A Phase I Clinical Trial Combining Nivolumab and Tumor Infiltrating Lymphocytes (TIL) for Patients with Advanced Non-Small Cell Lung Cancer

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PROTOCOL VERSION: 1.6

VERSION DATE: September 2, 2020

PROTOCOL NUMBERS:

CA209-9JA, MCC 19122

SPONSOR:

H. Lee Moffitt Cancer Center & Research Institute, Inc.
(Moffitt Cancer Center)

FUNDING: Stand Up to Cancer (SU2C)

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Participating Sites:
University of Florida

PROTOCOL SYNOPSIS
Clinical Protocol CA209-9JA

Study Title: A Phase I Clinical Trial Combining Nivolumab and Tumor Infiltrating Lymphocytes (TIL) for Patients with Advanced Non-Small Cell Lung Cancer

Protocol Number: MCC 19122, CA209-9JA

Clinical Phase: Phase I

Study Duration: 30 months

Investigational Product(s) and Reference Therapy:

Adoptive cell transfer with autologous tumor-infiltrating-lymphocyte will be supplied as a cellular product.

Primary Objectives:

• To evaluate the safety and tolerability of TIL administered following initial progression on nivolumab therapy in combination with nivolumab in subjects with advanced non-small cell lung cancer (NSCLC).

Secondary Objectives:

• To evaluate the efficacy of TIL administered in combination with nivolumab in subjects with NSCLC by assessing the objective response rate (ORR) per RECIST v1.1
• To evaluate the efficacy of TIL administered in combination with nivolumab in subjects with NSCLC by assessing duration of response .
• To evaluate the T-cell persistence following TIL and nivolumab when administered in combination.

Exploratory Objectives:

• To characterize the pharmacodynamics and evaluate biomarkers of TIL and nivolumab from tumor tissue and peripheral blood.
• To explore the antitumor activity of nivolumab in combination with TIL based on immune-related response criteria (irRC).
• To evaluate the overall survival (OS) of subjects with select advanced solid tumors treated with TIL administered in combination with nivolumab.
• To explore the proportion of reactivity of autologous T cells to tumor-specific antigens in subjects with NSCLC by assessing the correlation with response and survival.

Study Design:

This is a phase I open label clinical trial of TIL administered in combination with nivolumab in subjects with advanced NSCLC. Upon enrollment, subjects undergo tumor harvest for TIL. TIL is prepared and cryopreserved. Patients then receive 4 cycles of nivolumab. CT scans are performed after 2 and 4 cycles, and the patients with initial response to nivolumab continue to receive drug for up to 1 year. The TIL for patients without radiographic response to nivolumab will undergo rapid expansion protocol (REP) after completion of a pre-REP checklist. Prior to receiving TIL, patients will undergo cytoreductive chemotherapy with cyclophosphamide (60 mg/kg/day) on Day -7 and Day -6 with fludarabine (25 mg/m2 IV over 30 minutes) on Days -7 to -3. The TIL cell product is an intravenous (IV) infusion on Day 0. Subjects then receive IV intermediate-dose decrescendo IL-2 on Days +1 to +6 during an inpatient hospital stay. Patients receive a CT at Day 28, then every 6 weeks (+/- 7 days) for one year from the date of initial TIL infusion.
Patients will have clinic visits as outlined in the study calendar. Tumor biopsies will be taken at progression of disease, if accessible tumor is amenable to biopsy. Long term follow-up (LTFU) with the patient occurs after the patient has been confirmed to have progression of disease or discontinues from the study prior to progression.
Patients who have initial response to nivolumab and subsequently progress may be eligible for TIL treatment, if they meet eligibility requirements.

Number of Centers: 2. Sites: Moffitt Cancer Center, University of Florida

Number of Subjects:
The target enrollment for this trial is approximately 20 patients with the expectation of 14 evaluable subjects. In order to be considered evaluable, a patient must receive lymphodepletion/TIL and complete at least 70% of planned visits during the toxicity evaluation window. Unevaluable patients may be replaced.

Study Population:

Advanced non-small cell lung cancer

Investigational Product(s), Dose and Mode of Administration:

Nivolumab, 240 mg, IV infusion every 2 weeks for 8 weeks prior to TIL infusion, and then after TIL infusion 480 mg every 4 weeks for up to 12 months

Adoptive cell transfer with NSCLC TIL

IV decrescendo dose interleukin-2 (per Andersen CCR, 2016 22(15):3734-45)

Table of Contents:

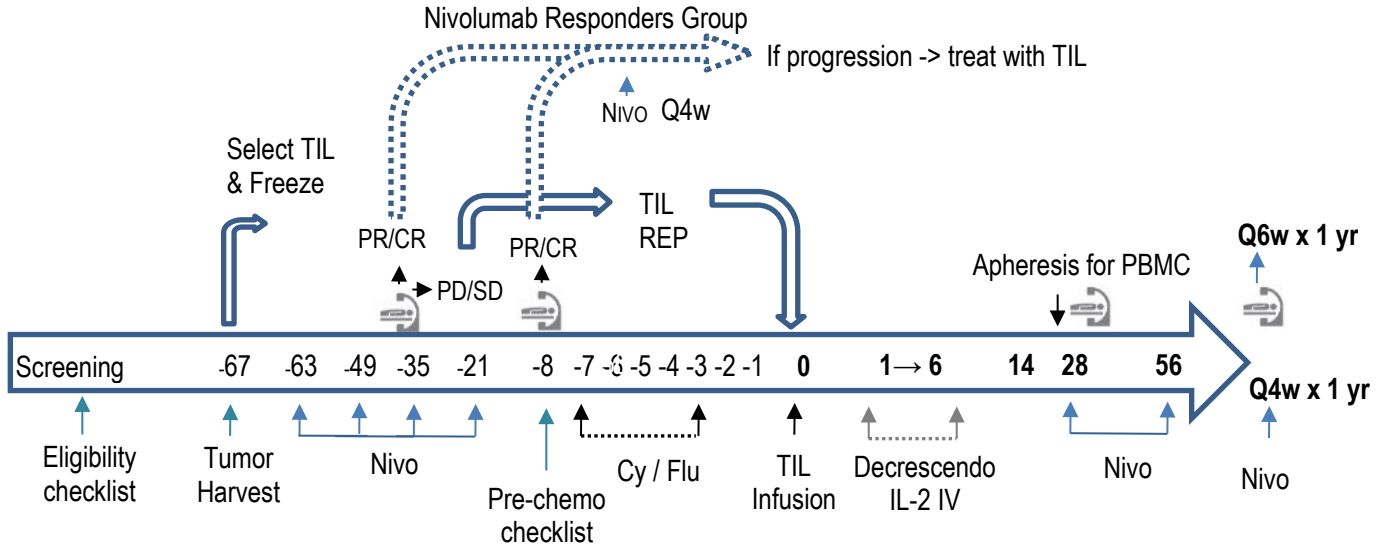
List of Abbreviations

ABBREVIATION	Term
α	anti
ab	antibody
ACT	Adoptive Cell Therapy
AE	Adverse Event
ALK	Anaplastic Lymphoma Kinase
ALT	Alanine Aminotransferase
ANC	Absolute Neutrophil Count
AST	Aspartate Aminotransferase
ATAC	Assay for Transposase-Accessible Chromatin
BID	Twice per Day (bis in die)
BMS	Bristol Myers Squibb
BP	Blood Pressure
BSA	Body Surface Area
BUN	Blood Urea Nitrogen
C	Celsius
CBC	Complete Blood Count
CD	Cluster of Differentiation
CEA	Carcinoembryonic Antigen
CEF	Cytomegalovirus, Epstein-barr virus and influenza virus peptide
cm	centimeter
CMP	Comprehensive Metabolic Panel
CMV	Cytomegalovirus
CNS	Central Nervous System
CO_2	Carbon Dioxide
CR	Complete Response
CRF	Case Report Form
CT	Computed Tomography
CTCAE v	Common Terminology Criteria for Adverse Events version
CTO	Clinical Trials Office
CLTA	Cytotoxic T-Lymphocyte Associated Antigen
D	Day
D5W	5% Dextrose in Water solution
DC	Dendritic Cell
dL	Deciliter
DLCO	Diffusion Lung Capacity for Carbon Monoxide
DLT	Dose-Limiting Toxicity
DNA	Deoxyribonucleic Acid
DS	Double Strength
DSMP	Data Safety & Monitoring Plan
EBV	Epstein Barr Virus
ECOG	Eastern Cooperative Oncology Group
EEG	Electroencephalogram
EGFR	Epidermal Growth Factor Receptor
EKG	Electrocardiogram
ELISA	Enzyme-Linked Immunosorbent Assay
ELISpot	Enzyme-linked Immune Absorbent Spot
EOP	Extended Outpatient
FACS	Fluorescence-Activated Cell Sorting
FDA	Food and Drug Administration
FEV1	Forced Expiratory Volume in 1 Second
FNA	Fine Needle Aspiration
FTA	Fluorescent Treponemal Antibody
G-CSF	Granulocyte Colony Stimulating Factor
GI	Gastrointestinal
gm	gram
HD	High Dose

HIV	Human Immunodeficiency Virus
HLA	Human Leukocyte Antigen
hr	hour
HSV	Human Simplex Virus
ICH	International Conference on Harmonisation
IFN	Interferon
IL	Interleukin
IM	Intramuscular
IND	Investigational New Drug
irAE	immune-related Adverse Event
irRC	immune-related Response Criteria
IRB	Institutional Review Board
IU	International Units
IULN	Institutional Upper Limit of Normal
IV	Intravenous
IVPB	Intravenous Piggyback
kg	kilogram
L	liter
LD	Longest Diameter
CEA	Carcinoembryonic antigen
LFT	Liver Function Test
LTFU	Long Term Follow-Up
m2	Square Meter
mAb	Monoclonal Antibody
MAGE	Melanoma Associated Gene
MCC	Moffitt Cancer Center
mcg	microgram
mcL	microliter
mg	milligram
MIU	Million International Units
MHC	Major Histocompatibility Complex
mL	milliliter
mm	millimeter
MRI	Magnetic Resonance Imaging
MSKCC	Memorial Sloan Kettering
MUGA	Multigated Acquisition Scan
NCI	National Cancer Institute
NIH	National Institutes of Health
NK	Natural Killer
NS	Normal Saline
NSCLC	Non-Small Cell Lung Cancer
OKT3	Muromonab-CD3
ORR	Overall Response Rate
OS	Overall Survival
PBL	Peripheral Blood Lymphocytes
PBMC	Peripheral Blood Mononuclear Cell
PA	Posteroanterior
PCP	Pneumocystis carinii Pneumonia
PD	Progressive Disease
PD-1	Programmed Cell Death Protein-1
PD-L	Programmed Death-Ligand
PFS	Progression-Free Survival
PFT	Pulmonary Function Testing
PI	Principal Investigator
PK	Pharmacokinetics
PMC	Protocol Monitoring Committee
PO	By Mouth (per os)
PR	Partial Response
PRBC	Packed Red Blood Cell
PT	Prothrombin Time

PTT	Partial Thromboplastin Time
q	Every
Q2W	Every 2 weeks
QD	Once per Day (quaque die)
QTc	QT interval corrected for heart rate
RECIST	Response Evaluation Criteria in Solid Tumors
REP	Rapid Expansion Protocol
RPR	Rapid Plasma Reagin
SAE	Serious Adverse Event
SC	Subcutaneous
SD	Stable Disease
SMX	Sulfamethoxazole
SOP	Standard Operating Procedure
SpO2	Peripheral Capillary Oxygen Saturation
SRC	Scientific Review Committee
SST	Serum Separator Tube
SU2C	Stand Up to Cancer
T3	Triiodothyronine
T4	Thyroxine
TCR	T Cell Receptor
TRVB	T Cell Receptor Variable Beta chain
TID	Three Times per Day (ter in die)
TIL	Tumor-infiltrating lymphocytes
TKI	Tyrosine Kinase Inhibitor
TMP	Trimethoprim
TNF	Tumor Necrosis Factor
TSH	Thyroid-Stimulating Hormone
PBMC	Peripheral blood mononuclear cells
ULN	Upper Limit of Normal
USP	United States Pharmacopeia
VATS	Video-Assisted Thoracoscopic Surgery
VZV	Varicella Zoster Virus
WBC	White Blood Cell Count

Study Schema



Study Calendar ***,** ALL +/- 3 DAYS** *; precise start for D0 will depend on rate of TIL growth and patient's clinical status.

Footnote								Window	(18)								IL2(12)	Week			(19)		(20)	Days post-EOT(21)		
Day	Screening	-67	-63	-49	-35	-21	-8	±7(8, 13)	-7	-6	-5	-4	-3	-2	-1	0 (TIL)	1 - 6	1	2	4	Q4w	Q6w	PD	0	+30	+90
Informed Consent	●																									
Medical history	●		●	●	●	●	●		●	●	●	●	●	●	●	●	●	●	●	●	●	●		●	●	●
Current medications	●		●	●	●	●	●		●	●	●	●	●	●	●						●	●		●	●	●
Physical Exam	●		●	●	●	●	●		●	●	●	●	●	●	●	●		●	●	●	●	●		●	●	●
Vital Signs including SpO2 (1)	●		●	●	●	●	●		●	●	●	●	●	●	●	●	●	●	●	●	●	●		●	●	●
Performance Status	●		●	●	●	●	●		●							●		●	●	●	●	●	●	●		
REP Eligibility Checklist						●																				
Chemotherapy Checklist							●																			
Cardiac Stress test (2)	●																									
PFTs, MUGA (3)	●																									
EKG	●						●									●										
Blood Tests																										
CBC, CMP	●		●	●	●	●	●		●	●			●	●		●	●	●	●	●	●	●		●	●	●
CEA	●		●	●	●	●	●		●							●		●	●	●	●	●		●	●	●
PT/PTT	●						●													●						
HLA sequencing	●																									
EBV ab	●																									
Hepatitis B and C	●																									
HIV, RPR (FTA if necessary) (4)	●																									
Thyroid Tests (T4, TSH) (5)	●			●		●			●							●		●		●	●	●				
Blood Pregnancy Test (6)	●		●	●	●	●	●									●				●	●	●				
Peripheral Blood Draw for Correlatives (7)	●		●		●		●											●	●	●		●	●	●	●	●
CT Chest-Abdomen-Pelvis (8)(9)	●				●		●													●		●				
Brain imaging (9)	●						●													●		●				
Chest X-Ray PA/lateral	●						●																			
Tumor surgical biopsy for TIL (10)		●																								
Tumor biopsy (Core/FNA biopsy) (10)																							●			
Adverse Event Assessment (11)			●	●	●	●	●		●	●	●	●	●	●		●	●	●		●	●	●		●	●	
Urinalysis	●						●									●				●		●				
Leukapheresis for Correlatives																				●						
Nivolumab			●	●	●	●														●	●	●				
Lymphodepletion:																										
Cyclophosphamide (Daily x 2)									●	●																
Fludarabine (Daily x 5)									●	●	●	●	●													
Adoptive Transfer of TIL																●										
Intermediate dose IL-2 (12)																●	●	●								
Preferred antibiotic (14)																				●						
Fluconazole (15)																	●	●	●							
Preferred antiviral (16)																	●	●	●	●						

Footnotes to the Study Calendar

1. Subjects will have their blood pressure and pulse measured before each nivolumab infusion. Subjects will have their vital signs measured during TIL infusion per section 5.1. Vital signs during IL-2 are recommended q4 hours or per institutional standard.
2. Cardiac stress test can be omitted if previously been done within 6 months of screening.
3. MUGA and PFTs can be omitted if previously done within 4 months of screening. Echocardiogram or stress echocardiogram is acceptable alternative for MUGA.
4. FTA only if RPR is positive per section 4.1.2 #8
5. If T4 and TSH are clinically significantly abnormal, thyroid or autoimmune workup will be done per family practice guidelines, *Am Fam Physician.* 2012 Aug 1; 86(3): 244.
6. Serum pregnancy test will be performed on women of child-bearing potential, and must be negative, within 7 days of screening, within 7 days prior to each dose of nivolumab, and within 14 days prior to chemotherapy.
7. Venipuncture for peripheral blood mononuclear cells (PBMCs) will be performed at day -63, -35, -8, +7, +14, then every 6 weeks +/- 7 days. PBMCs will be obtained by leukapheresis on day 28. If leukapheresis is not feasible at day 28, then PBMCs must be collected by peripheral blood draw. Samples will be used for immune monitoring purposes and will not be used for patient therapy. Blood draws will be omitted for patients with symptomatic anemia or deemed not clinically feasible by the PI or treating co-investigator.
8. A +/- 7 day window is included to account for variable preparation time for TIL and rapid expansion. Patients who achieve a PR/CR by day -8 will remain on nivolumab following q4w and q6w visits. For patients who experience clinical decline in ECOG PS due to cancer during Cycle 1-4 of nivolumab may elect to stop subsequent nivolumab and instead receive bridging systemic therapy if judged in the best patient interest by the treating clinical investigator. This bridging therapy group is described below.
9. CT Scans with IV contrast at screening, following 2 and 4 cycles of nivolumab, 6 weeks +/- 7 days after adoptive TIL transfer, then every 6 weeks (+/- 7 days) for 1 year, then every 12 weeks (+/- 14 days) for 3 years, then every six months (+/- 21 days) thereafter. CT pelvis after baseline may be omitted if patients do not have lesions identified in the pelvis.
   For brain imaging, MRI of the Brain with and without contrast is required for those patients with no contraindications; A CT of head with and without IV contrast (if not allergic) can be substituted for brain MRI at discretion of the PI. For patients with CT IV contrast allergy, appropriate premedication is preferred, and use of MRI or CT without IV contrast may be undertaken only at the discretion of the PI.
   The q6 week brain imaging should be omitted for patients without any lesions identified on brain imaging at screening and day -8 evaluation.
10. Tumor fragments from the harvest will be plated in the Cellular Therapies Core lab for TIL growth. At disease progression, a tumor core needle biopsy will be obtained. An FNA is an acceptable alternative to core biopsy only after discussion with the PI.
11. Adverse events will be collected until 30 days after both of the following conditions are met: progression of disease (PD) and end of treatment (EOT +0) has occurred. This is expected to correspond to the EOT +30 visit.
12. Inpatient stay for intermediate-dose IL-2. Patients are expected to be inpatient for TIL infusion (day 0) which will be followed within 12 hours by continuous IL-2 infusion administered in a decrescendo regimen (18 MIU/m^2 over 6, 12, and 24 hours followed by 4.5 MIU/m^2 over 24 hours for up to 3 days depending on tolerance)
13. *For nivolumab responders, if* subsequent disease recurrence is suspected, then the patient will be set up with Day -21 clinic visit, and then complete pre-REP eligibility checklist and procedures per Section 5.9, in preparation for subsequent Cy/Flu/TIL.

14. Daily oral TMP/SMX single-strength (SS) prescription is preferred. Alternative schedule, dose, or alternative antibiotics for pneumocystis prophylaxis are permitted at the discretion of the investigator. Preferred to continue for at least six (6) months or until CD4 > 200/mm^3
15. Continue until ANC > 1000/mm^3
16. Daily oral valcyclovir is preferred. Begins one day after Day 0. Alternative acyclovir prescription is permitted, or omission for patients with intolerance/allergy. Continue until 1 year or CD4 > 200/mm^3
17. *Placeholder.*
18. Lymphodepletion chemotherapy administered in inpatient or outpatient setting.
19. Follow-up visits will be conducted per study calendar after TIL infusion, then every 4 weeks (+/- 7 days) for 1 year (while on nivolumab therapy), then after nivolumab, every 12 weeks (+/- 14 days) for 3 years, then every six months (+/- 14 days) thereafter or until disease progression, withdrawal of consent, or death. The q4w and q6w calendar visit procedures are intended to overlap for patients who are receiving nivolumab treatment. For post-TIL non-treatment visits of patients facing transportation hardship, a virtual visit may be conducted as an alternative.
    Patients who have discontinued nivolumab for reasons other than confirmed progression of disease remain on regular trial schedule. These patients may forego q4w calendar visits after 6 weeks have elapsed from drug discontinuation. They will continue the q6w calendar visits.
20. PD: Progression of disease. Percutaneous biopsy after radiologic progression for subjects who have not suffered decline in performance status. This is further defined in Section 5, PD.
21. End of Treatment (EOT) procedures to take place within +0, +30 days and +90 days after the last regular study visit; including patients who go on to receive non-study therapies. EOT does not apply to patients who remain on-study follow-up after completing 1-year of nivolumab or require cessation of nivolumab for reasons other than progressive disease (see Footnote 19). These EOT visits are to continue to assess safety (including exam, CBC, CMP) as well as peripheral blood draw for PBMC collection. The EOT visits are further defined in Section 5, EOT.

1.0 Significance and Background

1.1 Non-small cell lung cancer as an immune target and non-small cell lung cancer differentiation antigens

Patients with stage IV metastatic non-small cell lung cancer have a five-year survival rate of less than 5% according to most published clinical series. While chemotherapy regimens have been shown to induce objective regressions in 10-40% of non-small cell lung cancer patients, anti-tumor responses are generally short-lived and rarely result in complete clinical responses. Over the past five years, immunotherapy has become a viable alternative to chemotherapy for the treatment of metastatic non-small cell lung cancer, largely because, like melanoma, non-small cell lung cancer is one of the most immunogenic of known human epithelial cancers (1-3).

The first indication that immune responses could alter the clinical course of established, invasive human cancers came from studies of the administration of interleukin-2 (IL-2) to patients with metastatic melanoma (4). While IL-2 has no direct impact on the growth of cancer cells, it does have a number of immune regulatory effects, including the expansion of T lymphocytes following activation by specific antigen and NK cells (5, 6). Approximately 15% of melanoma patients treated with IL-2 experience objective regressions, a portion of which are long-lasting and potentially curative (7, 8). This result suggested that activation of T lymphocytes with anti-tumor activity was responsible for the induction of tumor regressions (9).

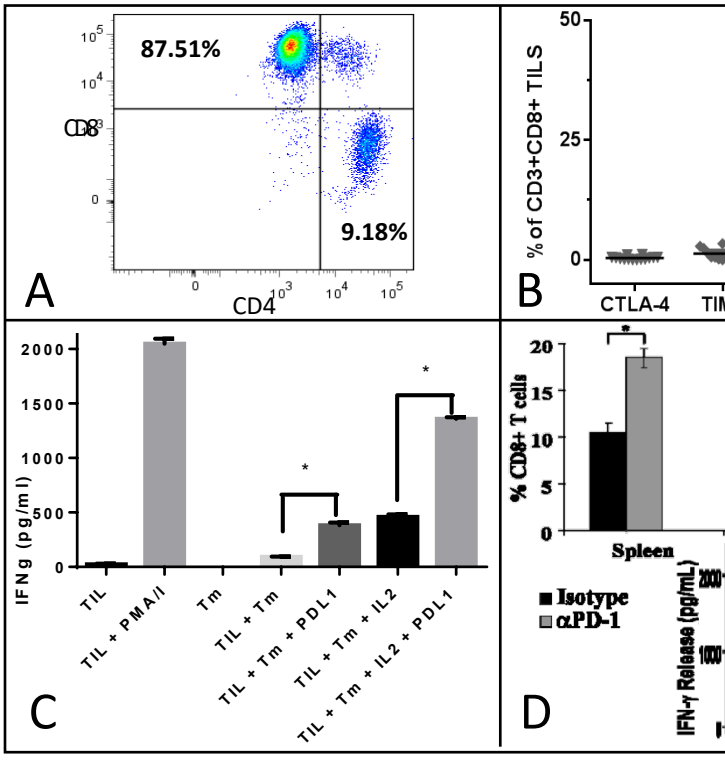
Subsequent studies in both humans and in animal models have borne out these predictions and have allowed for the elucidation of the mechanisms behind the immune recognition of tumor cells at the molecular level. T cells specifically recognize antigens presented as small peptides in association with surface human leukocyte antigen (HLA) molecules (10, 11). Peptide antigens expressed on the surface of cancer cells have been demonstrated in multiple studies to induce T cell recognition leading to tumor cell killing and/or the release of helper and other cytokines. In the case of non-small cell lung cancer, a number of antigens have now been identified that can be recognized by both CD8+ cytotoxic T cells and CD4+ T-helper cells, including mesothelin, MAGE-A3, and NY-ESO-1 (11 -13).

1.2 Adoptive cell therapy induces durable remissions for a subset of cancer patients:

Despite progress in T-cell immune checkpoint inhibitors for NSCLC, these agents fail to cause complete cancer remission for the majority of patients (14, 15). Further treatments which employ the exquisite specificity of memory T-cells are still needed for this pervasive cancer. Adoptive cell therapy (ACT) with tumor-infiltrating lymphocytes (TIL) is a promising T-cell-based immunotherapy that has evolved over the past sixty years into a tenable treatment for select solid tumors, particularly melanoma (16). In this process, TIL are first propagated from explanted minced tumor fragments and cultured with interleukin-2 (IL-2) to a target goal of >30 million cells. These TIL then undergo rapid clonal expansion (REP) by incubation with anti-CD3 monoclonal antibody (mAb), resulting in >500-fold expansion. Upon adequate TIL generation, the patients receive lymphodepleting chemotherapy prior to infusion of the TIL product and administration of IL-2. The National Cancer Institute has pioneered this treatment and reported response rates of 50% or more in metastatic melanoma; with 22% of patients achieving durable complete responses, many more than 9 years (1). The impressive durability of responses to ACT is a hallmark of this treatment. Our center at Moffitt has shown that ACT for metastatic melanoma is feasible and effective, with over 50 patients treated to date (17) Since TIL are derived from native genetically unmodified cells, complications due to engagement of normal host cells is uncommon. Durable regressions with TIL have previously been reported in a variety of epithelial malignancies, including cholangiocarcinoma, cervical, and colorectal cancer.

1.3 Lung cancer: a prime target for adoptive cell transfer:

Like melanoma, NSCLC arises from extreme environmental mutagenesis and therefore presents a rich array of tumor neoantigens for adaptive immunity. Once explanted, lung TIL are bereft of their suppressive tumoral mileu and may be free to recover antitumor capacity (18). A prior non-randomized trial of 131 patients with Stage I-III NSCLC yielded successful ACT in 86% of case, with a median of 20 x 10^9 cells infused and this product displayed autologous reactivity (19). However, because these were resections for curative intent, the subjects were not evaluable for response rate. TIL phenotype does not vary among histologies or metastastic location (20). Since metastatic deposits contain even richer amounts of TIL than primary lung tumors, metastatectomy samples may be expected to provide a reliable TIL source for NSCLC patients. Along these lines, a trial of ACT in metastatic NSCLC followed by high dose (HD) IL-2 has activated at NCI [NCT02133196]. Our center at Moffitt has shown that lung TIL has high proliferative capacity with a median of 1.9 x 10^9 CD3+ cells after REP. In our protocol, small pieces (~2mm x 2mm) of freshly resected tumor are aseptically cultured on separate wells of a 24 well plate containing 2 mL TIL media and 6000IU/mL of rhIL-2. Once ~5x10^7 TIL are propagated, cells are clonally expanded in IL-2 and 30ng/mL activating anti-CD3 mAb (OKT3) with irradiated feeder PBMCs at 200:1. Expansion is successful in over 75% of attempts (*n*=13) and these CD3+ cells displayed a predominantly CD8 phenotype [Figure 1A]. Moreover, these cells demonstrated moderate autologous tumor specificity which was enhanced in the presence of IL-2 [Figure 1C].



1.4 Initial studies of adoptive T cell transfer for the treatment of human cancer

The identification of T cells with the ability to specifically recognize epithelial cancer antigens, along with the technological capability to expand these tumor-reactive T cells to large numbers in the laboratory, has led to the development of adoptive transfer protocols for patients with metastatic epithelial cancers. Tumor-infiltrating lymphocytes (TIL) derived from resected tumors that were

expanded *in vitro* were shown to be capable of specifically recognizing tumor antigens, such as carcinoembryonic antigen (CEA), in over two-thirds of cancer patients (21, 22).

TIL growth requires two phases. In the first phase, called pre-rapid expansion, individual cancer tumor fragments (1-3 mm^3) are explanted in vitro in media containing interleukin-2. After 4-6 weeks, TIL propagated from fragments successfully yielding 30-60 million cells in total are pooled and used in the second phase, called rapid expansion. During rapid expansion, OKT and IL-2 as well as irradiated feeder cells result in 500-1000 fold proliferation. When TIL are expanded to large numbers (greater than 20 billion) and adoptively transferred intravenously to patients along with IL-2, objective response rates of 35-50% have been observed (23). This response rate is nearly twice that observed with IL-2 alone and was also seen in patients that were refractory to IL-2 treatment. However, most of the initial responses were transient and limited tumor reactivity and persistence of the transferred cells was observed, and TIL preparation typically required 6-8 weeks (24). Improving tumor reactivity, TIL persistence and reducing the time required for preparation can enhance patient outcomes.

1.5 Addition of non-myeloablative lymphodepletion to adoptive cell transfer

Mounting evidence suggests that the host immune environment can significantly impact the efficacy of adoptive cell transfer therapy. Data from mouse tumor models have demonstrated that sublethal doses of irradiation prior to adoptive transfer of tumor antigen-specific lymphocytes substantially increases the persistence and anti-tumor activity of the transferred cells (25). While the mechanisms leading to this enhanced T cell activity have not been precisely delineated, two non-mutually exclusive hypotheses may provide an explanation. A subset of $CD4^+$ T cells, expressing high levels of CD25 and the molecule FoxP3 and known as regulatory or suppressor T cells, is thought to have a negative impact on the activity of cytotoxic T cells *in vivo* (26, 27). It has been hypothesized that increased numbers of suppressor T cells in cancer patients may correlate with an unfavorable prognosis and that elimination of these cells may result in an improved efficacy of adoptive immunotherapy (28-31). Alternatively, prior depletion of lymphocytes may create ‘space’ for the adoptively transferred cells within the lymphocyte compartment (32). Under this model, homeostatic lymphocyte survival may result in increased proliferation and enhanced survival of transferred T cells, perhaps through a mechanism involving increased access to endogenous cytokines like IL-7 and IL-15 (33).

In a prior published protocol, patients with metastatic melanoma received a single dose of cyclophosphamide (Cytoxan) at 25 mg/kg prior to the administration of autologous TIL cells. Traffic of TIL to tumor sites was evaluated by labeling TIL with Indium-111 and performing sequential radionuclide scans. Of 26 patients that received cyclophosphamide, TIL trafficking to tumor was seen in 21 patients (81%) compared to TIL trafficking to tumor in 42% of patients that did not receive cyclophosphamide ($p=0.026$). No difference was seen in tumor regression rates. Thus, even with a mild and very transient leukopenia (about 5 days), evidence of increased lymphocyte trafficking to tumor was observed (34).

The animal and clinical studies cited above strongly suggest that the clinical effectiveness of these cells and their ability to survive and repopulate the host would be enhanced if patients were significantly immunosuppressed by the depletion of lymphocytes prior to the adoptive transfer of lymphocytes.

A recent clinical trial investigated the addition of a lymphodepleting conditioning regimen to adoptive cell transfer therapy in patients with metastatic melanoma. Patients received a lymphodepleting

chemotherapy regimen consisting of high dose cyclophosphamide and standard doses of fludarabine before administration of highly selected, expanded, tumor-reactive TIL and IL-2 (35, 36). The lymphodepletion step resulted in a transient myelosuppression and the elimination of all circulating lymphocytes for approximately one week, after which time patients recovered endogenous marrow function and reconstituted their lymphocyte compartments towards normal levels within two to three weeks (37).

Because of the immunosuppression of fludarabine, one of the patients who had clonal repopulation from infused TIL cells and a complete response of metastatic melanoma, developed Epstein-Barr virus (EBV) - associated B cell lymphoma. This patient was EBV-naïve prior to the treatments. The potential source of EBV was thought to be multiple blood product transfusions after chemotherapy. The patient later died of complications from the treatment of his lymphoma. Another patient developed polyneuropathy manifested by vision blindness, motor and sensory defects, approximately 2 months after chemotherapy. The etiology of this complication is unknown, but was possibly related to the fludarabine (35).

In a prior publication in *Science*, six patients of thirteen demonstrated objective tumor regression and four additional patients showed mixed responses with substantial shrinkage of some lesions after lymphoid depletion and adoptive transfer of highly selected, expanded, tumor-reactive TIL and IL-2. Significant levels of tumor regression were observed in metastatic deposits in the liver, lungs, cutaneous and subcutaneous tissues, and lymph nodes. One patient had dramatic regression of axillary, pelvic and intraabdominal metastases, on-going at 17 months and was rendered free of disease by a surgical removal of one residual intraperitoneal lesion. Two other patients had marked, persistent lymphocytosis up to 3 weeks after the TIL infusion. Molecular and immunological analyses confirmed that these lymphocytes from their peripheral blood were the progeny of the infused TIL (23). In particular, in one patient, it was shown that one specific clone repopulated this patient's peripheral blood lymphocytes (PBL) up to 2 months after infusion. Immunohistochemistry studies revealed that specific clones from infused bulk oligoclonal TIL cells infiltrated the regressing tumor nodules. Although the mechanism for the continued proliferation *in vivo* of these rapidly expanded (REPed) bulk TIL cells and their anti-tumor effects remain to be determined, it appears that some cells in the REPed bulk TIL might have provided necessary cytokines (such as IL-2) for cytotoxic T lymphocytes to persist and survive *in vivo* and eventually kill the tumor. Alternatively, the chemotherapy regimen used in this protocol might have depleted endogenous suppressive lymphocytes. Seven of these 13 patients received a second cycle of treatment with the same chemotherapy regimen plus bulk TIL and high dose IL-2. All seven patients recovered from a second cycle (35). Four out of the 13 patients developed vitiligo and one patient developed uveitis, which did not interfere with vision and reversed after steroid therapy (35).

More recent experience with lymphoid depletion, TIL and IL-2 at the Moffitt Cancer Center, MD Anderson Cancer Center, Sheba Medical Center in Israel and the National Cancer Institute suggest that so-called "young" TIL that are not selected for tumor reactivity but are grown from tumor fragments in IL-2 and pooled without an intermediate step to assay their tumor specific release of cytokine can be successfully used to treat patients. In a presentation at the American Society for Clinical Oncology annual meeting in June 2011, Shapira-Frommer et al described their experience with 42 patients treated with "young" TIL at the Sheba Medical Center, demonstrating a 40% ORR and significant regression of tumor in patients with extensive metastatic disease. At the MD Anderson Cancer Center, 35 patients have been treated with Cytoxan-Fludarabine followed by TIL and high-dose IL-2, with a 50% ORR. Tumor regression was associated with larger number of CD8 T cells administered (36, 37). However, the results were not reported on an intention-to-treat basis and would be significantly lower if the drop-out rate was taken into consideration.

Finally, the National Cancer Institute, which has pioneered TIL therapy for cancer, has described their long-term experience with up to 5-year follow-up in 93 patients treated with TIL that have had lymphoid depletion using the current Cytoxan-Fludarabine regimen, or using that regimen with 200 or 1200 rad of total lymphoid irradiation. They documented that 22% of their patients had complete responses, and that 93% of those patients were alive at 5 years, free of disease. Again, the results were not reported on an intention-to-treat basis. In the NCI experience, the level of anti-tumor response was associated with selection of tumor fragments plated in 24 well plates by their recognition of fresh tumor cells measured by gamma interferon release, with patients receiving so-called unselected "young TIL" having a lower response rate and fewer sustained complete responses. At Moffitt Cancer Center, in an ongoing adoptive cell therapy trial, we have successfully treated 10 patients (59%) of 17 patients with cytoxan-fludarabine followed by TIL with IL-2. Of these, we have observed 5 partial responses and two patients with stable disease (1), all with selected TIL.

The collective results suggest that the non-myeloablative lymphodepleting chemo-preparative regimen identical to the above regimen proposed in this current study can be tolerated and is potentially efficacious for the treatment of advanced metastatic disease. This may be due to the homeostatic pressure created by the chemotherapy regimen. After ablation of the endogenous lymphocyte compartment, the infused TIL cells may expand better *in vivo* without competition from endogenous lymphocytes. At the NIH, marked proliferation of the transferred TIL cells was not observed without this course of chemotherapy. To further optimize adoptive cell therapy in the proposed study, the plan is to administer nivolumab (PD-1 blocking antibody) as described below in order to enhance resulting TIL reactivity and persistence.

1.6 Intermediate (decrescendo) dose IL-2 as an alternative to high-dose IL-2:

The primary toxicities resulting from typical adoptive cell therapy regimens are related to high-dose IL-2 administered after cell transfer. IL-2 is administered to support in vivo proliferation and persistence of the infused cell product. In order to reduce this toxicity and maintain efficacy and persistence of the cell product, alternative IL-2 dosing regimens have been explored. One such regimen which has demonstrated efficacy in a melanoma population receiving TIL ACT product is an intermediate-dose decrescendo IL-2 infusion, as previously described by Keilholz et al. (38) and Andersen et al (39).

On day 0, patients receive a bolus infusion of TIL followed by a continuous IL-2 infusion administered in a decrescendo regimen (18 MIU/m2 over 6, 12, and 24 hours followed by 4.5 MIU/m2 over 24 hours for 3 days). IL-2 infusion is started within 12 hours after the TIL infusion. Maximum total dose of IL-2 administered is limited to 135 MIU, corresponding to a body surface area of 2 m^2. All patients receive prophylactic antibiotics and antiemetics. To reduce the neutropenic period and time in hospital a single subcutaneous injection of granulocyte colony-stimulating factor (G-CSF) is administered after the TIL infusion.

1.7 Anti-PD-1/L1 priming enhances TIL reactivity in vitro and in vivo:

ACT depends upon infiltration of T-cells into the tumor margin prior to harvest, and potent anti-tumor effector function after transfer. Lung tumors contain large numbers of regulatory T-cells and mediate potent inhibition of autologous T-cells (46). Aberrant expression of suppressive ligands such as PD-L1 may mediate failure of adoptive cell therapy. Specifically, PD-1 is upregulated on activated and exhausted T-cells. In an NCI study of CD8+ TIL from 6 melanoma patients, expression of PD-1 identified the autologous tumor-reactive repertoire, including mutated neoantigen-specific CD8+ cells, and TCRβ sequencing detected oligoclonal expansion of specific

TCRβ clonotypes which was only in PD-1+CD8+ TIL populations (47). Our group isolated TIL from disaggregated primary human NSCLC tumors (*n* =14) and performed flow cytometry (FACS) [Figure 1B], which demonstrated high PD-1 co-expression compared to other checkpoints. After clonal expansion, addition of 10 mcg/mL α-PD-L1 *in vitro* augmented TIL reactivity to autologous tumor and was synergistic with addition of IL-2 at 6,000 IU/mL [Figure 1C].

We have also shown that αPD-1 treatment prior to tumor resection improved the yield of TIL in a B16 murine melanoma model [Figure 1D]. Mice (n=3 per group) were injected subcutaneously with 10^5 B16 cells. Mice with established tumors received 30 mg/kg of αPD-1 or isotype control. Tumors and spleens were collected on day 24 and phenotyped for CD8+ T cells. αPD-1 caused a significant increase in CD8+ T cells infiltrating into the tumors and higher CD8+ counts in the spleen. These cultured TIL also had higher reactivity to B16 compared to isotype control [Figure 1D].

Since CD8+ T cells are thought to be the predominant mediator of anti-tumor function of TIL, we expect the addition of PD-1/PD-L1 blockade prior to tumor harvest will result in enhanced infiltration by anti-tumor effector T cells. This effect is expected to be durable in humans, as a single dose of αPD1 results in a mean occupancy of >70% of PD-1 co-receptors on circulating T-cells for more than two months after a single dose. Notably, post-treatment biopsies in humans with either αPD-1 or αPD-L1 show an significant increase in peritumoral T-cell infiltrate, substantiating its potential role as a partner with ACT.

1.8 Anti-PD-1 blocking antibody (nivolumab) as an agent to combine with adoptive cell therapy

Programmed death 1 (PD-1) is an immune checkpoint receptor expressed by activated T cells. Interaction between PD-1 and its ligand PD-L1 in peripheral tissues such as tumors and stromal cells leads to the immunosuppression of T cells (Dong, H Nat Med 2002; Topalian, S Curr Opin Immul 2012; Freeman, GL J Exp Med 1999). In murine models, blockade of PD-1/PD-L1 interactions results in enhanced T cell proliferation, persistence and anti-tumor activity (Pilon-Thomas, S JI 2010; Iwai, Y PNAS 2002). In recent Phase I clinical trials, treatment patients with anti-PD-1 or anti-PD-L1 antibodies led to an overall clinical response rates betweem 20 - 28% in patients with melanoma and 15 - 20% in non-small cell lung cancer (Topalian et al. NEJM 2012). Of note, a single dose of PD-1 antibody resulted in a mean occupancy of >70% of PD-1 co-receptors on circulating T cells for ≥ 2 months after a single dose (Brahmer et al. JCO 2010).

Published work in a murine model has shown that anti-PD-1 or anti-PD-L1 antibody treatment augments the infiltration of T cells within the tumor (Peng, Q Cancer Res 2012). Murine work has also demonstrated that pre-treatment of harvested B16 melanoma and other tumors with anti-PD-L1 antibody increases the likelihood that T cells with anti-tumor reactivity can be propagated from treated tumors (unpublished data). In the current trial, we will attempt to increase T cell trafficking to tumor by using anti-PD-L1 antibody prior to surgical resection of tumor and growth of TIL. The safety and feasibility of this approach will also be evaluated in the current trial.

To date, few investigators have examined ACT with autologous TIL for patients with advanced NSCLC. Moreover, the combination of aPD-1 mAb with ACT has not been reported in NSCLC. These drugs have a favorable side effect profile, and may re-activate the host's natural immune system to eliminate tumor cells. Thus, this project is original and significant, has intellectual merit, and has potential for broad impact in the field of immunotherapy.

1.9 Specifications of Nivolumab

Pharmacodynamics

Nivolumab binds with high affinity and specificity to human PD-1 and blocks its interaction with PD-L1 and PD-L2. In vitro studies demonstrate that nivolumab antagonizes the inhibitory effect of PD-L1 on primary human T cells, resulting in their restored proliferation and release of interferon gamma (IFN-γ). Additionally, nivolumab demonstrated a lack of antibody-dependent cell-mediated cytotoxicity and complement-dependent cytotoxicity in cell-based functional assays. *In vivo* studies show that nivolumab inhibits tumor growth in a xenograft model via a T lymphocyte (T-cell) dependent mechanism. Moreover, an anti-mouse PD-L1 antibody demonstrated improved survival in a syngeneic tumor model when given as monotherapy and resulted in complete tumor regression in > 50% of treated mice when given in combination with chemotherapy. Combination therapy (dual targeting of PD-L1 and cytotoxic T-lymphocyte-associated antigen 4 [CTLA-4]) resulted in tumor regression in a mouse model of colorectal cancer.

Cynomolgus monkeys were selected as the only relevant species for evaluation of the pharmacokinetics (PK)/pharmacodynamics and potential toxicity of nivolumab. Following intravenous (IV) administration, the PK of nivolumab in cynomolgus monkeys was nonlinear. Systemic clearance (CL) decreased and concentration half-life ($t_{1/2}$) increased with increasing doses, suggesting saturable target binding-mediated clearance of nivolumab. No apparent gender differences in PK profiles were observed for nivolumab.

In general, treatment of cynomolgus monkeys with nivolumab was not associated with any nivolumab-related adverse effects that were considered to be of relevance to humans. Adverse findings in the non-Good Laboratory Practice (GLP) PK/pharmacodynamics and dose range-finding study, and a GLP 4-week repeat-dose toxicity study were consistent with antidrug antibody (ADA)-associated morbidity and mortality in individual animals. The death of a single animal in the non-GLP, PK/pharmacodynamics, and dose range-finding study was consistent with an ADA-associated acute anaphylactic reaction. The spectrum of findings, especially the clinical signs and microscopic pathology, in a single animal in the GLP, 4-week, repeat-dose study was also consistent with ADA immune complex deposition, and ADA:nivolumab immune complexes were identified in a subsequent non-GLP, investigative immunohistochemistry study. Similar observations were reported in cynomolgus monkeys administered human mAbs unrelated to nivolumab. Given that immunogenicity of human mAbs in nonclinical species is generally not predictive of responses in humans, the ADA-associated morbidity and mortality were not considered for the determination of the no-observed-adverse-effect level (NOAEL) of nivolumab.

Finally, data from the pivotal 3-month GLP toxicity study with nivolumab in cynomolgus monkeys showed that subchronic dosing of nivolumab was not associated with any adverse effects. Therefore, the NOAEL of nivolumab in all the general toxicity studies was considered to be 100 mg/kg, the highest dose tested in these studies. In addition to the in vivo toxicology data, no unexpected membrane binding of nivolumab to human or cynomolgus monkey tissues was observed in GLP tissue cross-reactivity studies using normal human and cynomolgus monkey tissues.

Clinical experience with nivolumab is fully described in the current version of the Nivolumab Investigator's Brochure and drug package insert.

Pharmacokinetics and Product Metabolism

Nivolumab monotherapy exhibited nonlinear (dose-dependent) PK. The area under the concentration-time curve from 0 to 14 days (AUC0-14) increased in a greater than dose-

proportional manner over the dose range of 0.1 to 15 mg/kg and approached linearity at ≥ 3 mg/kg, suggesting that the nonlinear PK of nivolumab is likely due to saturable target-mediated clearance. Exposures following multiple doses (currently up to a maximum of 26 doses) demonstrated accumulation consistent with PK parameters estimated from the first dose.

Suppression of free soluble PD-L1 (sPD-L1) was correlated with nivolumab PK concentrations. Following administration of nivolumab monotherapy, free sPD-L1 levels were below the lower limit of quantitation (LLOQ) in the majority of subjects with available data (n = 38) at all timepoints following IV doses ≥ 1 mg/kg every 2 weeks (Q2W). Subsequent data has shown suppression of free sPD-L1 at higher doses using flat-dosing of 480 mg q4 weeks leading to FDA approval of this newer schedule for oncologic indications beginning in March 2018.

Overall, a low incidence of ADA was observed. Of the 220 subjects who received nivolumab monotherapy and for whom PK/ADA data were available, 5 were detected ADA positive, with an impact on PK/pharmacodynamics reported in 1 subject.

Safety

Based upon the package insert, important potential risks of nivolumab and its related molecules include immune-mediated reactions such as enterocolitis, dermatitis, hepatitis/hepatotoxicity, endocrinopathy, pneumonitis, and neuropathy. Additional important potential risks include infusion-related reactions, hypersensitivity, serious allergic reactions, serious infections, and immune complex disease.

The majority of the safety data are from the monotherapy study, CD-ON-nivolumab-1108, specifically the 10 mg/kg Q2W cohort (N = 393). In this cohort, the most frequently reported (≥ 10% of subjects) adverse events (AEs; all grades, regardless of causality) were fatigue (29.8%), nausea (20.1%), dyspnea (19.6%), decreased appetite (19.1%), constipation (14.0%), diarrhea and vomiting (12.5% each), cough (11.5%), pyrexia and back pain (10.4% each), and rash (10.2%). In approximately half of the subjects, the highest AE severity was Grade 1 (25.2% of subjects) or Grade 2 (22.9% of subjects). Most of these events were managed clinically without the need for dose modifications or delays. Grade 3 or higher AEs that occurred in > 1% of subjects were dyspnea (5.1%), increased gamma-glutamyltransferase (3.3%), fatigue, general physical health deterioration, increased aspartate aminotransferase, and back pain (2.3% each), anemia and dehydration (1.8% each), and abdominal pain, vomiting, sepsis, syncope, and hypotension (1.3% each). Treatment-related Grade 3 AEs in 2 or more subjects were fatigue (4 subjects), increased gamma-glutamyltransferase (3 subjects), and vomiting, increased alanine aminotransferase, increased aspartate aminotransferase, and arthralgia (2 subjects each). There were 2 subjects with treatment-related Grade 4 events (hypercalcemia, fatigue) and 1 subject with a treatment-related Grade 5 event (angiopathy). In general, Grade 3 or higher AEs were manageable and reversible with standard toxicity management guidelines.

Serious adverse events (SAEs) and other significant AEs occurred in less than one-third of subjects treated with nivolumab 10 mg/kg Q2W in Study CD-ON-nivolumab-1108. The most frequently reported SAEs (regardless of causality; > 5 subjects) were dyspnea (15 subjects), general physical health deterioration (9 subjects), pyrexia (8 subjects), back pain and abdominal pain (7 subjects each), and dehydration and pleural effusion (6 subjects each). One subject (with Stage IV lung cancer and a history of cardiac disease) died due to angiopathy considered by the investigator as related to nivolumab. Adverse events that resulted in permanent discontinuation of nivolumab in ≥ 2 subjects were dyspnea (7 subjects), general physical health deterioration (5 subjects), and death, increased transaminases, pulmonary embolism, and respiratory failure (2 subjects each).

No dose-limiting toxicities (DLTs) were reported in the dose-escalation cohorts of the monotherapy studies. Overall, the AE profile of nivolumab was consistent with the pharmacology of the target.

Efficacy

Nivolumab is FDA approved for multiple indications, including the second-line treatment of Metastatic Squamous and Non-squamous NSCLC. CHECKMATE-017 (NCT01642004) was a randomized (1:1), open-label study enrolling 272 patients with metastatic squamous NSCLC who had experienced disease progression during or after one prior platinum doublet-based chemotherapy regimen. Patients received 3 mg/kg of OPDIVO (n=135) by intravenous infusion every 2 weeks or docetaxel (n=137) administered intravenously at 75 mg/m2 every 3 weeks. The trial demonstrated a statistically significant improvement in OS for patients randomized to OPDIVO as compared with docetaxel at the prespecified interim analysis when 199 events were observed (86% of the planned number of events for final analysis). The Overall Response Rate (ORR) was 20% (95% CI 14-28) in the nivolumab arm compared to 9% (95% CI 5-15%) in the docetaxel arm, p value = 0.0083.

Nivolumab is also FDA approved for first-line treatment of Stage 4 metastatic NSCLC in combination with ipilimumab for patients with PD-L1 expression of 1% or higher (CHECKMATE-227). Nivolumab is also FDA approved for first-line treatment of Stage 4 NSCLC patients in combination with platinum-based chemotherapy and ipilimumab (CHECKMATE 9-LA) based upon superior overall survival (OS) compared to platinum doublet chemotherapy.

Nivolumab also has comparable, but not superior efficacy, to standard platinium-based chemotherapy in the first-line setting in patients. For example, the Phase III CHECKMATE-026 randomized patients (1:1) with NSCLC tumors with PD-L1 expression ≥1% of cells in an open-label to either nivolumab 3 mg/kg IV q2 weeks or control platinum-based chemotherapy of investigator's choice. In the primary intent-to-treat (ITT) population, the median PFS was 4.2 months with Opdivo and 5.9 months with platinum-based doublet chemotherapy (stratified hazard ratio [HR]=1.15 [95% CI: 0.91, 1.45, p=0.25]). Objective responses occurred in 26.1% of nivolumab patients and 33.5% of chemotherapy-treated patients. Overall survival was 14.4 months for Opdivo versus 13.2 months for chemotherapy (HR=1.02 [95% CI: 0.80, 1.30]). Eligible patients had stage IV or recurrent NSCLC and had received no prior systemic therapy for advanced disease and had no EGFR or ALK mutations sensitive to available targeted therapies. The trial was limited to patients whose tumors had ≥1% PD-L1 expression. The primary analysis did not show nivolumab superiority. The total rate of crossover at progression was 43.6% in the nivolumab arm and 64.2% in the chemotherapy arm. In the nivolumab arm, 1.4% of patients crossed over to other immunotherapy, as compared with 60.4%. At the end of the trial, 18.7% of treated patients remained on randomized therapy in the nivolumab group versus 5.3% of the chemotherapy arm.

2.0 Hypotheses and Objectives

The primary objective of this study will be to demonstrate that treatment with nivolumab in patients undergoing lymphodepletion/TIL/IL2 therapy is safe with a continuous Pocock-type stopping boundary for serious toxicity of < 17%, with safety reported based upon CTCAE version 4.0 criteria.

***Primary Hypothesis*:** The combination of TIL therapy and nivolumab for NSCLC patients will be feasible and achieve an acceptable toxicity profile.

Primary endpoint: To determine if serious toxicity rate (rate of DLT, defined in section 5.5) exceeds 17%

The secondary objectives of this study will be:

1. To determine the best objective response rate (BORR) associated with the treatment regimen

2. To determine the overall survival associated with the treatment regimen

Exploratory Hypothesis: The combination of TIL therapy with nivolumab for NSCLC will produce observed responses in a population of patients who are refractory to or progress on nivolumab monotherapy alone.

Secondary endpoint: Determine if the treatment produces a best overall radiologic response rate in radiographically evaluable patients who progress on nivolumab monotherapy in unselected NSCLC of 20% or more.

3.0 Clinical Pharmacology

3.1 Interleukin-2 (Aldesleukin, Proleukin)

Interleukin-2 (IL-2) is manufactured and supplied for in vitro use, and will be supplied for clinical use (aldesleukin). IL-2 is a 133 amino acid protein primarily secreted by T-cells in response to various antigenic stimuli. The cytokine acts through a specific IL-2 receptor consisting of α, β, γ subunits. In addition to T-cell proliferation, IL-2 leads to activation and proliferation of natural killer (NK) cells, increasing their tumoricidal activity. Other actions of IL-2 include augmentation of B-cell growth and immunoglobulin production, enhancement of interferon (IFN)-γ and tumor necrosis factor (TNF)-α production from T-cells, IL-6 production by monocytes, modulation of histamine release by basophils, and upregulation of IL-2 receptors. This triggers the release of various other cytokines leading to the total immune/ inflammatory reaction and resultant toxicity.

On day 0, following the infusion of TIL, IL-2 is administered as a continuous infusion in a decrescendo regimen (18 MIU/m^2 over 6 hours, then 18 MIU/m^2 over 12 hours, then 18 MIU/m^2 over 24 hours, followed by 4.5 MIU/m^2 over 24 hours daily for 3 days). IL-2 infusion is started within 12 hours after the TIL infusion. Maximum total dose of IL-2 administered is limited to 135 MIU, corresponding to a body surface area of 2.0 m^2.

Adverse events common to IL-2 include diarrhea, nausea, vomiting, hypotension, skin changes, anorexia, mucositis, dysphagia, constitutional symptoms, and laboratory changes. IL-2 administration has been associated with capillary leak syndrome (CLS) which is characterized by a loss of vascular tone and extravasation of plasma proteins and fluid into the extravascular space. CLS results in hypotension and reduced organ perfusion which may be severe and can result in death. CLS may be associated with cardiac arrhythmias (supraventricular and ventricular), angina, myocardial infarction, respiratory insufficiency requiring intubation, gastrointestinal bleeding or infarction, renal insufficiency, edema, and mental status changes.

IL-2 treatment is also associated with impaired neutrophil function (reduced chemotaxis) and with an increased risk of disseminated infection, including sepsis and bacterial endocarditis. Consequently, preexisting bacterial infections should be adequately treated prior to initiation of IL-2 therapy. Patients with indwelling central lines are particularly at risk for infection with gram positive microorganisms. Antibiotic prophylaxis with oxacillin, nafcillin, ciprofloxacin, or vancomycin has been associated with a reduced incidence of staphylococcal infections. IL-2 administration should be withheld in patients developing moderate to severe lethargy or somnolence; continued administration may result in coma.

The standard approach to the administration of high-dose IL-2 in all studies is to continue dosing until grade 3 or 4 events occur. The most commonly seen grade 4 events are pulmonary and renal

impairment, and mental status changes. These toxicities may sometimes require intubation for protection of the patient's airway. It is important to note that although these patients require significant supportive measures during this period, all toxicities are reversible and the overwhelming majority of patients have suffered no long term sequelae following this treatment regimen. However, fatal complications are possible and it is therefore only appropriate to carry out this experimental treatment in the context of life threatening metastatic cancer. Within this trial, IL-2 is given as a continuous infusion, which may partially abrogate the adverse effects due to high C_{max} of bolus dosing, while maintaining biologically relevant concentrations within the tissue and plasma compartments.

3.2 Fludarabine/Cyclophosphamide Conditioning Regimen

The animal and clinical studies cited above (section 1.5) strongly suggest that the clinical effectiveness of TIL and their ability to survive and repopulate the host would be enhanced if patients were significantly immunosuppressed by the depletion of lymphocytes prior to the adoptive transfer of lymphocytes. Based on this data, a non-myeloablative lymphodepleting chemo-preparative regimen consisting of fludarabine/cyclophosphamide, as proposed in this current study, is selected as it has been demonstrated it can be tolerated and can be efficacious for the adoptive cell therapy of advanced metastatic disease.

Cyclophosphamide will be administered at 60 mg/kg/day IV in 250 mL NS over approximately 2 hours. Cyclophosphamide will be initiated seven days prior to the anticipated TIL transfer, and the precise timing will depend on the rate of *in vitro* TIL growth. If a patient is obese (BMI >35), drug dosage will be calculated using practical weight as indicated below. Infusions may be slowed as medically indicated. Fludarabine will then be infused at 25 mg/m^2 IVPB daily over approximately 30 minutes starting 5 days prior to TIL transfer (Day -7 -6 -5, -4, -3). If a patient is obese (BMI >35), drug dosage will be calculated using practical weight. Infusions may be slowed as medically indicated.

Body Weight Determination

Actual body weight is used for dose calculations of study treatments (ie, cyclophosphamide, fludarabine, TIL, IL-2).
In subjects who are determined to be obese (BMI > 35), the practical body weight (see item 3 below) will be used.

1. BMI Determination:

- BMI = weight (kg) / [height (m)]2

2. Calculation of ideal body weight:

    - **Male** = 50 kg + 2.3 (number of inches over 60 inches)
        - *Example*: ideal body weight of 5'10" male: 50 + 2.3 (10) = 73 kg
    - **Female** = 45.5 kg + 2.3 (number of inches over 60 inches)

- *Example*: ideal body weight of a 5'3" female: 45.5 + 2.3 (3) = 57kg

3. Calculation of "***practical weight***": Calculate the average of the actual and the ideal body weights.

3.2.1 Fludarabine (Fludara)

Fludarabine phosphate is a fluorinated nucleotide analog of the antiviral agent vidarabine, 9-β-D-arabinofuranosyladenine (ara-A) that is relatively resistant to deamination. Fludarabine is a purine antagonist antimetabolite. Fludarabine phosphate is rapidly dephosphorylated to 2-fluoro-ara-A and then phosphorylated intracellularly by deoxycytidine kinase to the active triphosphate, 2-fluoro-ara-ATP. This metabolite appears to act by inhibiting DNA polymerase alpha, ribonucleotide reductase and DNA primase, thus inhibiting DNA synthesis. The mechanism of action of this antimetabolite is not completely characterized and may be multi-faceted.

Fludarabine will be purchased from commercial sources as a powder in the form of a white, lyophilized solid cake. The fludarabine powder is stable for at least 18 months at 2 – 8 degrees C; when reconstituted, fludarabine is stable for at least 16 days at room temperature. Specialized references should be consulted for specific compatibility information. Fludarabine is dephosphorylated in serum, transported intracellularly and converted to the nucleotide fludarabine triphosphate; this 2-fluoro-ara-ATP molecule is thought to be required for the drug's cytotoxic effects. Fludarabine inhibits DNA polymerase, ribonucleotide reductase, DNA primase, and may interfere with chain elongation, and RNA and protein synthesis.

Fludarabine is administered as an IV infusion in 100 mL 0.9% sodium chloride, USP over approximately 15 to 30 minutes. At doses of 25 mg/m^2/day for 5 days, the primary side effect is myelosuppression. However, thrombocytopenia is responsible for most cases of severe and life-threatening hematologic toxicity. Hemolytic anemia has been reported after one or more courses of fludarabine with or without a prior history of a positive Coomb's test; fatal hemolytic anemia has been reported. In addition, bone marrow fibrosis has been observed after fludarabine therapy. Other common adverse effects include malaise, fatigue, anorexia, and weakness. Irreversible and potentially fatal central nervous system toxicity in the form of progressive encephalopathy, blindness, and coma is rare at the currently administered doses. More common neurologic side effects at the current doses of fludarabine include weakness, pain, malaise, fatigue, paresthesias, visual or hearing disturbances, and sleep disorders. Adverse respiratory effects of fludarabine include cough, dyspnea, and allergic or idiopathic interstitial pneumonitis. Tumor lysis syndrome has been rarely observed in fludarabine treatment of chronic lymphocytic leukemia.

3.2.2 Cyclophosphamide (Cytoxan)

Cyclophosphamide is a synthetic anti-neoplastic drug chemically related to the nitrogen mustards. It is biotransformed principally in the liver to active alkylating metabolites by a mixed function microsomal oxidase system. These metabolites interfere with the growth of susceptible rapidly proliferating malignant cells. The mechanism of action is thought to involve cross-linking of tumor cell DNA.

Cyclophosphamide is well absorbed after oral administration with a bioavailability greater than 75%. The unchanged drug has an elimination half-life of 3 to 12 hours. It is eliminated primarily in the form of metabolites, but from 5% to 25% of the dose is excreted in urine as unchanged drug. Several cytotoxic and non-cytotoxic metabolites have been identified in urine and in plasma. Concentrations of metabolites reach a maximum in plasma 2 to 3 hours after an IV dose. Plasma protein binding of

unchanged drug is low but some metabolites are bound to an extent greater than 60%. It has not been demonstrated than any single metabolite is responsible for either the therapeutic or toxic effects of cyclophosphamide. Although elevated levels of metabolites of cyclophosphamide have been observed in patients with renal failure, increased clinical toxicity in such patients has not been demonstrated.

Following conversion to active metabolites in the liver, cyclophosphamide functions as an alkylating agent and possesses potent immunosuppressive activity. The serum half-life after intravenous administration ranges from 3 to 12 hours; the drug and/or its metabolites can be detected in the serum for up to 72 hours after administration.

Cyclophosphamide will be obtained from commercially available sources. It will be diluted in 250 mL NS and infused over approximately two hours. The dose will be based on the patient's body weight. Hematologic toxicity occurring with cyclophosphamide usually includes leukopenia and thrombocytopenia. Anorexia, nausea, and vomiting may occur, especially after high-doses. Diarrhea, hemorrhagic colitis, and mucosal and oral ulceration have been reported in patients. Sterile hemorrhagic cystitis occurs in about 20% of patients; severity can range from microscopic hematuria to extensive cystitis with bladder fibrosis. Although the incidence of hemorrhagic cystitis associated with cyclophosphamide appears to be lower than that associated with ifosfamide, mesna (sodium 2-mercaptoethanesulfonate) has been used prophylactically as an uroprotective agent Mesna may not be effective in all patients. Patients who receive high dose cyclophosphamide may develop interstitial pulmonary fibrosis, which can be fatal. Hyperuricemia due to rapid cellular destruction may occur, particularly in patients with hematologic malignancy. Hyperuricemia may be minimized by adequate hydration, alkalinization of the urine, and/or administration of allopurinol. If allopurinol is administered, patients should be watched closely for cyclophosphamide toxicity due to allopurinol induction of hepatic microsomal enzymes. At high doses, cyclophosphamide can also result in a syndrome of inappropriate antidiuretic hormone secretion; hyponatremia with progressive weight gain occurs. Cardiotoxicity has been observed at high doses of cyclophophamide. Deaths have occurred from diffuse hemorrhagic myocardial necrosis and from acute myopericarditis; in such cases, congestive heart failure may occur within a few days of the first dose. Other consequences of cyclophosphamide cardiotoxicity include arrhythmias, potentially irreversible cardiomyopathy, and pericarditis. Other reported adverse effects of cyclophosphamide include headache, dizziness, and myxedema; faintness, facial flushing, and diaphoresis. Patients may require loop diuretics such as furosemide as-needed during cyclophosphamide treatment for treatment of edema.

3.3 Mesna (Sodium 2-mercaptoethanesulfonate, Mesnum, Mesnex, NSC-113891)

Mesna (sodium 2-mercaptoethanesulphonate; given by IV injection) is a synthetic sulfhydryl compound that can chemically interact with urotoxic metabolites of cyclophosphamide (acrolein and 4-hydroxycyclophosphamide) to decrease the incidence and severity of hemorrhagic cystitis.

Mesna was developed as a prophylactic agent to reduce the risk of hemorrhagic cystitis. Analogous to the physiological cysteine-cystine, mesna is rapidly oxidized to its major metabolite, mesna disulfide (dimesna). Mesna disulfide remains in the intravascular compartment and is rapidly eliminated by the kidneys. In the kidney, the mesna disulfide is reduced to the free thiol compound, mesna, which reacts chemically with the urotoxic metabolites, resulting in their detoxification.

Mesna will be obtained commercially and is supplied as a 100 mg/mL solution. Intact ampules are stored at room temperature. Diluted solutions (1 to 20 mg/dL) are physically and chemically stable for at least 24 hours under refrigeration. Mesna is chemically stable at room temperature for 48-72 hours in D5W, 48-72 hour in D5W/0.45% normal saline, or 24 hours in normal saline. It will be diluted up to

20 mg Mesna/mL fluid in D5W or normal saline and will be administered intravenously as a continuous infusion. Toxicities include nausea, vomiting and diarrhea.

3.4 Granulocyte-Colony Stimulating Factor (G-CSF, Filgrastim, tbo-filgrastrim)

Filgrastim is a human granulocyte colony-stimulating factor (G-CSF), produced by recombinant DNA technology. Filgrastim, which has been selected as the name for recombinant methionyl human granulocyte colony-stimulating factor (r-metHuG-CSF). Biological equivalents, such as tbo-filgrastim or peg-filgrastim, are considered acceptable alternatives.

Filgrastim is a 175 amino acid protein manufactured by recombinant DNA technology, produced by Escherichia coli (E coli) bacteria into which has been inserted the human granulocyte colony-stimulating factor gene. Filgastrim is a sterile, clear, colorless, preservative-free liquid for parenteral administration. The product is available in single use vials and prefilled syringes. The single use vials or prefilled syringes contain either 300 mcg or 480 mcg Filgrastim.

The starting dose of Filgastrim is approximately 5 mcg/kg/day, usually rounded to either 300 mcg or 480 mcg prefilled syringes, administered as a single daily injection by SC injection. A CBC and platelet count should be obtained before instituting Filgastrim therapy, and monitored twice weekly during therapy. Doses may be increased, according to the duration and severity of the ANC nadir.

Filgastrim should only be administered if required based upon treating clinician discretion. Common reasons which may prompt initiation of filgrastrim include refractory infection, evidence of sepsis, or persistence of febrile neutropenia despite optimal supportive care. Filgrastrim should not be given earlier than 24 hours after the administration of cytotoxic chemotherapy. Filgastrim should not be administered in the period 24 hours before the administration of chemotherapy. Filgastrim can be administered daily for up to 2 weeks, until the ANC has reached 1,000/mm^3 following the expected chemotherapy-induced neutrophil nadir. The duration of Filgastrim therapy needed to attenuate chemotherapy-induced neutropenia may be dependent on the myelosuppressive potential of the chemotherapy regimen employed. Filgastrim therapy should be discontinued if the ANC surpasses 1,000/mm^3 after the expected chemotherapy-induced neutrophil nadir.

3.5 Levofloxacin (Levaquin) and Trimethoprim and Sulfamethoxazole double strength (TMP / SMX DS, Bactrim)

Levofloxacin is used to prevent infections caused by bacteria. It is a synthetic broad spectrum antibacterial agent. The mechanism of action of Levofloxacin involves inhibition of bacterial topoisomerase IV and DNA gyrase, enzymes required for DNA replication, transcription, repair, and recombination. An alternative antibiotic by mouth for patients who are allergic to Levofloxacin will be Keflex.

TMP/SMX SS will be obtained by from commercial sources. It will be used for the prevention of Pneumocystis carinii Pneumonia (PCP). The standard oral dose is 1 tablet PO SS daily, or DS tablet three times a week. Like other sulfa drugs, Bactrim (sulfamethoxazole-trimethoprim) can cause allergies, fever, nausea, and vomiting. Allergies typically develop as a widespread itchy red rash with fever eight to fourteen days after beginning the standard dose. Neutropenia, a reduction in the number of neutrophils, can also occur.

3.6 Acyclovir (Zovirax) and Valacyclovir Hydrochloride (Valtrex)

Valacyclovir or acyclovir will be obtained by from commercial sources.

Valtrex is the hydrochloride salt of *L*-valyl ester of acyclovir. It is rapidly converted to acyclovir, which has demonstrated antiviral activity against herpes simplex virus types 1 (HSV-1) and 2 (HSV-2) and varicella-zoster virus (VZV). The inhibitory activity of acyclovir is highly selective due to its affinity for the enzyme thymidine kinase (TK) encoded by HSV and VZV. This viral enzyme converts acyclovir into acyclovir monophosphate, a nucleotide analogue. The monophosphate is further converted into diphosphate by cellular guanylate kinase and into triphosphate by a number of cellular enzymes. Acyclovir triphosphate stops replication of herpes viral DNA.

Valtrex (valacyclovir) is the preferred antiviral to be used to prevent the occurrence of herpes virus infections. Acyclovir is an acceptable alternative. It is supplied as oral tablets of 200 and 800 mg. Reversible renal insufficiency has been reported with intravenous but not oral acyclovir. Neurologic toxicity including delirium, tremors, coma, acute psychiatric disturbances, and abnormal EEGs has been reported with higher doses of acyclovir. Should this occur, a dosage adjustment will be made, or the drug should be discontinued. Stomach upset, headache, nausea, rash, hives, diaphoresis, hematuria; hypotension, and thrombocytosis have been reported. Hair loss from prolonged use has also been documented. Acyclovir will not be used concomitantly with other nucleoside analogs that interfere with DNA synthesis, e.g. ganciclovir. In renal disease, the dose is adjusted as per product labeling.

3.7 Fluconazole (Diflucan)

Fluconazole will be obtained by from commercial sources. It will be used for prophylaxis against fungal infections. It is available in 200 mg tablets. It can cause headache, nausea, vomiting, diarrhea or abdominal pain, and liver damage that may be irreversible. It can cause rashes and itching, which in rare cases has caused Stevens Johnson Syndrome. It has several significant drug interactions. Voriconazole is a alternative for patients at very high risk for opportunistic fungal infections, especially those receiving prolonged courses of oral corticosteroids.

3.8 Ondansetron hydrochloride (Zofran)

Ondansetron hydrochloride will be obtained from commercial sources. It will be used to control nausea and vomiting during the chemotherapy preparative regimen. It can cause headache, dizziness, myalgias, drowsiness, malaise, and weakness. Less common side effects include chest pain, hypotension, pruritis, constipation and urinary retention. Consult the package insert for a complete list of side effects and specific dose instructions.

3.9 Furosemide (Lasix)

Furosemide, a loop diuretic, will be obtained from commercial sources. It will be used to enhance urine output during the chemotherapy preparative regimen with cyclophosphamide. Adverse effects include dizziness, vertigo, paresthesias, weakness, orthostatic hypotension, photosensitivity, rash and pruritis. Consult the package insert for a complete list of side effects and specific dose instructions.

3.10 Nivolumab

3.10.1 Identification

Nivolumab is available in concentrations of 100 mg/10mL. The sterile solution in the vial is clear and colorless. Nivolumab is administered via intravenous infusion only.

PRODUCT INFORMATION TABLE:

Product Description: (Nivolumab, Other names = MDX-1106, ONO-4538, anti-PD-1)

Product Description and Dosage Form	**Potency**	**Primary Packaging (Volume)/ Label Type**	**Secondary Packaging (Qty) /Label Type**	**Appearance**	**Storage Conditions (per label)**
Nivolumab (BMS-936558-01)* Injection drug product is a sterile, non-pyrogenic, single-use, isotonic aqueous solution	100 mg/Vial (10 mg/mL).	Carton of 5 or 10 vials	10-mL Type 1 flint glass vials stoppered with butyl stoppers and sealed with aluminum seals.	Clear to opalescent, colorless to pale yellow liquid. May contain particles	**BMS-936558-01 Injection** must be stored at 2 to 8 degrees C (36 to 46 degrees F) and protected from light and freezing.

3.10.2 Packaging and Labeling

Bristol Myers Squibb (BMS) will provide nivolumab antibody at no cost for this study. Nivolumab will be provided in open-label 10 mL vials containers at a concentration of 10 mg/mL. The labels will contain the protocol prefix, batch number, content, storage conditions, and dispensing instructions along with the Investigational New Drug (IND) caution statement.

3.10.3 Storage

Nivolumab must be stored in a secure area according to local regulations. The investigator must ensure that it is stored at a temperature $\geq 2°C$ and $\leq 8°C$.

3.10.4 Handling and Disposal

As with all injectable drugs, care should be taken when handling and preparing Nivolumab. Whenever possible, Nivolumab should be prepared in a laminar flow hood or safety cabinet using standard precautions for the safe handling of intravenous agents applying aseptic technique. Latex gloves are required. If Nivolumab concentrate or solution comes in contact with skin or mucosa, immediately and thoroughly wash with soap and water. After final drug reconciliation, unused Nivolumab solution should be disposed at the site following procedures for the disposal of anticancer drugs.

3.11 TIL Cell Preparation

The procedures and reagents for expanding human TIL cells will be followed according to Moffitt Cell Growth Facility SOPs. Briefly, TIL from the patient tumor biopsy are cultured and expanded in IL-2 (6000IU/ml) for an average of 21 days in 24 well culture plates. Briefly, patient tumor biopsies are dissected and 3mm tumor fragments were each cultured in separate wells of a 24 well culture plate with in RPMI media supplemented with 10% Human AB serum and 600IU/mL of IL-2. Each well is assessed every 2 to 3 days for TIL. TIL were fed every 2 to 3 days by removing half volume of media and replacing half volume of fresh media. Wells are split when TIL reach approximately >80% confluence. TIL cultures are tested by ELISA for tumor specific interferon-gamma (INF-γ) production after 24 hour stimulation with either tumor digest or CD3/CD28 beads. TIL are selected and pooled based on growth and IFN-γ production. Timing for patient dosing is facilitated by cryopreservation of the pooled TIL. When patients were ready for dosing, TIL are thawed and rapidly expanded in IL-2 (300IU/ml) and anti-CD3 (30ng/ml) with a layer of feeder cells in large flasks and incubated for approximately 14 days. Upon harvest, TIL product undergoes Quality Control testing and release testing.

Plastic adherence method is used to generate dendritic cells. Autologous PBMCs or apheresis samples are thawed and resuspended, incubated at 37°C, 5% CO2. After 90 minutes, non-adherent cells are collected and washed with media, then cultured over 7-10 days in media containing IL-4 and GM-CSF, then frozen in 10% DMSO for correlative assays.

4.0 Eligibility Assessment and Enrollment

Patient evaluation for eligibility and registration will occur utilizing a two-step design (at screening prior to tumor harvest and prior to lymphodepletion with chemotherapy). Patients with antibiotic allergies per se are not excluded; although the production of TIL for adoptive transfer includes antibiotics, extensive washing after harvest will minimize systemic exposure to antibiotics.

4.1 Screening, Initiation of Nivolumab, and TIL Expansion

Patients must fulfill all of the following screening criteria to be eligible for the study.

4.1.1 Screening Inclusion Criteria

1. Age greater than or equal to 18 years
2. Able to understand and give written informed consent.
3. Confirmed or suspected diagnosis of stage IV or recurrent non-small cell lung cancer (NSCLC). For suspected NSCLC, diagnosis must be histologically or cytologically confirmed prior to start of nivolumab treatment. Neuroendocrine cancers, or mixed neuroendocrine features in >10% of tumor cells, are excluded.
4. Tumor deemed accessible by metastatectomy (TIL harvest) which expects to yield >1.5 cm^3 of resectable tumor amount.
5. Measurable disease, even after resection of applicable lesion for TIL harvest. Defined as ≥1 lesion that is ≥10 mm in one dimension by CT scan, MRI, or calipers on clinical exam.
6. ECOG performance status of 0 or 1
7. Expected survival > 6 months
8. Patients with activating EGFR mutation or ALK rearrangement which is expected to be responsive to available tyrosine kinase inhibitor therapy, must have been previously treated with an applicable tyrosine kinase inhibitor.
9. Adequate normal organ and marrow function in an assessment performed within 7 days (+ 3 day window) of enrollment as defined below:
    - Hemoglobin ≥ 9.0 g/dL

- Absolute neutrophil count (ANC) ≥ 1.0 x 10^9/L (> 1000 per mm^3)
- Platelet count ≥ 100 x 10^9/L (>100,000 per mm^3)
- PT and PTT ≤ 1.5x the institutional upper limit of normal (ULN), (This will not apply to subjects with confirmed Factor XII deficiency.)
- Serum bilirubin ≤ 1.5x the institutional ULN, or ≤ 3x ULN if confirmed Gilbert's syndrome (persistent or recurrent hyperbilirubinemia that is predominantly unconjugated in the absence of hemolysis or hepatic pathology)
- AST (SGOT)/ALT (SGPT) ≤ 2.5x institutional ULN unless liver metastases are present, in which case it must be ≤ 5x ULN
- Serum creatinine of ≤ 1.5x institutional ULN.
- Albumin ≥ 2.5 g/dl.

10. Positive screening EBV IgG antibody titer on screening test.
11. Cardiac stress test within past 6 months without evidence of reversible ischemia.
12. Cardiac echocardiogram, stress test, or MUGA within past 6 months with demonstrated LVEF > 50%
13. Pulmonary function tests within past 6 months showing DLCO >50% of predicted. Adjusted DLCO based on hemoglobin concentration should be used, if available.

4.1.2 Screening Exclusion Criteria

1. More than 5 lines of prior systemic therapy in the preceding three years.
2. Any previous treatment with a PD-1 or PD-L1 inhibitor, including but not limited to: nivolumab, atezolizumab, pembrolizumab, avelumab, or durvalumab.
3. Current or prior use of any immunosuppressive medications, such as corticosteroids, within 14 days before enrollment.
    a. Oral hydrocortisone, only for the purposes of a documented and confirmed adrenal insufficiency diagnosis, is permitted if ≤ 25 mg daily total dose.
    b. Inhaled, intranasal, or topical corticosteroids are permitted.
4. Patients with untreated brain metastases. Treated brain metastases with radiation or surgery are allowed if: ≤ 3 cm in size AND ≤ 4 in number AND there is no evidence of progressive disease, on brain imaging ≥ 21 days after last day of CNS treatment.
5. History of leptomeningeal metastases.
6. Current or prior use of anticancer therapy before TIL collection:
    a. Chemotherapy within the past 4 weeks;
    b. Tyrosine kinase inhibitor (TKI) within the past 1 week;
    c. Investigational therapy within the past 4 weeks or 4 half-lives, whichever is shorter.
7. Uncontrolled intercurrent illness including, but not limited to, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia (other than stable atrial fibrillation), and significant carotid artery stenosis.
8. Patients known to be HIV positive, hepatitis B or C positive, or both RPR and FTA positive. (Hepatitis B surface or core antibody alone is not indicative of HBV infection).
9. Patients with rapidly progressing tumors, as judged by the investigator.
10. Mean QT interval corrected for heart rate (QTc) ≥470 ms calculated from electrocardiograms (ECGs) using Bazett's Correction
11. Known history of previous tuberculosis
12. Receipt of live attenuated vaccination within 30 days prior to first anticipated dose of nivolumab.
13. History of allogeneic organ transplant
14. History of primary immunodeficiency
15. History of severe hypersensitivity to nivolumab, cyclophosphamide, fludarabine, interleukin-2, or any excipient
16. Any condition that, in the opinion of the investigator, would interfere with evaluation of study treatment or interpretation of patient safety or study results

17. Patients with active systemic infections requiring intravenous antibiotics within 1 week prior to enrollment
18. Any unresolved toxicity (>CTCAE v4 grade 2) from previous anti-cancer therapy. Subjects with irreversible toxicity that is not reasonably expected to be exacerbated by the investigational product may be included (e.g., hearing loss, peripheral neuropathy).
19. History of pneumonitis or drug-related inflammatory lung disease
20. Patients who have a significant history of pulmonary disease that necessitates the use of supplemental oxygen, and patients with resting pulse oximetry <92% on room air.
21. Active or prior documented autoimmune disease within the past 2 years. NOTE: Subjects with vitiligo, Grave's disease, limited site eczema, or limited site plaque psoriasis not requiring systemic treatment (within the past 2 years), or other autoimmune conditions which are not expected to recur, are allowed after approval from the medical monitor or PI.
22. Patients with other prior malignancies must have had a ≥ 2-year disease-free interval, except for: *in situ* carcinoma of the cervix, *in situ* ductal carcinoma of the breast, *in situ* prostate cancer, *in situ* bladder cancer. These must have been deemed stable and not expected to relapse. In addition, early stage skin cancers, including basal, squamous cell cutaneous carcinoma, and melanoma, are permitted if previously treated with curative intent and not expected to relapse.
23. Women who are pregnant or lactating.
24. Women of childbearing potential and fertile men unwilling to use effective contraception during study until 4 months after conclusion of the treatment period.

4.1.3 Screening Procedures

All patients must sign an informed consent form and a negative pregnancy test (serum) must be documented for women of childbearing potential at screening and within 7 days before starting nivolumab. Screening procedures are outlined in the study calendar. If patients have had redundant laboratory screening studies performed for another reason within 14 days of enrollment, they need not be repeated, with the exception of pregnancy testing. If patients have had redundant imaging screening studies, such as CT scan or MRI, within 30 days of enrollment, then these need not be repeated. If a cardiac stress test is required and was done within the previous 6 months, the test will not be repeated unless in the judgment of the investigator at the time of screening, there has been a significant interval change in the patient's clinical status.

For patients who have a suspected but not confirmed diagnosis of metastatic NSCLC, the diagnosis must be confirmed cytologically or histologically prior to nivolumab treatment. This permits biopsy of patients for both histology confirmation and TIL harvest in a single procedure.

4.2 Adoptive Cell Treatment – Chemotherapy/TIL Infusion

4.2.1 Testing prior to Adoptive cell therapy

Patients must fulfill all of the following criteria to be eligible for the adoptive cell treatment phase. Laboratory testing must be complete and satisfactory within 14 days of initiation of chemotherapy. Imaging tests must be complete and satisfactory within 14 days of initiation of chemotherapy, and the identical criteria and management will be followed as discussed in section 4.1.1.

4.2.2 REP Eligibility Checklist

On Day -21, investigator will complete an eligibility checklist to confirm that it is appropriate to initiate the TIL REP by Cell Therapies Core.

1. Clinical performance status equivalent to ECOG 0-1 at the last calendar clinical visit.
2. Does not currently have a partial or complete response to nivolumab treatment
3. Absolute neutrophil count greater than or equal to 1000/mcL.
4. Platelet count greater than or equal to 100,000/mcL.
5. Hemoglobin greater than or equal to 8.0 g/dL(Note that this threshold is slightly lower than the initial trial screening parameter since most subjects will require blood transfusion at some point during the protocol treatment, and the established threshold for transfusion is 8 gm/dL).
6. Serum ALT and AST less than three times the institutional upper limit of normal.
7. Serum creatinine of ≤ 1.5x institutional ULN
8. Total bilirubin less than or equal to 2.0 mg/dL, except in patients with Gilbert's Syndrome who must have a total bilirubin less than 3.0 mg/dL
9. Patient has central IV access or request for central IV access submitted.

4.2.3 Chemotherapy/Cell Infusion Eligibility Checklist

On Day -8 (or within 7 days prior to Day -7), investigator must complete an eligibility checklist attesting that subject is fit to proceed with lymphodepletion and adoptive cell transfer.

1. Patients must have adequate TIL available as per Moffitt Cell Therapies current SOP.
2. Male patients with female partners of childbearing potential and female patients of childbearing potential must agree to use contraception for six months after receiving the preparative regimen
3. For women of child-bearing potential, a negative serum pregnancy testing will be verified within 7 days prior to treatment.
4. Clinical performance status equivalent to ECOG 0-1 at the clinical visit prior to lymphodepletion.
5. Absolute neutrophil count greater than or equal to 1000/mcL.
6. Platelet count greater than or equal to 100,000/mcL.
7. Hemoglobin greater than or equal to 8.0 g/dL(Note that this threshold is slightly lower than the initial trial screening parameter since most subjects will require blood transfusion at some point during the protocol treatment, and the established threshold for transfusion is 8 gm/dL).
8. Serum ALT and AST less than three times the institutional upper limit of normal.
9. Serum creatinine less than or equal to 1.7 mg/dL.
10. Total bilirubin less than or equal to 2.0 mg/dL, except in patients with Gilbert's Syndrome who must have a total bilirubin less than 3.0 mg/dL.
11. PT and PTT less than or equal to 1.5 times the institutional upper limit of normal
12. Patients with EKG within 14 days of initiation of chemotherapy demonstrating no new significant rhythm, axis or ST segment changes will be included. If clinically significant, new EKG changes are present, patients may be included if cardiac stress test indicates no reversible cardiac ischemia.
13. Urinalysis within 14 days demonstrating that no urinary tract infection is present.
14. Compete history and physical examination will be required within 2 weeks prior to initiation of chemotherapy.

5.0 Treatment Plan

5.1 Treatment Outline

TIL will be expanded in a state-of-the-art Good Medical Practices-certified cell growth facility at Moffitt Cancer Center that will allow compliance with all FDA regulations regarding investigational cell transfer products. The Moffitt adoptive cell therapy laboratory has demonstrated consistent TIL expansions of greater than 400 fold, which is ideal to treat patients with cell numbers consistent with earlier clinical

experiences at the National Cancer Institute (NCI), Moffitt Cancer Center and MD Anderson Cancer Center. TIL expansion will occur per Moffitt Cancer Center Cell therapy Standard Operating Procedures.

Venipuncture to obtain blood for correlative immunologic assays will be performed at screening. Nivolumab will be administered intravenously at a fixed dose of 240 mg for four doses beginning after tumor harvest (+/- 7 days). Patients will have a history and physical examination, vital signs and complete blood count and comprehensive metabolic panel prior to nivolumab. Women of child-bearing potential will have a confirmed negative serum pregnancy test prior to each nivolumab therapy. If patients are hospitalized for unanticipated reasons such as an adverse event, then patients are permitted to omit one nivolumab infusion (eg Day -49, Day -35, or Day -21) in order to remain on the calendar schedule.

Cyclophosphamide will be administered at 60 mg/kg/day IV in 250 mL NS over approximately 2 hours. Cyclophosphamide will be initiated seven days prior to the anticipated TIL transfer, and the precise timing will depend on the rate of in vitro TIL growth. The dose will be based on the patient's body weight. Fludarabine will then be infused at 25 mg/m^2 IVPB daily over approximately 30 minutes starting 7 days prior to TIL transfer. On day 0, all patients will receive TIL administered according to the current Moffitt Cell Therapy TIL SOP. Eight (8) to twelve (12) hours after completing the TIL infusion, all patients will begin intermediate-dose decrescendo interleukin-2 (IL-2). Doses will be skipped if patients reach Grade III or IV toxicity including diarrhea, nausea, vomiting, hypotension, skin changes, anorexia, mucositis, dysphagia, or constitutional symptoms and laboratory changes (i.e. platelets, creatinine, total bilirubin). If the toxicity is easily reversed by supportive measures, then additional doses may be continued. There will be no dose modification for IL-2, although dose holds are permitted as outlined in IL-2 section. Patients will discontinue IL-2 treatment for altered mental status, supra-ventricular arrhythmias that require medication, evidence of myocarditis, uncontrolled hypotension, urine output less than 600 mL per 24 hours and/or creatinine of ≥3.5 gm/dL in spite of maximal supportive measures, bilirubin of ≥8 gm/dL, positive blood culture, or evidence of infection. IL-2 will be permanently discontinued in patients who develop a life-threatening ventricular arrhythmia, life-threatening infection, myocardial infarction, or persistently altered mental status.

Of note, in a previous Moffitt trial, there were no DLTs related to adoptive cell therapy in melanoma (as defined in 34 days after TIL infusion; this time point was chosen as based on our previous experience as a majority of significant toxicity occurs within 2 weeks of the adoptive cell therapy. Therefore 3 – 4 weeks is a reasonable point to determine toxicity for safety assessment purposes). The dose of nivolumab will be fixed at 240 mg. Since nivolumab is FDA-approved at a fixed dose of 240 mg q2 weeks or 480 mg q4 weeks, there is no anticipated requirement to escalate the dosing of nivolumab. If at any point on the trial, toxicity probability is concluded to exceed 17% on the stopping boundary as defined in Section 8.0, the trial will stop accrual. In order to be evaluable for safety, patients must have received Cy/Flu chemotherapy and completed ≥70% of planned visits due the DLT evaluation period. Up to 20 subjects will be enrolled, with the goal of having 14 evaluable patients. Additional subjects may be replaced. No more than 4 subjects will be allowed into the DLT evaluation period at any given time.

Treatment:

Timing is based on days after day of TIL administration, defined as day 0. Note due to the variable time required to prepare TIL, the precise days that some of the treatment events will occur after the TIL transfer will be variable.

Nivolumab dose will be fixed at 240 mg for 4 doses every 2 weeks prior to TIL.

Nivolumab dose will be fixed at 480 mg every 4 weeks up to 12 months after TIL.

Nivolumab dose will be fixed at 480 mg every 4 weeks until progression for patients who have achieved PR/CR after their initial 4 doses. Reference Section 5.10

Nivolumab infusion rate will be infused over 30 minutes (or 60 minutes, per institutional standard).

The following is an outline intended to elaborate upon select procedures, in accordance with the study calendar. The study calendar is intended to serve as the primary management guide.

Day -67: Tumor harvest for TIL.

Day -63: Venipuncture for baseline sample to be used in correlative assays. First intravenous dose of nivolumab.

Day -49: Intravenous dose of nivolumab.

Day -35: Intravenous dose of nivolumab. Venipuncture for correlative assays (post 2 dose of nivolumab). Restaging scans will be done (and only those patients without PR/CR will proceed to have TIL expanded, those with PR/CR will continue nivolumab q4 weeks until progression, at which point they may then proceed to ACT after applicable checklist assessment)

Day -21: Intravenous dose of nivolumab. Completion of REP eligibility checklist prior to REP for eligible patients.

Day -8: Venipuncture for correlative assays (post 4 doses of nivolumab). Restaging scans will be done (and only those patients without PR/CR will proceed to TIL infusion, those with PR/CR will continue nivolumab q4 weeks until progression, at which point they may then proceed to TIL infusion). Completion of REP eligibility checklist (for those patients who had not previously undergone at day –21), as well as chemotherapy checklist assessment (for all patients receiving TIL infusion) prior to proceeding to conditioning chemotherapy (within 10 days of anticipated start of lymphodepletion).

Day -7 and -6 (timing will depend on rate of TIL growth):
Cyclophosphamide 60 mg/kg/day IV in 250 mL NS over approximately 2 hrs x 2 days. Mesna 20 mg/kg with D5W or NS at 125 mL/hr infused intravenously over 24 hours after each cyclophosphamide dose. Venipuncture for correlative assays (pre-lymphodepletion). Extended outpatient hospital stay.

Day -7 to Day -3 (timing will depend on rate of TIL growth):
Fludarabine 25 mg/m^2 IVPB daily over approximately 30 minutes for 5 days.

Day 0 (timing will depend on rate of TIL growth):

Venipuncture (10 mL only to confirm lymphodepletion). TIL infusion will be administered according to the Moffitt Cell Therapy TIL SOP. Vital signs (temperature, blood pressure, pulse and respiratory rate) and pulse oximetry will be monitored every 15 minutes during the TIL infusion, every 30 minutes for 1 hour after TIL infusion, and hourly thereafter for a total of 4 hours after completion of the infusion. G-CSF will be administered per protocol until ANC >1000/mcL or clinician discretion.

Day 0 - 10 (timing will depend on rate of TIL growth): Intermediate-dose decrescendo IL-2 will begin 8-12 hours after TIL infusion, and continue as per decrescendo regimen outlined in section 3.1 (18 MIU/m2 over 6, 12, and 24 hours followed by 4.5 MIU/m2 over 24 hours for up to 3 days depending on tolerance). Patients will receive IL-2 as part of an inpatient hospitalization starting Day 0 which is anticipated to be for approximately 7-10 days.

Day 14: (approximate; precise days will depend on rate of TIL growth): Toxicity assessment and PBMCs for correlatives.

Day 28 (approximate; precise days will depend on rate of TIL growth):
Toxicity assessment and assessment for response (restaging scans).Venipuncture (post TIL therapy). Apheresis catheter, if necessary, will be placed and up to 5 L of blood volume for apheresis product.
Day 28 (approximate) and onward: Patient proceed to receive nivolumab 480 mg IV every 4 weeks for up to one year.

5.2 Prophylaxis

5.2.1 Infection Prevention and Pneumocystis carinii Pneumonia (PCP) Prophylaxis

Patients will receive ciprofloxacin at 500 mg daily (or other preferred antibiotic if allergic to fluoroquinolone) until ANC recovers to greater than 1000/mcL and the fixed combination of trimethoprim (TMP) and sulfamethoxazole (SMX) as single strength (SS) tablet [SS tabs = TMP 80 mg/tab and SMX 400 mg/tab] po daily. TMP/SMX-SS will be taken by patients beginning on Day +28 and continuing until six months or CD4 count is >200 cells/mcL. Patients with sulfa allergies will receive aerosolized Pentamidine 300 mg per nebulizer within one week prior to admission and continued monthly for the same duration.

In addition to ANC specification above, patients will take ciprofloxacin (or preferred antibiotic) during intermediate dose IL-2 therapy.

5.2.2 Herpes Virus Prophylaxis

Within one day after TIL infusion, patients will be administered valacyclovir 500 mg po daily or acyclovir 800 mg twice daily, if patient is able to take oral medications, which is continued for one year for HSV prophylaxis. Acyclovir 5 mg/kg IVPB every 8 hours is given if patient needs intravenous medications. Reversible renal insufficiency has been reported with IV administered acyclovir but not with oral acyclovir. Neurologic toxicity including delirium, tremors, coma, acute psychiatric disturbances, and abnormal EEGs has been reported with higher doses of acyclovir. If symptoms occur, a dosage adjustment will be made or the drug be discontinued. Valacyclovir or acyclovir will not be used concomitantly with other nucleoside analogs (e.g. ganciclovir), which interfere with DNA synthesis. In patients with renal disease, the dose is adjusted as per product labeling.

5.2.3 Fungal Prophylaxis

Patients will begin fluconazole 200 mg po daily within one day after the TIL infusion (Day +1) and continue until the absolute neutrophil count is >1000/mcL or clinician discretion.

5.2.4 Empiric Antibiotics

If fever, patients will be admitted as inpatient status and start on broad-spectrum antibiotics, either a 3rd or 4th generation cephalsosporin or a fluoroquinolone for fevers ≥38.5 ºC with an ANC less than 500/mcL. Aminoglycosides should be avoided unless there is evidence of sepsis. Infectious disease consultation will be obtained for patients when deemed appropriate by the treating attending physician.

5.2.5 Blood Product Support

Using daily CBC's as a guide, the patient will also receive platelets and packed red blood cells (PRBC's) as needed. Attempts will be made to keep Hemoglobin >8.0 gm/dL, and platelets >20,000/mcL. Leukocyte reduction protocols or filters will be utilized for all blood and platelet transfusions to decrease sensitization to transfused WBC's and decrease the risk of CMV infection. Irradiated blood and blood products should be used.

5.3 Apheresis

Blood leukapheresis will take place at designated days as described in the study calendar. If required, apheresis catheters will be placed in interventional radiology on the designated day and removed after collection at the designated cell therapies apheresis suite by the designated nurse practitioner or physician. If site is incapable of performing an apheresis, then PBMC kit will be obtained instead.

5.4 TIL Harvest

Accessible tumors deemed safely accessible by a surgeon will be reviewed for eligibility. Tumor procedure must be expected to yield at least 1.5 cm^3 of tumor tissue. Tumor tissue will be collected by the cellular therapies core, selected, and expanded accordingly to their previously established TIL protocols.
Examples of potential sites for NSCLC TIL biopsy procedures include excisional supraclavicular or axillary lymph node biopsies, excisional biopsy of subcutaneous metastasis, cervical mediastinoscopy, or pleural biopsy via video-assisted thoracoscopic surgery (VATS).

5.5 Despite evidence of a metastasis on cross-section imaging, it is conceivable that initial TIL harvest obtained by excisional biopsy could yield no tumor tissue (negative biopsy). In this case, it is preferred that the TIL will be discarded and not used for manufacturing. Instead a second excisional biopsy of a different metastatic site may be arranged. This repeat biopsy is permitted to be obtained up until Day -35 of trial.

Definition of Dose-Limiting Toxicity (DLT)

Toxicity will be assessed using Common Terminology Criteria for Adverse Events version 4.0 (CTCAE v4). Dose-limiting toxicity will be assessed separately for nivolumab treatment and adoptive cell therapy (comprised of lymphodepletion, TIL transfer and intermediate dose IL-2).

DLT related to nivolumab will be defined as any grade ≥3 immune-related adverse event definitely attributable to nivolumab.

DLT related to adoptive cell therapy will be defined as:

- any grade 3 toxicity lasting more than 7 days, occurring upon or after the start of chemotherapy, that is not related to non-small cell lung cancer or other pre-existing condition, or
- any grade ≥ 4 toxicity, regardless of duration, occurring upon or after the start of chemotherapy, that is not related to non-small cell lung cancer or other pre-existing condition

With the following exceptions:

- Myelosuppression (includes bleeding in the setting of platelet count less than 50 $x10^9$/L and documented bacterial infections in the setting of neutropenia), defined as lymphopenia, neutropenia, decreased hemoglobin, and thrombocytopenia (since these are expected due to the chemotherapy preparative regimen)
- Immediate hypersensitivity reactions (excluding symptomatic bronchospasm and grade 4 hypotension) occurring within 2 hours of TIL infusion that are reversible to a ≤ grade 2 within 24 hours of TIL administration with standard therapy
- Grade 3 or 4 fever
- Tumor lysis syndrome (TLS) including associated manifestations attributable to TLS (eg, electrolyte abnormalities, renal function, hyperuricemia)
- Grade 3 transaminase, alkaline phosphatase, bilirubin or other liver function test elevation, provided there is resolution to ≤ grade 2 within 14 days
- Grade 4 transient serum hepatic enzyme abnormalities provided there is resolution to ≤ grade 3 within < 72 hours and ≤ grade 2 within 14 days
- Grade 3 cardiac adverse events (eg, arrhythmia, ventricular dysfunction) must resolve to grade 2 or better within 48 hours

5.6 Progressive disease (PD) procedures.

All patients with the occurrence of progressive disease after Day 0, including non-target lesions, must undergo a percutaneous core tumor biopsy. This biopsy should be performed within 14 days of the first occurrence of progression. An EUS, EBUS, FNA if an acceptable alternative only for patients whose lesion is not accessible to core biopsy. This tumor biopsy may be deferred for safety reasons only after documented discussion with the PI.

Before Day 0, patients with progressive disease who are changing treatment should also undergo a percutaneous core tumor biopsy. This tumor biopsy may be deferred for safety reasons only after documented discussion with the PI. Note that patients with symptomatic progressive disease prior to Day 0 are permitted to remain on study with expectation of receiving bridging therapy are not required to receive a PD biopsy at that timepoint, per Section 5.10 below.

5.7 Confirmation of progression (PD).

Patients with the occurrence progressive disease after Day 0 who are not experiencing symptoms of clinical deterioration may be permitted to continue on nivolumab treatment at the discretion of the investigator. A PD CRF and biopsy is still required in this instance. Tumor imaging should be repeated within a 4 to 6 week interval, and if additional increase in target lesions by >10% or unequivocal non-target progression is present, then the patient must discontinue treatment. If a diagnostic biopsy reveals only benign tissue in the radiographically progressing lesion(s), then the subject is also permitted to remain on nivolumab.

5.8 End of Treatment (EOT) procedures.

All patients who experience confirmed disease progression (PD) will enter into the EOT treatment phase. A +0 EOT should be completed at the time the patient is permanently discontinued from regular study follow-up. This +0 EOT is not applicable if a regular study visit, including PBMC blood draw, was performed within 3 days of the time that the subject was permanently discontinued from the study study. The +0 EOT can be combined with the PD procedure visit if clinically practical. After EOT +90, patients may occasionally be contacted by telephone for survival follow-up for up to five years.

The EOT visits do not apply to patients who have permanently stopped nivolumab for toxicity or drug intolerance. These patients must continue to receive scheduled imaging visits until confirmed disease progression is observed per the study calendar and footnotes.

5.9 Criteria for treatment with TIL after initial response to nivolumab.

Patients who experience initial response (PR/CR) with nivolumab and then have the occurrence of disease progression may be eligible to subsequently receive the TIL product. This eligibility is contingent upon an attestation from Cell Therapies Core that sufficient frozen pre-REP cell product remains available for the TIL manufacture, and that the patient meets the REP eligibility checklist as outlined. The investigator will also need to provide an attestation that the patient continues to meet eligibility requirements as outlined in 4.1, with the exception of items: 4.1.1 #4, 5, 9, 10, 11, 12, 13; and 4.1.2 #1, 2, 21. These patients will follow study procedures beginning on Day -21.

Patients who have achieved a response to initial nivolumab (PR/CR) may voluntarily elect to forego their subsequent trial-related visits and procedures at the study site if it imposes undue hardship. The rationale for this is that regular indefinite q28d nivolumab visits may result in cumulative burden for patients traveling from long distances, such as >300 miles. These patients are permitted to be seen and treated at the discretion of their local oncology team. If disease recurrence is suspected, then the patient will be set up with Day -21 clinic visit, and then complete pre-REP eligibility checklist and procedures per Section, in preparation for subsequent Cy/Flu/TIL.

5.10 Bridging therapy and criteria for TIL treatment

Patients who receive at least one dose of nivolumab, but experience clinical decline in ECOG PS attributable to cancer, as judged by the investigator, are eligible to stop nivolumab and instead receive bridging therapy with standard-of-care (SOC) systemic therapy. If the patients have improvement in ECOG PS and then have evidence of progression (PD) on subsequent CT scans, then these patients are then eligible to receive subsequent TIL product. This eligibility is contingent upon an attestation from Cell Therapies Core that sufficient frozen pre-REP cell product is available for a TIL manufacture, and that the patient meets the REP eligibility checklist as outlined. The investigator will also need to provide an attestation that the patient continues to meet eligibility requirements as outlined in 4.1, with the exception of items: 4.1.1 #4, 5, 9, 10, 11, 12, 13; and 4.1.2 #1, 2, 21. These bridging therapy patients will then follow study procedures beginning on Day -21.

1. Current or prior use of anticancer therapy before TIL collection:
    a. Chemotherapy within the past 4 weeks;
    b. Tyrosine kinase inhibitor (TKI) within the past 1 week;
    c. Investigational therapy within the past 4 weeks or 4 half-lives, whichever is shorter.

Patients who are started upon bridging therapy will follow the following study calendar:

Study Calendar during Bridging *,** ALL +/- 3 DAYS ***

Day	Bridging Therapy	-21	-8	Window ±7 (8, 17)	-7 (18)	Procedures after Day -7 follow main calendar. Pg 11
Medical history	-	●	●		●	
Current medications	–	●	●		●	
Physical Exam	-	●	●		●	
Vital Signs including SpO2 (1)	-	●	●		●	
Performance Status	-	●	●		●	
REP Eligibility Checklist		●				
Chemotherapy Checklist			●			
EKG	–	●	●			
Blood Tests						
CBC, CMP	-	●	●		●	
CEA	–	●	●		●	
PT/PTT	–		●			
Thyroid Tests (T4, TSH) (5)	–	●			●	
Blood Pregnancy Test (6)	--	●	●			
Peripheral Blood Draw for Correlatives (7)			●			
CT Chest-Abdomen-Pelvis (8)(9)	–	●				
Brain imaging (9)	–	●				
Chest X-Ray PA/lateral			●			
Adverse Event Assessment (11)	--	●	●		●	
Urinalysis			●			
Lymphodepletion:					●	
Cyclophosphamide, Fludarabine					●	

5.11 Failure to manufacture TIL

In some cases, it is possible that insufficient tumor is harvested for TIL, or insufficient TIL is able to be grown to manufacture the final TIL product. In these instances, the patient may continue to receive nivolumab at the discretion of their treating physician. However, if progression becomes evident while receiving nivolumab, the patient will discontinue trial treatment and receive appropriate therapy at the discretion of their treating physician.

Patients with initial response to nivolumab who subsequently progress on TIL are described in Section 5.9.

6.0 Evaluation during Study

During the preparative cyclophosphamide-fludarabine regimen therapy, patients will have a complete blood count (CBC) and comprehensive metabolic profile every 1 to 2 days of treatment. On the day of TIL infusion, patients will be monitored with vital signs at baseline and after cell infusion as described in section 5.1. Venipuncture will be performed at screening and on days as listed in the study calendar. In addition, apheresis will be performed on day 28 (+/- 7 days). These samples will be used to evaluate T cell persistence by comparing TCR clonotyping to the products of TIL infusion. Serum may be used to determine anti-tumor antibody formation using autologous tumor targets from excess, unused tumor obtained at the time of tumor harvest for TIL.

Clinical evaluation for tumor response with physical examinations and appropriate CT scans (routine chest, abdomen and pelvis, as well as any additional areas deemed appropriate by the study physician) and brain MRI will be performed pre nivolumab treatment, prior to lymphodepletion and 6 weeks following the adoptive TIL transfer. Then patients will have restaging CT scans with follow-up visits as

outlined in the study calendar. For patients with CT IV contrast allergy, appropriate premedication, use of MRI or CT without IV contrast will be undertaken at the discretion and judgment of the PI. Note CT of the head without and with IV contrast (if not allergic) will be substituted for brain MRI in cases of existing MRI contraindication. These follow-up assessments will be performed for all patients on the study until disease progression, withdrawal of consent, loss to follow-up or death.

Descriptive correlative laboratory studies:

Recovered circulating T cells will be assessed for TIL persistence by Vβ clonotyping T cell receptor. The TCR of the cells from PBMC collections will also be derived by sequencing. Persistence of TIL will be determined by comparing the clonotyping results of the TIL to the post-infusion blood samples. Persistence will be correlated to clinical outcome.

The generation of specific effector T cell activity in TIL capable of recognizing HLA-matched tumor cells will be assessed by the use of a TIL-target co-culture assay for 24 hours followed by an ELISA for gamma interferon as has been described previously (1). Successfully generated TIL from each discrete tumor fragment will be tested. TIL will be co-incubated with HLA-matched or autologous tumor targets at a ~2:1 ratio for a 24-hour period, and supernatants will be removed. Aliquots will be used for a gamma interferon ELISA assay, as well as multiplex cytokine bead array assay. Controls will include no effector and no target samples, as well as an HLA-mismatched target control. Assays will be performed in triplicate. Observation of more than 200 picograms per mL of gamma interferon and/or a statistically significant increase in pro-inflammatory cytokine release over the controls is desired for an assay to be declared positive at a given time point.

At the time of tumor harvest for TIL, a core or core-equivalent biopsy after tumor extirpation will be obtained. Immunohistochemistry will be performed to assay CD3, CD4, CD8 as well as T cell immune checkpoint expression. In addition, TCR sequencing will be performed on the TIL product and applicable PBMC samples, and whole-exome sequencing will be performed on the biopsy of harvested tumor. Due to the small sample size of this study, results will be reported descriptively. If sufficient material is available, peptide sequencing of the tumor will be performed by the Moffitt Proteomic Core. If sufficient material is available, single-cell RNA sequencing and/or bulk RNA sequencing of the tumor harvest sample or progressive biopsy will be performed. Tumor antigens will be predicted using existing MCC bioinformatics pipeline. Predicted peptides may be synthesized and tested for autologous reactivity with either cultured T cells or fresh T cells.

Collaborative laboratory studies

- Multi-color serial flow cytometry of PBMCs and TIL will be performed, including CD3, CD8, CD4, CD25, and T cell immune checkpoint markers to assess change in phenotype before/after TIL. For available research tumor samples, MultiOmyx, a multi-parameter immunohistochemistry, will be performed to assess for T cell subsets and activation status. This includes measurement of expression levels and spatial distribution and will utilize a finalized material transfer agreement. One to two unstained 5 um de-identified FFPE slides from each tumor will be sent externally to the vendor for this. Unused slides will be returned.

- In addition, some functional testing of T cells using de-identified samples may be performed at Fred Hutchison Cancer Center with the Riddell Lab, using a finalized material transfer agreement. Correlative analysis of circulating factors, such as Bioplex 27 platform, will be performed on serial serum samples to assess changes in cytokine production before/after TIL.

Single-cell cytokine profiling will be performing on CD8+ cells using Isolight ® to assess for change in polyfunctional T cells before/after TIL.

- Some de-identified PBMC samples may also be released to Pilon-Thomas laboratory for testing of MDSC phenotype.

- Additional *in vitro* testing will be performed to validate predicted antigens on correlative samples. Depending on HLA status, NFAT-GFP engineered Jurkat cells (NFAT-GFP-Jurkat) will be transfected with DNA encoding predicted epitopes together with the relevant HLA allele and the intracellular CD3/CD28 signaling domain. When recognized and bound with the epitope-specific TCR on T cells, HLA-epitope complex on NFAT-GFP-Jurkat cells are expected to transmit activation signals through CD3/CD28 intracellular domain to NFAT, which in turn causes the expression of GFP. Using this technique, the GFP+Jurkat cells will be sorted for sequencing of the epitope portion and antigen epitope sequence derived. These experiments will be performed through an academic collaboration which Dr Creelan has with Dr Xi Chen, PhD at Root Path Genomics in Cambridge MA. For an appropriate sample, Moffitt would provide 1 TIL sample, 1 vial of PBMCs, and information on the identified neoantigen to his laboratory. Moffitt will receive all the results from the experiments derived from these de-identified samples. Drs. Wang and Dr. Creelan, of Moffitt will supervise these projects and retain primary roles in the intellectual direction, interpretation and reporting, including writing academic papers or presentations.

- Dr Creelan is working with Dr Klebanoff to specifically explore KRAS mutations as an effective neoantigen. Moffitt may transfer one (1) vial of pre-TIL PBMC, one (1) vial of post-TIL PBMC, one (1) vial of random TIL fragment culture, and one (1) vial of post-REP TIL to Dr. Chris Klebanoff's laboratory at Memorial Sloan Kettering Cancer center. The team’s hypothesis is that several TCRs which Dr Klebanoff has already experience and identified in others patients a set of TCR alpha beta chains which may interact with KRAS. The analysis plan is to perform functional validation using the peptides and pMHC tetramer/pentamers that he has synthesized on the cells. He will incubate the peptide or pMHC and test for activation marker display by flow cytometry. He may sort cells based on memory or activation markers to determine if the specific TCR of interest is present before or after peptide stimulation in TIL and PBMCs. He may also perform paired single-cell VDJ seq on the cultured TIL, PBMCs, or uncultured tumor suspension immune cells. Moffitt will receive all the results from the experiments derived from these de-identified samples. This includes raw data and downstream interpretation files such as UMAP plots, tsne plots, volcano plots. Drs. Wang and Dr. Creelan, of Moffitt, expect to be maintained in an academic role and retain active collaborative roles in the intellectual direction, interpretation and reporting of the data, including citation as authors in academic papers or presentations.

- Dr Creelan works closely with Dr Antonia for this trial. Moffitt may transfer TIL samples to Dr Scott Antonia at Duke Cancer Institute for ATAC seq single-cell analysis to determine potential causes of resistance to adoptive cell transfer. This research is exploratory, and intended to identify signatures related to exhaustion of TIL or what phenotype may constitute an effective TIL product. Drs. Wang and Dr. Creelan, of Moffitt, expect to be maintained in an academic role for any projects involving this patient's data. They retain active collaborative roles in the

intellectual direction, interpretation and reporting of the data, including citation as authors in academic papers or presentations.

- Moffitt may transfer raw RNA-Seq or exome .bam or .vcf files through a collaboration with Dr Rabadan in bioinformatics at Columbia University. Dr Rabadan is the Gerald and Janet Carrus Professor in Systems Biology, Biomedical Informatics and Surgery at Columbia University. Dr Rabadan's group is experienced in detecting tumor allelic HLA loss of hetereozygosity and this is a hypothesized exploratory resistance mechanism for TIL. Dr Creelan is working with Dr Rabadan, to explore HLA mutation, down-regulation, or LOH as a mechanism of resistance for TIL treatment. Moffitt will receive all the results from the experiments derived from these de-identified samples. This includes downstream interpretation files such as plots, tsne plots, volcano plots. Drs. Wang and Dr. Creelan, of Moffitt, expect to be maintained in an academic role for any projects derived from this. They retain active collaborative roles in the intellectual direction, interpretation and reporting of the data, including citation as authors in academic papers or presentations.

7.0 Evaluation of Toxicity

7.1 Toxicity Monitoring

An ***Adverse Event (AE)*** is defined as any new untoward medical occurrence or worsening of a pre-existing medical condition in a patient or clinical investigation subject administered an investigational (medicinal) product and that does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign, symptom, or disease temporally associated with the use of investigational product, whether or not considered related to the investigational product.

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

This study will utilize the National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 for toxicity and Adverse Event reporting. A copy of the CTCAE version 4.0 can be found online at the NCI website. Careful evaluation to ascertain the toxicity and immunologic effects and anti-tumor efficacy of nivolumab and the cell infusion will be performed. Toxicities will be monitored and documented beginning at ~day -63 (nivolumab infusion) and continuing at regular intervals as outlined in the study calendar. Patients with treatment-limiting toxicity will continue to be followed as outlined in the study calendar, footnote 19. Dose limiting toxicity is defined above.

The sponsor, Moffitt Cancer Center, with the cooperation of the principal investigator, will monitor the data and toxicities to identify trends. The principal investigator will be responsible for revising the protocol as needed to maintain safety. The sponsor's contact for transmission of material to the FDA at Moffitt is:

Carrie Thomas
Moffitt Cancer Center
Ph 813 745 3467
FAX 813 745 3933

The principal investigator will also review serious adverse events and evaluate trends. Whenever a trend is identified, the sponsor, in cooperation with the principal investigator will determine an appropriate follow-up plan.

The use of nivolumab as well as the non-myeloablative chemotherapy regimen in this protocol entail serious potential discomforts and hazards for the patient. Although it is anticipated that this protocol is relatively safe because of the expected recovery of the patients' bone marrow within 2 to 4 weeks, fatal complications are possible. It is therefore only appropriate to carry out this experimental procedure in the context of life-threatening advanced cancer. The major hazards are infection, immune-related adverse events and disease progression. The major discomforts are nausea, mucositis, anorexia, diarrhea, fever and malaise.

Select side effects of common drugs used in this non-myeloablative regimen include, but are not limited to:

Cyclophosphamide: Marrow suppression, nausea, mucositis, rash, hemorrhagic cystitis, myocardial damage, alopecia, infertility, nausea and vomiting, and syndrome of inappropriate antidiuretic hormone (SIADH).

Fludarabine: Myelosuppression, fever and chills, nausea and vomiting, malaise, fatigue, anorexia, weakness, neurologic toxicity, and interstitial pneumonitis. Serious opportunistic infections have occurred in chronic lymphocytic leukemia (CLL) patients treated with fludarabine.

Antimicrobials in general: Allergic reactions, renal impairment, nausea, vomiting, hepatic damage, marrow suppression.

Intermediate-dose IL-2: A variety of side effects have been associated with intermediate-dose IL-2. Intermediate dose IL-2 is thought to have reduced toxicity profile as compared to high dose IL-2.

Adoptive cell therapy with TIL: a variety of side effects that potentially overlap with IL2 have been associated with adoptive cell therapy in our experience and at the NCI. Possible long-term side effects include: vitiligo, high frequency hearing loss and uveitis.

7.2 Immune-Related Adverse Events and Other Possible Toxicities Attributable to Nivolumab

Definition of immune-related adverse events (irAEs): Blocking PD-1 function may permit the emergence of autoreactive T cells and result in autoimmunity. Nivolumab can result in severe and even fatal immune-mediated reactions due to T-cell activation and proliferation.

For this proposal, an irAE is defined as a clinically significant (i.e requiring intervention) adverse event of any organ that is probably associated with nivolumab drug exposure, and is clinically consistent with an immune-mediated phenomenon. Serological, immunological, histological (biopsy) data where available as well as patient history and physical examination findings should be used to support an irAE diagnosis. Additionally the event should be of significant duration (e.g. ≥ 7 days) and/or grade to make it unlikely to be caused by non-autoimmune factors. Appropriate efforts should be made to rule out neoplastic, infectious, metabolic, toxin or other etiologic causes of the adverse event when clinically relevant. Patients will be carefully monitored for evidence of clinically significant systemic irAEs (e.g., symptoms associated with systemic lupus erythematosus-like diseases) or organ-specific irAEs (e.g., rash, colitis, uveitis, hepatitis or thyroid disease, see below).

Immune-related Pulmonary Toxicity

Nivolumab has been reported to cause an immune-mediated pneumonitis. Clinical manifestations include acute shortness of breath, chest pain, and increased work of breathing/supplemental oxygen requirement. Patients with a previous history of pulmonary disease that require supplemental oxygen or more than weekly inhaler therapy will be required to undergo pulmonary function tests at screening. If patients on this trial develop acute shortness of breath, a prompt clinical workup will ensue that may include CT scan of the chest if clinically warranted. If there is no clinical evidence of non-immune-mediated pulmonary disease (i.e. pneumonia, pulmonary embolus, or myocardial ischemia), patients will be considered for prompt corticosteroid treatment.

Immune-related Gastrointestinal Events

The clinical presentation of GI immune-related AEs included diarrhea, increase in the frequency of bowel movements, abdominal pain, or hematochezia. Patients will be carefully monitored for GI symptoms that may be indicative of immune-related colitis, diarrhea, or GI perforation. Diarrhea or colitis occurring after initiation of nivolumab therapy should be evaluated to exclude infectious and alternative etiologies. In clinical trials of other similar immunotherapies, immune-related colitis was associated with evidence of mucosal inflammation, with or without ulcerations, and lymphocytic infiltration.

Immune-related Hepatotoxicity

In previous trials of nivolumab, hepatic immune-related AEs were mostly clinically silent and manifested as transaminase or bilirubin laboratory abnormalities. Serum transaminase and bilirubin levels will be evaluated before dosing of nivolumab as early laboratory changes may be indicative of emerging immune-related hepatitis. Elevations in liver function tests (LFTs) may develop in the absence of clinical symptoms. Increase in LFTs or total bilirubin will be evaluated to exclude other causes of hepatic injury, including infections, disease progression, or medications, and laboratory abnormalities will be monitored until resolution. In trials of other similar immunotherapy, liver biopsies from patients who had immune-related hepatotoxicity showed evidence of acute inflammation (neutrophils, lymphocytes, and macrophages).

Immune-related Skin Toxicity

Skin immune-related AEs may present as a rash and/or pruritus. Some subjects reported vitiligo associated with nivolumab administration in previous trials. Pruritus can often be managed with oral antihistamines.

Immune-related Endocrinopathy

Nivolumab may cause inflammation of the endocrine system organs, specifically hypophysitis, hypopituitarism, and adrenal insufficiency. Patients may present with nonspecific symptoms, which may resemble other causes such as brain metastasis or underlying disease. The most common clinical presentation of immune-mediated endocrinopathy includes headache and fatigue. Symptoms may also include visual field defects, behavioral changes, electrolyte disturbances, and hypotension. Adrenal crisis as a cause of the patient's symptoms should be excluded. Based on the available data with known outcome, most of the subjects symptomatically improved with hormone replacement therapy. It is possible that longterm hormone replacement therapy will be required for subjects developing hypophysitis/hypopituitarism after treatment with nivolumab.

Immune-related Neurological Events

Neurological manifestations from nivolumab may include muscle weakness and sensory neuropathy. Fatal Guillan-Barré syndrome has been reported in clinical trials of immunotherapy agents similar to nivolumab. Patients may present with muscle weakness. Sensory neuropathy may also occur. Unexplained motor neuropathy, muscle weakness, or sensory neuropathy lasting more than 4 days will

be evaluated and non-inflammatory causes such as disease progression, infections, metabolic syndromes, and medications should be excluded.

Other Immune-related AEs

Other possible immune-related adverse events associated with nivolumab include ocular inflammation, manifesting as episcleritis or uveitis. Other presumed immune-related AEs reported include, but are not limited to, arthritis/arthralgias, pneumonitis, pancreatitis, autoimmune (aseptic) meningitis, autoimmune nephritis, pure red cell aplasia, noninfective myocarditis, polymyositis, and myasthenia gravis, of which have been individually reported for < 1% of subjects.

It is expected that some subjects may develop infusion-related reactions secondary to nivolumab, including diaphoresis, nausea, headache, or hypotension. Low-grade constitutional symptoms such as fever and fatigue, as well as headache, allergic rhinitis, nasal stuffiness, arthralgia, myalgia, and cough have occurred in prior clinical trials. Other low-grade adverse events include flushing, leukopenia, nausea, vomiting, thrombocytopenia, abdominal pain or cramping, anemia, pruritus, rash/ desquamation, vitiligo, and diarrhea.

7.3 Management of Toxicity Due to Nivolumab and/or TIL therapy

If an irAE is noted, appropriate workup (including biopsy if possible) should be considered, and steroid therapy may be considered if clinically necessary. It is unknown if systemic corticosteroid therapy will have an attenuating effect on nivolumab activity, and may attenuate the effects of IL-2 and TIL. However, clinical antitumor responses have been maintained in subjects with autoimmunity treated with corticosteroids and by discontinuing the immunotherapy, including nivolumab as monotherapy. In our recent experience with adoptive cell therapy, one patient required systemic corticosteroids for uveitis and still maintained a near complete response 12 months after being weaned from systemic steroids. If utilized, corticosteroid therapy should be individualized for each subject. Recommended dosing is 1 mg/kg of oral prednisone or intravenous methylprednisolone (as clinically warranted) followed by a taper over 4-6 weeks. Prior experience suggests that colitis manifested as a Grade 3 diarrhea requires systemic corticosteroid treatment.

7.4 Criteria that Require Permanent Discontinuation of Nivolumab and other study protocol treatment that are not related to DLT

7.4.1 Dose Delay Criteria for 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. Please see current Investigator Brochure for Nivolumab for citation examples.

Dose delay criteria for nivolumab apply for all drug-related adverse events (regardless of whether or not the event is attributed to nivolumab).

Subjects may resume treatment with nivolumab when the drug-related AE(s) resolve to Grade ≤ 1 or baseline value, with the following exceptions:

- Drug-related grade 2 fatigue, vitiligo, or arthropathy
- Subjects who have not experienced a Grade 3 drug-related skin AE may resume treatment in the presence of Grade 2 skin adverse event.

- 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
- Drug-related combined Grade 2 AST/ALT AND total bilirubin values meeting discontinuation parameters should have treatment permanently discontinued
- Drug-related endocrinopathies adequately controlled with physiologic hormone replacement may resume treatment
- Drug-related asymptomatic amylase or lipase elevations without clinical evidence of pancreatitis may continue drug treatment.
- Other conditions which, after discussion with PI, are not expected to recur, recrudesce, or worsen in the presence of additional nivolumab.

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 > 10 weeks, the subject must be permanently discontinued from study therapy, except as specified below.

7.4.2 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:

- Drug-related grade 2 fatigue, vitiligo, or arthropathy
- Subjects who have not experienced a Grade 3 drug-related skin AE may resume treatment in the presence of Grade 2 skin adverse event.
- 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
- Drug-related combined Grade 2 AST/ALT AND total bilirubin values meeting discontinuation parameters should have treatment permanently discontinued
- Drug-related endocrinopathies adequately controlled with physiologic hormone replacement may resume treatment
- Drug-related asymptomatic amylase or lipase elevations without clinical evidence of pancreatitis may continue drug treatment.
- Other conditions which, after discussion with PI, are not expected to recur, recrudesce, or worsen in the presence of additional nivolumab.

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 > 10 weeks, the subject must be permanently discontinued from study therapy, except as specified in discontinuation section.

7.5 Criteria that Require Permanent Discontinuation of Nivolumab and other study protocol treatment that are not related to DLT

Subjects MUST discontinue study protocol treatment for any of the following reasons that are not related to an immune-related adverse event/DLT:
• Withdrawal of informed consent (subject's decision to withdraw for any reason)
• Pregnancy (see Section 7.13.11)
• Loss of ability to freely provide consent through imprisonment or involuntary incarceration for treatment of either a psychiatric or physical (eg, infectious disease) illness.
• The development of unequivocal progressive disease accompanied by severe clinical deterioration of subject's condition such that a change of therapy to a different active therapy is immediately required. Investigators are permitted to continue treatment if pseudo-progression is suspected.

7.6 Nivolumab-related non-neurological adverse events that require permanent discontinuation and result in a nivolumab-related DLT

• Any Grade 3 or higher bronchospasm or other Grade 3 or higher hypersensitivity reaction will result in nivolumab-related DLT .
• Any other Grade 3 non-skin related adverse event will result in nivolumab-related DLT with the exception of laboratory abnormalities or events listed under "Exceptions to Permanent Discontinuation" (section 7.8).
• Any Grade 4 laboratory abnormalities, except abnormalities of amylase, lipase, AST, ALT, or Total Bilirubin will have different parameters that result in nivolumab-related DLT (see immediately below).
• AST or ALT > 8 x IULN will result in nivolumab-related DLT.
• Total Bilirubin > 5 x IULN will result in nivolumab-related DLT.
• Concurrent ALT > 4 X IULN **AND** total bilirubin > 2 X IULN will result in nivolumab-related DLT.
• Grade 4 elevation in amylase and lipase will **not** result in nivolumab-related DLT if the abnormality resolves to grade 1 or less within 2 weeks and if the subject is off steroids >10 mg of prednisone daily .
• Any other Grade 4 adverse event (including skin-related adverse events) will result in nivoluamab-related DLT.
• Any adverse event, laboratory abnormality or intercurrent illness that, in the judgment of the investigator, presents a substantial clinical risk to the subject with continued nivolumab dosing will result in nivolumab-related DLT.

7.7 Nivolumab-related neurological adverse events that require permanent discontinuation of the trial protocol and result in a nivolumab-related DLT

• Any Grade 2 eye pain or reduction of visual acuity that does not respond to topical therapy and does not improve to Grade 1 severity within 2 weeks of starting therapy or requires systemic treatment
• Any motor neurologic toxicity >/= Grade 3 regardless of causality

• Any>/= Grade 3 treatment-related sensory neurologic toxicity. Please refer to the Investigator Brochure for nivolumab for specific treatment algorithms.

7.8 Exceptions to Permanent Discontinuation of Nivolumab due to Toxicity:

• Potentially reversible inflammation (< Grade 4), attributable to a local antitumor reaction and a potential therapeutic response. This includes inflammatory reactions at sites of tumor resections or in draining lymph nodes, or at sites suspicious for, but not diagnostic of metastasis
• Hematologic and non-hematologic toxicities not felt due to an irAE and managed as above
• Hospitalization for Grade 2 adverse events where the primary reason for hospitalization is to expedite the clinical work-up
• Subjects with the following conditions where in the investigator's opinion continuing study drug administration is justified:

Ocular toxicity that has responded to topical therapy
Endocrinopathies where clinical symptoms are controlled with appropriate hormone replacement therapy.

Note: Nivolumab may not be restarted while the subject is being treated with systemic corticosteroids except for subjects on stable doses of physiologic/homeostatic hormone replacement therapy such as prednisone 10 mg daily or equivalent steroid dosing. All subjects who discontinue treatment prematurely due to a protocol treatment-related AE or symptomatic progression prior to disease assessment 12 weeks following adoptive TIL transfer will be asked to return for the applicable PD visit and series of EOT visits at 0, 30 and 90 days.

7.9 Management of gastrointestinal irAEs (diarrhea and colitis)

Patients with grade 1 diarrhea without blood may be managed symptomatically with loperamide, one tablet after each loose bowel movement up to 7 tablets in 24 hours or diphenoxylate/atropine four times a day until diarrhea subsides. For those with grade 2 diarrhea, or grade 1 diarrhea with blood, Budesonide may be added to the regimen for 14 days. If grade 3 diarrhea occurs, patients will be started on budesonide and tapering doses of prednisone over at least 30 days at the discretion of the treating physician. If there is bloody grade 3 diarrhea, intravenous methylprednisolone will be administered. For grade 4 diarrhea or any clinical concern for hemodynamic instability, patients will be hospitalized and given intravenous methylprednisolone, made NPO, and hydrated intravenously as clinically indicated. For all patients with grade 2 diarrhea or more, flexible sigmoidoscopy or colonoscopy will be performed with at least three random biopsies if normal appearing, and three or more directed biopsies if abnormal.

7.10 Non-DLT nivolumab-related Grade 3 IRAEs

For the purposes of this trial, non-DLT nivolumab drug-related irAEs are skin-related immune-mediated adverse events of Grade 3 or less and potentially reversible inflammation <Grade 4 that can be attributable to a local antitumor reaction that could potentially be a therapeutic response as judged by treating Moffitt physician will be considered an non-dose limiting irAE related to nivolumab. Grade IV elevations in amylase and/or lipase that resolve to grade 1 within 2 weeks, and if the subject is off steroids >10 mg of prednisone daily within 2 weeks will not result in nivolumab-related DLT.

7.11 Infusion Reactions Related to Nivolumab

Since nivolumab contains only human protein sequences, it is unlikely that any allergic reaction will be seen in subjects. However, it is possible that the infusion will induce a cytokine release syndrome that could be evidenced by fever, chills, rigors, rash, pruritus, hypo- or hypertension, bronchospasm, or other symptoms. No prophylactic premedication will be given unless indicated by previous experience in an individual subject (detailed below). Infusion reactions should be treated based upon the following recommendations:

For mild symptoms: (Localized cutaneous reactions such as mild pruritus, flushing, rash) the rate of infusion will be decreased until recovery from symptoms, then the infusion may be completed at the initial planned rate. Diphenhydramine 50 mg (or equivalent medication) and/or acetaminophen 325-1000 mg, may be administered at least 30 minutes before administration of nivolumab, at the discretion of the health care provider.

For moderate symptoms: For any symptom not listed above (mild symptoms) or below (severe symptoms) such as generalized pruritus, flushing, rash, dyspnea, hypotension with systolic BP >80 mm Hg, the infusion will be interrupted, the patient will receive diphenhydramine 50 mg IV (or equivalent medication) and/or acetaminophen 325-1000 mg, and the subject will be monitored until resolution of symptoms. Corticosteroid treatment may be authorized at the discretion of the treating physician. The infusion may be resumed after recovery of symptoms at the discretion of the treating physician. The infusion may be resumed at one-half the initial infusion rate, then increased incrementally to the initial infusion rate if no further complications ensue after 30 minutes. If symptoms develop after resumption of the infusion, the infusion should be discontinued and no additional treatment should be administered within 7 days. If clinical recovery after 7 days is complete, an additional attempt at treatment may be undertaken with monitoring, following the same treatment guidelines outline above. At the discretion of the treating physician, additional oral or IV diphenhydramine 50 mg (or equivalent medication) and/or acetaminophen 325-1000 mg ay be administered.

For severe symptoms: For any reaction such as bronchospasm, generalized urticaria, systolic blood pressure <80 mm Hg, mental status change, or angioedema, nivolumab infusion will be immediately discontinued. The treating physician will consider bronchodilators, epinephrine 1 mg IV or IM and/or diphenhydramine 50 mg IV with solumedrol as needed. Subjects will be monitored until complete resolution of symptoms or will be admitted to the hospital if the symptoms have not resolved after 6 hours or upon closure of the outpatient facility. Nivolumab will be permanently discontinued for severe symptoms.

In the case of late-occurring hypersensitivity symptoms (e.g. appearance within one week after treatment of a localized or generalized pruritus), symptomatic treatment may be given (e.g., oral antihistamine or corticosteroids).

7.12 Adverse Event Reporting to the FDA

All adverse events will be reported to the FDA in the annual report according to this table:

Attribution	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5
Unrelated			X	X	X
Unlikely			X	X	X
Possible	X	X	X	X	X

Probable	X	X	X	X	X
Definitive	X	X	X	X	X

Exceptions to this rule will include events and laboratory abnormalities that represent common symptoms and abnormalities of non-small cell lung cancer and chemotherapy and/or have no clinical significance:

- Abnormalities in hematologic parameters due to myelosuppressive therapeutic effect:
  i. Anemia, neutropenia, lymphopenia, thrombocytopenia
  ii. Epistaxis or bleeding except for catastrophic CNS or pulmonary hemorrhage
- Common symptoms of cancer (unless grade >/= 3) including:
  i. Fatigue
  ii. Weakness
  iii. Bone, joint or muscle pain
  iv. Alopecia
  v. Loss of appetite, nausea, vomiting
  vi. Chemistry abnormalities (phosphorus, calcium, glucose)
  vii. Coagulation abnormalities (shortened PT, PTT, increased fibrinogen)
- Laboratory abnormalities:
  i. CEA (increased or decreased)
  ii. Alkaline phosphatase (increased or decreased)
  iii. Low levels of the following: AST, ALT, creatinine, BUN, uric acid, bilirubin, albumin, total protein
  iv. Electrolyte abnormalities (sodium, potassium, bicarbonate, CO_2, magnesium
- General therapy related events:
  i. Catheter related events
  ii. Rash related to antibiotic use

7.13 ADVERSE EVENT REPORTING

7.13.1 Serious Adverse Events

A ***Serious AE (SAE)*** is any untoward medical occurrence that at any dose:

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

- Suspected transmission of an infectious agent (eg, any organism, virus or infectious particle, pathogenic or non-pathogenic) via the study drug is an SAE.

Although pregnancy, overdose, cancer are not always serious by regulatory definition, these events must be handled as SAEs.

NOTE: The following hospitalizations are not considered SAEs:

- a visit to the emergency room or other hospital department lasting less than 24 hours that does not result in admission (unless considered an “important medical event” or a life-threatening event)
- elective surgery planned before signing consent
- admissions as per protocol for a planned medical/surgical procedure
- routine health assessment requiring admission for baseline/trending of health status (eg, routine colonoscopy)
- medical/surgical admission for purpose other than remedying ill health state that was planned before study entry. Appropriate documentation is required in these cases.
- Admission encountered for another life circumstance that carries no bearing on health status and requires no medical/surgical intervention (eg, lack of housing, economic inadequacy, caregiver respite, family circumstances, administrative).

7.13.2 Collection and Reporting

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

The following categories and definitions of causal relationship to investigational product as determined by a physician should be used:

- **Related:** There is a reasonable causal relationship to investigational product administration and the adverse event.
- **Not Related:** There is not a reasonable causal relationship to investigational product administration and the adverse event.

The expression “reasonable causal relationship” is meant to convey in general that there are facts (eg, evidence such as dechallenge/rechallenge) or other arguments to suggest a positive causal relationship.

If known, the diagnosis of the underlying illness or disorder should be recorded, rather than its individual symptoms. The following information should be captured for all AEs: onset, duration, intensity, seriousness, relationship to investigational product, action taken, and treatment required. If treatment for the AE was administered, it should be recorded in the medical record.

The investigator shall supply the sponsor and Ethics Committee with any additional requested information, notably for reported deaths of subjects.

7.13.3 Serious Adverse Events

Following the subject’s written consent to participate in the study, all SAEs must be collected, including those thought to be associated with protocol-specified procedures. All SAEs must be collected that occur within 30 days of discontinuation of dosing of the investigational product. This 30-day period also

applies to subjects receiving bridging therapy as outlined in Section 5.10. Subsequent adverse events are not collected for patients receiving standard-of-care bridging therapy, until resumption of study procedures at Day 21 as specified in the calendar in Section 5.10. If applicable, SAEs must be collected that relate to any later protocol-specified procedure (eg, a follow-up skin biopsy). The investigator should notify the Sponsor of any SAE occurring after this time period that is believed to be related to the investigational product or protocol-specified procedure.

All SAEs whether related or unrelated to the trial treatment, must be immediately reported to the Sponsor (by the investigator or designee) within 24 hours of becoming aware of the event. If only limited information is initially available, follow-up reports are required. The original SAE form must be kept on file at the study site. Report SAEs by completing an SAE report in OnCore, the electronic data capture system. The SAE must be reported by email (affiliate.research@moffitt.org) to the External Site Coordination (ESC) office within 2 working days. If applicable, the site should also follow protocol guidelines for additional reporting to government agencies.

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

Serious adverse events that do not require expedited reporting to the FDA still need to be reported to the Sponsor preferably using the MedDRA coding language for serious adverse events. This information should be reported on a monthly basis and under no circumstance less frequently than quarterly.

For studies conducted under an Investigator IND, any drug-related event that is both serious and unexpected must be reported to the FDA as soon as possible and, in no event, later than 7 days (death or life-threatening event) or 15 days (all other SAEs) after the investigator's or institution's initial receipt of the information. SAEs should be reported on the MedWatch Form 3500A, which can be accessed at: http://www.accessdata.fda.gov/scripts/medwatch/.

MedWatch SAE forms should be sent to the FDA at:

MEDWATCH
5600 Fishers Lane
Rockville, MD 20852-9787
Fax: 1-800-FDA-0178 (1-800-332-0178)
http://www.accessdata.fda.gov/scripts/medwatch/

Any adverse event for which there is a reasonable possibility that the drug caused the adverse event, must be recorded on the SAE page and reported expeditiously to comply with regulatory requirements. An SAE report should be completed for any event where doubt exists regarding its status of seriousness.

All drug-related SAEs must be immediately reported by confirmed facsimile transmission (fax) and mailing of the completed SAE page. In some instances where a facsimile machine is not available, overnight express mail may be used. 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.) In selected circumstances, the protocol may specify conditions that require additional telephone reporting.

If the investigator believes that an SAE is not related to the investigational product, but is potentially related to the conditions of the study (such as withdrawal of previous therapy, or a complication of a study procedure), the relationship should be specified in the narrative section of the SAE page.

If an ongoing SAE changes in its intensity or relationship to the investigational product, a follow-up SAE report should be sent immediately to the sponsor. As follow-up information becomes available it should be sent immediately using the same procedure used for transmitting the initial SAE report. All SAEs should be followed to resolution or stabilization.

7.13.4 Handling of Expedited Safety Reports

In accordance with local regulations, the drug manufacturer will periodically notify investigators of all SAEs that are suspected (related to the investigational product) and unexpected (ie, not previously described in the Investigator Brochure) for nivolumab, (aka SUSARS).

Other important findings which may be reported by the sponsor as an expedited safety report include: increased frequency of a clinically significant expected SAE, an SAE considered associated with study procedures that could modify the conduct of the study, lack of efficacy that poses significant hazard to study subjects, important safety recommendations from a study data monitoring committee, or sponsor decision to end or temporarily halt a clinical study for safety reasons.

7.13.5 Nonserious Adverse Events

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

The collection of non-serious AE information should begin at initiation of investigational product.

If an ongoing nonserious AE worsens in its intensity, or if its relationship to the investigational product changes, a new nonserious AE entry for the event should be completed. 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 investigational product, or those that are present at the end of study participation. Subjects with nonserious AEs at study completion should receive post-treatment follow-up as appropriate.

All identified nonserious AEs must be recorded and described in the medical record.

7.13.6 Reporting of deaths

All deaths that occur during the study, or within the protocol defined 30-day post last dose of nivolumab safety follow-up period must be reported as follows:

Death that is clearly the result of disease progression should be documented but should not be reported as an SAE.

Where death is not due (or not clearly due) to progression of the disease under study, the AE causing the death must be reported to the Sponsor as a SAE within **24 hours**. The report should contain a comment regarding the co-involvement of progression of disease, if appropriate, and should assign main and contributory causes of death.

Deaths with an unknown cause should always be reported as a SAE. A post mortem maybe helpful in the assessment of the cause of death, and if performed a copy of the post-mortem results should be forwarded to the Sponsor within the usual timeframes.

Deaths that occur following the protocol-defined 30-day post-last-dose of nivolumab safety follow-up period will be documented as events for survival analysis, but will not be reported as an SAE

7.13.7 Overdose

1. An overdose is defined asf a subject receiving a dose of nivolumab in excess of that specified in the Investigator's Brochure, unless otherwise specified in this protocol.
2. Any overdose of a study subject with nivolumab, with or without associated AEs/SAEs, is required to be reported within 24 hours of knowledge of the event to the Sponsor or designee . If the overdose results in an AE, the AE must also be recorded as an AE. Overdose does not automatically make an AE serious, but if the consequences of the overdose are serious, for example death or hospitalization, the event is serious and must be recorded and reported as an SAE. There is currently no specific treatment in the event of an overdose of nivolumab.
3. The investigator will use clinical judgment to treat any overdose.

7.13.8 Hepatic function abnormality

1. Hepatic function abnormality in a study subject, with or without associated clinical manifestations, is required to be reported as "hepatic function abnormal" ***within 24 hours of knowledge of the event*** to the Sponsor or designee, unless a definitive underlying diagnosis for the abnormality (e.g., cholelithiasis or bile duct obstruction) that is unrelated to investigational product has been confirmed.
2. If the definitive underlying diagnosis for the abnormality has been established and is unrelated to investigational product, the decision to continue dosing of the study subject will be based on the clinical judgment of the investigator.
3. If no definitive underlying diagnosis for the abnormality is established, dosing of the study subject must be interrupted immediately. Follow-up investigations and inquiries must be initiated by the investigational site without delay.
4. Each reported event of hepatic function abnormality will be followed by the investigator and evaluated by the Sponsor.

7.13.9 Pregnancy

Pregnancy itself, or pregnancy of a subject's partner, is not regarded as an adverse event unless there is a suspicion that the investigational product under study may have interfered with the effectiveness of a contraceptive medication. Congenital abnormalities/birth defects and spontaneous miscarriages should be reported and handled as SAEs. Elective abortions without complications should not be handled as AEs. The outcome of any conception occurring from the date of the first dose until 90 days after the last dose (spontaneous miscarriage, elective termination, ectopic pregnancy, normal birth or congenital abnormality) should be followed up and documented even if the subject was withdrawn from the study.

1. Pregnancy in a female subject who has received investigational product is required to be reported ***within 24 hours of knowledge of the event*** to the Sponsor or designee.
2. Subjects who become pregnant during the study period must not receive additional doses of investigational product but will not be withdrawn from the study. The pregnancy will be followed for outcome of the mother and child (including any premature terminations) and should be reported to the Sponsor or designee after outcome.
3. Male subjects should refrain from fathering a child or donating sperm during the study and for 3 months following the last dose.
4. Should the investigator become aware of a pregnancy in the partner of a male study subject who has received investigational product this should be reported ***within 24 hours of***

knowledge of the event to the Sponsor or designee. The sponsor will endeavor to collect follow-up information on such pregnancies provided the partner of the study subject provides consent.

7.13.10 Other Safety Considerations

Any significant worsening noted during interim or final physical examinations, electrocardiograms, x-rays, and any other potential safety assessments, whether or not these procedures are required by the protocol, should also be recorded in the medical record.

7.13.11 Contraception

Females of childbearing potential who are sexually active with a nonsterilised male partner must use 2 methods of effective contraception from screening, and must agree to continue using such precautions for 90 days after the final dose of investigational product; cessation of birth control after this point should be discussed with a responsible physician. Periodic abstinence, the rhythm method, and the withdrawal method are not acceptable methods of birth control.

- Females of childbearing potential are defined as those who are not surgically sterile (i.e., bilateral tubal ligation, bilateral oophorectomy, or complete hysterectomy) or postmenopausal (defined as 12 months with no menses without an alternative medical cause).
- Subjects must use 2 acceptable methods of effective contraception as described in Table 1.
- Nonsterilised males who are sexually active with a female partner of childbearing potential must use 2 acceptable methods of effective contraception (see Table 1) from Day 1 and for 90 days after receipt of the final dose of investigational product.

Table Effective methods of contraception (two methods must be used)

Barrier Methods	Intrauterine Device Methods	Hormonal Methods
Male condom plus spermicide	Copper T	Implants
Cap plus spermicide	Progesterone T[a]	Hormone shot or injection
Diaphragm plus spermicide	Levonorgestrel-releasing intrauterine system (e.g., Mirena®)[a]	Combined pill
		Minipill
		Patch

[a] This is also considered a hormonal method.

7.13.12 Blood donation

Subjects should not donate blood while participating in this study

7.13.13 Permitted Concomitant Medications

Investigators may prescribe concomitant medications or treatments (e.g., acetaminophen, diphenhydramine) deemed necessary to provide adequate prophylactic or supportive care except for those medications identified as “excluded”.

7.13.14 Excluded Concomitant Medications

The following medications are considered exclusionary during the study.

1. Any investigational anticancer therapy <<other than the protocol specified therapies>>

2. Any concurrent chemotherapy, radiotherapy (except palliative radiotherapy), immunotherapy, biologic or hormonal therapy for lung cancer treatment, <<other than any stated comparator or combination regimens.>> Concurrent use of hormones for noncancer-related conditions (e.g., insulin for diabetes and hormone replacement therapy) is acceptable. <<NOTE: Local treatment of isolated lesions for palliative intent is acceptable (e.g., by local surgery or radiotherapy)>>

3. Immunosuppressive medications including, but not limited to systemic corticosteroids. Supplemental hydrocortisone or prednisone at doses not exceeding 10 mg/day of prednisone or equivalent are permitted for physiologic adrenal insufficiency only. Other excluded immunosuppressives include, but are not limited to, methotrexate, azathioprine, and TNF-α blockers. Use of immunosuppressive medications for the management of investigational product-related AEs or in subjects with contrast allergies is acceptable. In addition, use of inhaled and intranasal corticosteroids is permitted. A temporary period of steroids will be allowed for different indications, at the discretion of the principal investigator (e.g., chronic obstructive pulmonary disease, radiation, nausea, etc). This must be previously approved by the PI.

4. Live attenuated vaccines within 30 days of nivolumab dosing (ie, 30 days prior to the first dose, during treatment with nivolumab and for 30 days post discontinuation of nivolumab). Inactivated viruses, such as those in the influenza vaccine, are permitted.

8.0 Criteria for Stopping the Trial Due to Toxicity

The trial will be stopped early due to toxicity if a TIL-treated patient experiences a grade 5 event (i.e. death) that is reasonably attributable to the adoptive cell transfer (TIL) treatment. The trial will stopped due to toxicity if it crosses the Pocock boundary for toxicity as enumerated in Section 11.4 Table A1.

9.0 Criteria for Response

The initial disease assessment performed prior to TIL will be subsequently confirmed at the assessment immediately prior to TIL per the study calendar. Following TIL, subsequent disease assessments will occur regularly (+/- 7 days) for trial duration per Study Calendar. Following TIL, the interpretation of response will be based upon the baseline measurements obtained most proximal prior to administration of TIL. For example, a subject who received TIL will have their Day -8 CT scan

measurements serve as baseline for subsequent tumor worksheets and interpretation of subsequent CT scans.

For external sites. All tumor-specific imaging and reports performed for trial purposes, including CT scans, MRIs, and other relevant images, will be shipped to study coordinator and securely stored electronically for independent review by a designated Moffitt radiologists as applicable.

If, during trial treatment, subjects receive standard-of-care CT/MRI imaging that includes their tumor(s) at other centers or hospitals, then both the imaging report and imaging should be obtained. In this instance, RECIST measurements should be calculated and tumor worksheet completed, if applicable. .

9.1 Evaluation of target lesions[1]

Complete Response (CR): Disappearance of all target lesions

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

Progression (PD): At least a 20% increase in the sum of 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 references the smallest sum LD.

[1]All measurable lesions up to a maximum of 5 lesions representative of all involved organs should be identified as **target lesions** and recorded and measured at baseline. Mediastinal and abdominal lymphadenopathy should be considered as separate organ sites, since these receive different (regional v. metastatic) classifications in AJCC lung cancer staging. Target lesions should be selected on the basis of their size (lesions with the longest diameter) and their suitability for accurate repetitive measurements (either by imaging techniques or clinically). 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 to further characterize the objective tumor response of the measurable dimension of the disease. At least one lesion defined radiologically must be present. RECIST Version 1.1 will be referenced for determination of response.

9.2 Evaluation of non-target lesions[2]

Complete Response (CR): Disappearance of all non-target lesions and normalization of tumor marker level.

Non-Complete Response: Persistence of one or more non-target lesions.

Progression (PD): Appearance of one or more new lesions. Unequivocal progression of existing non-target lesions.

[2]All other lesions (or sites of disease) should be identified as **non-target lesions** and should also be recorded at baseline. Measurements are not required, and these lesions should be followed as “present” or “absent.”

9.3 Evaluation of best overall response

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

Target Lesions	Non-Target Lesions	New Lesions	Overall Response
CR	CR	No	CR
CR	Non-CR/Non-PD	No	PR
PR	Non-PD	No	PR
SD	Non-PD	No	SD
PD	Any	Yes or No	PD
Any	PD	Yes or No	PD
Any	Any	Yes	PD

10.0 Criteria for Removal from the Study

Patients will be taken off the regular study schedule if: (a) the patient voluntarily withdraws, (b) there is significant noncompliance, (c) there is progression of disease (PD) by RECIST 1.1 criteria that occurs after adoptive TIL transfer, or (d) death. These patients who are removed from the regular study should be scheduled for progression of disease (PD) assessments as outlined in the Study Calendar and followed according to the EOT schedule.

11.0 Statistical Considerations and Data Analysis

11.1 Overview

This is a phase I clinical trial that is designed to prospectively evaluate the safety and the feasibility of including nivolumab in the regimen of TIL administered after lymphodepletion and followed by a cycle of intermediate-dose decrescendo IL-2 (one dose cohort: TIL+ intermediate-dose IL-2). The primary objectives of this study will be: 1) to demonstrate that the combination of nivolumab with TIL therapy in patients with advanced NSCLC is safe, with a report of toxicity assessed by CTCAE v4 definitely attributed to the treatment regimen, and 2) to establish the feasibility of the combination treatment, as shown by the ability to successfully perform the TIL adoptive cell transfer in at least 67% of all patients accrued to this trial protocol. There is no dose escalation for the infused TIL cell product.

We will accrue up to 20 patients with the goal of having 14 patients who are evaluable (i.e., who receive lymphodepletion/TIL and complete at least 80% of planned visits during the toxicity evaluation window). The primary endpoints of the trial will be the safety and feasibility of administering nivolumab with TIL therapy.

11.2 Trial Design

The purpose of the study is evaluate the safety of the combination of TIL and IL-2 by employing the continuous monitoring method for toxicity using a Pocock-type stopping boundary. That is, each patient will be monitored to see any occurrence of DLT. If excessive numbers of patients with toxicity are seen, the trial will be stopped.

DLT related to nivolumab will be defined as any grade ≥3 immune-related adverse event definitely attributable to nivolumab. DLT related to adoptive cell therapy will be defined as a non-hematologic grade 4 or higher adverse event that is immediately life-threatening occurring upon or after the start of therapy that is immediately life-threatening and not related to non-small cell lung cancer or other pre-existing condition. For the purposes of safety assessment, toxicity will be assessed within 4 weeks of the adoptive TIL transfer.

11.3 Accrual

We anticipate that a maximum of 20 patients will be enrolled to obtain 14 evaluable patients for the safety assessment of the study. Being evaluable is defined as patients that receive lymphodepletion/TIL and complete at least 70% of planned visits during the toxicity evaluation window. Time from accrual to response confirmation is 4 months. Anticipated accrual rate is 2 patients per 3 months. We plan to have 30 months for accrual. This will result in a total of 20 patients (2/3*30=20) for enrollment. We expect a 30% of non-evaluable rate for toxicity and/or response. This will lead to an expected total of 14 evaluable patients for the study.

	Accrual Estimate								
months	3	6	9	12	15	18	21	24	27
total number of expected patients accrued	2	4	6	8	10	12	14	16	18

11.4 Sample Size Justification and Stopping Rules

We expect a total of 14 evaluable patients for the study. Sample size justification is based on continuous monitoring for toxicity using a Pocock-type stopping boundary. We consider the maximum-tolerated toxicity is 17%. We also consider the probability of early stopping to be at most 10%. With 14 patients, these settings result in a Pocock-type stopping boundary (Table A1). The accrual will be halted if excessive numbers of patients with toxicity are seen. For example, if there are 5 or more out of 10 patients (full follow-up) with toxicity, the trial will be stopped. This boundary is equivalent to testing the null hypothesis, after each patient, that the maximum-tolerated toxicity rate is equal to 17%, using a one-sided level 0.053 test. In sensitivity analysis (Table A2), if the true toxicity rate is 17%, the probability to stop the trial is 9% with about 13 expected patients (SD=2.59) for full follow-up and 2.25 patient with toxicity (SD=1.24). If the true toxicity rate is 50%, the chance to close the trial increases to 87%. For futility, we consider a response rate less than 5% as ineffective. A Bayesian approach is used to continuously monitor each patient for futility with a non-informative beta prior, beta(1,1), for response rate. If the posterior probability of response rate less than 5% is greater than 0.5, we will conclude the treatment is ineffective. This design converts into a stopping rule for futility: if there is no response in the first 13 patients or in all the 14 patients, we consider the treatment is ineffective and will not move forward the study. By simulation, the sensitivity analysis (Figure A1) shows if the true response rate is 1%, the chance as an ineffective treatment is 88%. For a true response rate of 5% and 20%, the probability as an ineffective treatment decreases to 51% and 6%, respectively.

Table A1:

Number of Patients, n	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Boundary, b_n	-	2	3	3	3	4	4	4	4	5	5	5	6	6

Table A2:

θ	φ^*	E[Y]	SD[Y]	E[N]	SD[N]	E[Y/N]	SD[Y/N]
0.17	0.0933	2.25	1.24	13.24	2.59	0.20	0.19
0.20	0.1445	2.57	1.24	12.87	3.07	0.24	0.21
0.30	0.3909	3.32	1.14	11.08	4.27	0.39	0.25
0.40	0.6696	3.54	1.08	8.84	4.61	0.53	0.26
0.50	0.8721	3.35	1.06	6.70	4.15	0.64	0.24
0.60	0.9681	3.01	0.96	5.02	3.25	0.73	0.22
0.70	0.9958	2.68	0.78	3.83	2.34	0.81	0.20
0.80	0.9998	2.42	0.60	3.02	1.58	0.88	0.17
0.90	1.0000	2.20	0.42	2.44	0.97	0.95	0.12
1.00	1.0000	2.00	0.00	2.00	0.00	1.00	0.00

Definitions:

Y = the number of patients with toxicity

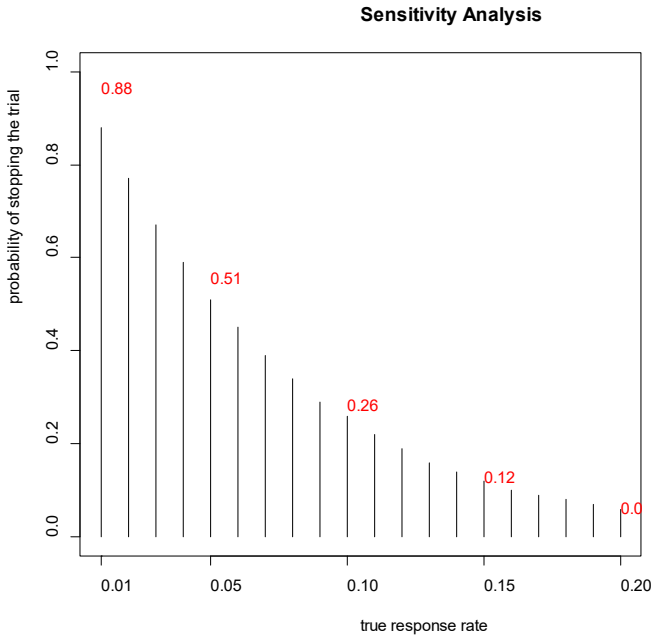
N = the number of patients, random, between 1 and K

φ* = the actual probability of early stopping (hitting the boundary)

E[ ] denotes the expected value (mean)

SD[ ] denotes the standard deviation

Figure A1



11.5 Outcomes and Data Analysis

The data analysis will mainly be descriptive, with inferential statistics provided for the primary and secondary endpoints. Summary statistics for adverse events, including the proportions of each preferred adverse event type will be tabulated and assembled into Tables. Adverse events will be categorized by Grade. Survival will be reported as a time-to-event outcome. All correlative study results will be treated as exploratory in nature due to the pilot status and sample size of the trial. The intention-to-treat approach will be used for data analysis of this trial. That means all patients who undergo tumor harvest will be included in the interim and final data analyses. Patients who are lost to follow-up or drop out of the study prior to their scheduled evaluation time due to any reason will be included for feasibility determination and censored for any time-to-event type of endpoints at the time of their last assessment/follow-up if no relevant event has occurred by then.

Safety will be determined by an assessment of all patients who initiate nivolumab infusion based upon CTCAE v4. It will be reported using both a point estimate and its exact confidence interval based on the binomial distribution. The rate of nivolumab dose-limiting toxicity will be reported similarly.

Overall response (OR) is assessed based upon timepoints after the adoptive TIL transfer, and the tumor size evaluated using the RECIST 1.1 criteria including a complete response (CR) or partial response (PR). Evaluations will be made for patients by CT scan approximately 4 weeks after the cell infusion, and by clinical evaluation during this time. The best overall response (CR+PR) (BORR) rate will be summarized using both a point estimate and its exact 95% confidence interval based on the binomial distribution.

Baseline characteristics including patient age, histology, gender, tumor PD-L1 status, and tumor-mutation burden will be summarized in a descriptive table including relevant average. Prior lines of systemic therapy will be listed. Survival will be plotted on a Kaplan-Meier curve. Confidence intervals for the median and survival rates at different time points will be constructed if needed and appropriate. This secondary endpoint will be reported descriptively. BORR will be plotted on applicable ‘Waterfall’

plots using percent change from nadir = (total length – nadir in total length) / (nadir in total length) x 100%. Depth of response will be recorded over time using applicable “Spider” plots showing the change in size of lesions at a given visit after nivolumab and after TIL.

For correlative analysis, we will explore the extent to which changes between pre- and post-treatment levels correlate with response,. This will be done on the percent change from the pre-treatment values for the biomarkers using the paired tests at α = .05, reported only for exploratory testing.

12.0 Biological sampling procedures

Blood flow cytometry and nucleic acid sequencing

Peripheral blood will be collected at visits as specified in the Study Calendar. The collection kits consist of four-green top (sodium heparin) tubes, one tiger-top (SST) tube and one lavender-top (citrate) tube at each time-point. These tubes will be used for multi-color flow cytometry, nucleic acid sequencing, and cytokine profiling, respectively. The screening blood draw will also include two yellow-top (acid dextrose-citrate) tubes for HLA Class I & II sequencing.

Tumor nucleic acid sequencing and Proteomics

In summary, residual tumor specimen that is not used for TIL will be processed for DNA, RNA, and proteomic sequencing, including whole-exome sequencing (WES). These samples will be used to evaluate potentially predictive biomarkers and complete other correlative studies. Approximately 50 g of tumor sample is required for this purpose, snap frozen and stored in liquid nitrogen. In addition, post-treatment tumor biopsies will be obtained on at least 7-10 total patients during the study. The biopsies may be performed under image guidance (including but not limited to CT or ultrasound-guided core biopsies) as determined by the location of tumor and risks associated with each procedure.

The tumor collected through these methods will be analyzed to explore whether positive vs. negative biomarkers could predict response and resistance to the nivolumab-TIL combination. More specifically for the fresh tumor biopsies, on-site evaluations for tissue quality will be performed by the cytotechnologist to ensure viable tissue and for collection of adequate tumor sample.

Immunohistochemistry will be used to assess the levels of the proposed molecules in histological tumor collected before and after therapy where appropriate. Additional biomarkers may be explored including but not limited to other immune checkpoint molecule expression. We will utilize advanced NGS DNA analyses to test and identify putative mutational load of tumors (tumor mutation burden, TMB) and candidate neoantigens. Further, exploratory correlative studies may be completed based on the additional data obtained from phenotypic characterization of the manufactured TIL product and the patients’ peripheral blood over time. The collection and interpretation of additional data has the potential to help address some of the critical barriers for effective personalized treatment. We will also use mRNA sequencing to verify expression of relevant genes.

The results from DNA sequencing do not get returned to patients or their medical files. Assays are for research purposes with coded samples, not diagnostic purposes. Not defined as an investigational device. This follows ICH E15 and Pharmacogenetics Working Group (PWG) recommendations, and thus genetic counseling for patients on study will not be required.

Polyfunctional Strength Index

PSI measures the ability of single CD8+ cells to secrete multiple different cytokines after stimulation. CD8+ T cells are sorted from TIL and peripheral blood, and stimulated with CD28 and CD3 antibody.

Testing is performed using the Isoplexis IsoCode chip using the 32 human cytokine single-cell proteome panel. Pre- and post- infusion timepoints will be compared using a mixed-effects model (REML) with stacked matching with Geisser-Greenhouse correction, in an exploratory comparison. Multiplicity adjusted p value using Dunnett's control.

Screening T cells for reactivity to neoantigens using the ELISpot assay

Effector T cells will be co-cultured with autologous DCs cells loading 1-10 μg/mL tumor antigen peptides. CEF peptides and/or plate-bound OKT3 (1 μg/mL) will be used as positive controls. Effector T cells only and/or effector T cells co-cultured with unloaded DCs are used as negative controls. After antibody staining, plates are scanned and auto-counted using an ImmunoSpot ELISpot plate reader (Cellular Technologies, Ltd).

Molecular Statistical Analyses

Molecular testing is considered exploratory for the interpretation of tumor biomarkers. Where applicable, biomarkers will be tested separately and considered significant if $P < 0.05$. Analysis will be carried out using either SAS statistical software (SAS, Inc, Cary, NC) or Prism.

Handling, storage and destruction of biological samples

The handling of the tissue samples will be per the laboratory manual.

13.0 Data Safety Monitoring Plan

13.1 Recruitment and Informed Consent

Patients who present with advanced NSCLC may be offered participation in the clinical trial described in this proposal. The trial is explained in detail to the patients by one of the investigators on the trial. The patients are given the opportunity to read the informed consent document and are given a chance to ask questions. If they wish to participate the patient will then sign the informed consent document in the presence of a witness. The study team member who participates in the informed consent process also documents, in a clinic note, the nature of the consent process that occurred.

All non-Moffitt subjects must be registered with the External Site Coordination (ESC) office to be able to participate in the trial. The participating site must fax or email the completed study specific eligibility checklist and registration forms, supporting documents and signed informed consent to the Coordinating Center. Unsigned or incomplete forms will be returned to the site. Once documents are received, the ESC Research Coordinator will review them to confirm eligibility and to complete the registration process. If eligibility cannot be confirmed, the research coordinator will query the site for clarification or additional documents as needed. Subjects failing to meet all study eligibility requirements will not be registered and will be unable to participate in the trial.

Upon completion of registration, the ESC Research Coordinator will provide the participating site with the study sequence number and randomization information, if indicated. Within 24-48 hours after registration, it is the site's responsibility to:

- Enter the demographic and on-study patient information into the Oncore database
- Order investigational agent(s) if indicated per protocol

It is the responsibility of the participating Investigator or designee to inform the subject of the research treatment plan and to conduct the study in compliance with the protocol as agreed upon with Moffitt Cancer Center and approved by the site's IRB.

To register a patient send the completed signed eligibility checklist along with supporting documentation to the ESC via email at affiliate.research@moffitt.org or via fax at 813-745-5666, Monday through Friday between 8:00AM and 5:00PM (EST).

13.2 Protection Against Risk

To protect participants from excess risk, the above-mentioned study procedures were instituted. Additional protection is provided through the data safety and monitoring plan described below. The complete care of each patient, including the clinical management of all toxicities, is provided to the patient by their physicians. The clinical data are kept in the patient's individual electronic hospital record. Research study documentation charts are kept in a locked secure room with limited access and through Oncore (a Web-based, password-protected database), with privacy protected to the full extent of the law. Authorized research investigators, the Department of Health and Human Services, and the Institutional Review Board may inspect the records. Final protocol and ICF approvals are to be obtained from the IRB.
Additional protection is provided through the data safety and monitoring plan described below.

13.3 Importance of the Knowledge to be Gained

The development of a well-tolerated and effective regimen in a disease could potentially at worst add to the armamentarium of available regimens and at best change standard of care. Specific strategies to improve the care of patients relapsing following chemotherapy for lung cancer are direly needed.

13.4 Data Safety and Monitoring Plan

The Data Safety & Monitoring Plan (DSMP) will ensure that this trial is well designed, responsibly managed, appropriately reported, and that it protects the rights and welfare of patients. The following internal and external review and monitoring processes provide oversight and active monitoring of this trial:

- The Principal Investigators (PI)
- The Clinical Trials Office (CTO)
- The Scientific Review Committee (SRC)
- The Protocol Monitoring Committee (PMC);
- The Research Compliance Division (RCD) of the Cancer Center's Compliance Office;
- Institutional Review Board (IRB).

The protocol includes a section that specifies the following with respect to Adverse Event reporting: what constitutes an adverse event (versus what is a serious adverse event), the entities to which adverse events should be reported, the timing of this reporting, and the person or persons responsible for reporting. This includes prompt (within one day of knowledge of the event) reporting to the IRB for unanticipated risks to subjects and reporting in writing within five working days to the IRB and sponsor.

13.5 Scientific Review Committee (SRC)

Each SRC conducts a formal internal peer review of all clinical protocols and general scientific oversight of interventional clinical research. Protocols are reviewed for scientific merit, adequate study design, safety, availability of targeted study population, and feasibility of timely completion of all proposed research projects to be conducted by its assigned programs at each Cancer Center. The SRC is responsible for evaluating the risk/benefit assessment and corresponding data and safety monitoring plan as part of the scientific review and approval process.

13.6 PI Responsibility

The PI of each study is ultimately responsible for every aspect of the design, conduct and actions of all members of the research team. This includes the final analysis of the protocol.

All protocols include a DSMP and procedures for its implementation commensurate with the risk and complexity of the study. The DSMP must include a structured adverse event determination, monitoring and reporting system, including standardized forms and procedures for referring and/or treating subjects experiencing adverse events. The plan must include data and safety-monitoring procedures for subjects enrolled who may be receiving a part of their protocol-required treatment at community sites.

In all cases, the PI of the study will have primary responsibility for ensuring that the protocol is conducted as approved by the SRC and IRB. The PI will ensure that the monitoring plan is followed, that all data required for oversight of monitoring are accurately reported to a DSMB and/or to the PMC and IRB as required, that all adverse events are reported according to protocol guidelines, and that any adverse actions reflecting patient safety concerns are appropriately reported.

13.7 The Protocol Monitoring Committee (PMC)

The PMC reviews and evaluates safety and/or efficacy data for all physician authored clinical intervention trials. The PMC ensures the safety of patients and the validity and integrity of data. PMC reviews SAEs, deviations, Interim analysis, interim and final reports from the external Data Monitoring Committee (DMC) as well as audits both internally and externally. The PMC can make the following determinations, Accepted, Acceptable with Corrective Action and Tabled.

Investigators of studies, which are designated to be reviewed by the PMC for data and safety monitoring, shall provide an interim analysis report of the study's progress and summary of adverse events and deviations based on the phase of the study and the associated risk of the study or more often if applicable. The external DSMB (if applicable) shall forward its report for high-risk studies designated for external review at least annually or more often if applicable.

13.8 Suspension/Termination

The PMC and/or the IRB may vote to suspend or terminate approval of a research study not being conducted in accordance with the IRB, the Cancer Center and/or regulatory requirements or that has been associated with unexpected problems or serious harm to subjects. The PMC/IRB will notify the PI in writing of such suspension or terminations. It is the responsibility of the PMC/IRB Chairperson to ensure prompt written notification of any suspensions or terminations of PMC/IRB approval to the

relevant Federal Agencies, including OHRP, FDA, the study sponsor/funding source and if applicable, the Affiliate Program.

13.9 Trial Discontinuation

For reasonable cause the Investigator and/or sponsor may terminate this study prematurely. Conditions that may warrant termination include, but are not limited to: the discovery of an unexpected, significant, or unacceptable risk to the patients enrolled in the study or if the accrual goals are met. A written notification of termination will be issued.

13.10 Monitoring of the Study and Regulatory Compliance

The Principal Investigator and the Clinical Research Coordinator assigned to the case will be primarily responsible for maintaining all study related documents including the clinical research forms. Oncore is the database of record for all CRF entries and will be verified with source documentation. The review of medical records within PowerChart will be done in a manner to assure that patient confidentiality is maintained.

Monitoring will be performed regularly to verify data is accurate, complete, and verifiable from source documents; and the conduct of the trial is in compliance with the currently approved protocol/amendments, Good Clinical Practice (GCP), and applicable regulatory requirements.

To obtain access to OnCore, the External Site Coordination (ESC) office Coordinator will supply forms required to be completed by the external site staff. Once the completed forms are received, the site coordinator will receive DUO access, logon/password, and information on how to access OnCore. The ESC office will provide OnCore training to the site once initial access is granted and on an ongoing basis, as needed.

Required Documentation

Before the study can be initiated at any site, the site will be required to provide regulatory documentation to the External Site Coordination (ESC) office at Moffitt Cancer Center. Sites must provide a copy of their informed consent to the ESC office for review and approval prior to submission of any documents to the site's IRB. Any changes requested by the site's IRB must be provided to the ESC staff for review and approval prior to resubmission to the IRB.

The ESC office must receive the following trial specific documents either by hardcopy, fax, or email before a site can be activated for any trial:

1. IRB Approval Letter that includes the protocol version and date
2. FDA Related Forms 1572/1571/310 as appropriate
3. Signed Protocol Title Page
4. IRB Approved Consent Form
5. Site Delegation of Authority Log
6. Signed Financial Interest Disclosure Forms (principal and sub investigators)
7. Updated Investigator/Personnel documents (CVs, licenses GCP and HSP training certificates, etc.) as needed
8. Updated Laboratory Documents (certifications, normal ranges, etc.) as needed

9. Signed protocol specific Task Order

A study initiation teleconference will be held prior to the start of any study related activity at the site. Attendance is required for:

- The site PI and appropriate research staff
- Moffitt PI and ESC research coordinator

The requirements of the protocol and all associated procedures and processes will be reviewed and agreed upon prior to the activation of the study. The ESC utilizes the EDC system, OnCore. OnCore training will be scheduled, if indicated, with the appropriate staff from the site.

13.11 Internal Monitoring Plan

Data will be captured in Oncore.
Regulatory documents and case report forms will be reviewed routinely by the MCC Clinical Research Monitoring Core for accuracy, completeness and source verification of data entry, validation of appropriate informed consent process, adherence to study procedures, and reporting of SAEs and protocol deviations according to MCC Monitoring Policies.

Regulatory documents and case report forms will be monitored internally according to Moffitt Cancer Center Monitoring Policies. Both internal and external site monitoring will be performed regularly by the MCC Clinical Monitoring Core for accuracy, completeness, and source verification of data entry, validation of appropriate informed consent process, reporting of SAEs, and adherence to the protocol, Good Clinical Practice (GCP) guidelines, and applicable regulatory requirements.

13.12 Protocol Modifications

No modifications will be made to the protocol without the agreement of the investigators. Changes that significantly affect the safety of the patients, the scope of the investigation, or the scientific quality of the study will require Scientific Review Committee and Institutional Review Board approval prior to implementation, except where the modification is necessary to eliminate apparent immediate hazard to human subjects. Any departures from the protocol must be fully documented in the case report form and the source documentation.

13.13 The Institutional Review Board (IRB)

The trial will not be initiated without approval of the appropriate Institutional Review Board (IRB). All administrative requirements of the governing body of the institution will be fully complied with. This protocol, consent procedures, and any amendments must be approved by the IRB in compliance with current regulations of the Food and Drug Administration. A letter of approval will be sent to the institution(s) funding the study prior to initiation of the study and when any subsequent modifications are made. The IRB will be kept informed by the investigator as to the progress of the study as well as to any serious or unusual adverse events.

13.14 Patient Privacy

In order to maintain patient confidentiality, all case report forms, study reports and communications relating to the study will identify patients by initials and assigned patient numbers. The US Food and Drug Administration (FDA) may also request access to all study records, including source documentation for inspection.

13.15 Records Retention

U.S. FDA regulations (21 CFR §312.62[c]) require that records and documents pertaining to the conduct of this study and the distribution of investigational drug, including CRFs, consent forms, laboratory test results, and medication inventory records, must be retained by the Principal Investigator for 2 years after marketing application approval. If no application is filed, these records must be kept 2 years after the study is discontinued and the U.S. FDA and the applicable national and local health authorities are notified.

13.16 Compliance with Trial Registration and Results Posting Requirements

Under the terms of the Food and Drug Administration Modernization Act (FDAMA) and the Food and Drug Administration Amendments Act (FDAAA), the Sponsor of the trial is solely responsible for determining whether the trial and its results are subject to the requirements for submission to the Clinical Trials Data Bank, http://www.clinicaltrials.gov. Information posted will allow subjects to identify potentially appropriate trials for their disease conditions and pursue participation by calling a central contact number for further information on appropriate trial locations and trial site contact information.

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