≥ 3 amylase or lipase abnormalities.

Any AE, laboratory abnormality, or intercurrent illness which, in the judgment of the investigator, warrants delaying the dose of study medication.

Subjects who require delay of nivolumab should be re-evaluated weekly or more frequently if clinically indicated and resume nivolumab dosing when re-treatment criteria are met.

Criteria to Resume Treatment

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 2-grade shift in AST/ALT or total bilirubin may resume treatment in the presence of Grade 2 AST/ALT OR total bilirubin
- Subjects with combined Grade 2 AST/ALT AND total bilirubin values meeting discontinuation parameters should have treatment permanently discontinued
- Drug-related pulmonary toxicity, diarrhea, or colitis, must have resolved to baseline before treatment is resumed. Subjects with persistent Grade 1 pneumonitis after completion of a steroid taper over at least 1 month may be eligible for retreatment if investigator allows.
- Drug-related endocrinopathies adequately controlled with only physiologic hormone replacement may resume treatment if investigator allows.

If the criteria to resume treatment are met, the subject should restart treatment at the next scheduled timepoint per protocol. However, if the treatment is delayed past the next scheduled timepoint per protocol, the next scheduled timepoint will be delayed until dosing resumes.

If treatment is delayed or interrupted for > 6 weeks, the subject must be permanently discontinued from study therapy, except as specified in discontinuation section.

6.1.2 Dose Omissions: TG4010

- The hematological threshold to allow the administration of TG4010 is defined as: Neutrophils $\geq 0.5 \times 10^9$/L
- If a grade 1-2 AE occurs TG4010 should be administered as planned.
- If you suspect a $\geq$ grade 3 AE possibly, probably or definitely related to TG4010 please call the principal investigator for guidance.
- TG4010 does not have to be delayed for Nivolumab related AEs. Please call the principal investigator for guidance.

6.2 Management of Nivolumab-Specific Adverse Events

Toxicities associated or possibly associated with nivolumab treatment should be managed according to standard medical practice. Additional tests, such as autoimmune serology or biopsies, may be used to determine a possible immunogenic etiology.

Although most immune-related adverse events observed with immunomodulatory agents have been mild and self-limiting, such events should be recognized early and treated promptly to avoid potential major complications. Discontinuation of nivolumab may not have an immediate therapeutic effect and in severe cases, immune-related toxicities may require acute management with topical corticosteroids, systemic corticosteroids, mycophenolate, or TNF-α inhibitors.

The primary approach to Grade 1-2 immune-related adverse events is supportive and symptomatic care with continued treatment with nivolumab; for higher grade immune-related adverse events, nivolumab should be held and oral/parental steroids administered. Recurrent Grade 2 immune-related adverse events may also mandate holding nivolumab or the use of steroids. Consideration for benefit/risk balance should be made by the investigator, with consideration of the totality of information as it pertains to the nature of the toxicity and the degree of clinical benefit a given patient may be experiencing prior to further administration of nivolumab. Nivolumab should be permanently discontinued in patients with life-threatening irAEs.

6.2.1 Guidelines for Treatment Interruption or Discontinuation

Nivolumab treatment will be given as long as the patient continues to experience clinical benefit in the opinion of the investigator, unacceptable toxicity, symptomatic deterioration attributed to disease progression, or any of the other reasons for treatment discontinuation.

Patients may temporarily suspend study treatment for up to 42 days from the last dose if they experience toxicity that is considered related to the study drug and requires a dose to be held. If nivolumab is held because of adverse events for >42 days beyond the last dose, then the patient will be discontinued from nivolumab.

If a patient must be tapered off steroids used to treat adverse events, nivolumab may be held for additional time beyond 42 days from the last dose until steroids are discontinued or reduced to

prednisone dose (or dose equivalent) ≤10 mg/day. The acceptable length of interruption will depend on an agreement between the investigator and the Principal Investigator.

Dose interruptions for reason(s) other than toxicity, such as surgical procedures, may be allowed with Principal Investigator approval. The acceptable length of interruption will depend on agreement between the investigator and the Principal Investigator.

Any toxicities associated or possibly associated with nivolumab treatment should be managed according to standard medical practice. Additional tests, such as autoimmune serology or biopsies, may be used to determine a possible immunogenic etiology. Although most irAEs observed with immunomodulatory agents have been mild and self-limiting, such events should be recognized early and treated promptly to avoid potential major complications. Discontinuation of nivolumab may not have an immediate therapeutic effect, and there is no available antidote for nivolumab. In severe cases, immune-related toxicities may be acutely managed with topical corticosteroids, systemic corticosteroids, mycophenolate, or TNFα inhibitors.

Patients should be assessed clinically (including review of laboratory values) for toxicity prior to, during, and after each infusion. If unmanageable toxicity due to nivolumab occurs at any time during the study, treatment with nivolumab should be discontinued.

Discontinuation Criteria

Treatment should be permanently discontinued for the following:

- Any Grade 2 drug-related uveitis or eye pain or blurred vision that does not respond to topical therapy and does not improve to Grade 1 severity within the re-treatment period OR requires systemic treatment
- Any Grade 3 non-skin, drug-related adverse event lasting > 7 days, with the following exceptions for drug-related laboratory abnormalities, uveitis, pneumonitis, bronchospasm, hypersensitivity reactions, and infusion reactions, and endocrinopathies:
    - Grade 3 drug-related uveitis, pneumonitis, bronchospasm, 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 those noted below
- 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 > 8 x ULN
    - Total bilirubin > 5 x ULN
    - Concurrent AST or ALT > 3 x ULN and total bilirubin > 2 x ULN
- Any Grade 4 drug-related adverse event or laboratory abnormality, except for the following events which do not require discontinuation:

- Isolated Grade 4 amylase or lipase abnormalities that are not associated with symptoms or clinical manifestations of pancreatitis and decrease to < Grade 4 within 1 week of onset.
- 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
- Grade 4 lymphopenia or leucopenia
- Grade 4 drug-related endocrinopathy adverse events, such as adrenal insufficiency, ACTH deficiency, hyper- or hypothyroidism, or glucose intolerance, which resolve or are adequately controlled with physiologic hormone replacement (corticosteroids, thyroid hormones) or glucose-controlling agents, respectively, may not require discontinuation after discussion with and approval from the Investigator [as allowed by protocol]
- Any dosing interruption lasting > 6 weeks with the following exceptions:
- Dosing delays or interruptions to allow for prolonged steroid tapers to manage drug-related adverse events are allowed. Prior to re-initiating treatment in a subject with a dosing interruption lasting > 6 weeks, the Investigator must be consulted. Tumor assessments should continue as per protocol even if dosing is interrupted or delayed
- Dosing interruptions or delays lasting > 6 weeks that occur for non-drug-related reasons may be allowed if approved by the Investigator. Prior to re-initiating treatment in a subject with a dosing interruption lasting > 6 weks, the Investigator must be consulted. Tumor assessments should continue as per protocol even if dosing is interrupted
- 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

Management of potentially immune related AEs are described below.

6.2.2 Management of infusion-related reaction

No premedication will be allowed for the first dose of nivolumab. Premedication may be administered for Cycles ≥2 at the discretion of the treating physician. Patients who experience infusion-associated symptoms may be treated symptomatically with antipyretics (ibuprofen preferred), diphenhydramine, and/or cimetidine or another H2 receptor antagonist, as per standard practice.

The management of infusion-related reactions will be according to severity as follows:

- In the event that a patient experiences a mild (NCI CTCAE Grade 1) infusion-related event, the infusion rate should be reduced to half the rate being given at the time of event onset. Once the event has resolved, the investigator should continue to deliver the infusion at the reduced rate for 30 minutes. If tolerated, the infusion rate may then be increased to the original rate.

- In the event that a patient experiences a moderate infusion-related event (NCI CTCAE Grade 2) or flushing, fever, or throat pain, the patient should have his or her infusion immediately interrupted and should receive aggressive symptomatic treatment. The infusion should be restarted only after the symptoms have adequately resolved to the baseline grade. The infusion rate at restart should be half of the rate that was in progress at the time of the onset of the infusion-related event.
- For severe or life-threatening infusion-related events (NCI CTCAE Grade 3 or 4), the infusion should be stopped immediately, and aggressive resuscitation and supportive measures should be initiated. Serious infusion-associated events manifested by dyspnea, hypotension, wheezing, bronchospasm, tachycardia, reduced oxygen saturation, or respiratory distress should be managed with supportive therapies as clinically indicated (e.g. supplemental oxygen and β_2-adrenergic agonists).
- Patients experiencing severe or life-threatening infusion-related events will not receive further infusion and will be further managed as clinically indicated until the event resolves.
- Please see Appendix 5 for anaphylaxis precautions

6.2.3 Gastrointestinal Toxicity

Immune-mediated colitis has been associated with the administration of nivolumab. Diarrhea or colitis was reported in 21% of patients in two trials of nivolumab. Immune-mediated colitis occurred in 2.2% of patients in one trial- of these one patient had Grade 2 colitis, and five had Grade 3 colitis. In the other trial, Grade 3 immune-mediated colitis occurred in 0.9% of patients.

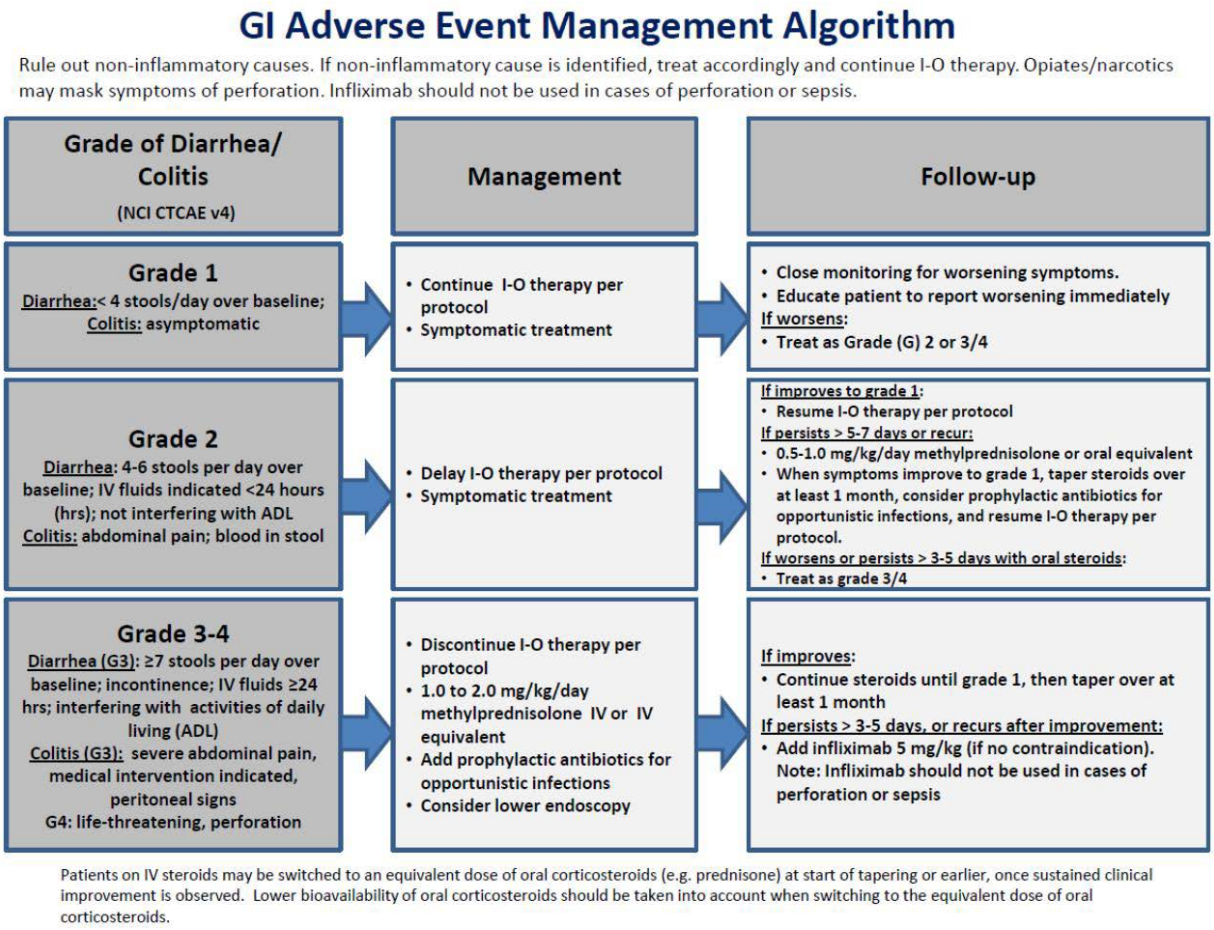
Patients should be advised to inform the investigator if any diarrhea occurs, even if it is mild. Range for time to onset of symptoms of colitis has been 1-6 months.

If the event is of significant duration or magnitude or is associated with signs of systemic inflammation or acute phase reactants (e.g. increased CRP or platelet count or bandemia), it is recommended that sigmoidoscopy (or colonoscopy, if appropriate) with colonic biopsy with three to five specimens for standard paraffin block be performed. If possible, one or two biopsy specimens should be snap frozen and stored.

Treatment may be restarted following the resolution of colitis. In addition, if the patient is being managed with corticosteroids, treatment should not be restarted until the steroids have been tapered down to a prednisone dose $\leq$ 10 mg/day. Patients who resume treatment should be monitored closely for signs of renewed diarrhea.

Table 3 provides a summary of dose modification guidelines for gastrointestinal toxicities.

Table 3. Dosing Guidelines for Gastrointestinal Toxicity



6.2.4 Hepatotoxicity

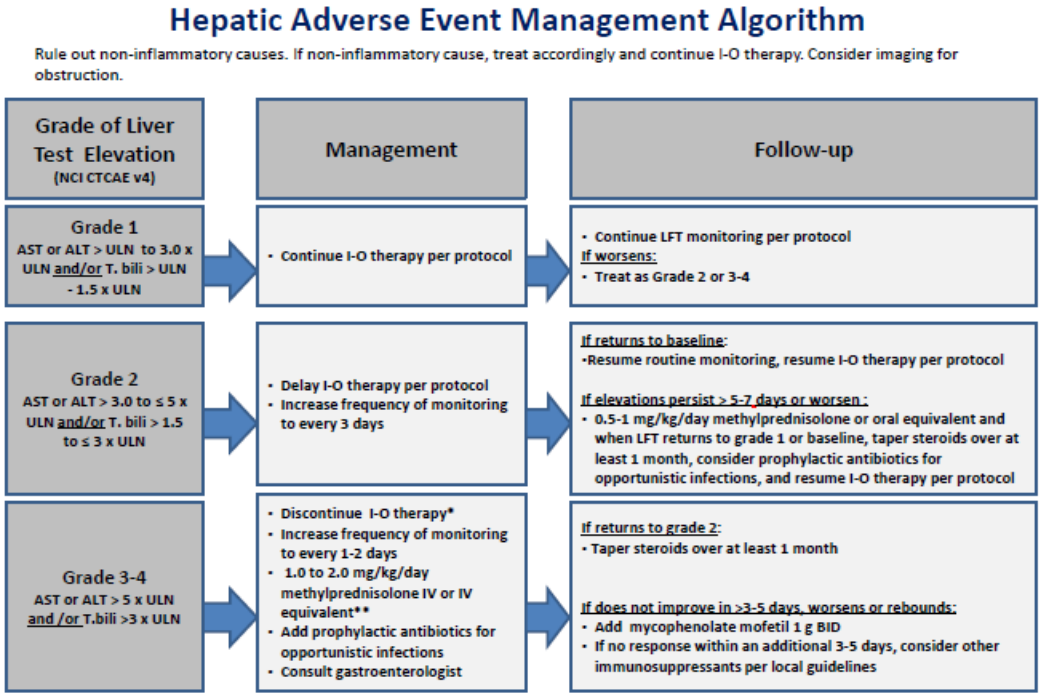
Immune-mediated hepatitis has occurred with the administration of nivolumab.

Patients should be monitored for abnormal liver tests prior to and periodically during treatment. While on this study, patients presenting with right upper-quadrant abdominal pain and/or unexplained nausea or vomiting should have LFTs performed immediately and LFTs should be reviewed before administration of the next dose of study drug.

If LFTs increase, neoplastic, concurrent medications, viral hepatitis, and toxic etiologies should be considered and addressed, as appropriate. Imaging of the liver, gall bladder, and biliary tree should be performed to rule out neoplastic or other causes for the increased LFTs. Anti-nuclear antibody, perinuclear anti-neutrophil cytoplasmic antibody, anti-liver-kidney microsomal antibodies, and anti-smooth muscle antibody tests should be performed in an autoimmune etiology is considered.

Patients with LFT abnormalities should be managed according to the guidelines in Table 4.

Table 4. Dosing Guidelines for Hepatotoxicity



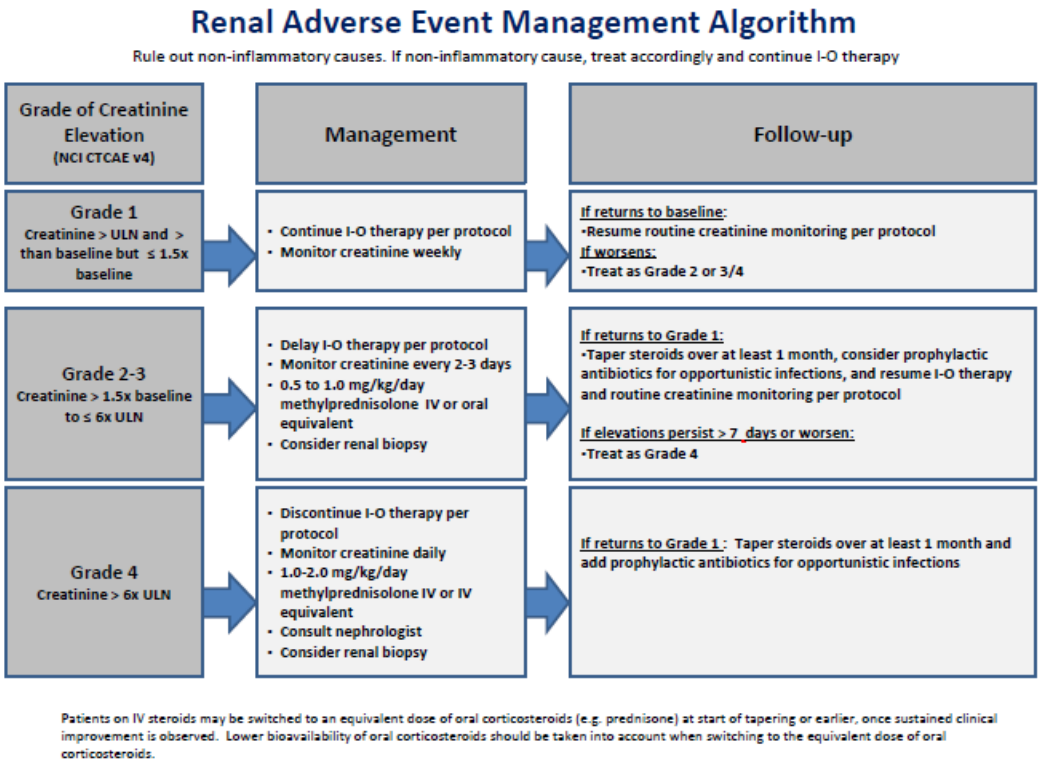
6.2.5 Renal Toxicity

Immune-mediated nephritis or renal dysfunction occurred with nivolumab treatment. Patients should be monitored for elevated serum creatinine prior to and periodically during treatment and clinically monitored for symptoms of decreased volume of urination, hematuria, peripheral edema, loss of appetite.

In one trial, there was increased incidence of elevated creatinine in the nivolumab-treated group as compared to chemotherapy (13% v 9%). Grade 2 or 3 immune-mediated nephritis or renal dysfunction occurred in 0.7% (2/268) of patients. In another trial, the incidence of elevated creatinine was 22%, with Grade 2 immune-mediated renal dysfunction occurring in 0.9% (1/117) patients.

Renal toxicity should be managed according to the guidelines in Table 5.

Table 5. Dosing Guidelines for Renal Toxicity



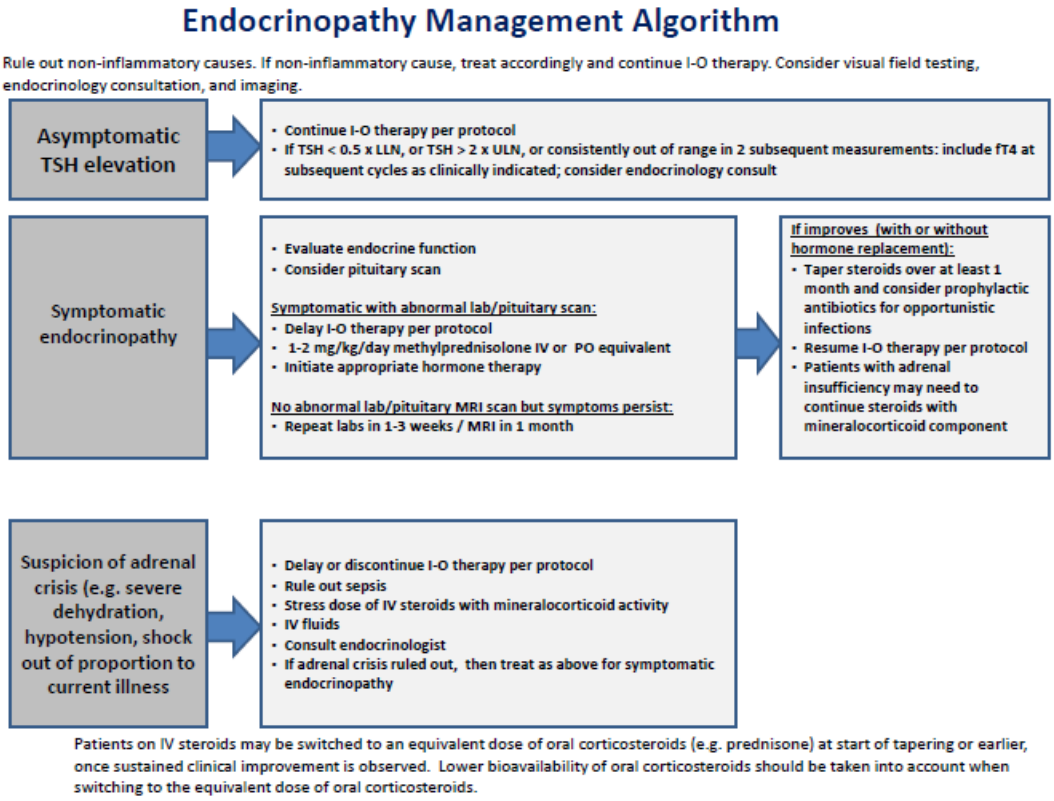
6.2.6 Endocrine Toxicity

Immune-mediated hypothyroidism and hyperthyroidism has been associated with the administration of nivolumab. In one trial, Grade 1 or 2 hypothyroidism occurred in 8% (21/268) of patients receiving nivolumab. Grade 1 or 2 hyperthyroidism occurred in 3% (8/268) of patients on nivolumab on that trial, and in 1% (1/102) patients receiving chemotherapy. In another trial, hypothyroidism occurred in 4.3% (5/117) of patients. Hyperthyroidism occurred in 1.7% (2/117) of patients. One patient experienced Grade 2 hyperthyroidism.

Patients with unexplained symptoms such as fatigue, headaches, myalgias, impotence, mental status changes, or constipation should be investigated for the presence of thyroid, pituitary, or adrenal endocrinopathies, as well as for hyponatremia or hyperkalemia. An endocrinologist should be consulted if an endocrinopathy is suspected. TSH and free T4 levels should be obtained to determine if thyroid abnormalities are present. TSH, prolactin, and a morning cortisol level will help to differentiate primary adrenal insufficiency from primary pituitary insufficiency.

Hypothyroidism should be managed according to the guidelines in Table 6.

Table 6. Dosing Guidelines for Endocrine Toxicity

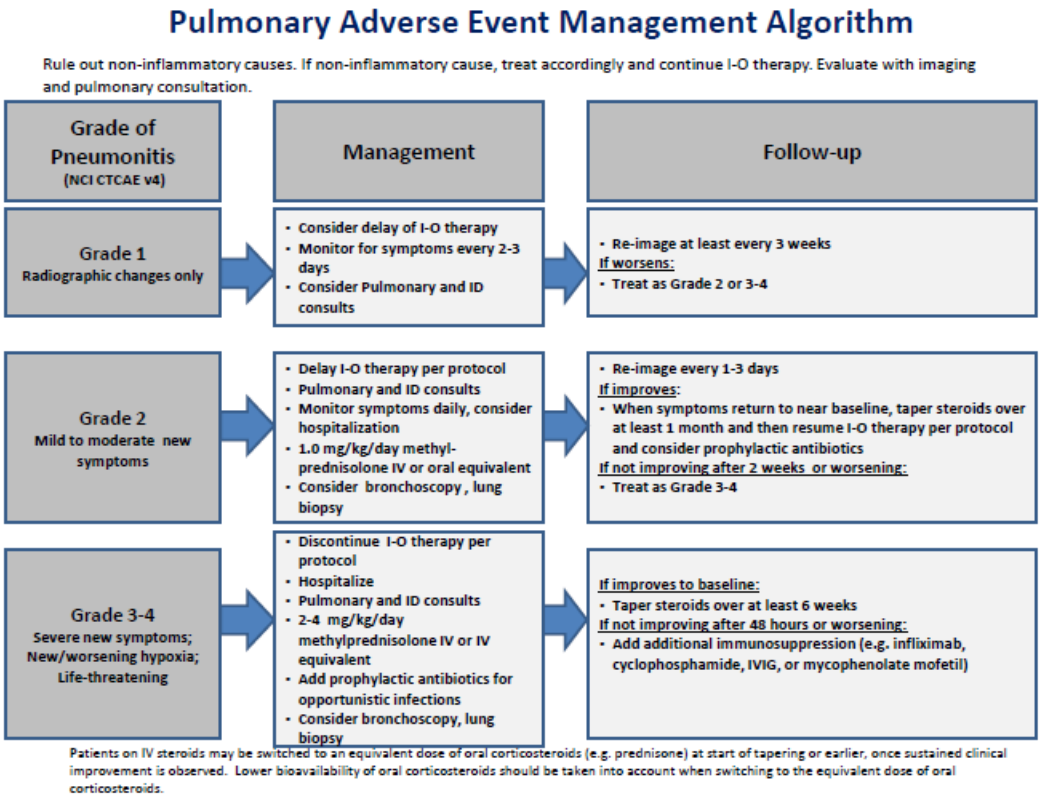


6.2.7 Pulmonary Toxicity

Severe pneumonitis or interstitial lung disease, including fatal cases, have occurred with nivolumab treatment. As such, patients must be monitored for signs and symptoms of pneumonitis. Across clinical trial experience in 691 patients with solid tumors, fatal immune mediated pneumonitis occurred in 0.7% (5/691) of patients receiving nivolumab. In one trial 2.2% (6/268) of patients receiving nivolumab developed immune-mediated pneumonitis, one with Grade 3 and vie with Grade 2 cases. In another trial, immune-mediated pneumonitis occurred in 6% (7/117) of patients receiving nivolumab including five Grade 3 and two Grade 2 cases.

Pulmonary toxicity should be managed according to the guidelines in Table 7.

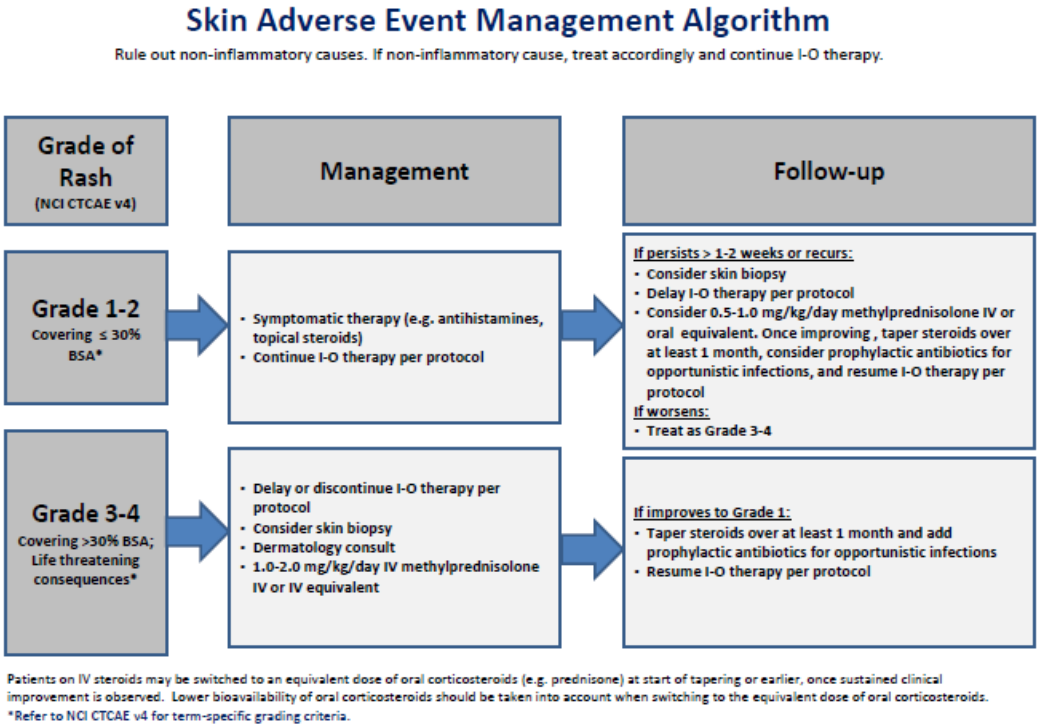
Table 7. Dosing Guidelines for Pulmonary Toxicity



6.2.8 Pericardial and Pleural Effusions

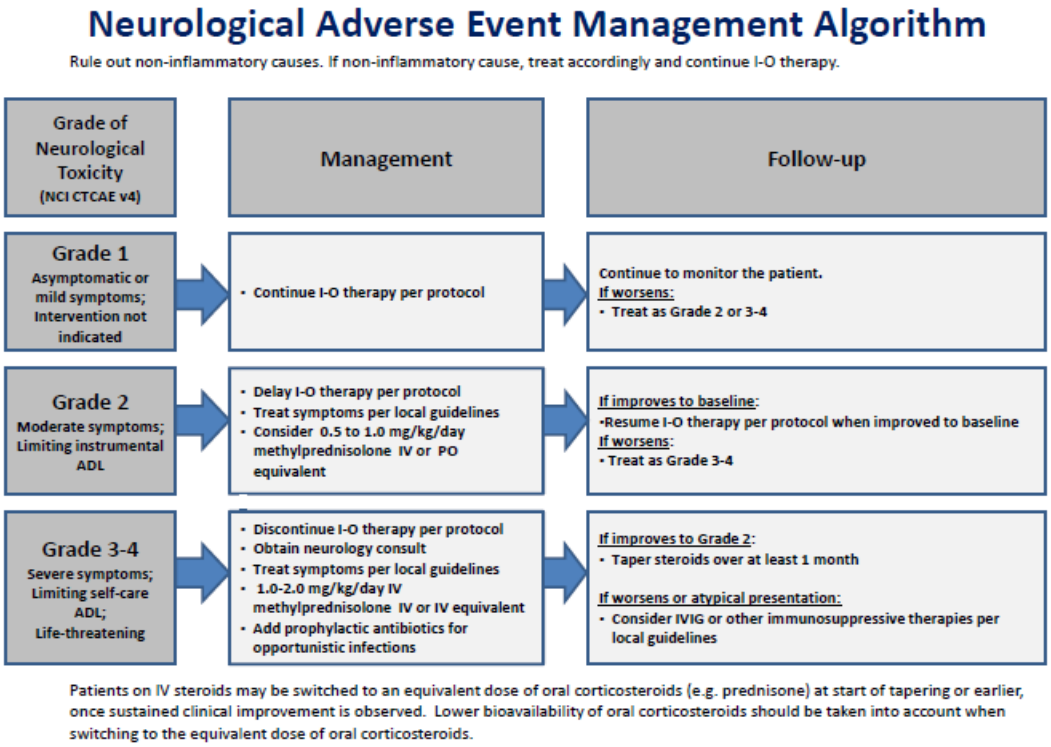
Pericardial and pleural involvement with associated effusions is common in patients with NSCLC and have the theoretical potential to be exacerbated by inflammation associated with antitumor immunity following PD-L1 blockade. Patients presenting with dyspnea, chest pain, or unexplained tachycardia should be evaluated for the presence of a pericardial effusion. Patients with preexisting pericardial effusion should be followed closely for pericardial fluid volume measurements and impact on cardiac function. When intervention is required for pericardial or pleural effusions, appropriate workup includes cytology, LDH, glucose, cholesterol, protein concentrations (with pleural effusions), and cell count. For patients with a pericardial effusion causing end-diastolic right ventricular collapse, treatment may be restarted following the placement of a pericardial window, demonstrations of hemodynamic stability, and resolution of right ventricular dysfunction.

6.2.9 Skin



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 v4 for term-specific grading criteria.

6.2.10 Nervous System



6.3 Management of TG4010-Specific Adverse Events

Most TG4010 related adverse events observed in clinical trials have been of low grade. In case of a specific TG4010-related AE that does not resolve before the next dose and according to investigator decision, that dose may be held until the next scheduled administration. Any questions about AEs should be discussed with the Principal Investigator.

7.0 Concomitant Therapy

The patient must be told to notify the investigational site about any new medications he/she takes after the start of the study drug. All medications (other than study drug) and significant non-drug therapies (blood transfusions) administered during the study should be recorded.

Concomitant therapy includes any prescription medications or over-the-counter preparations used by a patient from the time of consent until 100 days after treatment discontinuation. All medications (International Nonproprietary Names [INN]), dosage, route of administration, frequency, duration of administration, and the indication will be recorded in the appropriate sections of the CRF. Concomitant medications or non-drug therapy used or performed to treat a SAE, an AE related to study treatments or any other significant AE as recommended by the Sponsor (UC Davis Comprehensive Cancer Center), should be recorded beyond the AE reporting period as described in safety section 11.0.

7.1 Permitted Therapy

Systemic corticosteroids may attenuate potential beneficial immunologic effects of treatment with nivolumab + TG4010 but may be administered at the discretion of the treating physician. Alternatives to corticosteroids should be considered if feasible, but premedication may be administered for Cycles ≥2. The use of inhaled corticosteroids and mineralocorticoids (e.g. fludrocortisone) is allowed.

Megastrol administered as an appetite stimulant is acceptable while the patient is enrolled in the study.

Patients who use hormonal therapy such as oral contraceptives, hormone-replacement therapy, prophylactic or therapeutic anticoagulation therapy (such as low molecular weight heparin or warfarin at a stable dose level), or other allowed ongoing therapies or medications (see Section 4.2 (Exclusion Criteria)) should continue their use. Males and females of reproductive potential should use a highly effective means of contraception and for 5 months after end of treatment.

8.0 Study Calendar

Assessment Window (Days)	Screening	Cycle 1 (2 wks)	Cycle 2 (2 wks)	Cycle 3 (2 wks)	Subsequent Cycles (2 wks)	Safety follow-up visit after Treatment Discontinuation	PFS follow-up	OS Follow up
	Days -28 to -1	Day 1 (± 1 Days)			Day 1 (± 3 Days)	Within 28-60 Days after Last Dose		Every 2 months (± 7 days)
Medical, surgical and cancer histories, including prior anti-cancer therapies and demographic information	X							
HIV, HBV, HCV, HLA I and II typing by PCR[a]	X							
Concomitant medications	X	X	X	X	X	X		
Further anti-neoplastic therapies							X	X
Adverse events		X	X	X	X	X		
Tumor assessment[b,c]	X	Every 6 weeks (approximately every three cycles) ± 3 business days						
Brain MRI	X							
Complete physical examination	X							
Limited physical examination		X[d]	X[d]	X[d]	X[d]	X		
ECOG performance status	X	X[d]	X[d]	X[d]	X[d]	X		
Vital signs[e]	X	X	X	X	X	X		
12-lead electrocardiogram[f]	X	X[f] (if clinically indicated)						
Echocardiogram[g]	X	X (if clinically indicated) between Day 15-21 of Cycle 2 (for review prior to dosing on Cycle 3 Day 1)						
Weight	X	X	X	X	X	X		
Height	X							
Hematology	X	X[d]	X[d]	X[d]	X[d]	X		
Serum chemistry[h]	X	X[d]	X[d]	X[d]	X[d]	X		
Coagulation panel (aPTT, INR)	X							
C-reactive protein testing	X	X[d]	X[d]	X[d]	X[d]			
Urinalysis[i]	X			X[j]	X[j]	X		
Serum pregnancy test[k]	X			X[k]	X[k]	X[k]		
TSH, free T3, free T4	X			X[p]	X[p]	X		
Correlative Studies (Section 10.0)								
Blood sample for immune assays[l]	X	M2 (or W8), at M4 and at M6						
Diagnostic tumor tissue specimen or 15 unstained slides[m] (optional)	X							
Fresh biopsy prior to treatment and during treatment[n]	X (mandatory)			X (mandatory) 8 wks	X (optional) at progression			
Treatment								
Nivolumab infusion[o]		X	X	X	X			
TG4010 (subcutaneous injection)[o]		weekly	weekly	weekly	X			

anti-HBc=antibody against hepatitis B core antigen ECOG=Eastern Cooperative Oncology Group; FFPE=formalin fixed paraffin embedded; HBV=hepatitis B virus; HCV=hepatitis C virus; PD-L1=programmed death-ligand 1; RECIST=Response Evaluation Criteria in Solid Tumors; TSH=thyroid-stimulating hormone.

Note: Assessments scheduled on the days of study treatment infusions should be performed before the infusion unless otherwise noted.

[a] HIV testing to be performed in accordance with national and/or institutional guidelines. HBV DNA must be collected on or before Cycle 1, Day 1 in patients who have negative serology for hepatitis B surface antigen and positive serology for anti-HBc. HLA I and II typing must be done by PCR.

[b] Tumor assessments performed as standard of care prior to obtaining informed consent and within 28 days of Cycle 1, Day 1 may be used rather than repeating tests. All measurable and evaluable lesions should be assessed and documented at the screening visit. The same radiographic procedure must be used throughout the study for each patient. Results must be reviewed by the investigator before dosing at the next cycle. Tumor assessments will be performed at screening and every 6 weeks (approximately every three cycles) after the start of study treatment.

[c] Patients who came off for toxicity should have a scan every 6 weeks until progression

[d] ECOG performance status, limited physical examination, local laboratory assessments, and C-reactive protein assessment may be obtained ≤ 96 hours before the start of each cycle.

[e] Vital signs include heart rate, respiratory rate, blood pressures, and temperature. For the first infusion, vital signs will be collected 60 minutes prior to the infusion, (every 15 [± 5] minutes) and 30 [± 10] after the infusion. For subsequent infusions, vital signs will be collected within 60 minutes prior to the infusion and should be collected during or after the infusion if clinically indicated or if symptoms occurred in the prior infusion.

[f] ECG recordings will be obtained during screening and at the end of treatment visit plus when clinically indicated. Patients should be resting and in a supine position for at least 10 minutes prior to each ECG collection.

[g] Echocardiograms will be performed at screening on all patients. For patients with a known history of pericardial effusions or with evidence of pleural or pericardial effusion on screening chest CT, an ECHO will be performed between Day 15-21 of Cycle 2 (for review prior to dosing on Cycle 3 Day 1), and as clinically indicated or per standard of care thereafter during treatment, and at the treatment discontinuation visit if pleural or pericardial effusions are not resolved during the study. Patients who develop new pleural or pericardial effusions while on study must be followed by echocardiography.

[h] Serum chemistry includes BUN, creatinine, sodium, potassium, magnesium, chloride, bicarbonate, calcium, phosphorus, glucose, total bilirubin, ALT, AST, alkaline phosphatase, LDH, total protein, and albumin.

[i] Urinalysis (specific gravity, pH, glucose, protein, ketones, and blood).

[j] On Day 1 of Cycle 3 and every 4 cycles thereafter.

[k] Serum pregnancy test (for women of childbearing potential, including women who have had a tubal ligation) must be performed and documented as negative within 14 days prior to Day 1 and repeated during study treatment q3 cycles (i.e., every 6 weeks). After discontinuation from nivolumab these should be repeated at approximately 30 days and 70 days.

[l] Blood samples for translational studies will be collected, as described in section 10.

[m] Diagnostic tumor tissue (if available) and if it was a biopsy (no fine needle aspirates, FNA)

[n] Collection of pre- and 8 week treatment biopsies (no FNAs) are **mandatory unless it is unsafe or undesirable**. Discussion with the study chair is required to allow patient to go on without the biopsy. An optional biopsy at progression may be performed. The pre-treatment samples will be collected within 42 days prior to treatment implementation and the "on- treatment" sample at 8 weeks [±1 week]. For core needle biopsy specimens, at least three cores should be submitted for evaluation.

[o] Nivolumab will be administered by IV infusion q2w [±1 days] for cycles 1 to 3 and [±3 days] for subsequent cycles. TG4010 will be administered subcutaneously weekly for 6 weeks [±1 day] and then every 2 weeks [± 3 days]. TG4010 is to be administered **prior** to Nivolumab. Nivolumab will be administered over 60 minutes for the first infusion but can be reduced to 30 minutes for subsequent cycles if tolerated.

[p] TSH, T3 and T4 must be repeated during study treatment q3 cycles (i.e., every 6 weeks).

9.0 Assessment Types

9.1 Efficacy

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) (21). 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.

Disease Parameters

Measurable Disease
The presence of at least one measurable lesion. If the measurable disease is restricted to a solitary lesion, its neoplastic nature should be confirmed by cytology/histology.

Measurable Lesions
Lesions that can be accurately measured in at least one dimension with longest diameter 20mm using conventional techniques or 10mm with spiral CT scan.

Non-Measurable Lesions
All other lesions, including small lesions (longest diameter <20 mm with conventional techniques or <10 mm with spiral CT scan), i.e., bone lesions, leptomeningeal disease, ascites, pleural/pericardial effusion, inflammatory breast disease, lymphangitis cutis/pulmonis, cystic lesions, and also abdominal masses that are not confirmed and followed by imaging techniques**.**

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.

Clinical lesions will only be considered measurable when they are superficial (e.g., skin nodules and palpable lymph nodes). For the case of skin lesions, documentation by color photography, including a ruler to estimate the size of the lesion, is recommended.

Methods of Measurement

CT and MRI are the best currently available and reproducible methods to measure target lesions selected for response assessment. Conventional CT and MRI should be performed with cuts of 10 mm or less in slice thickness contiguously. Spiral CT should be performed using a 5 mm contiguous reconstruction algorithm. This applies to tumors of the chest, abdomen and pelvis. Head and neck tumors and those of extremities usually require specific protocols.

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

When the primary endpoint of the study is objective response evaluation, ultrasound (US) should not be used to measure tumor lesions. It is, however, a possible alternative to clinical measurements of superficial palpable lymph nodes, subcutaneous lesions and thyroid nodules. US might also be useful to confirm the complete disappearance of superficial lesions usually assessed by clinical examination.

The utilization of endoscopy and laparoscopy for objective tumor evaluation has not yet been fully and widely validated. Their uses in this specific context require sophisticated equipment and a high level of expertise that may only be available in some centers. Therefore, the utilization of such techniques for objective tumor response should be restricted to validation purposes in specialized centers. However, such techniques can be useful in confirming complete pathological response when biopsies are obtained.

Cytology and histology can be used to differentiate between PR and CR in rare cases (e.g., after treatment to differentiate between residual benign lesions and residual malignant lesions in tumor types such as germ cell tumors).

Baseline Documentation of "Target" and "Non-Target" Lesions

As per RECIST 1.1: When more than one measurable lesion is present at baseline all lesions up to a maximum of five lesions total (and a maximum of two lesions per organ) representative of all involved organs should be identified as target lesions and will be recorded and measured at baseline (this means in instances where patients have only one or two organ sites involved a maximum of two and four lesions respectively will be recorded).

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.

Lymph nodes merit special mention since they are normal anatomical structures which may be visible by imaging even if not involved by tumor. Pathological nodes which are defined as measurable and may be identified as target lesions must meet the criterion of a short axis of >=15mm by CT scan. Only the short axis of these nodes will contribute to the baseline sum.

A sum of the longest diameter (LD) for all target lesions will be calculated and reported as the baseline sum LD. The baseline sum LD will be used as reference by which to characterize the objective tumor.

All other lesions (or sites of disease) should be identified as non-target lesions and should also be recorded at baseline. Measurements of these lesions are not required, but the presence or absence of each should be noted throughout follow-up.

Response Criteria

Evaluation of Target Lesions

- **Complete Response (CR):** Disappearance of all target lesions
- **Partial Response (PR):** At least a 30% decrease in the sum of the LD of target lesions, taking as reference the baseline sum LD
- **Progressive Disease (PD):** At least a 20% increase in the sum of the LD of target lesions, taking as reference the smallest sum LD recorded since the treatment started or the appearance of one or more new lesions
- **Stable Disease (SD):** Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum LD since the treatment started

Evaluation of Non-Target Lesions

- **Complete Response (CR**): Disappearance of all non-target lesions and normalization of tumor marker level
- **Incomplete Response/ Stable Disease (SD):** Persistence of one or more non-target lesion(s) or/and 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

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

Evaluation of Timepoint Response

Table 8. Overall Response by Timepoint

Target Lesion	Non-Target Lesion	New Lesion	Overall Response
CR	CR	No	CR
CR	Non-CR / non-PD	No	PR
CR	Not evaluated	No	PR
PR	Non-PD or not all evaluated	No	PR
SD	Non-PD or not all evaluated	No	SD
Not all evaluated	Non-PD	No	NE
PD	Any	Yes or No	PD
Any	PD	Yes or No	PD
Any	Any	Yes	PD

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 PD the smallest measurements recorded since the treatment started). In general, the patient's best response assignment will depend on the achievement of both measurement and confirmation criteria.

The main goal of confirmation of objective response is to avoid overestimating the response rate observed. In cases where confirmation of response is not feasible, it should be made clear when reporting the outcome of such studies that the responses are not confirmed.

To be assigned a status of PR or CR, changes in tumor measurements must be confirmed by repeat assessments that should be performed no less than 4 weeks after the criteria for response are first met. Longer intervals as determined by the study protocol may also be appropriate.
In the case of SD, follow-up measurements must have met the SD criteria at least once after study treatment at a minimum interval (in general, not less than 6-8 weeks) that is defined in the study protocol.

The best overall response for each patient is determined from the sequence of overall lesion responses at each timepoint according to the following rules:
- CR: at least two determinations of CR at least 4 weeks apart before progression.
- PR: at least two determinations of PR (or better) at least 4 weeks apart before progression.
- SD: at least one SD assessment (or better) > after a minimum duration of SD
- PD: progression before any evaluation that would qualify for CR, PR or SD.

Table 9: Best overall response when confirmation of CR or PR is required

Overall response first timepoint	**Overall response subsequent timepoint**	**Best Overall Response**
CR	CR	CR
CR	PR	SD, PD or PR[a]
CR	SD	SD provided minimum duration for SD is met, otherwise PD
CR	PD	SD provided minimum duration for SD is met, otherwise PD
CR	NE	SD provided minimum duration for SD is met, otherwise NE
PR	CR	PR
PR	PR	PR
PR	SD	SD
PR	PD	SD provided minimum duration for SD is met, otherwise PD
PR	NE	SD provided minimum duration for SD is met, otherwise PD
NE	NE	NE

CR = complete response, PR = partial response, SD = stable disease, PD = progressive disease, and NE = inevaluable.
a If a CR is *truly* met at first time point, then any disease seen at a subsequent time point, even disease meeting PR criteria relative to baseline, makes the disease PD at that point (since disease must have reappeared after CR). Best response would depend on whether minimum duration for SD was met. However, sometimes 'CR' may be claimed when subsequent scans suggest small lesions were likely still present and in fact the patient had PR, not CR at the first time point. Under these circumstances, the original CR should be changed to PR and the best response is PR.

Patients with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be classified as having

"symptomatic deterioration". Every effort should be made to document the objective progression even after discontinuation of treatment.

In some circumstances it may be difficult to distinguish residual disease from normal tissue. When the evaluation of complete response depends on this determination, it is recommended that the residual lesion be investigated (fine needle aspirate/biopsy) to confirm the complete response status.

Immune Related Response Criteria

A growing body of literature indicates that radiographic responses to immunotherapy may have different patterns and kinetics than what would be expected with traditional cytotoxic therapies. To account for these differences we will also characterize radiographic outcomes using the immune related response criteria outlined by Wolchok and colleagues (22). See Appendix 6.

Duration of Overall Response

The duration of overall response is measured in patients with confirmed response from the time measurement criteria are met for CR or PR (whichever status is recorded first) until the first date that recurrence or PD is objectively documented, taking as reference for PD the smallest measurements recorded since the treatment started. The occurrence of the first responses over time will be described graphically.

Duration of Stable Disease

Duration of SD is measured from the start of the treatment until the criteria for disease progression are met.

Disease Control Rate (DCR)

DCR is defined as the percentage of patients that achieve an objective tumor response or stable disease to therapy.

Progression-Free Survival (PFS)

PFS is defined as the duration of time from start of treatment to time of progression or death, whichever occurs first.

Overall Survival (OS)

OS is defined as the duration of time from the start of treatment to death from any cause.

9.2 Safety

Safety assessments will consist of monitoring and recording all adverse events, including serious adverse events, the monitoring of hematology, blood chemistry, coagulation parameters, urinalysis and the regular monitoring of vital signs, and physical condition. AE will be reported per CTCAE v4.0.

10.0 Correlative Studies

10.1 Endpoints

Pre- and post-treatment tumor biopsies and blood samples will be obtained as outlined in the study calendar (Section 8.0). We will evaluate immunologic changes systemically and in the tumor microenvironment within patient's pre- to post- therapy and across the cohort of patients to identify predictive biomarkers and elucidate the mechanistic immunologic effects of therapy.

Correlative studies will include (in order of priority):

Tissue

a) IHC to assess tumor infiltrating immune cells (CD8, CD4, FoxP3) and expression of other markers with potential prognostic and/or predictive value on efficacy outcomes including MUC-1 and PD-L1 as well as new biomarkers.
b) qRT-PCR evaluation of gene signatures in the tumor microenvironment including: cytokines, T-cell activation markers, immunosuppressive enzymes and molecules (IDO, arginase, CTLA4, PD-1/PD-L1), macrophage polarization, etc.
c) RNAseq for identification of tumor neo-antigens
d) Flow Cytometry quantification, immunophenotyping, and activation / functional assessment of tumor infiltrating immune cells including myeloid-derived suppressor cells (MDSC), regulatory T (Treg) cells, T/B/NK cell immunophenotyping and activated T cells

Blood

a) Flow cytometry assessment of Natural Killer (NK) cells and Triple Positive Activated Lymphocytes (TrPAL) levels in order to analyze their value as a predictive biomarker of TG4010 activity in patients who received prior chemotherapy
b) Flow Cytometry quantification, immunophenotyping, and activation / functional assessment of tumor infiltrating immune cells including myeloid-derived suppressor cells (MDSC), regulatory T (Treg) cells, T/B/NK cell immunophenotyping and activated T cells
c) Evaluation of MUC-1, MVA, known TAA and neo-antigen specific T-cell responses using a HLA-A*02:01restricted tetramers
d) Evaluation of MUC-1 and MVA specific humoral responses
e) qPCR evaluation of gene signatures in circulating cells including: cytokines, T-cell activation markers, immunosuppressive enzymes and molecules (IDO, arginase, CTLA4, PD-1/PD-L1), macrophage polarization, etc.
f) Peripheral blood cytokine / chemokine profiling

10.2 Methodology

10.2.1 Blood

Blood samples for translational studies will be collected at baseline (prior to treatments), at M2 (or W8), at M4 and at M6.

Blood will be used to prepare PBMCs (from 50 mL of blood), and serum/plasma (from 10 mL of blood). In addition, whole blood will also be collected into PAXgene® RNA tubes (5 mL) and Cyto-Chex® tubes)(5 mL).

Immunophenotyping:

PBMCs will be stained for FACS analysis using well characterized antibodies against markers such as CD45, CD3, CD4, CD8, CD19, CD56, CD69, FOXP3, CD25, CD127, Ki67 and CD45RA for T/B/NK, activated T cells and Treg cells immunomonitoring (2).

In a separate set of cytometry experiments, fresh PBMC will be used for MDSC immunophenotyping with the following markers (LIN-(CD3-, CD19-, CD20-, CD56-)/HLA-DR-/CD11b, CD14, CD33, CD15).

For TrPAL assessment, fresh blood will be collected into Cyto-Chex® tubes. TrPAL levels will be performed at a centralize lab ie LabCorp.

T-cell Responses:

T cells will also be monitored for PD-1 and PD-L1 expression. Additionally, MUC-1 specific, MVA-specific, other TAA specific and neo-epitope specific T cells will be identified using combinatorial encoding of HLA-A*02:01 restricted tetramers. Briefly, PBMCs will be pulsed with MVA, MUC-1, TAAs or other identified neoantigen peptide sequences, incubated, and then assessed by FACS for functional responses including IFN-gamma production.

Humoral Responses:

Antibodies against MUC-1 and MVA will be assessed by ELISA on -80°C stored serum samples.

Transcriptomic analysis:

Whole blood collected in PAXgene RNA tubes and an aliquot of PBMCs will also be set aside for RNA isolation and batched analysis of gene expression. mRNA (messenger ribonucleic acid) will be extracted and reverse transcribed to cDNA. Extracted RNA will be used to perform transcriptomic arrays and identify differentially expressed RNAs. qRT-PCR (real-time reverse transcription-polymerase chain reaction) using verified primers will be then performed to investigate the expression of newly identified genes and already known ones including IDO, arginase, iNOS (inducible Nitric oxide synthases), CTLA-4, PD-1, PD-L1 and others.

Cytokine / chemokine profiling:

Plasma stored at -80°C will be evaluated for systemic cytokine and chemokine signatures using multiplex assays. Markers such as IL-2, IL-6, IL-10, IL-12p70, GM-CSF (granulocyte-

macrophage colony-stimulating factor), TNF alpha, IFN (interferon) gamma, CXCL10 (C-X-C motif chemokine 10), RANTES (regulated on activation, normal T cell expressed and secreted), MIP1 (Macrophage Inflammatory Protein) alpha, MIP1 beta, IFN alpha, IFN beta, and others will be evaluated.

10.2.2 Tissues

Tissue biopsies will be formalin fixed and paraffin embedded using standard protocols. Tumors will undergo IHC staining such as MUC1, PD-1, PD-L1, FOXP3, CD8, and CD4 markers. If sufficient biopsy material is available then a 10mg tissue sample will be frozen for batched analysis by qRT-PCR and RNAseq. If sufficient tissue is available an aliquot will also be weighed and immediately processed into a single cell suspension, stained, and analyzed by FACS as described above.

11.0 Safety Reporting of Adverse Events

11.1 Assessment of Safety - Definitions

Safety assessments will consist of monitoring and reporting of all adverse events (AEs) and serious adverse events (SAEs). The relationship to each of the study treatment should be evaluated.

11.1.1 Adverse Events

An AE for the purposes of this protocol is a medical occurrence or deterioration of a pre-existing medical condition occurring after signing the informed consent and even if the event is not considered to be related to the study treatment(s).

Preexisting medical conditions (other than the condition being studied) judged by the investigator to have worsened in severity or frequency or changed in character during the protocol-specified AE reporting period.

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

Nonserious Adverse Event Collection and Reporting

The collection of nonserious AE information should begin at initiation of study drug. All nonserious adverse events (not only those deemed to be treatment-related) should be collected continuously during the treatment period and for a minimum of 100 days following the last dose of study treatment.

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

11.1.2. Adverse drug reaction

All noxious and unintended responses to a medicinal study product (TG4010 or Nivolumab) related to any dose.

11.1.3 Serious Adverse Events

An AE should be classified as an SAE if the following criteria are met:

- It results in death (i.e., the AE actually causes or leads to death).
- It is life threatening (i.e., the AE, in the view of the investigator, places the subject at immediate risk of death. It does not include an AE that, had it occurred in a more severe form, might have caused death.).
- It requires or prolongs inpatient hospitalization: the hospitalization is an action taken to treat the event. It should not be reported as a SAE, but the AE leading to hospitalization. It results in persistent or significant disability/incapacity (i.e., the AE results in substantial disruption of the subject's ability to conduct normal life functions).
- It results in a congenital anomaly/birth defect: it relates to events occurring to babies born after their mother and/or father have taken an IMP at the time of pregnancy confirmation or during pregnancy.
- It is considered a significant medical event by the investigator based on medical judgment (e.g., may jeopardize the subject or may require medical/surgical intervention to prevent one of the outcomes listed above).

11.2 Methods and Timing for Assessing and Recording Safety Variables

The investigator is responsible for ensuring that all AEs and SAEs that are observed or reported during the study are collected and reported to the FDA, appropriate IRB(s), and Transgene/BMS, Inc. in accordance with CFR 312.32 (IND Safety Reports).

11.2.1 Adverse Event and Serious Adverse Event Reporting Period

The study period during which all AEs and SAEs must be reported begins after informed consent is obtained and ends 100 days following the last administration of study treatment. After this period, investigators should only report SAEs that are considered as related to study treatment (TG4010 and/or nivolumab).

11.2.2 Assessment of AEs and SAEs

All AEs and SAEs whether volunteered by the subject, discovered by study personnel during questioning, or detected through physical examination, laboratory test, or other means will be reported appropriately. Each reported AE or SAE will be described by its:

- AE term (ie, the nature of the event with self-explanatory and concise medical terminology indicating a diagnosis or syndrome instead of symptoms),
- date of onset and date of end (ie, actual dates when the event starts and is resolved rather than dates when the Investigator is informed),

- outcome,
- intensity,
- relation to TG4010 and/or nivolumab,
- action taken regarding the event,
- action taken regarding TG4010 / nivolumab,
- evaluation of seriousness

Evaluation of the causality:

To ensure consistency of AE and SAE causality assessments, investigators should apply the following general guideline:

Yes

There is a plausible temporal relationship between the onset of the AE and administration of nivolumab and /or TG4010, and the AE cannot be readily explained by the subject's clinical state, intercurrent illness, or concomitant therapies; and/or the AE follows a known pattern of response to nivolumab and /or TG4010; and/or the AE abates or resolves upon discontinuation of the nivolumab and/or TG4010 or dose reduction and, if applicable, reappears upon re-challenge.

No

Evidence exists that the AE has an etiology other than nivolumab + TG4010 (e.g., preexisting medical condition, underlying disease, intercurrent illness, or concomitant medication); and/or the AE has no plausible temporal relationship to nivolumab + TG4010 administration (e.g., cancer diagnosed 2 days after first dose of study drug).

Expected adverse events are those adverse events that are listed or characterized in the Package Insert or current Investigator Brochure.

Unexpected adverse events are those not listed in the Package Insert (P.I.) or current Investigator Brochure (I.B.) or not identified. This includes adverse events for which the specificity or severity is not consistent with the description in the P.I. or I.B. For example, under this definition, hepatic necrosis would be unexpected if the P.I. or I.B. only referred to elevated hepatic enzymes or hepatitis.

Evaluation of the intensity

The intensity of AE/SAE is graded according to the NCI CTCAE (version 4.03), which will be provided to the Investigators.
Should an event be missing in the CTCAE, the following 5-point scale is to be used:

- Mild: Discomfort noticed, but no disruption of normal daily activity
- Moderate: Discomfort sufficient to affect normal daily activity
- Severe: Inability to work or perform normal daily activity
- Life-threatening: Risk of death at the time of the event
- Fatal: The patient died

The correspondence between the two scales is as follows:

CTCAE	5-point scale
1	Mild
2	Moderate
3	Severe
4	Life-threatening
5	Fatal

11.3 Procedures for Eliciting, Recording, and Reporting Adverse Events

11.3.1 Eliciting Adverse Events

A consistent methodology for eliciting AEs at all subject evaluation timepoints should be adopted. Examples of non-directive questions include:

- "How have you felt since your last clinical visit?"
- "Have you had any new or changed health problems since you were last here?"

11.3.2 Specific Instructions for Recording Adverse Events

Investigators should use correct medical terminology/concepts when reporting AEs or SAEs. Avoid colloquialisms and abbreviations.

a. Diagnosis vs. Signs and Symptoms

If known at the time of reporting, a diagnosis should be reported rather than individual signs and symptoms (e.g., record only liver failure or hepatitis rather than jaundice, asterixis, and elevated transaminases). However, if a constellation of signs and/or symptoms cannot be medically characterized as a single diagnosis or syndrome at the time of reporting, it is ok to report the information that is currently available. If a diagnosis is subsequently established, it should be reported as follow-up information.

b. Deaths

All deaths that occur during the protocol-specified AE reporting period will be recorded on data collection form (Appendix 3). Reports of deaths that are associated with the study treatment must be communicated promptly to the FDA and the appropriate Institutional Review Board (IRB) and/or reported in accordance with local law and regulations. When recording a death, the event or condition that caused or contributed to the fatal outcome should be reported as the single medical concept. If the cause of death is unknown and cannot be ascertained at the time of reporting, report "Unexplained Death".

c. Preexisting Medical Conditions

A preexisting medical condition is one that is present at the start of the study. Such conditions should be reported as medical and surgical history. A preexisting medical condition should be re-assessed throughout the trial and reported as an AE or SAE only if the frequency, severity, or character of the condition worsens during the study. When reporting such events, it is important

to convey the concept that the preexisting condition has changed by including applicable descriptors (e.g., “more frequent headaches”).

d. Hospitalizations for Medical or Surgical Procedures

Any AE that results in hospitalization or prolonged hospitalization should be documented and reported as an SAE. If a subject is hospitalized to undergo a medical or surgical procedure as a result of an AE, the event responsible for the procedure, not the procedure itself, should be reported as the SAE. For example, if a subject is hospitalized to undergo coronary bypass surgery, record the heart condition that necessitated the bypass as the SAE.

Hospitalizations for the following reasons do not require reporting:

- Hospitalization or prolonged hospitalization for diagnostic or elective surgical procedures for preexisting conditions
- Hospitalization or prolonged hospitalization required to allow efficacy measurement for the study or
- Hospitalization or prolonged hospitalization for scheduled therapy of the target disease of the study.

e. Pregnancy

If, following initiation of the investigational product, it is subsequently discovered that a study subject is pregnant or may have been pregnant at the time of investigational product exposure, including during at least 6 half lives after product administration, the investigational product will be permanently discontinued in an appropriate manner (eg, dose tapering if necessary for subject safety).

The investigator must immediately notify Worldwide Safety @BMS of this event via the Pregnancy Surveillance Form in accordance with SAE reporting procedures.

Follow-up information regarding the course of the pregnancy, including perinatal and neonatal outcome and, where applicable, offspring information must be reported on the Pregnancy Surveillance Form [provided upon request from BMS]

Any pregnancy that occurs in a female partner of a male study participant should be reported to BMS. Information on this pregnancy will be collected on the Pregnancy Surveillance Form.

If a female subject becomes pregnant while receiving investigational therapy or within 120 days after the last dose of study drug, a report should be completed and expeditiously submitted to the Transgene/BMS. Follow-up to obtain the outcome of the pregnancy should also occur. Abortion, whether accidental, therapeutic, or spontaneous, should always be classified as serious, and expeditiously reported as an SAE. Similarly, any congenital anomaly/birth defect in a child born to a female subject exposed to nivolumab + TG4010 should be reported as an SAE.

f. Overdose

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

g. Laboratory values, vital signs, physical findings and other safety data

Clinically relevant abnormal laboratory values or test results will be reported by the Investigator and followed until normal, or the safety follow-up visit if they are not related to IMP. They should be recorded on the AE eCRF pages under the signs, symptoms, or diagnosis associated with them.

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

The following laboratory abnormalities should be documented and reported appropriately:

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

h. Post-Study Adverse Events

The investigator should expeditiously report any SAE occurring after a subject has completed or discontinued study participation if attributed to prior nivolumab and/or TG4010 exposure. If the investigator should become aware of the development of cancer or a congenital anomaly in a subsequently conceived offspring of a female subject who participated in the study, this should be reported as an SAE.

i. Reconciliation

The Sponsor-Investigator agrees to conduct reconciliation for the product. Transgene/BMS and the Sponsor-Investigator will agree to the reconciliation periodicity and format, but agree at minimum to exchange monthly line listings of cases received by the other party. If discrepancies are identified, the Sponsor-Investigator and Transgene/BMS will cooperate in resolving the discrepancies. The responsible individuals for each party shall handle the matter on a case-by-case basis until satisfactory resolution.

j. AEs of Special Interest (AESIs)

AEs of Special Interest are defined as a potential safety problem, identified as a result of safety monitoring of the Product. An AESIs is reported as an SAE.

The nivolumab Events of Special Interest are:

- Conditions suggestive of an autoimmune disorder, including, but not limited to, thyroiditis, colitis, rheumatoid arthritis, diabetes, vasculitis, neuritis, systemic lupus, erythematosus, Sjögren's syndrome, multiple sclerosis, etc.
- Grade ≥ 3 acute infection (bacterial, viral, zoonotic, or fungal)

- Grade ≥ 3 events suggestive of hypersensitivity, cytokine release, systemic inflammatory response, or infusion reaction syndromes, including, but not limited to, fever, chills, rash, urticaria, dyspnea, wheezing, angioedema, tachycardia, hypotension.
- Grade ≥ 2 rash or pruritis
- Grade ≥ 2 diarrhea or colitis
- Grade ≥ 3 AST/ALT/total bilirubin elevations lasting > 48 hours, asymptomatic
- Grade ≥ 2 AST/ALT/total bilirubin elevations lasting > 48 hours, with constitutional symptoms
- Grade ≥ 2 dyspnea not attributable to lung cancer or other pulmonary disease present at baseline (e.g., chronic obstructive pulmonary disease)
- Grade ≥ 2 hypoxia not attributable to lung cancer or other pulmonary disease present at baseline (e.g., chronic obstructive pulmonary disease)
- Grade ≥ 2 pleural effusion
- Grade ≥ 2 pericardial effusion

Additional data will be collected for the following selected adverse events:

- Grade≥ 2 pleural effusion
- Grade≥ 2 pericardial effusion
- Infusion-related reactions
- Flu-like symptoms

k. SAE Collection and Reporting

Following the subject's written consent to participate in the study, all SAEs, whether related or not related to study drug, must be collected, including those thought to be associated with protocol-specified procedures. All SAEs must be collected that occur within 100 days of discontinuation of dosing.

All SAEs must be collected that occur during the screening period. If applicable, SAEs must be collected that relate to any protocol-specified procedure (eg, a follow-up skin biopsy). The investigator should report any SAE that occurs after these time periods that is believed to be related to study drug or protocol-specified procedure.

SAEs, whether related or not related to study drug, and pregnancies must be reported to Transgene/BMS within 24 hours. SAEs must be recorded on BMS or an approved form; pregnancies on a Pregnancy Surveillance Form.

Global Pharmacovigilance & Epidemiology
Bristol-Myers Squibb Company
SAE Email Address: Worldwide.Safety@BMS.com
SAE Facsimile Number: 609-818-3804

Fax: (33) 03 69 22 75 07 it is specific to the PV and linked to the safety email box. Alternatively email the reports to E-mail: safety@transgene.fr

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

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

All SAEs should be followed to resolution or stabilization.

The Sponsor/Investigator will ensure that all SAEs in the clinical database are reported to Transgene/BMS and any applicable health authority during the conduct of the study including periodic reconciliation.

Note: Investigators should also report events to their IRB as required.

11.4 MedWatch 3500A Reporting Guidelines

In addition to completing appropriate patient demographic and suspect medication information, the report should include the following information within the Event Description of the MedWatch 3500A form:

- Protocol description (and number, if assigned)
- Description of event, severity, treatment, and outcome if known
- Supportive laboratory results and diagnostics
- Investigator's assessment of the relationship of the adverse event to each investigational product and suspect medication

Follow-up Information

- Additional information may be added to a previously submitted report by any of the following methods:
- Adding to the original MedWatch 3500A report and submitting it as follow-up
- Adding supplemental summary information and submitting it as follow-up with the original MedWatch 3500A form
- Summarizing new information and faxing it with a cover letter including patient identifiers (i.e. D.O.B. initial, patient number), protocol description and number, if assigned, brief adverse event description, and notation that additional or follow-up information is being submitted (The patient identifiers are important so that the new information is added to the correct initial report)

Occasionally Transgene/BMS may contact the reporter for additional information, clarification, or current status of the patient for whom and adverse event was reported. For questions regarding SAE reporting, you may contact the Transgene/BMS Drug Safety representative noted above or the MSL assigned to the study. Relevant follow-up information should be submitted to Transgene/BMS Drug Safety as soon as it becomes available and/or upon request.

MedWatch 3500A (Mandatory Reporting) form is available at
http://www.fda.gov/medwatch/getforms.html

11.5 Additional Reporting Requirements for IND Holders

For Investigator-Sponsored IND Studies, some additional reporting requirements for the FDA apply in accordance with the guidance set forth in 21 CFR § 600.80.

Events meeting the following criteria need to be submitted to the Food and Drug Administration (FDA) as expedited IND Safety Reports according to the following guidance and timelines:

7 Calendar Day Telephone or Fax Report:

The Investigator is required to notify the FDA of any fatal or life-threatening adverse event that is unexpected and assessed by the investigator to be possibly related to the use of nivolumab and/or to TG4010. An unexpected adverse event is one that is not already described in the nivolumab Investigator Brochure and the TG4010 Investigator Brochure. Such reports are to be telephoned or faxed to the FDA within 7 calendar days of first learning of the event. Transgene and BMS will be provided with a simultaneous copy of all adverse events filed with the FDA.

15 Calendar Day Written Report

The Investigator is also required to notify the FDA and all participating investigators, in a written IND Safety Report, of any serious, unexpected AE that is considered reasonably or possibly related to the use of nivolumab and/or TG4010. An unexpected adverse event is one that is not already described in the nivolumab Investigator Brochure and the TG4010 investigator brochure.

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

Written IND safety reports with Analysis of Similar Events are to be submitted to the FDA, and all participating investigators within 15 calendar days of first learning of the event. Transgene/BMS will be provided with a simultaneous copy of all adverse events filed with the FDA.

Under requirements of 21 CFR312.23, the completed MedWatch form and FDA Form 1571 must be sent to the FDA. If assistance is needed with completing filing the IND safety report, you may contact the Clinical Trial Support Unit (CTSU) Protocol Development Officer/IND Manager.

FDA fax number for IND Safety Reports:

Fax: 1 (800) FDA 0178
All written IND Safety Reports submitted to the FDA by the Investigator must also be faxed to Transgene/BMS Drug Safety:

Fax: (33) 03 69 22 75 07 it is specific to the PV and linked to the safety email box. Alternatively email the reports to E-mail: safety@transgene.fr

Global Pharmacovigilance & Epidemiology/Bristol-Myers Squibb Company
Fax Number: 609-818-3804
Email: Worldwide.safety@bms.com

And to the Site IRB per institutional policies:

For questions related to safety reporting, please fax to Transgene/BMS Drug Safety:
(+ (33) (0)3.88.27.91.41 (EU) or + (1) (617) 679 8050 (USA)**)**

11.5.1 IND Annual Reports

Copies to Transgene/BMS:

All IND annual reports submitted to the FDA by the Sponsor-Investigator should be copied to Transgene/BMS. Copies of such reports should be faxed to Transgene/BMS Drug Safety:

All IND annual reports submitted to the FDA by the Sponsor-Investigator should be copied to Transgene/BMS. Copies of such reports should be faxed to Transgene/BMS Drug Safety:

Global Pharmacovigilance & Epidemiology/Bristol-Myers Squibb Company
Fax Number: 609-818-3804
Email: Worldwide.safety@bms.com

Kelly H. Reich, M.S., RAC
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Biologics Consulting
(703) 739-5695 – Main
(703) 548-7457 – Fax
kreich@biologicsconsulting.com
BiologicsConsulting.com

11.6 Study Close-Out

Any study report submitted to the FDA by the Sponsor-Investigator should be copied to Transgene/BMS. This includes all IND annual reports and the Clinical Study Report (final study report). Additionally, any literature articles that are a result of the study should be sent to Transgene/BMS. Copies of such reports should be emailed to the assigned Clinical Operations contact for the study:

Global Pharmacovigilance & Epidemiology/Bristol-Myers Squibb Company
Fax Number: 609-818-3804
Email: Worldwide.safety@bms.com

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12.0 Statistics

12.1 Sample size

The primary endpoint of this trial is the Objective Response Rate (ORR) defined by RECIST 1.1

The null hypothesis for response rate H0 is set at 20% corresponding to the response rate observed for the class of PD-1 blockers in patients with NSCLC who have progressed after at least one line of systemic treatment for the advanced stage of the disease (23). The alternative hypothesis of efficacy is set at HA=40%. The type I error α is set at 5% one-sided; the power is set at 80%. Under these hypotheses, an interim analysis for futility and efficacy is planned after 15 evaluable patients. If less than 3/15 patients are considered responders corresponding to α_1=0.002, stop for futility, if more than 8 patients are considered responders stop for efficacy, otherwise the enrollment will be continued.

For the second part of the trial, enough patients will be treated to obtain a total of 29 evaluable patients. The study will be considered positive if at least 10/29 of them are considered responders (α_2=0.05). To account for patients who are not evaluable (10%), 33 patients will be recruited.

The ORR analysis will be performed 18 weeks after last patient-in.

12.2 Analysis populations

12.2.1 Intent-To-Treat (ITT) set

The ITT set will contain all included patients. ITT set will be the primary population for summaries of demographic and baseline variables.

12.2.2 Evaluable patients set

The evaluable patients set will contain all included patients who are evaluable for efficacy, ie a patient who:

- has at least one baseline and one post-baseline evaluable scan before the time of analysis or,
- had at least one cycle of treatment (2 injections of TG4010 and 1 infusion of nivolumab).

The evaluable patients set will be the primary population for the analysis of the primary endpoint.

12.2.3 Safety set

The safety set will contain all patients who entered the study and received at least one dose of TG4010 or Nivolumab.

12.3 Study endpoints

Please see Table 2 Section 2.0

12.4 Methods of analysis

12.4.1.1 General considerations

Statistical summaries will be produced using SAS® software version 9.3 or higher. Continuous variables will be described using the number of observations (N), arithmetic mean (Mean), standard deviation (SD), minimum (MIN), median (Median), and maximum (MAX). Means will be further described with 95% confidence intervals (CIs) where appropriate. Categorical variables will be summarized by frequency (N) and percentage (%). Proportions will be estimated with their exact (binomial) 95% CIs when appropriate.

12.4.2 Disposition of patients

The number of screen failure patients and reasons for screen failure will be summarized. A patient listing will be provided with the reason of screen failure.

The disposition data will be presented by patient in data listings and the following items will be summarized based on the ITT population:

- The number of patients included in each population,
- The number of patients excluded from the populations and reasons for exclusion,

- The number of patients who are still on treatment,
- The number of patients who discontinued TG4010 only and reasons for discontinuation,
- The number of patients who discontinued Nivolumab only and reasons for discontinuation,
- The number of patients who discontinued all study treatments and reasons for discontinuation

12.4.3 Demographic and baseline characteristics

Baseline demographics and patient and disease characteristics data will be listed and summarized. Qualitative data (e.g., gender, ethnic origin, PS, smoking status) will be summarized by means of contingency tables, and quantitative data (e.g., age and body weight) will be summarized by appropriate descriptive statistics (mean, standard deviation, median, minimum, and maximum).

Summaries will be provided overall for the ITT population and for key subpopulations, i.e. evaluable population, safety population, and per protocol population.

12.4.4 Treatments

12.4.4.1 Prior anti-cancer therapies

Prior systemic therapy for advanced disease will be summarized and listed.

Prior antineoplastic therapies (excluding 1st line for advanced disease) will be listed and summarized (surgery, radiotherapy, chemotherapy neo/adjuvant for early stage…).

The ITT set will be used for all summaries and listings of prior medications.

12.4.4.2 Study treatments

Exposure to TG4010 will be provided by listing and summarizing the number of injections and the duration of treatment.

Exposure to Nivolumab will be provided by listing and summarizing the number of infusions, the cumulated dose and the duration of treatment.

The temporal relationship of both agents will be determined from the above information.

12.4.4.3 Concomitant medications

Medications and / or significant non-drug therapies taken between ICF signature and start of study will be listed.

The concomitant medications (i.e., ongoing at the start of study treatment or taken during the course of the study) will be coded using the WHO Drug Dictionary and will be listed and

summarized by active ingredient and treatment arm by means of frequency counts and percentages.

The safety set will be used for all summaries and listings of study treatment.

12.4.5 Analysis of efficacy

12.4.5.1 Primary efficacy analysis: Overall Response Rate

The Overall Response Rate (ORR) is defined as the proportion of patients whose Best Overall Response (BOR) is either CR or PR according to RECIST 1.1.

Proportions of patients with a best overall response of CR or PR will be presented with exact 95% confidence intervals and p-value associated to the binomial test (with null proportion as reference). Patients with response 'unknown' will be summarized by reason for having unknown status.

The primary analysis will be performed on the Evaluable patient set. The analysis will be repeated on ITT for confirmation.

12.4.5.2 Secondary efficacy analyses

All secondary efficacy endpoints will be analyzed on evaluable patients set and repeated on the ITT populations.

- **Progression-Free Survival (PFS)**

PFS is defined as the time from enrollment to the date of first documented radiographic tumor progression or death due to any cause, whichever occurs first. If a patient has not had a PFS event at the cut-off date for analysis or before withdrawal, PFS will be censored at the date of last evaluable tumor assessment before the cut-off or before withdrawal.

PFS will be presented using a Kaplan-Meier curve. Summary statistics from the Kaplan Meier distributions will be determined, including median PFS and 25% and 75% quartiles with corresponding 95% confidence intervals (CIs). The proportions of patients remaining progression free at 3, 6 and 9 months, along with 95% CIs, will also be provided.

Analysis will be performed based on RECIST 1.1.

- **Overall Survival (OS)**

OS is defined as the time from enrollment until death from any cause. For patients not known to have died at the time of the analysis, overall survival will be censored on the date they were last known to be alive.

OS will be presented using a Kaplan-Meier curve. Summary statistics from the Kaplan Meier distributions will be determined, including median OS and 25% and 75% quartiles with corresponding 95% confidence intervals (CIs) using the method of Klein and Moeschberger (1997) as implemented in SAS PROC LIFETEST.. The proportions of patients remaining alive at 6, 9, 12, 15, 18 and 24 months, along with 95% CIs will also be provided.

- **Duration of response**

Duration of response (DoR) applies only to patients whose best overall response is CR or PR (confirmed response). The DoR is defined as the time from the first documented response (CR or PR) until the event defined as first documented disease progression. If a patient has not had a DoR event at the cut-off date for analysis, DoR will be censored at the date of last evaluable tumor assessment before the cut-off or before withdrawal.

DoR will be presented using a Kaplan-Meier curve. Summary statistics from the Kaplan Meier distributions will be determined, including median DoR and 25% and 75% quartiles with corresponding 95% confidence intervals (CIs), using the method of Klein and Moeschberger (1997) as implemented in SAS PROC LIFETEST.

Analysis will be performed based on RECIST 1.1.

- SD

The SD Rate (SDR) is defined as the proportion of patients whose Best Overall Response (BOR) is SD. Proportions of patients with a best overall response of SD will be presented with exact 95% confidence intervals and p-value associated to the binomial test (with null proportion as reference). Patients with response 'unknown' will be summarized by reason for having unknown status.

Analysis will be performed based on RECIST 1.1.

- Duration of SD

Duration of SD (DSD) applies only to patients whose best overall response is SD. The DSD is defined as the time from the first documented stable disease status (SD) until the event defined as first documented disease progression. If a patient has not had a DSD event at the cut-off date for analysis, DSD will be censored at the date of last evaluable tumor assessment before the cut-off.

DSD will be presented using a Kaplan-Meier curve. Summary statistics from the Kaplan Meier distributions will be determined, including median DSD and 25% and 75% quartiles with corresponding 95% confidence intervals (CIs) using the method of Klein and Moeschberger (1997) as implemented in SAS PROC LIFETEST.

Analysis will be performed based on RECIST 1.1.

- Disease Control Rate

The Disease Control Rate (DCR) is defined as the proportion of patients whose Best Overall Response (BOR) is either CR, PR or SD. Proportions of patients with a best overall response of CR, PR or SD will be presented with exact 95% confidence intervals and p-value associated to the binomial test (with null proportion as reference). Patients with response 'unknown' will be summarized by reason for having unknown status.

Analysis will be performed based on RECIST 1.1.

12.4.5.3 Exploratory efficacy analyses

- **ORR by irRC**

The Overall Response Rate (ORR) by irRC is defined as the proportion of patients whose Immune Related Best Overall Response (irBOR) is either irCR or irPR according to irRC.
Proportions of patients with a irBOR of irCR or irPR will be presented with exact 95% confidence intervals and p-value associated to the binomial test (with null proportion as reference).

- **PFS and DOR analyses will be repeated using irRC.**

- **Evolution of tumor size over time**

Tumor size is defined as the sum of the longest diameters for target tumors (SLD) as identified at baseline when the same method of evaluation is used. For each time point, tumor size will be calculated and summarized along with the relative change from baseline.

Waterfall plots will be used to represent the best change. These plots will display the best percentage change from baseline in the sum of the longest diameter of target tumors for each patient.

- **Biomarkers**

Descriptive analyses will be performed to analyze the impact of biomarkers on ORR, OS or PFS.
Relationships between TG4010 plus Nivolumab and biomarkers at single time points and/or over time will be described for the below mentioned parameters and put into perspective with the clinical outcome:

Tissue

a) IHC to assess tumor infiltrating immune cells (CD8, CD4, FoxP3) and expression of other markers with potential prognostic and/or predictive value on efficacy outcomes including MUC-1 and PD-L1 as well as new biomarkers.
b) qRT-PCR evaluation of gene signatures in the tumor microenvironment including: cytokines, T-cell activation markers, immunosuppressive enzymes and molecules (IDO, arginase, CTLA4, PD-1/PD-L1), macrophage polarization, etc.
c) RNAseq for identification of tumor neo-antigens
d) Flow Cytometry quantification, immunophenotyping, and activation / functional assessment of tumor infiltrating immune cells including myeloid-derived suppressor cells (MDSC), regulatory T (Treg) cells, T/B/NK cell immunophenotyping and activated T cells

Blood

a) Flow cytometry assessment of Natural Killer (NK) cells and Triple Positive Activated Lymphocytes (TrPAL) levels in order to analyze their value as a predictive biomarker of TG4010 activity in patients who received prior chemotherapy
b) Flow Cytometry quantification, immunophenotyping, and activation / functional assessment of tumor infiltrating immune cells including myeloid-derived suppressor cells (MDSC), regulatory T (Treg) cells, T/B/NK cell immunophenotyping and activated T cells
c) Evaluation of MUC-1, MVA, known Tumor Associated Antigens (TAA) and neo-antigens specific T-cell responses using a HLA-A*02:01 restricted tetramers
d) Evaluation of MUC-1 and MVA specific humoral responses
e) qRT-PCR evaluation of gene signatures in circulating cells including: cytokines, T-cell activation markers, immunosuppressive enzymes and molecules (IDO, arginase, CTLA4, PD-1/PD-L1), macrophage polarization, etc.
f) Peripheral blood cytokine / chemokine profiling

Continuous variables (e.g., laboratory values or cytokines levels) may be further classified into categorical variables using quartiles, normal ranges, or limit of detection as appropriate.

Exploratory analyses will assess the association between biomarker result and clinical outcome (ORR, OS, PFS). For ORR, the distribution of biomarker will be compared for responders with that for non-responders. For biomarkers with discrete distributions, distributions will be compared by Fisher's exact test or by exact contingency table methods if the biomarker has more than 2 categorical outcomes. For continuous biomarker data, Student's t test will be used to compare biomarker distributions in responders to non-responders when appropriate. Biomarker data will be transformed (e.g., logarithms) prior to analysis if transformation is needed to meet the assumptions of normality and homogeneity of variances that underlie these methods. Nonparametric methods, such as the Wilcoxon Mann Whitney test, will be used if biomarker data fail to meet these assumptions even after transformation. For OS and PFS, biomarkers will be considered individually as predictors of time-to-event using Cox proportional hazards models. For ORR, logistic regression model will be used including treatment arm, biomarker levels and interaction as explanatory parameters.

In order to explore or discover potentially important relationships between various markers, other exploratory statistical methods may be used, such as multivariate data reduction or statistical learning. All analyses will be at significance level error $\alpha=0.05$, with any statistically significant findings viewed as exploratory and requiring confirmation in further studies. Since these analyses are considered as hypothesis-generating, there will be no formal correction for multiple testing.

12.4.6 Analysis of safety

Safety analyses will be based on the Safety population. The safety summary tables will include all safety assessments collected up to 100 days after last study treatment administration. All safety data will be listed and those collected later than 100 days after study treatment discontinuation will be flagged in the listings.

The assessment of safety will be based mainly on the frequency of AEs and on the number of laboratory values that fall outside of predetermined ranges. Other safety data (e.g., ECG, vital signs) will be considered as appropriate.

Overall incidence of AEs and SAEs will be evaluated.

Adverse events/SAEs occurring after signing the ICF but before starting study treatment will be listed separately from those occurring after treatment start.

Adverse events

All AEs, significant AEs, AEs leading to discontinuation, and SAEs recorded during the study will be listed and summarized by body system organ class and preferred terms, intensity (based on the NCI CTCAE grades, also referred as CTCAE), relationship to each study treatment component (TG4010 or Nivolumab or the combination).

AEs will be summarized by presenting the number and percentage of patients having at least one AE. Data will be presented by System organ Class (SOC) and preferred term using MedDRA coding. A patient with multiple occurrence of an AE will be counted only once in the AE category.

Separate AE summaries will be presented by SOC, preferred terms and maximum CTCAE grade. The frequency of CTCAE grade 3 and 4 AEs will be summarized separately.

Written narratives will be produced for all SAEs and unexpected or other important AEs that are judged to be of special interest because of their clinical importance.

Fatal Events

All fatal events will be listed and summarized by System Organ Class (SOC) and Preferred Term (PT), intensity (based on the NCI CTCAE grades, also referred as CTCAE).

Fatal events will be summarized by presenting the number and percentage of patients who died. Data will be presented by SOC and PT using MedDRA coding.

Fatal events occurring after signing the Inform Consent Form (ICF) but before starting study treatment, including those observed in patients randomized but never treated with the IMP, will be listed separately from those occurring after treatment start.

Laboratory abnormalities

The summaries will include all laboratory assessments collected no later than 100 days after treatment discontinuation. All laboratory assessments will be listed and those collected during 100 days after study treatment discontinuation will be flagged in the listings.

All laboratory values will have a severity grade calculated using appropriate common terminology criteria for AEs (NCI CTCAE, version 4.03). A listing of laboratory values will be provided by laboratory parameter and patient. A separate listing will display notable laboratory

abnormalities (i.e., newly occurring CTCAE Grade 3 or 4 laboratory toxicities). The frequency of laboratory abnormalities will be displayed by parameter.

Other safety data

Other safety data (e.g., vital signs, electrocardiogram) will be listed, notable values will be flagged, and any other information collected will be listed as appropriate. Any statistical procedures performed in order to explore the data will be used only to highlight any interesting comparisons that may warrant further consideration.

13.0 Ethical Considerations and Administrative Procedures

13.1 Ethics and Good Clinical Practice

This study must be carried out in compliance with the protocol and Good Clinical Practice, as described in:

1. ICH Harmonized Tripartite Guidelines for Good Clinical Practice 1996.
2. US 21 Code of Federal Regulations dealing with clinical studies (including parts 50 and 56 concerning informed consent and IRB regulations).
3. Declaration of Helsinki, concerning medical research in humans (Recommendations Guiding Physicians in Biomedical Research Involving Human Subjects, Helsinki 1964, amended Tokyo 1975, Venice 1983, Hong Kong 1989, Somerset West 1996).

The investigator agrees, when signing the protocol, to adhere to the instructions and procedures described in it and thereby to adhere to the principles of Good Clinical Practice that it conforms to.

13.2 Institutional Review Board/Independent Ethics Committee

Before implementing this study, the protocol, the proposed informed consent form and other information to subjects, must be reviewed by a properly constituted Institutional Review Board/Independent Ethics Committee (IRB/IEC/REB). A signed and dated statement that the protocol and informed consent have been approved by the IRB/IEC/REB must be given to Transgene/BMS before study initiation. Any amendments to the protocol, other than administrative ones, must be approved by this committee.

13.3 Informed Consent

The investigator must explain to each subject (or legally authorized representative) the nature of the study, its purpose, the procedures involved, the expected duration, the potential risks and benefits involved and any discomfort it may entail. Each subject must be informed that participation in the study is voluntary and that he/she may withdraw from the study at any time

and that withdrawal of consent will not affect his/her subsequent medical treatment or relationship with the treating physician.

This informed consent should be given by means of a standard written statement, written in non-technical language. The subject should read and consider the statement before signing and dating it, and should be given a copy of the signed document. If the subject cannot read or sign the documents, oral presentation may be made or signature given by the subject's legally appointed representative, if witnessed by a person not involved in the study, mentioning that the patient could not read or sign the documents. No patient can enter the study before his/her informed consent has been obtained.

The informed consent form is considered to be part of the protocol, and must be submitted by the investigator with it for IRB/IEC/REB approval.

Fertile men and women of child bearing potential should be informed that taking the study medication may involve unknown risks to the fetus if pregnancy were to occur during the study and agree that in order to participate in the study they must adhere to the contraception requirement for the duration of the study. If there is any question that the patient will not reliably comply, they should not be entered in the study.

13.4 Discontinuation of Study Support

Transgene/BMS reserves the right to discontinue support for any study under the conditions specified in the clinical trial agreement.

13.5 Amendments to the Protocol

Any change or addition to this protocol requires a written protocol amendment that must be approved by Transgene/BMS and the investigator before implementation. Any protocol amendment requires approval by the IRB/IEC/REB. A copy of the written approval of the IRB/IEC/REB, must be sent to Transgene/BMS.

13.5.1 Publication of Results

Any formal presentation or publication of data from this trial may be published after review and comment by Transgene/BMS and prior to any outside submission. Transgene/BMS must receive copies of any intended communication in advance of publication (at least ten working days for presentational materials and abstracts and fifteen working days for manuscripts). These requirements acknowledge Transgene/BMS's responsibility to provide peer input regarding the scientific content and conclusions of such publications or presentations. Principal Investigator/Institution shall have the final authority to determine the scope and content of its publications, provided such authority shall be exercised with reasonable regard for the interests of Transgene/BMS and, in accord with the trial contract and shall not permit disclosure of Transgene/BMS confidential or proprietary information.

13.5.2 Disclosure and Confidentiality

The investigator agrees to keep all information provided by Transgene/BMS in strict confidence and to request similar confidentiality from his/her staff and the IRB/IEC/REB. Study documents provided by Transgene/BMS (investigators' brochures and other material) will be stored appropriately to ensure their confidentiality. The information provided by Transgene/BMS to the investigator may not be disclosed to others without direct written authorization from Transgene/BMS, except to the extent necessary to obtain informed consent from patients who wish to participate in the trial.

13.5.3 Declaration of Helsinki

The investigator must conduct the trial in accordance with the principles of the Declaration of Helsinki. Copies of the Declaration of Helsinki and amendments will be provided upon request or can be accessed via the website of the World Medical Association at http://www.wma.net/e/policy/17-c_e.html.

13.6 Protocol Deviations

Protocol deviations will be reported in accordance with UCD IRB Administration and UCD Cancer Center CTSU policies or the participating site's IRB policies.

14.0 Data Monitoring Committee

In addition to the requirements for adverse event reporting as outlined in Section 13.0, this protocol is subjected to the UC Davis Cancer Center's (UCDCC) Data and Safety Monitoring Plan. The UCDCC is committed to pursuing high-quality patient-oriented clinical research and has established mechanisms to ensure both scientific rigor and patient safety in the conduct of clinical research studies. The UCDCC relies on a multi-tiered committee system that reviews and monitors all cancer clinical trials and ensures the safety of its participants, in compliance with institutional and federal requirements on adverse event (AE) reporting, verification of data accuracy, and adherence to protocol eligibility requirements, treatment guidelines, and related matters. The Scientific Review Committee (SRC) assumes overall oversight of cancer studies, with assistance and input from two independent, but interacting, committees: the Quality Assurance Committee and the Data and Safety Monitoring Committee. A multi-level review system strengthens the ability of the UCDCC to fulfill its mission in conducting high quality clinical cancer research.

14.1 Multisite monitoring

Participating sites are required to follow their institutional guidelines for clinical trial conduct. In addition, quality assurance audits of selected patients and source documents will be conducted by the UC Davis Cancer Center Quality Assurance Committee as outlined in the UC Davis Cancer Center Data and Safety Monitoring Plan.

Quality control will be maintained by the CTSU Quality Assurance team according to CTSU policy. Quality assurance audits may be accomplished in one of two ways: (1) source documents and research records for selected patients are mailed/sent from participating sites to the Coordinating Center for audit, or (2) selected patient records may be audited on-site.

15.0 References

1. McCoach CE and Kelly K. Treatment Paradigms in Advanced Non-Small Cell Lung Cancer. Clin Adv Hematol Oncol 11:629-39, 2013.
2. Soria J-C, Marabelle A, Brahmer JR, Gettinger, S. Immune Checkpoint Modulation for Non-Small Cell Lung Cancer. Clin Cancer Res 21:2256-62, 2015
3. Antonia SJ, Larkin J, Ascierto PA. Immuno-oncology combinations: A review of clinical experience and future prospects. Clin Cancer Res 20:1-11, 2014.
4. Chen DS and Mellman I. Oncology meets immunology: the cancer-immunity cycle. Immunity 39:1-10, 2013.
5. Dong H, Strome SE, Salomao DR, et al. Tumor associated B7-H1 promotes T-cell apoptosis: a potential mechanism of immune evasion. Nat Med 2002; 8: 793-800.
6. Iwai Y, Ishida M, Tanka Y, et al. Involvement of PD-L1 on tumor cells in the escape from host immune system and tumor immunotherapy by PD-L1 blockade. Proc Natl Acad Sci U S A 2002; 99: 12293-7.
7. Topalian SL, Hodi FS, Brahmer JR et al. Safety, Activity, and Immune Correlates of Anti-PD-1 Antibody in Cancer. N. Engl. J Med. 2012: 366: 2443-2454
8. Rizvi et al, Latin America Lung Cancer Meeting 2014.
9. Rizvi NA, Mzieres J, Planchard D, et al. Activity and safety of nivolumab, an anti-PD-1 immune checkpoint inhibitor, for patients with advanced, refractory squamous non-small-cell lung cancer (CheckMate 063): a Phase 2, single-arm trial. Lancet Oncol 2015; 16: 257-265
10. Press release, Bristol-Myers Squibb, 1/11/15
11. Brahmer J, Reckamp K, Baas P et al. Nivolumab versus Docetaxel in Advanced Squamous-Cell Non-Small-Cell Lung Cancer. N Engl J Med 2015 Epub: 1-15
12. Paz-Ares L, Horn L, Borghaei H et al. Phase III, randomized trial (CheckMate 057) of Nivolumab versus Docetaxel in Advanced Non-squamous (non-SQ) Cell Non-small Cell Lung Cancer (NSCLC). ASCO Annual meeting 2015
13. Cohen S, Kaufman HL. TG-4010 Transgene. Curr Opin Investig Drugs 5:1319-1328, 2004.
14. Ho SB, Niehans GA, Lyftog C, et al. Heterogeneity of mucin gene expression in normal and neoplastic tissues. Cancer Res 53:641-651, 1993.
15. Transgene TG4010 Investigators Brochure Edition 11, July 2013.
16. Ramlau R, Quoix E, Rolski J, et al. A phase II study of TG4010 (Mva-Muc1-IL2) in association with chemotherapy in patients with stage III/IV non-small cell lung cancer. J Thorac Oncol. 3:735-744, 2008.
17. Quoix E, Ramlau R, Westeel V, et al. Therapeutic vaccination with TG4010 and first-line chemotherapy in advanced non-small cell lung cancer: a controlled phase 2B trial. Lancet Oncology 12:1125-1135, 2011.
18. Vivier E, Tomaselo E, Baratin M, et al. Functions of natural killer cells. Nat Immunolog 9:503-510, 2008.

19. Quoix E, Forget F, Papai Z, et al. Results of the Phase 2B part of TIME study evaluating TG4010 immunotherapy in Stage IV non-small cell lung cancer (NSCLC) patients receiving first-line chemotherapy. Abstract #3034, ASCO 2015.
20. Rittner K, Remy-Ziller C, Hortelano J et al. Immune checkpoint inhibitors enhance benefits of modified vaccinia virus Ankara to improve survival in preclinical models of cancer. Abstract #2497, AACR 2015
21. Eisenhauer EA, Therasse P, Boggerts J, et al. New response evaluation criteria in solid tumors: revised RECIST guideline (version 1.1). Eur J Cancer 45:228-247, 2009.
22. Wolchok JD, Hoos A, O'Day S, Weber JS, Hamid O, Lebbé C, Maio M, Binder M, Bohnsack O, Nichol G, Humphrey R, Hodi FS. et al. Guidelines for the evaluation of immune therapy activity in solid tumors: immune-related response criteria. Clin Cancer Res. 2009;15(23):7412–7420.
23. Borghaei, H, Paz-Ares L, Horn L., et al. Nivolumab versus Doectaxel in Advanced NonSquamous Non-Small-Cell Lung Cancer. N Engl J Med 373:1627-1639, 2015.

Appendix 1: Performance Status Scale

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

Appendix 2: Study Registration

A. Registrations for this protocol must be made through the Clinical Trials Support Unit (CTSU) of the University of California, Davis Cancer Center between normal business hours (Pacific Time), Monday through Friday (except holidays). Documentation of current IRB approval of this protocol by non-UCD institutions must be on file prior to registration of patients at these institutions.

B. Pre-study laboratory tests, scans, and x-rays (if applicable), must be completed prior to registration, within the time frame specified in the protocol. The eligibility checklist must be completed. Patients must sign an informed consent prior to registration.

C. If the patient is to be registered the same day as the proposed treatment start date, the UC Davis Protocol Coordinator must be notified by fax ([redacted]) 24 hrs prior to proposed treatment start date that the site has a patient to register.

D. Patients may be registered up to 72 hrs prior to treatment initiation. The signed consent, completed checklist and reports from all pre-study laboratory tests, scans and x-rays (if applicable) must be faxed to the University of California, Davis Cancer Center Clinical Trials Support Unit in order to register the patient. The UC Davis Protocol Coordinator will review these documents and fax a registration confirmation within 3 hours. **NOTE: Administration of study medication may not be initiated until the registration confirmation has been received.**

E. A patient failing to meet all protocol requirements may not be registered. If you have any questions regarding eligibility, please contact the coordinating site PI or Study Coordinator

Appendix 3: Data Submission Schedule

All data will be collected using UC Davis data collection forms. Copies of the completed forms will be submitted to UC Davis data coordinating center for data entry and storage in a secure location. The original data collection forms will reside at the originating institution in secure location.

- SUBMIT WITHIN 24 HOURS OF REGISTRATION:
  Patient Registration Form

- SUBMIT WITHIN 14 DAYS OF REGISTRATION:
  In-House Pre-Study Evaluation Form (IH-102)

- SUBMIT WITHIN 7 DAYS OF SCREENING FAILURE:
  Patient Screen Failure Form

- SUBMIT WITH 14 DAYS OF CYCLE COMPLETION:
  Adverse Event/Drug Relationship Form

- SUBMIT WITHIN 14 DAYS OF END OF EACH TREATMENT CYCLE:
  In-House Treatment Cycle Form – Infusion

- SUBMIT WITHIN 14 DAYS OF EACH RESPONSE ASSESSMENT:
  Tumor Measurement Log

- SUBMIT WITHIN 14 DAYS OF OFF TREATMENT:
  Off Treatment/In Follow-up/Off Study/Expiration Form (IH-301)

- SUBMIT WITHIN 14 DAYS OF KNOWLEDGE OF DEATH IF PATIENT IS STILL ON STUDY OR 30–DAYS IF OFF STUDY:
  Off Treatment/In Follow-up/Off Study/Expiration Form (IH-301)

- SUBMIT WITHIN 2 DAYS OF KNOWLEDGE OF PROTOCOL DEVIATION:
  Clinical Trials Support Unit: Notice of Protocol Deviation

- SUBMIT WITHIN 14 DAYS OF EACH REQUIRED FOLLOW-UP ENCOUNTER:
  Follow-Up Form (IH-302)

- ALL SERIOUS ADVERSE EVENTS MUST BE REPORTED AS OUTLINED IN THE PROTOCOL.

Appendix 4: Molecular Correlative Sample Handling

It is required that paraffin-embedded tissue blocks or slides from time of diagnosis (or subsequent, but prior to therapy) as well as blood specimens and fresh tumor biopsies, as outlined below, be submitted for expression of relevant molecular targets. A specimen submission form should be filled out for each specimen obtained. All specimens must be labeled with protocol number, patient registration number, date of specimen collection, and number of cells (for PBMCs) or weight of tissue (for tissue biopsies).

For correlative studies the following samples should be collected:

1. Archival tissue specimen or 15 unstained slides.
2. Peripheral blood samples obtained pre-treatment and at 2, 4, and 6 months. Optional blood sample at progression.
3. Fresh tumor biopsy pre-treatment and at 2 months. Optional biopsy at progression.

Correlative studies will include (in order of priority):

Blood

1. Flow cytometry assessment of Natural Killer (NK) cells and Triple Positive Activated Lymphocytes (TrPAL) levels in order to analyze their value as a predictive biomarker of TG4010 activity in patients who received prior chemotherapy
2. Flow Cytometry quantification, immunophenotyping, and activation / functional assessment of tumor infiltrating immune cells including myeloid-derived suppressor cells (MDSC), regulatory T (Treg) cells, T/B/NK cell immunophenotyping and activated T cells
3. Evaluation of MUC-1, MVA, known TAA and neo-antigen specific T-cells using a HLA-A*02:01 restricted tetramers
4. Transcriptome analysis by RNA deep sequencing
5. Whole exome sequencing (WES) as a normal tissue control to identify putative neo-antigens
6. Evaluation of MUC-1 and MVA specific humoral responses
7. Peripheral blood cytokine / chemokine profiling

Tissue

1. IHC to assess tumor infiltrating immune cells (CD8, CD4, FoxP3) and expression of other markers with potential prognostic and/or predictive value on efficacy outcomes including MUC-1 and PD-L1 as well as new biomarkers.
2. Transcriptome analysis of tumor tissues by RNA deep sequencing.
3. Identification of tumor neo-antigens by RNA deep sequencing in conjunction with peripheral blood WES data.

1.0 Archival Tumor Specimens

1.1 Archival Tumor Samples

If available, 1 - 2 paraffin-embedded tissue blocks containing formalin-fixed tumor from time of diagnosis (or subsequent, but prior to therapy) should be submitted. Paraffin blocks may be processed according to standard institutional protocols. If blocks are unavailable, 15 unstained slides are acceptable alternatives.

1.2 Archival Tumor Specimen Handling

Please refer to specimen handling and shipping section below. Briefly paraffin embedded tissues or slides should be shipped overnight to UC Davis at the indicated address. UC Davis will section slides from FFPE tissue blocks in a batched manner for IHC/IF analysis and 4 slides will be sent to Transgene for Muc-1 staining. Every attempt should be made to stain slides within 30 days of sectioning. If FFPE tissue is not available but slides are then these slides will not be batched but will be immediately stained and sent to Transgene for Muc-1 staining to avoid loss of antigenicity in the sectioned tissues.

1.3 Archival Tumor Analysis

Archival tumor samples will be stained by multi-plex IHC/IF at UC Davis or collaborating laboratories to immunologically profile the tumor microenvironment. Markers examined will include CD3, CD8, CD4, FoxP3, Ki-67, PD-1, and PD-L1. Additionally, 4 slides will be sent to Transgene to stain for Muc-1 expression.

2.0 Peripheral Blood

2.1 Blood Collection

Blood specimens (3-4 x 5ml lavender top EDTA tubes; 1 x 5mL Cyto-Chex tube) will be collected from each patient prior to initiating treatment and at months 2, 4, and 6 as indicated in the study calendar. **If possible, 4 x 5mL lavender top EDTA tubes should be collected for the first blood draw**. It is important that each tube be filled to 5mL to ensure sufficient specimens for the planned analyses. In the event that a patient does not start Cycles 2 or 3, it is important that a blood specimen be obtained at the time the patient is removed from protocol treatment or if the patient's disease progresses.

2.2 Blood Specimen Processing and Handling – Cyto-Chex Tubes

One 5mL Cyto-Chex tube will be collected from each patient at each time point. Cyto-Chex tubes will be provided directly to each site by Covance. Cyto-Chex tubes should be stored at room temperature and shipped directly to Transgene / Covance from each participating site the same day as collection. Tubes and shipping instructions are provided to each site in a kit from Covance. This is the top priority sample from the peripheral blood.

2.3 Blood Specimen Processing and Handling – Lavender Top EDTA Tubes

Three to four 5mL lavender top EDTA tubes will be collected from each patient at each time point. If possible four tubes should be collected for the first pre-treatment draw and three tubes thereafter. Each purple-top (EDTA) tube should be inverted several times, placed on wet ice, and delivered to the appropriate lab at each participating site as quickly as possible. Every effort should be made to process blood samples within 1 hour of collection. Samples should be processed in sterile fashion.

The tube should be centrifuged as soon as possible at approximately 1,000–1,500 rpm (400 x g) for 10 minutes. The Plasma layer should be removed and placed in 0.5 mL aliquots in labeled cryotubes. The plasma will be frozen (snap frozen with liquid nitrogen if possible) and stored at -70 to -80°C. Samples should be batched and shipped overnight on dry ice to UC Davis at the address indicated. One to two tubes of plasma per patient will be shipped to Transgene in a batched manner from UC Davis.

PBMCs should be separated using sterile and endotoxin free Ficoll density gradient solution. The peripheral blood mononuclear cells (PBMCs) should be removed and pooled. The PBMC layer should be resuspended in three times the volume of cold sterile PBS. Cells should be counted using a hemocytometer and the total cell number should be noted. The expected yield is 1–2 million cells per mL of blood.

For the first pretreatment blood draw, the PBMCs collected from 4 lavender top tubes will be divided into three aliquots. Roughly half of the cells should be cryopreserved for future analysis. The second aliquot consisting of roughly 25% will be placed in RNAlater and snap frozen with liquid nitrogen and stored at -70 to -80°C for future RNA extraction and the third aliquot will be snap frozen in liquid nitrogen and stored at -70 to -80°C for future DNA extraction. **For the 2, 4, and 6 month blood draws the PBMCs collected from 3 lavender top tubes will be divides into two aliquots**. Roughly 66% of the cells should be cryopreserved and roughly 33% should be placed in RNA later. **DNA extraction is performed only for the pre-treatment sample and not for subsequent samples**. The order of priority for these samples is cryopreservation, RNA later, and then snap frozen for DNA extraction.

2.3.1 Blood Plasma

Plasma should be distributed into 0.5mL aliquots, snap frozen and stored at -70 to -80°C. Samples should be batched and shipped overnight on dry ice to UC Davis at the address indicated. One to two tubes of plasma per patient will be shipped to Transgene in a batched manner from UC Davis.

2.3.2 Cryopreservation

After counting, PBS washed PBMCs should be divided into aliquots of 5 million cells and spun down at approximately 1,000-1,500 rpm (400 x g) for 10 minutes. Each aliquot should be resuspended in 1mL of cold sterile cryopreservation media consisting of 10% DMSO and 90%

FBS. Each 1mL aliquot should be placed in a cryopreservation tube and the lid should be tightly secured. Tubes should be placed into a "Mr. Frosty" or other slow freeze container and placed into a -70 to -80°C freezer for 12–24 hours. Tubes should then be transferred to and stored in vapor phase liquid nitrogen (-135°C). In a batched manner tubes should be shipped overnight in liquid nitrogen to UC Davis at the indicated address.

2.3.3 RNAlater

After counting, PBS washed PBMCs should be spun down at approximately 1,000–1,500 rpm (400 x g) for 10 minutes. The cell pellet should be re-suspended in 1mL of RNAlater solution snap frozen in liquid nitrogen and stored at -70 to -80°C. In a batched manner tubes should be shipped overnight on dry ice to UC Davis at the indicated address. Please note that PAXgene tubes will not be used for collection of PBMCs for RNA analysis.

2.3.4 Snap Frozen for DNA Extraction

This specimen will be collected from the pre-treatment blood draw only. After counting, PBS washed PBMCs should be spun down at approximately 1,000-1,500 rpm (400 x g) for 10 minutes. The cell pellet should be snap frozen in liquid nitrogen and stored at -70 to -80°C. In a batched manner tubes should be shipped overnight on dry ice to UC Davis at the indicated address.

2.4 Peripheral Blood Analysis

2.4.1 Cyto-Chex Tubes

Blood in Cyto-Chex tubes will be shipped on the same day of collection to Covance / Transgene directly from each participating site. These samples will be analyzed for CD56, CD16, CD69 triple positive activated lymphocytes (TrPAL).

2.4.2 Plasma

Plasma samples will be stored at UC Davis for batched analysis. Plasma will be interrogated for chemokine and cytokine levels using the luminex platform. Additionally plasma samples will be sent to transgene to evaluate for anti-MUC-1and MVA humoral immunity.

2.4.3 Cryopreserved Samples

Cryopreserved samples will be stored at UC Davis for batched analysis. Samples will be thawed and stained with fluorophore-conjugated antibodies against CD4, CD8, CD25, CD62L, CD45RA, CD127, ICOS, PD-1, PD-L1, FoxP3, CD3, CD56, CD16, CD83, TIM-3, Ki-67, CD19, CD20, CD33, CD15, CD11b, HLA-DR and others. Stained cells will be interrogated by flow cytometry and results analyzed using FlowJo software. Additionally, MUC-1 specific, MVA-specific, other TAA specific and neo-epitope specific T cells will be identified using combinatorial encoding of HLA-A*02:01 restricted tetramers. Putative TAA and neo-epitopes

will be determined from whole exome sequencing data and tetramers will be developed at Transgene.

2.4.4 RNAlater Samples

Cells in RNA later will be stored at UC Davis for batched analysis. Samples will be thawed and RNA will be extracted. The transcriptome will be analyzed by RNA deep sequencing (RNAseq) using the Illumina HiSeq platform. Targeted RT-PCR will be used to validate genes of interest identified by RNAseq. TCR deep sequencing data will also be obtained from the RNAseq.

2.4.5 Snap Frozen Samples

Snap frozen cell pellets will be stored at UC Davis for batched analysis. Samples will be thawed and genomic DNA will be extracted. Whole exome sequencing will be performed using the Illumina HiSeq platform.

3.0 Fresh Tumor Biopsy

3.1 Tumor Biopsy Collection

Fresh tumor biopsy is collected pre-treatment and at 2 months. Optional biopsy can be obtained at progression. Tumor biopsies should be collected by core needle biopsy using the largest bore needle and number of passes deemed safe. The same lesions should be targeted for all fresh tissue biopsies. Confirmation that the core biopsies contain tumor should be performed at the time of biopsy using touch prep.

3.2 Tumor Biopsy Processing and Handling

On site at the time of the biopsy tumor tissues should be aliquoted into two portions. The top priority assay is IHC/IF and biopsy samples should be placed in formalin fixative and later embedded into FFPE blocks. In general samples should be placed in fixative at a 10:1 ratio and fixed for at least 48 hours but thicker tissue samples may require longer fixation. FFPE blocks will be shipped to UC Davis in a batched manner.

If sufficient tissue is available (which there generally should be if more than one biopsy pass has been undertaken) then no less than 10mg and but preferably 20 - 50mg of tumor tissue should be placed in 1mL of RNAlater snap frozen in liquid nitrogen and stored at -70 to -80°C. Samples should be batched and shipped overnight on dry ice to UC Davis at the address indicated.

3.3 Tumor Biopsy Analysis

FFPE tissue biopsy blocks will be collected and stored at UC Davis. Tissues will be sectioned, prepared, and mounted for IHC/IF using standard procedures. IHC/IF should be performed within two weeks of slide sectioning. Biopsy samples will be stained by multi-plex IHC/IF at UC Davis or collaborating laboratories to immunologically profile the tumor microenvironment. Markers examined will include CD3, CD8, CD4, FoxP3, Ki-67, PD-1 and PD-L1.

Tissue in RNA later will be stored at UC Davis for batched analysis. Samples will be thawed and RNA will be extracted. The transcriptome will be analyzed by RNAseq using the Illumina HiSeq platform. Targeted RT-PCR will be used to validate genes of interest identified by RNAseq. TCR deep sequencing data will also be obtained from the RNAseq. RNAseq data will also be used in conjunction with PBMC WES to identify putative neoantigens.

4.0 Shipping of Specimens

All Cyto-Chex tubes should be shipped on the day of collection at ambient temperatures using the shipping kit provided.

All other samples should be batched and submitted directly to UC Davis. All archival paraffin block or slide specimens should be sent at ambient temperature. Cryopreserved PBMCs should be shipped in liquid nitrogen. All other frozen specimens should be shipped on dry ice. All samples should be shipped by overnight courier Monday through Wednesday only, to the following address:

UC Davis Comprehensive Cancer Center
4501 X Street, [redacted]
Sacramento, CA 95817
Phone: [redacted]

A Specimen Submission Form must be submitted with each specimen. Institutions should notify the recipient by either phone or fax prior to shipping specimens. This will allow the recipient to track the package in the event that there are any problems in delivery.

The Federal Guidelines for Shipment are as follows (these periodically change, please check for the most current guidelines):

1. The specimen must be wrapped in an absorbable material
2. The specimen must then be placed in an AIRTIGHT container (resealable bag)
3. Pack the resealable bag and specimen in a styrofoam shipping container
4. Pack the styrofoam shipping container in a cardboard box
5. The cardboard box should be labeled “UN3373 Biological Substance, Category B” “BIOHAZARD”

Appendix 5: Anaphylaxis Precautions

EQUIPMENT NEEDED

Tourniquet

Oxygen

Epinephrine for subcutaneous, intravenous, and/or endotracheal use in accordance with standard practice

Antihistamines

Corticosteroids

Intravenous infusion solutions, tubing, catheters, and tape

PROCEDURES

In the event of a suspected anaphylactic reaction during study drug infusion, the following procedures should be performed:

1. Stop the study drug infusion.

2. Apply a tourniquet proximal to the injection site to slow systemic absorption of study drug. Do not obstruct arterial flow in the limb.

3. Maintain an adequate airway.

4. Administer antihistamines, epinephrine, or other medications as required by patient status and directed by the physician in charge.

5. Continue to observe the patient and document observations.

Appendix 6: Immune-Related Response Criteria (irRC)

Increasing clinical experience indicates that traditional response criteria (e.g., Response Evaluation Criteria in Solid Tumors, Version 1.1 [RECIST v1.1] and World Health Organization [WHO]) may not be sufficient to characterize fully activity in the new era of target therapies and/or biologics. In studies with cytokines, cancer vaccines, and monoclonal antibodies, complete response, partial response, or stable disease has been shown to occur after an increase in tumor burden as characterized by progressive disease by traditional response criteria. Therefore, conventional response criteria may not adequately assess the activity of immunotherapeutic agents because progressive disease (by initial radiographic evaluation) does not necessarily reflect therapeutic failure. Long-term effect on the target disease must also be captured. The immune-related response criteria[1] (irRC) are criteria that attempt to do that by enhancing characterization of new response patterns that have been observed with immunotherapeutic agents (i.e., ipilimumab). (Note: The irRC only index and measurable new lesions are taken into account.)

GLOSSARY

Term	Definition
SPD	sum of the products of the two largest perpendicular diameters
Tumor burden	$SPD_{\text{index lesions}} + SPD_{\text{new, measurable lesions}}$
Nadir	minimally recorded tumor burden
irCR	immune-related complete response
irPD	immune-related progressive disease
irPR	immune-related partial response
irSD	immune-related stable disease
irBOR	immune-related best overall response

BASELINE ASSESSMENT USING irRC

Step 1. Identify the index lesions (five lesions per organ, up to ten visceral lesions and five cutaneous lesions).

Step 2. Calculate the SPD of all of these index lesions:

$$SPD = \sum_{i} \text{(Largest diameter of lesion i)} \times \text{(Second largest diameter of lesion i).}$$

[1] Wolchok JD, Hoos A, O'Day S, et al. Guidelines for the evaluation of immune therapy activity in solid tumors: immune-related response criteria. Clin Can Res 2009;15:7412–20.

POST-BASELINE ASSESSMENTS USING irRC

Step 1. Calculate the SPD of the index lesions.

Step 2. Identify new, measurable lesions ($\geq 5 \times 5$ mm; up to five new lesions per organ: five new cutaneous lesions and ten visceral lesions).

Step 3. Calculate the SPD of the new, measurable lesions.

Step 4. Calculate the tumor burden:

$$\text{Tumor burden} = \text{SPD}_{\text{index lesions}} + \text{SPD}_{\text{new, measurable lesions}}$$

Step 5. Calculate the change in tumor burden relative to baseline and the change in tumor burden relative to nadir.

Step 6. Derive the overall response at each timepoint using the table below.

Overall Response by timepoint	Criterion
irCR	Complete disappearance of all lesions (whether measurable or not, and no new lesions)
irPR	Decrease in tumor burden $\geq 50\%$ relative to baseline
irSD	Criteria for irCR, irPR, and irPD are not met; does not require confirmation
irPD	Increase in tumor burden $\geq 25\%$ relative to nadir

irCR = immune-related complete response; irPD = immune-related progressive disease; irPR = immune-related partial response; irSD = immune-related stable disease.

DETERMINATION OF irBOR

Once a patient has completed all tumor assessments, his/her irBOR may be determined:

Condition	irBOR
At least one irCR confirmed by a repeat, consecutive assessment ≥ 4 weeks from the date first documented	irCR
At least one irPR and no irCR confirmed by a repeat, consecutive assessment ≥ 4 weeks from the date first documented	irPR
At least one irSD and no irCR and no irPR	irSD
At least one irPD and no irCR, no irPR, and no irSD confirmed by a repeat, consecutive assessment ≥ 4 weeks from the date first documented	irPD

irBOR = immune-related best overall response; irCR = immune-related complete response; irPD = immune-related progressive disease; irPR = immune-related partial response; irSD = immune-related stable disease.