A PHASE I/II OPEN LABEL MULTI-CENTER STUDY OF IMMUNE CHECKPOINT THERAPY WITH NIVOLUMAB FOR PATIENTS WITH LOCALLY ADVANCED ESOPHAGEAL SQUAMOUS CELL CARCINOMA

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INVESTIGATOR SIGNATURE PAGE

I have read this protocol, including all appendices, and I agree to conduct the study in compliance with all applicable regulations (including 21 CFR Part 312). I will also make a reasonable effort to complete the study within the time designated. I will provide all study personnel under my supervision copies of the protocol and access to all information provided by Bristol-Myers Squibb. I will discuss this material with them to ensure that they are fully informed about the drug and the study.

I am aware that, prior to the commencement of this study, the Institutional Review Board must approve this protocol and the informed consent document associated with the clinical facility where the study will be conducted. I agree to make all reasonable efforts to adhere to the attached protocol. I agree to provide all subjects with a signed and dated copy of their informed consent document, as required by FDA and ICH regulations. I further agree to report to Bristol-Myers Squibb any adverse events in accordance with the terms of this protocol and FDA regulation 21 CFR 312.64.

Nothing in this document is intended to limit the authority of a physician to provide emergency medical care under applicable regulations.

Investigator Signature	Date of Signature (DD/MM/YYYY)

Name of Investigator (please print)

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LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

Abbreviation	Explanation
%	Percent
˚C	Degrees Celsius
5-FU	5-Fluorouracil
ADL	Activities of Daily Living
AE	Adverse Event
AKR	An esophageal squamous cell cancer line with cyclin-D1 overexpression and p53 deletion
Alk Phos	Alkaline Phosphatase, a non-specific metalloenzyme which hydrolyzes many types of phosphate esters, found in highest amounts in liver, bile ducts, and bone
ALT/SGPT	Alanine transaminase or alanine aminotransferase/serum glutamate-pyruvate transaminase, a transaminase enzyme found in plasma and various body tissues, but most common in the liver.
ANC	Absolute Neutrophil Count
AP	Anteroposterior
AST/SGOT	Aspartate transaminase or aspartate aminotransferase/Serum glutamic oxaloacetic transaminase, an enzyme that is normally present in liver and heart cells.
AUC	Area Under the Curve
BMS	Bristol-Myers Squibb
BRAF	A human gene that encodes the B-Raf protein, which is involved in directing cell growth.
CBC	Complete Blood Count
Cc	Cubic centimeter(s)
cCR	Clinical Complete Response
CD279	Programmed cell death protein 1 (PD-1), a cell surface receptor of the immunoglobulin superfamily and expressed on T cells and pro-B cells. It binds two ligands: PD-L1 and PD-L2.
CD3	Cluster of Differentiation 3: a T-cell co-receptor that helps to activate the cytotoxic T-Cell.
CD4	Cluster of Differentiation 4: a glycoprotein found on the surface of immune cells such as T helper cells, monocytes, macrophages, and dendritic cells.
CD8	Cluster of Differentiation 8: a transmembrane glycoprotein that serves as a co-receptor for the T cell receptor (TCR). Like the TCR, CD8 binds to a major histocompatibility complex (MHC) molecule, but is specific for the class I MHC protein.
CFR	Code of Federal Regulations
cGy	centi-Gray (see Gy below)
CMP	Complete Metabolic Panel
CR	Complete Response
CrCl	Creatinine Clearance

CRF	Case Report Form
CRO	Clinical Research Organization
CROSS	ChemoRadiotherapy for Oesophageal cancer followed by Surgery Study
CT	Computed Tomography
CTCAE	Common Terminology Criteria for Adverse Events
CTLA-4	Cytotoxic T-lymphocyte associated antigen 4: a protein receptor that, functioning as an immune checkpoint, downregulates the immune system.
CTV	Clinical Target Volume
DHEP	di-2-ethylhexyl phthalate: A plastic form of polyvinyl chloride used to manufacture intravenous (IV) tubing and containers
dL	deciliter(s)
DRR	Digitally reconstructed radiographs
DVH	Dose volume histograms
ECG	Electrocardiogram
ECOG	Eastern Cooperative Oncology Group
ELISA	Enzyme-Linked Immunosorbent Assay
EOT	End of Treatment Visit
ESCC	Esophageal Squamous Cell Carcinoma
et al.	et alia, a Latin phrase meaning "and others"
etc.	et cetera, used at the end of a list to indicate that further, similar items are included
EUS	Endoscopic Ultrasound
FDA	The US Food and Drug Administration
FDG	Fluorodeoxyglucose, a radiopharmaceutical used in the medical imaging modality positron emission tomography (PET)
FOXP3	Forkhead box P3, a protein involved in immune system responses and regulation of the development and function of regulatory T cells.
FSH	Follicle Stimulating Hormone
G	Gram(s)
GTV	Gross tumor volume
Gy	Gray: a derived unit of ionizing radiation dose in the International System of Units (SI). It is defined as the absorption of one joule of radiation energy per kilogram of matter.
H1 Blocker	Histamine 1 antagonist: a class of medications that block the action of histamine at the H1 receptor, helping to relieve allergic reactions
H2 Blocker	Histamine 2 antagonist: a class of medications that block the action of histamine at the histamine H2 receptors of the parietal cells in the stomach, decreasing production of stomach acid.
Hb	Hemoglobin
HCG	Human Chorionic Gonadotropin
HBV	Hepatitis B
HCV	Hepatitis C
HIV	Human Immunodeficiency Virus

HRT	Hormone Replacement Therapy
i.e.	id est, a Latin phrase meaning “that is”
IC_{50}	The concentration of an inhibitor required to reduce the rate of an enzymatic reaction by 50%. It is a measure of how effective a drug is.
ICH	The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
ICOS	Inducible T Cell Co-stimulator
ICRU	International Commission on Radiation Units & Measurements
IFNγ	Interferon Gamma
IgG4	Immunoglobulin G4
IL-13	Interleukin 13
IL-1β	Interleukin 1 Beta
IMF	Immune Monitoring Facility
IMRT	Intensity-Modulated Radiation Therapy
IU	International Units
IUD	Intrauterine Device
IUS	Intrauterine hormone releasing System
IV	Intravenous
IVPB	Intravenous piggy-back
K_D	Dissociation Constant
Kg	kilogram(s)
Ki-67	A protein that is a cellular marker for proliferation.
L	Liter
LAC	Los Angeles County
LAG-3	Lymphocyte-activation gene 3 protein: a cell surface molecule with diverse biologic effects on T cell function. It is an immune checkpoint receptor.
M	Meter(s)
MAGE-A3	Melanoma-associated antigen 3. The presence of the antigen on tumor cells has been associated with worse prognosis.
mcL or µL	Microliter(s)
Mg	Milligram(s)
Min	Minute(s)
mL	Milliliter(s)
Mm	Millimeter(s)
MRI	Magnetic Resonance Imaging
MSKCC	Memorial Sloan Kettering Cancer Center
MV	millivolt
NCI	National Cancer Institute
nmol/L	Nano molar (10^{-9} molar)
NY-ESO-1	A cancer-testis antigen found in various tumors; may serve as a marker for metastasis
NYU	New York University

O_2 Sat.	Oxygen Saturation
OS	Overall Survival
PA	Posteroanterior
PBMCs	Peripheral Blood Mononuclear Cells
pCR	Pathological Complete Response
PD-1	Programmed Death-1
PD-L1/2	Programmed Death Ligand ½
PET	Positron Emission Tomography
PFS	Progression-Free Survival
PI	Principal Investigator
Plt	Platelets
PVC	Polyvinylchloride
PO	Per os, referring to the oral administration of medications
PTV	Planning target volume
PVC	Polyvinylchloride
Q	Every
ROI	Regions of Interest
RPC	Radiological Physics Center
RT	Radiation Therapy
RTOG	Radiation Therapy Oncology Group
SAE	Serious Adverse Event
SUVmax	Maximum PET/CT Standardized Uptake Value
Th1	Type 1 T Helper Cells
Th2	Type 2 T Helper Cells
TIM-3	T-cell immunoglobulin and mucin-domain containing-3: a protein that in humans is encoded by the HAVCR2 gene. It is an immune checkpoint, Th1-specific cell surface protein that regulates macrophage activation.
TMA	Tumor Microarray
TNFα	Tumor Necrosis Factor Alpha
TPN	Total Parenteral Nutrition
Treg	Regulatory T Cell
Tx.	Treatment
ULN	Upper Limit of Normal
USC	University of Southern California
US	United States
USP	United States Pharmacopeia
UT	Unacceptable Toxicity
Vs	Versus
WOCBP	Women of Child-Bearing Potential
Wt	Wild Type
3D	3-dimensional
4DCT	4-dimentional CT

SYNOPSIS

Study Title	A Phase I/II Open Label Multi-Center Study of Immune Checkpoint Therapy with Nivolumab in Patients with Locally Advanced Esophageal Squamous Cell Carcinoma (ESCC)
Short Title	Nivolumab in ESCC
Study Sponsor	New York University (NYU)
Funding Sponsor	Bristol-Myers Squibb (BMS)
NYU Protocol Number	s16-00971
BMS Protocol Number	CA209-791
Phase	I/II
Study Type	Multi-Center, Single-arm, Open Label Study
Study Duration	1.5 - 2 years
Study Center(s)	1. NYU: Laura and Isaac Perlmutter Cancer Center and Bellevue Hospital 2. Memorial Sloan Kettering Cancer Center (MSKCC) 3. University of Southern California (USC): Norris Cancer Center and Los Angeles County (LAC) + USC Medical Center 4. Oregon Health Sciences University Knight Cancer Institute 5. University of San Diego: Moores Cancer Center
Objectives	Phase I Objective 1. Assess the safety of induction nivolumab followed by chemoradiation with nivolumab followed by surgery when necessary and adjuvant nivolumab for patients with locally advanced squamous cell cancer of the esophagus Phase II Objectives 1. Estimate the clinical complete response (cCR) + pathological complete response (pCR) rate of induction nivolumab followed by chemoradiation with nivolumab. 2. Describe Progression-Free Survival (PFS) and Overall Survival (OS) with nivolumab and chemoradiation, followed by adjuvant nivolumab 3. Describe the major toxicities encountered with this treatment Exploratory Objectives 1. Assess whether PET/CT clinical response to nivolumab can predict the following outcomes: CR, PFS and OS 2. Assess the association between the outcomes CR, PFS and OS and immune correlative assays

Number of Patients	50-56
Diagnosis	Histologically or cytologically confirmed treatment-naïve T3-T4N_{any}M0 or T1-2N+ squamous cell carcinoma of the esophagus
Inclusion Criteria	**Inclusion Criteria** 1. Histologically or cytologically confirmed, treatment-naive esophageal squamous cell carcinoma 2. Previously obtained archival tumor tissue, or tissue obtained by endoscopically-guided core biopsy at screening 3. $T_{any}N_{1-3}$ or $T_{3-4}N_0$ as determined by EUS or PET/CT. All palpable or PET/CT visible lymph nodes outside the usual surgical field must be biopsy-proven negative for cancer. 4. All patients must have locoregional staging determined by endoscopic ultrasound (EUS) if technically feasible. Endoscopy reports or subsequent GI clinic note should clearly state both the T and N stage. 5. All patients must have initial PET/CT scans to document no evidence of metastatic or unresectable squamous cell cancer 6. All patients with tumors involving the thoracic esophagus must undergo bronchoscopy to document the absence of a fistula 7. No known contraindication to the use of taxanes or platinum compounds. 8. No history of severe hypersensitivity reaction to Cremophor® EL. 9. Patients who are ≥ 18 years old are eligible for this study. No specific gender distribution, nor specific racial or ethnic origins are necessary for enrollment in this study. 10. Eastern Cooperative Oncology Group (ECOG) Performance Status 0-1 11. A patient must be capable of giving informed consent or have an acceptable surrogate capable of giving consent on the subject's behalf 12. Deemed a suitable candidate for esophagectomy by the treating surgeon 13. Deemed a suitable candidate for radiation therapy by the treating radiation oncologist. 14. Patient must be non-pregnant and non-nursing. Women of child bearing potential (WOCBP) must have a negative serum or urine pregnancy test (minimum sensitivity 25 IU/L or equivalent units of HCG) within 72 hours prior to C1D1. 15. Screening Laboratory Values must meet the following criteria and should be obtained within 14 days prior to C1D1 (see Table 1 below) 16. All patients have to agree to participate in the correlative study of sample collection for immune correlative assays in order to participate in the main study. However, if the sample cannot be obtained due to feasibility issues, the patient will be allowed to continue on treatment

Table 1. Screening Laboratory Values

Test	**Acceptable Result**
WBC	≥ 2000/μL
Neutrophils	≥ 1500/μL
Platelets	≥ 100,000 /μL
Hemoglobin	> 9.0 g/dL
Serum Creatinine	≤ 1.5 x ULN **OR**
Creatinine Clearance (CrCl)*	≥ 40 mL/min
AST	≤ 3 x ULN
ALT	≤ 3 x ULN
Total Bilirubin**	≤ 1.5 x ULN
Oxygen Saturation (O_2 Sat.)	≥92% on ambient air
Hepatitis B status	
HBV Surface Antigen	Negative
HBV Surface Antibody	Positive or Negative
HBV Core Antibody	Positive or Negative***
HBV Viral Load	Negative
Hepatitis C status	
Anti-HCV Total Antibody	Negative
HCV RNA analysis	Negative
HIV status	
Rapid HIV 1/2 Antibodies	Negative

***Creatinine Clearance** Calculated using the Cockcroft-Gault formula

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

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

	Total Bilirubin ≤ 1.5 x ULN, except subjects with Gilbert Syndrome, who can have total bilirubin < 3.0 mg/dL * Subjects whose HBV core antibody is positive must have a negative HBV viral load measurement
Exclusion Criteria	***Exclusion Criteria*** 1. $T_{1-2}N_0$ as determined by EUS or PET/CT. 2. Pregnant or lactating women 3. Active or prior documented autoimmune or inflammatory disorders including but not limited to diabetes mellitus Type 1, inflammatory bowel disease; systemic lupus erythematosus; Wegener syndrome [granulomatosis with polyangiitis]; myasthenia gravis; Graves' disease; rheumatoid arthritis, hypophysitis, uveitis) within the past 3 years prior to the start of treatment. The following are exceptions to this criterion: 3a.Subjects with vitiligo or alopecia 3b.Subjects with hypothyroidism (e.g., following Hashimoto syndrome) stable on hormone replacement or psoriasis not requiring systemic treatment 4. History of idiopathic pulmonary fibrosis, organizing pneumonia, drug-induced pneumonitis, idiopathic pneumonitis, or evidence of active pneumonitis on screening chest Computed Tomography (CT) scan 5. The use of immunosuppressive medication within 28 days prior to the first dose of nivolumab. The following are exceptions to this criterion: 5a. Intranasal, topical, inhaled corticosteroids or local steroid injections (e.g. intra-articular injection) 5b. Systemic corticosteroids at physiologic doses ≤10 mg/day of prednisone or equivalent 5c. Steroids as premedication for hypersensitivity reactions (e.g. CT scan premedication 6. Positive test for Human Immunodeficiency Virus (HIV) 7. Prior treatment with any immunotherapy 8. Any other factors, including psychiatric or social, that in the opinion of the treating physician makes the patient an inappropriate candidate for a study. 9. Patients are excluded if they have active brain metastases or leptomeningeal metastases. Subjects with brain metastases are eligible if metastases have been treated and there is no magnetic resonance imaging (MRI) evidence of progression for [lowest minimum is 4 weeks or more] after treatment is complete and within 28 days prior to the first dose of nivolumab administration. There must also be no requirement for immunosuppressive doses of systemic corticosteroids (> 10 mg/day

	prednisone equivalents) for at least 2 weeks prior to study drug administration. 10. Patients should be excluded if they have an active, known or suspected autoimmune disease. Subjects are permitted to enroll if they have vitiligo, residual hypothyroidism due to autoimmune condition only requiring hormone replacement, psoriasis not requiring systemic treatment, or conditions not expected to recur in the absence of an external trigger 11. Patients should be excluded if they have a condition requiring systemic treatment with either corticosteroids (> 10 mg daily prednisone equivalents) or other immunosuppressive medications within 14 days of study drug administration. Inhaled or topical steroids and adrenal replacement doses < 10 mg daily prednisone equivalents are permitted in the absence of active autoimmune disease. 12. As there is potential for hepatic toxicity with nivolumab or nivolumab non-drugs with a predisposition to hepatoxicity should be used with caution in patients treated with nivolumab-containing regimen. Patients with a history of allergy to the study drug components are excluded.
Study Product, Dose, Route, Regimen	Nivolumab 240 mg (flat dose) IV administered every 2 weeks
Duration of administration	2 cycles pre-chemotherapy and radiotherapy (1 Cycle = 14 Days) 3 cycles concurrently with chemotherapy and radiotherapy 6 cycles post-chemotherapy, radiotherapy, and surgery as applicable
Endpoints	**Phase I Primary Endpoint:** Unacceptable Toxicity (defined in a later section) at 28 days after last dose of carboplatin and paclitaxel on day 64. **Phase II Endpoints:** 1. Primary endpoint 2. Clinical complete response (cCR) by endoscopic and PET/CT evaluation or pathologic complete response (pCR) for those undergoing surgery 2.Secondary endpoints 3. Median overall survival (OS) using Kaplan Meier curves *OS is defined as time from treatment to death from any cause.*

	4. Median progression free survival (PFS) using Kaplan Meier curves. *PFS is defined as time from treatment to disease recurrence after all therapy is complete or death from any cause* **3. Exploratory Endpoints:** Immune correlative assays Patients will have the following samples collected: • Paraffin embedded blocks from the primary tumor (at baseline, following induction nivolumab and at surgery, if available). • Whole blood will be collected at the following time-points for all patients: pre-induction nivolumab, post-nivolumab/pre-chemoradiation and post-chemoradiation/pre-surgery. Whole blood will be processed into peripheral blood mononuclear cells (PBMCs), serum and plasma on the day of collection and stored at -70°C for future analysis. • Whole blood will be collected at the following time points for patients who receive adjuvant nivolumab: pre-1st, 2nd and 4th dose.
Research Analyses	The following analyses will be performed on these samples: <u>Immunohistochemical staining of tumor tissue</u> Archived tumor tissue will be stained with an antibody against PD-L1 to characterize the PD-L1 status of the tumor cells. The antibody and scoring system will be determined at a later date. <u>Tumor microarray (TMA) immune microenvironment analysis</u> Our laboratory collaborator at MSKCC, Dr. Prasad Adusumilli, has extensive experience in investigating the immune microenvironment in tumors from >2,000 patients with lung adenocarcinomas, squamous cell carcinomas and neuroendocrine cancers. In addition, he is the principal investigator of a National Cancer Institute R21-funded prospective clinical trial in defining mesothelin, a cancer-associated antigen as a marker for esophageal cancer therapy response. His laboratory will construct a TMA from each tumor that will comprise ≥4 tumor cores from the most inflammatory region of the tumor as seen on H&E, ≥4 peritumoral stromal cores, along with normal tissue. Tissue immune markers staining will be graded according to the intensity and/or distribution of staining. Grading intensity will be categorized as follows: 0 (staining absent); 1 (weak expression); 2 (moderate expression); and 3 (strong expression). The distribution of staining will be graded as follows: 0 (staining absent); 1 (1% to 50%); and 2 (51% to 100%). The sum of the stain intensity and the distribution grade will be used to determine the total score, ranging from 0 to 5. Markers that will be analyzed include immune cell surface markers (CD3, CD4, CD8, CD45RO, FOXP3) and chemokines/cytokines (CCR7, CXCL12, CXCR4 and IL-7R).

	Serum cytokine measurement Cytokine profiles provide information about the status of immune activation and whether there is predominance of a Th1 or Th2 response. Th1 responses are associated with cytotoxic T cell responses and effective anti-tumor immunity, while Th2 responses are regarded as being anti-inflammatory and counteract Th1 responses. Cytokines can be measured using multiplexed ELISA kits, which can detect IL-1β, -2, -4, -6, -8, -10, -12p70, IL-13, tumor necrosis factor-α, interferon (IFN-γ) and others. These assays will be performed in the Immune Monitoring Facility (IMF) at MSKCC. PBMC phenotype characterization Multiparameter flow cytometry will be used to characterize the PBMC phenotype at various time points. Based on the time-intensive nature of these analyses, they may be restricted to patients with extreme outlier responses, e.g. complete vs. minimal pathologic response or rapid recurrence following surgery. The panel will include CD3, CD4, CD8, FOXP3, Ki67, ICOS, PD-1, LAG-3, TIM-3 and CTLA-4. Testing will be performed by the MSKCC Immune monitoring Facility (IMF) using this "T cell activation/exhaustion panel," which has previously been validated in other studies of anti-PD-1 and anti-PD-L1 antibodies performed at MSKCC. Serum measurement of antibody responses to >8,000 antigens The analysis of antibody responses offers additional insight into the immunomodulatory properties of nivolumab. These assays will be performed by the IMF. Because of the cost of the array, such analyses may again be restricted to patients with unusual/extreme responses. Commercially available protein microarrays (Invitrogen) now allow for concurrent serologic screening for antibody responses to >8,000 antigens with a single serum sample on an unprecedented scale (termed seromics). Matched baseline and post-therapy sera would be assayed on one array.
Statistical Methodology	This is a single-arm phase I/II study of nivolumab combined with carboplatin/paclitaxel and radiation for patients with locally advanced squamous cell carcinoma the esophagus. In the phase III CROSS study, pre-operative carboplatin/paclitaxel and radiation was associated with a grade 3/4 toxicity rate of 20% (7% hematologic and 13% non-hematologic grade 3-4 toxicities). The discontinuation rate for nivolumab as a single agent due to any toxicity has been reported to be approximate 9% {BMS, #81}. The hypothesis is that the addition of nivolumab to carboplatin/paclitaxel and radiation will not increase the aggregate grade 3/4 toxicity rate beyond 30%. In the phase I portion of the study, up to six patients will be treated (radiation will be 50.4 Gy (1.8 Gy/fraction × 28 fractions)) and then observed for 28 days

	(following last day of treatment (Day 64)). If there are 1 or less unacceptable toxicities (1/6 is less than 30%), the current regimen will be used in the phase II portion of the study. If there are 2 or more unacceptable toxicities (2/6 is more than 30%), the radiation will be reduced to 41.4 Gy (1.8 Gy/fraction × 23 fractions). Up to six additional patients will be treated at the reduced radiation regimen and then observed for 28 days (following last day of treatment (Day 64)). If there are 1 or less unacceptable toxicities, radiation of 41.4 Gy will be used in the phase II portion of the study. If there are 2 or more unacceptable toxicities, the trial will be discontinued. Unacceptable Toxicity (UT) is defined here as: • Recurrent grade 3 or 4 hematologic toxicity (despite 1 prior dose reduction in chemotherapy) • Any toxicity that results in a >2-week delay in chemoradiation If the regimen is found to be tolerable, the phase II portion of the study will be executed. An optimal two-stage design will be implemented. With an overall sample size of 44 patients, there is 80% power (actual power is 80%) to test the null hypothesis of clinical complete response (cCR) rate + pathologic complete response (pCR) rate ≤ 35%, versus the alternative hypothesis of cCR+pCR ≥ 55%, with significance level of 5% (actual significance level is 4.5%). In the first stage, 18 patients will be treated with the combination therapy. If there are 7 or fewer cCR+pCRs the trial will be terminated. If there are 8 or more cPR+pCRs, the trial will continue with the subsequent enrollment of 26 additional patients in phase 2. If the total cPR+pCRs out of 44 patients is 20 or less, the combination therapy will be rejected. cCR will be evaluated by endoscopic evaluation and CT/PET scan evaluation at the completion of radiation with concurrent nivolumab + carboplatin and radiation and pCR will be evaluated post-surgery. The trial will continue accruing patients in the second stage, while the 14 patients in the first stage are being evaluated for cCR or pCR. In an event that the interim analysis deems the combination therapy futile, accrual to the second stage will cease.

1.0 BACKGROUND AND RATIONALE

1.1 Esophageal Squamous Cell Cancer (ESCC)

In 2016, there will be an estimated 16,980 cases of esophageal cancer and 15,590 death as a result of the disease in the United States [1]. However, worldwide, there will be an estimated 455,800 cases over 400,000 deaths [2]. Despite advances in the multimodality care in esophageal cancers, in particular the combination of chemotherapy, radiation

therapy and surgery, the generally observed 5-year overall survival rate remains only 15-34%.

The two major histologies of esophageal cancer, adenocarcinoma and squamous cell carcinoma (ESCC), have distinct epidemiologic and etiologic characteristics. These entities also have distinct precursor lesions and distinct molecular properties. Indeed, there are enough differences in these histologies to suggest exploitable differences in future therapeutic approaches [3].

Worldwide, over 80 percent of the esophageal cancers are squamous histology [4]. In the past three decades, Western societies have seen the incidence of ESCC decline and the incidence of adenocarcinomas markedly increase. This has led to a proportional under-representation of ESCC patients included in US clinical trials. Indeed, recent US, NCI-sponsored clinical trials have focused only on adenocarcinomas. This has led to the observation that ESCC has become an understudied orphan disease [5].

For patients with either ESCC or adenocarcinoma of the esophagus, multiple randomized controlled studies and meta-analyses have conclusively demonstrated a survival benefit for those patients receiving neoadjuvant chemoradiation [6]. A new standard-of-care was established by van Hagen, et al., who performed the seminal Dutch CROSS trial, in which patients with esophageal adenocarcinomas and ESCC were treated with weekly carboplatin AUC 2 and paclitaxel 50 mg/m^2, administered concurrently with 41.4 Gy external radiation prior to surgery, versus surgery alone [7]. The combined modality arm was markedly superior to surgery alone. Although patients with either histology benefitted from combined modality treatment, patients with ESCC tumors (about 23% of the total) had a statistical benefit far greater than those with adenocarcinomas [7]. Specifically, ESCC patients treated with chemoradiation and surgery had a 49% pathologic complete response (pCR) rate and a median overall survival (OS) of 82 months [8]. It should be noted that these data (pCR rate and OS) are far superior to what has been the overall experience of those who routinely treat ESCC of the esophagus.

In the CROSS Trial, the investigators reported on “any” adverse event and grade ≥ 3 adverse events. Of the 171 patients who received carboplatin and paclitaxel with radiation, 30 (18%) developed diarrhea; two patients (1%) developed grade ≥ 3 diarrhea. Twenty-five patients (15%) developed some grade of neurotoxicity; no patient developed grade ≥ 3 neurotoxicity. Sixteen patients (9%) developed neutropenia; four patients developed grade ≥ 3 neutropenia. Ninety-two patients (54%) developed thrombocytopenia; one patient (15) had grade ≥ 3 thrombocytopenia. In their manuscripts describing the CROSS trial [7, 8], there were no reports of renal and/or hepatic dysfunction.

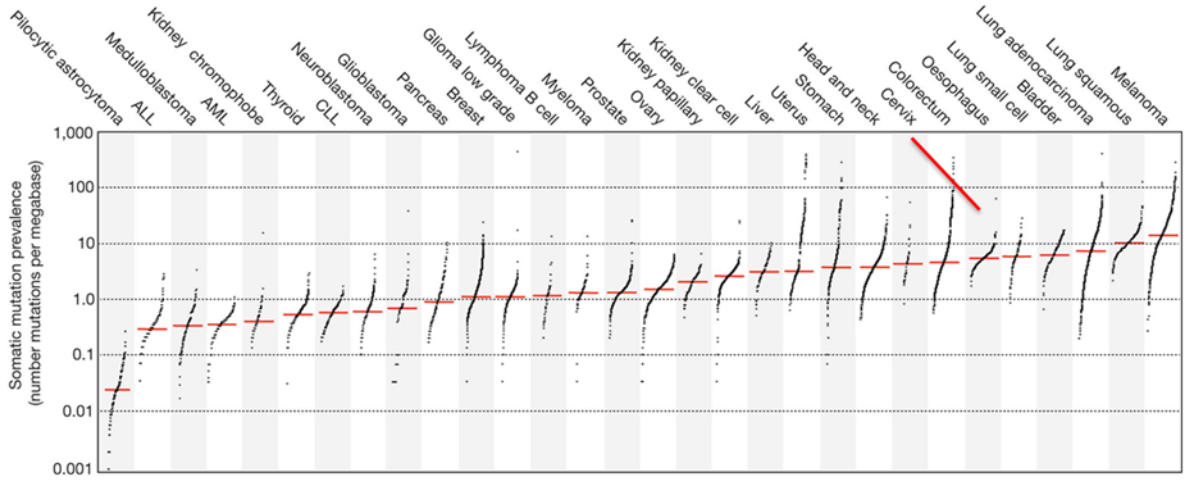
For patients receiving neoadjuvant combined modality therapy, survival is generally thought to be dependent on response, as those with complete pathologic response (pCR) or near pCR have the best chance of long-term survival [9]. In particular, in ESCC patients who have co-morbidities associated with alcohol and tobacco use, surgery carries with it a higher rate of post-operative mortality following neoadjuvant chemoradiation secondary to increased risks of cardiopulmonary complications [10]. Therefore, many clinicians opt to avoid surgery in such patients. Indeed, long-term survival data from RTOG 85-01 in which patients with esophageal ESCC were definitively treated with chemotherapy and radiation without surgery indicates that 27% of patients were alive without cancer 10 years after treatment [11].

In an effort to definitively address the issue regarding the need for an operation for ESCC patients, two randomized European trials compared definitive chemoradiation vs. chemoradiation followed by surgery in ESCC patients [12, 13]. Taken together, both studies suggest that local control is improved by subsequent surgery but that there is no clear improvement in survival for patients who respond to chemoradiation. This has led many investigators to the conclusion that those patients with clinical CR to definitive chemoradiotherapy (endoscopic clearance of the tumor and ^{18}F-fludeoxyglucose (FDG)-PET scan resolution of all FDG-avid areas) can safely forego surgery.

1.2 ESCC and Immunotherapy

Programmed cell death protein 1, also known as PD-1, is a protein transmembrane receptor expressed on activated lymphocytes, including tumor infiltrating lymphocytes that acts to shut down T cell responses in order to prevent unchecked inflammation. Thus, PD-1 functions as an immune checkpoint inhibitor. When PD-1 binds with its ligands, programmed cell death 1 ligand (PD-L1) or programmed cell death ligand 2 (PD-L2), effector T cells become inactive. Investigators have found that tumors with relatively high levels of PD-L1 have a poorer prognosis than those with relatively lower levels [14]. Another measure for the potential effectiveness for immune checkpoint inhibitors is the number of mutations found in a tumor. As noted by Alexandrov et al., squamous cell tumors of the esophagus have a relatively high number of somatic mutations (Figure 1) [15].

Figure 1. Somatic mutations, which are correlated with sensitivity to immunotherapy, are highly prevalent in esophageal cancer



Every dot represents a sample while the red horizontal lines are the median numbers of mutations in the respective cancer types. The vertical axis (log scaled) shows the number of mutations per megabase while the different cancer types are ordered on the horizontal axis based on their median numbers of somatic mutations

Alexandrov, et al. [15]

1.3 Nivolumab

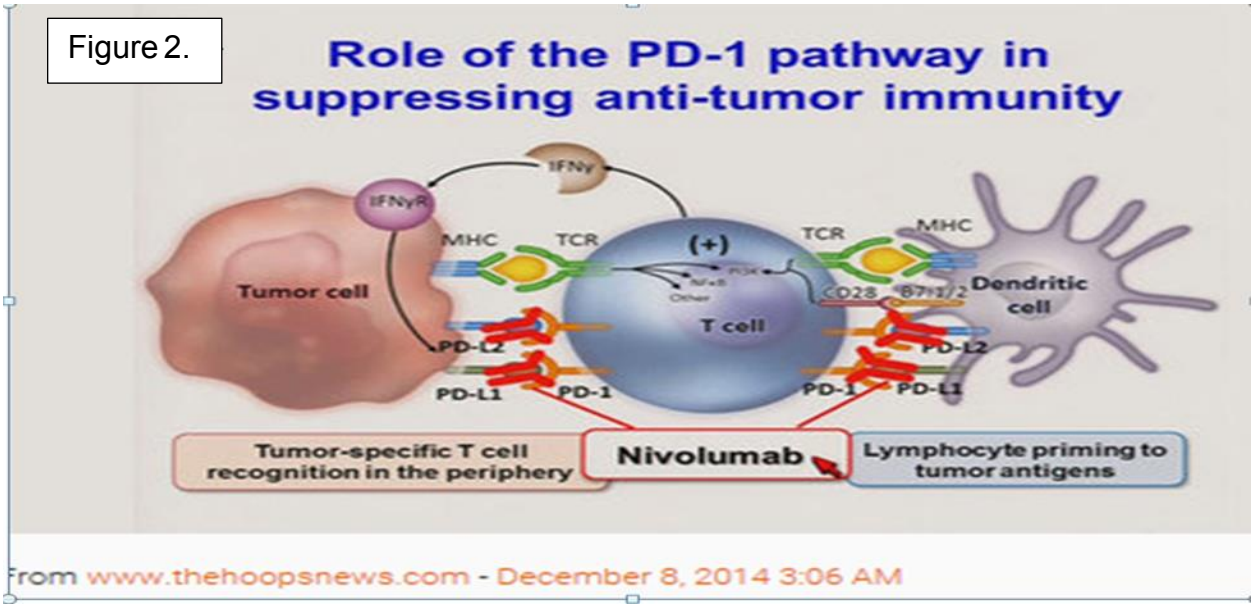
Nivolumab, a 146–kilodalton molecule is a fully human immunoglobulin G4 (IgG4) antibody with high affinity for the human programmed death-1 receptor (PD-1 or CD279). Nivolumab binds to PD-1 and blocks the interaction with its ligands (PD-L1 or PD-L2), thereby liberating T cells from suppression mediated by PD-1 signaling. Nivolumab is a genetically engineered monoclonal antibody with minimal cellular and complement-mediated cytolytic function by virtue of its IgG4 isotype. Preclinical models demonstrated that incubation with nivolumab augmented T-cell expansion and enhanced cytokine production and cytolytic function [16].

Nivolumab is administered intravenously every 2 weeks, and steady-state concentrations are reached by 12 weeks at a dose of 3 mg/kg. The serum half-life was estimated at 12 days for the lower-dosage cohorts (0.3-, 1- or 3-mg/kg dose) and at 20 days for the highest-dosage cohort (10 mg/kg). The mean volume of distribution at steady state was 8 L and the mean clearance was 9.5 mL/hour. The area under the curve and maximum concentrations were directly related to the dose level indicating that the pharmacokinetic properties remained linear across the dose ranges [17].

Blocking immune checkpoints that function as negative regulators of the immune system, is an approach that has proven to be most effective against solid tumors with relatively high mutational burdens such as melanoma [18], squamous cell lung cancers [19], and a subset of colon tumors (microsatellite instability-high tumors with a very high mutation burden) [20]. Squamous cell esophageal cancers, epidemiologically associated with cigarette smoking, have a high prevalence of somatic mutations, which may correlate with increased sensitivity to immune checkpoint inhibitors (Figure 2) [15, 21].

Nivolumab has been well-studied in solid tumors, demonstrating efficacy in melanoma, squamous cell and adenocarcinomas of the lung, and squamous tumors of the head and neck. Nivolumab is administered intravenously every 2 weeks and steady-state concentrations are reached by 12 weeks at a dose of 3 mg/kg [17, 22]. We will document the smoking status of patients of all patients registered for this protocol.

Figure 2.



*1.3.1 **Melanoma***

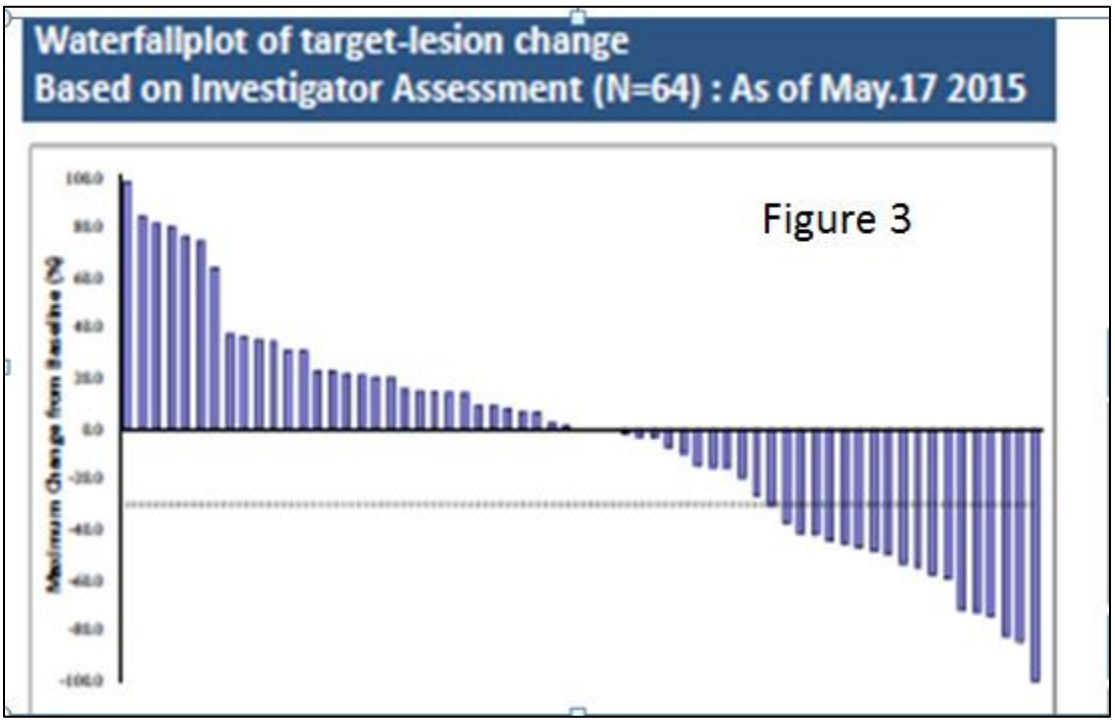
A phase 3 trial for patients with BRAFwt metastatic melanoma tested nivolumab at 3 mg/kg against dacarbazine at 1000 mg/m^2 + a nivolumab-matched placebo. The investigators reported that nivolumab-treated patients had a superior overall response rate of (40% vs. 13.9%), superior progression free survival (5.1 months vs. 2.2 months) and a higher percentage of those alive after one year (72.1% vs. 42.1%). Toxicities, generally non-cumulative occurred mostly in the first 6 months of therapy included pneumonitis, low-grade fatigue, diarrhea, pruritus, nausea and decreased appetite. Grade 3 or 4 adverse advents occurred in 11.7% of patients treated with nivolumab and 17.6% of those treated with dacarbazine [23].

*1.3.2 **Non-Small Cell Lung Cancers***

Two phase III non-small-cell lung cancer trials tested nivolumab 3 mg/kg against docetaxel 75 mg/m^2 for patients with squamous cell lung cancers and those with non-squamous cell lung cancers. In each trial, entry criteria included patients for whom initial platinum-based doublet therapy had failed. In each trial, the primary endpoint was overall survival. Both trials demonstrated superior survival for the nivolumab-treated patients. In the non-squamous cell trial, the median overall survival for the nivolumab-treated patients was 12.2 versus 9.4 months for the docetaxel-treated patients (p=0.002) [24]. The median overall survival for squamous cell lung cancer patients receiving nivolumab as second line therapy was 9.2 months versus 7.3 months for those receiving docetaxel (p=0.001) [25]. Grade 3 or4 or toxicities for patients who received nivolumab were 10% and 7% for non-squamous-cell and squamous-cell lung cancers.

1.3.3 Esophageal Cancer

Evidence for the benefit of nivolumab for ESCC comes from the results of a phase II trial reported as an abstract at the 2015 European Cancer Conference. Japanese investigators evaluated nivolumab in 65 patients with esophageal cancer who had up to 4 previous treatments. Although the trial allowed patients with adenocarcinoma or mixed adeno-squamous cell tumors to be treated, all 65 patients entered into this trial has squamous-cell histology. Two-thirds of patients had received ≥3 prior chemotherapy regimens. Fifty-four men and 11 women with a median age of 62 years were treated in this trial. One complete response and 12 partial responses total were found by the investigators (total response rate 20.4%) and one complete response and 10 partial responses were coded by central review (17.2%). Figure 3 is a waterfall plot of the target-lesion change as presented by Ura, et al. [26].



1.4 Combining Radiation Therapy with PD-1 Inhibition

Traditionally, radiation therapy has been used in cancer therapy as a means of controlling local disease. However, recent investigations have demonstrated that radiation to a specific tumor may prompt the release of tumor-associated antigens that prime the adaptive immune system [27, 28]. It is now postulated that the 'vaccine effect' of radiation is responsible for the abscopal effect whereby radiation to one tumor may induce a response in an area that was not irradiated. As a corollary, radiation may have the effect of turning an immunotherapy-insensitive tumor into one that is sensitive to radiation [29]. By releasing

tumor-associated antigens, radiation has been described as inducing a vaccine-like effect by priming the adaptive immune system [27, 30].

An abscopal response was observed in a patient with advanced melanoma previously treated with ipilimumab, an antibody against CTLA-4, who subsequently received palliative radiation to a painful metastatic site [31]. This patient's tumor expressed NY-ESO-1, an antigen expressed only in normal testicular germ cells and placenta and in 30-40% of advanced melanomas [31]. Significant regression was observed in tumors outside the radiation field [31]. Immune analyses revealed an increase in NY-ESO-1-specific antibody responses and in the proportion of NY-ESO-1-specific CD4+ T cells that expressed inducible co-stimulator (ICOS), a marker of T cell activation [31]. A second patient with melanoma treated with ipilimumab also experienced a similar abscopal response following radiation, with a corresponding increase in the antibody response against the cancer-testis antigen MAGE-A3 [32]. In addition to stimulating immune responses, the combination of radiotherapy and immunotherapy may also serve to reinvigorate pre-existing tumor-specific CD8+ T-cell populations [33].

1.5 NYU Preclinical Data for Radiation and PD-1 Inhibition

To examine the role of PD-1 in the radiation response of esophageal squamous cell carcinomas, investigators at the NYU Perlmutter Cancer Center used the AKR esophageal squamous cell cancer cell line in a mouse allograft model. The AKR mouse esophageal squamous cell cancer cell line is derived from the ED-L2-cyclin D1;p53−/− mouse model. Irradiation of AKR flank tumors demonstrated tumor reduction but also an upregulation in PD-1 expression, reflecting T-cell exhaustion. The addition of anti-PD-1 antibody to radiation leads to improved tumor reduction and increases in CD8+ effector T-cells and less exhaustion of PD-1+ T-cells. This pre-clinical data, summarized in figures 4 and 5 below, add to our enthusiasm in combining the PD-1 inhibitor nivolumab, radiation therapy and chemotherapy in a clinical trial for patients with squamous cell carcinomas of the esophagus [34].

Change in Tumor Volume **Change in Amount of CD8+ T Cells**

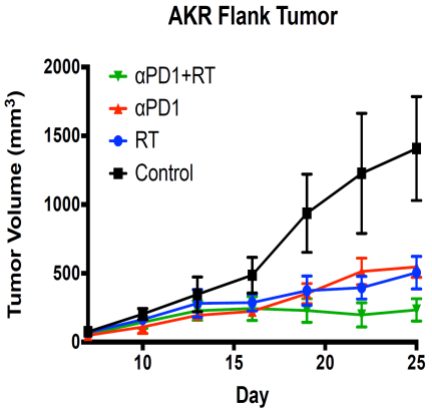


Figure 4

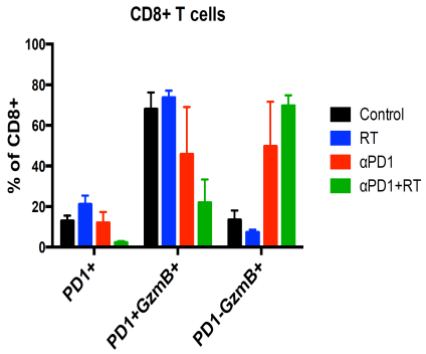


Figure 5

The investigators who designed this trial hypothesize that the combination of immune checkpoint blockade with radiation will exploit the abscopal effect.

1.6 Rationale for Combining Chemoradiation with Nivolumab

Although the potential synergy between radiation and PD-1 blockade has been demonstrated (see above), radiation by itself has not had an impact on survival for patients with ESCC. However, the curative benefit for combining external beam radiation therapy with chemotherapy for patients with ESCC has been clearly demonstrated [7]. Regardless, only a minority of patients treated obtain a pCR. Yet, even those who do achieve a pCR or near pCR have a distant recurrence rate close to 40% [35]. This is ample reason to investigate methods for producing a higher cure rate for the majority of patients with ESCC.

1.7 Rationale for Flat Dosing of NIVOLUMAB

Nivolumab monotherapy has been extensively studied in a number of tumor types including non-small cell lung cancer (NSCLC), melanoma, renal cell carcinoma (RCC), and colorectal cancer (CRC) with body weight normalized dosing (mg/kg). Nivolumab pharmacokinetics (PK) and exposures of subjects in these studies have been characterized by population pharmacokinetic (PPK) analysis of data collected in these studies, together with PK data from several Phase 1, 2, and 3 clinical studies of nivolumab monotherapy in solid tumors. PPK analyses have shown that the PK of nivolumab are linear, with dose proportional exposures over a dose range of 0.1 mg/kg to 10 mg/kg, and are similar across tumor types. Nivolumab clearance and volume of distribution were found to increase with increasing body weight, but the increase was less than proportional, indicating that a mg/kg dose represents an over-adjustment for the effect of body weight on nivolumab PK. Given the relationship between nivolumab PK and body weight, a flat dose is expected to lead to lower exposures in heavier patients, relative to the exposures in lighter patients.

Using the PPK model, nivolumab steady-state trough, peak and time-averaged concentration (Cminss, Cmaxss, and Cavgss, respectively) were predicted for a flat

nivolumab dose of 240 mg Q2W and compared to those following administration of 3 mg/kg Q2W in NSCLC, melanoma, and RCC subjects. A dose of 240 mg nivolumab is identical to a dose of 3 mg/kg for subjects weighing ~ 80 kg, which is the approximate median body weight of subjects in the Phase 2 and 3 BMS clinical studies of nivolumab monotherapy. From the simulations, the geometric mean values of Cminss, Cmaxss, and Cavgss with flat dosing are slightly (< 15%) higher than that produced by a 3 mg/kg dose, and the coefficient of variation (cv%) in these measures of exposure are only slightly (< 10%) greater than that of 3 mg/kg dosing. Given the similarity of nivolumab PK across tumor types and the similar exposures predicted following administration of a 240 mg flat dose compared to 3 mg/kg, it is expected that the safety and efficacy profile of nivolumab following a flat dose will be similar to that of 3 mg/kg nivolumab dose.

1.8 Adjuvant Nivolumab

In general, postoperative cytotoxic therapy following intensive chemo-radiation with or without surgery for patients with esophageal cancer has not been successful. Frequently, patient performance status declines following neoadjuvant therapy and surgery. This makes cytotoxic therapy quite difficult, if not impossible, for the majority of patients with esophageal cancer. The concept that continued stimulation of the immune system could be a successful method to wipe out microscopic cancer cells is being examined in a BMS trial for esophageal cancer patients that receive only chemotherapy and radiation prior to surgery. Thus, in this pilot trial, testing the toxicity of immunotherapy in the initial multi-modality therapy for squamous cell cancer of the esophagus, we will test whether continued treatment with nivolumab after definitive therapy with nivolumab and chemoradiation is well tolerated. Indeed, for most patients that have no or minimal toxicity to their first doses of nivolumab, continued use has generally been very well tolerated.

2. Study Hypotheses and Objectives

2.1 Hypotheses

This protocol will be testing the following hypotheses:

1) Nivolumab as induction single-agent therapy followed by nivolumab combined with chemoradiation is safe and feasible. (Phase I)

2) Nivolumab as induction single-agent therapy followed by nivolumab combined with chemoradiation will increase cCR + pCR rate beyond 55%. (Phase II)

3) Assessment of induction nivolumab by PET/CT and/or correlative analyses of tumor tissue and peripheral blood may identify biomarkers that correlate with outcomes. (Phase II)

2.2 Objectives

2.2.1 Phase I Objective

1. Assess the safety of induction nivolumab followed by chemoradiation with nivolumab and adjuvant nivolumab for patients with locally advanced squamous cell cancer of the esophagus

2.2.2 Phase II Objectives

2.2.2.1 Primary Objective

1. Estimate the cCR + pCR rate of induction nivolumab followed by chemoradiation with nivolumab.

2.2.2.2 Secondary Objectives

1. Describe the PFS and OS of nivolumab and chemoradiation, followed by adjuvant nivolumab
2. Describe the major toxicities encountered in this treatment

2.2.2.3 Exploratory Objectives

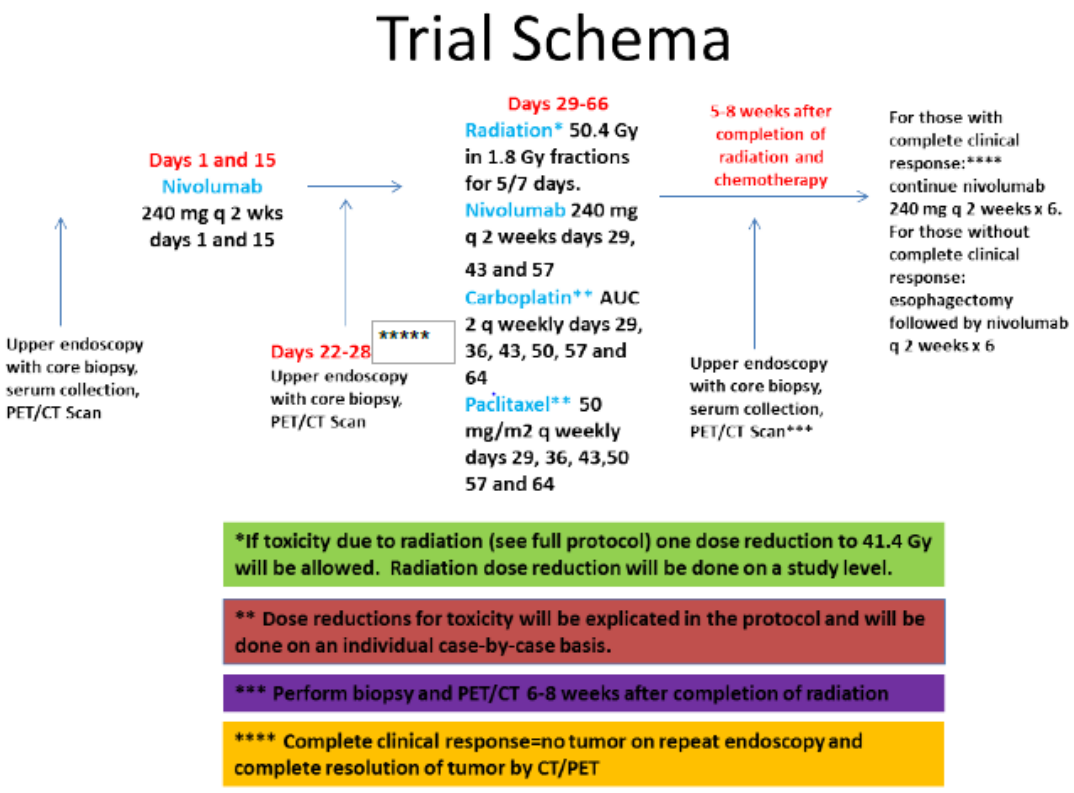
1. Assess whether PET/CT clinical response to nivolumab can predict the following outcomes: CR, PFS, and OS
2. Assess the association between the outcomes CR, PFS, and OS and immune correlative assays.

3. Study Design

3.1 General Design

In this multi-institution phase I/II trial, the investigators have chosen paclitaxel and carboplatin using a schedule and doses identical to those used in the CROSS trial. Following a run-in with nivolumab alone at 240 mg IVPB every 2 weeks for 2 doses, nivolumab at 240 mg every 2 weeks will be added to paclitaxel and carboplatin, which will be dosed according to the standard of care established by the CROSS trial: paclitaxel 50 mg/m^2 weekly for 6 weeks and carboplatin AUC 2 weekly for 6 weeks [7]. Concurrent radiation will be administered with chemotherapy at 1.8 Gy/fraction × 28 fractions to a total dose of 50.4 Gy, the standard radiation dose administered in the United States for trimodality therapy that includes concurrent therapy with carboplatin and paclitaxel [36, 37]. A decrease in dose to 41.4 Gy per the protocol established by van Hagen, et al. will be permitted before discontinuing therapy due to unacceptable toxicity [7]. While the CROSS study administered only 5 weekly doses of chemotherapy during the 5 weeks of radiation, the higher dose of 50.4 Gy (1.8 Gy/fraction ×28 fractions over 5½ weeks) utilized in this study permits for a sixth dose during the additional week of radiation.

Figure 6. Trial Schema



Footnotes
*****: Day 22-28 upper endoscopy with core biopsy is optional
*****: For those with clinical response, patients should go directly to nivo x6, but patients can be offered esophagectomies after discussion with the treating physician

Single-arm phase I/II open label multi-center study

Phase I: Up to 12 patients

6 patients treated and observed for 28 days after last day of treatment (Day 64)

- ≤ 1 unacceptable toxicity (UT): use current regimen for Phase II
- ≥ 2 UT: reduce total RT dose to 41.4 Gy and treat **6 additional patients**, then observe for 28 days after Day 64
    - ≤ 1 UT: use reduced RT for Phase II
    - ≥ 2 UT: discontinue trial

Phase II: 44 patients

Stage I: **18 patients** treated

- ≤ 7 CRs (either pCR or cCR): discontinue trial as combination therapy deemed futile
- ≥ 8 CRs (either pCR or cCR): continue on to Stage II.

Stage II: **26 additional patients** treated

- ≤ 20 cCR + pCR out of all 44 patients: combination therapy rejected

- ≥ 21 cCR + pCR out of all 44 patients: combination therapy should be studied further.

- Accrual to Stage II will continue while the 18 patients in Stage I are evaluated for cCR and pCR.

- Accrual to Stage II will stop if Stage I analysis deems combination futile

Unacceptable toxicity (UT) is defined here as:

- Recurrent grade 3 or 4 hematologic toxicity (despite 1 prior dose reduction in chemotherapy)
- Any toxicity related to chemotherapy, radiotherapy, or treatment with nivolumab that results in a >2-week delay in chemoradiation

See section 7.0 for the definition of toxicities and steps in dose reduction on an individual patient level.

3.2 Primary Study Endpoints

Phase I: Unacceptable toxicity at 28 days after last dose of carboplatin and paclitaxel (day 64). The primary endpoints of this phase are primary safety endpoints.

Phase II: cCR by endoscopic + PET/CT evaluation, pCR for patients undergoing surgery,

3.3 Secondary Study Endpoints

3.3.1 median OS (Kaplan Meier) + median PFS (Kaplan Meier)

3.3.2 Immune Correlative Assays

3.3.2.1 Sample Collection

The following samples will be collected**:**

- Paraffin embedded blocks from primary tumor (at baseline; after induction nivolumab(optional); and at surgery, if applicable)
- Whole blood will be collected at the following time points for all patients: pre-induction nivolumab, post-nivolumab/pre-chemoRT and post-chemoRT/pre-surgery. Whole blood will be processed into peripheral blood mononuclear cells (PBMCs), serum, and plasma on the day of collection and stored at -70°C for future analysis
- Whole blood will be collected at the following time-points for patients who receive adjuvant nivolumab: pre-1st, 2nd and 4th dose
  These assays that will be performed may include but are not limited to the assays discussed below. No genetic testing will be performed.

3.3.2.2 Immunohistochemical Staining of Tumor Tissue

Archived tumor tissue will be stained with an antibody against PD-L1 to characterize the PD-L1 status of the tumor cells. The antibody and scoring system will be determined at a later date.

3.3.2.3 Purpose of Immune Correlative Assays

Immune correlative assays will be conducted as part of this study in order to investigate changes that occur in cell surface markers, cytokine profiles, and PBMC phenotype before and after treatment with nivolumab, chemoradiation, and surgery (if applicable). Such information would lead to a better understanding of the effect of immunocheckpoint intervention on the subjects' esophageal squamous cell carcinoma. This information will not be of benefit to participating patients, but may influence future treatments with immunocheckpoint inhibitors in combination with chemoraditation therapy.

3.3.2.4 *Subject Participation*

Sample collection for the immune correlative assays described is mandatory for participation in this study. Sample collection is mandatory to report on one of the primary endpoints of the study. The study of this correlative information is essential for understanding the efficacy of immunotherapy and radiation therapy in this disease treatment application. If subjects do not agree to participate in this correlative study, they may not participate in the main study. If subjects decide not to participate, this will not affect the care the patients receive, and it will not result in any loss of benefits to which patients are otherwise entitled. Participating study subjects will be told of any significant new findings that may develop during the course of the research using their specimens and associated health information that may influence their willingness to continue to participate.

3.3.2.5 Rationale For Mandatory Correlative Study Specimen Collection

We hypothesize that these immune assays (CD3, CD4, CD8, CD45RO, FOXP3) and chemokines/cytokines (CCR7, CXCL12, CXCR4, and cytokine profiles) will statistically correlate with response and/or resistance to the protocol therapy.

3.3.2.6 Sample Labeling, Storage, and Handling

If a patient does give his/her permission (consent) to participate in this study, his/her samples will be kept for analysis indefinitely. This permission will never expire unless the subject withdraws it. The patient may withdraw (take back) his/her permission to use and share his/her specimens and health information at any time, without penalty. If the subject withdraws his/her consent, any unused specimens that have not been provided to researchers will be destroyed. If the subject withdraws his/her permission, we will not be able to take back information that has already been used or shared with others. To formally withdraw consent and/or revoke authorization, we ask that the subject contact Dr. Jennifer Wu in writing, and tell him of their wish to withdraw permission for specimens for research and/or authorization.

Only approved study personnel at NYU, MSKCC, and the study sub-sites will have access to the samples. Samples will be stored in a secure locked room at the laboratory at MSKCC:

Immune Monitoring Facility, Memorial Sloan Kettering Cancer Center
Zuckerman Research Center, 15th floor
417 East 68th Street
New York, NY 10065
Telephone: (646) 888-4588

Only authorized personnel will have access to them. Samples will only be studied for purposes relating to cancer and this study. The subject's name and personal information will not appear on the specimens. Samples will be labeled with a code (the subject's clinical study identification number) that can be linked to him/her only by the study doctor. Researchers who perform tests on these samples will only see the code, but will not see any personal information that specifically identifies the subject. Although complete privacy cannot be guaranteed, the study team will make every effort to protect subject identity and health information. The log to personal health information (PHI) will be maintained at each individual study site, only by authorized study personnel. Information will be stored in a secure locked room and in a secure password-protected database.

Any results from the research testing performed in this study will not be shared with the subjects, thereby greatly reducing the possibility of psychological or social risks that could arise from knowledge of this information, such as risk to the subject's employability or insurability, or the risk of discrimination.

For samples collected at NYU, the Center for Biospecimen Research and Development will ship processed samples (see Appendix II for shipment preparation) to MSKCC in batches. These batched samples will be labeled with a code (the subject's clinical study identification number) that can be linked to him/her only by the study doctor. Researchers who perform tests on these samples will only see the code, but will not see any personal information that specifically identifies the subject. Although complete privacy cannot be guaranteed, the study team will make every effort to protect subject identity and health information. The log to personal health information (PHI) will be maintained at each individual study site, only by authorized study personnel. Information will be stored in a secure locked room and in a secure password-protected database.

3.3.2.7 Tumor Microarray (TMA) Immune Microenvironment Analysis

The laboratory of our collaborator at MSKCC, Dr. Prasad Adusumilli, will construct a TMA from each tumor that will comprise ≥4 tumor cores from the most inflammatory region of the tumor as seen on H&E, ≥4 peritumoral stromal cores, along with normal tissue. Tissue immune markers staining will be graded according to the intensity and/or distribution of staining. Grading intensity will be categorized as follows: 0 (staining absent); 1 (weak expression); 2 (moderate expression); and 3 (strong expression). The distribution of staining will be graded as follows: 0 (staining absent); 1 (1% to 50%); and 2 (51% to 100%). The sum of the stain intensity and the distribution grade will be used to determine the total score, ranging from 0 to 5.

Markers that will be analyzed include immune cell surface markers (CD3, CD4, CD8, CD45RO, FOXP3) and chemokines/cytokines (CCR7, CXCL12, CXCR4 and IL-7R).

3.3.2.8 Serum Cytokine Measurement

Cytokine profiles provide information about the status of immune activation and whether there is predominance of a Th1 or Th2 response. Th1 responses are associated with cytotoxic T cell responses and effective anti-tumor immunity, while Th2 responses are regarded as being anti-inflammatory and counteract Th1 responses. Cytokines can be measured using multiplexed ELISA kits, which can detect IL-1β, -2, -4, -6, -8, -10, -12p70, IL-13, tumor necrosis factor-α, interferon (IFN)-γ and others. These assays will be performed in the Immune Monitoring Facility (IMF) at MSKCC.

3.3.2.9 PBMC Phenotype Characterization

Multiparameter flow cytometry will be used to characterize the PBMC phenotype at various time-points. Based on the time-intensive nature of these analyses, they may be restricted to patients with extreme outlier responses, e.g. complete vs. minimal pathologic response or rapid recurrence following surgery. The panel will include CD3, CD4, CD8, FoxP3, Ki67, ICOS, PD-1, LAG-3, TIM-3 and CTLA-4. Testing will be performed by the IMF using this "T cell activation/exhaustion panel", which has previously been validated in other studies of anti-PD-1 and anti-PD-L1 antibodies performed at MSKCC.

3.3.2.10 Serum Measurement of Antibody Responses to >8,000 Antigens

The analysis of antibody responses offers additional insight into the immunomodulatory properties of nivolumab. These assays will be performed by the IMF. Because of the cost of the array, such analyses may again be restricted to patients with unusual/extreme responses.

Commercially available protein microarrays (Invitrogen) now allow for concurrent serologic screening for antibody responses to >8,000 antigens with a single serum sample on an unprecedented scale (termed seromics). Matched baseline and post-therapy sera would be assayed on one array.

4.0 Patient Selection and Withdrawal

4.1 Inclusion Criteria

1. Histologically or cytologically confirmed, treatment-naive esophageal squamous cell carcinoma
2. Previously obtained archival tumor tissue, or tissue obtained by endoscopically-guided core biopsy at screening
3. TanyN_{1-3} or T_{3-4} N_0as determined by EUS or PET/CT. All palpable or CT/PET visible lymph nodes outside the usual surgical field must be biopsy-proven negative for cancer.
4. All patients must have locoregional staging determined by endoscopic ultrasound (EUS) if technically feasible. Endoscopy reports or subsequent GI clinic note should clearly state both the T and N stage.

5. All patients must have initial PET/CT scans to document no evidence of metastatic or unresectable squamous cell cancer
6. All patients with tumors involving the thoracic esophagus must undergo bronchoscopy to document the absence of a fistula No known contraindication to the use of taxanes or platinum compounds.
7. No history of severe hypersensitivity reaction to Cremophor® EL.
8. Patients who are ≥ 18 years old are eligible for this study. Neither specific gender distribution, nor specific racial or ethnic origins are necessary for enrollment in this study.
9. Eastern Cooperative Oncology Group (ECOG) Performance Status 0-1
10. A patient must be capable of giving informed consent or have an acceptable surrogate capable of giving consent on the subject's behalf
11. Deemed a suitable candidate for esophagectomy by the treating surgeon as documented in a pre-operative assessment visit per standard practice at each participating institution.
12. Deemed a suitable candidate for radiation therapy by the treating radiation oncologist as documented in a standard pretreatment visit per standard practice at each participating institution.
13. Patient must be non-pregnant and non-nursing. Women of child bearing potential (WOCBP) must have a negative serum or urine pregnancy test (minimum sensitivity 25 IU/L or equivalent units of HCG) within 72 hours prior to C1D1.
14. Screening Laboratory Values must meet the following criteria and should be obtained within 14 days prior to C1D1 (see Table 1 below)
15. Patients with a positive Hepatitis B viral load are allowed on the study as long as patients are getting active treatment for hepatitis B on or prior to the first dose of treatment on this trial and continue throughout this trial.
16. All patients have to agree to participate in the correlative study of sample collection for immune correlative assays in order to participate in the main study. However, if the sample cannot be obtained due to feasibility issues, the patient will be allowed to continue on treatment

Table 1. Screening Laboratory Values

Test	**Acceptable Result**
WBC	≥ 2000/μL
Neutrophils	≥ 1500/μL
Platelets	≥ 100,000 4/μL
Hemoglobin	> 9.0 g/dL
Serum Creatinine	≤ 1.5 x ULN **OR**

Creatinine Clearance (CrCl)*	≥ 40 mL/min
AST	≤ 3 x ULN
ALT	≤ 3 x ULN
Total Bilirubin**	≤ 1.5 x ULN
Oxygen Saturation (O_2 Sat.)	≥92% on ambient air

HIV status	
Rapid HIV 1/2 Antibodies	Negative

***Creatinine Clearance** Calculated using the Cockcroft-Gault formula

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

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

**Total Bilirubin ≤ 1.5 x ULN, except subjects with Gilbert Syndrome, who can have total bilirubin < 3.0 mg/dL

*** Subjects whose HBV core antibody is positive must have a negative HBV viral load measurement

4.2 Exclusion Criteria

1. T_{1-2} N_0 as determined by EUS and PET/CT.
2. Pregnant or lactating women
3. Active or prior documented autoimmune or inflammatory disorders including but not limited to inflammatory bowel disease; systemic lupus erythematosus; type I diabetes mellitus; Wegener syndrome [granulomatosis with polyangiitis]; myasthenia gravis; Graves' disease; rheumatoid arthritis, hypophysitis, uveitis) within the past 3 years prior to the start of treatment. The following are exceptions to this criterion:
   - 3a) Subjects with vitiligo or alopecia
   - 3b) Subjects with hypothyroidism (e.g., following Hashimoto syndrome) stable on hormone replacement or psoriasis not requiring systemic treatment
4. History of idiopathic pulmonary fibrosis, organizing pneumonia, drug-induced pneumonitis, idiopathic pneumonitis, or evidence of active pneumonitis on screening chest Computed Tomography (CT) scan

5. The use of immunosuppressive medication within 28 days prior to the first dose of nivolumab. The following are exceptions to this criterion:
   5a) Intranasal, topical, inhaled corticosteroids or local steroid injections (e.g. intra-articular injection)
   5b) Systemic corticosteroids at physiologic doses ≤10 mg/day of prednisone or equivalent
   5c) Steroids as premedication for hypersensitivity reactions (e.g. CT scan premedication
6. Positive test for Human Immunodeficiency Virus (HIV) or known acquired immunodeficiency syndrome (AIDS)
7. Prior treatment with any immunotherapy
8. Any other factors, including psychiatric or social, that in the opinion of the treating physician makes the patient an inappropriate candidate for a study.
9. Patients are excluded if they have active brain metastases or leptomeningeal metastases. Subjects with brain metastases are eligible if metastases have been treated and there is no magnetic resonance imaging (MRI) evidence of progression for [lowest minimum is 4 weeks or more] after treatment is complete and within 28 days prior to the first dose of nivolumab administration. There must also be no requirement for immunosuppressive doses of systemic corticosteroids (> 10 mg/day prednisone equivalents) for at least 2 weeks prior to study drug administration.
10. Patients should be excluded if they have an active, known or suspected autoimmune disease. Subjects are permitted to enroll if they have vitiligo, residual hypothyroidism due to autoimmune condition only requiring hormone replacement, psoriasis not requiring systemic treatment, or conditions not expected to recur in the absence of an external trigger
11. Patients should be excluded if they have a condition requiring systemic treatment with either corticosteroids (> 10 mg daily prednisone equivalents) or other immunosuppressive medications within 14 days of study drug administration. Inhaled or topical steroids and adrenal replacement doses < 10 mg daily prednisone equivalents are permitted in the absence of active autoimmune disease.
12. As there is potential for hepatic toxicity with nivolumab or nivolumab non-drugs with a predisposition to hepatoxicity should be used with caution in patients treated with nivolumab-containing regimen.
13. Patients with a history of allergy to the study drug components are excluded.

4.3 Patient Recruitment

Patients will be recruited from the investigators' clinical practices in Medical, Surgical and Radiation Oncology at the Perlmutter Cancer Center at NYU, Bellevue Hospital Center, Memorial Sloan-Kettering Cancer Center (MSKCC), USC-Norris Cancer Center, LAC-USC Medical Center, and Oregon Health Sciences University (OHSU) Knight Cancer Institute.

Prior to study entry, a patient's eligibility must be approved in writing (e-mail is allowed) by the NYU Clinical Trials Office study coordinator.

Target enrollment for this study is 50-56 patients. Patients will be recruited from physicians at the NYU Langone Perlmutter Cancer Center. Consenting, screening, and treatment will take place at the NYULMC PCC under the supervision of the Overall PI. Prospective subjects will receive detailed information regarding this study; its investigational nature, required study procedures, alternative treatments, risks and potential benefits of the study. They will also receive the informed consent document to read. All questions are answered by the PI and qualified research personnel.

The Principal Investigator will:

1. Obtain signed and dated informed consent from the potential subject before any study specific procedures are performed.
2. Determine patient eligibility See Section 4.1 and 4.2
3. Submit registration to NYU Langone Perlmutter Cancer Center CTO
4. Receive registration confirmation from NYU Perlmutter Cancer Center CTO, including a unique study identification number assigned to the patient that will be distributed to the study team upon registration of the patient.

Recruitment and consenting will take place in a private area such as an exam room to protect the patient's privacy. The informed consent process and documentation follows the established procedures of the NYULMC Perlmutter Cancer Center Clinical Trials Office.

4.3.1 Informed Consent

Consent will be obtained only by a participating investigator who has completed requisite training for human subject research and has been instructed by the Principal Investigator about the patients and address any questions or concerns prior to obtaining written informed consent for participation and HIPAA authorization.

Patients will be given adequate time to read the consent form. They will be given time to ask questions about the study in private exam rooms. Questions will be answered by a participating physician, or qualified research study team member all of whom have completed requisite training for human subject research. Investigators will review the informed consent form with patients and address any questions or concerns prior to obtaining written informed consent for participation. Investigators will stress that participation in the study is completely voluntary and will not affect the care patients receive or result in any loss of benefits to which patients are otherwise entitled.

The Investigator must explain to each subject the nature of the study, its purpose, the procedures involved, the expected duration, the potential risks and benefits involved and any discomfort it may entail. This informed consent should be given by means of standard

written statement, written in non-technical language. No patient can enter the study before his/her informed consent has been obtained.

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

All patients will be required to sign a written informed consent prior to being registered on this study. Every effort will be made to answer questions raised by patients and their families or advocates regarding the protocol and alternative therapies prior to asking a patient to sign the consent form.

For non-English speaking patients, institutional translation services will be utilized. For these patients the consent letter and all other information will be administered orally and a witness, not related to the research project, will be present while the oral presentation is given. A short form will be utilized for the subject to sign in his/her name and the translator and/or witness must sign the short form. The translator will also sign the main consent form.

For patients who cannot read. A witness, not related to the research study will be present. The consent will be read to the patient. The patient will also be allowed to ask any questions s/he may have. The investigator will ask the patient questions to ensure s/he understands the study. If the investigator determines the subject understands the study, the patient will mark an X where his/her name would go and the witness will sign the consent form.

4.3.2 *Documentation of Consent*

The Principal Investigator or IRB approved sub-investigator will be responsible for documentation in the medical record that consent has been obtained from all participants. A signed copy of the consent form will be given to each participant. Original consent forms will be stored in the subject's medical chart.

4.3.3 *Multi-Site Surveillance*

As the lead investigator in a multi-site trial, the Overall Principal Investigator is responsible for organizing and conducting monthly teleconferences with all participating sites. The PI will also be responsible for including data from all of the participating sites within the overall trial's quarterly Data and Safety Monitoring report to the DSMC to include minutes from monthly PI teleconferences. Each participating site will be responsible for submitting the results and recommendations from the DSMC's quarterly review to their IRB of record at the time of continuing review. Additionally, the NYULMC PCC Clinical Trial Office, Quality Assurance Unit will provide monitoring every 4-6 weeks to ensure completeness, accuracy, and consistency of the data.

4.3.4 Patient Informed Consent at Additional Sites

The Principal Investigator (PI) at each participating site will identify the physician members of the study team who will be obtaining consent and signing the consent form for therapeutic

protocols. It is NYULMC policy that Nurses and Fellows cannot obtain consent to greater than minimal risk trials, unless Fellows are listed as co-investigators.

The Investigator must ensure that each participant, or his/her legally acceptable representative, is fully informed about the nature and objectives of the study and possible risks associated with participation. All participants must be provided a consent form describing this study and providing sufficient information for participants to make an informed decision about their participation in this study. The formal consent of a participant, using the IRB approved consent form, must be obtained before the participant is involved in any study-related procedure. The consent form must be signed and dated by the participant or the participant's legally authorized representative (if applicable), and by the person obtaining the consent. The participant must be given a copy of the signed and dated consent document. The original signed copy of the consent document must be retained in the medical record or research file.

All parties will ensure protection of participant personal data and will not include participant names on any sponsor forms, reports, publications, or in any other disclosures, except where required by laws. In case of data transfer, NYULMC Perlmutter Cancer Center (PCC) will maintain high standards of confidentiality and protection of participant personal data.

The informed consent form must be in compliance with ICH/GCP, local regulatory requirements, and legal requirements.

The informed consent form used in this study, and any changes made during the course of the study, must be prospectively approved by both the IRB and NYULMC before use.

4.4 Registration Procedures

4.4.1 *General Guidelines*

Each patient must sign and date an informed consent form before undergoing any study specific procedure unless a procedure is being performed as part of the patient's standard of care.
Enrollment in the study requires that all inclusion and exclusion criteria have been met. Enrollment occurs upon confirmation of registration from the NYULMC PCC Clinical Trials Office. The following materials must be submitted to the Research Coordinator for subject registration:

1. Complete signed and dated informed consent form
2. Complete signed and dated eligibility checklist
3. All supporting documentation verifying each criterion has been met

Registration will occur within 48 hours of research coordinator receipt of all of the above documents. A written confirmation of enrollment including a unique study identification number assigned by the research coordinator will be disbursed to the study team upon registration.

Once eligibility is verified, a unique patient study number will be issued within 24 hours of receiving all required registration material. The patient will not be identified by name. This is the point, at which, the patient is considered accrued on study.

4.4.2 *Patient Registration at Other Participating Institutions*

The Principal Investigator (PI) at each participating site will identify the physician members of the study team who will be obtaining consent and signing the consent form for therapeutic protocols. It is NYULMC policy that Nurses and Fellows cannot obtain consent to greater than minimal risk trials.

Enrollment at addition sites can begin once each site's IRB has approved this protocol, a copy of each site's IRB approval, Citi training certificates, Medical Licenses and signed CVs are provided to NYU Langone Health Perlmutter Cancer Center (PCC) Clinical Trials Office. Once, all required documents are provided to NYU Clinical Trials Office an activation notification will be sent to the PI and research coordinator of that site. Central registration for this study will take place at NYU Langone Health PCC Quality Assurance Unit (PCC-QAU@nyumc.org).

Each patient must sign and date an informed consent form before undergoing any study specific procedures unless a procedure is being performed as part of the patient's standard of care. Once a patient has signed consent, each site must notify the NYU Langone Health PCC Quality Assurance Unit and forward a copy of the signed consent to NYU Langone Health PCC Clinical Trials Office within 24 hours.

Enrollment in the study requires that all inclusion and exclusion criteria have been met. Enrollment occurs upon confirmation of registration from the NYU Langone Health PCC Clinical Trials Office. The following materials must be submitted to the Quality Assurance Unit at NYU Langone Health via email (PCC-QAU@nyumc.org):

1. Complete signed and dated informed consent form
2. Complete signed and dated informed consent checklist
3. Complete signed and dated eligibility checklist
4. All supporting documentation verifying each criterion has been met.

Registration will occur once the Senior Research Nurse for Quality Assurance conducts a central review of the submitted materials. Once eligibility is verified, a unique subject study number will be issued within 48 hours of receiving all required registration material. This number is unique to the participant and must be written on all data and correspondence for the participant. The NYU Langone Health PCC CTO will return a signed eligibility confirmation worksheet email with the subject's unique study number.

The subject will not be identified by name. This is the point, at which, the patient is considered accrued on study. Protocol treatment should begin within designated timeframe; issues that would cause treatment delays should be discussed with the overall PI, Dr. Wu. Pretreatment evaluation will therefore be dictated by standard clinical practice. Except in very unusual circumstances, each participating institution will order the study drug directly from the supplier. All screen failures/ineligible subjects, as well as subject's who withdraw consent prior to initiation of protocol therapy must be submitted to the CTO in a manner analogous to the procedures noted above. Applicable source documentation will be required within the corresponding submissions.

Each site is responsible for reporting all unexpected problems involving risks to participants or others to NYULMC PCC Clinical Trials Office and to their IRB as per site institutional policy.

Each site is responsible for reporting all unexpected problems involving risks to participants or others to NYU Langone PCC Clinical Trials Office and to their IRB as per site institutional policy.

Please email all SAEs to NYUPCCsafetyreports@nyumc.org, PCC Assigned Medical Monitor, Dr. Wu, and the NYU Langone Health CTO regulatory specialist.

4.5 Early Withdrawal of Subjects

4.5.1 *When and How to Withdraw Subjects*

A subject may withdraw consent and leave the trial at any time.

A subject who withdraws consent prior to receiving the combination of chemotherapy, immunotherapy and radiation may be replaced in the phase I portion of the trial.

A subject has the right to voluntarily discontinue study treatment or withdraw from the study at any time, for any reason, and without repercussion. The investigator and sponsor have the right to discontinue a patient from study treatment or withdraw a patient from the study at any time.

Reasons for subject withdrawal from the study may include, but are not limited to:

- Subject withdrawal of consent at any time.
- Disease progression
- Intolerable toxicity (If applicable)

- Any medical condition that the investigator or sponsor determines may jeopardize the patient's safety if s/he continues in the study or continues treatment with study drug.
- The investigator or sponsor determines it is in the best interest of the subject.
- Failure of the subject to adhere to protocol procedure requirements
- Pregnancy
- Study termination by Sponsor

4.5.2 *Data Collection and Follow-up for Withdrawn Subjects*

Subjects who withdraw consent following treatment with chemotherapy, immunotherapy and radiation will be followed for toxicity and survival. Such subjects will not be replaced.

The follow-up will depend on the staging of the subject's disease at the time he/she is withdrawn from the study. Subjects will be seen in the outpatient clinic at a minimum of every 3 months, for two years, or until death.

5.0 Description of Therapy

5.1 Systemic Therapy: Description, Preparation, Administration, and Toxicity

Table 2. Product Description

Product Description and Dosage Form	Potency	Primary Packaging (Volume)/ Label Type	Secondary Packaging (Quantity) / Label Type	Appearance	Storage Conditions (per label)
BMS-936558-01 (nivolumab) Solution for Injection	100mg (10 mg/mL)	10m per vial/ Open-label	5 or 10 vials per carton/Open-label	Clear to opalescent colorless to pale yellow liquid. May contain particles	2 to 8ºC. Protect from light and freezing
Carboplatin Solution for Injection	450 mg/vial (10 mg/mL)	45 mL per vial/ Open-label	4 vials per carton/Open-label	Clear, colorless or slightly yellow solution	Store at or below 25ºC. Protect from light.
Paclitaxel Solution for Injection	100 mg/vial (6 mg/mL)	16.7 mL vial/ Open-label	4 vials per carton/ Open-label	Clear colorless or slightly yellow viscous solution	Store at 15ºC-30ºC. Protect from light

5.1.1 Nivolumab

5.1.1.1 Dose and Schedule

Nivolumab 240 mg (flat dose) will be administered as an intravenous infusion on days 1 and 15 as a single agent. On Days 29, 43 and 57 nivolumab will be administered concurrently with chemotherapy and radiation.

For patients determined to have a complete clinical response continue nivolumab 240 mg (flat dose) q 2 weeks x 6 within two weeks of response determination

For patients undergoing surgery begin nivolumab 240 mg (flat dose) q 2 weeks x 6, within 12 weeks following esophagectomy

5.1.1.2 Nivolumab Description

Nivolumab is a sterile, preservative-free, non-pyrogenic, clear to opalescent, colorless to pale-yellow liquid that may contain light (few) particles. Nivolumab injection for intravenous infusion is supplied in single-use vials. Each mL of nivolumab 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. The solution may contain hydrochloric acid and/or sodium hydroxide to adjust pH to 6.

5.1.1.3 Storage Requirements and Stability

- The product does not contain a preservative.
- Protect from light by storing in the original package until time of use. Do not freeze or shake.
- After preparation, store the infusion either:
- At room temperature for no more than 4 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-46°F) for no more than 24 hours from the time of infusion preparation.
- If stored in a glass front refrigerator, vials should be stored in the carton. Recommended safety measures for preparation and handling of nivolumab include laboratory coats and gloves.
- For additional details on prepared drug storage and use time of nivolumab under room temperature/light and refrigeration, please refer to the BMS-936558 (nivolumab) Investigator Brochure section for “Recommended Storage and Use Conditions”

5.1.1.4 Preparation

- Nivolumab will be supplied in single-use injection vials at concentrations of 40 mg/4 mL (10 mg/mL) and 100 mg/10 mL (10 mg/mL).
- 240 mg IV diluted 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/L, to be infused over 30 minutes

- Nivolumab is available as follows:

Carton Contents	NDC
40 mg/4 mL single-use vial	0003-3772-11
100 mg/10 mL single-use vial	0003-3774-12

5.1.1.5 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). Fewer infusion reactions have been observed in clinical trials when decreasing nivolumab infusion time from 60

minutes to 30 minutes. It is not to be administered as IV push or bolus injection.

- Do not coadminister other drugs through the same intravenous line.
- Flush the intravenous line at end of infusion. Nivolumab can be infused undiluted (10 mg/mL) or diluted with 0.9% Sodium Chloride Injection, USP or 5% Dextrose Injection, USP to protein concentrations as low as 1 mg/mL. Care must be taken to assure sterility of the prepared solution as the product does not contain any antimicrobial preservative or bacteriostatic agent
- Subjects may be dosed no less than 12 days from the previous dose of drug. There are no premedications recommended for nivolumab on the first cycle.
- The dosing calculations should be based on the actual body weight at baseline. If the subject's weight on the day of dosing differs by > 10% from the weight used to calculate the original dose, the dose must be recalculated. All doses should be rounded to the nearest milligram. There will be no dose modifications allowed.
- Subjects should be carefully monitored for infusion reactions during nivolumab administration. If an acute infusion reaction is noted, subjects should be managed according to Protocol Section 7.4.

5.1.2 Carboplatin

5.1.2.1 Dose and Schedule

Carboplatin will be administered days 29, 36, 43, 50, 57 and 64 at AUC 2 q weeks as an IV infusion.

5.1.2.2 Carboplatin Description

Carboplatin is commercially available as a sterile lyophilized powder in single-dose vials containing 50 mg, 150 mg, and 450 mg of carboplatin. Each vial contains equal parts by weight of carboplatin and mannitol. It is also available as an aqueous solution in multidose vials containing 50 mg, 150 mg or 600 mg, at a concentration of 10 mg/mL, and as a preservative- free 10 mg/mL solution containing 50 mg, 150 mg, or 450 mg.

5.1.2.3 ***Storage Requirements and Stability***

Unopened vials of carboplatin powder or solution are stable for the life indicated on the package when stored at controlled room temperature and protected from light. Reconstituted solutions are reported to be stable for at least five days at room temperature, but if no preservative is employed, it is recommended that carboplatin solutions be discarded eight hours after reconstitution. The multidose vials of carboplatin aqueous solution are reported to maintain microbial and chemical stability at room temperature for up to 14 days following multiple needle entries.

5.1.2.4 ***Preparation***

Reconstitute carboplatin powder with sterile water for injection, USP, 5 % dextrose in water, or 0.9% sodium chloride injection, USP, according to the following schedule:

Table 3. Carboplatin Preparation

Vial Strength	Diluent Volume
50 mg	5 mL
150 mg	15 mL
450 mg	45 mL

5.1.2.5 *Administration*

Carboplatin will be administered intravenously (IV) over 30 minutes.

Note: Aluminum reacts with carboplatin causing precipitate formation and loss of potency: therefore, needles or intravenous sets containing aluminum parts that may come in contact with the drug must not be used for the preparation or administration of carboplatin.

5.1.3 Paclitaxel

5.1.3.1 Dose and Schedule

Paclitaxel will be administered on days 29, 36, 43, 50, 57 and 64 at 50 mg/m^2 as an IV infusion.

5.1.3.2 Paclitaxel Description

Paclitaxel is commercially available in a concentration of 6 mg/mL in 5 mL, 16.7 mL, 25 mL and 50 mL multidose vials. Each mL of solution also contains 527 mg of polyoxyethylated castor oil and dehydrated alcohol, USP. Please refer to the FDA-approved package insert for complete product information.

5.1.3.3 *Storage Requirements and Stability*

Intact vials should be stored at controlled room temperature (20° - 25°C; 68° - 77°F) in the original package to protect from light, and remain stable until the expiration date on the label. Refrigeration does not adversely affect stability. Upon refrigeration components in the paclitaxel vial may precipitate, but will re-dissolve upon reaching room temperature with little or no agitation. Diluted 0.3 – 1.2 mg/mL solutions are stable for up to 27 hours at room temperature under normal room lighting. Although solutions may be chemically stable for longer periods in some situations, precipitation may occur unpredictably. The mechanism of this precipitation has not been determined.

5.1.3.4 *Preparation*

Paclitaxel must be diluted prior to administration with 0.9% sodium chloride Injection, USP; 5% Dextrose Injection, USP; 5% Dextrose and 0.9% Sodium Chloride Injection, USP; or 5% Dextrose in Ringer's Injection to a final concentration of 0.3 to 1.2 mg/mL. Paclitaxel should be prepared and stored in glass, polypropylene, or polyolefin containers due to leaching of DEHP [di-(2ethylhexyl)phthalate] plasticizer from polyvinyl chloride (PVC) containers. Non-PVC containing tubing and connectors should also be used. Paclitaxel should be administered through an in-line < 0.22 micron filter.

5.1.3.5 Administration

In this study, during induction therapy, paclitaxel is administered intravenously over 2 hours on days 1 and 8 of each 21-day cycle of therapy. During combined therapy, paclitaxel is administered intravenously, over 2 hours weekly for 5 weeks.

5.2 Radiation Therapy

Radiation therapy will be started on the first day of combined chemoradiation, Day 29. The total dose of RT will be 50.4 Gy in 1.8 Gy fractions x 28 fractions. Patients can undergo simulation for radiotherapy planning prior to the second PET scan since the treatment volumes are based on pre-chemotherapy tumor involvement. The simulation should be performed at least 2 weeks prior to the start date for radiotherapy so that the treatment plan can undergo QA without delaying the start date of the radiotherapy.

5.2.1 Technical Factors

Linear accelerators with a minimum energy of 6-16 MV will be used. A multiple field 3-D conformal technique or IMRT technique will be used. All fields will be treated each day. The patient will be treated in the supine position. Radiation will be delivered 5 days/week, once per day.

5.2.2 Required Benchmarks and Pre-approval of 3D Treatment Plans

CT-based conformal planning is required on this study. In accordance with current guidelines for use of IMRT in clinical trials (see www.irocri.qarc.org), IMRT may be used.

5.2.3 Protocol Treatment Volumes

The nomenclature and definitions of ICRU Reports 50 and 62 shall be followed in this study.

A volumetric treatment planning CT study will be required for this study. Each patient will be positioned in an individualized immobilization device in the treatment position on a flat table. Contiguous CT slices, 3-5 mm thickness of the regions harboring gross tumor and grossly enlarged nodes and 8-10 mm thickness of the remaining regions, are to be obtained starting from the level of the cricoid cartilage and extending inferiorly through the liver. A measurement scale for the CT image shall be included.

Gross Tumor Volume (GTV): The GTV is based on the pre-chemotherapy extent of disease using the initial PET/CT scan, endoscopy report, and CT scan. The entire esophageal wall, including any disease that has extended through the wall should be contoured as the GTV as well as any PET/CT-avid or enlarged lymph nodes.

Clinical Target Volume (CTV): The intent of pre-operative treatment is to include the tumor bed plus the nodal groups at risk (whether clinically positive or negative). The clinical target volume (CTV) should encompass the peri-esophageal lymph nodes, mediastinal lymph nodes for mid- and upper- thoracic esophageal tumors, and the submucosal spread longitudinally along the esophagus. This is generally a 3-4 cm expansion on the GTV superiorly and inferiorly and 1.0 cm expansion radially. For distal esophageal tumors and GE junction tumors, the CTV should include the celiac lymph nodes. For tumors above the carina, the supraclavicular lymph nodes should be included in the CTV. Due to the variable margin expansion models used by different contouring and treatment planning systems, the radial expansion may be larger than 1 cm on each slice. The treating physician should modify the margins to minimize overlap with normal adjacent structures such as the heart, lungs, or liver.

Planning Target Volume (PTV): The PTV is established by expanding the CTV by 0.5 cm in all directions. This will result in a margin of up to 5 cm superior and inferior and approximately 1.0-2 cm radially to the extent of tumor (GTV). For distal esophageal and GE junction tumors where motion management is not being used, the superior-inferior expansion can be 0.7-1.0 cm to account for the respiratory motion.

Internal Target Volume (ITV): The ITV may be utilized with four-dimensional CT simulation. The ITV volume shall be contoured per the treating Radiation Oncologists standard practice. This is described in “Motion Management” section 5.2.26.

5.2.4 Target Dose Constraints

Dose Prescription

The prescribed dose to the PTV is 5040 cGy delivered in 180 cGy/day over 28 fractions.

Dose Uniformity

The dose to 99% of the PTV must be at least 93% of the prescribed dose, and no more than 2cc within the PTV may receive a dose greater than 120% of the prescribed dose.

Tissue Heterogeneity

Calculation shall take into account the effects of tissue heterogeneities. Planning must be performed using an approved dose calculation algorithm. Approved algorithms include: convolution superposition, collapsed cone convolution, and Monte Carlo.

5.2.5 Normal Tissue Dose Constraints

The normal structures to be contoured will depend on the level of the esophagus involved, but can include left and right lungs, heart, esophagus, brachial plexus, left and right kidneys, liver, stomach, small intestine, and spinal canal. The dose to normal tissues must be kept within the parameters described below.

1. Lungs
    1. $V_{20Gy} \leq 25\%$
    2. and $V_{30Gy} \leq 15\%$
    3. and $V_{40Gy} \leq 10\%$
    4. $V_{10Gy} \leq 40\%$
2. Cord
    1. Max ≤ 4500 cGy
3. Bowel
    1. Max bowel dose < Max PTV dose
    2. and $D_{05} \leq 4500$ cGy
4. Heart
    1. $V_{30Gy} \leq 30\%$ (closer to 20% preferred)
    2. Mean < 3000 cGy
5. Left kidney, Right kidney (evaluate each one separately):
    1. No more than 33% of the volume can receive 1800 cGy
6. Liver
    1. $V_{20Gy} \leq 30\%$
    2. $V_{30Gy} \leq 20\%$
    3. Mean < 2500 cGy
7. Stomach
    1. Mean < 3000 cGy (if not within PTV)
    2. Max dose <54 Gy

5.2.6 Treatment Planning

Simulation: Patients will be positioned supine with arms above the head in a mold for immobilization. A CT simulation will be performed using oral and/or IV contrast, when possible, using ≤3 mm slice thickness. In patients for whom treatment will be delivered using respiratory gating or tracking, the planning CT scan should be performed with the patient in a breath-hold in end-expiration.

Motion Management: For distal esophageal and GE junction tumors, respiratory motion can be significant, requiring an assessment of the degree of tumor motion at the time of simulation. Gold fiducial markers will be placed prior to simulation in patients for whom treatment will be delivered using respiratory gating or tracking to aid the visualization of the tumor motion during treatment. To determine the extent of respiratory motion, a respiratory correlated 4DCT scan will be obtained at the time of simulation: this scan will be performed throughout the breathing cycle (i.e., 4-dimensional CT) so that separate CT data sets associated with each phase of respiration can later be reconstructed. Treatment delivery can be done using the motion management technique available at the institution and can include treatment

during free-breathing as long as an internal target volume (ITV) has been designed based on the motion of the tumor on the 4DCT.

Beam Arrangements:
1. 3D Conformal Beam Arrangements
Beam arrangement selection for 3D conformal treatment will vary based on the shape, size, and location of the CTV and the resulting PTV in relation to normal organs. The most common arrangement is an AP/PA with Right and Left Lateral beams.

2. IMRT - Beam Arrangement
A five-field beam arrangement is preferred to minimize the low dose distributed to the lungs. Suggested beam arrangements are:

a. For distal esophagus the following beam arrangement is useful for minimizing dose to the heart and lungs:
LPO (155), LAO(70-80), AP(0), RAO(280-290), RPO(205)
Note that the range of gantry angles for the LAO and RAO fields is due to the fact that one needs to find the best compromise between the amount of heart and lung in the field.

b. For GE-junction esophagus, the following beam arrangement may be substituted if it is better for minimizing dose to the kidney. All beams are 15/16 MV:
PA (180o), close to LL (90o+/-10o), LAO (30-35o), RAO (325-330o), close to RL (270o+/-10o)

c. VMAT or RapidArc-like delivery approaches are acceptable

These recommended beam arrangements may be changed to one more fitting for the patient's particular anatomy.

Field verification: As a minimum requirement, institutions are required to obtain verification images at the start of treatment and each week thereafter. Prior to the first treatment images that verify the position of the isocenter placement must be obtained. For 3D-CRT this imaging can include individual portal views. Weekly imaging can consist of portal views for 3D-CRT and isocenter verification images. For IMRT orthogonal images, verifying isocenter position are required. More frequent (daily) imaging is allowed, particularly for patients treated with motion management techniques and/or arc-based treatment techniques, but is not required.

5.2.7 Portal Film Review

First day port films or portal images of each field must be obtained and kept by the treating institution. Weekly (at least 48 hours apart) verification films or images of orthogonal views (anterior to posterior and lateral projection) must be reviewed by the treating physician. The required accuracy of patient positioning and the use of

multi-leaf collimator apertures suggest the daily use of on-line imaging may be desirable. If on-line daily imaging is used, this must be documented.

5.2.8 Definitions of Deviations in Protocol Performance

Prescription Dose

- **No Deviation:** ≥ 99% of the PTV receives ≥ 93% of the prescribed dose, and a contiguous volume of no more than 2cc inside PTV exceeds 20% of the prescribed dose.
- **Minor Deviation:** Between 95% and 99% of the PTV receives 93% of the prescribed dose, or the dose to a volume of 2cc within the PTV exceeds the prescribed dose by 20- 25%.
- **Major Deviation** The dose to 1 cc of tissue outside the PTV exceeds 120% of the prescribed dose, or less than 95% of the PTV receives 93% of the prescribed dose, or the dose to a volume of 2cc within the PTV exceeds 125% of the prescribed dose.

Volume

- **Minor Deviation: Margins less than specified, or field(s) 1-3 cm greater than specified.**
- **Major Deviation: Fields transect tumor or specified target volume(s), or fields are more than 3 cm greater than specified.**
- **Critical Organ**
- **Major Deviation:** The maximum dose to the spinal cord exceeds 4500cGy; the heart mean dose exceeds 3000cGy; the lung V20 exceeds 30% or the V10 exceeds 50%.
- **Minor Deviation:** The lung V20 exceeds 20% or the V10 exceeds 40%.

Treatment Interruption

- **Minor Deviation:** Treatment interruptions between five and nine normally scheduled treatment days.
- **Major Deviations:** Treatment interruptions totaling more than nine normal scheduled treatment days.

5.3 Surgery: Esophagectomy

Patients who do not achieve a complete clinical response after study therapy will undergo esophagectomy followed by nivolumab 240 mg every two weeks for 6 weeks. Patients who achieve a complete clinical response after study therapy will go on to receive nivolumab 240 mg every two weeks for 6 weeks and will not undergo surgery. At the discretion of the treating physician and based on patient preference, patients who achieve a clinical complete response may still choose to undergo surgery.

5.3.1 Surgical Procedures

Surgical options (Types of Esophagectomies)

1. Ivor Lewis Esophagectomy
    a. Laparotomy with gastric mobilization. Feeding jejunostomy. Gastric emptying procedure at discretion of surgeon.
    b. Thoracotomy with esophageal mobilization and resection. Esophagogastric anastomosis at discretion of the surgeon.
2. Three Incision Esophagectomy
    a. Right thoracotomy with esophageal mobilization
    b. Laparotomy with gastric mobilization. Feeding jejunostomy. Gastric emptying procedure at discretion of surgeon.
    c. Left neck incision. Esophagogastric anastomosis at discretion of the surgeon.
3. Transhiatal Esophagectomy
    a. Laparotomy with gastric mobilization. Feeding jejunostomy. Gastric emptying procedure at discretion of surgeon.
    b. Transhiatal esophageal mobilization
    c. Left neck incision. Esophagogastric anastomosis at discretion of the surgeon.
4. Minimally Invasive Versions
    a. Minimally invasive versions of above procedures, which may include thoracoscopy, laparoscopy, robotics.

6.0 Study Procedures

6.1 *Study Visits*

TABLE 4: SCHEDULE OF EVENTS / STUDY CALENDAR

Activity	Screening	C1 (D 1)	C2 D1 (D15)	C2 W2/ (D22-28)	C3 D1 (D29)	C3 D8 (D36)	C4 D1 (D43)	C4 D8 (D50)	C5 D1 (D57)	C5 D8 (D64)	5-8 W Post RT	C 6	C 7	C 8	C 9	C10	C11	EOT	30D Post Tx.	60D Post Tx.	Follow-up@	EOS
Enrollment	X																					
Eligibility	X																					
Endoscopy*	X			X[1]							X									X	X	
Tumor core biopsy**	X			X[1]							X											
PET/CT scan**	X			X							X										X	
Disease staging	X																					
ECG	X																					
Medical history	X																					
Vital Signs	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Physical exam	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
CBC	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
CMP	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
TSH	X	X	X		X		X		X		X	X	X	X	X	X	X	X	X	X		
fT4	X	X	X		X		X		X		X	X	X	X	X	X	X	X	X	X		
fT3	X	X	X		X		X		X									X	X	X		
Amylase	X																					
Lipase	X																					
Cortisol	X																					
Infectious disease screen	X																					
B-HCG - serum (pregnancy test)	X	X	X		X		X		X			X	X	X	X	X	X		X	X		
HIV testing	X																					
Hepatitis panel	X																					
Correlative studies	X			X							X	X	X		X							
Nivolumab		X	X		X		X		X			X	X	X	X	X	X					
Carboplatin					X	X	X	X	X	X												
Paclitaxel					X	X	X	X	X	X												
Radiation therapy***					X	X	X	X	X	X												

AEs	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X			
Concurrent medications	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X			
Esophagectomy[#]											X											
Extent of disease evaluation				X							X									X	X^	
Off treatment																		X				
Protocol deviation	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Survival status																		X	X	X	X	X
Off study																						X

* Endoscopic ultrasound will be performed if technically feasible. Bronchoscopy will be performed for tumors involving the cervical or thoracic esophagus only at screening to rule out fistula.
** For those with allergy to CT IV contrast, PET alone, with MRI of abdomen and non-contrast CT of chest, is acceptable. Procedures done on C2W2 can be done beyond Day 28, as long as it is prior to C3D1. It can be done at time of surgery if endoscopy during 5-8 week post RT is not feasible.
*** Radiation Therapy (RT) will be administered at 1.8 Gy/fraction, Monday through Friday, × 28 fractions, on Days 29-66.
[****]Optional at C2W2(D22-28)
If no complete clinical response; for subjects with a complete clinical response, Esophagectomy can be offered at the discretion of the treating physician.
@ Every 3 months up to 2 years, then every 6 months until 5 years after the start of therapy; 11 clinic follow-up visits in all
^ Every 6 months up to 2 years, then annually until 5 years after the start of therapy; 5 follow-up scans in all
[1] Procedure can occur after 28 days, but must be prior to C3D1 drug administration

6.1.1 Screening

The following will be performed during the **Screening period** (28 days prior to study enrollment):

- Informed Consent
- Physical Examination
- Vital signs
- Electrocardiogram (ECG)
- Complete Blood Count (CBC) with Differential
- Complete Metabolic Panel (CMP) with liver function tests
- Thyroid Function Tests (TSH, fT4, fT3)
- Amylase
- Lipase
- Cortisol
- Hepatitis Panel
  - Hepatitis B Surface Antigen, Antibodies, and Core Antibody
  - Anti-Hepatitis C Total Antibody
- HIV Testing
  - Rapid HIV 1/2 Antibodies
- Upper Endoscopy
  - Endoscopic ultrasound will be performed if technically feasible
  - Bronchoscopy will be performed for tumors involving the cervical or thoracic esophagus only at screening to rule out fistula.
- Tumor Core Biopsy
- Correlative Studies
- PET/CT Scan
  - For those with allergy to CT IV contrast: PET alone is acceptable with MRI of abdomen and non-contrast CT of chest.
- Serum B-HCG for WOCBP

6.1.2 Pregnancy Precautions

Pregnancy Test: WOCBP prior to dosing nivolumab.

A serum or urine pregnancy testing is required within 24 hrs. of study enrollment, then prior to day 1 of each cycle. After discontinuation from nivolumab, these should be repeated at approximately 30 days and approximately 60 days [or more frequently if required by local standard].

Women of childbearing potential (WOCBP) must have a negative serum or urine pregnancy test (minimum sensitivity 25 IU/L or equivalent units of HCG) within 24 hours prior to the start of study drug and prior to day 1 of each cycle.

Women must not be breastfeeding.

WOCBP must agree to follow instructions for method(s) of contraception for the duration of treatment with study drug Nivolumab plus 5 months post-treatment completion

Men who are sexually active with WOCBP must agree to follow instructions for method(s) of contraception for the duration of treatment with study drug(s) nivolumab plus 7 months post-treatment completion. In addition, male subjects must be willing to refrain from sperm donation during this time.

Azoospermic men are exempt from contraceptive requirements. WOCBP who are continuously not heterosexually active are also exempt from contraceptive requirements, and still must undergo pregnancy testing as described in this section.

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

HIGHLY EFFECTIVE METHODS OF CONTRACEPTION

Highly effective methods of contraception have a failure rate of < 1% when used consistently and correctly. WOCBP and female partners of male subjects, who are WOCBP, are expected to use one of the highly effective methods of contraception listed below. Male subjects must inform their female partners who are WOCBP of the contraceptive requirements of the protocol and are expected to adhere to using contraception with their partner. Acceptable contraception methods of include:

- Progestogen: hormonal contraception associated with inhibition of ovulation
- Hormonal methods of contraception including oral contraceptive pills containing combined estrogen + progesterone
- Vaginal Ring
- Injectables
- Implants
- Intrauterine Devices (IUDs) such as Mirena®
- Non-hormonal IUDs, such as ParaGard ®
- Bilateral Tubal Occlusion
- Vasectomised partner with documented azoospermia 90 days after procedure.

A Vasectomised partner is a highly effective birth control method provided that partner is the sole sexual partner of the WOCBP trial participant and that the vasectomised partner has received medical assessment of the surgical success.

- Intrauterine Hormone-Releasing System (IUS).
- Complete Abstinence

Complete Abstinence is defined as the complete avoidance of heterosexual intercourse. Complete abstinence is an acceptable form of contraception for all study drugs and must be used throughout the duration of the study treatment (plus 5 half-lives of the investigational drug plus 30 days).

It is not necessary to use any other method of contraception when complete abstinence is elected. Subjects who choose complete abstinence must continue to have pregnancy tests. Acceptable alternate methods of highly effective contraception must be discussed in the event that the subject chooses to forego complete abstinence. The reliability of sexual abstinence needs to be evaluated in relation to the duration of the clinical trial and the preferred and usual lifestyle of the subject.

6.1.3 Cycle 1 Day 1

The following will be performed at the **Cycle 1 Day 1 Visit**:

- Physical Examination
- Administration of Nivolumab 240 mg IV (q 2 weeks)
- Complete Blood Count with Differential
- Complete Metabolic Panel with Liver Function Tests
- Thyroid Function Tests (TSH, fT4, fT3)
- Serum B-HCG for WOCBP

6.1.4 Cycle 2 Day 1 (Day 15)

The following will be performed at the **Cycle 2 Day 1 Visit**:

- Physical Examination
- Administration of Nivolumab 240 mg IV (q 2 weeks)
- Complete Blood Count with Differential
- Complete Metabolic Panel with Liver Function Tests
- Thyroid Function Tests (TSH, fT4, fT3)
- Serum B-HCG for WOCBP

Patients for whom nivolumab is not being held due to toxicity may begin Cycle 2 Day 1 on days 14-16 after Cycle 1 Day 1 without incurring a protocol deviation.

6.1.5 Cycle 2 Days 8-14 (Days 22 – 28)

The following will be performed during Cycle 2 between Days 8 and 14 (between **Days 22 and 28)**:

- Upper Endoscopy (optional)
- Tumor Core Biopsy (optional)

- PET/CT scan
- Correlative studies
- Endoscopy and tumor biopsy collections may occur after day 28, but must be done prior to Day1 of Cycle 3 administration.

6.1.6 Cycle 3 Day 1 (Day 29)

The following will be performed at **Cycle 3 Day 1 (Day 29) (± 2 Days *up to 14 days max*)**

- Physical Examination
- Administration of Nivolumab 240 mg (flat dose) IV(q 2 weeks)
- Administration of Carboplatin AUC 2 (weekly)
- Administration of Paclitaxel 50 mg/m^2 (weekly)
- Radiation Therapy commences (1.8 Gy/fraction Monday through Friday × 28 fractions)
- Complete Blood Count with Differential
- Complete Metabolic Panel with Liver Function Tests
- Thyroid Function Tests (TSH, fT4, fT3)
- Serum B-HCG for WOCBP

6.1.7 Cycle 3 Day 8 (Day 36)

The following will be performed at **Cycle 3 Day 8 (Day 36) (± 1 Day):**

- Physical Examination
- Administration of Carboplatin AUC 2 (weekly)
- Administration of Paclitaxel 50 mg/m^2 (weekly)
- Radiation Therapy
- Complete Blood Count with Differential
- Complete Metabolic Panel with Liver Function Tests

6.1.8 Cycle 4 Day 1 (Day 43)

The following will be performed at **Cycle 4 Day 1 (Day 43) (± 1 Day):**

- Physical Examination
- Administration of Nivolumab 240 mg (flat dose) IV (q 2 weeks)
- Administration of Carboplatin AUC 2 (weekly)
- Administration of Paclitaxel 50 mg/m^2 (weekly)
- Radiation Therapy
- Complete Blood Count with Differential
- Complete Metabolic Panel with Liver Function Tests
- Thyroid Function Tests (TSH, fT4, fT3)
- Serum B-HCG for WOCBP

6.1.9 Cycle 4 Day 8 (Day 50)

The following will be performed at **Cycle 4 (Day 50) (± 1 Day):**

- Physical Examination
- Administration of Carboplatin AUC 2 (weekly)
- Administration of Paclitaxel 50 mg/m^2 (weekly)
- Radiation Therapy
- Complete Blood Count with Differential
- Complete Metabolic Panel with Liver Function Tests

6.1.10 Cycle 5 Day 1 **(Day *57)***

The following will be performed at **Cycle 5 Day 1 (Day 57) (± 1 Day):**

- Physical Examination
- Administration of Nivolumab 240 mg (flat dose) IV (q 2 weeks)
- Administration of Carboplatin AUC 2 (weekly)
- Administration of Paclitaxel 50 mg/m^2 (weekly)
- Radiation Therapy
- Complete Blood Count with Differential
- Complete Metabolic Panel with Liver Function Tests
- Thyroid Function Tests (TSH, fT4, fT3)
- Serum B-HCG for WOCBP

6.1.11 Cycle 5 Day 8 (Day 64)

The following will be performed at **Cycle 5 (Day 64) (± 1 Day).**

- Physical Examination
- Administration of Carboplatin AUC 2 (weekly)
- Administration of Paclitaxel 50 mg/m^2 (weekly)
- Radiation Therapy
- Complete Blood Count with Differential
- Complete Metabolic Panel with Liver Function Tests

Radiation Therapy (RT) will be administered at 1.8 Gy/fraction, Monday through Friday × 28 fractions, on Days 29-66.

6.1.12 After Completion of Radiation Therapy

The following will be performed **5-8 weeks after completion of radiation therapy:**

- Physical Examination

- Complete Blood Count with Differential
- Complete Metabolic Panel with Liver Function Tests
- Thyroid Function Tests (TSH, fT4, fT3)
- Upper endoscopy
- Tumor Core Biopsy
- Correlative Studies
- PET/CT scan & Disease Assessment
- Surgical Consult for esophagectomy, to be performed in 5-8 weeks post-chemoradiation (for patients who do not achieve a CR or who elect to undergo surgery)
    - Delay beyond 8 weeks is reasonable if the patient has not yet recovered from chemoradiation and should be discussed with the study Principal Investigator.

Patients who do not achieve a radiographic/clinical complete response (CR) should undergo surgery.

Patients who achieve a clinical CR are not required to undergo surgery but, based on a discussion with their treating physician, can still undergo esophagectomy.

Patients who achieve a CR and who do not undergo surgery must initiate adjuvant nivolumab within 2 weeks of determination of their CR status and within 8 weeks of completing chemoradiation. Patients who do undergo surgery must initiate adjuvant nivolumab within 12 weeks of their surgery date.

6.1.13 Cycles 6-11

The following will be performed on Day 1 of **Cycles 6-11:**

- Physical Examination
- Administration of Nivolumab 240 mg (flat dose) mg/kg IV
- Complete Blood Count with Differential
- Complete Metabolic Panel with Liver Function Tests
- Thyroid Function Tests (TSH, fT4, fT3)
- Serum B-HCG for WOCBP
- Correlative studies will be done prior to treatment on Day 1 of Cycles 6, 7, and 9.

6.1.14 End of Treatment and 30-Day Post Treatment Visits

The following will be performed at the **End of Treatment (EOT) and 30-Day Post Treatment Visits:**

- Physical Examination
- Complete Blood Count with Differential
- Complete Metabolic Panel with Liver Function Tests
- Thyroid Function Tests (TSH, fT4, fT3)

- Serum B-HCG for WOCBP

The End of Treatment Visit is defined as the last day of protocol therapy. The 30- and 60-Day Post Treatment Visits will take place approximately 30 and 60 days after the last day of protocol therapy, respectively.

6.1.15 60-Day Post Treatment Visit

The following will be performed at the **60-Day Post Treatment Visit:**

- Physical Examination
- Complete Blood Count with Differential
- Complete Metabolic Panel with Liver Function Tests
- Thyroid Function Tests (TSH, fT4, fT3)
- Upper endoscopy (for patients who have not undergone surgery; it is optional for patients who have undergone esophagectomy)
- Serum B-HCG for WOCBP

6.1.16 Follow-up

Evaluations during **post-treatment surveillance** are summarized as follows:

Table 5. SCHEDULE OF EVENTS IN FOLLOW-UP

Clinic Visits	Visits every 3 months ±1 month until 2 years after the start of therapy, then every 6 months ±1 month weeks for 3 years until 5 years after the start of therapy CBC and standard comprehensive panel at each visit
CT or MRI of chest/abdomen	Every 6 months ±1 month for 2 years, then annually ±1 month for 3 years until 5 years after the start of therapy
Endoscopy	As clinically indicated (annually is recommended)

6.2 PET/CT Scans

6.2.1 *PET/CT Imaging Interpretation*

Baseline PET/CT Scan Interpretation

The baseline PET/CT scans will be interpreted by an experienced nuclear medicine physician at each participating site who will be responsible for image interpretation and clinical reporting for the local site. The images will be interpreted together with pertinent clinical findings and findings of other imaging modalities such as barium esophagogram, EUS, endoscopy and standard (i.e., contrast-enhanced) CT or MRI. Interpreting the PET/CT scans in this fashion mimics the usual clinical situation, in which this information is incorporated into the interpretation, especially in the case of an equivocal scan finding that may be easily explained

by the CT/MRI scan result (e.g., anatomic variation of the bowel or bladder) or clinical information (e.g., increased uptake at a site of recent surgery or biopsy).

6.3 Endoscopy and Biopsy

Endoscopy with tumor core biopsy will be assessed at screening, after 2 weeks of therapy with nivolumab (optional), and 6-8 weeks after completion of radiation therapy.

7.0 Dose Modifications and Management of Toxicity

Table 6. Adverse Event Definitions

Common Terminology for Adverse Events				
Condition	Grade			
	1	2	3	4
Hematologic				
Neutropenia	<1999-1500/mm3 (<1.99-1.5 x 10e9 /L)	<1500-1000/mm3 (<1.5-1.0 x 10e9 /L)	<1000 - 500/mm3 (<1.0-0.5 x 10e9 /L)	<500/mm3 (<0.5 x 10e9 /L)
Anemia	<LLN -10 g/dL (males) <LLN-10 g/dL (females)	<10.0-8.0 g/dL (males and females)	<8.0-7.0 g/dL (males and females)	<7.0 g/dL (males and females)*
Thrombocytopenia	<75,000/mm3 (<75.0 x 10e9 /L)	<75,000 - 50,000/mm3 (<75.0 - 50.0 x 10e9 /L)	<50,000 - 25,000/mm3 (<50,000-25.0x10e9 /L)	<25,000/mm3 (<25.0 x 10e9 /L)
Pulmonary				
Pneumonitis	Asymptomatic; clinical or diagnostic observations only; intervention not indicated	Symptomatic; medical intervention indicated; limiting instrumental ADL	Severe symptoms; limiting self-care ADL; oxygen indicated	Life-threatening respiratory compromise; urgent intervention indicated (e.g., tracheotomy or intubation)
Gastrointestinal				
Vomiting	1-2 episodes (separated by 5 minutes) in 24 hours	3-5 episodes (separated by 5 minutes) in 24 hours	≥ 6 episodes (separated by 5 minutes) in 24 hours	Life-threatening consequences; urgent intervention indicated
Colitis	Asymptomatic; clinical or diagnostic observations only; intervention not indicated	Abdominal pain; mucus or blood in stool	Severe abdominal pain; change in bowel habits; medical intervention indicated; peritoneal signs	Life-threatening consequences; urgent intervention indicated
Diarrhea	Increase of <4 stools per day over baseline; mild increase in ostomy	Increase of 4 - 6 stools per day over baseline; moderate increase in ostomy	Increase of >=7 stools per day over baseline; incontinence; hospitalization	Life-threatening consequences; urgent intervention indicated

	output compared to baseline	output compared to baseline	indicated; severe increase in ostomy output compared to baseline; limiting self-care ADL	
Esophagitis	Asymptomatic; clinical or diagnostic observations only; intervention not indicated	Symptomatic; altered eating/swallowing; oral supplements indicated	Severely altered eating/swallowing; tube feeding, TPN or hospitalization indicated	Life-threatening consequences; urgent operative intervention indicated
Dysphagia/ odynophagia	Symptomatic, able to eat regular diet	Symptomatic and altered eating/swallowing	Severely altered eating/swallowing; tube feeding or TPN or hospitalization indicated	Life-threatening consequences; urgent intervention indicated
Hepatitis	Asymptomatic, treatment not indicated AST, ALT, Alk Phos: 1.25-2.5 x ULN Bilirubin: >1.0-1.5 ULN	AST, ALT, Alk Phos: >2.5-5.0 x ULN Bilirubin: >1.5-2.5 x ULN	Symptomatic liver dysfunction; fibrosis by biopsy; compensated cirrhosis; reactivation of chronic hepatitis AST, ALT, Alk Phos: >5.0-10 x ULN Bilirubin: > 2.5-5 x ULN	Decompensated liver function (e.g., ascites, coagulopathy, encephalopathy, coma) AST, ALT, Alk Phos: >10 x ULN Bilirubin: >5 x ULN
Renal				
Creatinine elevation	>1-1.5 x baseline >ULN - 1.5 x ULN	>1.5 - 3.0 x baseline >1.5 - 3.0 x ULN	>3.0 baseline >3.0 - 6.0 x ULN	>6.0 x ULN
Neurologic				
Peripheral sensory neuropathy	Asymptomatic; loss of deep tendon reflexes or paresthesia	Moderate symptoms; limiting instrumental ADL	Severe symptoms; limiting self-care ADL	Life-threatening consequences; urgent intervention indicated
Endocrine				
Hyperthyroidism	Asymptomatic; clinical or diagnostic observations only; Intervention generally not indicated	Symptomatic: thyroid suppression therapy indicated; limiting instrumental ADL	Severe symptoms; limiting self care ADL; hospitalization indicated	Life-threatening consequences; urgent intervention indicated
Hypothyroidism	Asymptomatic; clinical or diagnostic observations only; intervention not indicated	Symptomatic: thyroid replacement indicated; limiting instrumental ADL	Severe symptoms; limiting self care ADL; hospitalization indicated	Life-threatening consequences; urgent intervention indicated
Adrenal insufficiency	Asymptomatic; clinical or diagnostic observations only; intervention not indicated	Moderate symptoms; medical intervention indicated	Severe symptoms; hospitalization indicated	Life-threatening consequences; urgent intervention indicated

Other endocrine disorders	Asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated	Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental ADL	Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of existing hospitalization indicated; disabling; limiting self care ADL	Life-threatening consequences; urgent intervention indicated
General				
Allergic reaction	Transient flushing or rash, drug fever <38 degrees C (<100.4 degrees F); intervention not indicated	Intervention or infusion interruption indicated; responds promptly to symptomatic treatment (e.g., antihistamines, NSAIDS, narcotics); prophylactic medications indicated for <=24 hours	Prolonged (e.g., not rapidly responsive to symptomatic medication and/or brief interruption of infusion); recurrence of symptoms following initial improvement; hospitalization indicated for clinical sequelae (e.g., renal impairment, pulmonary infiltrates)	Life-threatening consequences; urgent intervention indicated
For grading of all other toxicities, please refer to Common Terminology Criteria for Adverse Events (CTCAE) Version 4.03 [38] *There are no CTCAE criteria for grade 4 anemia.				

Table 7. Dose Modifications*

Chemotherapy			
Drug	**Starting Dose**	**Dose Modification**	
		Level -1	**Level -2**
Paclitaxel	***50 mg/m^{2}IV***	***37.5 mg/m^2***	***25 mg/m^2***
Carboplatin	***AUC = 2 IV***	***AUC = 1.5***	***AUC = 1***
Immunotherapy			
Nivolumab	***240 mg IV flat dose No dose adjustments. See management of toxicities below***		
Radiation			
	Starting Dose	**Dose Modification**	
	50.4 Gy (28 fractions)*	***41.4 Gy (23 fractions)*****	

***There are no dose reductions beyond level -2. Patients who require a dose reduction beyond dose level -2 should discontinue protocol therapy. Patients should also be taken off-study if they develop unacceptable toxicity. **Standard radiation dose administered in the United States for trimodality therapy that includes concurrent therapy with carboplatin and paclitaxel [36, 37]**
*****Per the protocol established by the CROSS regimen [7] Radiation dose level reduction will be done on a study level.**

7.1 Grade 1 and/or Grade 2 Hematologic Toxicities

- No delay in dosing of systemic agents or radiation therapy.

7.2 Hematologic Adverse Events

The dose of paclitaxel, carboplatin, and radiation will be modified according to blood counts on the day of treatment as shown in the table below. Dose reductions of paclitaxel and carboplatin are permanent.

Table 8. Treatment Modification for Hematologic Toxicity

Treatment Day Blood Counts			Dosage
ANC		Platelet Count	
> 1,000/mm^3 or (>1.0 x 10^9/L)	AND	> 75,000/mm^3 or > 75,000 x 10^9/L	Full dosage paclitaxel, carboplatin, and nivolumab.
500-999/mm^3	OR	50,000-75,000/mm^3	No change in nivolumab dose. Continue radiation without change in dose or schedule. Hold carboplatin and paclitaxel. Recheck CBC weekly. When ANC > 1,000 and Plt > 75,000, resume paclitaxel and carboplatin at level -1. If grade III hematologic toxicity persists after decreasing to dose level -1, hold for one week and restart at dose level -2. For persistent grade III hematologic toxicity after treating at level 2, discontinue chemotherapy: patient is off-study.
< 500/mm^3	OR	< 50,000/mm^3mcL	Hold RT, carboplatin, paclitaxel, and nivolumab. Recheck CBC weekly. When ANC > 500 and Plt >50,000 resume XRT, full-dose nivolumab. When ANC >1,000 and Plt >75,000 resume paclitaxel and carboplatin and reduce at dose level -2. For persistent grade III hematologic toxicity after treating at level 2, discontinue chemotherapy: patient is off-study.

For absolute neutrophil count less than 1000/mm^3, must use filgrastim 300 mcg subcutaneously (for patients < 70 kg) or 480 mcg (for patients > 70 kg) for one day and up to three consecutive days**.**

Missing doses of carboplatin and paclitaxel will not be made up.

Missing doses of radiation therapy will be made up.

7.3 Grade 1 Hepatic Toxicity

- No change in dosing of systemic therapy for grade 1 bilirubin elevation

- No change in dosing of systemic therapy for grade 1 AST/ALT elevation
- No change in dosing or delay in therapy of nivolumab

7.4 Grade 2 Hepatic Toxicity

- For grade 2 total bilirubin elevation OR grade 2 AST/ALT elevation, do not delay systemic therapy but decrease paclitaxel dose to level -1
- If grade 2 total bilirubin elevation OR grade 2 AST/ALT elevation persists despite 1 dose reduction in paclitaxel, decrease paclitaxel to dose level -2.
- No change in carboplatin or nivolumab dose.

- For grade 2 total bilirubin elevation AND grade 2 AST/ALT elevation, decrease paclitaxel to dose level -2
- No change in carboplatin or nivolumab dose or treatment schedule

7.5 Grade 3/4 Hepatic Toxicity

- For grade 3 hepatic toxicity discontinue paclitaxel, carboplatin and nivolumab. Begin steroids as per section 7.13.
- When bilirubin and AST/ALT return to grade 2 resume paclitaxel at Dose -1. Resume carboplatin and previous dose and resume nivolumab if reduction from grade 3 to grade 2 occurs within two weeks.
- See section 7.13 for changes in nivolumab administration.

7.6 Allergic Reactions

Paclitaxel premedication:

- Diphenhydramine (or equivalent) and ranitidine (or equivalent) are given according to institutional procedures. Patients should receive dexamethasone 10 mg intravenously immediately prior to receiving paclitaxel. The dexamethasone premedication dose can be tapered to 0 (zero) if no hypersensitivity reactions occur by the 4th dose of paclitaxel. If patients develop a hypersensitivity reaction despite this pre-medication schedule, other schedules (e.g. dexamethasone 20 mg immediately prior to chemotherapy or the night before and morning of chemotherapy) can be considered after discussion with the Principal Investigator.

- **For grade 1 or grade 2 allergic reactions during any drug administration:** stop infusion. Administer H1 and/or H2 blockers, and/or steroids according to institutional policy. Restart the infusion when symptoms resolve and pretreat before all subsequent doses. Treat according to institutional policy.

- **For grade 3 or 4 allergic reactions or anaphylaxis during any drug administration,** stop infusion and discontinue treatment completely. Patients should be taken off protocol.

7.7 Grade 1 Neurologic Toxicities

- For grade 1 peripheral sensory neuropathy that is not limiting activity of daily living, no change in dosing for systemic therapies or radiation therapy.

- No change in nivolumab dosing or treatment schedule

7.8 Grade 2 Neurologic Toxicities

- Delay all systemic therapy until toxicity resolves to ≤ grade 1
- Resume paclitaxel at dose level -1 and resume carboplatin at the previous dose level
- If recurrent grade 2 toxicity develops, decrease paclitaxel dose to level -2 and decrease carboplatin dose to level -1
- Continue radiation therapy
- See section 7.13 for changes in nivolumab administration

7.9 Grade 3/4 Neurologic Toxicities

- Discontinue all systemic therapy and take patient off study.

7.10 Gastrointestinal Toxicities

7.10.1 Grade 1 and 2 Vomiting

- Treat patient symptomatically with antiemetic therapy without holding or changing doses of systemic therapy or radiation.

7.10.2 Grade 3/4 Vomiting

- Hold radiation and systemic therapy until vomiting improves to ≤grade 2
- Decrease carboplatin and paclitaxel doses to level -1
- If recurrent toxicity develops, decrease carboplatin and paclitaxel doses to level -2
- No change in nivolumab dose.

7.10.3 Grade 1/2 Diarrhea

- No changes in carboplatin and paclitaxel dosing. Rule out *C. difficile*. If *C. difficile ele* is ruled out, treat symptomatically with anti-diarrheals (see section 7.13 for changes in nivolumab administration).

7.10.4 Grade 3/4 Diarrhea

- Hold carboplatin, paclitaxel, and radiation therapy until diarrhea improves to grade ≤2.
- Resume treatment with carboplatin at full-dose and reduce paclitaxel dose to level -1.
- If recurrent toxicity develops, decrease paclitaxel dose to level -2.
- See section 7.13 for changes in nivolumab administration.

7.10.5 Grade 1 Esophagitis

- No change in chemotherapy dose or schedule
- No change in radiation dose or schedule

- No change in nivolumab dosing or treatment schedule

7.10.6 Grade 2 Esophagitis

- No change in chemotherapy, radiation therapy, or nivolumab dosing or treatment schedules

  It is suggested that patients be advised to begin:
  - Soft diet, high-calorie with oral nutritional supplements
  - Oral analgesics such as hydrocodone with acetaminophen, liquid morphine, prolonged action opiates as necessary
  - Outpatient IV fluids at the discretion of the investigator
  - Continue chemoradiation at stated dosages and schedule

7.10.7 Grade 3/4 Esophagitis

- Discontinue all therapy until improvement to ≤ grade 2
- Hospitalization or daily outpatient IV hydration and IV pain control is recommended
- Total parenteral nutrition or feeding tube at the discretion of the investigator. Decrease carboplatin and paclitaxel dose to level -1
- If recurrent toxicity develops, decrease carboplatin and paclitaxel dose to level -2
- No change in nivolumab dose or treatment schedule

7.11 Grade 1 Pneumonitis

- No change in systemic therapy; no change in radiation therapy.

7.12 Grade 2-4 Pneumonitis

- Hold chemotherapy and radiation until symptoms improve to ≤ grade 2
- Resume chemotherapy paclitaxel and carboplatin and radiation without dose reduction.
- See section 7.13 for changes in nivolumab administration.

7.13 Dose Modifications for Other Non-hematologic Toxicities During Combined Therapy

For other grade 3 or 4 non-hematologic toxicities (excluding suboptimally controlled nausea or vomiting, and fatigue) related to treatment, hold carboplatin and paclitaxel until toxicity improves to ≤ grade 2. Then resume with one dose level reduction in carboplatin/paclitaxel. If treatment is held for >2 weeks, this is considered unacceptable toxicity and treatment should be discontinued. The patient will be followed off-protocol.

7.14 Nivolumab Immune-Mediated Toxicity

7.14.1 Immune-Mediated Pneumonitis

- Patients will be monitored weekly for symptoms and signs of immune-mediated pneumonitis
- For patients with new-onset symptoms of shortness of breath, cough or hemoptysis, a non-contrast chest CT is required at the time of symptoms or signs.
- For treatment days 1-28 (nivolumab induction)

    - **Grade 1**
        - No delay or change in schedule or dose

    - **Grade 2**
        - Hold nivolumab
        - Administer corticosteroids at a dose of 1-2 mg/kg/day prednisone equivalents. Upon improvement to grade 1, begin a corticosteroid taper over 1 month
        - Upon resolution of pneumonitis, continue nivolumab at the same dose and schedule if resolution occurs within two weeks of holding nivolumab

    - **Grade 3 or 4**
        - Permanently discontinue nivolumab for grade 3 or grade 4 pneumonitis
        - Administer corticosteroids at a dose of 1-2 mg/kg/day prednisone equivalents. Upon improvement to grade 1, begin corticosteroid taper over one month

- For days 29-64 (combination chemoradiotherapy and immunotherapy):
    - For patients with new-onset symptoms of shortness of breath, cough or hemoptysis, a non-contrast chest CT is required at the time of symptoms or signs.

    - **Grade 2-4**
        - **For grade 2 pneumonitis,** Hold nivolumab.
        - Administer corticosteroids at a dose of 1-2 mg/kg/day prednisone equivalents. Upon improvement to grade 1restart nivolumab and begin a corticosteroid taper over 1 month

        - **For grade 3 or 4 pneumonitis,** permanently discontinue nivolumab
        - Administer corticosteroids at a dose of 1-2 mg/kg/day prednisone equivalents. Upon improvement to grade 1, begin corticosteroid taper over one month.

7.14.2 Immune-Mediated Colitis

- Patients will be monitored weekly for symptoms and signs of immune-mediated colitis

    - **Grade 1**
        - No change in dose or schedule

- **Grade 2**
  - Delay nivolumab until colonoscopy is performed to confirm immune-colitis. If immune-colitis is diagnosed, administer corticosteroids at a dose of 0.5-1 mg/kg/day prednisone equivalents followed by a corticosteroid taper over 1 month.
  - For grade 2 colitis of more than 5 days duration, if worsening or no improvement occurs despite initiation of corticosteroids, increase corticosteroid dose to 1-2 mg/kg/day prednisone equivalents. Other immunosuppressants, e.g. infliximab, should also be considered
  - Upon improvement of diarrhea to grade 1 or less, initiate corticosteroid taper over 1 month and resume nivolumab without a change in dose or schedule.

- **Grade 3 and 4**
  - Permanently discontinue nivolumab for ≥grade 3 colitis or for recurrent colitis upon restarting nivolumab. For grade 3 colitis that recovers to grade 1 or less by day 28, can remain on treatment

7.14.3 Immune-Mediated Hepatitis

- Evaluation of serum liver function studies will be performed weekly while the patient is on therapy.

  - **Grade 1**
    - Continue nivolumab
    - Repeat liver function tests, including AST, ALT, and bilirubin prior to the next cycle of treatment

  - **Grade 2**
    - Delay nivolumab
    - Administer corticosteroids at a dose of 1 mg/kg/day prednisone equivalents
    - Upon resolution of bilirubin, AST, and ALT to grade ≤ 1, taper corticosteroids over 1 month
    - Resume therapy with nivolumab only upon resolution of bilirubin, AST, and ALT to grade ≤ 1.

  - **Grade 3-4**

    - Permanently discontinue nivolumab
    - Begin therapy with prednisone 1-2 mg/kg
    - Also begin therapy with mycophenolate mofetil 500-1000mg q12h

- If refractory and/or rapid clinical deterioration, the patient should be hospitalized with consideration of adding antithymocyte globulin 1.5 mg/kg for 2 consecutive days

7.14.4 Immune-Mediated Nephritis and Renal Dysfunction

- PD-1 inhibitors may cause acute interstitial nephritis (AIN). Although evaluation of serum renal function studies will be performed weekly while patients are receiving therapy, those developing grade 2 creatinine elevation will have a delay in nivolumab therapy if their urine shows evidence of AIN or immune-mediated nephritis with elevated white cells, white cell casts, and, in some cases, eosinophilia without bacteria.

    - Grade 2-4 elevation of serum creatinine
    - Delay nivolumab for grade 2 or grade 3 serum creatinine elevation, and administer corticosteroids at a dose of 0.5-1 mg/kg/day prednisone equivalents if U/A shows evidence of AIN or immune-mediated nephritis with elevated white cells, white cell casts, and, in some cases, eosinophilia without bacteria.

    - If no improvement or worsening of the creatinine level occurs, increase dose of corticosteroids to 1-2 mg/kg/day prednisone equivalents and permanently discontinue nivolumab
    - Upon improvement to grade 1 or less, initiate corticosteroid taper and continue to taper over at least 1 month
    - Drug-related elevation in creatinine must have resolved to baseline before treatment with nivolumab is resumed.

7.14.5 Immune-Mediated Neuropathy

- Evaluation of peripheral neuropathy will be performed weekly during protocol therapy by history and physical examinations.
    - Delay nivolumab for grade $\geq$2 neuropathy or any symptom that may be related to Guillain-Barre Syndrome.
    - For grade $\geq$ 2 neuropathy, administer corticosteroids at a dose of 1 to 2 mg/kg/day prednisone equivalents
        - For Guillan Barre Syndrome symptoms, the first line of therapy should be immune globulin administration.
    - Upon improvement to grade 1 or less, initiate corticosteroid taper and continue to taper over at least 1 month.
    - Upon improvement in neurotoxicity to grade $\leq$1, resume nivolumab. The decision to reinitiate nivolumab at the next scheduled treatment date will be based upon treating physician's clinical judgment.

7.14.6 Immune-Mediated Endocrinopathies

- **Hypothyroidism and Hyperthyroidism**

- Thyroid function will be monitored at baseline and every two weeks while the patient is on therapy; these studies will be measured every four weeks while the patient is being followed off active therapy

  - **Grade 1**
    - If TSH < 0.5 x lower limit of normal or TSH > 2 x ULN or consistently out of range in 2 subsequent measurements, check free thyroxine (fT4) at subsequent cycles
    - Continue nivolumab

  - **Grade 2**

    - **Hypothyroidism**
      - If TSH > 2 x upper limit of normal and the patient is symptomatic, initiate thyroid replacement therapy
      - Continue nivolumab

    - **Hyperthyroidism**
      - If TSH < 0.5 x lower limit of normal and the patient is asymptomatic, initiate thyroid suppression therapy
      - Continue nivolumab

  - **Grade 3**
    - If TSH < 0.5 x lower limit of normal or TSH > 2 x ULN and the patient is severely symptomatic (limiting activities of daily living):
      - Delay nivolumab
      - Initiate prednisone at 1 mg/kg/day
      - Initiate thyroid replacement therapy (hypothyroidism) or thyroid suppression therapy (hyperthyroidism)
      - Administer corticosteroids at a dose of 1 mg/kg/day prednisone equivalents
      - Upon improvement to grade 1 or less, initiate corticosteroid taper and continue to taper over at least 1 month.
      - Upon improvement in thyroid function to grade ≤1, resume nivolumab

- **Hypophysitis**
  - For symptoms including significant headache, visual impairment, nausea, vomiting, anorexia, fatigue, weakness, fever, lethargy, hypotension, hypoglycemia, hyponatremia, eosinophilia: check adrenocorticotropic hormone (ACTH), thyroid-stimulating hormone (TSH), free thyroxine (fT4), follicle-stimulating hormone (FSH),

luteinizing hormone (LH), insulin-like growth factor 1 (IGF-1), prolactin, plasma cortisol, ADH (antidiuretic hormone), testosterone/estradiol. Also perform pituitary MRI.

- **≤ Grade 1:** Asymptomatic TSH elevation
  - Hold nivolumab pending evaluation, Endocrinology consultation
  - Resume nivolumab after completion of the consultation

- **Grade 2-3**
  - For abnormal lab and/or pituitary MRI results and significant symptoms limiting activities of daily living
    - Delay nivolumab
    - Endocrinology consultation
    - Administer corticosteroids at a dose of 1 mg/kg/day prednisone equivalents
      - Also initiate appropriate hormone therapy
    - Upon resolution of symptoms, initiate corticosteroid taper and continue to taper over at least 1 month
    - Resume nivolumab
    - Patients with adrenal insufficiency may need to continue steroids with a mineralocorticoid component

    If no abnormal lab or pituitary MRI results but symptoms persist, repeat labs in 1 week or MRI in 1 month

- **Grade 4**
  - For life-threatening symptoms and abnormal lab and/or pituitary MRI results
    - Permanently discontinue nivolumab
    - Endocrinology consultation
    - Administer corticosteroids at a dose of 1 mg/kg/day prednisone equivalents
    - Also initiate appropriate hormone therapy

- **Adrenal insufficiency**

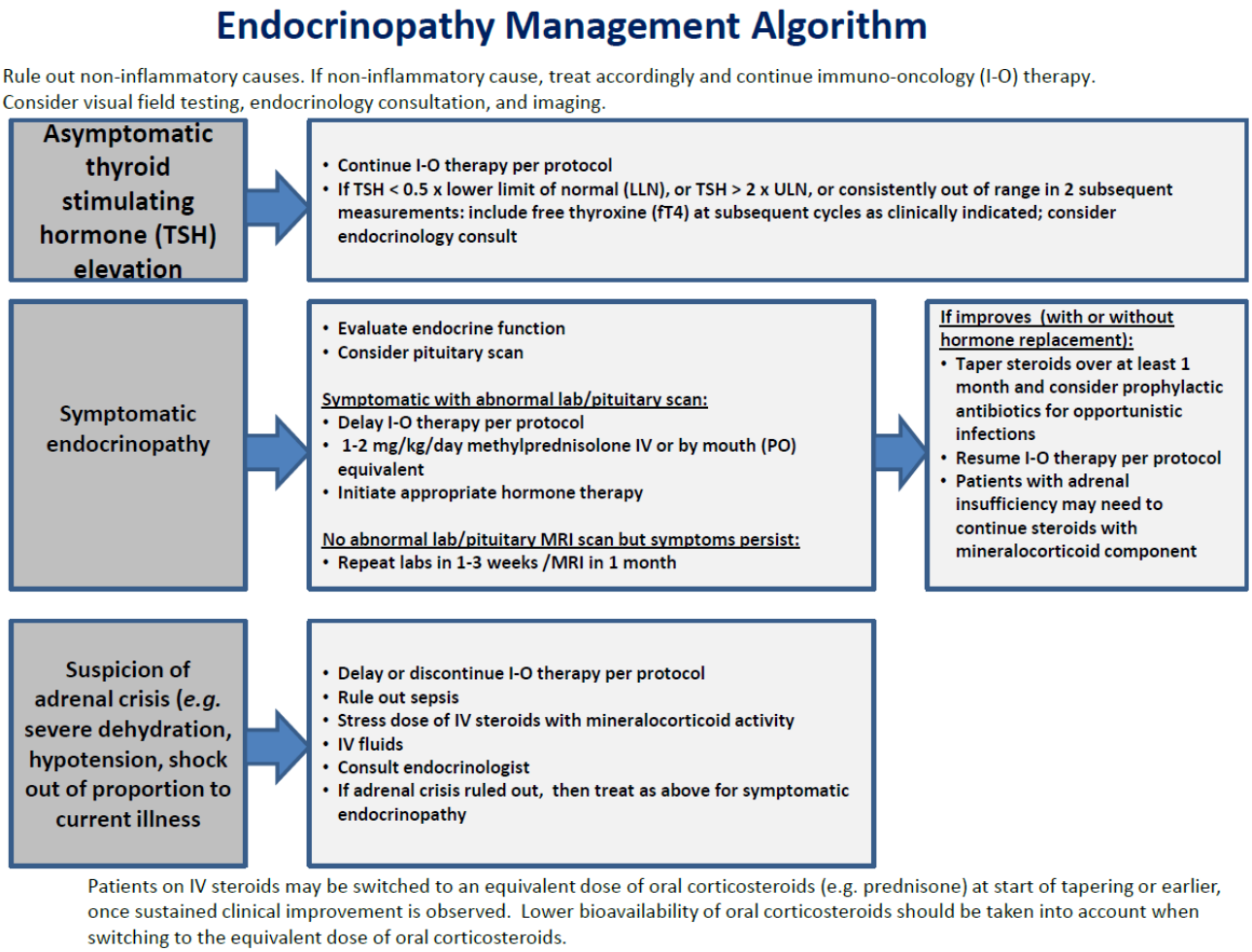
  - **≤ Grade 1**
    - Hold nivolumab pending evaluation, Endocrinology consultation
    - Resume nivolumab at the same dose level once the consultation has been completed

  - **Grade 2**
    - Delay nivolumab

- Initiate appropriate hormone replacement therapy (may require steroids with mineralocorticoid component)
- Upon resolution of symptoms and lab abnormalities to grade 1 or less, resume nivolumab

- **Grade 3-4**
  - For suspicion of adrenal crisis, including severe dehydration, hypotension, shock out of proportion to current illness
    - Discontinue nivolumab
    - Rule out sepsis
    - Administer stress-dose IV steroids with mineralocorticoid activity
    - IV fluids
    - Endocrinology consultation

Endocrine Hypophysitis Adrenal Insufficiency	**Management/Next Dose for Nivolumab + Ipilimumab**
≤ Grade 1	Asymptomatic TSH elevation. *Hold pending evaluation, endocrine consult
Grade 2	Hold until patients are on a stable replacement hormone regimen. If treated with steroids patients must remain stable after completion of any doses greater than replacement dose. Resume nivolumab at same dose level.
Grade 3	Off study treatment
Grade 4	Off study treatment
Note all patients with symptomatic pituitary enlargement, exclusive of hormone deficiency, but including severe headache or enlarged pituitary on MRI should be considered grade 3 events. Isolated thyroid or testosterone deficiency may be treated as grade 2 if there are no other associated deficiencies and adrenal function is monitored. Please evaluate pituitary function before beginning steroid therapy or replacement therapy of any kind. *Note patients with thyroiditis may be retreated on replacement therapy. Patients must be evaluated to rule out pituitary disease prior to initiating thyroid replacement.	
Recommended management: See Endocrine Management Algorithm below	



7.15 General Criteria to Resume Treatment (Dose Resumption) with Nivolumab

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

- Subjects may resume treatment in the presence of grade 2 fatigue
- Subjects who have experienced a Grade 3 drug-related skin AE may resume treatment in the presence of grade 2 skin toxicity
- Subjects with baseline grade 1 AST/ALT or total bilirubin who require dose delays for reasons other than a 2-grade shift in AST/ALT or total bilirubin may resume treatment in the presence of grade 2 AST/ALT OR total bilirubin
- Nivolumab-related grade 2 pulmonary toxicity or grade 2 diarrhea or colitis must have resolved to baseline before treatment is resumed

If treatment is interrupted for >4 consecutive weeks, patient's protocol treatment will be discontinued with the exception of the following outlined below (pg.102):

- Any dosing interruption lasting > 6 weeks with the following exceptions:
    - Dosing delays or interruptions to allow for prolonged steroid tapers to manage drug-related adverse events are allowed. Prior to re-initiating treatment in a subject with a dosing interruption lasting > 6 weeks, the Investigator must be consulted. Tumor assessments should continue as per protocol even if dosing is interrupted or delayed
    - Dosing interruptions or delays lasting > 6 weeks that occur for non-drug-related reasons may be allowed if approved by the Investigator. Prior to re-initiating treatment in a subject with a dosing interruption lasting > 6 weeks, the Investigator must be consulted. Tumor assessments should continue as per protocol even if dosing is interrupted

8.0 Criteria for Response and Progression

8.1 Clinical Response

All eligible patients who have received therapy on this study will be considered evaluable. Patients who discontinue study therapy because of progressive disease or serious drug-related adverse events are also considered evaluable for efficacy. Patients removed from the study prior to treatment will not be evaluable.

The assessment of clinical response is based on clinical staging of the patient five to eight weeks following the completion of radiation by PET/CT scan, upper GI endoscopy, and histopathologic evaluation of tumor at the time of endoscopic evaluation.
To be coded as a complete clinical response the following must occur:

1. A resolution of PET SUV evaluation to the lower value between SUV ≤75% of pre-treatment value or SUV ≤2.
2. No visible tumor by CT scan (Esophageal wall thickening does not indicate residual cancer).
3. No visible tumor at the time of endoscopy
4. No viable tumor at biopsy of the esophageal mucosa where tumor had previously been appreciated.

NB: Those patients achieving a complete clinical response will be allowed to forego surgery in a decision that will be made by the patient and his/her oncology team. Those patients achieving a complete clinical response who do not undergo surgery will be treated with adjuvant nivolumab as outlined in the protocol.

NB: Those patients who do not achieve a complete clinical response will be referred for esophagectomy and lymph node dissection. The procedure of choice will be left to the surgical team. Upon recovery from esophagectomy and nodal dissection patient will be placed on adjuvant nivolumab as outlined in the protocol.

8.2 Pathologic Response

Pathologic response will be based on anatomic pathologic evaluation of the biopsy or surgical specimen and be reported as complete pathologic response if all evidence of tumor is gone from the primary esophageal cancer and lymph nodes. Pathologic response will be categorized as either complete (T0N0M0) or near complete Tis/T1N0M0 or not complete T2-$T4N_{any}M_{any}$

8.3 Progression of Disease

Distant metastatic disease on CT or PET/CT imaging or evidence of locally recurrent disease or progressive disease documented by endoscopy, CT or PET/CT imaging. Progression of disease on PET/CT scan alone (new sites of metastatic disease) prior to surgery should be corroborated by biopsy whenever possible.

Patients who undergo an operation for following neoadjuvant combined modality therapy and are found to have an R1 or R2 resection will be declared as having progressive disease at that time.

9.0 Statistical Plan

This is a single-arm phase I/II study of nivolumab combined with carboplatin/paclitaxel and radiation for patients with locally advanced squamous cell carcinoma the esophagus.
In the phase III CROSS study, pre-operative carboplatin/paclitaxel and radiation was associated with a grade 3/4 toxicity rate of 20% (7% hematologic and 13% non-hematologic grade 3-4 toxicities). The discontinuation rate for nivolumab as a single agent due to any toxicity has been reported to be approximate 9% {BMS, #81}. The hypothesis is that the addition of nivolumab to carboplatin/paclitaxel and radiation will not increase the aggregate grade 3/4 toxicity rate beyond 30%.

In the phase I portion of the study, up to six patients will be treated (radiation will be 50.4 Gy (1.8 Gy/fraction × 28 fractions)) and then observed for 28 days (following last day of treatment (Day 64)). If there are 1 or less unacceptable toxicities (1/6 is less than 30%), the current regimen will be used in the phase II portion of the study. If there are 2 or more unacceptable toxicities (2/6 is more than 30%), the radiation will be reduced to 41.4 Gy (1.8 Gy/fraction × 23 fractions). Up to six additional patients will be treated at the reduced radiation regimen and then observed for 28 days (following last day of treatment (Day 64)). If there are 1 or less unacceptable toxicities, radiation of 41.4 Gy will be used in the phase II portion of the study. If there are 2 or more unacceptable toxicities, the trial will be discontinued.

Unacceptable Toxicity (UT) is defined here as:

- Recurrent grade 3 or 4 hematologic toxicity (despite 1 prior dose reduction in chemotherapy)
- Any toxicity that results in a >2-week delay in chemoradiation

If the regimen is found to be tolerable, the phase II portion of the study will be executed. An optimal two-stage design will be implemented. With an overall sample size of 44 patients,

there is 80% power (actual power is 80%) to test the null hypothesis of clinical complete response (cCR) rate + pathologic complete response (pCR) rate ≤ 35%, versus the alternative hypothesis of cCR+pCR ≥ 55%, with significance level of 5% (actual significance level is 4.5%). In the first stage, 18 patients will be treated with the combination therapy. If there are 7 or fewer cCR+pCRs the trial will be terminated. If there are 8 or more cPR+pCRs, the trial will continue with the subsequent enrollment of 26 additional patients in phase 2. If the total cPR+pCRs out of 44 patients is 20 or less, the combination therapy will be rejected [39]

cCR will be evaluated by endoscopic evaluation and CT/PET scan evaluation at the completion of radiation with concurrent nivolumab + carboplatin and radiation and pCR will be evaluated post-surgery. The trial will continue accruing patients in the second stage, while the 18 patients in the first stage are being evaluated for cCR or pCR. In an event that the interim analysis deems the combination therapy futile, accrual to the second stage will cease.

The median overall survival (OS) will be described using Kaplan Meier curves. OS is defined as the time from treatment to death from any cause. The median progression-free survival (PFS) will be described using Kaplan Meier curves. PFS is defined as time from treatment to disease progression or death from any cause.

Regression models will be used to assess whether the PET/CT clinical response to nivolumab can predict the outcomes pCR, PFS, and OS.

10.0 Safety and Adverse Events

10.1 Definitions

Unanticipated Problems Involving Risk to Subjects or Others
Any incident, experience, or outcome that meets all of the following criteria:

- Unexpected in nature, severity, or frequency (i.e. not described in study-related documents such as the IRB-approved protocol or consent form, the investigators brochure, etc.)
- Related or possibly related to participation in the research (i.e. possibly related means there is a reasonable possibility that the incident experience, or outcome may have been caused by the procedures involved in the research)
- Suggests that the research places subjects or others at greater risk of harm (including physical, psychological, economic, or social harm).

ADVERSE EVENTS

An Adverse Event (AE) is defined as any new untoward medical occurrence or worsening of a preexisting medical condition in a clinical investigation subject administered an investigational (medicinal) product and that does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (such as an abnormal laboratory finding),

symptom, or disease temporally associated with the use of investigational product, whether or not considered related to the investigational product.

The causal relationship to study drug is determined by a physician and should be used to assess all adverse events (AE). The casual relationship can be one of the following:

Related: There is a reasonable causal relationship between study drug administration and the AE.

Not related: There is not a reasonable causal relationship between study drug administration and the AE.

The term "reasonable causal relationship" means there is evidence to suggest a causal relationship.

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

SERIOUS ADVERSE EVENTS

A **S*erious Adverse Event (SAE)*** is any untoward medical occurrence that at any dose:

- Results in death
- Is life-threatening (defined as an event in which the subject was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe)
- Requires inpatient hospitalization or causes prolongation of existing hospitalization (see **NOTE** below)
- Results in persistent or significant disability/incapacity
- Is a congenital anomaly/birth defect
- Is an important medical event (defined as a medical event(s) that may not be immediately life-threatening or result in death or hospitalization but, based upon appropriate medical and scientific judgment, may jeopardize the subject or may require intervention [e.g., medical, surgical] to prevent one of the other serious outcomes listed in the definition above.) Examples of such events include, but are not limited to, intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalization.)
- Potential drug induced liver injury (DILI) is also considered an important medical event.
- Suspected transmission of an infectious agent (e.g., pathogenic or nonpathogenic) via the study drug is an SAE.
- Although pregnancy, overdose, and cancer are not always serious by regulatory definition, these events must be handled as SAEs.

Pregnancy

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

The investigator must immediately notify Worldwide Safety @BMS of this event via the Pregnancy Surveillance Form in accordance with SAE reporting procedures. Also, it must be reported immediately to the regulatory specialist, research coordinator, PCC Assigned Medical Monitor, and NYUPCCsafetyreport@nyumc.org in accordance with the procedures described below. Pregnancy in itself is not regarded as an adverse event unless there is a suspicion that the investigational product may have interfered with the effectiveness of a contraceptive medication. This will be reported to the IRB if necessary.

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

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

Overdose

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

10.2 Other Safety Considerations

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

All adverse events that do not meet any of the criteria for serious should be regarded as ***non-serious adverse events***.

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

Non-serious Adverse Event Collection and Reporting

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

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

Adverse Event Reporting Period

The study period during which adverse events must be reported is normally defined as the period from the initiation of any study procedures to the end of the study treatment follow-up. For this study, the study treatment follow-up is defined as 30 days following the last administration of study treatment.

Preexisting Condition
A preexisting condition is one that is present at the start of the study. A preexisting condition should be recorded as an adverse event if the frequency, intensity, or the character of the condition worsens during the study period.

General Physical Examination Findings
At screening, any clinically significant abnormality should be recorded as a preexisting condition. At the end of the study, any new clinically significant findings/abnormalities that meet the definition of an adverse event must also be recorded and documented as an adverse event.

Post-study Adverse Event
All unresolved adverse events should be followed by the investigator until the events are resolved, the subject is lost to follow-up, or the adverse event is otherwise explained. At the last scheduled visit, the investigator should instruct each subject to report any subsequent event(s) that the subject, or the subject's personal physician, believes might reasonably be related to participation in this study. The investigator should notify the study sponsor of any death or adverse event occurring at any time after a subject has discontinued or terminated study participation that may reasonably be related to this study. The sponsor should also be notified if the investigator should become aware of the development of cancer or of a congenital anomaly in a subsequently conceived offspring of a subject that has participated in this study.

Abnormal Laboratory Values
A clinical laboratory abnormality should be documented as an adverse event if <u>any one of the following</u> conditions is met:

- The laboratory abnormality is not otherwise refuted by a repeat test to confirm the abnormality
- The abnormality suggests a disease and/or organ toxicity
- The abnormality is of a degree that requires active management; e.g. change of dose, discontinuation of the drug, more frequent follow-up assessments, further diagnostic investigation, etc.

Hospitalization, Prolonged Hospitalization or Surgery
Any adverse event that results in hospitalization or prolonged hospitalization should be documented and reported as a serious adverse event unless specifically instructed otherwise in this protocol. Any condition responsible for surgery should be documented as an adverse event if the condition meets the criteria for and adverse event.

Neither the condition, hospitalization, prolonged hospitalization, nor surgery are reported as an adverse event in the following circumstances:

- Hospitalization or prolonged hospitalization for diagnostic or elective surgical procedures for a preexisting condition. Surgery should ***not*** be reported as an outcome of an adverse event if the purpose of the surgery was elective or diagnostic and the outcome was uneventful.
- Hospitalization or prolonged hospitalization required to allow efficacy measurement for the study.
- Hospitalization or prolonged hospitalization for therapy of the target disease of the study, unless it is a worsening or increase in frequency of hospital admissions as judged by the clinical investigator.

10.3 Recording of Adverse Events

At each contact with the subject, the investigator must seek information on adverse events by specific questioning and, as appropriate, by examination. Information on all adverse events should be recorded immediately in the source document, and also in the appropriate adverse event module of the case report form (CRF). All clearly related signs, symptoms, and abnormal diagnostic procedures results should recorded in the source document, though should be grouped under one diagnosis.

All adverse events occurring during the study period must be recorded. The clinical course of each event should be followed until resolution, stabilization, or until it has been determined that the study treatment or participation is not the cause. Serious adverse events that are still ongoing at the end of the study period must be followed up to determine the final outcome. Any serious adverse event that occurs after the study period and is considered to be possibly related to the study treatment or study participation should be recorded and reported immediately.

10.4 Reporting of Serious Adverse Events and Unanticipated Problems

Investigators and the protocol sponsor must conform to the adverse event reporting timelines, formats and requirements of the various entities to which they are responsible, but at a minimum those events that must be reported within 5 days of PI notification are those that are:

- related to study participation,
- unexpected, and
- Harmful or have the potential to cause harm (see definitions, section 10.1)

Events should be reported using the NYU CTO Medical Events Form (see section 10.4.1 below).

Adverse events that do not fit the above immediately reportable criteria must still be reported to the IRB at each annual review, either in a summary or tabular format.

10.4.1 Investigator reporting: notifying the study sponsor and Perlmutter Cancer Center Clinical Trials Office

The following describes events that must be reported to the study sponsor in an expedited fashion.

Initial Report: within 24 hours:
The following events must be reported to the study sponsor within 24 hours of awareness of the event using the NYU CTO Medical Events Form:

- Unanticipated problems related to study participation,
- Serious adverse events, regardless of whether they are unexpected.

Additionally, an FDA Form 3500A (MEDWATCH Form) must be completed by the investigator and faxed to the study sponsor within 24 hours. The investigator shall maintain a copy of the MEDWATCH Form on file at the study site. The study sponsor will then report all medical events to BMS directly.

All report forms must be signed and dated by the Principal Investigator. If the Principal Investigator is not available at the time of the initial report, then the form can be submitted by a Sub-Investigator. This form should be reviewed by the Principal Investigator, whom sign/date initial report upon return.

Report to:

NYUPCCsafetyreports@nyumc.org

AND

Jennifer Wu, MD
462 First Ave
New York, NY 10016
Phone: (212) 263-6530
Email: Jennifer.Wu@nyumc.org

AND

PCC Assigned Medical Monitor

Follow-up report: within 48 hours:
As a follow-up to the initial report, within the following 48 hours of awareness of the event, the investigator shall provide further information, as applicable, on the unanticipated device

event or the unanticipated problem in the form of a written narrative. This should include any other diagnostic information that will assist in the understanding of the event. Significant new information on ongoing unanticipated adverse device effects shall be provided promptly to the study sponsor.

Other external sites will be monitored and informed of other adverse events by the medical monitor within 7 days of toxicities and within 3 business days of SAE. Adverse events and SAEs occurring at external sites should be reported as detailed above. Scheduled conference calls will be conducted after 3 patients are enrolled and at each dose escalation point. Additional conference calls will be scheduled as indicated based on the recommendations from the medical monitor, the Overall PI of this study.

Other Reportable events:

- **Deviations from the study protocol**
  Deviations from the protocol must receive both Sponsor and the investigator's IRB approval before they are initiated. Any protocol deviations initiated without Sponsor and the investigator's IRB approval that may affect the scientific soundness of the study, or affect the rights, safety, or welfare of study subjects, must be reported to the Sponsor and to the investigator's IRB as soon as a possible, but ***no later than 5 working days*** of the protocol deviation.

- ***Withdrawal of IRB approval***
  An investigator shall report to the sponsor a withdrawal of approval by the investigator's reviewing IRB as soon as a possible, but ***no later than 5 working days*** of the IRB notification of withdrawal of approval.

10.4.2 Investigator reporting: notifying the IRB

Federal regulations require timely reporting by investigators to their local IRB of unanticipated problems posing risks to subjects or others. The following describes the NYULMC IRB reporting requirements, though Investigators at participating sites are responsible for meeting the specific requirements of their IRB of record. The NYU IRB address is:

NYU School of Medicine IRB
1 Park Avenue, 6th Floor
New York, NY 10016

Report promptly, but no later than 5 working days:
Researchers are required to submit reports of the following problems promptly but no later than 5 working days from the time the investigator becomes aware of the event:

- ***Unanticipated problems including adverse events*** **that are unexpected and related**
  - *Unexpected: An event is "unexpected" when its specificity and severity are not accurately reflected in the protocol-related documents, such as the IRB-approved research protocol, any applicable investigator brochure, and the current IRB-approved informed consent document and other relevant sources of information, such as product labeling and package inserts.*
  - *Related to the research procedures: An event is related to the research procedures if in the opinion of the principal investigator or sponsor, the event was more likely than not to be caused by the research procedures.*
  - *Harmful: either caused harm to subjects or others, or placed them at increased risk*

Other Reportable events:
The following events also require prompt reporting to the IRB, though ***no later than 5 working days***:

- ***Complaint of a research subject*** when the complaint indicates unexpected risks or the complaint cannot be resolved by the research team.

- ***Protocol deviations or violations*** (includes intentional and accidental/unintentional deviations from the IRB approved protocol) for any of the following situations:
  - *one or more participants were placed at increased risk of harm*
  - *the event has the potential to occur again*
  - *the deviation was necessary to protect a subject from immediate harm*
- ***Breach of confidentiality***
- ***Incarceration of a participant*** when the research was not previously approved under Subpart C and the investigator believes it is in the best interest of the subject to remain on the study.
- ***New Information indicating a change to the risks or potential benefits*** of the research, in terms of severity or frequency. (e.g. analysis indicates lower-than-expected response rate or a more severe or frequent side effect; Other research finds arm of study has no therapeutic value; FDA labeling change or withdrawal from market)

Reporting Process
The reportable events noted above will be reported to the IRB using the form: "Reportable Event Form" or as a written report of the event (including a description of the event with information regarding its fulfillment of the above criteria, follow-up/resolution and need for revision to consent form and/or other study documentation). The contact information for submitting IND safety reports is noted below:

Email: NYUPCCsafety@nyumc.org
Tel: 212-263-4427

Copies of each report and documentation of IRB notification and receipt will be kept in the Clinical Investigator's study file.

10.4.3 Sponsor reporting: Notifying BMS

Serious Adverse Event Collection and Reporting

All Serious Adverse Events must be reported to BMS Worldwide Safety.

BMS SAE forms should be used, or if performed under a US IND a MedWatch or CIOMS form can be used as required by regulatory authorities.

Site specific forms will be requested for review.

The sponsor/investigator will be required to reconcile SAEs reported in the clinical database with SAE cases transmitted to BMS Global Pharmacovigilance (GPV&E); worldwide.safety@bms. BMS requests this is initiated by the sponsor investigator up to quarterly and prior to the database lock or final data summary. The process will be further defined. During reconciliation, any events found to not be reported previously to BMS must be sent to Worldwide.Safety@BMS.com

Following the subject's written consent to participate in the study, all SAEs, whether related or not related to study drug, must be collected, including those thought to be associated with

protocol-specified procedures. All SAEs must be collected that occur within 100 days of discontinuation of dosing.

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

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

SAE Email Address: Worldwide.Safety@BMS.com

SAE Facsimile Number: 609-818-3804

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

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

All SAEs should be followed to resolution or stabilization.

The Sponsor/Investigator will ensure that all SAEs in the clinical database are reported to BMS and any applicable health authority during the conduct of the study including periodic reconciliation. For studies conducted under an Investigator IND in the US, any event that is both serious and unexpected must be reported to the Food and Drug Administration (FDA) as soon as possible and no later than 7 days (for a death or life-threatening event) or 15 days (for all other SAEs) after the investigator's or institution's initial receipt of the information. BMS will be provided with a simultaneous copy of all adverse events filed with the FDA.

SAEs should be reported on MedWatch Form 3500A, which can be accessed at: http://www.accessdata.fda.gov/scripts/medwatch/.

MedWatch SAE forms should be sent to the FDA at:
MEDWATCH
5600 Fishers Lane
Rockville, MD 20852-9787
Fax: 1-800-FDA-0178 (1-800-332-0178)
http://www.accessdata.fda.gov/scripts/medwatch/

10.4.4 *Sponsor reporting: Notifying Participating Investigators*

It is the responsibility of the study sponsor to notify all participating investigators of any adverse event that meets the FDA 15-day reporting requirement criteria as noted above. The same materials and timeline used to report to the FDA are used for notifying participating investigators.

10.5 Stopping Rules

Phase I: Up to 12 patients

6 patients treated and observed for 28 days after last day of treatment (Day 64)

- ≤ 1 unacceptable toxicity (UT): use current regimen for Phase II
- ≥ 2 UT: reduce total RT dose to 41.4 Gy and treat 6 additional patients, then observe for 28 days after Day 64
    - ≤ 1 UT: use reduced RT for Phase II
    - ≥ 2 UT: **discontinue trial**

Phase II: 44 patients

Stage I: 18 patients treated

- ≤ 7 CRs (either pCR or cCR): **discontinue trial** as combination therapy deemed futile
- ≥ 8 CRs (either pCR or cCR): continue on to Stage II.

Stage II: 30 additional patients treated

- ≤ 20 cCR + pCR out of all 44 patients: combination therapy rejected
- ≥ 21 cCR + pCR out of all 44 patients: combination therapy is promising

- Accrual to Stage II will continue while the 18 patients in Stage I are evaluated for cCR and pCR.

- **Accrual to Stage II will stop if Stage I analysis deems combination futile**

10.6 Medical Monitoring

It is the responsibility of the Principal Investigator at each site to oversee the safety of the study at his/her site. This safety monitoring will include careful assessment and appropriate reporting of adverse events as noted above, as well as the construction and implementation of a site data and safety-monitoring plan. The Data Safety and Monitoring Committee (DSMC) will review the study at least annually. Medical monitoring will include a regular assessment of the number and type of serious adverse events.

10.6.1 Data Monitoring Committee

This investigator initiated and sponsored study will be monitored by the Data Safety Monitoring Committee (DSMC) of the New York University Perlmutter Cancer Center. The DSMC operates based on the 2014 National Cancer Institute approved Charter. It is an existing and multidisciplinary committee (consisting of clinical investigators/oncologists, biostatisticians, nurses and design and in proper conduct of clinical trials) that is responsible for monitoring safety, conduct and compliance in accordance with protocol data monitoring plans for interventional clinical trials conducted in the New York University Langone Perlmutter Cancer Center that are not monitored by another institution or agency. The DSMC reports to the director of the New York University Perlmutter Cancer Center (Benjamin Neel, MD/PhD).

Per the New York University Perlmutter Cancer Center Institutional Data Safety and Monitoring Plan, this Phase I/II trial will be monitored by the DSMC quarterly (from the date the first patient is enrolled), at times of RT dose reduction, completion of Phase I, interim analysis and completion of Phase II. This review includes accrual data, subject demographics, and adverse events. Principal Investigators are required to attend the review of their studies. Additional reviews can be scheduled based on SAE reports, investigator identified issues, external information, etc. The DSMC will review safety data every 4 months.

Safety assessments will be performed at regular study intervals and include standard clinical and laboratory tests (hematology, serum chemistry, electrolytes,), physical examinations, pregnancy testing, concomitant medications, and reporting of AEs. The AE reporting period for a subject enrolled in the study will begin from the time the informed consent is signed through 90 days after the last dose.

Toxicity grade will be defined by the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) Version 4.03. Subjects will be monitored throughout the treatment and follow-up period for occurrence of AEs (acute, delayed, and/or cumulative), as well as for changes in clinical status, vital sign measurements, and laboratory data. Safety parameters to be measured/assessed include eligibility assessment, medical history, performance status evaluation, vital sign measurements, physical examinations, hematology and serum chemistries.

At the NYU Perlmutter Cancer Center, all investigator-initiated protocols are subject to a standardized data and safety monitoring, which includes scientific peer review, IRB review, Phase I/II committee review and DSMC review as well as internal auditing.
The review of AEs and trial conduct for this trial occurs at several levels:

1. Principal Investigator: Adverse events are evaluated monthly by the principal investigator in conjunction with the research nurses, data manager and research team.
2. DSMC, quarterly
3. IRB: An annual report to the IRB is submitted by the trial PI for continuation of the protocol. It includes a summary of all AEs, total enrollment with demographics, protocol violations, and current status of subjects as well as available research data.
4. In addition, the quality assurance unit will provide extensive monitoring, including real-time review of all eCRFs to ensure completeness and compliance with the protocol, with 100% source documentation verification; the completeness, accuracy and consistency of the data; and adherence to ICH Good Clinical Practice guidelines. Additionally, a first subject audit will be conducted within four weeks of enrollment.

Any overdose of a study subject with, with or without associated AEs/SAEs, is required to be reported within 24 hours of knowledge of the event to the sponsor and BMS Patient Safety.

11.0 Data Handling and Record Keeping

11.1 Data Collection

The NYU Clinical Trials Office will act as the clinical research organization for this Investigator-Initiated Trial (Jennifer Wu, PI). We anticipate accrual to take place in four centers: Perlmutter Cancer Center, NYU (Jennifer Wu, PI); Memorial Sloan Kettering Cancer Center (Geoffrey Ku, PI); USC Norris Cancer Center (Syma Iqbal, PI) and Oregon Health Sciences University (Charles Thomas, PI).

11.2 Confidentiality

All samples will be stored in a secure, locked room in the laboratory of Dr. Geoffrey Ku at MSKCC and Center for Biospecimen Research & Development at NYU:

Geoffrey Y. Ku, MD
Immune Monitoring Facility, Memorial Sloan Kettering Cancer Center
Zuckerman Research Center, 15th floor
417 East 68th Street
New York, NY 10065
Telephone: (646) 888-4588

Center for Biospecimen Research & Development

NYU Langone Medical Center: Medical Science Building
550 First Avenue, Berg 3rd Fl., Rm. 381
New York, NY 10016

Samples will be identified by a unique identifier. The patient's name will not be used on the sample. This unique identifier will be put into the sponsor database and will link in the sponsor database only to de-identified patient data (subject number). Personal health information (PHI) will not be identifiable in the sponsor database or on the stored samples. Besides protecting confidentiality, this system will allow the sponsor to destroy the sample if the subject requests this. Samples will be retained and used until they are exhausted. The log to PHI will be maintained at each individual study site. Information will be stored in a secure, locked room and in a secure, password-protected database. Only approved study personnel will have access to the samples.

11.3 Confidentiality and HIPAA

Information about study subjects will be kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act of 1996 (HIPAA). Those regulations require a signed subject authorization informing the subject of the following:

- What protected health information (PHI) will be collected from subjects in this study
- Who will have access to that information and why
- Who will use or disclose that information
- The rights of a research subject to revoke their authorization for use of their PHI.

In the event that a subject revokes authorization to collect or use PHI, the investigator, by regulation, retains the ability to use all information collected prior to the revocation of subject authorization. For subjects that have revoked authorization to collect or use PHI, attempts should be made to obtain permission to collect at least vital status (i.e. that the subject is alive) at the end of their scheduled study period.

11.4 Source Documents

Source data is all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents. Examples of these original documents, and data records include: hospital records, clinical and office charts, laboratory notes, memoranda, subjects' diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, x-rays, subject files, and records kept at the pharmacy, at the laboratories, and at medico-technical departments involved in the clinical trial. Source documentation should be consistent with data entered into Velos. Relevant source documentation to be reviewed by the DSMC throughout the study includes:

1. Baseline measures to assess pre-protocol disease status
2. Concurrent medications
3. Treatment records
4. Adverse events

11.5 Data and Source Documentation

Velos, an electronic database capture system will be created to record the data for this trial. Research coordinators will input clinical trial data into the database. This database is password protected and only the PI, assigned research coordinator, and CTO quality assurance specialists will have access to the database. Velos is the primary data collection instrument for the study. All data requested in Velos must be reported. All missing data must be explained. The quality assurance specialists will monitor this trial every 4-6 weeks for data entry accuracy.

11.6 Records Retention

It is the investigator's responsibility to retain study essential documents for at least 2 years after the last approval of a marketing application in their country and until there are no pending or contemplated marketing applications in their country or at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational product. These documents should be retained for a longer period if required by an agreement with the sponsor. In such an instance, it is the responsibility of the sponsor to inform the investigator/institution as to when these documents no longer need to be retained.

12.0 Study Monitoring, Auditing, and Inspecting

12.1 Study Monitoring Plan

This study will be monitored according to the monitoring plan. The investigator will allocate adequate time for such monitoring activities. The Investigator will also ensure that the monitor or other compliance or quality assurance reviewer is given access to all the above noted study-related documents and study related facilities (e.g. pharmacy, diagnostic laboratory, etc.), and has adequate space to conduct the monitoring visit.

12.2 Auditing and Inspecting

The investigator will permit study-related monitoring, audits, and inspections by the IRB/EC, the sponsor, government regulatory bodies, and University compliance and quality assurance groups of all study related documents (e.g. source documents, regulatory documents, data collection instruments, study data etc.). The investigator will ensure the capability for inspections of applicable study-related facilities (e.g. pharmacy, diagnostic laboratory, etc.).

Participation as an investigator in this study implies acceptance of potential inspection by government regulatory authorities and applicable University compliance and quality assurance offices. The investigator will contact the PCC CTO immediately if contacted by a regulatory agency about an inspection at the center.

13.0 Ethical Considerations

This study is to be conducted accordance with applicable US government regulations and international standards of Good Clinical Practice, and applicable institutional research policies and procedures.

This protocol and any amendments will be submitted to a properly constituted Institutional Review Board (IRB) in agreement with local legal prescriptions, for formal approval of the study conduct. The decision of the IRB/EC concerning the conduct of the study will be made in writing to the investigator and a copy of this decision will be provided to the sponsor before commencement of this study. The investigator should provide a list of IRB/EC members and their affiliate to the sponsor.

All subjects for this study will be provided a consent form describing this study and providing sufficient information for subjects to make an informed decision about their participation in this study. This consent form will be submitted with the protocol for review and approval by the IRB/EC for the study. The formal consent of a subject, using the IRB/EC-approved consent form, must be obtained before that subject undergoes any study procedure. The consent form must be signed by the subject or legally acceptable surrogate, and the investigator-designated research professional obtaining the consent.

14.0 Study Finances

14.1 Funding Source

This study is being financed by Bristol-Myers Squibb.

14.2 Conflict of Interest

Any investigator who has a conflict of interest with this study (patent ownership, royalties, or financial gain greater than the minimum allowable by their institution, etc.) must have the conflict reviewed by a properly constituted Conflict of Interest Committee with a Committee-sanctioned conflict management plan that has been reviewed and approved by the study sponsor prior to participation in this study. All NYULMC investigators will follow the applicable University conflict of interest policies.

15.0 Publication Plan

Neither the complete nor any part of the results of the study carried out under this protocol, nor any of the information provided by the sponsor for the purposes of performing the study, will be published or passed on to any third party without the consent of the study sponsor. Any investigator involved with this study is obligated to provide the sponsor with complete test results and all data derived from the study.

The results of the trial may not be published in part or in full without written consent of the PI and Co-PI.

16.0 Potential Risks and Potential Benefits of Study Treatment

16.1 Risks of Study Drugs

16.1.1 Potential Nivolumab Toxicities

Patients Reporting AEs n=334	Any	Grade 3-4
Treatment-related AE	228 (68%)	39 (11.7%)
Fatigue	86 (25.7%)	2 (0.6%)
Pruritis	55 (16.5%)	0
Rash	40 (12%)	1 (0.3%)
Diarrhea	41 (12%)	1 (0.3%)
Nausea	31 (9.3%)	0
Arthralgia	20 (6.0%)	0 (2.8%)
Vitiligo	18 (5.4%)	0
Rash, maculopapular	18 (5.4%)	0
Hypothyroidism	16 (4.8%)	0
Decreased appetite	17 (5.1%)	0
AEs leading to discontinuation	20 (6.0%)	13 (3.9)

From “Efficacy and Safety of Nivolumab for BRAF V600 Mutant and BRAF Wild-Type Advanced Melanoma: A Pooled Analysis of 4 Clinical Trials. JAMA Oncology July 2015 Volume 1 No. 4

Potential Nivolumab Toxicities:

- Immune-Mediated Pneumonitis
- Immune-Mediated Colitis
- Immune-Mediated Hepatitis
- Immune-Mediated Endocrinopathies
- Immune-Mediated Nephritis and Renal Dysfunction
- Immune-Mediated Rash
- Immune-Mediated Encephalitis
- Other Immune-Mediated Adverse Reactions
- Infusion Reactions
- Embryo-Fetal Toxicity

Patients should call or see their healthcare provider right away if they develop any symptoms of the following problems or these symptoms get worse:

Lung problems (pneumonitis). Symptoms of pneumonitis may include:

● new or worsening cough
● chest pain
● shortness of breath

Intestinal problems (colitis) that can lead to tears or holes in the intestine. Signs and symptoms of colitis may include:

● diarrhea (loose stools) or more bowel movements than usual
● blood in the stools or dark, tarry, sticky stools
● severe stomach-area (abdomen) pain or tenderness

Liver problems (hepatitis). Signs and symptoms of hepatitis may include:

● yellowing of the skin or the whites of the eyes
● severe nausea or vomiting
● pain on the right side of the stomach area (abdomen)
● drowsiness
● dark urine (tea colored)
● bleeding or bruising more easily than normal
● feeling less hungry than usual

Hormone gland problems (especially the thyroid, pituitary, adrenal glands, and pancreas). Signs and symptoms that hormone glands are not working properly may include:

● headaches that will not go away or unusual headaches
● hair loss
● feeling cold
● constipation
● voice gets deeper
● excessive thirst or lots of urine
● extreme tiredness
● weight gain or weight loss
● dizziness or fainting
● changes in mood or behavior, such as decreased sex drive, irritability, or forgetfulness

Kidney problems, including nephritis and kidney failure. Signs of kidney problems may include:
● decrease in the amount of urine
● swelling in the ankles
● blood in the urine
● loss of appetite

Skin Problems. *Signs of these problems may include:*

● *rash*
● *skin blistering*
● *itching*
● *ulcers in mouth or other mucous membranes*

Inflammation of the brain (encephalitis). Signs and symptoms of encephalitis may include:

● *headache*
● *fever*
● *tiredness or weakness*
● *confusion*
● *memory problems*
● *sleepiness*
● *seeing or hearing things that are not really there (hallucinations)*
● *seizures*
● *stiff neck*

Problems in other organs. Signs of these problems may include:

● *changes in eyesight*
● *severe muscle weakness*
● *severe or persistent muscle or joint pains*

Getting medical treatment right away may keep these problems from becoming more serious. The patient's healthcare provider will check for these problems during treatment with

nivolumab . Treatment may include corticosteroid or hormone replacement medicines. Treatment with nivolumab may also be delayed or stopped completely if the patient has severe side effects.

What are the possible side effects of nivolumab ?

Nivolumab can cause serious side effects, including:

● **Severe infusion reactions.** The patient should tell the doctor or nurse right away if he/she experiences these symptoms during an infusion of nivolumab :

○ chills or shaking
○ dizziness
○ itching or rash
○ fever
○ flushing
○ feeling like passing out
○ difficulty breathing

● **Complications of stem cell transplant that uses donor stem cells (allogeneic) after treatment with .** These complications can be severe and can lead to death, and will be monitored by the patient's healthcare provider.

The most common side effects of nivolumab when used alone in people with melanoma include:

● feeling tired
● rash
● pain in muscles, bones, and joints
● itchy skin
● diarrhea
● nausea

The most common side effects of nivolumab when used in combination with ipilimumab include:

● feeling tired
● rash
● diarrhea
● nausea
● fever
● vomiting
● shortness of breath

The most common side effects of nivolumab in people with non-small cell lung cancer include:

● feeling tired
● cough
● pain in muscles, bones, and joints
● constipation
● decreased appetite

The most common side effects of nivolumab in people with renal cell carcinoma include:

● feeling tired
● nausea
● shortness of breath
● diarrhea
● pain in muscles, bones, and joints
● constipation
● decreased appetite
● rash
● cough

The most common side effects of in people with classical Hodgkin lymphoma include:

● feeling tired
● diarrhea
● upper respiratory tract infection
● cough
● fever

Management Algorithms

Guidelines for the management of immune related events can be found in the current Investigator Brochure and in the approved USPI in the US. Investigators should decide the appropriate source of AE management for each protocol.

Immuno-oncology (I-O) agents are associated with AEs that can differ in severity and duration than AEs caused by other therapeutic classes. Nivolumab is considered an immuno-oncology agent in this protocol. Early recognition and management of AEs associated with immuno-oncology agents may mitigate severe toxicity. Management algorithms have been developed to assist investigators in assessing and managing the following groups of AEs:

Gastrointestinal, Renal, Pulmonary, Hepatic, Endocrinopathies, Skin, Neurological.

For subjects expected to require more than 4 weeks of corticosteroids or other immunosuppressants to manage an AE, consider recommendations provided in the algorithms. These algorithms are found in the Nivolumab IB [and in Appendix] of this protocol. The guidance provided in these algorithms should not replace the Investigator's medical judgment but should complement it.

Discontinuation Criteria

Treatment should be permanently discontinued for the following:

- Any Grade 2 drug-related uveitis or eye pain or blurred vision that does not respond to topical therapy and does not improve to Grade 1 severity within the re-treatment period OR requires systemic treatment
- Any Grade 3 non-skin, drug-related adverse event lasting > 7 days, with the following exceptions for drug-related laboratory abnormalities, uveitis, pneumonitis, bronchospasm, hypersensitivity reactions, and infusion reactions, and endocrinopathies:
    - Grade 3 drug-related uveitis, pneumonitis, bronchospasm, hypersensitivity reaction, or infusion reaction of any duration requires discontinuation
    - Grade 3 drug-related endocrinopathies adequately controlled with only physiologic hormone replacement do not require discontinuation
    - Grade 3 drug-related laboratory abnormalities do not require treatment discontinuation except those noted below
        - Grade 3 drug-related thrombocytopenia > 7 days or associated with bleeding requires discontinuation
        - Any drug-related liver function test (LFT) abnormality that meets the following criteria require discontinuation:
            - AST or ALT > 8 x ULN
            - Total bilirubin > 5 x ULN
            - Concurrent AST or ALT > 3 x ULN and total bilirubin > 2 x ULN
- Any Grade 4 drug-related adverse event or laboratory abnormality, except for the following events which do not require discontinuation:
    - Isolated Grade 4 amylase or lipase abnormalities that are not associated with symptoms or clinical manifestations of pancreatitis and decrease to < Grade 4 within 1 week of onset.
    - Isolated Grade 4 electrolyte imbalances/abnormalities that are not associated with clinical sequelae and are corrected with supplementation/appropriate management within 72 hours of their onset
    - Grade 4 lymphopenia or leucopenia
    - Grade 4 drug-related endocrinopathy adverse events, such as adrenal insufficiency, ACTH deficiency, hyper- or hypothyroidism, or glucose intolerance, which resolve or are adequately controlled with physiologic hormone replacement (corticosteroids, thyroid hormones) or glucose-controlling agents, respectively, may not require discontinuation after discussion with and approval from the Investigator [as allowed by protocol]
- Any dosing interruption lasting > 6 weeks with the following exceptions:

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

- Any adverse event, laboratory abnormality, or intercurrent illness which, in the judgment of the Investigator, presents a substantial clinical risk to the subject with continued nivolumab dosing

Treatment of Nivolumab Related Infusion Reactions

Since nivolumab contains only human immunoglobulin protein sequences, it is unlikely to be immunogenic and induce infusion or hypersensitivity reactions. However, if such a reaction were to occur, it might manifest with fever, chills, rigors, headache, rash, pruritis, arthralgias, hypo- or hypertension, bronchospasm, or other symptoms of allergic-like reactions.

All Grade 3 or 4 infusion reactions should be reported as an SAE if criteria are met. Infusion reactions should be graded according to NCI CTCAE (version 4.03) guidelines.

Treatment recommendations are provided below and may be modified based on local treatment standards and guidelines as appropriate:

For Grade 1 symptoms: (Mild reaction; infusion interruption not indicated; intervention not indicated)

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

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

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

be administered at least 30 minutes before additional nivolumab administrations. If necessary, corticosteroids (recommended dose: up to 25 mg of IV hydrocortisone or equivalent) may be used.

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

Immediately discontinue infusion of nivolumab. Begin an IV infusion of normal saline, and treat the subject as follows. Recommend bronchodilators, epinephrine 0.2 to 1 mg of a 1:1,000 solution for subcutaneous administration or 0.1 to 0.25 mg of a 1:10,000 solution injected slowly for IV administration, and/or diphenhydramine 50 mg IV with methylprednisolone 100 mg IV (or equivalent), as needed. Subject should be monitored until the investigator is comfortable that the symptoms will not recur. Nivolumab will be permanently discontinued. Investigators should follow their institutional guidelines for the treatment of anaphylaxis. Remain at bedside and monitor subject until recovery from symptoms.

In the case of late-occurring hypersensitivity symptoms (e.g., appearance of a localized or generalized pruritis within 1 week after treatment), symptomatic treatment may be given (e.g., oral antihistamine, or corticosteroids).

16.1.2 Potential Carboplatin Toxicities

Potential Adverse Reactions:

- Hematologic Toxicity
  - Thrombocytopenia
  - Neutropenia
  - Leukopenia
  - Anemia
  - Infection
  - Bleeding
  - Need for Blood Transfusion
- Gastrointestinal Toxicity
  - Nausea
  - Vomiting
  - Mucositis
  - Diarrhea
  - Constipation
- Neurologic Toxicity
  - Peripheral Neuropathy
  - Ototoxicity

- Central Nervous System Toxicity

- Renal Toxicity
  - Nephrotoxicity
  - Elevated Serum Creatinine
  - Elevated Blood Urea

- Hepatic Toxicity
  - Elevated Bilirubin
  - Elevated SGOT/AST
  - Elevated Alkaline Phosphatase

- Electrolyte Imbalance
  - Hyponatremia
  - Hypokalemia
  - Hypocalcemia
  - Hypomagnesemia

- Other Side Effects
  - Allergic Reaction
  - Respiratory Toxicity
  - Injection Site Reactions
  - Cardiovascular Toxicity
    - Cardiac Failure
    - Embolism
    - Hypertension
    - Cerebrovascular Accident
  - Cancer-Associated Hemolytic Uremic Syndrome
  - Pain
  - Asthenia
  - Malaise
  - Anorexia
  - Alopecia

16.1.3 Potential Paclitaxel Toxicities

Adverse Events of any grade during chemoradiotherapy n=171	No patients (%)
Anorexia	51 (30)
Alopecia	25 (15)
Constipation	47 (27)
Diarrhea	30 (27)

Esophageal perforation	1 (1)
Esopghagitis	32 (19)
Fatigue	115 (67)
Nausea	91 (53)
Neurotoxic effects	25 (15)
Vomiting	43 (25)
Leukopenia	103 (60)
Neutropenia	16 (9)
Thrombocytopenia	92 (54)

Potential Adverse Reactions:

- Bone Marrow Suppression
- Neutropenia
- Leukopenia
- Thrombocytopenia
- Anemia
- Infection
- Fever
- Bleeding
- Need for Red Cell and Platelet Transfusions
- Peripheral Neuropathy
- Autonomic Neuropathy
- Parasthesia
- Hyperesthesia
- Arthralgia
- Myalgia
- Nausea
- Vomiting
- Diarrhea
- Mucositis
- Alopecia
- Bradycardia
- Hypotension
- Elevated Bilirubin
- Elevated Alkaline Phosphatase
- Elevated AST/SGOT
- Anaphylaxis and Hypersensitivity Reactions
- Dyspnea
- Angioedema
- Urticaria
- Pneumonia
- Lung Fibrosis
- Pulmonary Embolism

- Pleural Effusion
- Respiratory Failure
- Severe Cardiac Conduction Abnormalities
- Edema
- Optic Nerve and/or Visual Disturbances (Scintillating Scotoma)
- Conjuctivitis
- Photopsia
- Visual Floaters
- Increased Lacrimation
- Seizures
- Syncope
- Ataxia
- Neuroencephalopathy
- Nail Changes
- Increased Myelotoxicity
- Paralytic Ileus
- Intestinal Obstruction
- Intestinal Perforation
- Pancreatitis
- Ischemic Colitis
- Dehydration
- Esophagitis
- Constipation
- Ascites
- Neutropenic Enterocolitis (Typhlitis)
- Infusion Site Reactions
- Stevens-Johnson Syndrome
- Toxic Dermal Necrolysis
- Swelling, Thickening and Sclerosis of the Skin at Injection Site
- Erythema
- Tenderness
- Skin Discolorations
- Phlebitis
- Induration
- Cellulitis
- Skin Exfoliation
- Necrosis
- Elevated Creatinine
- Renal Insufficiency
- Asthenia
- Anorexia
- Confusion
- Vertigo

What are the possible side effects of paclitaxel?

Patients should tell their healthcare provider right away if they have:

- severe stomach pain
- severe diarrhea

The most common side effects of Paclitaxel (those which occur in >10% of patients) include:

- low red blood cell count (anemia) feeling weak or tired
- hair loss
- numbness, tingling, or burning in the hands or feet (neuropathy)
- joint and muscle pain
- nausea and vomiting
- hypersensitivity reaction - trouble breathing; sudden swelling of the face, lips, tongue, throat, or trouble swallowing; hives (raised bumps) or rash
- diarrhea
- mouth or lip sores (mucositis)
- infections - if the patient has a fever (temperature above 100.4°F) or other sign of infection, he/she should tell their healthcare provider right away
- swelling of the hands, face, or feet
- bleeding events
- irritation at the injection site
- low blood pressure (hypotension)

16.2 Risks of Radiation Therapy

Potential adverse effects may include:

- Fatigue
- Nausea
- Vomiting
- Loss of Appetite

- Dysphagia
- Odynophagia
- Weight Loss
- Skin changes in the area being treated which can range from mild redness and soreness to blistering and peeling

16.3 Risks of Biopsy

Potential adverse effects may include:

- Bleeding
- Infection
- Fever
- Tearing/Perforation of the Esophagus
- Chest Pain
- Severe or Persistent Abdominal Pain
- Vomiting
- Black or Very Dark Colored Stool

16.4 Risks of Surgery

Short-term risks of esophagectomy include reactions to anesthesia, excess bleeding, blood clots in the lungs or elsewhere, and infections. Most people will have at least some pain after the operation, which can usually be helped with pain medicines.

Lung complications are common. Pneumonia may develop, leading to a longer hospital stay, and sometimes even death.

Some people might have voice changes after the surgery.

There may be a leak at the place where the stomach (or intestine) is connected to the esophagus, which might require another operation to fix.

Strictures (narrowing) can form where the esophagus is surgically connected to the stomach, which can cause problems swallowing for some patients. To relieve this symptom, these strictures can be expanded during an upper endoscopy procedure.

After the operation, the stomach may empty too slowly because the nerves that control its contractions can be affected by surgery. This can sometimes lead to frequent nausea and vomiting.

After surgery, bile and stomach contents can enter the esophagus because the muscle that normally controls this (the lower esophageal sphincter) is often removed or changed by the

surgery. This can cause symptoms such as heartburn. Sometimes antacids or motility drugs can help relieve these symptoms.

Some complications from this surgery can be life threatening.

16.5 Other Risks of Study Participation

16.5.1 Side Effects of Having Blood Taken

- Fainting or feeling faint
- Redness, pain, bruising, bleeding or infection at the needle site

16.5.2 Radiation Risks

During this study, patients will be exposed to radiation from imaging tests and radiation therapy. The risk from this amount of radiation is less than the risk from everyday exposure to the sun. The risks of receiving very small doses of radiation are thought to be low. These risks are not actually known. The use of radiation may involve a low risk of cancer. Pregnant women cannot be exposed to radiation. Women must have a negative pregnancy test before they can enter the study.

16.5.3 Electrocardiogram (ECG) Risks

The sticky pads (electrodes) that are placed on the patient's chest can sometimes cause discomfort, such as redness or itching. Shaving of the patient's chest before attaching these pads may be necessary. Irritation from shaving also may occur.

16.5.4 FDG-PET/CT Scan Risks

A small amount of radioactive sugar will be injected (shot) into the patient's blood through a vein in the patient's arm about 1 hour before the scan. The patient should not feel any effects from the sugar as it is a small, safe amount of radioactive material. There is a small risk of allergic reaction to the radioactive material and there is always a slight risk from being exposed to any radiation. The patient may have swelling, soreness, or infection at the injection site. The radioactive material is passed out of the body through the urine within 24 hours after the test is completed.

The patient will need to lie still for up to 1 hour in the PET/CT scanner. The patient will have a brief CT scan that takes about a minute followed by the PET imaging that takes between 25 and 45 minutes. There is a risk that the patient may feel anxious in the scanner due to the confined space. If the patient feels anxious in closed spaces, medication may be prescribed to help the patient relax.

16.5.5 Magnetic Resonance Imaging (MRI) Risks

Fear of Closed Spaces
When the patient is in the MRI scanner he/she will be in a relatively small, closed space. The patient will need to lie still for up to 1 hour inside the MRI scanner. If it is known that closed spaces make the patient anxious, the patient may be able to take medication to help him/her relax. Instruct the patient to tell the study staff or technologist if they become anxious or uncomfortable during the MRI, and the MRI will be stopped right away.

Noise Level
The MRI machine makes loud, tapping sounds. The patient will be given earplugs to help block the amount of noise heard during the MRI.

MRI System Failure
In rare cases, the magnet in the machine can stop working. If this happens, some cooling fluids could leak out and gas can form in the room. The gas is not dangerous. If this happens, the technologist will take the patient out of the room right away.

Body Twitching and Heating
In rare cases, the patient may have muscle twitching and/or tingling and a fever during the MRI. Instruct the patient to tell the technologist right away if he/she has any of these symptoms.

Receiving Contrast Material
The patient may need to have a dye called gadolinium injected (shot) into a vein in his/her arm during the MRI. This dye will make the pictures clearer. The patient may have bleeding, bruising, swelling, soreness or infection at the injection site. In rare cases, the patient may experience nausea and/or headache after this injection. These symptoms usually go away quickly and without medical treatment. Some systemic reaction may occur, such as a metallic taste, nausea, vomiting, and hives. This is usually limited. Very infrequently, there is difficulty in breathing, low blood pressure and dizziness that requires appropriate treatment. Severe reactions where death has occurred are extremely rare. If the patient has allergies, the possibility of reaction is higher than in a patient a without allergies. Instruct the patient to tell the study doctor if he/she has kidney disease or has had a reaction to intravenous contrast previously. Patients who have bad kidney function are at risk for worsening their kidney function. The patient will need a blood test to make sure it is safe for him/her to get the gadolinium and may need pre-medication to decrease the possibility of complications of allergic reaction. If the patient is diabetic, and on oral medication, it is recommended that he/she stop taking the medication for two days after the contrast injection.

Pregnancy
If the patient is pregnant or thinks she might be pregnant, instruct her to tell the study doctor or the study staff. It is not safe for the patient to have the MRI or receive gadolinium if she is pregnant.

Magnetic Field Risk

MRI uses a strong magnetic field to create images of the body. Because of the strong magnetic field, there are risks. These risks are detailed in this section.

One possible risk is burns to the skin. There is an increased risk of burns from devices that conduct electrical energy. These devices can include metallic objects, pulse oximeters, EKG leads, or skin tattoos. These devices can be either in or on the patient in order for a skin burn to occur. The FDA has found that 70% of all reported injuries from MRIs were burns to the skin.

To reduce this risk, all patients who are scanned in this study must complete thorough screening to ensure that no conductive materials are present in or on the patient's body. Additionally, the power limits of the magnet will be adjusted as necessary.

Another possible risk is that a metal object could be pulled into the scanner and hit the patient. The patient could be physically injured as a result.

To reduce this risk, everyone near the magnet will remove all metal from their clothing or pockets when in the scanning environment. The door to the scan room will remain closed during the exam for the patient's safety.

There are no known risks or adverse effects resulting directly from exposure to MRI. However, patients who have a pacemaker or metal objects in their body such as shrapnel or metal in the eye should not have the scan performed. Instruct the patient to inform the technologist or investigators before entering the magnet room if he/she has any question about metal implants or metal fragments in the body.

16.5.6 Unforeseeable Risks

This research study may involve risks that are currently unforeseeable, including death.

16.6 Potential Benefits

It is possible that some patients who receive the study therapies may experience an improvement in their cancer during the study.

Others with squamous cell carcinoma of the esophagus cancer may benefit in the future from what we learn in this study.

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APPENDIX I

TABLE 9: EASTERN COOPERATIVE ONCOLOGY GROUP (ECOG) PERFORMANCE STATUS SCALE

Grade	Description
0	Fully active, able to carry on all pre-disease performance without restriction
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light house work, office work
2	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	Capable of only limited self-care, confined to bed or chair more than 50% of waking hours
4	Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair
5	Dead

Source: *Oken 1982*.

APPENDIX II: SAMPLE PREPARATION AND SHIPMENT INSTRUCTIONS

Ship Samples to:

Geoffrey Y. Ku, MD
Immune Monitoring Facility, Memorial Sloan Kettering Cancer Center
Zuckerman Research Center, 15th floor
417 East 68th Street
New York, NY 10065
Telephone: (646) 888-4588

Isolation and Cryopreservation of Human Peripheral Blood Mononuclear Cells and Plasma from Whole Blood

I. SCOPE

A. This standard operating procedure provides instructions for the isolation and cryopreservation of peripheral blood mononuclear cells (PBMC), as well as the collection and freezing of plasma from human whole blood.

II. GENERAL

A. Human PBMC consists of nucleated white blood cells, including lymphocytes and monocytes, and can be separated from other components of whole blood by density gradient centrifugation.
B. Human PBMCs are widely used in clinical research and can be evaluated by a variety of immune monitoring methods to assess cellular components of immunity after vaccination or immunotherapy.
C. PBMC should be processed from whole blood as soon as possible and cryopreserved in liquid nitrogen to maintain long-term viability and allow downstream (batched) analyses of cellular immunity.
D. The PBMC isolation and plasma collection procedure here applies to whole blood collected in BD vacutainer CPT Cell Preparation Tubes containing heparin as anti-coagulant.

III. ROLES AND RESPONSIBILITIES

A. It is the responsibility of personnel performing this procedure to be familiar with this protocol and perform it in a consistent manner as described herein.
B. Assigned personnel should be proficient in basic sterile technique and tissue culture methods.

IV. SAFETY

A. All human blood-derived samples irrespective of donor status must be considered bio hazardous and handled minimally under Biosafety Level-2 conditions as defined in the CDC/NIH manual, “Biosafety in Microbiological and Biomedical Laboratories”, 5th edition, 2009. Human blood may contain infectious organisms and should be handled according to universal safety policies and procedures.

B. Appropriate personal protective equipment including lab coats or disposable gowns, safety glasses, and disposable gloves should be worn when handling human specimens. Change gloves as frequently as needed to minimize contamination of work areas.
C. Work should be performed in a certified biosafety cabinet. Spray work areas with 70% ethanol and/or 10% bleach to minimize contamination, before and after working.
D. Used pipettes, tips, and tubes exposed to human blood are recommended to be soaked in 10% bleach solution at least 1 hr. prior to discarding in biohazard waste containers. Personnel should wash hands following completion of procedures.

V. DEFINITIONS AND ABBREVIATIONS

A. CFM cell freezing medium
B. CPT Cell Preparation Tubes for PBMC isolation from BD Biosciences
C. ddH2O deionized distilled water
D. DMSO dimethyl sulfoxide
E. FBS fetal bovine serum
F. PBMC peripheral blood mononuclear cells
G. PBS phosphate-buffered saline
H. RBC red blood cells
I. RCF relative centrifugal force (g force)
J. RPM revolutions per minute
K. RT room temperature (18-25°C)
L. TC tissue culture

VI. MATERIALS AND REAGENTS

A. Human whole blood collected in BD Vacutainer CPT tubes with sodium heparin (16x125 mm size, 8 ml draw capacity, BD Biosciences Cat. 362753)
B. Sterile disposable pipettes (25 ml, 10 ml, 5 ml, and 2 ml sizes; BD Falcon Cat. 357525, 357551, 357543, 357507 or equivalent)
C. Sterile 15-ml polypropylene conical tubes (BD Falcon, Cat. 352096, or equivalent)
D. Sterile disposable 50-ml centrifuge tubes (Fisher Scientific, Cat. 06-443-19, or equivalent)
E. Corning polypropylene 2.0-ml cryogenic vials (sterile, self-standing, silicone washer, internal thread, Fisher Scientific Cat. 03-374-21, Corning Ref #430488, or equivalent)
F. Cryolabels for laser printers (GA International, Cat. CL-9T1)
G. Corning sterile universal fit pipette tips, size 1-200 µl (Fisher Scientific Cat. 07-200-301, Corning Ref #4864, or equivalent)
H. Dulbecco's Phosphate Buffered Saline without magnesium or calcium (1X DPBS, Cellgro Cat. 21-031-CM, or equivalent), stored at 4°C.
I. Heat inactivated fetal bovine serum (Gemini Bioproducts, Cat. 100-106, sterile, triple 0.1µm filtered)
J. Cell wash buffer (D-PBS containing 10% FBS)

- Prepare by adding 100 ml heat-inactivated FBS into 900 ml D-PBS. Store at 4°C, this can be used for up to 1 month after preparation

K. Dimethyl sulfoxide (DMSO, ACS spectrophotometric grade, Sigma Aldrich Cat. 154938, or equivalent)
L. Cell freezing medium (FBS containing 10% DMSO)

- Prepare by adding 10 ml DMSO into 90 ml heat-inactivated FBS. Store at

4°C in the dark, this can be used for up to 1 month after preparation
M. Distilled, deionized water (ddH2O) (Cellgro, Cat. 25-055-CM, or equivalent)
N. Cryogenic vial storage boxes (10 x 10), Taylor Wharton (Cat. R24K-9C44, Fisher Scientific Cat. 50-902-0656, or equivalent)
O. CoolCell LX-1 freezing containers, 12- and 30-vial capacities (Fisher Scientific, Cat. 13900856 and 13900679) or equivalent (e.g., NALGENE Mr. Frosty Cryo
1°C Freezing Container)
P. Ethanol (95%, Decon Labs Cat. 2801, Fisher Scientific Cat. 04-355-226)
Q. Bleach diluted to 10% in distilled water (Clorox germicidal bleach, VWR, Cat. 21899-504, or equivalent)
R. Powder-free nitrile gloves (Fisher Scientific, Cat. 19-130-1597, or equivalent)
S. Non-sterile cotton gauze sponges, 4” x 4” 12 ply (Fisher Scientific, Cat. 13-761-52, or equivalent)

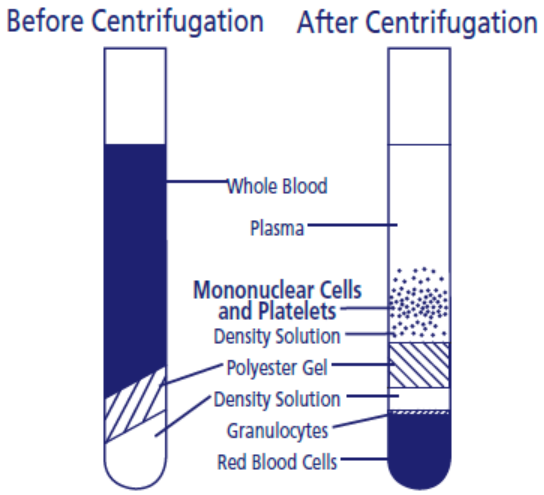
VII. EQUIPMENT
A. Certified biological laminar flow safety cabinet rated at BSL-2A
B. Refrigerated centrifuge (Allegra 6KR with swinging bucket rotor GH3.8 and appropriate adapters for conical tubes, Beckman Coulter, or equivalent)
C. Multipurpose lab rotator (Thermo Scientific Model #2314, Fisher Scientific Cat. 2314-1CEQ)
D. Single channel micropipetters (Finnpipette, Cat. 21-377-816, 21-377-818, Fisher Scientific, or equivalent)
E. Muse™ Cell Analyzer with Count & Viability Kit (Cat. 0500-3115, MCH100102, EMD Millipore) for counting cells and measuring viability using 2 DNA-binding dyes
F. Inverted microscope with 10X magnification (Eclipse TS100, Nikon, or equivalent), hemacytometer (BrightLine Hemacytometer, Cat. 15170-168, Hausser Scientific, or equivalent), and hand tally counter (Cat. 23609-102, VWR, or equivalent) for counting cells (if automated counting instrument unavailable)

VIII. PROCEDURE
The following procedures are conducted in a biosafety hood except for centrifugation. The BD Vacutainer® CPT Cell Preparation Tube with Sodium Heparin combines a blood collection tube containing a sodium heparin anticoagulant with a FICOLL Hypaque density fluid and a polyester gel barrier which separates the two liquids. The result is a convenient, single tube system for the collection of whole blood and the separation of mononuclear cells.

NOTE: After blood collection, store CPT tubes (gently rocking on a plate rotator) at room temperature until centrifugation. Blood samples should be centrifuged within 2-4 hours of blood collection for best results. For this procedure, generally 4 CPT tubes or ~32 ml of blood (for PBMC and plasma) are collected per subject.

A. Sample database entry
1. For each sample, note the time and date of blood draw indicated on the sample requisition form for each sample, record time & date of receipt in the core lab, date of actual processing, patient ID number, patient initials, patient visit time point, study protocol number, items

received (number of CPT tubes and red top tubes), and processor's initials in both **laboratory notebook/records/sample worksheet and on original requisition form.**
2. Log the sample into **electronic clinical database,** including date and time of collection, date and time of processing, and items received. Generate and print labels for cryovials and sample processing tubes.
B. Density gradient centrifugation of whole blood in CPT tubes
1. Remix the blood samples immediately prior to centrifugation by gently inverting the tubes 8 to 10 times.
2. Carefully balance and centrifuge CPT tubes at 2500 rpm (~1500 x g),
room temperature, **no brakes,** for 20 minutes. **NOTE**: Ensure that the tubes are in the proper centrifuge carrier/adapter and are balanced. Excessive centrifuge speed (over 2000 RCF) may cause tube breakage and exposure to blood and possible injury.
3. During centrifugation, print barcoded sample ID labels for the vials into
which plasma samples will be distributed. These labels will provide information regarding date of sample processing, study protocol number, patient ID, and study time point information.
4. Place barcoded sample ID labels vertically (e.g., top to bottom of tube) onto the 6 cryovials into which the plasma will be later distributed.
5. Aliquot 50 ml of cell wash buffer into one 50-ml conical for each sample and label each with sample ID label. This wash buffer will be used for all subsequent washes of PBMC samples (~50 ml buffer per sample).
6. Place sample ID label on one 15 ml conical tube per sample for collection of PBMC monolayer from centrifuged CPT tubes.
7. Place sample ID label on one 15 ml conical tube per sample for pooling of plasma from centrifuged CPT tubes.
NOTE: Labeling of tubes for PBMC and plasma samples with proper sample ID information is important to minimize potential for incorrect identification of samples especially when multiple blood samples from different subjects are being processed simultaneously.
8. Once the centrifuge has come to a complete stop, remove CPT tubes, place in rack, and place tubes and rack in sterile biological safety cabinet. Collection of cells immediately following centrifugation will yield best results. After centrifugation, mononuclear cells and platelets will be in a whitish layer just under the yellow plasma layer, with RBC at the bottom of the tube below the polyester gel.



C. Plasma collection from centrifuged CPT tubes
1. Sterilize the exterior of the centrifuged CPT tubes with 70% ethanol. Wipe the CPT tubes with a clean disposable gauze sponge or Kimwipes.
2. Carefully open the caps of the CPT tubes and pipet a final total of at least 9 ml of the yellow plasma layer collectively from all tubes per sample into the appropriate sample ID-labeled 15-ml conical tube using 10-ml serological pipet without disturbing the cell layer.
3. Remove as much as possible and discard any remaining plasma from CPT tubes above the PBMC monolayer without disturbing the PBMC monolayer.
4. Pooled plasma from all blood collection tubes collected into the 15-ml conical should then distributed into **6** x 1.5 ml aliquots in the 6 labeled cryovials set aside for each sample. If collecting from >6 CPT tubes, distribute plasma into **12** x 1.5 ml aliquots instead.
5. Store the plasma aliquots per sample in the appropriate protocol boxes in -20°C freezer, recording location of vials on the requisition form. Record volume of plasma collected (per vial and total).

D. PBMC isolation from centrifuged CPT tubes
1. After plasma collection, carefully collect the white PBMC layer from the CPT tubes using a 10-ml serological pipet or smaller sterile 1-2 ml transfer pipet (being careful not to touch the gel barrier of the CPT tube) and place into appropriately labeled 15-ml conical tube set aside earlier. Add cell wash buffer (PBS containing 10% FBS from 50-ml conical tube set aside above) to bring volume in each tube up to 15 ml.
2. Centrifuge tube containing collected PBMC @ 1350 rpm (~400 x g) at 4°C, brakes on, for 15 minutes.
3. Pour off wash buffer supernatant from tube.
4. Resuspend cells into a total of 15 ml fresh wash buffer (PBS/10% FBS) per tube, and centrifuge @ 1150 rpm (~300 x g), 4°C, brakes on, for 10 minutes.
5. After spin is completed for 2nd wash of cells, pour off wash supernatant and resuspend cells in 10 ml fresh wash buffer. Keep cell suspension cold on ice.
6. With sterile micropipet tip, remove 10 µl of the resulting cell suspension and dilute in the appropriate buffer to the proper concentration for counting cells using automated counting instrument or hemacytometer.

7. Count cells (taking into account dilution factor) and record viability. Calculate the number of cryovials of cells to be frozen based on a final cell concentration of **6-8x10^6 viable cells/ml/vial**.
NOTE: It may be necessary to refer to specific study protocol for appropriate cell concentration to be used for freezing.
8. After counting, centrifuge the cell sample sitting on ice in 15-ml conical at 1050 rpm (~250 x g), 4°C, brakes on, for 10 minutes.
9. While cells spin down, enter post-processing sample information into electronic clinical database and sample requisition form (including cell yields, concentration, viability) and generate freezing labels for cryovials. Affix barcoded sample labels vertically (e.g., top to bottom) on the appropriate number of cryovials (based on cell yield and target cell concentration).
10. After centrifugation, pour off supernatant, invert conical tube and tap gently on gauze to remove excess wash buffer.
11. Resuspend cells at the target concentration of 6-8x106 viable cells/ml (up to a maximum of 1.5x107/ml for samples with excessive cell numbers) in ice-cold cell freezing media (FBS containing10% DMSO) and aliquot 1 ml into labeled cryovials for each sample.
NOTE: If total cell counts for a sample are low and are between 6-12x10^6 cells, distribute cells equally into 2 individual cryovials instead of one, noting final cell volume per vial. For total cell counts below 6x106 cells, freeze in only 1 cryovial per sample to preserve cell viability upon thaw.
12. Place cryovials in CoolCell -1°C/min controlled rate freezing container (stored at room temperature), making sure all remaining empty slots in vials' containing 1 ml/vial of freezing medium (no cells).
13. Place CoolCell container in -80°C Revco #3 for **24-72 hours (generally one business day)**.
14. After 24-72 hours (next business day), transfer frozen PBMC vials from CoolCell container to cryobox in -80°C freezer to await transfer to liquid nitrogen storage (or frozen shipment to central lab if liquid nitrogen storage is not possible) within one week of initial freezing of PBMC.
NOTE: Some study protocols may require the transfer of frozen PBMC from -80°C to liquid nitrogen within as soon as 24 hrs. so be sure to verify requirements for each particular study protocol.
15. When samples have been transferred to liquid nitrogen, record location information including liquid nitrogen tank, rack, slot, box, and position numbers in clinical database for tracking of frozen PBMC specimens.

IX. REFERENCES

- BD Vacutainer CPT Cell Preparation Tube product insert
- CoolCell freezing container product brochure

X. ADDITIONAL RESOURCES

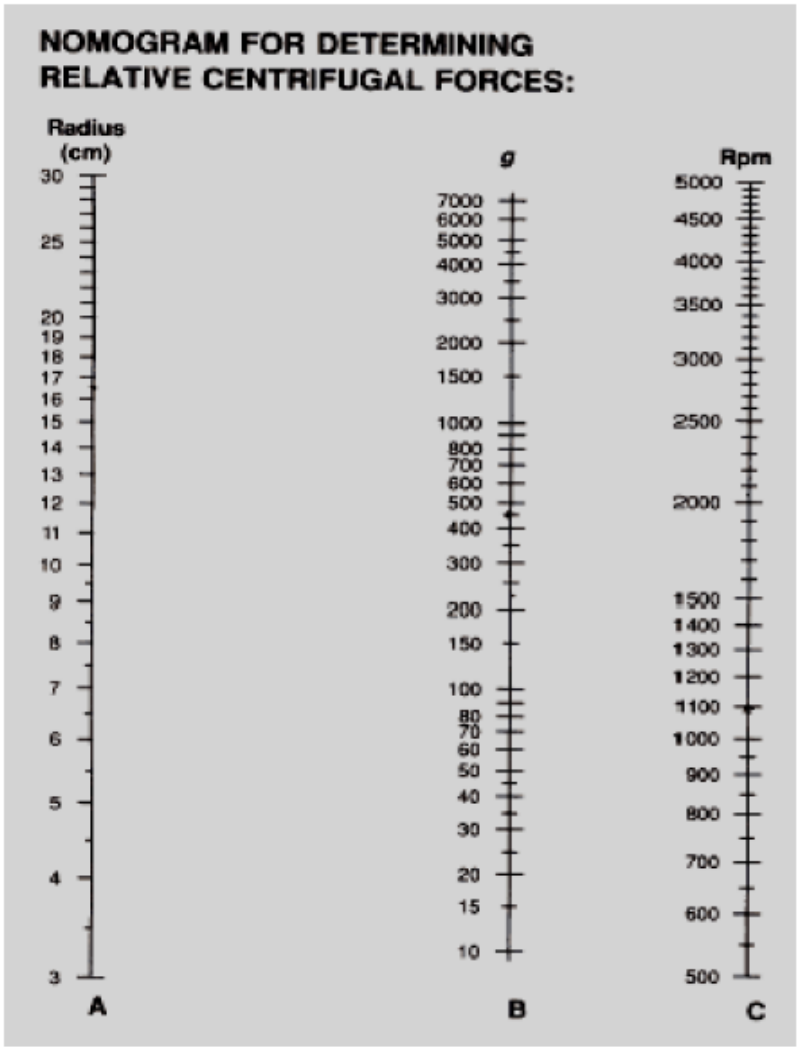
A. Nomogram for converting g force (RCF) to RPM
B. Isolation and Cryopreservation of Human Peripheral Blood Mononuclear Cells and Plasma from Whole Blood – Mini Protocol Worksheet

* * * * *

A. Nomogram for converting g force (RCF) to RPM

- rcf = 11.18 x r x $(rpm/1000)^2$

where rcf is the relative centrifugal force (in g), r is the radius of the rotor in centimeters (from center of rotation axis to bottom of test tube), and rpm is the speed of the centrifuge in revolutions per minute.



B. Isolation and Cryopreservation of Human Peripheral Blood Mononuclear Cells and Plasma from Whole Blood – Mini Protocol Worksheet

Record information for each sample:

Study Protocol	Patient Initials	Patient ID #	Time point	Date of Sample Dropoff at IMF (DD-MMM-YYYY)	Date of Sample Processing (if diff from Sample Dropoff Date)

Hrs Elapsed Post Blood Draw to PBMC Processing	___hrs or O/N stored	# of CPT Tubes			

Check off boxes when steps are completed

Density gradient centrifugation of whole blood in CPT tubes

☐Gently invert CPT tubes 8 to 10 times and centrifuge at **2500rpm (~1500 x g), room temperature, no brakes, 20 min.**

☐During centrifugation, print sample ID labels for plasma samples with information below, and label 6 cryovials for plasma:

Protocol # - Patient ID # - Patient Initials - Time Point - Date of Collection
Sample Type (e.g., Plasma) - # of vial out of total (e.g. #1 of 6)

☐Aliquot 50 ml of cell wash buffer (PBS + 10% FBS) into conical tube.

☐Place sample ID label on one 15 ml tube for collection of PBMC.

Plasma collection from centrifuged CPT tubes

☐Sterilize exterior of centrifuged CPT tubes, carefully open the caps, and pipette total of 9 ml of the yellow plasma layer collectively from all tubes per sample into the labeled 15-ml conical tube. Do not disturb cell layer!

☐Discard any remaining plasma from CPT tubes above the PBMC monolayer. Do not disturb PBMC monolayer!

☐Distribute pooled plasma as 6 x 1.5 ml aliquots in labeled cryovials, store at -20°C in appropriate protocol box, and record location of vials.

PBMC isolation from centrifuged CPT tubes

☐After plasma collection, carefully collect the white PBMC layer from CPT tube and place into labeled 15-ml conical tube.

☐Add cell wash buffer into tube to reach 15 ml total, and centrifuge tube **@ 1350 rpm (~400 x g) at 4°C, brakes on, 15 min.**

☐Pour off wash buffer supernatant from tube and resuspend cells into a total of 15 ml fresh cell wash buffer.

☐Centrifuge **@ 1150 rpm (~300 x g), 4°C, brakes on, for 10 min.**

☐Pour off wash buffer supernatant from tube and resuspend cells into a total of 10 ml of fresh cell wash buffer, place on ice.

☐Count and record total live cell yield & viability. Calculate number of cryovials of cells to be frozen based on a target cell concentration of **6-8x10^6 viable cells/ml/vial**. **keep sample cold while counting**

NOTE: If total cell counts for a sample are between 6-12x10^6 cells, distribute cells equally into 2 individual cryovials instead of one. For total cell counts below 6x10^6 cells, freeze in only 1 cryovial per sample.

☐After counting, centrifuge PBMC sample at **1050 rpm (~250 x g), 4°C, brakes on, for 10 min.**

☐While cells spin down, enter cell yields, concentration, viability, number of tubes, vol per tube into clinical sample database and generate freezing labels for cryovials using format above.

☐After centrifugation, pour off supernatant and resuspend cells at the target concentration of 6-8x10^6/ml in ice-cold cell freezing media (FBS + 10% DMSO).

NOTE: If total cell counts for a sample are between 6-12x10^6 cells, distribute cells equally into 2 individual cryovials instead of one. For total cell counts below 6x10^6 cells, freeze in only 1 cryovial per sample. Samples exceeding 8x10^7 viable cells should be frozen at up to 1-1.5x10^7 cells/ml/vial in a maximum of 10 vials (discard remainder unless otherwise instructed).

☐Aliquot 1 ml into labeled cryovials for each sample (or <1 ml if cell yields are low as described above).

☐Place cryovials in CoolCell controlled rate freezing container and place CoolCell container in -80°C for 24-72 hours.

☐After 24-72 hours transfer frozen PBMC vials into cryobox in -80°C to await transfer to liquid N_2 (or shipment to central lab if no liquid N_2 freezer) within <u>one week</u> of initial freezing of PBMC.

Analyst _____ Date _____