DUKE CANCER INSTITUTE

A National Cancer Institute-designated Comprehensive Cancer Center

A Randomized Pilot Study to Investigate the Safety and Immunologic Impact of Evolocumab when Given in Combination with Ipilimumab and Nivolumab in Treatment-Naïve Patients with Metastatic Non-Small Cell Lung Cancer

TOP 2101

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LIST OF ABBREVIATIONS

ACD	Acid-citrate-dextrose
AE	Adverse Events
AFS	Acoustic Focusing Sampler
ALK	Anaplastic Large Cell Kinase
ALT	Alanine Transaminase
ANC/AGC	Absolute Neutrophil Count/Absolute Granulocyte Count
APC	Antigen Presenting Cell
aPTT	Activated Partial Thromboplastin Time
ASCO	American Society of Clinical Oncology
AUC	Area Under the Curve
B/P	Blood Pressure
B2M	Beta-2 Microglobulin
BMS	Bristol Myers Squibb
BSA	Body Surface Area
C1D1	Cycle 1; Day1
CAP	College of American Pathologists
CBC	Complete Blood Count
CD4	CD4 Molecule
CD8	CD8 Molecule
cHL	Classic Hodgkin's Lypmhoma
CLIA	Clinical Laboratory Improvement Amendments
CPC	Cancer Protocol Committee
CR	Complete Response
CrCl	Creatinine Clearance
CRF	Case Report Form (sometimes referred to as Clinical Report Form). A printed or electronic form for recording study participants' data during a clinical study, as required by the protocol.
CT	Computed Tomography
CTCAE	Common Terminology Criteria for Adverse Events; National Cancer Institute, Version 5.0
CTL	Cytotoxic T-Lymphocyte
CTLA4	Cytotoxic T-lymphocyte Antigen
CTQA	Duke School of Medicine Clinical Trials Quality Assurance
CVD	Cardiovascular Disease
DC	Dendritic Cell
DCI	Data and Safety Monitoring Plan
DFS	Disease Free Survival
DIPC	Duke Immune Profiling Core
DKA	Diabetic Ketoacidosis

DLCO	Diffusing capacity of the lungs for carbon monoxide
DLT	Dose Limiting Toxicity
DNA	Deoxyribonucleic Acid
DSMB	Data Safety Monitoring Board
DSMP	Data and Safety Monitoring Plan
DUHS	Duke University Health System
EBUS	Endobronchial Ultrasound
ECOG	Eastern Cooperative Oncology Group
EGFR	Epidermal Growth Factor Receptor
EOT	End of Treatment
EPO	Erythropoietin
EU	European Union
Evo	Evolocumab
FACS	Fluorescence-activated cell sorting
FDA	Food and Drug Administration
FDR	False Discovery Rate
FEV1	Forced Expiratory Volume in One Second
FFPE	Formalin-Fixed Paraffin-Embedded
FNA	Fine Needle Aspirate
GCP	Good Clinical Practice
GFR	Glomerular Filtration Rate
HBsAg	Hepatitis B Surface Antigen
HCV	Hepatitis C Virus
HeFH	Familial Hypercholesterolemia
HR	Hazard Ratio
HRPP	Human Research Protection Program
HuMAb	Human monoclonal antibody
IB	Investigator's Brochure
ID	Identification
IFN-γ	Interferon Gamma
IHC	Immunohistochemistry
INR	International Normalized Ratio
IRB	Institutional Review Board
IT	Information Technology
IV	Intravenous
JAK	Janus Kinase
KEAP1	Kelch Like ECH Associated Protein 1
KLH	keyhole limpet hemocyanin

LD	Longest Diameter
LDL	Low Density Cholesterol
LIHC	Liver Hepatocellular Carcinoma
mDC	Mature Dendritic Cell
MDSC	Myeloid Derived Suppressor Cell
MHC-I	Major Histocompatibility Complex, Class I
MPR	Major Pathologic Response
MRI	Magnetic Resonance Imaging
mRNA	Messenger Ribonucleic Acid
MRR	Major Response Rate
MTD	Maximum Tolerated Dose
MW	Molecular Weight
mWHO	Modified World Health Organization
NCG	NOD CRISPR Prkdc Il2r Gamma Triple-Immunodeficient Mouse
NCI	National Cancer Institute
NFE2L2	Nuclear Factor, Erythroid 2 Like 2 (also known as NRF2)
NGS	Next Generation Sequencing
Nivo	Nivolumab
NK	Natural Killer
NSAIDS	Non-steroidal Anti-inflammatory Drugs
NSCLC	Non-Small Cell Lung Cancer
NSG	NOD scid gamma mouse
ORR	Overal Response Rate
OS	Overal Survival
OTC	Over the Counter
PAAD	Pancreatic Adenocarcinoma
PBMC	Peripheral Blood Mononuclear Cell
PCSK9	Proprotein Convertase Subtilisin/Kexin Type 9
PD	Progressive Disease
PD1	Programmed Cell Death 1 (Gene Name PDCD1)
pDC	Plasmacytoid dendritic cell
PDL1	Programmed Cell Death 1 Ligand (Gene Name CD274)
PDX	Patient Derived Xenograft
PET	Positron Emission Tomography
PFS	Progression Free Survival
PFT	Pulmonary Function Test
PI	Principal Investigator
PR	Partial Response

PS	Performance Status
PT	Preferred Term
PT	Prothrombin Time
Q2W	Every Two Weeks
Q4W	Every Four Weeks
RCC	Renal Cell Carcinoma
RDSP	Research Data Security Plans
RNA	Ribonucleic Acid
RNAseq	RNA sequencing (using next generation sequencing technology)
RP2D	Recommended Phase II Dose
RT-PCR	Reverse-Transcriptase Polymerase Chain Reaction
SAE	Serious Adverse Event
SD	Standard Deviation
SEER	Surveillance, Epidemiology, and End Results Program
SKCM	Skin Cutaneous Melanoma
SOC	Safety Oversight Committee
SOP	Standard Operating Procedure
STAT	Signal Transducer And Activator Of Transcription
STING	Stimulator Of Interferon Genes Protein (Gene Name TMEM173)
STK11	Serine Threonine Kinase 11 (also known as LKB1)
SubQ	Subcutaneous
T1DM	Type 1 Diabetes Mellitus
TAA	Tumor Associated Antigen
TB	Bacillus Tuberculosis
TCGA	The Cancer Genome Atlas
TCR	T-Cell Repertoire
TIL	Tumor Infiltrating Lymphocytes
TIME	Tumor Immune Microenvironment
TSH	Thyroid Stimulating Hormone
ULN	Upper Limit of Normal
US	United States
UVM	Uveal Melanoma
WES	Whole Exome Sequencing
WOCBP	Women of Child Bearing Potential

PROTOCOL SYNOPSIS

OBJECTIVES

Primary Objectives:

1) To evaluate the safety and tolerability of evolocumab in combination with ipilimumab and nivolumab
2) To characterize treatment-related changes in tumor infiltrating lymphocytes (TIL) using immunohistochemistry analysis

Secondary Objectives:

1) To assess the preliminary efficacy of the combination treatment
    a. Objective response rate (ORR) by immune RECIST
    b. Progression free survival (PFS)
    c. Overall Survival (OS)
2) To assess the change in the degree of surface expression of MHC-I molecules on tumor cells within each patient comparing on-treatment versus pre-treatment biopsy specimens

Exploratory Objectives:

1) To determine the correlation between baseline PCSK9 (serum PCSK9 concentration and tumor expression via RNAseq) and treatment response
2) To evaluate treatment response in molecularly defined subgroups (obtained from standard of clinical care next generation DNA sequencing assays), specifically STK11 or KEAP1 mutated tumors
3) To assess ability to successfully generate viable patient-derived MicroOrganosphere™ (MOS) tumor organoids from core biopsy specimens and to evaluate drug response in MOS

PATIENT POPULATION

Subjects with histologically confirmed stage IV or recurrent non-curable NSCLC of squamous or non-squamous histology, with no prior systemic anticancer chemotherapy or immunotherapy given as primary treatment for advanced incurable or metastatic disease.

STUDY DESIGN

This open-label, randomized, pilot trial is designed to assess whether the addition of evolocumab increases tumor infiltrating lymphocytes in NSCLC treated with nivolumab and ipilimumab. The safety of nivolumab and ipilimumab in combination with evolocumab will be assessed every 2 weeks and efficacy will be assessed every 6 weeks in patients with treatment naïve NSCLC. All subjects with metastatic or recurrent non-curable NSCLC enrolled will complete an extensive medical history, baseline physical examination and clinical assessment to ensure subject eligibility requirements (see Eligibility Criteria for key inclusion and exclusion criteria) prior to starting study treatment. Patients will be treated with nivolumab, and ipilimumab with or without evolocumab. Patients initially on the nivolumab and ipilimumab without evolocumab control arm will have the option of adding evolocumab to the nivolumab and ipilimumab regimen after 6 weeks.

NUMBER OF SUBJECTS

Up to 38 patients will be enrolled.

ESTIMATED LENGTH OF PARTICIPATION

Estimated duration of subject enrollment is 24 months. Patients will continue to receive study treatment until they experience unacceptable drug-related toxicity, disease progression, or 24 months. Subjects that discontinue

study treatment with no documented disease progression and no subsequent anti-cancer treatment will be followed every 12 weeks with tumor evaluations until disease progression or start of new anti-cancer therapy is documented. All subjects will be followed for survival until death, loss to follow-up, or study completion.

Study completion is 2 years after the last subject starts study drug regimen.

STUDY DRUG REGIMEN

The dosing regimen for the study arm will be nivolumab 240 mg every 2 weeks, ipilimumab 1 mg/kg every 6 weeks, with evolocumab 140 mg SQ every 2 weeks; and nivolumab 240 mg every 2 weeks, ipilimumab 1 mg/kg every 6 weeks for the control arm. Patients initially on the nivolumab and ipilimumab without evolocumab arm will have the option of adding evolocumab after 6 weeks.

STUDY ASSESSMENTS

The first 6 patients treated with nivolumab, ipilimumab, and evolocumab will analyzed for safety. After 6 patients have initiated therapy with evolocumab (including those who have crossed over to receive evolocumab), enrollment will be paused for 30 days to assess for DLTs. If two or more of the first six patients develop a DLT, further accrual will be paused. The study team will evaluate the cause of toxicity and determine if the trial should be revised and if accrual can resume.

Each cycle is 6 weeks in length. Safety assessments will be performed every 2 weeks. These assessments will include vital signs, ECOG performance status, medical history, physical examination, complete blood count (CBC), biochemistry, creatinine, AST, ALT, and bilirubin. Thyroid stimulating hormone testing will be performed at regular intervals. Adverse events will be recorded using National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE) version 5. General symptom management and supportive care as clinically indicated to ensure optimal patient care will be provided.

TUMOR ASSESSMENTS

Efficacy will be assessed by radiographic imaging (CT and/or MRI) every 6 weeks using immune response RECIST for 48 weeks and thereafter every 12 weeks.

CORRELATIVE STUDIES

Fresh tumor will be obtained pre- and on- treatment (between days 21 and 29). Peripheral blood samples will be collected from each subject for correlative studies at specified time points.

1. *Tumor tissue for immunohistochemistry, flow cytometry, molecular profiling and organoid analyses*. Fresh tumor will be obtained before and after therapy is initiated. A research biopsy will be done if fresh tumor is not available from a standard of care surgical or core needle biopsy.
2. *Circulating immune cells for flow cytometry*. Subjects will have peripheral blood mononuclear cells (PBMC) collected at baseline, after one treatment (two weeks), at first restaging (six weeks), and at disease progression.
3. *Plasma for circulating proteins*. Subjects will have plasma collected at baseline, after one treatment (two weeks), at first restaging, and at disease progression.

Hypothesis:
This protocol is intended to test the following hypothesis: Inhibition of PCSK9 with evolocumab will increase tumor infiltrating lymphocytes in advanced stage, treatment naïve NSCLC receiving nivolumab plus ipilimumab therapy.

STUDY CALENDAR

Study Evaluation Cycle = 6 weeks	Screen [A]	Day 1 Cycle 1	Day 15 Cycle 1	Day 29 Cycle 1	Day 1 Cycles 2+	Day 15 Cycles 2+	Day 29 Cycles 2+	Tumor Assessment	F/U #1 [N]	F/U #2 [N]	Progression	Long Term F/U [M]
Required Evaluations												
Informed Consent	X											
Eligibility Criteria	X											
Demographics	X											
Medical and Smoking History	X											
Cancer Treatment History	X											
Physical Exam	X	X	X	X	X	X	X		X	X		
Height	X											
Weight	X	X	X	X	X	X	X		X	X		
Vital Signs	X	X	X	X	X	X	X		X	X		
Performance Status	X	X	X	X	X	X	X		X	X		
Concomitant Medications	X	X	X	X	X	X	X		X	X		
Adverse Events	X	X	X	X	X	X	X		X	X		
Laboratory Evaluations												
CBC with differential [C,D,F]	X	X	X	X	X	X	X		X	X		
Serum Chemistry [C,E,F]	X	X	X	X	X	X	X		X	X		
Calculated Creatinine Clearance [F,G]	X											
Thyroid Functions (FT4, TSH) [F,H]	X	X			X							
Urinalysis [F]	X											
Serum or Urine Pregnancy Test [I]	X	X										
Other Evaluations												
ECG	X											
Disease Evaluations												
MRI [J]	X							X [J]				
CT Scan [J]	X							X [J]				
Survival Status	X								X	X		X
Correlative Evaluations												
Tumor Tissue Collection	X [B]			X [O]								
Research Blood Collection [K]		X	X					X [K]			X	
Treatment [L]												
Nivolumab		X	X	X	X	X	X					
Ipilimumab		X			X							
Evolocumab [L]		X	X	X	X	X	X					

To allow for patient and investigator schedules, holidays, weather or other emergencies requiring facilities to be closed, visits can be performed ±3 days of scheduled visit and -3/+ 7 days for all treatment days in Cycle 2 onward.

A. Pre-enrollment baseline (screen) assessments are to be performed within -30 to 0 days unless otherwise specified.

B. Patients without a pre-existing pathologic diagnosis of malignancy prior to enrollment must undergo a diagnostic biopsy within appropriate standard of care, from which at least three (and preferably four) additional core needle biopsy samples (size 19 gauge needle or larger) should be obtained for dedicated research use, OR agree to undergo an elective research only biopsy either by bronchoscopic approach with endobronchial ultrasound (EBUS) core needle biopsy or percutaneous CT guided core biopsy approach. At least three and preferably four core biopsy needle samples of at least 19 gauge in size are required. Patients who agree to undergo an elective research only biopsy must have case and images assessed by procedural specialist (pulmonologist or interventional radiologist, respectively), and the procedure must be deemed feasible and without excessive risk.

If the research biopsy has insufficient tissue for the primary outcome analysis, archived tissue may be accessed if available. Archived FFPE samples would be utilized for the primary outcome of T cell infiltration measured by a CD3 immunohistochemical stain.

C. May be obtained within 3 days of nivolumab dosing after Cycle 1 Day 1.

D. Hematology values to include Hgb/Hct, WBC with auto or manual differential, platelets.

E. Chemistries to include Na+, K+, Cl-, total protein, albumin, calcium, glucose, BUN, creatinine, total bilirubin, alkaline phosphatase, AST, ALT.

F. Screening required labs to be performed within 30 days of enrollment. Screening tests may be used for Cycle 1 Day 1 if obtained within 14 days of Cycle 1 Day 1 treatment.

G. Calculated creatinine clearance (see Appendix C).

H. Thyroid profile (TSH & Free T4) to be performed every 6 weeks.

I. All WOCBP MUST test negative for pregnancy at screening and within 72 hours of first study treatment dose done by urine pregnancy test.

J. Baseline tumor imaging will be performed with the relevant imaging modalities to include CT scan (or MRI) of chest/abdomen/pelvis with or without contrast and all known or suspected sites of disease. MRI brain or CT brain with contrast required at baseline. Restaging scans CT of chest and known sites of disease (e.g., abdomen with or without pelvis) required for subjects with metastasis in those areas identified at baseline or if clinically indicated, will be performed every 6 weeks after the start of study treatment for the first 48 weeks, then every 12 weeks thereafter (unless patient is being treated past progression, in which case CT scans should be obtained every 6 weeks even if >48 weeks into study treatment). The same method for tumor assessment is to be employed at every assessment. Subjects with a history of brain metastasis to have surveillance MRI approximately every 12 weeks of sooner if clinically indicated. Subjects who discontinue study treatment for any reason (e.g., toxicity) other than disease progression will have disease status followed per standard of care until disease progression, or start of new anti-cancer regimen.

K. Peripheral blood collection for circulating immune cells (PBMC), and circulating proteins (plasma) are to be done at the following time points: 1) baseline (prior to dose Cycle 1 Day 1); 2) Cycle 1 Day 15; 3) first re-imaging (six weeks); and 4) at progression. Progression defined as iCPD or iUPD if discontinuing study without progression confirmation per provider discretion. Research blood will be drawn at end of treatment for subjects that discontinue treatment for toxicity. Subjects completing 2 years on treatment without progression will be followed for progression and have research blood drawn at progression. Subjects who discontinue treatment without progression and start new treatment will **not** have research blood drawn.

L. Patients will be randomized to receive nivolumab 240mg IV every two weeks and ipilimumab 1 mg/kg IV every 6 weeks with or without evolocumab 140mg subcutaneous injection every 2 weeks.

M. Subjects are followed for survival up to 2 years or until the study is closed (whichever comes first). Survival status may be collected by personal interviews or review of medical or public records every 12 weeks.

N. Subjects to return for follow-up visit #1 at 35 days after last dose of study drug (+/- 7 days) or coinciding with the date of discontinuation of the study drug if the date of discontinuation is > 42 days from last dose for an off treatment visit. Follow-up visit #2 to occur 80 days from Follow-up #1 (+/- 7 days) or 100 days from discontinuation of the study drug.

O. On treatment biopsy may be obtained between Cycle 1 Days 21 and 29.

1.0 BACKGROUND

1.1 Non-Small Cell Lung Cancer

Incidence, prevalence, and mortality

Lung cancer is the leading cause of cancer mortality in the United States with an estimated 225,000 new cases in 2017 and approximately 160,000 deaths annually in the US (1). Non-small cell lung cancer (NSCLC) encompasses the vast majority of new lung cancer diagnoses. More than half of patients have distant metastases at time of initial diagnosis, indicating stage IV disease which is considered to be incurable in the vast majority of cases (2).

Current standard of care

Standard of care treatment of stage IV NSCLC without actionable alterations has advanced significantly in recent years with histology directed chemotherapy utilizing pemetrexed in non-squamous NSCLC, and the addition of bevacizumab to platinum doublet chemotherapy for non-squamous NSCLC (3-6). PD1 is a protein expressed on the surface of certain immune cells that exert inhibitory effects on immune activation and function, while PDL1 is the ligand that interacts with PD1 to enact this inhibitory effect. PDL1 is often upregulated on the surface of cancer cells to inhibit anti-cancer immune activity in the tumor microenvironment (7). Immune checkpoint inhibitors were first proven effective in the second line treatment of recurrent or metastatic lung cancer (8,9). Efficacy in the first line treatment setting was first demonstrated in metastatic lung cancer patients selected for high expression of PDL1 (greater than 50% of tumor cells as assessed by IHC) (10). Patients were randomized to previous standard of care platinum-based doublet chemotherapy vs single agent pembrolizumab, and pembrolizumab demonstrated significantly higher response rate, duration of response, progression free and overall survival, while also having less toxicity than chemotherapy. Subsequent studies have since demonstrated that the addition of pembrolizumab to a regimen of chemotherapy is superior to chemotherapy alone, irrespective of PDL1 expression (11). Checkmate 227 is a large randomized trial in which nivolumab and ipilimumab produced superior survival compared to standard platinum doublet chemotherapy in untreated, advanced stage NSCLC, and the combination is now FDA approved (12).

1.2 Mechanisms of Resistance to Immune Checkpoint Inhibitors in NSCLC

Certain genetic subgroups seem to exhibit resistance or sensitivity to immune checkpoint inhibition. Specifically, mutations in STK11 and/or KEAP1 have been found to confer resistance to immune checkpoint inhibition across multiple studies (13-16). Loss of STK11 seems to induce an ‘immune cold’ tumor environment, with significant reduction in infiltration by multiple immune populations, decreased PDL1 expression, and poor outcomes when treated with immunotherapy (17). Mechanistic evaluation has shown reduced expression of STING in LKB1 deficient lung cancer, which serves as a key immune modulatory protein integrating signals from the innate and adaptive immune system (18).

Other mechanistic and clinical studies have demonstrated many key signaling pathways that are essential to the development of an effective anti-cancer immune response. Among these are interferon signaling including intracellular JAK/STAT pathways, innate immune signals mediated by STING and other proteins, and regulation of antigen presenting machinery. Studies of acquired resistance to PD1 blockade in patients have confirmed these findings, with mutations in JAK1 and JAK2 evolving to confer PD1 resistance and progressive disease by abrogating intra-tumoral interferon signaling, and B2M mutations evolving to eliminate expression of MHC-1 antigen presentation (19).

1.3 Molecular, Genetic, and Cellular Basis of PCSK9 Inhibition

PCSK9 (Proprotein Convertase Subtilisin/Kexin Type 9) is a member of the subtilisin-like proprotein convertase family, which includes proteases that process protein and peptide precursors via endocytic trafficking. PCSK9

has been determined to be a key determinant of LDL cholesterol levels and cardiovascular risk. Gain of function mutations in PCSK9 result in familial hypercholesterolemia (20); whereas loss of function mutations result in reduced levels of LDL-C and lower risk for cardiovascular disease (21). Based on these population based genetic studies implicating PCSK9 in cholesterol homeostasis and atherosclerotic risk, monoclonal antibodies to PCSK9 were developed and subsequently proven effective in cholesterol management and cardiovascular risk reduction (22,23).

We demonstrate that PCSK9 has additional roles in endocytic trafficking, specifically mediating downregulation of MHC class-I molecules by targeting them to endocytic vesicles and degradation. We demonstrate that abrogation of this pathway via genetic abrogation of PCSK9 or via drug mediated (PCSK9 monoclonal antibody) inhibition of PCSK9 is sufficient to increase MHC-I antigen presentation, and moreover, to potentiate efficacy of PD1 immune checkpoint inhibition in murine models of cancer (Figure 1A). Based on these pre-clinical observations we propose investigation of the combination of PD1 and PCSK9 inhibition in humans (24).

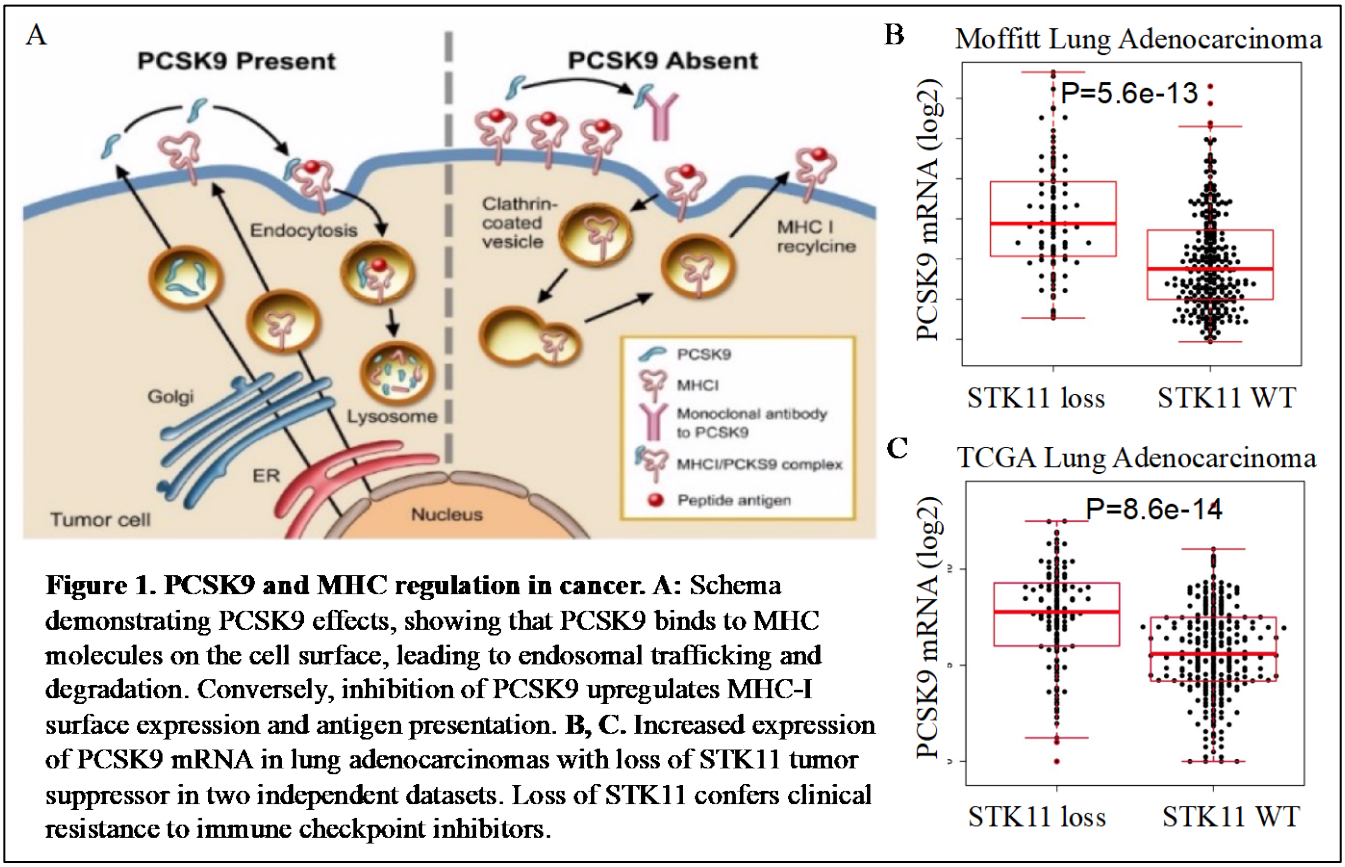

Figure 1. PCSK9 and MHC regulation in cancer. A: Schema demonstrating PCSK9 effects, showing that PCSK9 binds to MHC molecules on the cell surface, leading to endosomal trafficking and degradation. Conversely, inhibition of PCSK9 upregulates MHC-I surface expression and antigen presentation. **B, C.** Increased expression of PCSK9 mRNA in lung adenocarcinomas with loss of STK11 tumor suppressor in two independent datasets. Loss of STK11 confers clinical resistance to immune checkpoint inhibitors.

Furthermore, we show that PCSK9 mRNA expression is significantly increased in lung adenocarcinomas with loss of the STK11 tumor suppressor, which we demonstrate to a high degree of confidence in two independent datasets (Figure 1B,C). Synergy between PCSK9 and PD1 inhibition to improve response to immune checkpoint inhibition may thus have great potential to improve patient outcomes in this difficult to treat patient population.

Data that supports the conduct of this research

Using a genetic screen we identified proprotein convertase subtilisin/kexin type 9 serine protease (PCSK9) as being important in tumor escape from immune mediated rejection. PCSK9 is a secreted protein that binds to the LDL receptor redirecting it to the lysosome for degradation rather than recycling to the plasma membrane (25-29). In our preclinical experiments, we discovered that genetic depletion of PCSK9 in murine tumor cells significantly attenuated their abilities to form tumors in a T cell dependent manner. Furthermore, PCSK9

depletion overcame resistance to PD1 immune checkpoint therapy with a high fraction of mice free of tumors and resistant to later challenges with parental tumor cells (24). We found a significant increase both in the total number and diversity of T cells in the PCSK9 deficient tumors. Furthermore, we showed that PCSK9 deficiency led to significantly enhanced MHC I expression on the surface of tumor cells. These striking preliminary data formed the basis for this clinical trial.

Preliminary Data

Successful knockout of PCSK9 and its influence on tumor cell growth in vitro. In order to assess any potential roles of PCSK9 on tumor growth, we generated 4T1 (a murine breast cancer line), B16F10 (a murine melanoma line), and CT26 (a murine colon cancer line) with *PCSK9* knockout (**Fig. 1**). *PCSK9* deletion had minimal effect on the rate of tumor growth in tissue culture (**Fig. 2**) and in soft agar (**Fig. 3**).

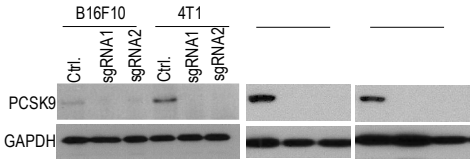

Fig. 1. CRISPR/Cas9 mediated knockout of PCSK9 gene in murine tumor lines. Shown are western blot analyses of the expression of PCSK9 in murine tumor lines with PCSK9 knockout.

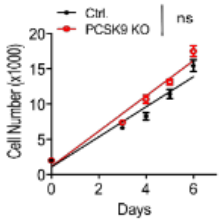

Fig. 2. Growth rates of control or PCSK9 KO B16F10 tumor cells. n=5. Error bars represent SEM.

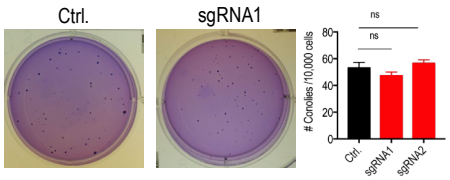

Fig. 3. The abilities of PCSK knockout B16F10 to form colonies in soft agar.

The influence of PCSK9 knockout on tumor formation in immunocompetent mouse hosts. When *PCSK9*-deficeint tumor cells were injected into syngeneic hosts, significant growth delays were observed for *PCSK9* deficient 4T1 and B16F10 cells (**Fig 4A & B**). In fact, 13 of 26 *PCSK9KO* 4T1 injected Balb/C mice (**Fig. 4C**) and 2 out of 12 *PCSK9KO* B16F10 injected C57BL/6 mice (**Fig. 4D**) remained tumor free more than 60 days after tumor cell inoculation. These data showed that PCSK9 deficiency caused significant growth attenuation in immune-competent syngeneic hosts.

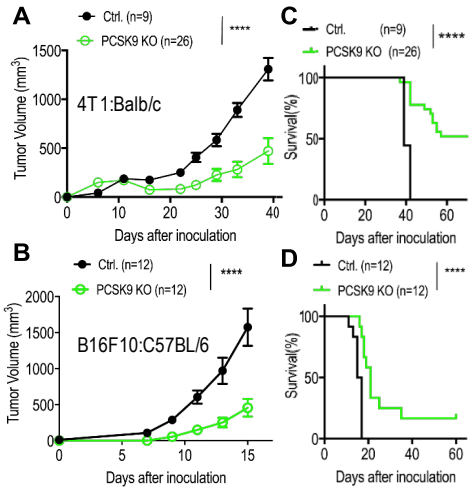

Fig. 4. PCSK9 depletion attenuates tumor growth in syngeneic mice. About $1x10^5$ vector control and PCSK9 knockout murine tumor cells were inoculated subcutaneously into syngeneic mice and observed for tumor formation. Both tumor size and tumor free survival were monitored. **A & C.** 4T1 breast cancer line grown in Balb/C mice. **B & D.** B16F10 melanoma line grown in C57BL/6 mice. In panels A,C and B,D respectively, data were from two independent experiments. In all panels, error bars represent standard error of the mean (SEM). TF: Tumor Free. ****p<0.0001, as determined by 2-way ANOVA (A, B) or log-rank test (C, D).

Lack of tumor growth delay from PCSK9 deficient tumor cells grown in immune-deficient NCG mice. In order to determine if the growth attenuation in immune-competent syngeneic hosts has anything to do with the host's immune system, we inoculated *PCSK9* deficient 4T1 and B10F10 cells into NCG mice (similar to NSG mice) that are deficient in T cells, B cells, and NK cells. Strikingly, our results indicated that these cells formed tumors at

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similar rates with vector control cells (**Fig. 5**). These results thus strongly suggest that the host immune system was responsible for mediating the tumor-suppressing effect of PCSK9 deficiency.

CD8$^+$ T cells are responsible PCSK9 deficiency mediated anti-tumor immunity. We also attempted to determine the immune effector cell subsets that were responsible for the PCSK9 deficiency induced tumor growth suppression. Antibodies against specific immune cell subsets were used to deplete CD8$^+$ T, CD4$^+$ T, and NK1.1$^+$NK cells in C57BL/6 mice. Our results indicated that CD8$^+$ T cells were mostly responsible for the observed growth suppression (**Fig. 6**).

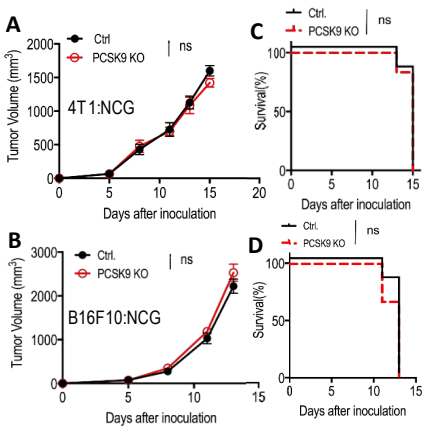

Fig. 5. Growth rates of control and PCSK9 knockout tumor cells in immuno-deficient NCG mice. Growth rate, mouse survival and endpoint tumor weight of vector control and PCSK9 knockout 4T1 (**A & C**) and B16F10 (**B & D**) tumors. In each case, about 1 x 10^5 tumor cells were injected subcutaneously and observed for tumor formation in NCG mice. n=5.

PCSK9 deficiency in tumor cells overcomes tumor resistance to PD1 inhibitor therapy. After generating preliminary data that PCSK9 deficiency led to tumor growth suppression through the immune system, we next examined if PCSK9 deficiency could influence PD1 immune checkpoint therapy. Our results indicated that when PD1 antibody was administered to mice bearing PCSK9 deficient B16F10 tumors, 13 of 20 mice remained tumor free 100 days post initial tumor cell inoculation, whereas none of the mice treated with PD1 inhibition alone were cured of their tumors (**Fig. 7**). These data indicated that PCSK9 deficiency could overcome resistance to PD1 inhibition.

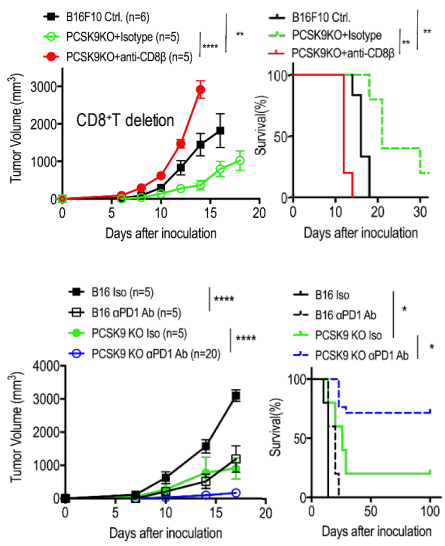

Fig. 6 Requirement for CD8$^+$ T cells in growth delay observed in PCSK9-deficient tumors. C57BL/6 mice were depleted of T cell and NK cells by intraperitoneally (i.p.) injection of an anti-CD8b, andti-CD4, and anti-NK(1.1) antibodies respectively following established protocols. Tumor growth (left panels) and mouse survival (right panels) were then observed after inoculation of control and PCSK9KO B16F10 tumor cells inoculation.

Fig.7 PCSK9 depletion overcomes tumor resistance to anti-PD1 therapy. Control and PCSK9 knockout B16F10 melanomas were treated with an anti-PD1 antibody in syngeneic mice. Experimental protocol (A), tumor growth curve (B), as well tumor-free fraction (C) were shown. Data were from two independent experiments. An isotype control antibody was used to control for the anti-PD1 antibody. Error bars in b, e, h, k represent SEM. TF: Tumor Free. *p<0.05, ****p<0.0001, as determined by 2-way ANOVA (B) or log-rank test (C).

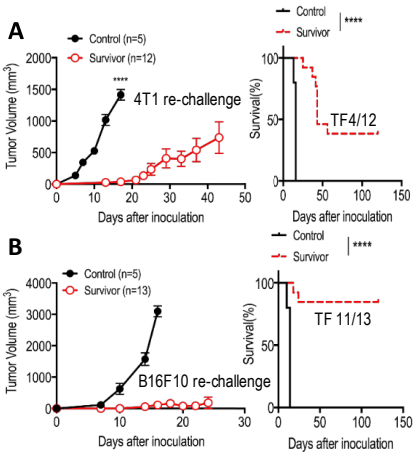

Fig.8. Tumor growth resistance after re-challenge in mice that were cured of their initial tumor inoculation. **(A).** Survivor mice challenged with WT 4T1. (**B**). Survivor mice challenged with WT B16F10.

Resistance to re-challenge in mice cured of initial challenge from PCSK9-deficient tumors. We also examined if mice cured of their initially inoculated tumor cells developed anti-tumor immune memory. Mice that remained tumor free after initial 4T1-PCSK9KO cell inoculation and mice that were cured of their B16F10-PCSK9KO challenge with PD1 inhibition were re-challenged with wild type 4T1 and B16F10 tumor cells, respectively. Our results indicated 4 out of 12 Balb/C mice (tumor free after initial 4T1-PCSK9KO challenge) and 11 of 13 C57BL/6 mice (tumor free after initial B16F10-PCSK9KO challenge and PD1 inhibition) were resistant to re-challenge to wild type parental tumor cells (**Fig.8**). These data indicated that Pcsk9 deficiency stimulated anti-tumor immune memory.

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PCSK9 deficiency led to increased intratumoral infiltration of immune effector cells. In order to assess the influence of PCSK9 deficiency on the tumor immune microenvironment, we analyzed PCSK9-deficient tumors for their immune cell composition. Flow cytometry analysis indicated that there was a significant increase in $CD8^+$ T cells, $CD4^+$T cells, γδT cells, and NK cells (**Fig. 9**). There was also a significant increase in the ratio of $CD8^+$CTL vs $CD4^+FoxP3^+$ Treg cells (**Fig.10**). Furthermore, there was a significant increase in activated $CD8^+$ T cells, as measured by the amount of $IFN\gamma^+$ and $GZMB^+$ CD8+ T cells (**Fig. 11**). These data suggest that PCSK9 deficiency made the tumor microenvironment significantly "hotter" immunologically.

TCR repertoire analysis indicate both increased total number of T cells as well as the diversity of TCRs in the tumor microenvironment. In order to further characterize T cell immune infiltration into the tumor mass, we analyzed control and Pcsk9-deficient tumors for their T cell repertoire. Our analysis (with help from *Adaptive Biotechnologies*) indicated that both the total number of TCRs and the number of unique TCRs were increased in PCSK9-deficient tumors, as was productive clonality (**Fig. 12**). Our data therefore suggest a significant enhancement of the TCR repertoire in *PCSK9* deficient tumors.

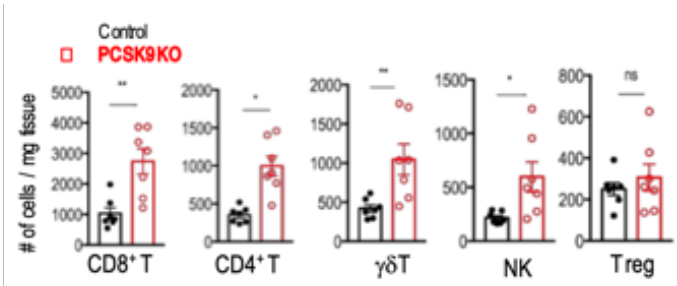

Fig.9. PCSK9 depletion enhances intratumoral T-cell infiltration. Quantitative estimate of various immune effector cells in vector control and PCSK9 knockout B16F10 tumors as determined by flow cytometry. ns: not significant, *p<0.05, **p<0.01 as determined by unpaired t test.

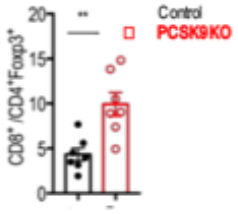

Fig.10. Ratio of $CD8^+$ cytotoxic T cell vs $CD4^+Foxp3^+$ Treg cells in control and PCSK9KO B16F10 tumors. n=7 tumors per group.

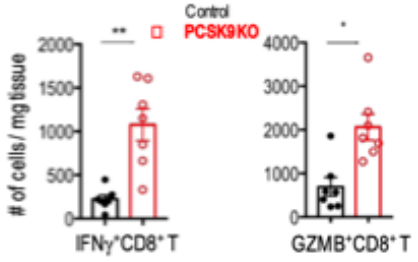

Fig.11. Average numbers of tumor-infiltrating $IFN\gamma^+$ $CD8^+$ T and $Gzmb^+CD8^+$T cells per mg of tumor tissue in control or PCSK9 KO tumors. n=7 tumors.

PCSK9 deficiency led to increased MHC I mediated tumor antigen presentation in vitro. Since PCSK9 is known to be able to influence the cell surface expression levels of its targets such as LDLR and VLDLR (28), we decided to examine if PCSK deficiency could influence the function of surface MHC I, the key antigen presenting complex in the B16F10OVA cells. We then used an antibody that could recognize the well characterized OVA antigen SIINFEKL in complex with surface MHC I to determine the level of the complex on the surface of B16F10OVA cells. Our flow cytometry data clearly indicate that SIINFEKL-MHC I complex levels was significantly increased on the surface of PCSK9 deficient B16F10OVA cells (**Fig. 13**), suggesting strongly that PCSK9 could indeed down-regulate MHC I.

PCSK9 deficiency leads to increased MHC I expression on tumor cells in vivo. We further analyzed the level of MHC I (H2-b) levels in B16F10 tumor cells derived from dis-aggregated control and PCSK9 deficient B16F10 tumors. Our data indicated that there was a significant increase in H2-b levels on the surface of B16F10-*Pcsk9KO* cells grown *in vivo* when compared with vector control B16F10 cells (**Fig. 14**). Our data therefore provide strong evidence that at least one way that Pcsk9 could influence anti-tumor immunity in the tumor microenvironment is through down-regulating the MHC I levels on the surface of tumor cells.

High PCSK9 expression levels correlates with low CTL signature in human patient tumors and poor overall survival in multiple human malignancies. We also assessed the levels of PCSK9 expression and their correlations with cytotoxic T cell signature (as indicated by *CD8A* expression levels) and overall patient survival through the TCGA database. Our data indicated that, consistent with our preliminary data, high PCSK9 expression levels were correlated with lower CTL signature and poor patient survival (**Fig. 15**).

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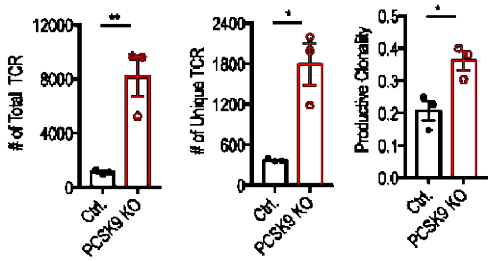


Fig.12. TCR repertoire analysis of intratumoral T cells. Shown are data for total # of TCRs (left panel), # of unique TCRs (middle panel), and relative productive clonality.

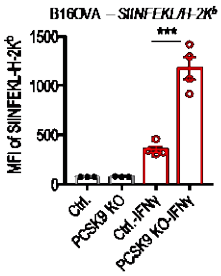


Fig. 13. Quantitative estimates of SIINFEKL-H-2K^b levels in B16F10-OVA cells with or without IFNγ treatment. Enhanced presentation of a model OVA antigen (SIINFEKL) by MHC I in tissue cultured B16F10 cells with PCSK9 deficiency. Control and PCSK9-deficeint B16F10 cells transduced with the chicken OVA gene were treated with IFNγ and assayed for cell surface H-2K^b-SIINFEKL complex by use of flow cytometry. n=4.

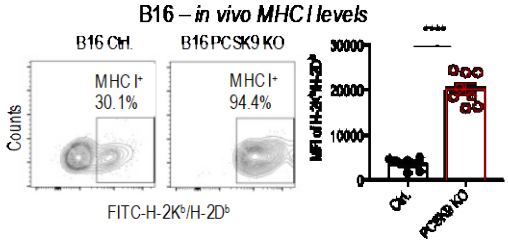


Fig. 14. Vector control and PCSK9 knockout B16F10 stably express tdTomato were inoculated into B16F10 mice and grown for 10 days. After tumor excision and disaggregation, tdTomato positive tumor cells were analyzed for surface MHC I expression by use of an antibody against H2^b and flow cytometry. Quantitative estimates of MHC I levels on the surface of subcutaneously grown control and PCSK9-deficient B16F10 cells were shown in the right panels.. n=8.

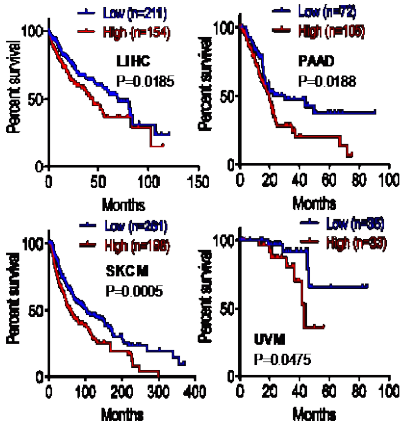


Fig. 15. Higher levels of PCSK9 mRNA levels correlate with worse survival in human liver hepatocellular carcinoma (LIHC), pancreatic adenocarcinoma (PAAD), skin cutaneous melanoma (SKCM), and uveal melanoma (UVM). P values calculated from log-rank test. Data from the TCGA database.

1.4 Nivolumab

PD-1 (or CD279), a 55-kilodalton Type 1 transmembrane protein, is a member of the CD28 family of T-cell co-stimulatory receptors that include immunoglobulin super family members CD28, CTLA-4, ICOS, and BTLA. PD-1 is highly expressed on activated T cells and B cells. PD-1 expression can also be detected on memory T-cell subsets with variable levels of expression. Two ligands specific for PD-1 have been identified: PD-L1 (also known as B7-H1 or CD274) and PD-L2 (also known as B7-DC or CD273). PD-L1 and PD-L2 have been shown to down-regulate T-cell activation upon binding to PD-1 in both murine and human systems. The interaction of PD-1 with its ligands, PD-L1 and PD-L2, which are expressed on antigen-presenting cells (APCs) and DCs, transmits negative regulatory stimuli to down-modulate the activated T-cell immune response. The absence or inhibition of PD-1 in murine models has resulted in the development of various autoimmune phenotypes and autoimmune diseases. Taken together, these results suggest that inhibition of PD-1 binding to its ligands has the potential to activate T-cell responses. Since these responses are variable and dependent upon various host genetic factors, PD-1 deficiency or inhibition is not accompanied by a universal loss of tolerance of self-antigens.

Tumors can express tumor-specific antigens as a result of non-synonymous gene mutations, and ongoing immune surveillance is believed to control the development of many tumors. Tumor progression may depend on the acquisition of mechanisms that permit them to evade an effective immune response. One such mechanism of evasion may be the expression of ligands, which engage inhibitory receptor(s) on anti-tumor T-cells of many tumors. PD-L1 expression has been found on a number of tumors and may be a mechanism by which tumors can directly engage PD-1 to evade an effective anti-tumor immune response. Expression of IFN-γ by activated T cells is known to induce PD-L1 expression in tumors. PD-L1 expression has been associated with poor prognosis in renal, esophageal, gastric, ovarian, pancreatic, and lung cancers. PD-1 engagement on T-cells by PD-L1-positive APC or PD-L1-positive tumor cells in the tumor microenvironment may limit effective immune responses. Conversely, PD-L1 expression may be a positive prognostic factor as it may indicate infiltration of

tumor-specific T cells that secrete IFN-γ, which upregulates PD-L1 expression. Consistent with this hypothesis is the co-localization of lymphoid cell infiltrates and PD-L1 staining observed in human melanoma lesions.

Studies in multiple tumor models using a chimeric murine PD-1 antibody showed that PD-1 blockade has anti-tumor activity. Blocking PD-1 in PD-L1-positive tumors may reverse the inactivation of tumor-specific effector T-cells at the tumor site, as well as activate anti-tumor responses that are limited by PD-L1 expression on "host" DC or APC. The anti-tumor effects of PD1 inhibition observed in several murine models suggest that both PD-L1-positive and PD-L1-negative tumors may be targeted using this approach. In addition, in several tumor models in which PD1 inhibition has proved ineffective, PD1 blockade can be combined with vaccines or other immunomodulatory antibodies for improved therapeutic efficacy.

Nivolumab (also referred to as BMS-936558 or MDX1106) is a human monoclonal antibody (HuMAb; immunoglobulin G4 [IgG4]-S228P) that targets the programmed death-1 (PD-1) cluster of differentiation 279 (CD279) cell surface membrane receptor. PD-1 blockade by nivolumab is a promising avenue to pursue as an anti-tumor therapy for recurrent or treatment-refractory malignancies or as an anti-viral therapy for chronic viral infections.

Nivolumab monotherapy is approved in multiple countries, including the US and EU, for the treatment of advanced melanoma, advanced non-small cell lung cancer, advanced small cell lung cancer, advanced renal cell carcinoma, classical Hodgkin lymphoma, advanced squamous cell carcinoma of the head and neck, urothelial carcinoma, MSI-H or dMMR metastatic colorectal cancer, and hepatocellular carcinoma, and is approved in combination with ipilimumab for the treatment of advanced melanoma, previously treated MSI-H/dMMR metastatic colorectal cancer, first-line treatment for patients with intermediate- and poor-risk advanced renal cell carcinoma.

Preclinical and Clinical Trial Experience

For complete study information, refer to the current Nivolumab Investigator's Brochure (IB).

Non-Clinical Toxicology Summary

Nivolumab has been shown to bind specifically to the human PD-1 receptor and not to related members of the CD28 family. Nivolumab inhibits the interaction of PD-1 with its ligands, PD-L1 and PD-L2, resulting in enhanced T-cell proliferation and interferon-gamma (IFN-γ) release in vitro. Nivolumab binds with high affinity to activated human T-cells expressing cell surface PD-1 and to cynomolgus monkey PD-1. In a mixed lymphocyte reaction (MLR), nivolumab promoted a reproducible concentration-dependent enhancement of IFN-γ release.

In intravenous (IV) repeat-dose toxicology studies in cynomolgus monkeys, nivolumab was well tolerated at doses up to 50 mg/kg, administered weekly for 5 weeks, and at doses up to 50 mg/kg, administered twice weekly for 27 doses. While nivolumab alone was well tolerated in cynomolgus monkeys, combination studies have highlighted the potential for enhanced toxicity when combined with other immunostimulatory agents.

In addition, an enhanced pre- and postnatal development (ePPND) study in pregnant cynomolgus monkeys with nivolumab was conducted. Administration of nivolumab at up to 50 mg/kg 2QW was well tolerated by pregnant monkeys; however, nivolumab was determined to be a selective developmental toxicant when administered from the period of organogenesis to parturition at ≥ 10 mg/kg (area under the concentration-time curve [AUC] from time zero to 168 hours [AUC(0-168 h)] 117,000 μ•h/mL). Specifically, increased developmental mortality (including late gestational fetal losses and extreme prematurity with associated neonatal mortality) was noted in the absence of overt maternal toxicity. There were no nivolumab-related changes in surviving infants tested throughout the 6-month postnatal period. Although the cause of these pregnancy failures was undetermined, nivolumab-related effects on pregnancy maintenance are consistent with the established role of PD-L1 in maintaining fetomaternal tolerance in mice.

Clinical Trial Summary

The PK, clinical activity, and safety of nivolumab have been assessed in approximately 70 clinical studies sponsored by BMS, ONO, or other partners. Across these studies, approximately 20,200 subjects have received nivolumab monotherapy in single- or multiple-dose phase 1/2/3 studies or studies with nivolumab in combination with other therapeutics (ipilimumab, cytotoxic chemotherapy, anti-angiogenics, and targeted therapies). Results from the ongoing studies are preliminary and are subject to change.

Nivolumab has demonstrated clinical activity in NSCLC, melanoma, RCC, and cHL (approved indications) and other tumor types as monotherapy or in combination with ipilimumab. The majority of responses were durable and exceeded 6 months. In randomized, controlled studies, nivolumab monotherapy demonstrated statistically significant improvement in OS over standard of care in subjects with advanced or metastatic melanoma, in subjects with advanced or metastatic NSCLC, and in subjects with advanced RCC.

All available data suggest that nivolumab monotherapy has a consistent AE profile across tumor types. The safety profile is generally consistent across completed and ongoing clinical trials, with no maximum tolerated dose (MTD) reached at any monotherapy dose tested up to 10mg/kg. There was no pattern in the incidence, severity, or causality of AEs to nivolumab dose level. Across all studies conducted to date, drug-related AEs have included pulmonary toxicity, renal toxicity (including acute renal failure), endocrine abnormalities, GI toxicity, dermatologic toxicity (including rash) and hepatotoxicity. For nivolumab monotherapy the majority of these AEs have been managed successfully with supportive care and, in more severe cases, a combination of dose delay, permanent discontinuation, and/or use of corticosteroids or hormone replacement therapy (endocrinopathies) as instructed in the management guidelines provided.

Safety Profile

The overall safety experience with nivolumab is based on experience in approximately 12,300 subjects as either monotherapy or in combination with other therapeutics. In general, for monotherapy, the safety profile is similar across tumor types. The only exception is pulmonary inflammation AEs, which may be numerically greater in subjects with NSCLC, possibly because in some cases, it can be difficult to distinguish between nivolumab-related and unrelated causes of pulmonary symptoms and radiographic changes. The most frequently reported treatment-related AE is fatigue, which is almost always of low grade.

Most related AEs are thought to be due to the effects of inflammatory cells on specific tissues. A variety of preferred terms (PTs) have been used to describe similar kinds of organ-related AEs, with the result being that AE frequency tables organized by PTs can lead to underestimation of the frequency of similar kinds of organ-related AEs. To address this issue, select AE categories were created. Select AE categories group together the most common and impactful PTs by organ category. These categories include the following: pulmonary, GI, hepatic, skin, endocrine, hypersensitivity/infusion reaction, and renal AEs. It is also useful to consider the management of nivolumab-related AEs by organ category as the diagnostic work-up often requires excluding other potential diagnoses and, when appropriate, instituting specific management principles.

In general, the approach to suspected nivolumab-related AEs is similar across any involved organ system. Subjects should have a thorough diagnostic work-up to evaluate potential drug- and non-drug-related diagnoses. For suspected nivolumab-related AEs, based on the severity of the event, management with immunosuppressants may be necessary. In general, dose delays and observation are adequate for low-grade AEs. For moderate- and high-grade AEs, immunosuppression with corticosteroids should be utilized. Once the AE has begun to improve, corticosteroids can be tapered over approximately 3 weeks to 6 weeks (depending on the severity of the AE).

It is rare for a patient receiving immunosuppression for nivolumab-related AEs to develop an opportunistic infection. Subjects with inflammatory events of any organ category expected to require more than 4 weeks of corticosteroid or other immunosuppressive agents to manage the AE should be considered for antimicrobial/antifungal prophylaxis, per institutional guidelines, to prevent opportunistic infections such as P. jiroveci (formerly P carinii) and fungal infections. Early consultation with an infectious disease specialist should

be considered. Depending on the presentation, consultation with a pulmonologist for bronchoscopy or a gastroenterologist for endoscopy may also be appropriate. In addition, a concomitant opportunistic infection should be considered in the differential diagnosis if a patient develops recurrent AEs in the setting of ongoing or prior immunosuppressive use. Nivolumab should not be used in subjects with active autoimmune disease given the mechanism of action of the antibody.

Dose Selection

A flat dose of nivolumab 240 mg Q2W was selected for this study since it is identical to a dose of 3 mg/kg for subjects weighing 80 kg, the observed median body weight in nivolumab treated cancer patients. Using a PPK model, the overall distributions of nivolumab exposures (Cavgss, Cminss, Cmaxss, and Cmin1) are comparable after treatment with either 3 mg/kg or 240 mg nivolumab. The predicted range of nivolumab exposures (median and 90% prediction intervals) resulting from a 240 mg flat dose across the 35 to 160 kg weight range is maintained well below the corresponding exposures observed with the well tolerated 10 mg/kg nivolumab Q2W dosage. Across the various tumor types in the clinical program, nivolumab has been shown to be safe and well tolerated up to a dose level of 10 mg/kg, and the relationship between nivolumab exposure produced by 3 mg/kg and efficacy and safety has been found to be relatively flat. Given the similarity of nivolumab PK across tumor types and the similar exposures predicted following administration of 240 mg flat dose compared to 3 mg/kg Q2W regimen, it is expected that the safety and efficacy profile of 240 mg Q2W nivolumab will be similar to that of 3 mg/kg nivolumab. Hence, a flat dose of 240 mg nivolumab is now a clinical standard of care, and will be utilized for the duration of this trial given the dosing schedule with Ipilimumab.

1.5 Ipilimumab

Ipilimumab *(*BMS-734016, MDX010, MDX-CTLA4) is a fully human monoclonal immunoglobulin (Ig) G1κ specific for human cytotoxic T-lymphocyte antigen 4 (CTLA-4, cluster of differentiation [CD] 152), which is expressed on a subset of activated T cells. CTLA-4 is a negative regulator of T-cell activity. Ipilimumab is a monoclonal antibody (mAb) that binds to CTLA-4 and blocks the interaction of CTLA-4 with its ligands, CD80/CD86. Blockade of CTLA-4 has been shown to augment T-cell activation and proliferation, including the activation and proliferation of tumor-infiltrating T-effector cells. Inhibition of CTLA-4 signaling can also reduce T-regulatory cell (Treg) function, which may contribute to a general increase in T-cell responsiveness, including the anti-tumor response.

Yervoy (ipilimumab) 3 mg/kg has been approved for use in advanced melanoma in over 47 countries, including the United States (US, 25-Mar-2011), the European Union (EU, 13-Jul-2011), and Australia (Jul-2011). Yervoy 10 mg/kg is approved as adjuvant treatment of unresectable or metastatic melanoma in the US.

Ipilimumab has specificity and high affinity for human CTLA-4. The calculated dissociation constant value from an average of several studies was 5.25 nM. Binding of ipilimumab to purified, recombinant human CTLA-4 antigen was also demonstrated by enzyme-linked immunosorbent assay with half-maximal binding at 15 ng/mL, whereas saturation was observed at approximately 0.1 g/mL. No cross-reactivity was observed against human CD28. Ipilimumab completely blocked binding of B7.1 and B7.2 to human CTLA-4 at concentrations higher than 6 and 1 g/mL, respectively.

Clinical Trial Experience

For complete study information, refer to the current ipilimumab Investigator's Brochure (IB). The combination of nivolumab with ipilimumab 1 mg/kg every 6 weeks was found to be safe and effective in the large phase 3 randomized trial checkmate 227.Tthe combination is FDA approved for treatment naïve PD-L1 positive advanced stage NSCLC (12).

Clinical Trial Summary

Bristol-Myers Squibb (BMS) and Medarex, Inc. (MDX, acquired by BMS in Sep-2009) have co-sponsored an

extensive clinical development program for ipilimumab, encompassing more than 19,500 subjects (total number of subjects enrolled in ipilimumab studies) in several cancer types in completed and ongoing studies, including a compassionate use program. The focus of the clinical program is in melanoma, prostate cancer, and lung cancer, with advanced melanoma being the most comprehensively studied indication. Ipilimumab is being investigated both as monotherapy and in combination with other modalities such as chemotherapy, radiation therapy, and other immunotherapies. Phase 3 programs are ongoing in melanoma, prostate cancer, and lung cancer.

In melanoma, completed Phase 3 studies (MDX010-20, CA184024, CA184029, and CA184169) have demonstrated a clinically meaningful and statistically significant survival benefit in pretreated advanced melanoma, in previously untreated advanced melanoma, and in adjuvant melanoma. Ipilimumab monotherapy or in combination with chemotherapy has not prolonged survival in prostate cancer, NSCLC, and SCLC (Studies CA184043, CA184095, CA184104, and CA184156). Outside melanoma, combination with other checkpoint inhibitors (e.g. PD-1 inhibitors) may be required to achieve clinically meaningful activity.

The unique immune-based mechanism of action is reflected in the clinical patterns of anti-cancer activity in some patients. Ipilimumab induces an immunologic response, and measurable clinical effects emerge after the immunological effects. Tumor infiltration with lymphocytes and the associated inflammation (documented by biopsy in some subjects) is likely the cornerstone of the effect of ipilimumab and can manifest in various patterns of clinical activity leading to tumor control. In some cases, inflammation may not be noted by radiological examination, and objective response is observed with the first tumor assessment in a manner seen in patients receiving other types of anti-cancer treatments. In other cases, response may be preceded by an apparent increase in initial tumor volume and/or the appearance of new lesions, which may be mistaken for tumor progression on radiological evaluations. Therefore, in patients who are not experiencing rapid clinical deterioration, confirmation of progression is recommended (at the investigator's discretion) to better understand the prognosis, as well as to avoid unnecessarily initiating potentially toxic alternative therapies in subjects who might be benefiting from treatment. Immune-related response criteria were developed based on these observations to systematically categorize novel patterns of clinical activity and are currently being prospectively evaluated in clinical studies.

In metastatic diseases, stabilization is more common than response and in some instances is associated with a slow, steady decline in tumor burden over many months, sometimes improving to partial and/or complete responses (CRs). Thus, the immune-based mechanism of action of ipilimumab results in durable disease control, sometimes with novel patterns of response, which contribute to its unique improvement in overall survival (OS).

Safety Profile

The unique immune-based mechanism of action is also reflected in the safety profile. The most common treatment-related AEs are inflammatory in nature, consistent with the mechanism of action of the drug and generally medically manageable with topical and/or systemic immunosuppressants. Such immunological safety events are described as immune-related adverse events (irAEs) or immune-mediated adverse reactions (imARs). The irAEs are described as AEs of unknown etiology, which were consistent with an immune phenomenon and considered causally related to drug exposure by the investigators. The irAEs primarily involve the GI tract and skin. Immune-related AEs in the liver were also observed, particularly in subjects receiving 10 mg/kg. Endocrinopathy and neuropathy were important irAEs that were observed less frequently. The imARs were adjudicated in a blinded fashion based on sponsor-physician data review to exclude noninflammatory etiologies, such as infection or tumor progression, and to consider available evidence of inflammation, such as tumor biopsies or responsiveness to steroids, in an effort to determine whether specific AEs or abnormal hepatic laboratory values were likely to be immune mediated and associated with ipilimumab treatment.

The early diagnosis of inflammatory events is important to initiate therapy and minimize complications. Inflammatory events are generally manageable using symptomatic or immuno-suppressive therapy as recommended through detailed diagnosis and management guidelines. The management guidelines for general irAEs and ipilimumab-related GI toxicities, hepatitis, endocrinopathy, and neuropathy are described in this

protocol.

In summary, ipilimumab offers clinically meaningful and statistically significant survival benefit to patients with pretreated advanced melanoma (as 3 mg/kg monotherapy compared to the melanoma peptide vaccine gp100), previously untreated advanced melanoma (at 10 mg/kg in combination with dacarbazine [DTIC] compared to DTIC alone), and adjuvant melanoma. In addition, ipilimumab shows evidence of enhanced clinical activity when combined with nivolumab in melanoma and in other tumor types including NSCLC. These findings, together with evidence of a safety profile that is manageable with careful monitoring and appropriate intervention for treatment of immune-related toxicities, suggest an acceptable benefit to risk ratio.

1.6 Nivolumab and Ipilimumab Combination

The combination of nivolumab with ipilimumab 1 mg/kg every 6 weeks was found to be safe and effective in patients with untreated advanced NSCLC (12). Ipilimumab 1 mg/kg every 6 weeks with nivolumab was studied in Checkmate 227 is a large randomized phase 3 trial in treatment naïve advanced stage NSCLC. The FDA approved the nivolumab plus ipilimumab regimen in PD-L1 positive NSCLC.

1.7 Evolocumab

PCSK9 (Proprotein Convertase Subtilisin/Kexin Type 9) is a member of the subtilisin-like proprotein convertase family, which includes proteases that process protein and peptide precursors via endocytic trafficking. PCSK9 has been determined to be a key determinant of LDL cholesterol levels and cardiovascular risk. Gain of function mutations in PCSK9 result in familial hypercholesterolemia (20); whereas loss of function mutations result in reduced levels of LDL-C and lower risk for cardiovascular disease (21). Based on these population based genetic studies implicating PCSK9 in cholesterol homeostasis and atherosclerotic risk, monoclonal antibodies to PCSK9 were developed and subsequently proven effective in cholesterol management and cardiovascular risk reduction (22,23).

Evolocumab (also known as Repatha) is approved by the FDA and indicated as an adjunct to diet, alone or in combination with other LDL-C-lowering therapies, in adults with primary hyperlipidemia, including heterozygous familial hypercholesterolemia (HeFH), to reduce LDL-C or in adults with established cardiovascular disease (CVD) to reduce the risk of myocardial infarction, stroke, and coronary revascularization. It is also indicated as an adjunct to other LDL-C-lowering therapies in patients with homozygous familial hypercholesterolemia (HoFH), to reduce LDL-C.

There are no published studies evaluating effects of evolocumab in cancer patients, and no studies evaluating the combination of evolocumab or other PCSK9 inhibitors with any PD1 inhibitor.

Preclinical and Clinical Trial Experience

For complete study information, refer to the current Evolocumab US Package Insert.

Non-Clinical Toxicology Summary (30)

Carcinogenesis, Mutagenesis, Impairment of Fertility: The carcinogenic potential of evolocumab was evaluated in a lifetime study conducted in the hamster at dose levels of 10, 30, and 100 mg/kg administered every 2 weeks.

There were no evolocumab-related tumors at the highest dose at systemic exposures up to 38- and 15-fold the recommended human doses of 140 mg every 2 weeks and 420 mg once monthly, respectively, based on plasma AUC. The mutagenic potential of evolocumab has not been evaluated; however, monoclonal antibodies are not expected to alter DNA or chromosomes.

There were no adverse effects on fertility (including estrous cycling, sperm analysis, mating performance, and embryonic development) at the highest dose in a fertility and early embryonic developmental toxicology study

in hamsters when evolocumab was subcutaneously administered at 10, 30, and 100 mg/kg every 2 weeks. The highest dose tested corresponds to systemic exposures up to 30- and 12-fold the recommended human doses of 140 mg every 2 weeks and 420 mg once monthly, respectively, based on plasma AUC. In addition, there were no adverse evolocumab-related effects on surrogate markers of fertility (reproductive organ histopathology, menstrual cycling, or sperm parameters) in a 6-month chronic toxicology study in sexually mature monkeys subcutaneously administered evolocumab at 3, 30, and 300 mg/kg once weekly. The highest dose tested corresponds to 744- and 300-fold the recommended human doses of 140 mg every 2 weeks and 420 mg once monthly, respectively, based on plasma AUC.

Animal Toxicology and/or Pharmacology (30)

During a 3-month toxicology study of 10 and 100 mg/kg once every 2 weeks evolocumab in combination with 5 mg/kg once daily rosuvastatin in adult monkeys, there were no effects of evolocumab on the humoral immune response to keyhole limpet hemocyanin (KLH) after 1 to 2 months exposure. The highest dose tested corresponds to exposures 54- and 21-fold higher than the recommended human doses of 140 mg every 2 weeks and 420 mg once monthly, respectively, based on plasma AUC. Similarly, there were no effects of evolocumab on the humoral immune response to KLH (after 3 to 4 months exposure) in a 6-month study in cynomolgus monkeys at dose levels up to 300 mg/kg once weekly evolocumab corresponding to exposures 744- and 300-fold greater than the recommended human doses of 140 mg every 2 weeks and 420 mg once monthly, respectively, based on plasma AUC.

Clinical Trial Summary (30)

Repatha received FDA approval in 2015 based on clinical trial data supporting efficacy in lowering LDL cholesterol levels. In a combined analysis of 4,465 patients with hyperlipidemia across 12 studies, Repatha reduced the level of LDL cholesterol by 61% from a median of 120 mg per deciliter to 48 mg per deciliter (P<.001) (22). Repatha was then studied in a randomized controlled phase III study of 27,564 patients to assess a primary outcome of reduction in cardiovascular events. This trial demonstrated significant reduction in LDL cholesterol and the primary endpoint of cardiovascular death, myocardial infarction, stroke, hospitalization for unstable angina, or coronary revascularization (23) (11.3% vs 9.8% in placebo; HR 0.85, P<0.001).

Safety Profile (30)

Adverse Reactions in Adults with Primary Hyperlipidemia

The data described below reflect exposure to Repatha in 8 placebo-controlled trials that included 2651 patients treated with Repatha, including 557 exposed for 6 months and 515 exposed for 1 year (median treatment duration of 12 weeks). The mean age of the population was 57 years, 49% of the population were women, 85% White, 6% Black, 8% Asians, and 2% other races.

Adverse Reactions in a 52-Week Controlled Trial

In a 52-week, double-blind, randomized, placebo-controlled trial, 599 patients received 420 mg of Repatha subcutaneously once monthly (31). The mean age was 56 years (range: 22 to 75 years), 23% were older than 65 years, 52% were women, 80% White, 8% Black, 6% Asian, and 6% identified with Hispanic ethnicity. Adverse reactions reported in at least 3% of Repatha -treated patients, and more frequently than in placebo-treated patients, are shown in Table 1.71. Adverse reactions led to discontinuation of treatment in 2.2% of Repatha-treated patients and 1% of placebo-treated patients. The most common adverse reaction that led to Repatha treatment discontinuation and occurred at a rate greater than placebo was myalgia (0.3% versus 0% for Repatha and placebo, respectively).

Table 1.71. Adverse Reactions Occurring in Greater than or Equal to 3% of Repatha-Treated Patients and More Frequently than with Placebo in a 52-Week Trial

	Placebo (N = 302) %	REPATHA (N = 599) %
Nasopharyngitis	9.6	10.5
Upper respiratory tract infection	6.3	9.3
Influenza	6.3	7.5
Back pain	5.6	6.2
Injection site reactions[†]	5.0	5.7
Cough	3.6	4.5
Urinary tract infection	3.6	4.5
Sinusitis	3.0	4.2
Headache	3.6	4.0
Myalgia	3.0	4.0
Dizziness	2.6	3.7
Musculoskeletal pain	3.0	3.3
Hypertension	2.3	3.2
Diarrhea	2.6	3.0
Gastroenteritis	2.0	3.0

[†]includes erythema, pain, bruising

Table 1.72. Adverse Reactions Occurring in Greater than or Equal to 1% of Repatha-Treated Patients and More Frequently than with Placebo in Pooled 12-Week Trials

	Placebo (N = 1224) %	REPATHA[†] (N = 2052) %
Nasopharyngitis	3.9	4.0
Back pain	2.2	2.3
Upper respiratory tract infection	2.0	2.1
Arthralgia	1.6	1.8
Nausea	1.2	1.8
Fatigue	1.0	1.6
Muscle spasms	1.2	1.3
Urinary tract infection	1.2	1.3
Cough	0.7	1.2
Influenza	1.1	1.2
Contusion	0.5	1.0

[†]140 mg every 2 weeks and 420 mg once monthly combined

Adverse Reactions in Seven Pooled 12-Week Controlled Trials

In seven pooled 12-week, double-blind, randomized, placebo-controlled trials, 993 patients received 140 mg of Repatha subcutaneously every 2 weeks and 1059 patients received 420 mg of Repatha subcutaneously monthly. The mean age was 57 years (range, 18 to 80 years), 29% were older than 65 years, 49% women, 85% White, 5% Black, 9% Asian, and 5% identified as Hispanic ethnicity. Adverse reactions reported in at least 1% of Repatha-treated patients, and more frequently than in placebo-treated patients, are shown in Table 1.72.

Adverse Reactions in Eight Pooled Controlled Trials (Seven 12-Week Trials and One 52-Week Trial)

The adverse reactions described below are from a pool of the 52-week trial and seven 12-week trials. The mean and median exposure durations of Repatha in this pool of eight trials were 20 weeks and 12 weeks, respectively.

Local Injection Site Reactions

Injection site reactions occurred in 3.2% and 3.0% of Repatha-treated and placebo-treated patients, respectively. The most common injection site reactions were erythema, pain, and bruising. The proportions of patients who discontinued treatment due to local injection site reactions in Repatha-treated patients and placebo-treated patients were 0.1% and 0%, respectively.

Hypersensitivity Reactions

Hypersensitivity reactions occurred in 5.1% and 4.7% of Repatha-treated and placebo-treated patients, respectively. The most common hypersensitivity reactions were rash (1.0% versus 0.5% for Repatha and placebo, respectively), eczema (0.4% versus 0.2%), erythema (0.4% versus 0.2%), and urticaria (0.4% versus 0.1%).

Adverse Reactions in the Cardiovascular Outcomes Trial

In a double-blind, randomized, placebo-controlled cardiovascular outcomes trial, 27,525 patients received at least one dose of Repatha or placebo. The mean age was 62.5 years (range: 40 to 86 years), 45% were 65 years or older, 9% were 75 years or older, 25% women, 85% White, 2% Black and 10% Asian; 8% identified as Hispanic ethnicity. Patients were exposed to Repatha or placebo for a median of 24.8 months; 91% of patients were exposed for ≥ 12 months, 54% were exposed for ≥ 24 months and 5% were exposed for ≥ 36 months. The safety profile of evolocumab in this trial was generally consistent with the safety profile described above in the 12- and 52-week controlled trials involving patients with primary hyperlipidemia. Common adverse reactions (> 5% of patients treated with Repatha and occurring more frequently than placebo) included diabetes mellitus (8.8% Repatha, 8.2% placebo), nasopharyngitis (7.8% Repatha, 7.4% placebo), and upper respiratory tract infection (5.1% Repatha, 4.8% placebo). Among the 16,676 patients without diabetes mellitus at baseline, the

incidence of new-onset diabetes mellitus during the trial was 8.1% in patients treated with Repatha compared with 7.7% in patients that received placebo.

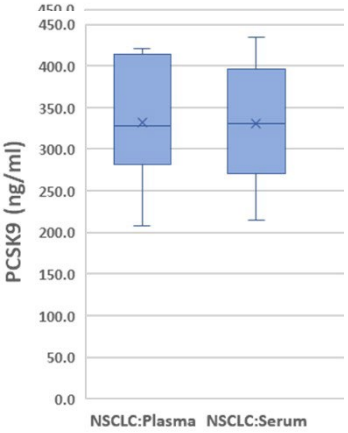
Due to previous concerns regarding the effects of lipid lowering agents on neurocognitive function, the rate of neurocognitive adverse events was prospectively assessed in a subgroup of 1204 patients from a randomized, placebo-controlled trial of Repatha added to statin therapy. This concluded that Repatha was non-inferior to placebo with respect to the primary endpoint of change from baseline over time in the raw score for the spatial working memory strategy index of executive function (p-value for non-inferiority <0.001). Repatha was also non-inferior in secondary end points of scores for working memory, episodic memory, or psychomotor function. In an exploratory analysis, there were no associations between LDL cholesterol levels and cognitive changes. No additional safety concerns were identified (32).

Dose Selection
The recommended subcutaneous dosage of evolocumab in patients with heterozygous familiar hypercholesterolemia or patients with primary hyperlipidemia with established clinical atherosclerotic cardiovascular disease is either 140 mg every 2 weeks OR 420 mg once monthly. For the purpose of this study, the 140 mg dose will be given every 2 weeks of treatment, concurrent with nivolumab 240 mg every 2 weeks, and ipilimumab 1 mg/kg every 6 weeks.

2.0 STUDY RATIONALE

2.1 Serum PCSK9 Level as a Potential Prognostic Biomarker

The Human Protein Atlas RNAseq data from 994 lung cancers reveal a median expression level of 2 fragments per kilobase million (FPKM) with a range of 0-110. Additionally, Bonaventura *et al* recently published a study correlating serum PCSK9 levels with clinical outcome in NSCLC patients treated with nivolumab. Using receiver operating characteristic (ROC) analysis, they determined that the subset of patients who had a serum PCSK9 concentration above a cut point of 95 ng/ml had a very poor OS (HR 0.35) (33). We measured circulating levels of PCSK9 in matched serum and EDTA plasma samples from 10 NCSLC cancer patients using the PCSK9 ELISA assay employed by Bonaventura and colleagues (33). Levels were consistent between plasma and serum preparations, and we observed that PCSK9 was elevated in this cohort of NSCLC patients. The median PCSK9 levels were 332 ng/ml (EDTA plasma) and 330 ng/ml (serum). The assay performed very well, all samples were measurable within the expected range of the assay and the average %CV was below 2%.



Circulating PSCK9 levels in matched serum and plasma preparations from NCSLC patients (n=10).

2.2 Clinically Unmet Need

There remains an urgent unmet need for well tolerated, high efficacy therapies for frontline, treatment-naive NSCLC. Standard of care treatment of stage IV disease has advanced significantly over the past 10 years with the development of small molecule inhibitors active against many forms of lung cancer with oncogenic actionable molecular alterations such as EGFR, ALK, ROS1, and others. The majority of patients have cancers that do not exhibit targetable oncogenic mutations, and immune based treatments are typically used in the first line setting for advanced stage lung cancer (stage IIIb and IV) (10-12). The combination of CTLA-4 and PD1 checkpoint antibody-based therapy produces a significant number of patients with advanced lung cancer who are long term survivors. Still the majority of patients with advanced NSCLC without an actionable molecular alteration live less than 2 years. Improving immune based medical therapy for advanced stage NSCLC is one of the most important unmet clinical needs in cancer care.

Why the stated objectives of the research are important

Decrease in MHC1 on the tumor cell surface is an important mechanism by which cancers evade the cellular immune system. Preclinical data indicates that PCSK9 inhibition increases MHC1 on the cell surface. This study proposes a highly novel strategy using a repurposed PCSK9 inhibitor evolocumab to increase MCH1 expression on tumor cells in the context of combination immune therapy with nivolumab plus ipilimumab. The increase in MHC1 expression was ultimately shown to increase T cell infiltration in tumor cells in the murine models and promote anti-tumor immunity. The primary objective of this study is to determine if PCSK9 inhibition with evolocumab increases T cell infiltration, and identify a new strategy to overcome immune checkpoint resistance in NSCLC.

2.3 Study Purpose

Nivolumab plus ipilimumab 1 mg/kg every 6 weeks produced superior survival compared to standard chemotherapy in untreated advanced stage NSCLC, and produced a high number of durable responses (12). The clinical experience with PD-1 inhibition in lung cancer is extensive, with numerous phase three clinical trials showing benefit of nivolumab and pembrolizumab for the treatment of advanced, stage IV lung cancer. Despite improved efficacy for combination immune therapy compared to chemotherapy many tumors exhibit primary resistance to immune based treatment strategies through multiple mechanisms that are incompletely understood. The majority of advanced lung cancer patients succumb to their disease either due to initial resistance to first line treatment or to subsequent development of acquired resistance. Efforts to understand resistance mechanisms and strategies to overcome them will be crucial to improve clinical treatment of lung cancer. Nivolumab and ipilimumab is now an approved option for advanced stage NSCLC. The combination of PD-1 inhibition with PCSK9 inhibition is based on preclinical studies demonstrating that expression of PCSK9 leads to resistance to the effects of PD-1 blockade by downregulating MHC-I antigen presentation and decreasing anti-tumor immune responses induced by PD-1 inhibition. The combination of PD-1 blockade and PCSK9 inhibition was synergistic in pre-clinical animal models, leading to significant reduction in tumor growth compared to either agent alone. Analysis of gene expression patterns in NSCLC demonstrates that PCSK9 is significantly over-expressed in NSCLC patients with STK11 mutations, which have been shown previously to exhibit primary resistance to immunotherapy. Thus, the combination of PD-1, CTLA4 and PCSK9 inhibition may be particularly important in this refractory patient population.

We will test the combination of PD-1 and CTLA4 inhibition with PCSK9 inhibition in the setting of untreated, advanced stage non-small cell lung cancer. The primary endpoint is assessment of pharmacodynamic biomarkers to directly assess the proposed mechanism of synergy of these agents.

We have demonstrated in preclinical models that PCSK9 has previously undescribed role in down-regulating MHC class I antigen presentation through endocytosis. We show that this effect can be reversed using genetic or pharmacologic inhibition of PCSK9 and that applying these approaches in animal models upregulates tumor expression of MHC class I expression in vivo and synergizes with PD-1 immune checkpoint inhibition to significantly improve tumor control. Hence, we propose testing this mechanism in vivo, using a biomarker based randomized clinical trial studying an approved immune combination therapy with or without the PCSK9 inhibitor evolocumab. We will assess both tumor response and pharmacodynamic determination of the increase in tumor infiltrating lymphocytes and change in MHC class I expression in response to immune combination treatment with or without evolocumab.

The purpose of this study is to investigate the novel combination of PD-1 inhibitor monoclonal antibody, nivolumab, CTLA4 inhibitor, ipilimumab, and a PCSK9 inhibitory monoclonal antibody, evolocumab in patients with treatment naïve metastatic NSCLC.

How conclusions derived from this research will be used

Conclusions from this work will serve to establish a novel synergistic anti-cancer effect for the combination of PD-1 and PCSK9 inhibition in the treatment of NSCLC, and moreover, to validate the proposed mechanism of

increased MHC class I expression induced by PCSK9 inhibitors. The demonstration of increased MHC class I expression (primary outcome) would prompt the evaluation of this combination in larger prospective phase II and/or phase III clinical trials to evaluate efficacy both in the early stage and metastatic NSCLC disease setting.

2.4 Investigational Plan

This is a 38 patient two arm, open label, randomized pilot trial of nivolumab and ipilimumab with or without evolocumab in patients with no previous treatment for advanced stage NSCLC. Patients will undergo research biopsy of cancer prior to treatment and after initiation of treatment to evaluate for effect of evolocumab on tumor infiltrating lymphocytes. Tissue biopsy specimens will be used for organoid generation. Serial blood-based biomarker analysis will be performed. Patients will receive therapy for up 2 years, if cancer is controlled and there is no excess toxicity. Patients initially on the nivolumab and ipilimumab without evolocumab arm will have the option of receiving evolocumab after 6 weeks. Allowing the control arm to receive evolocumab after week 6 will not interfere with the primary endpoint, provide the investigational therapy to all patients, and provide additional efficacy and toxicity data for the nivolumab, ipilimumab, and evolocumab combination.

3.0 STUDY OBJECTIVES

3.1 Primary Objectives

1) To evaluate the safety and tolerability of evolocumab in combination with ipilimumab and nivolumab
2) To characterize treatment-related changes in tumor infiltrating lymphocytes (TIL) using immunohistochemistry analysis

3.2 Secondary Objectives

1) To assess the preliminary efficacy of the combination treatment
    a) Objective response rate (ORR) by immune RECIST
    b) Progression free survival (PFS)
    c) Overall Survival (OS)
2) To assess the change in the degree of surface expression of MHC-I molecules on tumor cells within each patient comparing on-treatment versus pre-treatment biopsy specimens

3.3 Exploratory Objectives

1) To determine the correlation between baseline PCSK9 (serum PCSK9 concentration and tumor expression via RNAseq) and treatment response
2) To evaluate treatment response in molecularly defined subgroups (obtained from standard of clinical care next generation DNA sequencing assays), specifically STK11 or KEAP1 mutated tumors
3) To assess ability to successfully generate viable patient-derived MicroOrganosphere^{TM} (MOS) tumor organoids from core biopsy specimens and to evaluate drug response in MOS

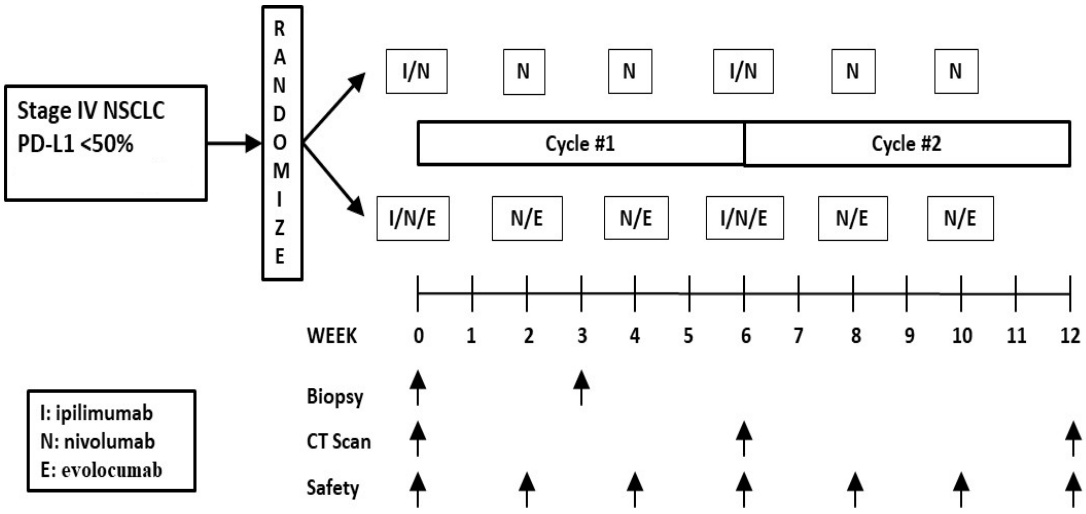
4.0 STUDY DESIGN

Assessment of eligibility will include assessment of expected biopsy safety. Patients who have a pathologic diagnosis of malignancy at time of study enrollment will undergo a dedicated research biopsy through CT guided percutaneous or endobronchial ultrasound (EBUS) guided approach. Upon confirmation of eligibility, patients will then be randomized to receive nivolumab 240mg IV every two weeks and ipilimumab 1 mg/kg IV every 6

weeks with or without evolocumab 140mg subcutaneous injection every 2 weeks. CT scans to assess response to therapy will be obtained every 6 weeks for 48 weeks and every 12 weeks thereafter until disease progression.

Patients initially on the nivolumab and ipilimumab without evolocumab arm will have the option of adding evolocumab after 6 weeks. The addition of evolocumab will be determined jointly by the treating physician and the patient. Evolocumab can be added so long as the patient has not developed toxicity, and/or progressive disease meeting criteria for study discontinuation (the first restaging scan will also obtained at 6 weeks) while on nivolumab plus ipilimumab. Patients in a partial response or with stable disease in the control arm may also elect to continue on trial without adding evolocumab.

This is an up to 38 patient two arm, open label, randomized pilot trial of nivolumab and ipilimumab with or without evolocumab in patients with no previous treatment for stage IV NSCLC. Patients will undergo research biopsy of cancer prior to treatment and after initiation of treatment to evaluate for effect of evolocumab on tumor cell MHC1 expression. Tissue will also be sent to Xilis, Inc. (Durham, NC) for MicroOrganosphere™ (MOS) generation. Serial blood-based biomarker analysis will be performed. After 6 patients have initiated therapy with nivolumab, ipilimumab, and evolocumab enrollment will pause for 30 days to assess for DLTs and safety. Patients who have crossed over to receive evolocumab will be included in this safety analysis if they are among the first 6 patients treated with evolocumab. If two or more of the first six patients develop a DLT, further accrual will be paused. The study team will evaluate the cause of toxicity and determine if the trial should be revised and if accrual can resume. Patients will receive therapy for up 2 years, if cancer is controlled and there is no excess toxicity.



4.1 Assessment of Primary Endpoint

All patients who received at least one dose of nivolumab and ipilimumab +/- evolocumab, and who have adequate tissue for immunohistochemical analysis from both their pre-treatment biopsy and on treatment specimen will be considered evaluable for the primary endpoint of change in tumor infiltrating lymphocytes. Tumor infiltrating lymphocytes will be assessed on standard sections of FFPE tissues with CD3 immunohistochemical stains. TILs will be reported as a continuous variable measured as the number of TIL per mm2 of tissue as previously described (34).

4.2 Assessment of Key Secondary Endpoints

All patients who received at least one dose of nivolumab and ipilimumab with or without evolocumab, and who have adequate tissue for flow cytometry analysis from both their pre-treatment biopsy and on treatment specimen

will be considered evaluable for the secondary endpoint of MHC-I expression analysis. Multidimensional flow cytometry will be used to simultaneously assess MHC-I tumor cell expression, suitable lineage and population markers to allow tumor cells to be distinguished from non-tumor stromal cells, and immune phenotypic markers to assess the secondary endpoint of correlative immunophenotyping of TILs. Matched pre-treatment and post-treatment specimens will be assessed on the same day when possible to minimize variation due to reagents or instrument effects. Positive and negative controls for MHC-I expression (e.g. MHC-I coated beads) will be included, which will facilitate comparison of MHC-I between samples. For each tumor specimen analyzed the fraction of tumor cells with positive cell-surface expression of MHC-I will be determined, as well as the MHC-I mean fluorescence intensity relative to controls.

All patients who received at least one dose of nivolumab and ipilimumab will be evaluable for the key secondary endpoint of radiographic response. We will estimate the objective response rate (ORR=CR+PR) using Immune Response Evaluation Criteria in Solid Tumors (Appendix A). The ORR along with its 80% exact confidence interval will be estimated. The small sample size and the heterogeneous patient population associated restrict the ability to evaluate treatment efficacy. The analysis on efficacy endpoints will be viewed as exploratory.

4.3 Safety Monitoring and Reporting

The Investigator is responsible for the identification and documentation of adverse events and serious adverse events as defined below. At each study visit, the Investigator or designee must assess, through non-suggestive inquiries of the subject or evaluation of study assessments, whether an AE or SAE has occurred.

4.4 Definition of Dose-Limiting Toxicity (DLT)

Toxicities will be graded according to the NCI CTCAE version 5.0 criteria. DLTs will be defined as any grade ≥ 3 toxicity at least (possibly, probably, or definitely) attributable to nivolumab, ipilimumab, and/or evolocumab, except electrolyte abnormality which can be correct with oral supplementation, or diarrhea persisting for < 48 hours. The incidence of DLT(s) assessed in the first 6 evaluable subjects will be used to initially determine whether the combination is tolerable. A subject will be considered evaluable for DLT if they have received at least one dose of nivolumab, ipilimumab and evolocumab. DLTs will be assessed for 30 days after Cycle 1 Day 1. DLT should not be AE, if considered by the investigator to be disease related.

4.5 Definition of Evaluable Subjects, On Study, and End of Study

- **Enrolled subjects** are defined as subjects who give informed consent.
- **Safety evaluable patients** include all enrolled subjects who undergo any procedure or receive any study treatments.
- **Toxicity evaluable patients** include all enrolled subjects who receive any study treatments.
- **Primary efficacy evaluable patients** include all enrolled patients who receive any study treatments and have pre-treatment and post-treatment tumor specimens adequate for flow cytometry analysis.
- Patients are considered to be **on study** if they have received any dose of nivolumab, ipilimumab and/or evolocumab as part of this trial, until the date of their last patient visit.
- **End of study** is defined as the date of the last patient's last visit, unless the study is terminated early.

4.6 Early Study Termination

This study can be terminated at any time for any reason by the Sponsor PI. If this occurs, all subjects on study should be notified as soon as possible. Additional procedures and/or follow up should occur in accordance with the study document, which describes procedures and process for prematurely withdrawn patients.

5.0 PATIENT ELIGIBILITY

5.1 Inclusion Criteria

1) All patients must have histologically documented or suspected metastatic squamous or non-squamous stage 4 NSCLC or recurrent NSCLC following therapy for locally advanced disease. Patients with suspected lung cancer without pathological diagnosis are eligible. Patients may not have received prior systemic anti-cancer treatment given as primary therapy for advanced or metastatic disease.
2) **NO** prior chemotherapy, radiation therapy or biologic/targeted therapy for current diagnosis recurrent/metastatic NSCLC. Medical therapy (including adjuvant or maintenance immune therapy) for early stage NSCLC allowed if completed ≥ 6 months prior to study enrollment. Palliative radiation to non-target lesions for symptom management is allowable, as long as there remains measurable non-radiated lesions.
3) TPS PD-L1 < 50%
4) Performance Status ECOG 0-1 (Appendix B).
5) Age >18 years old.
6) No active invasive malignancy in the past 2 years other than non-melanoma skin cancer. Cancers that are in-situ are not considered invasive.
7) No autoimmune disease that would constitute contraindication to receive nivolumab or ipilimumab
8) Patients must have core needle biopsy tissue that is available and adequate for dedicated research purposes or agree to undergo an elective 'research only' core needle biopsy. Core needle biopsy must be fresh biopsy in order to be processed at the time of biopsy for dissociation into single cell suspension.
9) No excessive risk for CT or ultrasound guided percutaneous biopsy to obtain research biopsy specimen. Risk assessment is to be determined by the treating oncologist and the interventional radiologist.
10) Patients who do not have an indication for a diagnostic biopsy must undergo an elective 'research only' core needle biopsy.
11) Signed written informed consent including HIPAA according to institutional guidelines.
12) Patients are eligible to be included in the study only if they meet **all** of the following criteria. For time limits on imaging and blood tests see study calendar:

REQUIRED LABORATORY DATA

System	Laboratory Value
Hematological	
Absolute neutrophil count (ANC) or AGC	≥1500 per uL
Platelets	≥100,000 per uL
Hemoglobin	≥9 g/dL or ≥5.6 mmol/L without transfusion or EPO dependency (within 7 days of assessment)
Renal	
Serum creatinine **OR** Measured or calculated[a] creatinine clearance (GFR can also be used in place of creatinine or CrCl)	≤1.5 X upper limit of normal (ULN) **OR** ≥50 mL/min for subject with creatinine levels > 1.5 X institutional ULN
Hepatic	
Serum total bilirubin	≤ 1.5 X ULN **OR**

System	Laboratory Value
	Bilirubin < 3.0 ml/dL and Direct bilirubin ≤ ULN for subjects with Gilbert's syndrome with total bilirubin levels > 1.5 ULN
AST (SGOT) and ALT (SGPT)	≤ 2.5 X ULN
Albumin	≥2.5 mg/dL
[a]Creatinine clearance should be calculated per institutional standard. (Appendix C)	

5.2 Exclusion Criteria

Patients will be excluded from the study if they meet **any** of the following criteria:

1) Is currently participating and receiving study therapy or has participated in a study of an investigational agent and received study therapy or used an investigational device within 4 weeks of the first dose of treatment.
2) Has a known history of active TB (Bacillus Tuberculosis)
3) Hypersensitivity to nivolumab or ipilimumab or any of its excipients
4) Hypersensitivity to evolocumab or any of its excipients
5) Patient does not have a site of suspected malignancy that is accessible to pre-treatment biopsy.
6) Concurrent administration of any other anti-tumor therapy.
7) Has received prior therapy with a PD1, PDL1, or PDL2 inhibitor.
8) Has received therapy with PCSK9 inhibitor within 90 days of study entry.
9) Known active CNS metastases which are symptomatic. Eligible if metastases have been locally treated 14 days prior to cycle 1 day 1, are clinically controlled, and asymptomatic off high dose steroids on cycle 1 day 1(≤ 2 mg decadron or 10 mg prednisone daily or equivalent allowed). Untreated, asymptomatic brain metastases allowed if subject does not require corticosteroids or anticonvulsant therapy.
10) Has known active Hepatitis B (e.g., HBsAg reactive) or Hepatitis C (e.g., HCV RNA [qualitative] is detected).
11) Inability to comply with protocol or study procedures.
12) Active infection requiring antibiotics, antifungal or antiviral agents, that in the opinion of the investigator would compromise the patient's ability to tolerate therapy.
13) Has known history of, or any evidence of, active, non-infectious pneumonitis. Patients with a history of immunotherapy-induced pneumonitis and radiation pneumonitis are ineligible regardless of whether they have current evidence of active pneumonitis.
14) Has a known history of Human Immunodeficiency Virus (HIV) (HIV 1/2 antibodies)
15) Has active autoimmune disease that has required systemic treatment in the past 2 years (i.e. with use of disease modifying agents, corticosteroids or immunosuppressive drugs). Replacement therapy (e.g. thyroxine, insulin or physiologic corticosteroid replacement therapy for adrenal or pituitary insufficiency etc) is not considered a form of system treatment. Patients with a history of inflammatory bowel disease, including ulcerative colitis and Crohn's Disease, are excluded from this study, as are patients with a history of symptomatic disease (e.g., rheumatoid arthritis, systemic progressive sclerosis [scleroderma], systemic lupus erythematosus, autoimmune vasculitis [eg, Wegener's Granulomatosis]); motor neuropathy considered of autoimmune origin (e.g. Guillain-Barre Syndrome and Myasthenia Gravis).
16) Subjects with interstitial lung disease that is symptomatic or may interfere with the detection or management of suspected drug-related pulmonary toxicity

17) Has a diagnosis of immunodeficiency or is receiving systemic steroid therapy or any other form of immunosuppressive therapy within 7 days prior to the first dose of trial treatment.

18) Is pregnant or breastfeeding, or expecting to conceive or father children within the projected duration of the trial, starting with the pre-screening or screening visit through 120 days after the last dose of trial treatment.

19) Major surgery (other than definitive lung cancer surgery) within two weeks of study or other serious concomitant disorders that in the opinion of the investigator would compromise the safety of the patient of compromise the patient's ability to complete the study.

20) Any non-oncology live-attenuated vaccine therapy used for prevention of infectious diseases (for up to 30 days before or after any dose of nivolumab, ipilimumab, and/or evolocumab). *Note: Seasonal influenza vaccines for injection are generally inactivated flu vaccines and are allowed; however, intranasal influenza vaccines (e.g., Flu-Mist®) are live attenuated vaccines and are not allowed. COVID19 vaccines will be allowed on protocol.* Recombinant, polysaccharide and RNA vaccines including for COVID19, shingles and pneumonia are allowed.

21) Myocardial infarction having occurred less than 6 months before inclusion, any known uncontrolled arrhythmia, symptomatic angina pectoris, active ischemia, or cardiac failure not controlled by medications. Patients with CAD recently treated with surgery and/or stent, if stable without symptomatic angina pectoris, active ischemia are eligible.

22) Has known psychiatric or substance abuse disorders that would interfere with cooperation with the requirements of the trial.

23) Prisoners or subjects who are compulsorily detained (involuntarily incarcerated) for treatment of either psychiatric or physical (e.g., infectious) illness.

24) Patient takes daily prednisone > 10 mg or the equivalent dose of a different steroid.

5.3 Inclusion of Women and Minorities

There are no exclusions based on gender, race or ethnicity in this trial. There is no evidence to suggest that outcomes will differ.

5.4 Protocol Eligibility Waivers

No waivers of inclusion or exclusion criteria will be granted. All prospective patients must meet all entry criteria. If there are any questions regarding the interpretation of a criterion for a potential patient, contact the Principal Investigator to discuss.

5.5 Registration Procedure

Patient registration for all patients signing informed consent will be completed through the Duke Cancer Institute (DCI) Clinical Research Unit (CRU) into Epic (electronic medical record) and OnCore (clinical trials management) systems within one (1) business day of obtaining consent. Patients will be enrolled only after all pre-treatment evaluations are completed and all eligibility criteria are met.

Registration for Outside Sites

All patients signing informed consent at outside sites (including screen failures) must be registered with the Coordinating Center. The following documents are to be completed by the Investigator and/or designee and submitted to the Coordinating Center by email:

- TOP 2101 Subject Registration Form
- Signed Informed Consent

6.0 STUDY ASSESSMENTS

The protocol Study Calendar summarizes the trial procedures to be performed at each visit. Individual trial procedures are described in detail below. It may be necessary to perform these procedures at unscheduled time points if deemed clinically necessary by the investigator. For the purpose of scheduling evaluations and to allow for patient and investigator schedules, holidays and weather or other emergencies requiring clinical facilities to be closed, a window of +/-3 days for Cycle 1 and -3 days/+ 7 days for Cycle 2+ will be applied to all Day 1 study visits unless otherwise noted. Nivolumab may not be given <12 days from previous dose. Furthermore, additional evaluations/testing may be deemed necessary by the Sponsor PI for reasons related to subject safety.

Medical History

A medical history will be obtained by the investigator or qualified designee. Medical history will include all active conditions, and any condition diagnosed within the prior 10 years that are considered to be clinically significant by the Investigator. Details regarding the disease for which the subject has enrolled in this study will be recorded separately and not listed as medical history.

Full Physical Exam

The Investigator or qualified designee will perform a complete physical exam during the screening period. Clinically significant abnormal findings should be recorded as medical history. A full physical exam should be performed during screening.

Vital Signs

The Investigator or qualified designee will take vital signs at screening, prior to the administration of each dose of trial treatment and at treatment discontinuation as specified in the Study Calendar. Vital signs should include temperature, pulse, respiratory rate, weight and blood pressure unless otherwise specified. Height will be measured at screening only.

6.1 Screening Period

During the Screening Period, subjects are consented and screened for the study. Informed consent must be obtained before initiation of any screening procedure that is performed solely for the purpose of determining eligibility for this study. Evaluations performed as part of routine care before informed consent can be considered as screening evaluations if done within the defined screening period, and if permitted by the local Institutional Review Board (IRB)/ Independent Ethics Committee policies. Study eligibility is based on meeting all of the inclusion criteria and none of the exclusion criteria (refer to Section 5.0) before the first dose of study drug on Cycle 1 Day 1.

Prior to approaching a patient for consent, the treating physician or primary investigator will review imaging studies with the procedural team (interventional radiology or interventional pulmonology) to determine if the patient has a tumor amenable for research biopsy. If the procedural team determines the patient to be an appropriate candidate for the research biopsy, the patient can then be approached for screening and enrollment into the trial.

The following study procedures must be done within 30 days prior to Cycle 1 Day 1 (unless otherwise specified). Baseline and Cycle 1 Day 1 procedures may be completed on the same day, however, screening assessment to confirm eligibility MUST have already been determined:

- Informed consent
- Eligibility criteria review (inclusion/exclusion)
- Demographics/baseline characteristics
- Smoking history

- Medical and cancer treatment history
- Physical examination
- Height
- Vital signs (temperature, blood pressure, pulse and respiratory rate) and weight
- Concomitant medications
- ECOG performance status
- Adverse event assessment (review of baseline symptoms).
- Tumor assessment (CT and/or MRI) of chest, abdomen, pelvis with or without contrast and all known or suspect sites of disease. MRI of brain or CT brain with contrast is required for all subjects.
- ECG
- Blood collection for circulating immune cells (PBMC) and circulating proteins (plasma) - may be collected pre-dose on Cycle 1 Day 1
- Tumor tissue collection

The following study procedures must be done within 14 days prior to Cycle 1 Day 1:

- CBC with differential*
- Chemistries including liver function tests (LFTs)*
- Thyroid Function (TSH, Free T4)
- Calculated Creatinine Clearance
- Urinalysis

The following must be done at screening and within 72 hours (or institutional standards) of the first study drug administration:

- Serum or urine pregnancy test (women of childbearing potential)

*Subject eligibility is determined using lab results obtained up to 14 days prior to Cycle 1 Day 1. Any laboratory assessments repeated on Cycle 1 Day 1 must meet eligibility requirements. The Screening Period ends upon receipt of the first dose of study drug or final determination that the subject is ineligible for the study.

6.2 Treatment Period

Treatment Study Day 1: Nivolumab, Ipilimumab +/- Evolocumab

To be performed prior to receiving treatment:

- Weight
- Vital signs (temperature, blood pressure, and pulse)
- ECOG performance status
- Physical exam
- Adverse events assessment
- Concomitant medication assessment
- CBC with diff, chemistries with LFTs, TSH, FT4
- Pregnancy test ≤ 3 days (72hrs) prior to first dose of nivolumab (for WOCBP only) (only required prior to first study cycle)
- Blood collection for circulating immune cells (PBMC) and circulating proteins (plasma)
- Dose #1 Nivolumab 240 mg IV
- Dose #1 Ipilimumab 1 mg/kg IV
- Dose #1 Evolocumab 140 mg SubQ

Treatment Study Day 15: Nivolumab +/- Evolocumab

- Weight
- Vital signs (temperature, blood pressure, and pulse)
- ECOG performance status
- Physical exam
- Adverse events assessment
- Concomitant medication assessment
- CBC with diff, chemistries with LFTs
- Dose #2 Nivolumab 240 mg IV
- Dose #2 Evolocumab 140 mg SubQ
- Blood collection for circulating immune cells (PBMC) and circulating proteins (plasma) – Cycle 1 Day 15 only

Treatment Study Day 29: Nivolumab +/- Evolocumab

- Weight
- Vital signs (temperature, blood pressure, and pulse)
- ECOG performance status
- Physical exam
- Adverse events assessment
- Concomitant medication assessment
- CBC with diff, chemistries with LFTs
- Dose #3 Nivolumab 240 mg IV
- Dose #3 Evolocumab 140 mg SubQ

6.3 End of Treatment

The Treatment Period ends when a subject receives his or her last dose of study treatment; the subject then enters the Follow-up Period.

6.4 Follow-up Period

Post study treatment follow-up is of critical importance and is essential to preserving subject safety and integrity of the study. Subjects are to return for Follow-Up Visit #1 35 days after their last dose of study drug (±7 days) or coinciding with the date of discontinuation of study drug (±7 days) if the date of discontinuation is greater than 42 days from the last dose for an off-treatment follow up visit for adverse event monitoring. Follow-up Visit #2 to occur 80 days from Follow-Up Visit #1 ±7 days or 100 days from discontinuation of study drug (serious adverse events will be collected for 100 days after the end of treatment as described in Section 11.2).

Subjects who discontinue for reasons other than progressive disease will have post-treatment follow-up for disease status until disease progression, initiating a non-study cancer treatment, withdrawing consent or becoming lost to follow-up (subjects to be followed for up to 2 years). This follow up should occur every 12 weeks by personal interview or review of medical records. Restaging scans for these patients will be obtained per standard of care, and if available, scans will be submitted for iRECIST until progressive disease is confirmed.

For all other subjects outcome data will be collected for 2 years after enrollment or until the study is closed (whichever comes first). Every effort should be made to collect information regarding disease during this follow-up which may include the start of new anti-neoplastic therapy, disease progression, death, or end of study. This should collected every 12 weeks by personal interviews or review of medical or public records.

Subjects are to be monitored for at least 100 days after the last dose of study drug and complete the following study procedures during Follow-Up Visits #1 and #2:

- Physical examination
- Vital signs and weight
- Concomitant medications
- ECOG performance status
- Adverse event assessment
- CBC with differential
- Chemistries including LFTs

Additional follow-up may occur for subjects with adverse events (AEs) related to study drug that are ongoing at the time of this off-treatment visit unless AE is deemed unresolvable or subject has started a new anti-cancer treatment regimen.

For subjects that are discontinued from study treatment for reasons other than disease progression, subjects will have restaging scans per standard of care schedule followed until disease progression or start of new anti-cancer treatment regimen, at the discretion of the treating physician. Disease status may be collected by personal interviews or review of medical records.

6.5 End of Study

Patient care and patient study visits are planned to continue until all patients have completed all phases of treatments and have completed Follow-Up Visit #1 and Follow-Up Visit #2 as above. Subjects will continue to be followed for survival up to two years after study therapy or until the study is closed. The study will close when two years of survival follow up is completed for all patients after their last treatment dose. Data lock will occur when two years of survival follow up is completed for all patients.

If subjects are lost to follow-up, attempts will be made to contact them via phone, email if available, and letter. Four attempts will be made by phone on a weekly basis, after which three additional attempts will be made by phone, email if available, and by letter on a monthly basis. If a subject is lost to follow-up prior to completion of Follow up Visit #1, i.e. while still actively receiving treatment on study, then initial attempts to contact patient by phone will be made daily for five days and emergency contact number will also be contacted. Subjections lost to follow up will be censored to time of last contact. Public records may be interrogated to identify patient death.

The study may also be terminated early for the following reasons:

- The investigator, for any reason, stops the study
- In the event of Amgen decision to no longer supply study drug
- Clinical criteria for Early Trial Termination
- Quality or quantity of data recording is inaccurate or incomplete
- Poor adherence to protocol and regulatory requirements
- Incidence or severity of adverse drug reaction in this or other studies indicates a potential health hazard to subjects
- Plans to modify or discontinue the development of the study drug

In the event decision by Amgen to no longer supply study drug, ample notification will be provided so that appropriate adjustments to subject treatment can be made.

6.6 Early Withdrawal of Subject(s)

Criteria for Early Withdrawal

When a subject discontinues/withdraws prior to trial completion, all applicable activities scheduled for the final trial visit should be performed at the time of discontinuation. Any adverse events which are present at the time of discontinuation/withdrawal should be followed in accordance with the safety requirements outlined in Section 11.0.

A subject must be discontinued from the trial for any of the following reasons:

- The subject or legal representative (such as a parent or legal guardian) withdraws consent
- Immune confirmed radiographic disease progression

Note: In certain cases, immune response unconfirmed progressive disease (iUPD) may require discontinuation. Specific criteria must be met to continue therapy (see Section 6.8).

- There is toxicity deemed by the investigator or subject as unacceptable
- Intercurrent illness that prevents further administration of treatment
- Investigator's decision to withdraw the subject
- The subject has a confirmed positive serum pregnancy test
- Noncompliance with trial treatment or procedure requirements
- The subject is lost to follow-up.
- Administrative reasons
- The investigator, for any reason, stops the study
- Termination of the study by Amgen
- The patient, for any reason, requires treatment with another systemic agent potentially effective for treatment of the study indication. In this case, discontinuation from the study occurs immediately upon introduction of the new agent.
- A patient who cannot be administered the study drug after a 6 week delay must be discontinued from the study treatment
- The compulsory detention for the treatment of either a psychiatric or physical (e.g. infectious disease) illness
- There is clear evidence of progressive disease

Follow-up Requirements for Early Withdrawal

When a subject discontinues/withdraws prior to trial completion, all applicable activities scheduled for the final trial visit should be performed at the time of discontinuation. Any adverse events which are present at the time of discontinuation/withdrawal should be followed in accordance with the safety requirements outlined in Section 11.0.

Subjects who discontinue for reasons other than progressive disease will have post-treatment follow-up for disease status until disease progression, initiating a non-study cancer treatment, withdrawing consent or becoming lost to follow-up (subjects to be followed for up to 2 years).

For all other subjects, outcome data will be collected for 2 years after the end of treatment. Every effort should be made to collect information regarding disease during this follow-up which may include the start of new anti-neoplastic therapy, disease progression, death, or end of study.

6.7 Subject Replacement

Subjects may be replaced during this study if they did not receive nivolumab or ipilimumab for any reason. Additionally, patients who do not have at least one pre-treatment core biopsy (or archived FFPE tissue) dedicated for the primary TIL outcome analysis or if they do not obtain an on treatment biopsy for determination of the primary outcome will also be replaced. Otherwise replacement decisions will be made between the Sponsor PI and Investigator on a case-by-case basis.

If a patient undergoes a core biopsy procedure at enrollment but there is insufficient tissue for at least one core research biopsy, the case must be discussed with the Sponsor PI. The patient may receive study treatment but will be considered non-evaluable for the primary outcome unless archived tissue is deemed sufficient for the primary TIL assessment.

6.8 Treatment beyond Progression

Accumulating evidence indicates a minority of subjects treated with immunotherapy may derive clinical benefit despite initial evidence of progressive disease (PD).

Subjects will be permitted to continue on nivolumab, ipilimumab, and/or evolocumab in cases of immune response unconfirmed progressive disease (iUPD) at the discretion of the treating physician or confirmed progression as long as they meet the following criteria:

- Investigator-assessed clinical benefit and no rapid disease progression
- Subject is tolerating study treatment
- Stable performance status
- Treatment beyond progression will not delay an imminent intervention to prevent serious complications of disease progression (e.g. CNS metastases)
- Subject provides written informed consent prior to receiving additional nivolumab, ipilimumab and/or evolocumab treatment using an informed consent form describing any reasonably foreseeable risks or discomforts, or other alternative treatment options.

The decision to continue treatment beyond initial investigator-assessed progression should be discussed and approved by the Sponsor PI and documented in the study records. Treatment should be not be delayed > 6 weeks for intervention per protocol dose delay. If treatment is not able to resume within 6 weeks, treatment continuation should be discussed with Sponsor PI.

Tumor assessments are to continue on Q6 week schedule during treatment beyond progression.

A radiographic assessment/scan should be performed within 6 weeks of initial investigator-assessed progression to determine whether there has been a decrease in the tumor size or continued progressive disease unless > 6 weeks approved by Sponsor PI.

For subjects who continue study therapy beyond progression, further progression is defined as an additional 10% increase in tumor burden with a minimum 5 mm absolute increase from time of initial progressive disease from the time of initial PD. This includes an increase in the sum of diameters of all target lesions and/ or the diameters of new measurable lesions compared to the time of initial PD. Treatment should be discontinued permanently upon documentation of further progression.

New lesions are considered measurable at the time of initial progression if the longest diameter is at least 10mm (except for pathological lymph nodes which must have a short axis of at least 15 mm). Any new lesion considered non-measurable at the time of initial progression may become measurable and therefore included in the tumor burden if the longest diameter increases to at least 10 mm (except for pathological lymph nodes which must have

a short axis of at least 15 mm). In situations where the relative increase in total tumor burden by 10% is solely due to inclusion of new lesions which become measurable, these new lesions must demonstrate an absolute increase of at least 5 mm.

7.0 DOSE MODIFICATIONS

Adverse events (both non-serious and serious) associated with nivolumab, ipilimumab, and/or evolocumab exposure may represent an immunologic etiology. These adverse events may occur shortly after the first dose or several months after the last dose of treatment. Nivolumab, ipilimumab and evolocumab must be withheld for drug-related toxicities and severe or life-threatening AEs as per **Table 7.0**. All three study drugs will be withheld together. See Section 7.1 for supportive care guidelines, including use of corticosteroids. If treatment is held, doses are not skipped but study calendar is resumed after resolution of toxicity (ie. if treatment held C3D15, therapy restarts on C3D15 once toxicity has resolved to level appropriate for treatment per Table 7.0). Imaging studies should not be delayed and should be kept on every 6 week interval.

Table 7.0: Dose Modification Guidelines for Drug-Related Adverse Events

Toxicity	Hold Treatment For Grade	Timing for Restarting Treatment[1]	Discontinue Subject
Diarrhea/Colitis lasting >48 hours	2	Toxicity resolves to Grade 0-1.	Toxicity does not resolve within 6 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 6 weeks.
	3-4	Permanently discontinue	Permanently discontinue
AST, ALT, or Increased Bilirubin	2	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 6 weeks of last dose.
	3-4	Permanently discontinue (see exception below)[2]	Permanently discontinue
Type 1 diabetes mellitus (if new onset) or Hyperglycemia	T1DM or 3-4	Hold nivolumab for new onset Type 1 diabetes mellitus or Grade 3-4 hyperglycemia associated with evidence of beta cell failure.	Resume therapy when patients are clinically and metabolically stable (including on stable insulin dose). Therapy does not need to be discontinued.
Hypophysitis	2-3	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 6 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 6 weeks.
	4	Permanently discontinue	Permanently discontinue
Hyperthyroidism	3	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 6 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 6 weeks.
	4	Permanently discontinue	Permanently discontinue
Hypothyroidism	2-4	Therapy with nivolumab can be continued while treatment for the thyroid disorder is instituted	Therapy with can be continued while treatment for the thyroid disorder is instituted.
Infusion Reaction	3-4	Permanently discontinue	Permanently discontinue
Pneumonitis	2	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 6 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 6 weeks.
	3-4	Permanently discontinue	Permanently discontinue
Renal Failure or Nephritis	2	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 6 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 6 weeks.
	3-4	Permanently discontinue	Permanently discontinue

Toxicity	Hold Treatment For Grade	Timing for Restarting Treatment[1]	Discontinue Subject
All Other Drug-Related Toxicity[3]	3 or Severe	Toxicity resolves to Grade 0-1	Toxicity does not resolve within 6 weeks of last dose or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 6 weeks.
	4	Permanently discontinue	Permanently discontinue

Note: Permanently discontinue for any severe or Grade 3 drug-related AE that recurs or any life-threatening event.

[1] In cases where treatment is held for toxicity and subsequently restarted, the initial treatment should consist of one treatment of nivolumab with or without ipilumumab at the appropriate study dose without administration of evolocumab. If toxicity has not recurred after a cycle of nivolumab with or without ipilimumab alone then subsequent cycles will include nivolumab with or without ipilimumab and evolocumab. For subjects on the control arm, not receiving evolocumab treatment will be restarted when parameters in Table 7.0 are met.

[2] For patients who begin treatment with Grade 2 AST or ALT, if AST or ALT increases by greater than or equal to 50% relative to baseline and lasts for at least 1 week then patients should be discontinued.

[3] Patients with intolerable or persistent Grade 2 drug-related AE may hold study medication at physician discretion. Permanently discontinue study drug for persistent Grade 2 adverse reactions for which treatment with study drug has been held, that do not recover to Grade 0-1 within 6 weeks of the last dose.

7.1 Supportive Care Guidelines

Subjects should receive appropriate supportive care measures as deemed necessary by the treating investigator. Suggested supportive care measures for the management of adverse events with potential immunologic etiology are outlined below. Where appropriate, these guidelines include the use of oral or intravenous treatment with corticosteroids as well as additional anti-inflammatory agents if symptoms do not improve with administration of corticosteroids. Note that several courses of steroid tapering may be necessary as symptoms may worsen when the steroid dose is decreased. For each disorder, attempts should be made to rule out other causes such as metastatic disease or bacterial or viral infection, which might require additional supportive care. The treatment guidelines are intended to be applied when the investigator determines the events to be related to nivolumab, ipilimumab and evolocumab.

It may be necessary to perform conditional procedures such as bronchoscopy, endoscopy, or skin photography as part of evaluation of the event.

7.1.1 Pneumonitis

For **Grade 2 events**, treat with systemic corticosteroids. When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks.
For **Grade 3-4 events**, immediately treat with intravenous steroids. Administer additional anti-inflammatory measures, as needed.

Add prophylactic antibiotics for opportunistic infections in the case of prolonged steroid administration.

7.1.2 Diarrhea/Colitis

Subjects should be carefully monitored for signs and symptoms of enterocolitis (such as diarrhea, abdominal pain, blood or mucus in stool, with or without fever) and of bowel perforation (such as peritoneal signs and ileus). All subjects who experience diarrhea/colitis should be advised to drink liberal quantities of clear fluids. If sufficient oral fluid intake is not feasible, fluid and electrolytes should be substituted via IV infusion.

For **Grade 2 or higher diarrhea**, consider GI consultation and endoscopy to confirm or rule out colitis.
For **Grade 2 diarrhea/colitis** that persists greater than 3 days, administer oral corticosteroids.
For **Grade 3 or 4 diarrhea/colitis** that persists > 1 week, treat with intravenous steroids followed by high dose oral steroids.

When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks.

7.1.3 Hyperglycemia

Type 1 diabetes mellitus (if new onset, including diabetic ketoacidosis [DKA]) or ≥ Grade 3 Hyperglycemia, if associated with ketosis (ketonuria) or metabolic acidosis (DKA)

For **T1DM** or **Grade 3-4** Hyperglycemia
Insulin replacement therapy is recommended for Type I diabetes mellitus and for Grade 3-4 hyperglycemia associated with metabolic acidosis or ketonuria.

Evaluate patients with serum glucose and a metabolic panel, urine ketones, glycosylated hemoglobin, and C-peptide.

7.1.4 Hypophysitis

For **Grade 2** events, treat with corticosteroids. When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks. Replacement of appropriate hormones may be required as the steroid dose is tapered.
For **Grade 3-4** events, treat with an initial dose of IV corticosteroids followed by oral corticosteroids. When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks. Replacement of appropriate hormones may be required as the steroid dose is tapered.

7.1.5 Hyperthyroidism or Hypothyroidism

Thyroid disorders can occur at any time during treatment. Patients will be monitored for changes in thyroid function (at the start of treatment, periodically during treatment, and as indicated based on clinical evaluation) and for clinical signs and symptoms of thyroid disorders.

Grade 2 hyperthyroidism events (and **Grade 3-4** hypothyroidism):
- In hyperthyroidism, non-selective beta-blockers (e.g. propranolol) are suggested as initial therapy.
- In hypothyroidism, thyroid hormone replacement therapy, with levothyroxine or liothyroinine, is indicated per standard of care.

Grade 3-4 hyperthyroidism
- Treat with an initial dose of IV corticosteroid followed by oral corticosteroids.

When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks. Replacement of appropriate hormones may be required as the steroid dose is tapered.

7.1.6 Hepatic

For **Grade 2** events, monitor liver function tests more frequently until returned to baseline values (consider weekly). Treat with IV or oral corticosteroids
For **Grade 3-4** events, treat with intravenous corticosteroids for 24 to 48 hours.

When symptoms improve to Grade 1 or less, a steroid taper should be started and continued over no less than 4 weeks.

7.1.7 Renal Failure or Nephritis

For **Grade 2** events, treat with corticosteroids.
For **Grade 3-4** events, treat with systemic corticosteroids.

When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks.

7.1.8 Infusion Reactions

Signs and symptoms usually develop during or shortly after drug infusion and generally resolve completely within 24 hours of completion of infusion.

Table 7.1.8 shows treatment guidelines for subjects who experience an infusion reaction associated with administration of nivolumab, ipilimumab, or evolocumab.

Table 7.1.8 Infusion Reaction Treatment Guidelines

NCI CTCAE Grade	Treatment	Premedication at subsequent dosing
Grade 1 Mild reaction; infusion interruption not indicated; intervention not indicated	Increase monitoring of vital signs as medically indicated until the subject is deemed medically stable in the opinion of the investigator.	None
Grade 2 Requires infusion interruption but responds promptly to symptomatic treatment (e.g., antihistamines, NSAIDS, narcotics, IV fluids); prophylactic medications indicated for < =24 hrs	**Stop Infusion and monitor symptoms.** Additional appropriate medical therapy may include but is not limited to: IV fluids Antihistamines NSAIDS Acetaminophen Narcotics Increase monitoring of vital signs as medically indicated until the subject is deemed medically stable in the opinion of the investigator. If symptoms resolve within one hour of stopping drug infusion, the infusion may be restarted at 50% of the original infusion rate (e.g., from 100 mL/hr to 50 mL/hr). Otherwise dosing will be held until symptoms resolve and the subject should be premedicated for the next scheduled dose. **Subjects who develop Grade 2 toxicity despite adequate premedication should be permanently discontinued from further trial treatment administration.**	Subject may be premedicated 1.5h (± 30 minutes) prior to infusion with: Diphenhydramine 50 mg po (or equivalent dose of antihistamine). Acetaminophen 500-1000 mg po (or equivalent dose of antipyretic).
Grades 3 or 4 Grade 3: Prolonged (i.e., not rapidly responsive to symptomatic medication and/or brief interruption of infusion); recurrence of symptoms following initial improvement; hospitalization indicated for other clinical sequelae (e.g., renal impairment, pulmonary infiltrates) Grade 4: Life-threatening; pressor or ventilatory support indicated	**Stop Infusion.** Additional appropriate medical therapy may include but is not limited to: IV fluids Antihistamines NSAIDS Acetaminophen Narcotics Oxygen Pressors Corticosteroids Epinephrine Increase monitoring of vital signs as medically indicated until the subject is deemed medically stable in the opinion of the investigator. Hospitalization may be indicated. **Subject is permanently discontinued from further trial treatment administration.**	No subsequent dosing
Appropriate resuscitation equipment should be available in the room and a physician readily available during the period of drug administration.		

7.1.9 Restarting Treatment after Resolved Toxicity

In certain cases, as defined in **Table 7.0**, treatment may be restarted after resolution of toxicity or appropriate treatment of certain toxicities (e.g. endocrine disorders). In such cases treatment should be restarted by administering one treatment of nivolumab and/or ipilimumab (if day 1 of a cycle) at the appropriate study dose without administration of evolocumab. If toxicity has not recurred after a treatment of nivolumab and/or ipilimumab then subsequent treatments will include evolocumab.

If a cycle is delayed, that same cycle will begin when subject meets protocol parameters to resume treatment (with the exception of holding evolocumab with the first dose).

Of note, ipilimumab should be discontinued and not resumed for any grade 3 or higher immune related toxicity, with the exception of endocrinopathies that are treated with hormone replacement.

If a grade 3 or 4 infusion/hypersensitivity reaction occurs to either nivolumab, ipilimumab, or evolocumab, treatment will be discontinued.

7.2 Safety Considerations

Nivolumab and ipilimumab are approved for the treatment of metastatic NSCLC and toxicity profiles of nivolumab and other immunotherapy agents are well established especially with regard to immune mediated toxicities considered to be typical of this class of medications.

Evolocumab and other PCSK9 inhibitors have been studied in large randomized phase III clinical trials and have demonstrated a favorable safety profile. No suggestion of increased autoimmune events or other immune mediated toxicity has been reported with these agents. However, the combination of PCSK9 and PD1 inhibition has never been reported in the setting of a clinical trial, and thus the study will pause after the first 6 patients receive evolocumab in addition to ipilimumab and nivolumab (including those that cross over).

7.2.1 Missed Doses

Dosing interruptions are permitted in the case of medical / surgical events or logistical reasons not related to study therapy (e.g., elective surgery, unrelated medical events, patient vacation, and/or holidays). Subjects should resume study therapy within 3 weeks of the scheduled interruption, unless otherwise discussed with the Sponsor PI. The reason for interruption should be documented in the patient's study record.

If dose interruptions are the result of a drug-related adverse event then further treatment should follow the guidelines for dose modification given in **Table 7.0**. In cases of resolved or adequately treated toxicity in which restarting study treatment is acceptable according to **Table 7.0**, the first treatment should consist of nivolumab and/or ipilimumab without evolocumab. If no further adverse events are noted after nivolumab and/or ipilimumab, then subsequent treatments will include evolocumab.

7.2.2 Concomitant Medications

Concomitant medications will be documented throughout the study. Medications specifically prohibited in the exclusion criteria are not allowed during the ongoing trial. If there is a clinical indication for one of these or other medications or vaccinations specifically prohibited during the trial, discontinuation from trial therapy may be required. The Investigator should discuss any questions regarding this with the Sponsor PI.

Acceptable Concomitant Medications

All treatments that the Investigator considers necessary for a subject's welfare may be administered at the discretion of the Investigator in keeping with the local standards of medical care. All concomitant medication received from the date of signed informed consent through 30 days after the last dose of study drug should be recorded in the electronic medical records including all prescription, over-the-counter (OTC), herbal supplements, and IV medications.

Prohibited Concomitant Therapy

Subjects are prohibited from receiving the following therapies during the Screening and Treatment Period of this study:

- Antineoplastic systemic chemotherapy or biological therapy
- Immunotherapy not specified in this protocol

- Systemic immunosuppressive agents > equivalent dose of 2 mg decadron daily or > 10 mg prednisone or equivalent
- Other investigational agents
- Live vaccines within 30 days prior to the first dose of study drug and while participating in the study. Examples of live vaccines include, but are not limited to, the following: measles, mumps, rubella, varicella/zoster, yellow fever, rabies, BCG, and typhoid vaccine. *Recombinant, polysaccharide and RNA vaccines including for COVID19, shingles and pneumonia are allowed.*
- Systemic glucocorticoids for any purpose other than to modulate symptoms from an event of clinical interest of suspected immunologic etiology. The use of physiologic doses of corticosteroids per **Table 7.0** is permitted.
- Palliative XRT to non-target lesion **is allowed** on study.

Subjects who, in the assessment by the Investigator, require the use of any of the aforementioned treatments for clinical management should be removed from the trial. Subjects may receive other medications that the Investigator deems to be medically necessary.

There are no prohibited therapies during the Follow-Up Period.

8.0 STUDY DRUGS

Nivolumab is given prior to ipilimumab per standard of care. There is no waiting period between infusions. Evolocumab will be given after ipilimumab on Day 1 of treatment and after nivolumab on Day 15 & Day 29. There is no observation period after evolocumab.

8.1 Randomization

Up to 38 eligible patients will be randomized to nivolumab & ipilimumab or nivolumab, ipilimumab and evolocumab with equal allocations to reach a target sample size of 30 evaluable patients for the efficacy outcome.

This is an open-label study; therefore, each patient will be aware of his or her own assigned treatment. All staff involved in treating and caring for study patients will have full knowledge of treatment assignments for those patients under their care.

8.2 Rationale for Selection of Dose, Regimen, and Treatment Duration

Dose selection for nivolumab, ipilimumab, and evolocumab are chosen from standard FDA recommended dosing suggestions on the basis of randomized phase III trials. The dose of nivolumab 240 mg IV Q2W and ipilimumab 1 mg/kg IV every 6 weeks is from the FDA approved regimen, and evolocumab 140 mg Q2W via subcutaneous administration is a standard of care dose.

8.3 Nivolumab

Names, Classification, and Mechanism of Action

Nivolumab (also referred to as Opdivo, BMS-936558 or MDX1106) is a human monoclonal antibody (HuMAb; immunoglobulin G4 [IgG4]-S228P) that targets the programmed death-1 (PD-1) cluster of differentiation 279 (CD279) cell surface membrane receptor. PD-1 blockade by nivolumab is a promising avenue to pursue as an anti-tumor therapy for recurrent or treatment-refractory malignancies or as an anti-viral therapy for chronic viral infections.

Packaging and Labeling

Please see Investigational Brochure for Nivolumab for complete information.

Supply, Receipt, and Storage
Nivolumab is standard of care therapy on this trial.

Nivolumab is a sterile, preservative-free, non-pyrogenic, clear to opalescent, colorless to pale-yellow liquid that may contain light (few) particles. OPDIVO injection for intravenous infusion is supplied in single-dose vials. Each mL of OPDIVO solution contains nivolumab 10 mg, mannitol (30 mg), pentetic acid (0.008 mg), polysorbate 80 (0.2 mg), sodium chloride (2.92 mg), sodium citrate dihydrate (5.88 mg), and Water for Injection, USP. May contain hydrochloric acid and/or sodium hydroxide to adjust pH to 6. After receipt, nivolumab is stored under refrigeration at 2°C to 8°C (36°F to 46°F). It should be protected from light by storing in the original package until time of use. Do not freeze or shake.

Dispensing and Preparation
Please see Investigational Brochure for Nivolumab for complete information.

Visually inspect drug product solution for particulate matter and discoloration prior to administration. OPDIVO is a clear to opalescent, colorless to pale-yellow solution. Discard the vial if the solution is cloudy, discolored, or contains extraneous particulate matter other than a few translucent-to-white, proteinaceous particles. Do not shake the vial.

Preparation
- Withdraw the required volume of nivolumab and transfer into an intravenous container.
- Dilute nivolumab with either 0.9% Sodium Chloride Injection, USP or 5% Dextrose Injection, USP to prepare an infusion with a final concentration ranging from 1 mg/mL to 10 mg/mL. The total volume of infusion must not exceed 160 mL.
- Mix diluted solution by gentle inversion. Do not shake.
- Discard partially used vials or empty vials of nivolumab.

Storage of Infusion
The product does not contain a preservative. After preparation, store the nivolumab infusion either: at room temperature for no more than 8 hours from the time of preparation. This includes room temperature storage of the infusion in the IV container and time for administration of the infusion or under refrigeration at 2°C to 8°C (36°F to 46°F) for no more than 24 hours from the time of infusion preparation. Do not freeze.

Administration
Administer the infusion over 30 minutes through an intravenous line containing a sterile, non-pyrogenic, low protein binding in-line filter (pore size of 0.2 micrometer to 1.2 micrometer). Do not coadminister other drugs through the same intravenous line. Flush the intravenous line at end of infusion.

8.4 Ipilimumab

Recommended Storage and Use Conditions
Ipilimumab is standard of care therapy on this trial.

Ipilimumab 5mg/ml vials is supplied packaged 4 vials per carton. Ipilimumab injection (5 mg/mL) can be used for intravenous (IV) administration without dilution after transferring to a polyvinyl chloride (PVC), non-PVC/non-di-(2-ethylhexyl) phthalate (DEHP), or glass container and is stable for 24 hours at 2°C to 8°C or room temperature/room light. Ipilimumab injection may be diluted in 0.9% Sodium Chloride Injection, United States Pharmacopeia (USP) or 5% Dextrose Injection, USP to concentrations between 1 and 4 mg/mL and stored in PVC, non-PVC/non-DEHP, or glass containers for up to 24 hours at 2°C to 8°C or room temperature/room light. The product may be infused using a volumetric pump at the protocol-specific dose(s) and rate(s) through a PVC

IV solution infusion set with an in-line, sterile, nonpyrogenic, low-protein-binding filter (pore size of 0.2 to 1.2 m). Ipilimumab injection must not be administered as an IV push or bolus injection. Care must be taken to assure sterility of the prepared solutions since the drug product does not contain any antimicrobial preservatives or bacteriostatic agents.

Ipilimumab injection, 50 mg/10 mL (5 mg/mL) or 200 mg/40 mL (5 mg/mL), must be stored refrigerated (2°C to 8°C) and protected from light. Ipilimumab injection must not be frozen. Partially used vials or empty vials of ipilimumab injection should be discarded at the site according to appropriate drug disposal procedures.

Ipilimumab injection may be stored undiluted (5 mg/mL) or following dilution in 0.9% Sodium Chloride Injection, USP or 5% Dextrose Injection, USP in PVC, non-PVC/non-DEHP, or glass containers for up to 24 hours at 2°C to 8°C or room temperature/room light.

Recommended safety measures for preparation and handling include protective clothing, gloves, and safety cabinets.

8.5 Evolocumab

8.5.1 Names, Classification, and Mechanism of Action

Evolocumab (also known as Repatha) is a human monoclonal immunoglobulin G2 (IgG2) directed against human proprotein convertase subtilisin kexin 9 (PCSK9). Evolocumab has an approximate molecular weight (MW) of 144 kDa and is produced in genetically engineered mammalian (Chinese hamster ovary) cells.

Evolocumab binds to PCSK9 and inhibits circulating PCSK9 from binding to the low-density lipoprotein (LDL) receptor (LDLR), preventing PCSK9-mediated LDLR degradation and permitting LDLR to recycle back to the liver cell surface. By inhibiting the binding of PCSK9 to LDLR, evolocumab increases the number of LDLRs available to clear LDL from the blood, thereby lowering LDL-C levels.

In the context of malignancy, we have demonstrated in pre-clinical models that PCSK9 additionally binds to MHC-I antigen presentation molecules and results in their endosomal degradation, thus decreasing tumor antigen presentation and facilitating tumor immune evasion. In preclinical models of immunologic response to cancer, we show that the downregulating PCSK9 either with genetic approaches or with the use of blocking antibodies to PCSK9 increases antigen presentation and synergizes with PD1 blockade to improve tumor immune responses.

8.5.2 Packaging and Labeling

Please see US package insert for Repatha for complete information.

8.5.3 Supply, Receipt, and Storage

Repatha will be supplied by Amgen as a 140 mg/mL single-dose prefilled SureClick® autoinjector, containing 140 mg evolocumab for subcutaneous injection. Evolocumab is a sterile, preservative-free, clear to opalescent, colorless to pale yellow solution. Each 1 mL single-dose prefilled SureClick® autoinjector contains 140 mg evolocumab, acetate (1.2 mg), polysorbate 80 (0.1 mg), proline (25 mg) in water for Injection, USP. Sodium hydroxide may be used to adjust to a pH of 5.0. Store refrigerated at 2° to 8°C (36° to 46°F) in the original carton to protect from light. Do not freeze. Do not shake.

8.5.4 Dispensing and Preparation

Please see US package insert for Repatha for complete information.

Each 1 mL single-dose prefilled SureClick® autoinjector contains 140 mg evolocumab. Evolocumab is administered by subcutaneous injection to the thigh, abdomen, or upper arm. In this study evolocumab will be administered in the treatment room after nivolumab and ipilimumab.

- To administer the 140 mg dose, give 1 evolocumab injection by subcutaneous injection to the thigh, abdomen, or upper arm.
- Evolocumab will be given after ipilimumab on Day 1 of each cycle and after nivolumab on Day 15 and Day 29. May be given immediately following these infusions and no observation time is required following administration.
- Keep evolocumab in the refrigerator until ready to use. Prior to use, remove the SureClick® autoinjector from the package and allow to warm to room temperature for at least 30 minutes. Do not warm in any other way.
- Visually inspect the SureClick® autoinjector. Check the medicine in the window for particles and discoloration prior to administration. Evolocumab is a clear to opalescent, colorless to pale yellow solution. Do not use if the solution is cloudy or discolored or contains particles.
- Gather all materials needed for the injection.
- Prepare and clean the injection site.
- Administer evolocumab by subcutaneous injection into areas of the abdomen, thigh, or upper arm that are not tender, bruised, red, or hard.
- Do not co-administer evolocumab with other injectable drugs at the same injection site.
- Rotate the injection site with each injection.

8.5.5 Compliance and Accountability

It is the responsibility of the investigator to ensure that a current record of evolocumab disposition is maintained at each study site where evolocumab is inventoried and disposed. This inventory must be available for monitoring. Records or logs must comply with applicable regulations and guidelines, and should include:

- Amount received and placed in storage area.
- Amount currently in storage area.
- Label ID number or batch number and use date or expiry date.
- Dates and initials of person responsible for each evolocumab inventory entry/movement.
    - Amount dispensed to and returned by each subject, including unique subject identifiers.
    - Amount transferred to another area/site for dispensing or storage.
    - Non-study disposition (e.g., lost, wasted, broken).
    - Amount destroyed at study site.

All supplies, including unused, partially used or empty containers will be destroyed according to sites drug destruction policy.

8.5.6 Disposal and Destruction

All supplies, including unused, partially used or empty containers will be destroyed in accordance to Duke's Investigational Drug Services Drug Disposal Policy and Procedure. At completion of the study, to satisfy regulatory requirements regarding drug accountability, all unused study drug will be reconciled and destroyed in accordance with applicable state and federal regulations. The Duke Department of Pharmacy is responsible for ensuring that these procedures are performed and documented in accordance to federal regulations and Duke Policies and Procedures.

9.0 CORRELATIVE STUDIES

9.1 Rationale for Correlative Studies

A series of correlative analyses will be conducted to elucidate to what extent evolocumab plus nivolumab and ipilimumab treatment increases MHC class I expression on tumor cells and how the combination influences T cell activity within the tumor microenvironment. Pre-treatment tumor biopsy and on-treatment tumor biopsy samples will be assessed by FACS to evaluate the pharmacodynamic change of MHC-I on the tumor cells in response to treatment. Tumor samples will be further examined for immune cell infiltration and FACS analyses of the disaggregated tumors will be conducted. Based on the flow analyses, additional IHC analyses can be performed to evaluate specific key immune cell populations and gain further insights into the spatial relationship between the immune cell populations. RNA profiling will also be performed on the pre- and on-treatment tumor samples to determine if certain gene expression patterns associate with the presence of radiographic tumor response by RECIST. Further, PCSK9 expression levels (as well as other key markers of interest) will be directly assessed using RT-PCR methodology, generating more specific and quantifiable gene expression data. Biopsy specimens will additionally be sent to Xilis, Inc. for MOS generation. Patient-derived organoids will be exposed to the same pharmacologic agents utilized in this study in order to evaluate drug performance in MOS.

9.2 Tumor Tissue Collection

All patients MUST have pre-treatment and on-treatment biopsy to allow analysis of TIL and MHC-1 expression. Patients must have a research biopsy to allow analysis of tumor cell MHC-I expression by flow cytometry, which can be obtained while undergoing a standard of care diagnostic biopsy, OR undergo an elective pre-treatment research only biopsy. Biopsy alternatives include bronchoscopic biopsy, percutaneous CT guided biopsy, or surgical biopsy (i.e. mediastinoscopic biopsy) as indicated by the clinical case and the imaging findings – anatomic location and clinical staging – of the suspected malignancy. If a clinically indicated diagnostic biopsy is performed, at least three and preferably four additional core needle biopsy samples (needle gauge 19 or larger) should be obtained for research use. A fine needle aspirate (FNA) will not be considered sufficient.

Patients who agree to undergo evaluation for elective pre-treatment 'research only' biopsy will be evaluated for one of two procedures, depending on the results of their thoracic imaging results and the corresponding anatomic location of their lung cancer or suspected lung cancer. 1) Patients with centrally located tumors or lymph nodes that are considered potentially accessible by bronchoscopy will have case and imaging reviewed by interventional pulmonologist and if the procedural specialist considers the target lesion to be both accessible and without excessive procedural risk the patient may choose to undergo an elective, research only biopsy via bronchoscopy using endobronchial ultrasound guided (EBUS) biopsy, with at least three (and preferably four) core needle biopsy samples (needle gauge 19 or larger). 2) Patients with peripherally located tumors considered potentially accessible by percutaneous CT guided approach will have case and imaging reviewed by interventional radiologist and if the procedural specialist considers the target lesion to be both accessible and without excessive procedural risk the patient may choose to undergo an elective, 'research only' biopsy by CT guided core needle biopsy, with at least three and preferably four core needle biopsy samples (needle gauge 19 or larger). Tissue processing and handling information is provide in Lab Manual.

If the research biopsy has insufficient tissue for the primary outcome analysis, archived tissue may be accessed if available. Archived FFPE samples would be utilized for the primary outcome of T cell infiltration measure by a CD3 immunohistochemical stain.

Patients are ineligible for the study if biopsy is thought to confer excessive procedural risk due to either anatomic location of the tumor or due to patient specific factors.

Tumor tissue will be evaluated for treatment effects of nivolumab, ipilimumab and evolocumab on both tumor and infiltrating immune cells. Pre- and on-treatment (Day 21) samples will be analyzed for multiple endpoints

using multiple methods. For FACS and scRNAseq analyses, up to 2 fresh needle core biopsies will be obtained from all at both time points. Tissues will be processed within hours and disaggregated cells will be cryopreserved and stored in liquid nitrogen freezers for subsequent batch testing in flow-based assays. In addition, up to 2 additional cores will be obtained and preserved in FFPE for downstream IHC and RNA expression analyses. Core(s) will be sent either fresh or frozen to Xilis, Inc. for organoid generation using their MOS platform.

9.3 Blood Collection

Research peripheral blood specimens will be collected at baseline (prior to receipt of study treatment on Cycle 1 Day 1), Cycle 1 Day 15, first re-imaging (6 weeks), and progression.

Progression research blood defined as:

- iCPD or iUPD if discontinuing study without confirmation per provider discretion.
- Research blood will be drawn at progression for subjects that discontinue treatment for toxicity.
- Subjects completing 2 years on treatment without progression will be followed for progression and have research blood drawn at progression.
- Subjects who discontinue treatment without progression and start new treatment will **NOT** have research blood drawn.

Blood will be collected to assess longitudinal analyses of T cell and MDSC phenotypes and blood based biomarkers. Blood will be collected in three 8.5 ml ACD (yellow top) and one 10 ml EDTA (purple top) tubes at each time point. Blood tubes will be processed for PBMC and plasma isolation by the Duke Substrate Services Core Research Support (SSCRS) Laboratory and stored until transfer to the Duke Immune Profiling Core (DIPC) Laboratory for analysis led by Dr. Kent Weinhold.

9.4 Analyses

Assess the pharmacodynamic change in MHC class I protein expression on tumor cells in patients treated with nivolumab, ipilimumab and evolocumab.

A secondary endpoint of this study is the change in MHC-I levels in tumor cells after treatment. Tumor-infiltrating lymphocytes (TILs) and MDSC's will be isolated from tumor tissues harvested prior to treatment and on-treatment biopsies. Cells will be obtained from individual patients using acid citrate dextrose (ACD) tubes, cryopreserved, and stored in LN2 freezers for downstream batch analysis. All FACS-based immune cell correlates will be performed in collaboration with the Duke Immune Profiling Core (DIPC) laboratory under the direction of Dr. Kent J. Weinhold. The DIPC represents one of only a few facilities nationwide offering highly standardized/validated assays for polyfunctional T-cell analysis. Following isolation, TILs and MDSCs will be cryopreserved and stored in liquid nitrogen freezers for subsequent batch testing in flow-based assays.

All FACS analyses will be conducted on a FAC Symphony A5 analyzer from BD Biosciences, capable of measuring up to 44 fluorophores simultaneously and equipped with an Acoustic Focusing Sampler Option (AFS). The AFS optimizes acquisition of samples with limited numbers of cells (such as TILs) and is designed with microfluidics to wash and introduce cells to the cytometer to reduce background fluorescence and coincidence. Use of this technology allows the limited amounts of pre-treatment tissue biopsy material to be comprehensively profiled. As high dimensional arrays are developed, we maintain open channels to easily incorporate additional assays using not already dedicated to the core markers within any panel. For the evaluation of MHC-I levels on tumor cells, we will incorporate CD45 and HLA ABC (MHC-I) assays into a validated, pre-existing panel. Based on our preliminary findings, we hypothesize that MHC-I levels will increase upon treatment.

Characterize treatment-related changes in tumor infiltrating immune cells using FACS analysis.

A custom, high-dimensional (HD) polychromatic flow cytometry panel will be used to analyze changes in the immune cell subtypes that are expected to be altered by treatment. The HD panel will include markers to identify CD4+ & CD8+ T cells, T cell activation, maturation, regulation, proliferation, inhibition, transcription factors; Tfh cells, B cells, NK cells, NK T cells, MDSCs, monocytes (conventional and non-conventional), DCs (pDC, mDC), plus additional markers for activation and inhibition (**Table 9.4**). As discussed above, a key feature in this panel is our ability to incorporate additional assays using the two open channels (shown in yellow). The markers included in this panel have been reported to be of value in the context of prognostic or predictive immune markers in cancer studies (34-37). Comparisons between pre- and post-treatment tumor will be used to identify potential tissue-specific responses to therapy. Data will be analyzed using novel computational methods developed at Duke that can be used to identify multi-dimensional populations and evaluate changes in these populations across treatment (38, 39).

	Marker	Stain	Purpose	Clone
30-color Tumor Reactive T cells, Exhaustion, Treg, MDSC, B, NK, NKT Panel	Zombie	Surface	Dead cell exclusion	*na*
	CD16	Surface	Lineage exclusion, NK cells, Monocytes	3G8
	CD56	Surface	Lineage exclusion, NK cells	HCD56
	CD19	Surface	Lineage exclusion	HIB19
	CD20			2H7
	CD3	Intracellular	T- cells	SK7
	CD4	Intracellular	CD4+ (helper) T cells	SK3
	CD8α	Intracellular	CD8+ (cytotoxic) T cells	SK1
	CD28	Surface	Co-stimulation	CD28.2
	CD38	Surface	Activation	HB7
	CD197 (CCR7)	Surface	Maturation	G043H7
	CD45RA	Surface	Maturation	HI100
	CD279 (PD1)	Surface	Activation/Exhaustion	EH12.2H7
	CD152 (CTLA4)	Intracellular	Regulation	BNI3
	Ki67	Intracellular	Proliferation	Ki67
	CD95	Surface	Activtion, Pre-apoptosis	DX2
	CD278 (ICOS)	Surface	Activation	DX29
	CD366 (TIM3)	Surface	Activation/Exhaustion	7D3
	CD223 (Lag3)	Surface	Activation/Exhaustion	3DS223H
	CD274 (PDL1)	Surface	Activation/Exhaustion	MIH1
	CD273 (PDL2)	Surface	Activation/Exhaustion	MIH18
	CD276 (B7H3)	Surface	Activation/Exhaustion	7-517
	CD25	Surface	Activation, Tregs	2A3
	CD127	Surface	Tregs	A019D5
	CD194 (CCR4)	Surface	Tregs, MDSC's	L291H4
	CD14	Surface	MDSC's, Monocytes	61D3
	HLA-DR	Surface	Surface	G46-6
	CD45	Surface	Maturation	2D1
	HLA ABC	Surface	MHC class I	*G46-2.6*

Table 9.4. Expanded FACS panels for flow-based analysis of circulating immune cell subpopulations and tumor MHC-I

Characterize treatment-related changes in RNA expression using RNAseq and RT-PCR analyses.

The application of high throughput sequencing for the study of the transcriptome continues to be a powerful and versatile approach for quantifying RNA expression (41). RNA sequencing offers the advantages of an increased dynamic range, increased sensitivity, and increased accuracy in the quantification of RNA expression levels. We will evaluate the expression of various tumor and immune regulators in tumor samples before and during evolocumab and nivolumab treatment using RNAseq technology. Tumor sections will be macro-dissected, mRNA extracted, NGS libraries constructed and RNAseq performed. In addition, RNAseq results will be confirmed using quantitative RT-PCR approaches for specific measurement of PCSK9 and any other key biomarker identified in our analysis. Results of the RNAseq/RT-PCR analyses will be associated with clinical variables, flow cytometric data, and other biomarker results to further characterize this patient population. Changes in specific genes or gene signatures may provide additional insights into the molecular pathways affected by PSCK9 inhibition and MHC-I upregulation. Specifically, we will measure baseline PCSK9 expression by RNAseq to assess the difference in expression between patients with biologic response vs patients without biologic response (based on the MHC primary outcome).

Evaluate the impact of genomic subgroups on the biologic primary outcome of change in MHC expression.

NGS based DNA sequencing will be obtained on tumors per clinical standard of care. In exploratory analysis, patients with STK11 or KEAP1 mutated tumors will be evaluated as a subgroup to assess the primary biologic outcome (change in TIL), progression free survival and response rate. Patients will be included in this analysis if they have standard of care next generation sequencing results to detect somatic DNA mutations.

Explore strategies to further the impact of PCSK9 inhibition using fully autologous matched human TIL, organoid and PDX lines.

Generation of patient-derived organoid and TIL models. We will use our organoids from lung cancer patients 42). We will conduct the RNA-seq and whole exome sequencing (WES) to fully characterize the organoids transcriptionally and genetically. Previously, it has been demonstrated that *ex vivo* cultured organoids could re-capitulate both the histopathological as well as genetic features of the human tumors. RNA-seq will yield information on the key molecular pathways present in the tumor cells as well within the TIME. Those information will be invaluable in allowing us to identify the key molecular factors that determine the responsiveness of patients' tumors to immunotherapy. Organoids established from both tumor tissues as well as from adjacent normal tissues will be analyzed.

We will generate tumor infiltrating lymphocyte (TIL) cell lines derived from patient tumor specimens with matched organoid models. TILs will characterized phenotypically using FACS analysis using the following panels of antibodies listed in **Table 9.4** that we have already optimized. The various immune checkpoint proteins and costimulatory molecule receptors will be assessed on T cell subsets.

TIL plus organoid analysis. Initial testing of the various immune-engineering approaches proposed will be done with the high throughput *in vitro* model where we will co-culture TILs with organoids. TILs will be added to wells of a 24 well plate containing a droplet of organoids embedded in Matrigel under varying experimental conditions. Tumor cell killing will be assessed using the IncuCyte Cytotox Red reagent and imaged with the Incucyte Zoom HD live cell imaging system (Essen Bioscience, Michigan, USA). The system is installed in a 37^{o}C/5% CO_2 cell culture incubator and the wells will be imaged with 4X objective lens at 6 hour time intervals for a total of 7 days. Phase and red fluorescence images will be captured during the experiment. Tiled 4X images from each well will be stitched together by the Incucyte software to provide whole well images. In addition, the supernatants from each of the wells will be recovered and the amounts of various cytokines and chemokines secreted will be determined using Bio-Plex system (17-plex analysis of the following cytokines: G-CSF, GM-CSF, IFNγ, IL-1 β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12, IL-13, IL-17, MCP-1, MIP-1β, and TNF-α).

Assess ability to successfully generate viable patient-derived MOS tumor organoids from core biopsy specimens and to evaluate drug response in MOS.

Tissue samples will be shipped fresh or frozen to Xilis, Inc. Frozen tissue samples will be stored in Antonia Lab until shipment. Once tissue sample is received by Xilis, Inc., the tissue core will be processed per standard operating procedure. Established MOS will be treated with/without ipilimumab/nivolumab and evolocumab and assessed over time for T-cell mediated potency. Utilizing these various data generated from the MOS assay, the relationship between MOS drug response and patient outcome will be examined. Given small sample size, this will be purely exploratory.

10.0 STATISTICAL METHODS AND DATA ANALYSIS

All statistical analysis will be performed under the direction of the statistician designated in key personnel. Any data analysis carried out independently by the investigator must be approved by the statistician before publication or presentation.

Analysis Sets

Patients with adequate pretreatment and post treatment biopsies will be evaluated for the efficacy outcome, in the efficacy evaluable set.

All evaluable patients treated by at least one dose of nivolumab and ipilimumab with or without evolocumab combination therapy will be included in the analysis of safety and response rate, PFS, and OS.

Patient Demographics and Other Baseline Characteristics

Descriptive statistics on patient demographics and other baseline characteristics will be provided. Mean, standard deviation, median, IQR, and range will be calculated for continuous variables, such as age at enrollment or at disease diagnosis. Frequencies and percentages will be provided for categorical variables, such as race, sex, ECOG performance status, smoking status, disease clinical stage, and histological type.

10.1 Statistical Considerations

The randomized pilot study is to evaluate the safety and the efficacy of the experimental therapy evolocumab in combination with ipilimumab and nivolumab (arm A) relative to the control ipilimumab and nivolumab (arm B) among treatment naive, stage IV NSCLC with tumor PD-L1 expression <50%.

The primary objectives are (1) to assess the change of CD3+ TILs on tumor cells between on-treatment versus pre-treatment biopsy specimens, and (2) to evaluate the safety and tolerability of the experimental therapy; Secondary objectives are to assess the change of MHC-I expression on tumor cells, objective response rate (ORR) by immune RECIST and progression free survival (PFS) of the experimental therapy. P values reported for the secondary objectives are exploratory in nature and meant for future hypothesis generation.

We assume that 80% of randomized patients meet eligibility criteria and have evaluable measures at pre-treatment and on-treatment. Therefore, up to 38 patients will be randomized to arm A and arm B with equal allocation to reach a target sample size of 30 evaluable patients for the efficacy outcome. The evaluable rate (percentage of patients with adequate pre and post treatment biopsies) will be monitored throughout the duration of the study. If the rate is below 80%, enrollment may be extended to reach 30 evaluable patients.

10.2 Sample Size Justification

Sample size will be justified for the primary efficacy endpoint. We are interested in testing the increase CD3+ TILs of the experimental therapy (arm A) over the control therapy (arm B). Let $E_A = \log(T_{A1}/T_{A0})$, where T_{A1} is the on-treatment CD3+ TILs for arm A and T_{A0} is the pre-treatment CD3+ TILs for arm A. Let $E_B = \log(T_{B1}/T_{B0})$, where T_{B1} is the on-treatment CD3+ TILs for arm B and T_{B0} is the pre-treatment CD3+ TILs for arm B. E_A and E_B represent random draws from the continuous distributions of the change of CD3+ TILs on log scale for arm A and arm B, respectively. Wilcoxon rank sum test is used to test $\eta = P(E_A > E_B)$, where η is the parameter that Wilcoxon rank sum test is assessing. In particular, we are interested in testing the hypothesis.

$$H_0: \eta \leq 0.5 \text{ versus } H_a: \eta > 0.5$$

Based on Cascone et al (2021) (Ref 41) Figure 4f and our expectation for the combination therapy, we make the following assumptions for the sample size justification: $E_A \sim norm(0.85,0.35)$ and $E_B \sim norm(0.5,0.35)$ and $\eta = \Phi(\frac{\delta}{\sqrt{0.35}})$, where $\delta = \mu_A - \mu_B = 0.35$. Wilcoxon rank sum test is used to test the change of CD3+ TILs from pre-treatment to on-treatment. With 30 patients (15 patients on each arm), the trial has approximately 90% power rejecting the null hypothesis $\eta = P(E_A > E_B) = 0.5$. at one-sided significance level of 0.1. If the assumptions of the 2-sample t-test is met, the power of 2-sample t-test is slightly higher that of Wilcoxon rank sum test.

10.3 Safety Endpoint

The first 6 patients treated with nivolumab, ipilimumab, and evolocumab will analyzed as a safety cohort, including those that cross over to receive evolocumab. After 6 patients have initiated therapy with evolocumab, enrollment will be paused for 30 days to assess for DLTs. If two or more of the first six patients develop a DLT, further accrual will be paused. The study team will evaluate the cause of toxicity and determine if the trail should be revised and if accrual can resume.

Toxicities will be graded according to the NCI CTCAE version 5.0 criteria. DLTs will be defined as any grade ≥ 3 toxicity at least (possibly, probably, or definitely) attributable to nivolumab, ipilimumab, and~~/or~~ evolocumab, except electrolyte abnormality which can be correct with oral supplementation, or diarrhea persisting for < 48 hours. The incidence of DLT(s) assessed in the first 6 evaluable subjects will be used to determine whether the combination is tolerable. A subject will be considered evaluable for DLT if they have received at least one dose of nivolumab, ipilimumab and evolocumab. DLTs will be assessed for 30 days after cycle 1 day 1. DLT should not be AE, if considered by the investigator to be disease related.

10.4 Statistical Methods

The primary efficacy analysis will be conducted with a efficacy evaluable cohort in which all randomized patients who have adequate pre-treatment and post-treatment biopsy specimens will be included. Two-sided p-values will be reported. The statistical analyses will be performed on SAS 9.4 (SAS Inc., Cary, NC) and R (R Core Team, 3.6.3).

The primary efficacy endpoint is the change of the CD3+ TILs between pre-treatment and on-treatment. The mean and the standard deviation of the pre-treatment CD3+ TILs and the on-treatment CD3+ TILs as well as those of the difference of these ratios will be estimated. When the distribution of CD3+ TILs is clearly skewed, median and interquartile range will be used to summarize its distribution. The primary comparison between treatment arms will be done with Wilcoxon rank sum test. As a supplementary analysis, transformation will be used to make the difference of the CD3+ TILs from pre-treatment and on-treatment into normal distributions. If normal distribution assumption is satisfied after transformation, standardized difference between baseline and post treatment will be estimated as well as the p-value from 2-sample t-test. The secondary analysis of the change of MHC-I between on-treatment and pre-treatment is similar to that of the change of the CD3+ TILs, and will also use the efficacy evaluable cohort.

For the secondary endpoints of response rate, progression free survival and overall survival, the modified intention to treat cohort will be used which includes all patients randomized and receiving at least one dose of the protocol treatment. The dates of response and progression are determined by immune RECIST. Objective response rates (ORR) of two arms and their confidence intervals as well as their difference will be estimated. Progression-free survival (PFS) is the time from the first dose of study treatment to disease progression or death, whichever comes first. Per iRECIST progression is counted at the time of iUPD in the disease assessment immediately prior to iCPD. If a patient does not continue on therapy beyond iUPD, then this is counted as the time of disease progression. Overall survival (OS) is the time from the first dose of study treatment to death. If the patient does not experience disease progression or death, then the data will be censored at the date of the last disease assessment. Survival endpoints will be analyzed by Kaplan-Meier methodology (Kaplan and Meier, 1958), and compared between arm A and arm B, using a log-rank test (Mantel, 1966). Hazard ratio and its 95% confidence interval will be estimated using Cox PH regression with treatment effect as the single variable. Median PFS and its 95% confidence interval will be presented for each arm. Multivariable Cox models will be used to evaluate the treatment effect on survival time and its interaction with baseline covariates, including stage, histology and performance status (Cox, 1972). Restricted mean survival time (RMST) ratio could be a useful measure to quantify the treatment effect for immunotherapies (Uno, et al, 2011). We will choose the last observed event time as tau. Regression analyses with baseline prognostic factors adjusted will be conducted for RMST ratio.

The association of the pre-treatment MHC-I expression and the difference between the on-treatment and the pre-treatment MHC-I expressions with clinical outcomes will be evaluated with multivariable regression models with Cox PH regression for PFS and OS and logistic regression for ORR.

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11.0 SAFETY MONITORING AND REPORTING

All patients who receive at least one dose of nivolumab, ipilimumab, and/or one dose of evolocumab will be considered evaluable for safety parameters. Additionally, any occurrence of non-SAE or SAE first dose of study drug to 100 days after the last dose will be reported as per Adverse Event Reporting sections below. Safety will be evaluated for all treated patients using the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE), Version 5.0 (http://ctep.cancer.gov). Safety assessments will be based

on medical review of adverse event reports and the results of vital sign measurements, physical examinations, and clinical laboratory tests.

The Investigator is responsible for the identification and documentation of adverse events and serious adverse events, as defined below. At each study visit, the Investigator or designee must assess, through non-suggestive inquiries of the subject or evaluation of study assessments, whether an AE or SAE has occurred.

11.1 Adverse Events

An adverse event (AE) is any untoward medical occurrence in a subject receiving study drug and which does not necessarily have a causal relationship with this treatment. For this protocol, the definition of AE also includes worsening of any pre-existing medical condition. An AE can therefore be any unfavorable and unintended or worsening sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of nivolumab, ipilimumab, or evolocumab, whether or not related to use of the nivolumab, ipilimumab, or evolocumab. Abnormal laboratory findings without clinical significance (based on the Investigator's judgment) should not be recorded as AEs. But laboratory value changes that require therapy or adjustment in prior therapy are considered adverse events.

From the time of the first dose of study drug to 100 days after the last dose, all AEs must be recorded in the subject medical record and adverse events case report form.

All grade 1-5 AEs will be assessed according to the CTCAE version 5.0. If CTCAE grading does not exist for an AE, the severity of the AE will be graded as mild (1), moderate (2), severe (3), life-threatening (4), or fatal (5).

Attribution of AEs will be indicated as follows:
- Definite: The AE is clearly related to the study drug
- Probably: The AE is likely related to the study drug
- Possible: The AE may be related to the study drug
- Unlikely: The AE is doubtfully related to the study drug
- Unrelated: The AE is clearly NOT related to the study drug

11.1.1 Reporting of Adverse Events

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

Abnormal laboratory results are to be records as AEs if any of the following conditions are met:
- The abnormal laboratory value leads to a therapeutic intervention (e.g., corrective therapy).
- The abnormal laboratory value is considered to be clinically significant by the Investigator.
- Any lab test result that is clinically significant or meets the definition of an SAE.
- Any laboratory test result abnormality that required the subject to have study treatment discontinued or interrupted.

11.2 Serious Adverse Events

An AE is considered "serious" if in the opinion of the investigator it is one of the following outcomes:
- Fatal
- Life-threatening
- Constitutes a congenital anomaly or birth defect

- A medically significant condition (defined as an event that compromises subject safety or may require medical or surgical intervention to prevent one of the three outcomes above).
- Requires inpatient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant incapacity or substantial disruption to conduct normal life functions.

SAEs and/or follow up to SAEs including death due to any cause other than progression of the cancer under study, that occurs from the date of the first dose of study drug through 100 days following the last dose of study drug, whether or not related to study drug(s), must be recorded on the CRF and must be reported within 24 hours to the Sponsor PI and within 2 business days to supporting companies.

All SAEs must be followed until resolution, return to baseline condition, or stabilization. Any SAEs that are ongoing at the time the clinical database is closed will be reported to supporting companies as unresolved.

11.2.1 Reporting of Serious Adverse Events

The initial report for each SAE or death should include at minimum the following information:

- protocol number and title
- patient initials, study identification number, sex, age
- date the event occurred
- description of the event
- seriousness criteria
- event causality or causal relationship
- study drug name(s)
- dose level and cycle number at the time the event occurred
- description of the patient's condition
- study status of patient at time of report
- responsible investigator name and contact details

The Investigator should report a diagnosis or a syndrome rather than individual signs or symptoms. The Investigator should also try to separate a primary AE considered as the foremost untoward medical occurrence from secondary AEs which occurred as complications. Whenever possible, the Investigator should also provide the batch or lot number of the study drug(s).

SAE Reporting Procedure for Investigators:
Immediately upon awareness of a SAE, the Investigator (or designee) completes and submits the **DCI Serious Adverse Event Report Form – Investigator-Initiated Trials (IITs)** within 24 hours of knowledge of the event to the Sponsor PI as instructed below:

Email to Dr. Scott Antonia at scott.antonia@duke.edu with copy to christy.arrowood@duke.edu

Note: It is imperative that initial SAE reports are submitted as soon as possible (within 24 hours of knowledge of the event) with available information to the Sponsor PI. Missing and/or clarified event information may be provided in a follow-up report.

De-identified source documentation (i.e. admission notes, discharge notes, applicable laboratory results, radiology/diagnostic testing results, etc.) must be sent with the DCI SAE Report Form.

Follow-up information including severity, action taken, concomitant medications, and outcome should be communicated to the Sponsor PI as soon as possible using the same forms mentioned above.

SAE Reporting Procedure for Sponsor PI and Coordinating Center:
Upon receipt, the Sponsor PI will review the submitted DCI SAE Report Form with available source documents and complete the PI Medical Review Assessment on the DCI SAE Report Form. If the event meets the Duke University Health System (DUHS) IRB reporting requirements, the Sponsor PI (or designee) will submit information about the SAE including the PI Medical Review Assessment as a safety event to the DUHS IRB.

The Sponsor PI (or designee) will report SAEs to supporting companies as described below:

Safety Data	Timeframe for Submission	Fax to
Suspected Unexpected Serious Adverse Reaction (SUSARs)	At time of regulatory submission	Amgen Global Safety 1-888-814-8653
Pregnancy/Lactation exposure and any associated reports/outcomes (i.e. unexpected pregnancy, pregnancy of partner, spontaneous abortion, congenital anomaly etc.)	Within one (1) business day of Sponsor awareness, for reports meeting serious criteria Not to exceed 15 calendar days of Sponsor awareness, for non-serious reports	Amgen Global Safety 1-888-814-8653

Duke Cancer Institute as the coordinating center for this study is responsible for reporting SAEs to the FDA in accordance with 21CFR 312.32. Any SAE that is possibly related and unexpected must be submitted to the FDA attached to the IND. If the SAE meets criteria for reporting to the FDA, the Sponsor PI (or designee) will complete the Form FDA 3500A (MedWatch) and send to the supporting companies that are noted above. This submission of the Form FDA 3500A to the FDA attached to the IND will be completed by the Sponsor PI (or designee).

1. All unexpected, drug related SAEs that are fatal or life-threatening will be reported to the FDA by phone or fax within 7 calendar days of initial receipt of the information and will provide a complete report within 8 days of the initial report submission (by calendar day 15).
2. All unexpected, treatment-related SAEs that are not fatal or life-threatening will be reported in a written report to the FDA within 15 days of initial receipt of the information.

The Sponsor PI (or designee) will forward all expedited reports to participating Investigators in the form of an Investigator Alert.

11.3 Other Reportable Information

Select (non-serious and serious) adverse events called Events of Clinical Interest (ECI) that occur from the date of the first dose of study drug through 30 days following the last dose of study drug, must be recorded on the CRF and must be reported within 2 business days to Amgen.

Special reporting circumstances include exposure via a parent during pregnancy or breast-feeding, overdose, potential drug-induced liver injury, and cancer, medication error, misuse, abuse, off-label use or occupational exposure are ECIs for this study and must be recorded on the CRF and must be handled as SAEs.

11.4 Special Warnings and Precautions

Special warning and precautions include the risk of fetal harm. Advise of potential risk to a fetus and use of effective contraception.

12.0 REGULATORY, ETHICAL, AND STUDY OVERSIGHT CONSIDERATIONS

This protocol was designed and will be conducted and reported in accordance with the International Conference on Harmonization (ICH) Harmonized Tripartite Guidelines for Good Clinical Practice, the Declaration of Helsinki, and applicable federal, state, and local regulations.

12.1 Institutional Review Board and Scientific Review Committee

The protocol, informed consent form, advertising material, and additional protocol-related documents must be submitted to the Duke University Health System (DUHS) Institutional Review Board (IRB) and Duke Cancer Institute (DCI) Protocol Review and Monitoring Committee (PRMC) for review. The study may be initiated only after the Principal Investigator has received written and dated approval from the PRMC and IRB.

The Principal Investigator must submit and obtain approval from the IRB for all subsequent protocol amendments and changes to the informed consent form. The PRMC should be informed about any protocol amendments that potentially affect research design or data analysis (i.e. amendments affecting subject population, inclusion/exclusion criteria, agent administration, statistical analysis, etc.).

The Principal Investigator must obtain protocol re-approval from the IRB within one year of the most recent IRB approval. The Principal Investigator must also obtain protocol re-approval from the PRMC within 1 year of the most recent IRB approval, for as long as the protocol remains open to subject enrollment.

12.2 Informed Consent

The informed consent form must be written in a manner that is understandable to the subject population. Prior to its use, the informed consent form must be approved by the IRB.

The Investigator or authorized key personnel will discuss with the potential subject the purpose of the research, methods, potential risks and benefits, subject concerns, and other study-related matters. This discussion will occur in a location that ensures subject privacy and in a manner that minimizes the possibility of coercion. Appropriate accommodations will be made available for potential subjects who cannot read or understand English or are visually impaired. Potential subjects will have the opportunity to contact the Investigator or authorized key personnel with questions, and will be given as much time as needed to make an informed decision about participation in the study.

Before conducting any study-specific procedures, the Investigator must obtain written informed consent from the subject or a legally acceptable representative. The original informed consent form will be stored with the subject's study records, and a copy of the informed consent form will be provided to the subject. The Investigator is responsible for asking the subject whether the subject wishes to notify his/her primary care physician about participation in the study. If the subject agrees to such notification, the Investigator will inform the subject's primary care physician about the subject's participation in the clinical study.

12.3 Protocol Amendments

The Principal Investigator must submit and obtain approval from the IRB for all subsequent protocol amendments and changes to the informed consent form. IRB approval is not required for protocol changes that occur to protect the safety of a subject from an immediate hazard. However, the Principal Investigator must inform the IRB and all other applicable regulatory agencies of such action immediately.

The PRMC should be informed about any protocol amendments that potentially affect research design or data analysis (i.e. amendments affecting subject population, inclusion/exclusion criteria, agent administration, etc.).

12.4 Protocol Deviations and Violations

A protocol deviation is non-adherence to protocol specific study procedures or schedules that does not involve inclusion/exclusion criteria, primary objective evaluation criteria, and/or GCP guidelines.

A protocol violation is any significant divergence from the protocol such as non-adherence on the part of the subject, the Investigator, or the sponsor-investigator to protocol specific inclusion/exclusion criteria, primary objective evaluation criteria, and/or GCP guidelines.

As a matter of policy, the sponsor-investigator (i.e. Principal Investigator) will not grant exceptions to protocol specific entry criteria to allow subjects to enter a study. If it is found that a subject who did not meet protocol eligibility criteria was entered in a study (a protocol violation), the Principal Investigator and/or designee must be informed immediately. Such subjects will be discontinued from the study, except in an exceptional instance following review and written approval by the Principal Investigator and the IRB.

Protocol deviations and violations must be reported according to IRB policy.

12.5 Safety Oversight Committee

The Duke Cancer Institute Safety Oversight Committee (SOC) is responsible for annual data and safety monitoring of DUHS sponsor-investigator phase I and II, therapeutic interventional studies that do not have an independent data safety monitoring board (DSMB).

The primary focus of the SOC is review of safety data, toxicities and new information that may affect subject safety or efficacy. Annual safety reviews includes but may not be limited to review of safety data, enrollment status, stopping rules if applicable, accrual, toxicities, reference literature, and interim analyses as provided by the sponsor-investigator. The SOC in concert with the Duke Cancer Institute Monitoring Team oversees the conduct of DUHS cancer-related, sponsor-investigator greater-than-minimal-risk intervention studies that do not have an external monitoring plan, ensuring subject safety and that the protocol is conducted, recorded and reported in accordance with the protocol, standing operating procedures (SOPs), Good Clinical Practice (GCP), and applicable regulatory requirements.

12.6 Data and Safety Monitoring Board

There is no independent data and safety monitoring board (DSMB) for this study. Neither the Principal Investigator nor DCI has a potential conflict of interest with conduct of this protocol.

12.7 Monitoring and Audits/Inspections

The study will be monitored both internally by the Principal Investigator and institutionally by the Duke Cancer Institute (DCI), through the Protocol Review and Monitoring Systems (PRMS) and Protocol Review and Monitoring Committee (PRMC).

In terms of internal review, the Principal Investigator and/or designee(s) will continuously monitor and tabulate adverse events. Appropriate reporting to the IRB will be made. If an unexpected frequency of Grade 3 or 4 adverse events occurs, depending on their nature, action appropriate to the nature and frequency of these adverse events will be taken. This may require a protocol amendment, dose de-escalation, or potentially closure of the study. The Principal Investigator will also continuously monitor the conduct, data, and safety of this study to ensure that:

- Interim analyses occur as scheduled (if applicable);
- Stopping rules for toxicity and/or response are met;
- Risk/benefit ratio is not altered to the detriment of the subjects;
- Appropriate internal monitoring of adverse events and outcomes is done;
- Over-accrual does not occur;
- Under-accrual is addressed with appropriate amendments or actions;
- Data are being appropriately recorded on the CRF in a reasonably timely manner.

The DCI PRMS protocol review begins with an initial review by the PRMC. The PRMC new protocol review focuses on scientific relevance, study design, biostatistical input adequacy, protocol prioritization, feasibility of study completion within a reasonable time frame and trial risk assessment. The Principal Investigator will abide by PRMC risk level assessment. PRMC also conducts annual scientific progress reviews on protocols that are open to enrollment and focus on protocol prioritization, accrual and scientific progress. The PRMC conducts reviews at the time of IRB annual renewals and maintains documentation in eIRB/iRIS (online IRB application).

During the initial PRMC approval, the PRMC determines the monitoring risk level which will commensurate with the type and level of intervention, phase, endpoints, degree of risk, size and protocol complexity. The DCI monitoring team will conduct formal, independent monitoring according to the risk level and the PRMC monitoring plan until the study is closed to enrollment or subjects are no longer receiving study drug or other interventions that are more than minimal risk.

Findings from monitoring visits, unexpected frequency of serious and/or unexpected toxicities, or other concerns may prompt additional monitoring. DUHS and DCI Leadership, PRMC, SOC, a sponsor, an investigator, or the IRB may also request additional monitoring visits.

The DCI monitoring team reviews informed consent adequacy, eligible patient enrollment, protocol-specified procedures and treatment implementation, data collection adequacy, and adverse event monitoring and reporting appropriateness. The DCI monitoring team presents final monitoring reports to the DCI SOC highlighting safety concerns and unresolved issues. The SOC, at a convened meeting, assigns an overall rating of satisfactory, marginal, or unsatisfactory to reflect the overall quality of data, regulatory, consent, eligibility, study conduct and AE reporting. Corrective action plans (CAPs) are developed, implemented, and evaluated as indicated. The SOC will notify the sponsor-investigator and DUHS IRB when significant safety concerns are identified.

The SOC in concert with DCI monitoring team conducts data and safety monitoring for DUHS sponsor-investigator phase I and II, therapeutic interventional oncology studies that do not have an independent DSMB. These reviews occur at a minimum annually and possibly more frequently based on risk level. The SOC safety reviews include review of safety, data accuracy, enrollment status, stopping rules if applicable, accrual, toxicities, reference literature, and interim analyses as the sponsor-investigator provides. The SOC, at a convened meeting, assigns a “satisfactory” rating when adequate accrual with lack of excessive toxicity is present.

The Duke Cancer Institute (DCI) Monitoring Team will conduct monitoring visits to ensure subject safety and to ensure that the protocol is conducted, recorded, and reported in accordance with the protocol, standard operating procedures, good clinical practice, and applicable regulatory requirements. As specified in the DCI Data and Safety Monitoring Plan, the DCI Monitoring Team will conduct routine monitoring after the third subject is enrolled, followed by annual monitoring of 1 – 3 subjects until the study is closed to enrollment and subjects are no longer receiving study interventions that are more than minimal risk.

An external site monitoring plan addendum describes monitoring at participating sites.

Regulatory authorities may also audit an Investigator during or after the study. The Investigator should contact the Principal Investigator and designee(s) at the Duke Cancer Institute as well as their local IRB, immediately if this occurs, and must fully cooperate with governmental (e.g., FDA) audits conducted at a reasonable time in a reasonable manner.

The Duke University Compliance Program - Human Subject Research Compliance (HSRC) section may conduct confidential audits to evaluate compliance with the protocol and the principles of GCP. The Principal Investigator agrees to allow the HSRC auditor(s) direct access to all relevant documents and to allocate his/her time and the time of the study personnel to the auditor(s) in order to discuss findings and any relevant issues.

12.8 Source and Study Documentation

Source documents include all original recordings of observations or notations of clinical activities and all reports and records necessary for the evaluation and reconstruction of the clinical study. Accordingly, source documents include, but are not limited to, laboratory reports (including normal and abnormal results), radiology reports, subject diaries, biopsy reports, ultrasound photographs, subject progress notes, hospital charts or pharmacy records and any other similar reports or records of any procedure performed in accordance with the protocol.

Whenever possible, the original recording of an observation should be retained as the source document; however, a photocopy is acceptable provided that it is a clear, legible, and exact duplication of the original certified document. For paper source documents, errors will crossed out with a single line, and this line will not obscure the original entry. Changes or corrections will be dated, initialed, and explained (if necessary).

When clinical observations are entered directly into an electronic medical record system (i.e. in lieu of original hardcopy records), the electronic record can serve as the source document if the system has must be validated to meet the FDA requirements for electronic records and signatures (i.e. meets 21 CFR Part 11 compliant).

Regulations require that Investigators maintain information in the study subject's medical records which corroborate data recorded on the CRF.

Study documentation includes but is not limited to source documents, CRFs, monitoring logs, correspondence with sponsors or regulatory bodies/committees, and regulatory documents maintained in the 'regulatory binder', which includes but is not limited to approved protocols, approved and signed subject consent forms, FDA Form 1572, laboratory certifications, and clinical supplies receipts and distribution records.

12.9 Case Report Forms

The case report form (CRF) will be the primary data collection document for the study. Subject data will be entered (ie. CRFs completed) and an audit trail maintained into an electronic data capture (EDC) system. The EDC system is maintained on a secure Duke University server.

The CRFs will be updated within two weeks of acquisition of new source data. Only authorized key personnel are permitted to make entries, changes, or corrections in the CRF. In the event of discrepant data, the study monitor or designee will request data clarification from the Investigator or authorized key personnel for which may be resolved electronically in the EDC system.

12.10 Privacy, Confidentiality, and Data Storage

The Principal Investigator will ensure that subject privacy and confidentiality of the subject's data will be maintained. Research Data Security Plans (RDSPs) will be approved by the appropriate institutional Site Based Research group.

To protect privacy, every reasonable effort will be made to prevent undue access to subjects during the course of the study. Prospective participants will be consented in an exam room where it is just the research staff, the patient and his family, if desired. For all future visits, interactions with research staff (study doctor and study coordinators) regarding research activities will take place in a private exam room. All research related interactions with the participant will be conducted by qualified research staff who are directly involved in the conduct of the research study.

To protect confidentiality, subject files in paper format will be stored in secure cabinets under lock and key accessible only by the research staff. Subjects will be identified only by a unique study number and subject initials. Electronic records of subject data will be maintained using a dedicated web-access secure database, which is housed in an encrypted and password-protected server behind the Duke firewall. Access to electronic databases will be limited to delegated personnel. The security and viability of the IT infrastructure will be managed by the DCI and/or Duke Medicine.

Subject names or identifiers will not be used in reports, presentations at scientific meetings, or publications in scientific journals.

12.10 Records Retention

Upon completion of the study, research records will be retained and stored per DUHS Human Research Protection Program (HRPP) policies.

The Principal Investigator will maintain study-related records for the longer of a period of:

- at least two years after the date on which a New Drug Application is approved by the FDA; or
- at least two years after formal withdrawal of the IND associated with this protocol, at least six years after study completion.

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Appendix A: RECIST 1.1 and iRECIST

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

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

Definitions
Response and progression will be evaluated in this study using the international criteria proposed by the Response Evaluation Criteria in Solid Tumors (RECIST) Committee (version 1.1). Changes in only the largest diameter (uni-dimensional measurement) of the tumor lesions are used in the RECIST criteria. Note: Lesions are either measurable or non-measurable using the criteria provided below. The term "evaluable" in reference to measurability will not be used because it does not provide additional meaning or accuracy.

Measurable Disease
Tumor lesions: Must be accurately measured in at least one dimension (longest diameter in the plane of measurement is to be recorded) with a minimum size of:

- 10mm by CT scan (irrespective of scanner type) and MRI (no less than double the slice thickness and a minimum of 10mm)
- 10mm caliper measurement by clinical exam (when superficial)
- 20mm by chest X-ray (if clearly defined and surrounded by aerated lung)

Malignant lymph nodes: To be considered pathologically enlarged and measurable, a lymph node must be ≥15mm in short axis when assessed by CT scan (CT scan slice thickness recommended to be no greater than 5 mm). At baseline and in follow-up, only the short axis will be measured and followed.

Non-measurable Disease
All other lesions (or sites of disease), including small lesions (longest diameter ≥10 to <15 mm with conventional techniques or <10 mm using spiral CT scan), are considered non-measurable disease. Leptomeningeal disease, ascites, pleural or pericardial effusion, inflammatory breast disease, lymphangitic involvement of skin or lung, abdominal masses/abdominal organomegaly identified by physical exam that is not measurable by reproducible imaging techniques are all non-measurable.
Bone lesions:

- Bone scan, PET scan or plain films are not considered adequate imaging techniques to measure bone lesions. However, these techniques can be used to confirm the presence or disappearance of bone lesions.
- Lytic bone lesions or mixed lytic-blastic lesions, with identifiable soft tissue components, that can be evaluated by cross sectional imaging techniques such as CT or MRI can be considered as measurable lesions if the soft tissue component meets the definition of measurability described above.
- Blastic bone lesions are non-measurable.

Cystic lesions:

- Lesions that meet the criteria for radiographically defined simple cysts should not be considered as malignant lesions (neither measurable nor non-measurable) since they are, by definition, simple cysts.

- 'Cystic lesions' thought to represent cystic metastases can be considered as measurable lesions, if they meet the definition of measurability described above. However, if noncystic lesions are present in the same patient, these are preferred for selection as target lesions.

Lesions with prior local treatment:

- Tumor lesions situated in a previously irradiated area, or in an area subjected to other loco-regional therapy, are usually not considered measurable unless there has been demonstrated progression in the lesion. Study protocols should detail the conditions under which such lesions would be considered measurable.

Target Lesions

All measurable lesions up to a maximum of 2 lesions per organ and 5 lesions in total, should be identified as target lesions and recorded and measured at baseline. Target lesions should be selected on the basis of their size (lesions with the longest diameter), be representative of all involved organ, but in addition should be those that lend themselves to reproducible repeated measurements.

Lymph nodes merit special mention since they are normal anatomical structures which may be visible by imaging even if not involved by tumor. Pathological nodes which are defined as measurable and may be identified as target lesions must meet the criterion of a short axis of ≥15mm by CT scan. Only the short axis of these nodes will contribute to the baseline sum. The short axis of the node is the diameter normally used by radiologists to judge if a node is involved by solid tumor. Nodal size is normally reported as two dimensions in the plane in which the image is obtained (for CT scan this is almost always the axial plane; for MRI the plane of acquisition may be axial, sagittal or coronal). The smaller of these measures is the short axis. For example, an abdominal node which is reported as being 20mm x 30mm has a short axis of 20mm and qualifies as a malignant, measurable node. In this example, 20mm should be recorded as the node measurement. All other pathological nodes (those with short axis ≥10mm but <15 mm) should be considered non-target lesions. Nodes that have a short axis <10mm are considered non-pathological and should not be recorded or followed.

A sum of the diameters (longest for non-nodal lesions, short axis for nodal lesions) for all target lesions will be calculated and reported as the baseline sum diameters. If lymph nodes are to be included in the sum, then as noted above, only the short axis is added into the sum. The baseline sum diameters will be used as reference to further characterize any objective tumor regression in the measurable dimension of the disease.

Non-target Lesions

All other lesions (or sites of disease) including pathological lymph nodes 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', 'absent', or in rare cases 'unequivocal progression' (more details to follow). In addition, it is possible to record multiple non-target lesions involving the same organ as a single item on the case record form (e.g. 'multiple enlarged pelvic lymph nodes' or 'multiple liver metastases').

Guidelines for Evaluation of Measurable Disease

All measurements should be recorded in metric notation, using calipers if clinically assessed. All baseline evaluations should be performed as close as possible to the treatment start and never more than 4 weeks before the beginning of the treatment.

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

Clinical lesions: Clinical lesions will only be considered measurable when they are superficial and >10mm diameter as assessed using calipers (e.g. skin nodules). For the case of skin lesions, documentation by color photography including a ruler to estimate the size of the lesion is suggested. As noted above, when lesions

can be evaluated by both clinical exam and imaging, imaging evaluation should be undertaken since it is more objective and may also be reviewed at the end of the study.

Chest X-ray: Chest CT is preferred over chest X-ray, particularly when progression is an important endpoint, since CT is more sensitive than X-ray, particularly in identifying new lesions. However, lesions on chest X-ray may be considered measurable if they are clearly defined and surrounded by aerated lung.

CT, MRI: CT is the best currently available and reproducible method to measure lesions selected for response assessment. This guideline has defined measurability of lesions on CT scan based on the assumption that CT slice thickness is 5mm or less. When CT scans have slice thickness greater than 5 mm, the minimum size for a measurable lesion should be twice the slice thickness. MRI is also acceptable in certain situations (e.g. for body scans).

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

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

Tumor markers: Tumor markers alone cannot be used to assess objective tumor response. If markers are initially above the upper normal limit, however, they must normalize for a patient to be considered in complete response. Because tumor markers are disease specific, instructions for their measurement should be incorporated into protocols on a disease specific basis. Specific guidelines for both CA-125 response (in recurrent ovarian cancer) and PSA response (in recurrent prostate cancer), have been published. In addition, the Gynecologic Cancer Intergroup has developed CA125 progression criteria which are to be integrated with objective tumor assessment for use in first-line trials in ovarian cancer.

Cytology, histology: These techniques can be used to differentiate between PR and CR in rare cases if required by protocol (for example, residual lesions in tumor types such as germ cell tumors, where known residual benign tumors can remain). When effusions are known to be a potential adverse effect of treatment (e.g. with certain taxane compounds or angiogenesis inhibitors), the cytological confirmation of the neoplastic origin of any effusion that appears or worsens during treatment can be considered if the measurable tumor has met criteria for response or stable disease in order to differentiate between response (or stable disease) and progressive disease.

Response Criteria

Evaluation of Target Lesions

- Complete Response (CR): Disappearance of all target lesions. Any pathological lymph nodes (whether target or non-target) must have reduction in short axis to <10 mm.
- Partial Response (PR): At least a 30% decrease in the sum of diameters of target lesions, taking as reference the baseline sum diameters.
- Progressive Disease (PD): At least a 20% increase in the sum of diameters of target lesions, taking as reference the smallest sum on study (this includes the baseline sum if that is the smallest on study). In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5 mm. (Note: the appearance of one or more new lesions is also considered progression).
- Stable Disease (SD): Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum diameters while on study.

Lymph nodes. Lymph nodes identified as target lesions should always have the actual short axis measurement recorded (measured in the same anatomical plane as the baseline examination), even if the nodes regress to below 10 mm on study. This means that when lymph nodes are included as target lesions, the 'sum' of lesions may not be zero even if complete response criteria are met, since a normal lymph node is defined as having a short axis of <10 mm. Case report forms or other data collection methods may therefore be designed to have target nodal lesions recorded in a separate section where, in order to qualify for CR, each node must achieve a short axis <10 mm. For PR, SD and PD, the actual short axis measurement of the nodes is to be included in the sum of target lesions.

Target lesions that become 'too small to measure'. While on study, all lesions (nodal and non-nodal) recorded at baseline should have their actual measurements recorded at each subsequent evaluation, even when very small (e.g. 2 mm). However, sometimes lesions or lymph nodes which are recorded as target lesions at baseline become so faint on CT scan that the radiologist may not feel comfortable assigning an exact measure and may report them as being 'too small to measure'. When this occurs it is important that a value be recorded on the case report form. If it is the opinion of the radiologist that the lesion has likely disappeared, the measurement should be recorded as 0mm. If the lesion is believed to be present and is faintly seen but too small to measure, a default value of 5mm should be assigned (Note: It is less likely that this rule will be used for lymph nodes since they usually have a definable size when normal and are frequently surrounded by fat such as in the retroperitoneum; however, if a lymph node is believed to be present and is faintly seen but too small to measure, a default value of 5 mm should be assigned in this circumstance as well). This default value is derived from the 5 mm CT slice thickness (but should not be changed with varying CT slice thickness). The measurement of these lesions is potentially non-reproducible, therefore providing this default value will prevent false responses or progressions based upon measurement error. To reiterate, however, if the radiologist is able to provide an actual measure, that should be recorded, even if it is below 5 mm.

Lesions that split or coalesce on treatment. When non-nodal lesions 'fragment', the longest diameters of the fragmented portions should be added together to calculate the target lesion sum. Similarly, as lesions coalesce, a plane between them may be maintained that would aid in obtaining maximal diameter measurements of each individual lesion. If the lesions have truly coalesced such that they are no longer separable, the vector of the longest diameter in this instance should be the maximal longest diameter for the 'coalesced lesion'.

Evaluation of Non-target Lesions
While some non-target lesions may actually be measurable, they need not be measured and instead should be assessed only qualitatively at the time points specified in the protocol.

- Complete Response (CR): Disappearance of all non-target lesions and normalization of tumor marker level. All lymph nodes must be non-pathological in size (<10mm short axis).
- Non-CR/Non-PD: Persistence of one or more non-target lesion(s) and/or maintenance of tumor marker level above the normal limits.
- Progressive Disease (PD): Unequivocal progression (see comments below) of existing non-target lesions. (Note: the appearance of one or more new lesions is also considered progression).

When the patient also has measurable disease. In this setting, to achieve 'unequivocal progression' on the basis of the non-target disease, there must be an overall level of substantial worsening in non-target disease such that, even in presence of SD or PR in target disease, the overall tumor burden has increased sufficiently to merit discontinuation of therapy. A modest 'increase' in the size of one or more non-target lesions is usually not sufficient to quality for unequivocal progression status. The designation of overall progression solely on the basis of change in non-target disease in the face of SD or PR of target disease will therefore be extremely rare.

When the patient has only non-measurable disease. This circumstance arises in some phase III trials when it is not a criterion of study entry to have measurable disease. The same general concept apply here as noted above, however, in this instance there is no measurable disease assessment to factor into the interpretation of an increase in non-measurable disease burden. Because worsening in non-target disease cannot be easily quantified (by definition: if all lesions are truly non-measurable) a useful test that can be applied when assessing patients for unequivocal progression is to consider if the increase in overall disease burden based on the change in non-measurable disease is comparable in magnitude to the increase that would be required to declare PD for measurable disease: i.e. an increase in tumor burden representing an additional 73% increase in 'volume' (which is equivalent to a 20% increase diameter in a measurable lesion). Examples include an increase in a pleural effusion from 'trace' to 'large', an increase in lymphangitic disease from localized to widespread, or may be described in protocols as 'sufficient to require a change in therapy'. If 'unequivocal progression' is seen, the patient should be considered to have had overall PD at that point. While it would be ideal to have objective criteria to apply to non-measurable disease, the very nature of that disease makes it impossible to do so, therefore the increase must be substantial.

New Lesions
The appearance of new malignant lesions denotes disease progression; therefore, some comments on detection of new lesions are important. There are no specific criteria for the identification of new radiographic lesions; however, the finding of a new lesion should be unequivocal: i.e. not attributable to differences in scanning technique, change in imaging modality or findings thought to represent something other than tumor (for example, some 'new' bone lesions may be simply healing or flare of pre-existing lesions). This is particularly important when the patient's baseline lesions show partial or complete response. For example, necrosis of a liver lesion may be reported on a CT scan report as a 'new' cystic lesion, which it is not.

A lesion identified on a follow-up study in an anatomical location that was not scanned at baseline is considered a new lesion and will indicate disease progression. An example of this is the patient who has visceral disease at baseline and while on study has a CT or MRI brain ordered which reveals metastases. The patient's brain metastases are considered to be evidence of PD even if he/she did not have brain imaging at baseline.

If a new lesion is equivocal, for example because of its small size, continued therapy and follow-up evaluation will clarify if it represents truly new disease. If repeat scans confirm there is definitely a new lesion, then progression should be declared using the date of the initial scan.

While FDG-PET response assessments need additional study, it is sometimes reasonable to incorporate the use of FDG-PET scanning to complement CT scanning in assessment of progression (particularly possible 'new' disease). New lesions on the basis of FDG-PET imaging can be identified according to the following algorithm:

- Negative FDG-PET at baseline, with a positive FDG-PET at follow-up is a sign of PD based on a new lesion.
- No FDG-PET at baseline and a positive FDG-PET at follow- up: If the positive FDG-PET at follow-up corresponds to a new site of disease confirmed by CT, this is PD. If the positive FDG-PET at follow-up is not confirmed as a new site of disease on CT, additional follow-up CT scans are needed to determine if there is truly progression occurring at that site (if so, the date of PD will be the date of the initial abnormal FDG-PET scan). If the positive FDG-PET at follow-up corresponds to a pre-existing site of disease on CT that is not progressing on the basis of the anatomic images, this is not PD.

Evaluation of Best Overall Response

The best overall response is the best response recorded from the start of the study treatment until the end of treatment taking into account any requirement for confirmation. On occasion a response may not be documented until after the end of therapy so protocols should be clear if post-treatment assessments are to be considered in determination of best overall response. Protocols must specify how any new therapy introduced before progression will affect best response designation. The patient's best overall response assignment will depend on the findings of both target and non-target disease and will also take into consideration the appearance of new lesions. Furthermore, depending on the nature of the study and the protocol requirements, it may also require confirmatory measurement. Specifically, in non-randomized trials where response is the primary endpoint, confirmation of PR or CR is needed to deem either one the "best overall response."
The best overall response is determined once all the data for the patient is known. Best response determination in trials where confirmation of complete or partial response IS NOT required: Best response in these trials is defined as the best response across all time points (for example, a patient who has SD at first assessment, PR at second assessment, and PD on last assessment has a best overall response of PR). When SD is believed to be best response, it must also meet the protocol specified minimum time from baseline. If the minimum time is not met when SD is otherwise the best time point response, the patient's best response depends on the subsequent assessments. For example, a patient who has SD at first assessment, PD at second and does not meet minimum duration for SD, will have a best response of PD. The same patient lost to follow-up after the first SD assessment would be considered inevaluable.

The iRECIST guidelines have been developed to adequately characterize additional patterns of response and progression specific to patients treated with immunotherapy, that cannot be captured by the conventional criteria such as such as Response Evaluation Criteria in Solid Tumors (RECIST).

*Seymour L et al, iRECIST: guidelines for response criteria for use in trials testing immunotherapeutics. *Lancet Oncol*, 2017.

	Timepoint response with no previous iUPD in any category	Timepoint response with previous iUPD in any category*
Target lesions: iCR; non-target lesions: iCR; new lesions: no	iCR	iCR
Target lesions: iCR; non-target lesions: non-iCR/non-iUPD; new lesions: no	iPR	iPR
Target lesions: iPR; non-target lesions: non-iCR/non-iUPD; new lesions: no	iPR	iPR
Target lesions: iSD; non-target lesions: non-iCR/non-iUPD; new lesions: no	iSD	iSD
Target lesions: iUPD with no change, or with a decrease from last timepoint; non-target lesions: iUPD with no change, or decrease from last timepoint; new lesions: yes	Not applicable	New lesions confirm iCPD if new lesions were previously identified and they have increased in size (≥5 mm in sum of measures for new lesion target or any increase for new lesion non-target) or number; if no change is seen in new lesions (size or number) from last timepoint, assignment remains iUPD
Target lesions: iSD, iPR, iCR; non-target lesions: iUPD; new lesions: no	iUPD	Remains iUPD unless iCPD is confirmed on the basis of a further increase in the size of non-target disease (does not need to meet RECIST 1.1 criteria for unequivocal progression)
Target lesions: iUPD; non-target lesions: non-iCR/non-iUPD, or iCR; new lesions: no	iUPD	Remains iUPD unless iCPD is confirmed on the basis of a further increase in sum of measures ≥5 mm; otherwise, assignment remains iUPD
Target lesions: iUPD; non-target lesions: iUPD; new lesions: no	iUPD	Remains iUPD unless iCPD is confirmed based on a further increase in previously identified target lesion iUPD in sum of measures ≥5 mm or non-target lesion iUPD (previous assessment need not have shown unequivocal progression)
Target lesions: iUPD; non-target lesions: iUPD; new lesions: yes	iUPD	Remains iUPD unless iCPD is confirmed on the basis of a further increase in previously identified target lesion iUPD sum of measures ≥5 mm, previously identified non-target lesion iUPD (does not need to be unequivocal), or an increase in the size or number of new lesions previously identified
Target lesions: non-iUPD or progression; non-target lesions: non-iUPD or progression; new lesions: yes	iUPD	Remains iUPD unless iCPD is confirmed on the basis of an increase in the size or number of new lesions previously identified

Appendix B: ECOG Performance Status

The ECOG Scale of Performance Status, developed by the Eastern Cooperative Oncology Group, Robert L. Comis, MD, Group Chair*, describes a patient's level of functioning in terms of their ability to care for themselves, daily activity, and physical ability (walking, working, etc.).

**Oken, M.M., Creech, R.H., Tormey, D.C., Horton, J., Davis, T.E., McFadden, E.T., Carbone, P.P.: Toxicity And Response Criteria Of The Eastern Cooperative Oncology Group. Am J Clin Oncol 5:649-655, 1982.* The Eastern Cooperative Oncology Group, Robert Comis M.D., Group Chair.

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

Appendix C: Standard Cockcroft and Gault Formula for Calculated Creatinine Clearance

Standard Cockcroft and Gault Formula for Calculated Creatinine Clearance

For serum creatinine concentration in mg/dL:

Creatinine clearance (CrCl) will be calculated using the Cockroft-Gault equation as follows:

$$\text{CrCl (ml/min)} = \frac{(140 - \text{age}) \times (\text{actual weight in kg})}{72 \times \text{serum creatinine (mg/dl)}} \times 0.85 \text{ for females}$$

For serum creatinine concentration in mol/L:

CrCl = [(140 - age) x (wt in kg)] / [0.81 x serum creatinine (mol/L)]
Females: Multiply the result x 0.85
Units: age in years, weight in kilograms.

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Appendix D: Research Blood Specimens for Correlative Sciences

Blood will be collected to assess CD8+ T cells with specificity against tumor antigens. Blood will also be utilized to assess the presence of circulating populations of regulatory T cells, myeloid-derived suppressor cells (MDSC), as well as activated and exhausted T cells.

Blood Volumes and Collection Time Points:

At each blood collection, three 8.5ml ACD anti-coagulated (yellow top) and one 10 ml EDTA (purple top) tubes will be collected. The following time points will have blood collections:

Collection 1: Baseline prior to treatment initiation
Collection 2: Cycle 1 Day 15
Collection 3: 1st re-imaging (six weeks)
Collection 4: Progression

Progression research blood defined as:

- iCPD or iUPD if discontinuing study without confirmation per provider discretion.
- Research blood will be drawn at progression for subjects that discontinue treatment for toxicity.
- Subjects completing 2 years on treatment without progression will be followed for progression and have research blood drawn at progression.
- Subjects who discontinue treatment without progression and start new treatment will **NOT** have research blood drawn.

Refer to Lab Manual associated with this study for further details.

Appendix E: Research Tissue Specimens for Correlative Sciences

Patients may also be approached to participate in the "DUHS Biospecimen Repository and Processing Core (BRPC)" eIRB 35974 protocol prior to biopsy procedure. Tissue will be collected and released as described in this protocol to ensure proper involvement of pathology to minimize the chance that a tissue collection event interferes with appropriate clinical tissue processing and diagnosis.

PRINCIPLE:
Patients undergoing a standard of care biopsy with added research component will undergo a bronchoscopy with endobronchial ultrasound (EBUS) biopsy, a percutaneous CT imaging guided biopsy, or surgical biopsy (e.g. mediastinoscopy), depending on anatomic site of suspected malignancy and appropriate standard of care. Procedural specialist obtaining biopsy will first obtain as many core biopsy samples as indicated for usual standard of care needed for diagnostic purposes. After collecting this he or she will collect an additional at least three, but preferably up to four core biopsy specimens using a core needle biopsy needle 19 gauge or larger. These specimens will be transported fresh to the Duke Immune Profiling Core (DIPC) for disaggregation and viable cell preservation to optimize the use of these samples for the correlative analyses outlined in this protocol.

Patients undergoing an elective biopsy procedure will undergo either a bronchoscopy with EBUS or a percutaneous CT imaging guided biopsy. Procedural specialist obtaining biopsy will obtain at least three but preferably four core biopsy specimens using a core needle biopsy needle 19 gauge or larger. These specimens will be transported fresh to DIPC for disaggregation and viable cell preservation to optimize the use of these samples for future correlative analyses.

SPECIMEN: Pre-treatment biopsy specimen and on treatment biopsy specimen

LOCATION: Interventional Pulmonology Suite, Interventional Radiology Suite, or Surgical Pathology Suite

QUALITY CONTROL:
Lab guidelines for safe handling of all samples must be followed. All tubes used for specimen processing must be labeled with the unique patient identifier or sample number before transfer of the tissue sample.

Refer to Lab Manual associated with this study for further details.

Appendix F: Adverse Device Effects

Adverse Device Effects for Amgen-marketed devices and device constituents in combination products[b,c] and Product complaints:

Safety Data	Timeframe for submission to Amgen	Send to
Unanticipated Serious Adverse Device Effects, (USADEs), Serious Adverse Device Effects (SADEs) and Non-serious Adverse Device Effect (Non-serious ADEs)	Within 1 business day of Sponsor awareness	Amgen Safety
Product Complaints[d]	Within 1 business day of Sponsor awareness	Amgen Quality

[b] Amgen combination products and devices that are marketed anywhere in the world (regardless of the status of the combination product/device in the country where the study is carried out).

[c] Adverse device effect (ADE) is any adverse effect caused by or associated with the use of a device constituent of a combination product or medical device. Adverse device effects include, but are not limited to, adverse effects resulting from insufficient or inadequate instructions for use, any malfunction of the device, or use error or intentional misuse of the device.
Unanticipated serious adverse device effect (USADE)

- ISO14155/MEDDEV2.7.3 definition: Serious adverse device effect which by its nature, incidence, severity or outcome has not been identified in the current version of the risk analysis report
    - Note**:** The 'risk analysis report' refers to the most current risk documents for a given investigational device. The essence of the risk documents is captured under the Risk Assessment section of the device IB or the device section of a combination product IB.

- US FDA definition (Unanticipated adverse device effect (UADE)): Any serious adverse effect on health or safety, any life-threatening problem or death caused by, or associated with a device, if that effect, problem, or death was not previously identified in nature, severity, or degree of incidence in the application; or any other unanticipated serious problem associated with a device that relates to the rights, safety, or welfare of subjects.

[d] Product Complaint is: Any written, electronic or oral communication that alleges deficiencies related to the identity, quality, durability, reliability, safety, effectiveness, or performance of a drug, combination product, or device after it is released for distribution to market or clinic by either: (1) Amgen or (2) distributors or partners for whom Amgen manufactures the material. This includes all components distributed with the drug, such as packaging, drug containers, delivery system, labelling, and inserts. Examples include:

- Device that is damaged or broken
- Bent or blunt needles
- Missing or illegible labeling
- Inability of customer to administer the product
- Product with an unexpected color, appearance, or particles
- Use error (i.e, an act or omission of an act that results in a different combination product or medical device response than intended by the manufacturer or expected by the user, where the user attempted to use the combination product or medical device in good faith and experienced difficulty or deficiency administering the product).

Reports of misuse of a combination product or medical device (i.e, the intentional and improper use of a combination product or medical device not in accordance with the authorized product information) are not considered Product Complaints.

Appendix G: Aggregate Reports

Aggregate reports[a](as applicable):

Safety Data	Timeframe for submission to Amgen	Send to
Listing for Safety data reconciliation[b]	Once per year and at the end of the study	NASCR Manager
Annual Safety Report (eg, EU Clinical Trial Directive DSUR and US IND Annual Report)	Annually	NASCR Manager
Other aggregate analyses (any report containing Safety data generated during the course of the study)	At the time of Sponsor submission to any body governing research conduct (eg. RA, IRB etc.)	NASCR Manager
Final (End of Study Report, including): • Unblinding data for blinded studies • Reports of unauthorized use of a marketed product	At the time of Sponsor submission to any body governing research conduct (eg. RA, IRB etc.) but no later than 1 calendar year of study completion	NASCR Manager

[a] Specific requirements are to be outlined in the Research Agreement.

[b] Listing for reconciliation should include all ICSRs submitted to Amgen Safety per contract (i.e. for studies in Table 1 listing should include ADRs, SADRs, Other Safety Findings, USADEs, SADEs and non-serious ADEs ; for studies in Table 2 listing should contain SUSARs, pregnancy and lactation exposure (and any associated reports/outcomes), USADEs, SADEs and non-serious ADEs; studies in table 3 do not require reconciliation).