EProst # 20150932

NCT# **NCT02636725**

A Phase II Trial of Concurrent Axitinib and Pembrolizumab in Subjects with Advanced Alveolar Soft Part Sarcoma (ASPS) and Other Soft Tissue Sarcomas (STS)

VERSION #:	7.0
VERSION DATE:	15 April, 2020

PRINCIPAL INVESTIGATOR: Jonathan C. Trent, MD
Professor of Medicine
Medicine/ Hematology Oncology

CONTACT INFORMATION

PRINCIPAL INVESTIGATOR:	Jonathan C. Trent, MD, PhD Professor of Medicine Medicine/ Hematology Oncology [redacted]
STATISTICIAN:	Deukwoo Kwon, PhD Associate Scientist Biostatistics and Bioinformatics Core [redacted] Email: dkwon@med.miami.edu
COLLABORATOR(S):	Krishna Komanduri, PhD Hematology/Oncology [redacted] Ty Subhawong, MD Radiology [redacted] Efrosyni Sfakianaki, MD Radiology/Nuclear Medicine [redacted] Eric Wieder, PhD Hematology/Oncology [redacted]
CLINICAL TRIAL MANAGEMENT	Clinical Research Services (CRS) Fox Cancer Center, [redacted]

	[redacted]
FUNDING SOURCE(S):	Merck Sylvester Comprehensive Cancer Center (SCCC) Clinical Trials Acceleration Grant

Regulatory Sponsor	**Sylvester Comprehensive Cancer Center (SCCC)** **PI: Jonathan C. Trent, MD**
Investigational Agent(s)	Axitinib (Inlyta®) – provided by Pfizer Pembrolizumab (Keytruda®) – provided by Merck
Other Agent(s)	**N/A**
IND Status	IND#: **128722** **IND Sponsor/Investigator:** Jonathan C. Trent, MD Professor of Medicine Medicine/ Hematology Oncology [redacted]

INVESTIGATOR AGREEMENT

I confirm that I have read this protocol, I understand it, and I will work according to this protocol and to the ethical principles stated in the latest version of the Declaration of Helsinki, the applicable ICH guidelines for good clinical practices, and the applicable federal, state, and local laws, rules, and regulations relating to the conduct of the protocol.

I have read and understand the information in the Investigators' Brochure (and/or other such pertinent safety information) regarding the risks and potential benefits.

I agree to inform all those who assist/collaborate with me in the conduct of this study of their responsibilities and obligations.

Once the protocol has been reviewed and approved by the Institutional Review Board (IRB) I understand that any change(s) made during the course of the study must also (first) be approved by the IRB prior to implementation, except when such modification is made to remove any immediate hazard(s) to the subject(s).

I certify that I and the study staff responsible, have received the requisite training to conduct this research protocol.

I agree to maintain adequate and accurate records in accordance with the University of Miami policies, federal, state and local laws and regulations.

I agree to maintain the confidentiality of all information received and/or developed in connection with this protocol.

eProst Number:	
Protocol Version Number:	Protocol Version Date:

Signature of Investigator:	Date:
Name of Investigator (printed):	Institution:

PROTOCOL REVISION HISTORY

Version #	Summary of Changes	Version Date

1.1	**(Pre-Approval Changes)**
	• Administrative, editorial and formatting changes:
	  o Clarification provided in Note under Figure 3.
	  o Labels for Figure(s) 2, 3 and 4 corrected.
	• Secondary Objectives and corresponding Endpoints modified to include clinical benefit rate (CBR), objective response rate (ORR) and progression-free survival (PFS); removing time to progression (TTP).
	• Section 7.1 Dose Escalation/Dose De-Escalation language modified to incorporate traditional 3+3 design.
	• Stopping Rules using objective response rate (ORR) and clinical benefit rate (CBR)
	• Section 16.0 (Statistical Considerations) language modified to clarify analysis
		02/JUL/2015

1.2	**(Pre-Approval Changes)**
	• Administrative, editorial, numbering and formatting changes
	  o Removed Sub-Investigator (John Goldberg, MD) who is no longer working at the Institution
	  o Labels for Figures and Tables corrected
	• Phase II with safety lead-in
	  o Single-arm survival design; axitinib intrapatient dose titration
	  o Sample size adjusted from 38 patients to 30 patients (see Section 16.4 Patient Enrollment and Sample Size, for justification)
	• Primary Objective modified to determine recommended phase 2 dose (RP2D); originally for progression-free survival (PFS) after 3 months
	• Ordering of Secondary Objectives modified
	• Exploratory Objectives modified and additional (exploratory) objectives added to identify further immunological associations with clinical status.
	• Section 19.0 STUDY MONITORING, modified wording to incorporate applicable monitoring by UM CRORS and link.
	• Addressed Merck comments
	  o Pembrolizumab dosing schedule changed to every 3 weeks
		30/JUL/2015

	○ Inclusion Criterion 6.1.3 clarified (as numbering was changed for administrative reasons 6.1.3 is now Inclusion Criterion #9.) ○ Prohibited concomitant medication information from Merck-provided template is missing partial information ○ Section 5.7.1 (Diet) from Merck-provided template is missing ○ Section 7.2.3 (AST, ALT elevation) from Merck template is missing ○ Section 13.1 reference to Appendix D corrected	
1.3	**(Pre-Approval Changes)** • Section 4.0 Subject Recruitment & Screening ○ Added statement with regards to study recruitment of minors: "The intervention in the study holds out a prospect of direct benefit that is important to the health or well-being of minors and is available only in the context of the research. Thus minors 16 years of age and older are included in the subject population." • Section 5.0 Patient Selection ○ Inclusion Criterion 3: Added "malignant myoepithelioma". ○ Inclusion Criterion 17: Prior history of vasectomy does NOT replace requirement for contraceptive use. ○ Exclusion Criterion 1: Patients may have received prior PD-1/PD-L1 directed therapy. • Section 10.2 Dose Titration Guidelines for Axitinib ○ Management of Axitinib-related Hypertension: Clarified calcium channel blockers (CCB) and beta-blocker examples • Appendix A: Expedited Adverse Event (AE) Reporting Requirements ○ Added Pfizer Expedited AE Reporting Requirements • Appendix F: Biomarker, Correlative and Special Studies (C.) Exploratory Objective 3: Measurement of circulating tumor cells pre- and post-treatment, and correlation with tumor response and time to progression ○ Added statement to Approach (e): "Where appropriate, additional immunocytochemistry will be performed using cell surface vimentin or other marker based on specific histology." • Appendix K: Guidelines for Preparation and Administraton/ Keytruda for Injection (Lyophilized Powder) added	28/SEP/2015
1.4	**PRC Determination Letter dated 30/OCT/2015**	05/NOV/2015

	• Section 3.2 Evaluable Patients clarified for response and toxicity • PFS, OS and TTP will be evaluated from study treatment initiation • The statements incorporating the term "single arm survival design" have been retained/rephrased in the Study Design and Statistical Consideration sections, after discussion with Statistics • Section 17.0 title corrected to Data and Safety Monitoring, Table 13: (last row) modified from 27 to 29 • Section 16.3 Statistical Analysis (Analysis for Exploratory Endpoints) "summarized in endpoints" clarified • Inclusion criteria includes parental consent **and** assent requirement for minors 16 to 18 years of age	
2.0	**Administrative/editorial changes for consistency:** • Consistent language between Protocol Synopsis and body of the protocol • Consistent language between Section 12.0 "Schedule of Clinical and Laboratory Evaluations" and Section 12.7 "Calendar of Clinical and Laboratory Evaluations" • Appendix I: Patient Dosing Diary adjusted for longer cycle and for Cycle 1 **FDA Office of Hematology and Oncology Products Comments** *Email correspondence with FDA Division of Oncology Products 2 Regulatory Project Manager dated 10/DEC/2015* <u>Protocol</u>: • Section 12.0 "Schedule of Clinical and Laboratory Evaluations" o Added weekly assessments of physical exams, performance status, vital signs, weight and labs through the first 2 cycles; o Revised physical exam, performance status, vital signs, weight. every 6 weeks (i.e. Day 1 of each cycle) for cycle 3 and subsequent cycles and then labs every 3 weeks thereafter (i.e. Day 1 and 22); o Added comment that if a patient is dose-escalated (on axitinib) on day 1 of a cycle they should be evaluated at least once, on the week 4 (i.e. Day 22) visit, or as clinically indicated. Main, Minors' Assent and Parents of Minors' Informed Consent Forms (ICFs) Version 2 (dated: 11/DEC/2015):	11/DEC/2015

	• "Alternatives" to study treatment with specific examples provided and more detailed descriptions of known benefits
	• Revised wording on the schedule of Clinical and Laboratory Evaluations (as described above) to maintain consistency
	• Administrative/editorial changes for consistency
	
	Sponsor-Designated (Merck)
	
	Reference Safety Information as provided in Version 10 (date: 31/AUG/2015) of Pembrolizumab Investigator's Brochure (IB)
	
	Section 9.1.4 (Management of Agent-Specific Adverse Events):
	
	• Updated safety information for patients with melanoma based on KEYNOTE-006 (i.e. AEs of Special Interest or AEOSI)
	• "Clinical Trials Experience"
	• "Immune-Related Adverse Reactions (irAEs)"
	• "Identification and Treatment of irAEs"
	• "Other Immune-Mediated Adverse Reactions":
	  o added Guillain-Barré syndrome and vitiligo
	• "Immune-Mediated Pneumonitis"
	  o Updated "pneumonitis" text
	
	Reference Risk Language as provided in Version 1.0 (date: 03/SEP/2015) of Risk Language Template for MK-3475
	
	Main, Minors' Assent and Parents of Minors' Informed Consent Forms (ICFs) Version 2 (dated: 11/DEC/2015):
	
	• Updated pembrolizumab side effects	

3.0	**Additional warnings on Keytruda® added after recommendation from drug sponsor (Merck)** Revisions after letter from sponsor indicated cases of Stevens-Johnson syndrome, Toxic Epidermal Necrolysis and Immune-mediated Myocarditis. Descriptions were added to Section 9.2.4 Management of Agent-Specific Adverse Events and to Section 10.3 Dose Modification Guidelines for Pembrolizumab. Updated study enrollment number to 45 to account for screen failures consented as well as additional patients to replace non-evaluable patients	26APR2017

	Updated windows for follow-up visits and safety visit for consistency throughout protocol Clarification regarding concomitant medication usage. Substrates are allowed, inhibitors and inducers should be avoided	
4.0	**Post Approval Amendment** In response to Merck's Dear Investigator Letter of Nov 28 2017 to update the dose modification table 10 on page 67 Additional changes to this version include: 1. Expansion of clinical trial by 10 additional patients with ASPS only. 2. Addition and clarification of new exploratory endpoints and objectives, including outside collaborators requiring transfer of deidentified samples for analysis 3. Section 1.5, addition of rationale for expansion cohort in ASPS 4. Edited requirements for blood pressure (BP) log monitoring – patients will monitor BP daily during the entire first cycle., For subsequent cycles, if a dose escalation of axitinib is performed, patients will keep BP logs for 3 weeks until their day 22 visit for that cycle. If stable, they do not need to continue to log BP for the duration of the cycle. 5. Section 10.1 - Clarified management of grade 4 toxicities – subjects with grade 4 adverse events are generally required to discontinue all study treatment except in the following conditions - 1) clear evidence that the toxicity was attributable to axitinib alone, resolved upon cessation of axitinib, and there is rationale to continuing pembrolizumab monotherapy OR 2) Grade 4 lab abnormalities that are not immune-related, where the patient is asymptomatic, and the abnormalities can be reversed with medical treatment. Example: grade 4 hypertriglyceridemia that responds to antilipid drugs. 6. Modification of Table 9 - Changed management of axitinib-related elevated liver enzymes or bilirubin.– For grade 3 LFTs, it is no longer required to discontinue axitinib, the drug will be held until LFTs resolve to grade 1 and then dose reduce axitinib. Grade 4 LFTs still requires discontinuation of axitinib. 7. Management of proteinuria – Grade 1 proteinuria (1+) in the ABSENCE of infection or contaminated sample will require microalbuminuria or creatinine clearance at screening, without affecting eligibility. Follow up urinalyses are decreased in frequency to day 1 of each cycle. Repeat microalbumin or creatinine clearance in the absence of infection or contaminated sample, for grade 2 (2+ urine protein) or greater.	20 MAR 2018

	8. Added additional correlative peripheral blood sample collection on cycle 1 day 8 prior to Pembrolizumab dose (expansion patients only). 9. Clarified requirements and timing for imaging studies in setting of treatment delays – if patient gets off schedule, as long as within three weeks, scans should be done at day 1 as normally scheduled based on pembrolizumab dosing. If greater than three weeks of consecutive delays, scans should be done at the 12 week mark. 10. Omitted day 22 telephone calls, patients are seen every visit so this was erroneous. 11. 12 lead EKGs are required on day 22 of each cycle ONLY if axitinib dose escalation was performed on day 1. 12. In table of assessments – deleted erroneous coagulation tests on C2D22, coagulation factors are only to be drawn on day 1 of each cycle), removed D22 urinalysis and clarified requirements for microalbumin/Creatinine clearance as detailed above, EKGs corrected to D22 only if new dose of axitinib begun on day 1, corrected BP log requirements as above, added additional peripheral blood collection on Day 8, omitted day 22 telephone calls. 13. Section 13.0 – additions to the correlative evaluations. Omitted transfer of a core to Dr. Trent lab for RNA analysis (insufficient tissue supplies). . 14. Section 16 – added additional language regarding correlative statistical analysis. Edited sample size calculations for the larger number of patients. 15. Revised references 16. Appendix F section A to reflect new correlative methodology and collaborations, added Figure F2. Miscellaneous other formatting/grammatical corrections.	
5.0	**Change in PI** 1. Replaced Breelyn Wilky, MD as Principal Investigator (PI) with Matteo Trucco, MD. 2. Removed Matteo Trucco, MD as Sub-Investigator. 3. Added contact information for Matteo Trucco, MD. 4. Increased expected enrollment from 35 participants to 45 participants.	15OCT2018
6.0	**Correlative Studies** 1. Section 1.7- added language regarding samples being transferred to University of Colorado per collaboration agreement between University of Miami and University of Colorado. 2. Section 1.6.1 corrected to 1.7.1	

	3. Section 1.6.2 corrected to 1.7.2 4. Section 1.6.3 corrected to 1.7.3	
6.1	**Change in PI** **1. Replaced Matteo Trucco, MD as Principal Investigator (PI)** **2. Removed Jonathan C. Trent, MD, PhD as Sub-Investigator** **3. Added contact information for Jonathan C. Trent, MD, PhD**	18SEP2019
7.0	**1. Second course treatment:** Added details for second course treatment (axitinib monotherapy and pembrolizumab re-introduction) following the completion of the first two years of axitinib/pembrolizumab combination treatment. Changes reflected in sections 8.1 and 8.2 2. Section 8.0 was updated to reflect the length of cycles and schedule for axitinib monotherapy and pembrolizumab re-introduction for patients who progress while on axitinib monotherapy. 3. Section 8.5 and 8.5.1 was updated to reflect axitinib dispensation during axitinib monotherapy and during the re-introduction of pembrolizumab for patients that progress. 4. Schedule of assessments was updated to reflect the assessments that will be performed during axitinib monotherapy (section 12.2.18) and during pembrolizumab re-introduction (section 12.2.19 and 12.2.20) 5. Descriptive superscripts were added to the study calendar in section 12.7. 6. The following warnings from the investigator's brochure (IB) were added to section 9.1.4, Management of Agent-Specific Adverse Events: • Aneurysms and artery dissections • Arterial Thromboembolic Events • Venous Thromboembolic Events • Elevation of Hemoglobin or Hematocrit • Hemorrhage • Gastrointestinal Perforation and Fistula Formation • Wound Healing Complications • Reversible Posterior Leukoencephalopathy Syndrome/Posterior Reversible Encephalopathy Syndrome • Hepatic Impairment	15 APR 2020

TABLE OF CONTENTS

CONTACT INFORMATION 2
INVESTIGATOR AGREEMENT 4
PROTOCOL REVISION HISTORY 5
TABLE OF CONTENTS 12
ABBREVIATIONS & DEFINITIONS 16
PROTOCOL SYNOPSIS 17
PROTOCOL SCHEMA....24
1.0 BACKGROUND AND RATIONALE....25
1.1 Soft Tissue Sarcomas (STS) 25
1.2 PD-1/PD-L1 25
1.3 Rationale for Combination Anti-PD-1 with anti-VEGFR Therapy in Sarcomas 26
1.4 Rationale for Intrapatient Dose Titration of Axitinib 30
1.5 Rationale for Expansion Cohort in Alveolar Soft Part Sarcoma 31
1.6 Hypothesis....31
1.7 Correlative Studies....31
2.0 OBJECTIVES....35
2.1 Primary Objective 35
2.2 Secondary Objectives....35
2.3 Exploratory Objectives 35
3.0 ENDPOINTS 36
3.1 Primary endpoint....36
3.2 Secondary endpoints 37
3.3 Exploratory endpoints 37
4.0 SUBJECT RECRUITMENT & SCREENING 38
5.0 PATIENT SELECTION....38
5.1 Inclusion Criteria....38
5.2 Exclusion Criteria 41
6.0 ENROLLMENT PROCEDURES 43
6.1 Cancellation Guidelines 43
6.2 Emergency Registration....43
6.3 Enrollment Procedure(s) 43

7.0 STUDY DESIGN 43
7.1 Axitinib Dose Titration with Flat Dose of Pembrolizumab 44
7.2 Dose-Limiting Toxicity (DLT) 45
8.0 TREATMENT PLAN 47
8.1 Second Course Axitinib (Inlyta®) 50
8.2 Second Course Pembrolizumab 50
8.3 Axitinib (Inlyta®) 50
8.4 Pembrolizumab (Keytruda®) 50
8.5 Treatment Dispensation, Compliance and Accountability 51
8.6 Supportive Care Guidelines 52
8.7 Concomitant Medications/ Vaccinations 54
8.8 Duration of Treatment 55
8.9 Duration of Follow-Up 55
8.10 Telehealth 55
8.11 Shipping of Investigational Product 55
9.0 AGENTS (DRUG FORMULATION AND PROCUREMENT) 55
9.1 Axitinib 55
9.2 Pembrolizumab 61
10.0 TREATMENT/ DOSE MODIFICATIONS 70
10.1 Unacceptable Toxicity 70
10.2 Dose Titration Guidelines for Axitinib 71
10.3 Dose Modification Guidelines for Pembrolizumab 74
11.0 TREATMENT DISCONTINUATION 79
12.0 SCHEDULE OF CLINICAL & LABORATORY EVALUATIONS 80
12.1 Pre-Treatment Evaluation (Screening) 80
12.2 Evaluations on Treatment 81
12.3 Off-Treatment Evaluations 89
12.4 End of Treatment (EOT) Safety Evaluations 89
12.5 Follow-up Evaluations 90
12.6 Survival Assessment Evaluations 91
12.7 Calendar of Clinical and Laboratory Evaluations (continued on following page) 92
13.0 SCHEDULE OF CORRELATIVE EVALUATIONS 96

13.1 Pre-Treatment Specimen Collection 96
13.2 On Treatment Specimen Collection 98
13.3 Off-Treatment Specimen Collection (End of Treatment or EOT visit) 99
13.4 Radiology correlative studies 100
13.5 Calendar of Specimen Collection for Correlative Studies 101
13.6 Banking of Additional Specimens 101
14.0 MEASUREMENT OF EFFECT 101
14.1 Antitumor Effect in Solid Tumors 101
14.2 Progression-Free Survival (PFS) 108
14.3 Overall Survival (OS) 108
15.0 ADVERSE EVENTS 108
15.1 Purpose 108
15.2 Adverse Event 108
15.3 Serious Adverse Events (see also Appendix A) 109
15.4 Adverse Event Collection Period 110
15.5 Adverse Event Reporting Requirements 111
15.6 Expedited Adverse Event Reporting Requirements 112
16.0 STATISTICAL CONSIDERATIONS 112
16.1 Overview 112
16.2 Definitions and Endpoints 112
16.3 Statistical Analysis 114
16.4 Patient Enrollment and Sample Size 116
17.0 DATA AND SAFETY MONITORING (Interim monitoring) 117
18.0 DATA REPORTING 119
19.0 STUDY MONITORING 119
20.0 INVESTIGATOR RESPONSIBILITIES 119
20.1 Investigator Responsibility/Performance 119
20.2 Confidentiality 119
20.3 Informed Consent and Permission to Use Protected Health Information 119
20.4 Source Documentation and Investigator Files 120
20.5 Recording and Processing of Data 120
20.6 Non-Protocol Research 121

20.7 Ethics 121
20.8 Essential documents for the conduct of a clinical trial 121
REFERENCES 122
APPENDIX A: EXPEDITED ADVERSE EVENT (AE) REPORTING REQUIREMENTS 125
APPENDIX B: DATA SUBMISSION SCHEDULE 128
APPENDIX C: PERFORMANCE STATUS SCALES 129
APPENDIX D: NYHA CLASSIFICATION OF HEART DISEASE 130
APPENDIX E: INFORMATION ON POSSIBLE DRUG INTERACTIONS [AXITINIB] 131
APPENDIX F: BIOMARKER, CORRELATIVE AND SPECIAL STUDIES 134
APPENDIX G: PROTOCOL APPROVED METHODS OF CONTRACEPTION 146
APPENDIX H: EVENTS OF CLINICAL INTEREST & SUPPORTIVE CARE GUIDELINES FOR PEMBROLIZUMAB 148
APPENDIX I: PATIENT DOSING DIARY AND BLOOD PRESSURE LOG FOR AXITINIB 153
APPENDIX J: CYP1A2, CYP2C8, CYP2C19, CYP3A4 SUBSTRATES, INHIBITORS & INDUCERS * 159
APPENDIX K: GUIDELINES FOR PREPARATION AND ADMINISTRATION/ KEYTRUDA FOR INJECTION (LYOPHILIZED POWDER) 162

Table 1. Presence of PD-L1/PD-1 expression and tumor infiltrating lymphocytes in initial cohort of sarcoma patients. NOTE: Bold/Italicized patients had paired biopsies evaluated. 30
Table 2. **Adequate Organ Function Laboratory Values.** 41
Table 3. **Axitinib Dose Levels.** *Fixed dose based on simulations of population PK model for pembrolizumab suggesting consistency with previous 2 mg/kg dose (see Merck Investigator's Brochure for pembrolizumab). 46
Table 4. Summary of Lab DLTs. 47
Table 5. Summary of Non-Lab DLTs. 47
Table 6. Treatment Schema: Axitinib and Pembrolizumab Concurrent Therapy 48
Table 7. **Treatment Schema: Axitinib Monotherapy** 49
Table 8. Treatment Schema: Axitinib/Pembrolizumab Combination Treatment for Patients who Progress while on Axitinib Monotherapy 49
Table 9. Axitinib and Pembrolizumab Regimen Description 50
Table 10. Dose Titration of Axitinib 73
Table 11. Additional Dose Modification Guidelines for Axitinib. 75
Table 12. Dose Modification and Toxicity Management Guidelines for Immune-Related AEs Associated with Pembrolizumab. 77

Table 13. Correlative Studies' Timepoints 102
Table 14. RECIST 1.1 For Patients with Measurable Disease (i.e. Target Disease) 108

Figure 1. Representative staining for CD45/PD-1 on TIL and PD-L1. 27
Figure 2. Known signaling pathways of selected checkpoint molecules 32
Figure 3. Schematic diagram of the study. 44

ABBREVIATIONS & DEFINITIONS

Term	Abbreviation	Definition
Clinical Benefit Rate	CBR	The percentage of patients with advanced or metastatic cancer who have achieved complete response (CR), partial response (PR) and stable disease (SD) to a therapeutic intervention in clinical trials of anti-cancer agents.
Dose-Limiting Toxicity	DLT	Describes side effects of a drug or other treatment that are serious enough to prevent an increase in dose or level of that treatment. (DLTs are defined before beginning the trial and are protocol-specific.)
Objective Response Rate	ORR	The percentage of patients with advanced or metastatic cancer who have achieved complete response (CR) or partial response (PR) to a therapeutic intervention in clinical trials of anti-cancer agents.
Overall Survival	OS	The length of time from either the date of diagnosis or the start of treatment for a disease, that patients diagnosed with the disease are still alive.
Progression-Free Survival	PFS	The length of time during and after the treatment of a disease that a patient lives with the disease but it does not get worse.
Time to Progression	TTP	The length of time from the date of diagnosis or the start of treatment for a disease until the disease starts to get worse or spread to other parts of the body.

Reference: National Cancer Institute (NCI) Dictionary of Cancer Terms
http://www.cancer.gov/dictionary

PROTOCOL SYNOPSIS

<table>
<tr><td>Protocol Title</td><td>A Phase II Trial of Concurrent Axitinib and Pembrolizumab in Subjects with Advanced Alve
Soft Part Sarcoma (ASPS) and Other Soft Tissue Sarcomas (STS)</td></tr>
<tr><td>Targeted Patient Population</td><td>Young adult and adult patients (age ≥ 16 years) with histologically confirmed sarcoma, who demonstrate either refractory disease, radiographic progression of disease and/or refusal of standard/routine care options. Sarcoma(s) must be unresectable and measurable by RECIST</td></tr>
<tr><td>Study Design</td><td>This is an open-label, single-institution, non-randomized, Phase II study of concurrent axitin with pembrolizumab therapy. After a safety lead-in consisting of the initial five patients, intrapatient dose escalation of axitinib will be permitted based on the absence of predefined toxicities (Section 7.1, Table 3 and Section 10.2, Table 8, respectively).</td></tr>
<tr><td>Treatment Schema</td><td>Concurrent Axitinib and Pembrolizumab Treatment:
<table>
<tr><td>Cycle(s)</td><td colspan="7">1</td><td colspan="6">2</td><td rowspan="6">Imaging prior to every subsequent odd cycle (i.e. 3, 5, 7, 9, etc.)</td><td colspan="6">3, 4, 5, etc. until progression</td></tr>
<tr><td>Week(s)</td><td>1</td><td>2</td><td>3</td><td>4</td><td>5</td><td>6</td><td>7</td><td>1</td><td>2</td><td>3</td><td>4</td><td>5</td><td>6</td><td>1</td><td>2</td><td>3</td><td>4</td><td>5</td><td>6</td></tr>
<tr><td>Day(s)</td><td>1</td><td>8</td><td>15</td><td>22</td><td>29</td><td>36</td><td>43</td><td>1</td><td>8</td><td>15</td><td>22</td><td>29</td><td>36</td><td>1</td><td>8</td><td>15</td><td>22</td><td>29</td><td>36</td></tr>
<tr><td>Axitinib oral, BID continuously (uptitration permitted beginning in cycle 2)</td><td>X</td><td>X</td><td>X</td><td>X</td><td>X</td><td>X</td><td>X</td><td>X</td><td>X</td><td>X</td><td>X</td><td>X</td><td>X</td><td>X</td><td>X</td><td>X</td><td>X</td><td>X</td><td>X</td></tr>
<tr><td>Pembrolizumab IV q21 days</td><td></td><td>X</td><td></td><td></td><td>X</td><td></td><td></td><td>X</td><td></td><td></td><td>X</td><td></td><td></td><td>X</td><td></td><td></td><td>X</td><td></td><td></td></tr>
</table>
Treatment Schema: Axitinib Monotherapy
<table>
<tr><td>Cycle(s)</td><td colspan="12">1, 2, 3, etc. until progression</td><td rowspan="4">Imaging every 12 weeks (+/- 7 days)</td></tr>
<tr><td>Week(s)</td><td>1</td><td>2</td><td>3</td><td>4</td><td>5</td><td>6</td><td>7</td><td>8</td><td>9</td><td>10</td><td>11</td><td>12</td></tr>
<tr><td>Day(s)</td><td>1</td><td>8</td><td>15</td><td>22</td><td>29</td><td>36</td><td>43</td><td>50</td><td>57</td><td>64</td><td>71</td><td>78</td></tr>
<tr><td>Axitinib oral, BID continuously</td><td>X</td><td>X</td><td>X</td><td>X</td><td>X</td><td>X</td><td>X</td><td>X</td><td>X</td><td>X</td><td>X</td><td>X</td></tr>
</table></td></tr>
</table>

<table>
<tr><td></td><td>Treatment Schema: Axitinib/Pembrolizumab Combination Treatment for Patients who Progress while on Axitinib Monotherapy
<table>
<tr><td>Cycle(s)</td><td colspan="6">1, 2, 3, etc. up to 17 cycles (approximately 1 year)</td><td rowspan="5">Imaging prior to every subsequent odd cycle (i.e. 3, 5, 7, 9, etc.)</td></tr>
<tr><td>Week(s)</td><td>1</td><td>2</td><td>3</td><td>4</td><td>5</td><td>6</td></tr>
<tr><td>Day(s)</td><td>1</td><td>8</td><td>15</td><td>22</td><td>29</td><td>36</td></tr>
<tr><td>Axitinib oral, BID continuously</td><td>X</td><td>X</td><td>X</td><td>X</td><td>X</td><td>X</td></tr>
<tr><td>Pembrolizumab IV q21 days</td><td>X</td><td></td><td></td><td>X</td><td></td><td></td></tr>
</table></td></tr>
<tr><td>Duration of Treatment</td><td>Trial therapy will last until withdrawal of consent, disease progression and/or unacceptable toxicity, whichever occurs first.</td></tr>
<tr><td>Follow-up Required Post-Treatment</td><td>All subjects will be followed at approximately 30-days (±5 days) after the last dose of trial treatment or before the initiation of a new anti-cancer treatment, whichever occurs first.

Subjects who discontinue trial treatment for any reason other than disease progression will b assessed every 12 weeks (±7 days) to monitor disease status.

Following confirmed disease progression or initiation of new anti-cancer therapy, survival w assessed (at a minimum) by telephone contact every 12 weeks (±7days).</td></tr>
<tr><td>Objectives</td><td><u>Primary Objective</u>: To estimate progression-free survival rate at 3 months after concurrent axitinib and pembrolizumab therapy in patients with alveolar soft part sarcoma (ASPS) and o advanced soft tissue sarcomas.

<u>Secondary Objectives</u>:

• To estimate the objective response rate (ORR), clinical benefit rate (CBR), progressior survival (PFS) using RECIST 1.1, as well as overall survival (OS) in patients with ASP other advanced soft tissue sarcomas treated with concurrent axitinib and pembrolizumab.

• To describe the safety and toxicity profile of axitinib with intrapatient dose titration com with pembrolizumab therapy in patients with ASPS and other advanced soft tissue sarc treated with concurrent axitinib and pembrolizumab.

<u>Exploratory Objective(s)</u>:

• To identify associations between clinical benefit status (CR/PR/SD vs. PD) and a) T infiltration; b) immune markers including PD-1, PD-L1, PD-L2, TIM3, LAG3, and CT expression categories in tumor tissue.</td></tr>
</table>

	• To identify associations between progression-free status at 3 months (no progressio progression) and a) T-cell infiltration; b) immune markers including PD-1, PD-L1, PI TIM3, LAG3, and CTLA-4 expression categories in tumor tissue. • To identify associations between clinical benefit status (CR/PR/SD vs. PD) and the abs change (baseline to cycle 3, baseline to off-study, and cycle 3 to off-study) in each c following T-cell subsets in peripheral blood and tumor tissue: (CD8+, CD4+, T-regula memory phenotype, naïve phenotype, Ki67+, PD-1+, TIM3+, LAG3+, CTLA4+). • To identify associations between progression-free status at 3 months (no progressio progression) and the absolute change (baseline to cycle 3, baseline to off-study, and cycl off-study) in each of the following T-cell subsets in peripheral blood and tumor tissue: (C CD4+, T-regulatory, memory phenotype, naïve phenotype, Ki67+, PD-1+, TIM3+, LA CTLA4+). • To describe the relationship between assessments of tumor response according to RECIS and tumor response according to alternative radiologic methods including: a) Choi criter immune-related response criteria (irRC), c) MRI volumetrics, and d) PERCIST 1.0. • To identify associations between clinical benefit status (CR/PR/SD vs. PD) by RECIST 1. the absolute change (baseline to cycle 3, baseline to off-study, and cycle 3 to off-stu baseline) in quantity of circulating tumor cells (CTCs). • To identify associations between the progression-free status at 3 months (no progressio progression) and the absolute change (baseline to cycle 3, baseline to off-study, and cycl off-study at baseline) in quantity of circulating tumor cells (CTCs). • To identify associations between the progression-free status at 3 months (no progression progression) and the change in genomic expression of angiogenesis and immune-related genes, as assessed by RNA expression in tumor biopsies and peripheral blood samples. • To identify associations between the progression-free status at 3 months (no progression progression) and T cell clonality in an expansion cohort of ASPS tumor biopsies and peripheral blood cells • To identify associations between the progression-free status at 3 months (no progression progression) and baseline plasma angiogenic activity and the change in angiogenic activi occurring with treatment

Expected Number of Patients	45, including screen failures and non-evaluable patients Expansion cohort: up to 10 additional patients with alveolar soft part sarcoma
Expected Number of Centers	Sylvester Comprehensive Cancer Center (SCCC) *Note: SCCC is inclusive of the constituent satellite sites.*
Expected Duration of the Protocol	The estimated duration of the protocol is 3 years from screening/accrual to end of follow-up.
Inclusion Criteria	1. Patients must have histologically confirmed sarcoma with pathology review required for outside samples. 2. The following histologies may be enrolled without prior treatment: a. alveolar soft part sarcoma, (NOTE: expansion only permits patients with ASPS) b. clear cell sarcoma, c. epithelioid hemangioendothelioma, and d. chordoma. 3. The following histologies may be enrolled ONLY if refractory to anthracycline-based chemotherapy or if the patient refuses to undergo standard of care treatment: a. synovial sarcoma, b. rhabdomyosarcoma, c. malignant peripheral nerve sheath tumors, d. dedifferentiated , pleomorphic, or myxoid/round cell liposarcoma, e. leiomyosarcoma, f. malignant phylloides tumor, g. high grade undifferentiated pleomorphic sarcomas (HGUPS/MFH), h. angiosarcoma, i. spindle cell sarcoma NOS j. malignant myoepithelioma 4. The following histologies may be enrolled ONLY if refractory to at least one line of chemotherapy or if the patient refuses to undergo standard of care treatment: a. solitary fibrous tumor/hemangiopericytoma. 5. The following histologies may be enrolled ONLY if refractory to at least first-line targete therapy or if the patient refuses to undergo standard of care treatment: a. gastrointestinal stromal tumors, b. extraskeletal myxoid chondrosarcoma, c. PEComa 6. Primary tumors of bone including Ewing's sarcoma, osteosarcoma, and dedifferentiated chondrosarcoma may only be enrolled if there are measurable target lesions occurring in tissue and they are refractory to standard of care anthracycline-based chemotherapy.

7. Any other histology or standard of care treatment not specifically addressed will be revie by the principal investigator and pathologist for final determination of eligibility.
8. Measurable disease as defined by RECIST v1.1 (provided in Section 14.0).
9. Radiographic progression as defined by RECIST v1.1, based on comparison between two radiographic studies no greater than 6 months apart.
10. Inability to undergo complete resection of the disease by surgery.
11. Adequate organ function as defined below (Section 5.0, Inclusion Criterion 11).

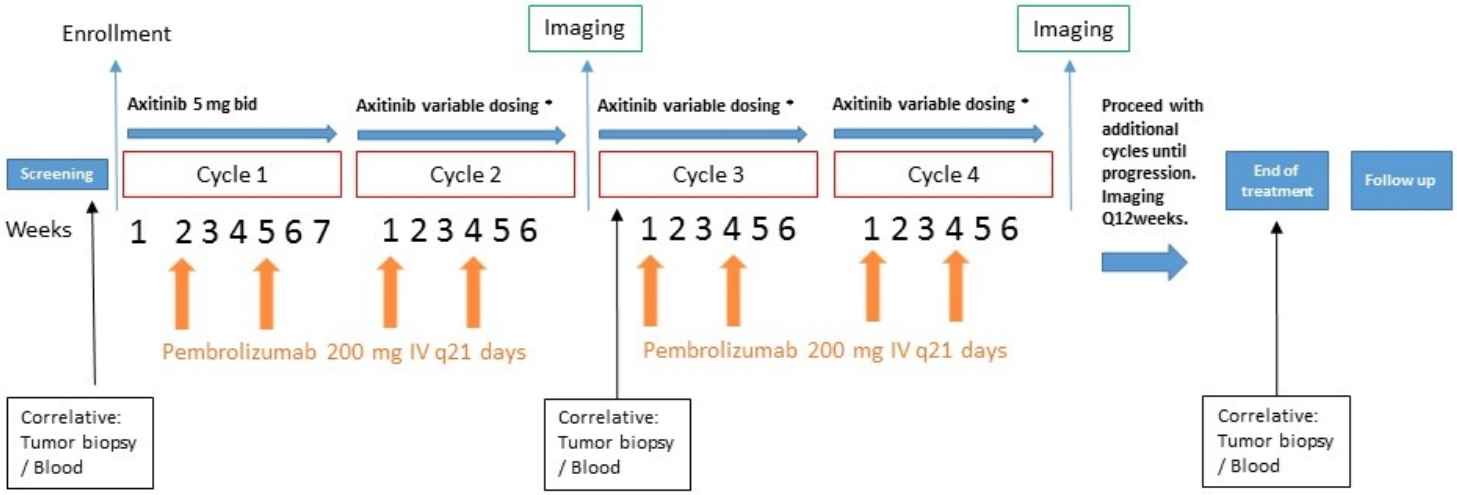
System	Laboratory Value
Hematological	
Absolute neutrophil count (ANC)	≥1,000 /mcL
Platelets	≥75,000 / mcL
Hemoglobin	≥8 g/dL without transfusion or EPO dependency (within 7 days of assessment)
Renal	
Serum creatinine **OR** Measured or calculated[a] creatinine clearance (GFR can also be used in place of creatinine or CrCl)	≤1.5 X upper limit of normal (ULN) **OR** ≥60 mL/min for subject with creatinine levels > 1.5 X institutional ULN
Hepatic	
Serum total bilirubin	≤ 1.5 X ULN **OR** Direct bilirubin ≤ ULN for subjects with total bilirubin levels > 1.5 ULN
AST (SGOT) and ALT (SGPT)	≤ 2.5 X ULN **OR** ≤ 5 X ULN for subjects with liver metastases
Albumin	≥2.5 mg/dL
Coagulation	
International Normalized Ratio (INR) or Prothrombin Time (PT) Activated Partial Thromboplastin Time (aPTT)	≤1.5 X ULN unless subject is receiving anticoagulant therapy as long as PT or PTT is within therapeutic range of intended use of anticoagulants ≤1.5 X ULN unless subject is receiving anticoagulant therapy as long as PT or PTT is within therapeutic range of intended use of anticoagulants
[a] Creatinine clearance should be calculated per institutional standard.	

12. Age ≥ 16 years.

	13. ECOG performance status of 0 or 1. 14. Patients must consent and be willing to undergo tumor core needle biopsies at three timepoints (1. baseline, 2. cycle 3, and 3. off-study). At least one tumor site must be amenable to biopsy in the judgment of the interventional radiologist. 15. Female subjects of childbearing potential should have a negative urine or serum pregnanc within 72 hours prior to receiving the first dose of study medication. If the urine test is positive or cannot be confirmed as negative, a serum pregnancy test will be required. 16. Females of child bearing potential that are sexually active must agree to either practice 2 medically accepted highly effective methods of contraception at the same time or abstain from heterosexual intercourse from the time of signing the informed consent through 120 days after the last dose of study drug. See Appendix G for protocol-approved highly effe methods of contraceptive combinations. Subjects of childbearing potential are those who not been surgically sterilized or have not been free from menses for > 1 year. a. Negative test for pregnancy is required of females of child-bearing potential; A fe of child bearing potential is any woman, regardless of sexual orientation or wheth they have undergone tubal ligation, who meets the following criteria: 1. has not undergone a hysterectomy or bilateral oophorectomy; or 2. has not been naturally postmenopausal for at least 24 consecutive months (i.e., has had menses at any ti the preceding 24 consecutive months or 730 days). b. Conception while on treatment must be avoided 17. Male subjects should agree to use an adequate method of contraception starting with the dose of study therapy through 120 days after the last dose of study therapy. Prior history vasectomy does NOT replace requirement for contraceptive use. 18. Suitable venous access to allow for all study related blood sampling. 19. Ability to understand and willingness to sign a written informed consent document. 20. For minors that are 16 to 18 years of age, assent **and** parental (or legally accep representative) written informed consent must be obtained.
Exclusion Criteria	1. Prior therapy with axitinib. Patients are permitted to have received prior tyrosine kinase inhibitor (TKI) therapy including imatinib, sunitinib, pazopanib, or similar. Patients may have received prior PD-1 or PD-L1 directed therapy. 2. Hypersensitivity to axitinib, pembrolizumab or any of its excipients. 3. Patients may not be receiving any other investigational agents (within 4 weeks prior to C 1, day 1). 4. Prior anti-cancer monoclonal antibody (mAb) within 4 weeks prior to Cycle 1, day 1 or h not recovered (i.e., ≤ Grade 1 or at baseline) from adverse events due to agents administe more than 4 weeks earlier. 5. Patient has had prior chemotherapy, targeted small molecule therapy, or radiation therapy within 2 weeks prior to Cycle 1, Day 1 or has not recovered (i.e., ≤ Grade 1 or at baseline from adverse events due to agents administered more than 4 weeks earlier. Subjects with Grade 2 neuropathy are an exception to this criterion and may qualify for the study. Note

	subject received major surgery, they must have recovered adequately from the toxicity ar complications from the intervention prior to starting therapy. 6. Additional known malignancy that is progressing or requires active treatment. Exceptior include basal cell carcinoma of the skin, or squamous cell carcinoma of the skin that has undergone potentially curative therapy, or in situ cervical cancer. 7. Patients with end-organ dysfunction (detailed in Section 5.1, Inclusion Criterion 11). 8. Patients with bone-only lesions. 9. Patients with underlying immune deficiency, chronic infections including HIV, hepatitis, tuberculosis (TB) or autoimmune disease. 10. Patients with underlying hematologic issues including bleeding diathesis, known previou bleeding requiring intervention within the past 6 months, active pulmonary emboli or DV that are not stable on anticoagulation regimen. 11. Has known history of, or any evidence of active, non-infectious pneumonitis. 12. Has known active central nervous system (CNS) metastases, and/or carcinomatous menir or leptomeningeal disease. Subjects with previously treated brain metastases may partici provided they are stable (without evidence of progression by imaging for at least four we prior to the first dose of trial treatment and any neurologic symptoms have returned to baseline), have no evidence of new or enlarging brain metastases, and are not using stero for at least 7 days prior to trial treatment. This exception does not include carcinomatous meningitis which is excluded regardless of clinical stability. 13. Concomitant (or receipt of) treatment with medications that may affect the metabolism o pembrolizumab and/or axitinib within 7 days prior to Cycle 1, day 1 of axitinib. (CYP3A substrates are not exclusionary) 14. Has received a live vaccine within 30 days of planned start of study therapy. Note: Seas influenza vaccines for injection are generally inactivated flu vaccines and are allowed; however intranasal influenza vaccines (e.g., Flu-Mist®) are live attenuated vaccines, and not allowed. 15. Is pregnant or breastfeeding, or expecting to conceive or father children within the projec duration of the trial, starting with the pre-screening or screening visit through 120 days a the last dose of trial treatment. 16. Any uncontrolled, intercurrent illness including but not limited to ongoing or active infec symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia 17. Prolonged QTc interval on Screening EKG >475 ms. 18. Ejection Fraction <40% by 2D ECHO at Screening. 19. Any serious medical or psychiatric illness/condition including substance use disorders lik in the judgment of the Investigator(s) to interfere or limit compliance with study requirements/treatment. 20. Has active autoimmune disease that has required systemic treatment in the past 2 years (i with use of disease modifying agents, corticosteroids or immunosuppressive drugs). Replacement therapy (eg., thyroxine, insulin, or physiologic corticosteroid replacement therapy for adrenal or pituitary insufficiency, etc.) is not considered a form of systemic treatment.

PROTOCOL SCHEMA



The first cycle of treatment will be seven weeks, with one week of axitinib alone prior to initiation of pembrolizumab. Subsequently, six-week cycles of treatment consist of twice daily oral doses of axitinib administered continuously with intravenous (I.V.) administration of pembrolizumab on day 1 and 22. The first five evaluable patients will be considered a safety lead-in, and will not have axitinib dose escalation above 5 mg PO bid. In subsequent cycles, patients will begin cycle 1 with axitinib 5 mg PO twice daily (BID) and be monitored for DLT; these patients may then have axitinib dose-escalated as tolerated for the individual patient starting with cycle 2. Dose de-escalations may occur at any time for toxicity.

1.0 BACKGROUND AND RATIONALE

1.1 Soft Tissue Sarcomas (STS)

Soft tissue sarcomas (STS) are rare, heterogeneous and highly aggressive neoplasms that often affect young and otherwise healthy individuals. Although aggressive chemotherapy has improved outcomes for pediatric histologies such as embryonal rhabdomyosarcoma, very few targeted therapies have been approved for refractory and metastatic STS; median overall survival is dismal at only 12-18 months. Additionally, some histologies, such as alveolar soft part sarcoma (ASPS) and clear cell sarcoma (CCS) do not respond at all to traditional chemotherapy, and inevitably progress with distant metastases and death. Gastrointestinal stromal tumors still ultimately develop resistance to imatinib and other tyrosine kinase inhibitors despite initial dependence on KIT, due to the development of escape mutations and alternative pathways. Thus, novel treatment strategies are desperately needed for this challenging group of cancers.

Sarcomas have long been suspected to have an immunogenic phenotype; as early as 1891, William Coley noted resolution of sarcoma after a severe bacterial infection (1). Although sarcoma's rapid and aggressive course has limited the efficacy of most previous immunotherapy approaches, a few remarkable responses have been seen in synovial sarcomas expressing NY-ESO-1 antigens with adoptive T-cell immunotherapy (2-3). Unlike most solid tumors, some sarcomas can be "cured" by resection of all metastatic disease even after an incomplete response to chemotherapy, suggesting that immune surveillance and suppression may play a role in prevention of recurrence.

1.2 PD-1/PD-L1

The importance of intact immune surveillance in controlling outgrowth of neoplastic transformation has been known for decades. Accumulating evidence shows a correlation between tumor-infiltrating lymphocytes (TIL) in cancer tissue and favorable prognosis in various malignancies. In particular, the presence of CD8+ T-cells and the ratio of CD8+ effector T-cells / FoxP3+ regulatory T-cells seems to correlate with improved prognosis and long-term survival in many solid tumors.

The PD-1 receptor-ligand interaction is a major pathway hijacked by tumors to suppress immune control. The normal function of PD-1, expressed on the cell surface of activated T-cells under healthy conditions, is to down-modulate unwanted or excessive immune responses, including autoimmune reactions. PD-1 (encoded by the gene Pdcd1) is an Ig superfamily member related to CD28 and CTLA-4 which has been shown to negatively regulate antigen receptor signaling upon engagement of its ligands (PD-L1 and/or PD-L2). The structure of murine PD-1 has been resolved. PD-1 and family members are type I transmembrane glycoproteins containing an Ig Variable-type (V-type) domain responsible for ligand binding and a cytoplasmic tail which is responsible for the binding of signaling molecules. The cytoplasmic tail of PD-1 contains 2 tyrosine-based signaling motifs, an immunoreceptor tyrosine-based inhibition motif (ITIM) and an immunoreceptor tyrosine-based switch motif (ITSM). Following T-cell stimulation, PD-

1 recruits the tyrosine phosphatases SHP-1 and SHP-2 to the ITSM motif within its cytoplasmic tail, leading to the dephosphorylation of effector molecules such as CD3ζ, PKCθ and ZAP70 which are involved in the CD3 T-cell signaling cascade. The mechanism by which PD-1 down modulates T-cell responses is similar to, but distinct from that of CTLA-4 as both molecules regulate an overlapping set of signaling proteins. PD-1 was shown to be expressed on activated lymphocytes including peripheral CD4+ and CD8+ T-cells, B-cells, T regs and Natural Killer cells. Expression has also been shown during thymic development on CD4-CD8- (double negative) T-cells as well as subsets of macrophages and dendritic cells. The ligands for PD-1 (PD-L1 and PD-L2) are constitutively expressed or can be induced in a variety of cell types, including non-hematopoietic tissues as well as in various tumors. Both ligands are type I transmembrane receptors containing both IgV- and IgC-like domains in the extracellular region and contain short cytoplasmic regions with no known signaling motifs. Binding of either PD-1 ligand to PD-1 inhibits T-cell activation triggered through the T-cell receptor. PD-L1 is expressed at low levels on various non-hematopoietic tissues, most notably on vascular endothelium, whereas PD-L2 protein is only detectably expressed on antigen-presenting cells found in lymphoid tissue or chronic inflammatory environments. PD-L2 is thought to control immune T-cell activation in lymphoid organs, whereas PD-L1 serves to dampen unwarranted T-cell function in peripheral tissues. Although healthy organs express little (if any) PD-L1, a variety of cancers were demonstrated to express abundant levels of this T-cell inhibitor. PD-1 has been suggested to regulate tumor-specific T-cell expansion in subjects with melanoma (MEL). This suggests that the PD-1/PD-L1 pathway plays a critical role in tumor immune evasion and should be considered as an attractive target for therapeutic intervention.

1.3 **Rationale for Combination Anti-PD-1 with anti-VEGFR Therapy in Sarcomas**

Inhibitors of PD-1/PD-L1 interactions, including the anti-PD-1 antibody pembrolizumab, have been recently approved for melanoma and non-small cell lung cancer, but are largely unexplored in sarcomas. However, recent studies have reported PD-L1 expression in sarcomas, particularly GIST and osteosarcoma, as well as the presence of tumor-infiltrating T-cells that express PD-1 ((4-6), Figure 1.) The first single-agent trial of pembrolizumab for STS patients has just been initiated (NCT02301039). However, many have questioned whether anti-PD-1 monotherapy will be sufficient in aggressively-growing sarcomas; response to anti-PD-1 can take time to manifest. It is recognized that therapy efficacy and prolongation of response are often enhanced by combining two or more treatments targeting compensatory signaling pathways, and given the complexity of immune checkpoint inhibition, many studies of combination treatments with PD-1 targeted therapies are ongoing.

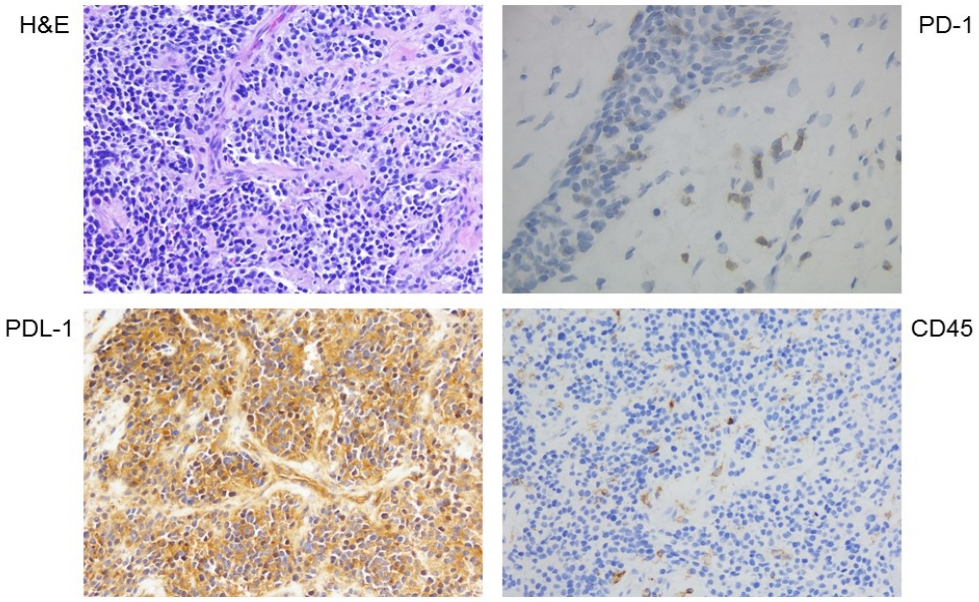


Figure 1. Representative staining for CD45/PD-1 on TIL and PD-L1. Performed on patient with recurrent alveolar rhabdomyosarcoma. (Courtesy of Andrew Rosenberg, MD, University of Miami Department of Pathology, unpublished data.)

Studies of tumor immunology in melanoma and renal cell carcinoma cells have shown that HIF-1α and related angiogenic proteins contribute to the failure of the immune system to recognize and attack tumor cells. These pro-angiogenic factors lead to suppression of dendritic cell maturation and antigen presentation, suppression of migration of lymphocytes across endothelium into tumor deposits, and encourage accumulation of myeloid derived suppressor cells (7-9). Additionally, preclinical work has suggested that anti-VEGF treatment may increase PD-L1 expression in renal cell carcinoma cells, facilitating inhibitory interactions with PD-1 on tumor-infiltrating lymphocytes (10). Based on these observations, a compelling rationale exists for combined therapy with immunostimulatory agents like checkpoint inhibitors, and anti-VEGFR tyrosine kinase inhibitors (TKI). A study of combination CTLA-4 blockade with ipilimumab and VEGF inhibitor bevacizumab produced a disease control rate of 67.4% and mean overall survival of 25.1 months (11). Tumor biopsies revealed marked trafficking of CD8+ T cells and dendritic macrophages across the tumor vasculature, and increased circulating memory T-cells in patients treated with the combination. A phase 1 study of combined sunitinib or pazopanib with PD-1 inhibitor nivolumab in renal cell carcinoma was recently reported (ASCO 2014, Abstract #5010). Although the combination was toxic, with high rates of hepatic and renal failure leading to discontinuation of the pazopanib containing arm, response rates with both regimens were higher than previously reported with the TKI alone.

A Phase I trial of combination axitinib, another pan-VEGFR TKI, and anti-PD-1 antibody pembrolizumab in renal cell carcinoma is ongoing (NCT02133742). The initial dose-finding cohort has been completed. Remarkably, the combination of axitinib 5 mg PO twice daily with pembrolizumab 2 mg/kg every three weeks was well-tolerated.

Three DLTs occurred in 10 evaluable patients, including one transient ischemic attack, one headache, and one patient with arthralgias, hypertension and fatigue (Atkins et al, Abstract #370, Society of Immunotherapy for Cancer 2015 Annual Meeting, National Harbor, MD). An expansion cohort at the dose combination of axitinib 5 mg PO bid and pembrolizumab 2 mg/kg every three weeks, permitting intrapatient dose escalation of axitinib to 7 mg PO twice daily and 10 mg PO twice daily is ongoing.

The rationale for combined anti-angiogenic therapy and immunotherapy is similarly compelling in sarcomas. Two of the most immunogenic sarcomas, ASPS and CCS, share marked similarity with melanoma based on pathologic translocations that activate members of the MITF transcription factor family. Downstream targets of these aberrant fusion proteins include HIF-1α, the critical mediator of tumor angiogenesis. High expression of pro-angiogenic factors including VEGF, PDGF, angiopoietin-1/2, and FGF have been implicated in sarcomas, portending worse prognosis and resistance to chemotherapy [1]. Interestingly, the most promising responses in ASPS and CCS to date have occurred with the pan-VEGF tyrosine kinase inhibitors cediranib and sunitinib [2, 3]. Anti-angiogenic TKI have also been effective in other chemotherapy-resistant sarcomas, including gastrointestinal stromal tumor and solitary fibrous tumor/hemangiopericytoma, and pazopanib was recently approved by the FDA after improving overall survival in non-adipocytic STS refractory to chemotherapy. However, most patients eventually develop resistance to anti-angiogenic therapy.

Goldberg *et al* recently reported the results of a Phase I trial of autologous GM-CSF-secreting vaccines in patients with ASPS and CSS (15). Interestingly, although no tumor regressions were seen, correlative studies did reveal intra-tumor influx of lymphocytes, T-regulatory cells, and dendritic cells. Additionally, sera from vaccinated patients showed increased antibodies against angiogenic cytokines TIE1 and TIE2, suggesting that immune stimulation increased the host response to angiogenic factors thought to drive tumor growth. In an exploratory analysis of vaccinated patients, 2 ASPS tumors showed expression of PD-1 by tumor-infiltrating lymphocytes as well as PD-L1 expression by neighboring tumor cells. This suggests that ASPS may express immune-evading markers at baseline, or perhaps have the ability to upregulate PD-L1 in response to vaccination, preventing effective destruction. Regardless, this would suggest a role for PD-1 blockade as a potential sensitizing strategy to permit immune recognition of ASPS, in combination with an anti-angiogenic strategy already shown to have activity in ASPS.

As a pilot investigation, we evaluated PD-1/PD-L1 expression and the presence of tumor infiltrating lymphocytes in 10 patients with sarcoma from our institution (Table 1.) Patient 10 is a current patient with alveolar soft part sarcoma who recently progressed on cediranib, a tyrosine kinase inhibitor similar to axitinib. In the baseline biopsy, his tumor was negative for PD-L1 expression in the tumor tissue, but exhibited 2-5 TIL per 40X HPF, with a low fraction of the TIL positive for PD-1 expression. However, on the post-cediranib resection specimen, tumor cells were diffusely positive for PD-L1, with increased TIL and greater than 10% positivity for PD-1. We also evaluated two other sarcoma patients from our institution with pre-and post-treatment biopsies. Patient 9

(alveolar rhabdomyosarcoma) was biopsied upon recurrence several months after completion of adjuvant chemotherapy. Patient 3 (Ewing's sarcoma) was biopsied as part of an ongoing clinical trial combining a dendritic cell vaccine with tumor lysate injections (Eprost 20110462, PI, John Goldberg). All of these patients showed upregulation of PD-L1 on tumor cells and increased lymphocyte infiltration in the post-treatment biopsies relative to their own baseline, suggesting that exposure to chemotherapy or immune therapy increases expression of PD-L1 in resistant, viable cells, and increases lymphocyte infiltration.

Patient ID	**Tumor type**	**PD-L1 expression**	**CD45+ TIL**	**PD-1 expression (% of TIL)**
1	Osteosarcoma	Diffuse	Dense	Focal (1%)
2	Osteosarcoma	Focal	Positive	Negative
3	Ewing's sarcoma (pre-treatment)	Diffuse	Scattered	Focal
	Post-treatment (dendritic cell vaccine protocol)	Diffuse	Increased relative to baseline	Negative
4	Osteosarcoma	Weakly positive	Few intratumoral, greater peritumoral	Negative
5	Rhabdomyosarcoma (Alveolar)	Diffuse	Scattered	Negative
6	Ewing's sarcoma	Diffuse	Scattered	Focal
7	Histiocytic sarcoma	Diffuse	Dense	10% positive
8	Rhabdomyosarcoma (Alveolar)	Diffuse	Positive	Scattered
9	Rhabdomyosarcoma (Alveolar) – initial	Weakly positive	Scattered	Negative
	Rhabdomyosarcoma (Alveolar) – recurrence after chemotherapy	Diffuse	Scattered	Scattered
10	ASPS (initial)	Negative	Scattered	Focal
	ASPS (progressing tumor on cediranib)	Diffuse	Positive	10% positive

Table 1. Presence of PD-L1/PD-1 expression and tumor infiltrating lymphocytes in initial cohort of sarcoma patients. NOTE: Bold/Italicized patients had paired biopsies evaluated.

Based on the compelling rationale for combined inhibition of immune checkpoints and tumor angiogenesis in sarcomas, we propose treatment of patients with ASPS, CCS and other soft tissue sarcomas with concurrent axitinib with pembrolizumab. While the activity of pembrolizumab as monotherapy in sarcomas is currently being studied,

axitinib has shown activity in soft tissue sarcomas in early clinical trials. Axi-STS, a phase II trial of axitinib at 5 mg PO twice daily in soft tissue sarcomas is ongoing in the UK and was reported at the NCRI conference in November 2014 (Abstract #B48) and CTOS 014 (Paper 031, Berlin, Germany). Progression-free survival rates at 12 weeks were 47% in leiomyosarcoma, and 38% with other soft tissue sarcomas. Results for the other cohorts studying synovial sarcoma and angiosarcoma are still pending. Grade 3/4 toxicities included fatigue (12/60 patients), hypertension (7/60 patients), arthralgia (2/60 patients), diarrhea (6/60 patients), and hypercalcemia (Grade 4, 1/60 patients).

We hypothesize that by using the anti-angiogenic agent axitinib to "prime" the sarcoma tumor microenvironment, producing an influx of immune cells into the area and possible upregulation of PD-L1 on the tumor cells, this may intensify the immune-directed cytotoxicity resulting from subsequent pembrolizumab therapy.

This trial will provide pilot data for subsequent randomized trials should activity in the combination be observed.

1.4 Rationale for Intrapatient Dose Titration of Axitinib

Oral tyrosine kinase inhibitors are well-documented to show significant variability in plasma concentrations, pharmacodynamic on-target effects, toxicity, and tumor response in individual patients despite fixed dosing (16). In particular, axitinib has been studied extensively with regards to pharmacokinetic variability in patients. In renal cell carcinoma, increased axitinib AUC correlates with superior tumor response rate, (17) which has led to incorporation of dose escalation above the standard approved dose of 5 mg twice daily in subsequent phase III clinical trials and inclusion on the FDA label as accepted practice. As evidence of the variable tolerance of axitinib, in the phase III trial of axitinib vs. sorafenib, 31% of patients required at least one dose reduction of axitinib from the starting dose of 5 mg bid, while 37% of patients were treated above 5 mg bid (18). However, the most commonly utilized dose titration schemes result in significant changes to expected AUC, which may result in undertreating or overtreating the individual patient. Unlike standard chemotherapy which may be dose reduced by 10 or 20%, the usual dose reductions for axitinib to either 3 mg bid or 2 mg bid leads to 60% and 40% respectively of the starting dose level. Similarly, with the standard dose escalation to 7 mg bid followed by 10 mg bid patients receive 140% and 200% of the starting dose respectively. A clinical trial of more stringent dose titration in smaller intervals is planned in renal cell carcinoma to better customize axitinib dosing, with the hypothesis that patients that can be treated closer to their optimal AUC will show improved toxicity profiles and superior clinical responses (Brian Rini, personal communication).

The Axi-STS trial of axitinib in sarcoma did not utilize dose escalation above 5 mg bid but thus far has shown similar rates of grade 3 and 4 DLT to the larger studies in renal cell carcinoma. Thus, we propose to include stringent titration guidelines and permit dose escalation in our patients to ensure optimal exposure to the anti-angiogenic effects.

1.5 Rationale for Expansion Cohort in Alveolar Soft Part Sarcoma

We have completed accrual of the first 30 patients in this study, including 12 patients with ASPS. Out of 10 evaluable patients, all of whom enrolled on study with progressing disease, 5 patients achieved partial response, and 2 patients achieved minor responses and remain on study therapy. One patient had PD at 3 months and withdrew consent at that time. One patient progressed prior to 3 month imaging. Two patients progressed after the 3 month timepoint. One patient was discontinued for axitinib-related toxicity. The final patient has not yet reached 3 month assessments.

Based on the significant and largely durable responses in ASPS patients, we will expand the study to enroll up to 10 additional patients to obtain additional data regarding safety and efficacy.

1.6 Hypothesis

We hypothesize that combination axitinib with pembrolizumab will prolong the time to tumor progression in patients with ASPS and other advanced soft tissue sarcomas, with favorable progression-free survival and clinical benefit rate relative to historical controls.

1.7 Correlative Studies

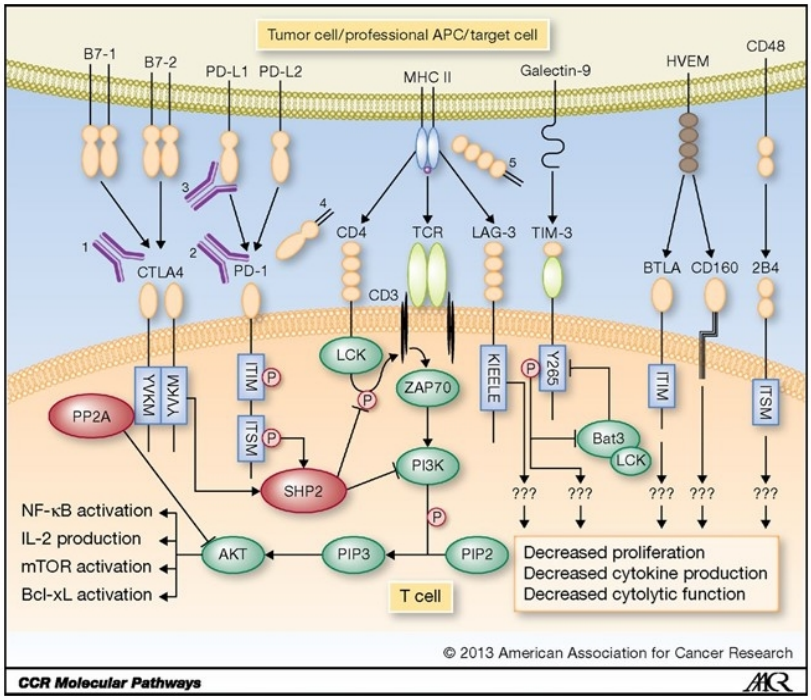
All samples collected will be transferred to the University of Colorado for assays and final analysis with clinical outcomes. This is supported by a collaboration agreement between the University of Colorado and the University of Miami. All samples will be de-identified prior to transfer and labeled only with the subject ID.

1.7.1 Characterization of immunomodulatory factors in patients treated with concurrent axitinib/pembrolizumab

Although the immune system is suspected to play a role in sarcoma development and recurrence, the specific mediators of immune surveillance and suppression of tumor growth are largely unknown. Additionally, we have learned from analysis of patients treated thus far with PD-1-directed therapy that biomarkers of response to immunotherapy are extremely complex. For example, in an elegant analysis of 227 patients treated with MPDL3280A (anti PD-L1 antibody), treatment response appeared to correlate with high tumor PD-L1 expression, pre-existing tumor lymphocyte infiltration with PD-L1 expression, but also with CTLA4 expression, absence of fractalkine, and T-helper 1 gene expression (19). Data from Taube et al in patients with multiple tumor types treated with nivolumab also suggested a geographic tendency for PD-L1 expression at the interface of lymphocyte-rich infiltrations, with PD-L1 expression linked to other markers, including PD-1 expression on lymphocytes and PD-L2 expression (20). Although PD-L1 expression in tumor cells and the presence of lymphocyte infiltration with PD-1/PD-L1 expression seem to be the best predictors of response, 10-15% of patients without tumor PD-L1 expression have responded to therapy, suggesting that other immunomodulatory factors must be playing a role. In addition to CTLA4 expression, other negative regulatory receptors have also been implicated, including LAG-3 and TIM-3, with pre-clinical blockade of some of these pathways

resulting in improved anti-tumor response (21, Figure 2). Finally, the presence of myeloid-derived suppressor cells (MDSCs) and regulatory T-cells have been shown to negatively impact response to therapy in many cancers, though these are also understudied in sarcomas.

Thus, a key correlative component of our study will be to profile sarcoma tumors and infiltrating tumor lymphocytes using pre- and post-treatment tumor biopsies, and analysis of circulating peripheral blood T-cells to better understand the immune environment in sarcoma patients. **We aim to define changes in intra-tumoral and peripheral blood T-cell phenotypes, differentiation and activation status, and checkpoint ligand expression after treatment with axitinib/pembrolizumab, and correlate with progression-free survival and radiographic tumor response to therapy.**



Christopher J. Nirschl, and Charles G. Drake Clin Cancer Res 2013;19:4917-4924

Clinical Cancer Research

Figure 2. Known signaling pathways of selected checkpoint molecules and current therapeutics.

1.7.2 Assessment of tumor response using non-conventional radiologic criteria

Sarcomas often display atypical radiographic changes in response to treatment. Traditional size-based criteria, such as RECIST and WHO criteria, in

musculoskeletal tumors has been shown to be relatively insensitive to biological response as measured by other markers, such as histologic necrosis (22). This is probably due the tendency of sarcomas to undergo internal hemorrhage and necrosis, and as a result enlarge in size (pseudoprogression) despite the fact that the tumor is actually exhibiting positive treatment response. Comparable findings have been reported in other large STS treated with pazopanib (23). With current advances in radiographic imaging, tumor responses or progression can be detected much earlier, and eliminate some of the clinical problems with RECIST. For example, some patients may exhibit obvious three-dimensional progression, yet would be continued on ineffective therapy if the largest diameter does not reach 20% increase. Additionally, "stable disease" spans from 30% regression to 20% growth- generally requiring a waterfall plot or other description of response to get a sense of overall drug activity.

To avoid erroneously classifying pseudoprogression as RECIST progression and premature discontinuation of therapy in clinical trials, alternative criteria for tumor response such as Choi criteria have been developed (24). These criteria assess response in GIST tumors by measuring the degree of lesional enhancement; a modified approach has been proposed for assessment of enhancement pattern on conventional post-contrast MRI (25). PET/CT has also been a useful modality in sarcomas that are initially FDG-avid, with a metabolic response (decrease in SUV) prior to radiographic changes in size (26). PERCIST criteria for response have been developed and validated in solid tumors (27-28).

Additionally, the use of novel functional MRI techniques, such as dynamic contrast enhanced imaging protocols, has been shown to be a useful non-invasive measure of response to therapy in sarcomas (29). Recent advances have enabled functional MRI techniques to interrogate tissues at the cellular level. This capability is particularly relevant in sarcomas since morphological parameters alone do not necessarily correlate with treatment response and prognosis. Diffusion-weighted imaging (DWI) is based on the Brownian motion of molecules and of delineates areas of tissue with restricted diffusion; in general, areas of more restricted diffusion correlate to areas of higher cellularity. DWI is particularly well suited to longitudinal evaluation of sarcomas with multiple studies in both osseous and soft tissue sarcomas showing significant decreases in restricted diffusion (30-31).

Dynamic contrast-enhanced (DCE) MRI is based on rapid acquisition of post-contrast sequences allowing for a map of tissue perfusion kinetics. Recent trials have shown significant drops in DCE parameters after treatment of soft tissue sarcoma with targeted agents after two weeks of treatment (32); recent phase I trials have also confirmed corresponding drops in tumor with higher histologic necrosis, but these promising results have been based on very few subjects ($n<10$) (33). In addition to more conventional measures of enhancing tumor fraction and kinetics, tissue heterogeneity has been shown to be a discriminator of responders from non-responders (34-35). Accurate quantification of these imaging parameters will likely require a volumetric approach to tumor segmentation, which will provide

optimal data for maximizing sensitivity for any real change in tumor composition or percentage necrosis. Utilization of advanced MRI techniques will thus ensure that the most sensitive imaging parameters are those that will be employed to measure tumor response in this trial.

Interestingly, tumor imaging after the first weeks of anti-PD-1 therapy often shows an increase in tumor size, thought to be related to intra-tumor inflammation (36). In light of these paradoxical responses in the setting of checkpoint inhibitor therapy, immune-related response criteria (irRC) have been developed to more accurately account for early post-treatment changes in tumor size, and avoid erroneously discontinuing therapy in patients who will ultimately benefit.

While the official endpoint for this trial of concurrent axitinib/pembrolizumab in sarcomas will utilize RECIST v1.1, tumor response will also be assessed in parallel using Choi criteria and immune-related response criteria for patients utilizing CT for serial imaging, or modified Choi criteria and MRI volumetrics using dynamic contrast enhanced MRI sequences for patient being followed with MRI. All patients will also have a pre-treatment PET/CT, and those patients with a positive PET at baseline will undergo additional on-treatment PET imaging. The results from these non-traditional methods will be used to design future clinical trials in sarcoma in order to more accurately assess tumor response, particularly in the setting of immunotherapy.

1.7.3 Measurement of circulating tumor cells pre- and post-treatment, and correlation with tumor response and time to progression.

Circulating tumor cells (CTC) have been detected in peripheral blood in patients with epithelial tumors, including metastatic breast, colon, and prostate cancer. Recent data suggests that the presence of CTC can be predictive and prognostic, and the CellSearch assay platform has been approved by the FDA to collect CTC before and after therapy, and assess response to treatment (37-38). However, aside from high associated costs, this assay depends on enrichment of CTC based on their expression of EpCAM, a variably expressed cell surface marker, adversely affecting CTC enrichment. It is also likely that enumeration of CTCs alone may be inadequate as prognostic and predictive marker for clinical outcome and response to therapy; particular biomarkers expressed on CTCs may provide a wealth of additional information.

Importantly, although the utility of CTC analysis in other common malignancies has been widely demonstrated, very few studies have looked for circulating tumor cells in sarcoma patients and there are no studies evaluating their prognostic or predictive impact. Previous reports have detected Ewing sarcoma CTC in blood and bone marrow using flow cytometry to isolate CD99+CD45- cells (39-40). Most recently, Satelli et al demonstrated that CTC with cell surface vimentin expression were detectable in human peripheral blood samples from patients with osteosarcoma, undifferentiated pleomorphic sarcoma, leiomyosarcoma, Ewing's

sarcoma, and angiosarcoma (41). Interestingly, patients with primary tumors had fewer CTC than patients with metastatic tumors.

Utilizing a unique, microfabricated parylene membrane-based device in collaboration with Ram Datar (42), we are currently collecting CTCs at various timepoints in sarcoma patients exploring whether CTC could serve as a marker of early micrometastatic disease in sarcomas, and may portend worse prognosis as seen in other tumor types. Effects on circulating tumor cells are particularly interesting in light of immunotherapy, in hopes that a more effective CD8+ T-cell anti-tumor effect could decrease the presence of circulating tumor cells and decrease development of new metastatic sites. Thus, peripheral blood samples will be drawn at selected timepoints and filtered to detect the numbers of circulating tumor cells using this technology. The presence or absence of CTC will be correlated with tumor response.

2.0 **OBJECTIVES**

2.1 **Primary Objective**

- To estimate progression-free survival rate at 3 months after concurrent axitinib and pembrolizumab therapy in patients with alveolar soft part sarcoma and other advanced soft tissue sarcomas.

2.2 **Secondary Objectives**

- To estimate the objective response rate (ORR), clinical benefit rate (CBR), and progression-free survival (PFS) using RECIST 1.1, as well as overall survival (OS) in patients with ASPS and other advanced soft tissue sarcomas treated with concurrent axitinib and pembrolizumab.

- To describe the safety and toxicity profile of axitinib with intrapatient dose titration combined with pembrolizumab therapy in patients with ASPS and other advanced soft tissue sarcomas.

2.3 **Exploratory Objectives**

- To identify associations between clinical benefit status (CR/PR/SD vs. PD) and a) T-cell infiltration; b) PD-1, PD-L1, PD-L2, TIM3, LAG3, and CTLA-4 expression categories in tumor tissue.

- To identify associations between progression-free status at 3 months (no progression vs. progression) and a) T-cell infiltration; b) PD-1, PD-L1, PD-L2, TIM3, LAG3, and CTLA-4 expression categories in tumor tissue.

- To identify associations between clinical benefit status (CR/PR/SD vs. PD) and the absolute change (baseline to cycle 3, baseline to off-study, and cycle 3 to off-study) in each of the following T-cell subsets in peripheral blood and tumor tissue: (CD8+,

CD4+, T-regulatory, memory phenotype, naïve phenotype, Ki67+, PD-1+, TIM3+, LAG3+, CTLA4+).

- To identify associations between progression-free status at 3 months (no progression vs. progression) and the absolute change (baseline to cycle 3, baseline to off-study, and cycle 3 to off-study) in each of the following T-cell subsets in peripheral blood and tumor tissue: (CD8+, CD4+, T-regulatory, memory phenotype, naïve phenotype, Ki67+, PD-1+, TIM3+, LAG3+, CTLA4+).

- To identify associations between the progression-free status at 3 months (no progression vs. progression) and the change in genomic expression of angiogenesis and immune-related genes, as assessed by RNA expression in tumor biopsies and peripheral blood samples (collaboration with Nanostring, Simpatica Medicine).

- In expansion cohort of ASPS patients, to identify associations between the progression-free status at 3 months (no progression vs. progression) with T cell clonal populations in tumor biopsies and peripheral blood cells.

To identify plasma angiogenic activity at baseline, cycle 1 day 8 representing axitinib monotherapy (ASPS expansion cohort only), and cycle 3 day 1.

- To describe the relationship between tumor response according to RECIST 1.1 and tumor response according to alternative radiologic methods including: a) Choi criteria, b) immune-related response criteria (irRC), c) MRI volumetrics, and d) PERCIST 1.0.
- To identify associations between clinical benefit status (CR/PR/SD vs. PD) by RECIST 1.1 and the absolute change (baseline to cycle 3, baseline to off-study, and cycle 3 to off-study) in quantity of circulating tumor cells (CTCs).
- To identify associations between the progression-free status at 3 months (no progression vs. progression) and the absolute change (baseline to cycle 3, baseline to off-study, and cycle 3 to off-study) in quantity of circulating tumor cells (CTCs).

3.0 **ENDPOINTS**

3.1 **Primary endpoint**

- The progression-free survival by RECIST v1.1 of sarcoma patients treated with concurrent axitinib/pembrolizumab therapy.

Evaluable criteria:
Eligible subjects enrolled into the study that received 80% of scheduled axitinib doses and two infusions of pembrolizumab, have measurable disease at baseline and at least one post-baseline disease assessment.

3.2 **Secondary endpoints**

- The objective response (CR/PR), clinical benefit (CR/PR/SD), time to progression (TTP), and overall survival (OS) defined as time from study treatment initiation to first occurrence of progression by RECIST v1.1 or death of sarcoma patients treated with concurrent axitinib/pembrolizumab therapy.

Evaluable criteria:
Eligible subjects enrolled into the study that received at least 80% of scheduled axitinib doses and two infusions of pembrolizumab, have measurable disease at baseline and at least one post-baseline disease assessment.

- Adverse events, including dose-limiting toxicity and serious adverse events (grade 3 or 4) will be graded using NCI CTCAE version 4.03.

Evaluable criteria:
Eligible subjects enrolled into the study that receive at least one dose of both axitinib and pembrolizumab. Patients who withdraw from the study prior to week 2 for any reason other than DLT or other significant toxicity will not be considered evaluable for toxicity.

3.3 **Exploratory endpoints**

- The quantity of CD3+ T-cells in peripheral blood and in tumor biopsies at each timepoint (baseline, cycle 3, and off-study).

- The expression category in tumor tissue (none (0%), low (<5%), intermediate (5-50%), or high (>50%) of PD-1, PD-L1, PD-L2, CTLA-4, TIM-3, LAG-3 at each timepoint (baseline, cycle 3, and off-study).

- The following T cell subsets will be studied in peripheral blood and tumor tissue: (CD4, CD8, T-reg, CTLA4, TIM3, LAG3, memory, naïve, PD-1, Ki67). For each subset, the absolute change in the relevant marker(s) value will be calculated:
  - Cycle 3 marker value minus Baseline marker value
  - Off-study marker value minus Cycle 3 marker value
  - Off-study marker value minus Baseline marker value

Evaluable criteria:
Eligible subjects who receive at least one cycle of each study drug, and successfully undergo at least one post-treatment core biopsy and blood draw.

- Utilizing each of the alternative (non-RECIST) radiological criteria we will categorize clinical benefit status (CR/PR/SD vs PD). CT and/or MRI with dynamic contrast enhanced sequences will be collected throughout the study at every disease evaluation and analyzed using Choi criteria, MRI volumetrics, and immune-related response criteria. PET/CT will be obtained at baseline, Cycle 3, and off-study and tumor response determined by PERCIST 1.0.

Evaluable criteria:
Eligible patients who receive at least one cycle of each study drug and undergo at least one post-treatment radiographic imaging study.

- The quantity of circulating tumor cells (CTCs) in peripheral blood will be measured at three timepoints: 1. Baseline, 2. Cycle 3, and 3. Off-study.

Evaluable criteria:
Eligible patients who receive at least one cycle of each study drug and undergo at least one post-treatment blood sampling.

- RNA expression of a panel of angiogenesis and immune related genes in tumor biopsies and peripheral blood samples. Expression profiles will be obtained at baseline and at Cycle 3 Day 1 on treatment.

Evaluable criteria: Eligible patients who receive at least one cycle of study drug and undergo at least one post-treated radiographic imaging study.

- T cell clonality indices in tumor and peripheral blood obtained at baseline and at Cycle 3 Day 1.

Evaluable criteria: Eligible patients who receive at least one cycle of study drug and undergo at least one post-treated radiographic imaging study.

- Plasma angiogenic activity at baseline and the ratio between on-treatment (either Cycle 1 Day 8 or Cycle 3 Day 1) and baseline.

Evaluable criteria: Eligible patients who receive at least one cycle of study drug and undergo at least one post-treated radiographic imaging study.

4.0 **SUBJECT RECRUITMENT & SCREENING**

Subjects will be recruited at Sylvester Comprehensive Cancer Center via clinical practice offices. Both men and women of all races and ethnic groups are eligible for this trial. Informed consent documents will be translated and available in English and Spanish.

The intervention in the study holds out a prospect of direct benefit that is important to the health or well-being of minors and is available only in the context of the research. Thus minors 16 years of age and older are included in the subject population.

5.0 **PATIENT SELECTION**

5.1 **Inclusion Criteria**

1. Patients must have histologically confirmed sarcoma with pathology review required for any outside samples.
2. The following histologies may be enrolled without prior treatment:

   a. alveolar soft part sarcoma, (NOTE: expansion only permits patients with ASPS)
   b. clear cell sarcoma,
   c. epithelioid hemangioendothelioma, and
   d. chordoma.
3. The following histologies may be enrolled ONLY if refractory to anthracycline-based chemotherapy or if the patient refuses to undergo standard of care treatment:
   a. synovial sarcoma,
   b. rhabdomyosarcoma,
   c. malignant peripheral nerve sheath tumors,
   d. dedifferentiated, pleomorphic or myxoid/round cell liposarcoma,
   e. leiomyosarcoma,
   f. malignant phylloides tumor,
   g. high grade undifferentiated pleomorphic sarcomas (HGUPS/MFH),
   h. angiosarcoma,
   i. spindle cell sarcoma NOS
   j. malignant myoepithelioma.
4. The following histologies may be enrolled ONLY if refractory to at least one line of chemotherapy or if the patient refuses to undergo standard of care treatment:
   a. solitary fibrous tumor/hemangiopericytoma.
5. The following histologies may be enrolled ONLY if refractory to at least first-line targeted therapy or if the patient refuses to undergo standard of care treatment:
   a. gastrointestinal stromal tumors,
   b. extraskeletal myxoid chondrosarcoma,
   c. PEComa.
6. Primary tumors of bone including Ewing's sarcoma, osteosarcoma, and dedifferentiated chondrosarcoma may only be enrolled if there are measurable target lesions occurring in soft tissue and they are refractory to standard of care anthracycline-based chemotherapy.
7. Any other histology or standard of care treatment not specifically addressed will be reviewed by the principal investigator and pathologist for final determination of eligibility.
8. Measurable disease as defined by RECIST v1.1 (provided in Section 14.0).
9. Radiographic progression as defined by RECIST v1.1, based on comparison between two radiographic studies no greater than 6 months apart.
10. Inability to undergo complete resection of the disease by surgery.
11. Adequate organ function as defined in Table 2 (below):

System	Laboratory Value
Hematological	
Absolute neutrophil count (ANC)	≥1,000 /mcL

Platelets	≥75,000 / mcL
Hemoglobin	≥8 g/dL without transfusion or EPO dependency (within 7 days of assessment)
Renal	
Serum creatinine **OR** Measured or calculated[a] creatinine clearance (GFR can also be used in place of creatinine or CrCl)	≤1.5 X upper limit of normal (ULN) **OR** ≥60 mL/min for subject with creatinine levels > 1.5 X institutional ULN
Hepatic	
Serum total bilirubin	≤ 1.5 X ULN **OR** Direct bilirubin ≤ ULN for subjects with total bilirubin levels > 1.5 ULN
AST (SGOT) and ALT (SGPT)	≤ 2.5 X ULN **OR** ≤ 5 X ULN for subjects with liver metastases
Albumin	≥2.5 mg/dL
Coagulation	
International Normalized Ratio (INR) or Prothrombin Time (PT) Activated Partial Thromboplastin Time (aPTT)	≤1.5 X ULN unless subject is receiving anticoagulant therapy as long as PT or PTT is within therapeutic range of intended use of anticoagulants ≤1.5 X ULN unless subject is receiving anticoagulant therapy as long as PT or PTT is within therapeutic range of intended use of anticoagulants
[a] Creatinine clearance should be calculated per institutional standard.	

Table 2. **Adequate Organ Function Laboratory Values.**

12. Age ≥ 16 years.
13. ECOG performance status of 0 or 1.
14. Patients must consent and be willing to undergo tumor core needle biopsies at three timepoints (1. baseline, 2. prior to starting Cycle 3, and 3. at off-study). At least one tumor site must be amenable to biopsy in the judgment of the interventional radiologist.
15. Female subject of childbearing potential should have a negative urine or serum pregnancy within 72 hours prior to receiving the first dose of study medication. If the urine test is positive or cannot be confirmed as negative, a serum pregnancy test will be required.
16. Females of child bearing potential that are sexually active must agree to either practice 2 medically accepted highly effective methods of contraception at the same time or abstain from heterosexual intercourse from the time of signing the informed consent through 120 days after the last dose of study drug. See Appendix G for

protocol-approved highly effective methods of contraceptive combinations. Subjects of childbearing potential are those who have not been surgically sterilized or have not been free from menses for > 1 year.

a. Negative test for pregnancy is required of females of child-bearing potential; A female of child bearing potential is any woman, regardless of sexual orientation or whether they have undergone tubal ligation, who meets the following criteria: 1. has not undergone a hysterectomy or bilateral oophorectomy; or 2. has not been naturally postmenopausal for at least 24 consecutive months (i.e., has had menses at any time in the preceding 24 consecutive months or 730 days).
b. Conception while on treatment must be avoided

17. Male subjects should agree to use an adequate method of contraception starting with the first dose of study therapy through 120 days after the last dose of study therapy. Prior history of vasectomy does NOT replace requirement for contraceptive use.
18. Suitable venous access to allow for all study related blood sampling
19. Ability to understand and willingness to sign a written informed consent document.
20. For minors that are 16 to 18 years of age, assent **and** parental (or legally acceptable representative) written informed consent must be obtained.

5.2 **Exclusion Criteria**

1. Prior therapy with axitinib. Patients are permitted to have received prior tyrosine kinase inhibitor (TKI) therapy including imatinib, sunitinib, pazopanib, or similar. Patients may have received prior PD-1 or PD-L1 directed therapy.
2. Hypersensitivity to axitinib, pembrolizumab or any of its excipients.
3. Patients may not be receiving any other investigational agents (within 4 weeks prior to Cycle 1, day 1).
4. Prior anti-cancer monoclonal antibody (mAb) within 4 weeks prior to Cycle 1, day 1 or has not recovered (i.e., ≤ Grade 1 or at baseline) from adverse events due to agents administered more than 4 weeks earlier.
5. Patient has had prior chemotherapy, targeted small molecule therapy, or radiation therapy within 2 weeks prior to Cycle 1, Day 1 or has not recovered (i.e., ≤ Grade 1 or at baseline) from adverse events due to agents administered more than 4 weeks earlier. Subjects with ≤ Grade 2 neuropathy are an exception to this criterion and may qualify for the study. Note: If subject received major surgery, they must have recovered adequately from the toxicity and/or complications from the intervention prior to starting therapy.
6. Additional known malignancy that is progressing or requires active treatment. Exceptions include basal cell carcinoma of the skin, or squamous cell carcinoma of the skin that has undergone potentially curative therapy, or in situ cervical cancer.
7. Patients with end-organ dysfunction as defined in inclusion criterion (i.e. #11 above).
8. Patients with bone-only lesions.
9. Patients with underlying immune deficiency, chronic infections including HIV, hepatitis, or tuberculosis (TB) or autoimmune disease.

10. Patients with underlying hematologic issues including bleeding diathesis, known previous GI bleeding requiring intervention within the past 6 months, active pulmonary emboli or DVT that are not stable on anticoagulation regimen.
11. Has known history of, or any evidence of active, non-infectious pneumonitis.
12. Has known active central nervous system (CNS) metastases and/or carcinomatous meningitis or leptomeningeal disease. Subjects with previously treated brain metastases may participate provided they are stable (without evidence of progression by imaging for at least four weeks prior to the first dose of trial treatment and any neurologic symptoms have returned to baseline), have no evidence of new or enlarging brain metastases, and are not using steroids for at least 7 days prior to trial treatment. This exception does not include carcinomatous meningitis which is excluded regardless of clinical stability.
13. Concomitant (or receipt of) treatment with medications that may affect the metabolism of pembrolizumab and/or axitinib within 7 days prior to Cycle 1, day 1 of axitinib.
14. Has received a live vaccine within 30 days of planned start of study therapy. Note: Seasonal influenza vaccines for injection are generally inactivated flu vaccines and are allowed; however intranasal influenza vaccines (e.g., Flu-Mist®) are live attenuated vaccines, and are not allowed.
15. Is pregnant or breastfeeding, or expecting to conceive or father children within the projected duration of the trial, starting with the pre-screening or screening visit through 120 days after the last dose of trial treatment.
16. Any uncontrolled, intercurrent illness including but not limited to ongoing or active infection, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia
17. Prolonged QTc interval on Screening EKG >475 ms.
18. Ejection Fraction <40% by 2D ECHO at Screening.
19. Any serious medical or psychiatric illness/condition including substance use disorders likely in the judgment of the Investigator(s) to interfere or limit compliance with study requirements/treatment.
20. Has active autoimmune disease that has required systemic treatment in the past 2 years (i.e. with use of disease modifying agents, corticosteroids or immunosuppressive drugs). Replacement therapy (eg., thyroxine, insulin, or physiologic corticosteroid replacement therapy for adrenal or pituitary insufficiency, etc.) is not considered a form of systemic treatment.

6.0 ENROLLMENT PROCEDURES

To enter a patient, the Investigator or Study Team will contact the Clinical Research Services' (CRS) Representative. All eligibility requirements must be reviewed prior to the patient entering the study. The following information must be provided to the CRS Representative:

1) Completed and signed protocol-specific eligibility checklist;
2) All pages of the original signed informed consent form (ICF) including HIPAA Form B;
3) Relevant source documents including but limited to: subject medical history and physical exam, concomitant therapy, admission or discharge notes, diagnostic reports, pathologic confirmation of diagnosis, and relevant subject-specific written communication.

6.1 Cancellation Guidelines

If a patient does not receive protocol therapy, the patient may withdraw. Contact the CRS Representative, or e-mail the information including the reasons for withdrawal within 10-business days.

6.2 Emergency Registration

If an emergency registration takes place after business hours, the items listed above must be submitted by the next business day.

6.3 Enrollment Procedure(s)

After all Enrollment Guidelines are met as described (above) a CRS Clinical Research Coordinator (CRC) will enroll the patient by entering the requisite information into the informatics software system (e.g. CIERRA) developed by the SCCC-Informatics Office to implement enrollment. This system will automatically generate a secure e-mail message to the appropriate study team members, i.e. CRC, CRORS, and the Research Pharmacist(s).

Study treatment must be initiated within seven (7) days of enrollment.

7.0 STUDY DESIGN

The study will be a single-institution, open-label, single-arm phase II study. Since the primary endpoint is survival outcome (PFS) sample size calculation is based on a single-arm survival design. We will employ early stopping rules for lack of efficacy, based on previously reported historical controls (19% PFS at 3 months) and a large database suggesting that a progression-free rate at 3 months of >40% correlates with an active drug in the second-line setting for patients with advanced sarcoma (43). Patients will be treated with twice daily dosing of axitinib alone for the first 7 days, followed by concurrent axitinib administered twice daily at 5 mg PO twice daily, plus intravenous administration of pembrolizumab every 21 days (Figure 3). Patients will be assessed every three weeks for toxicity. After the first five patients are enrolled, we will assess safety of the combination. If 2 or fewer patients exhibit DLT, we will

then proceed with intrapatient titration of axitinib dosing at each cycle based on the presence or absence of predefined toxicities (Section 7.1, Table 3). Correlative studies characterizing T-cells in tumor tissue and in peripheral blood will be performed at three timepoints: 1. pre-treatment, 2. on-treatment on cycle 3 day 1, and 3. off-study. Additional exploratory imaging investigations, and assessment of circulating tumor cells are included for all patients (See Appendix F for complete description of correlative studies).

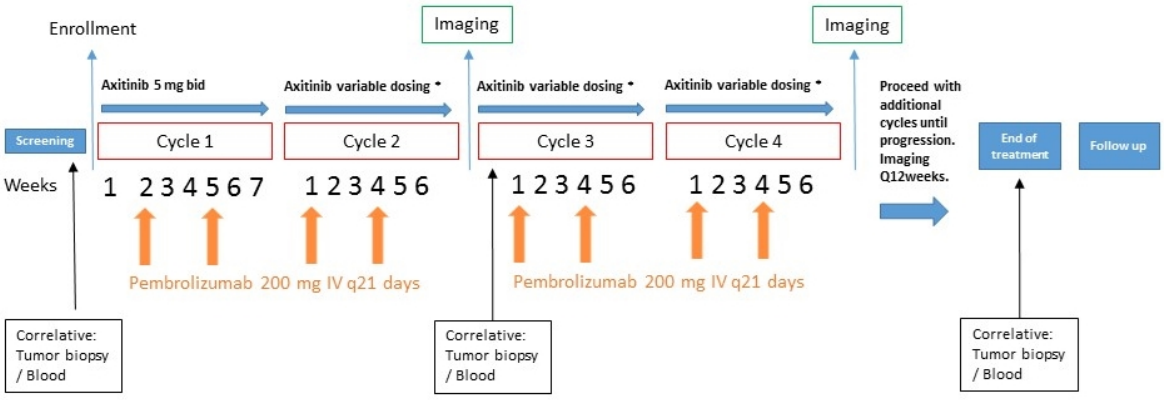


Figure 3. **Schematic diagram of the study.** The first cycle of treatment will be seven weeks, with one week of axitinib alone prior to initiation of pembrolizumab. Subsequently, six-week cycles of treatment consist of twice daily oral doses of axitinib administered continuously with intravenous (I.V.) administration of pembrolizumab on day 1 and 22. The first five evaluable patients will be considered a safety lead-in, and will not have axitinib dose escalation above 5 mg PO bid. If 2 or fewer patients in the safety lead-in develop DLT, axitinib will then be individually titrated based on toxicity beginning on cycle 2 day 1.

7.1 Axitinib Dose Titration with Flat Dose of Pembrolizumab

In trials with dose escalation of pembrolizumab, no difference in response nor toxicity was noted in melanoma patients randomized to either 2 mg/kg every three weeks versus 10 mg/kg every three weeks (44) [4]. Thus, this trial will utilize flat dosing of pembrolizumab based on equivalent pharmacokinetics with 200 mg flat dose compared to FDA approved dose of 2 mg/kg (Merck, Investigators Brochure). However, increasing response rates were noted in patients who were escalated on axitinib therapy (17) [5]. Given the younger age in general of sarcoma patients and lack of other comorbid medical conditions, we will incorporate individualized dose titration of axitinib based on toxicity to maximize exposure and likelihood of response. Dose levels are summarized in Table 3. See Section 10.2 for toxicity criteria and dose titration rules.

Dose Level	Axitinib dose	Pembrolizumab dose
-3	2 mg PO bid	200 mg flat dose *
-2	3 mg PO bid	
-1	4 mg PO bid	
1 (Starting dose level)	5 mg PO bid (FDA-approved)	
2	6 mg PO bid	
3	7 mg PO bid	
4	8 mg PO bid	
5	10 mg PO bid (maximum)	

Table 3. **Axitinib Dose Levels.** *Fixed dose based on simulations of population PK model for pembrolizumab suggesting consistency with previous 2 mg/kg dose (see Merck Investigator's Brochure for pembrolizumab).

Based on the recently completed dose escalation trial of concurrent axitinib and pembrolizumab in renal cell carcinoma, estimated DLT rate is 30%. Thus, we will conduct a safety review after the first 5 patients enrolled on the study during the first stage to ensure similar rates of DLT are noted in our patient population.

7.2 Dose-Limiting Toxicity (DLT)

Dose-Limiting Toxicity (DLT) is defined as any one of the following Adverse Events (AEs) that occurs during the first cycle (49 days) and is considered by the Investigator to be related (possible, probable, or definite) to study drug, specifying attribution to axitinib, pembrolizumab, or both. Management and dose modifications associated with the above AEs are outlined in Section 10.0 (Treatment/Dose Modifications).

DLT is defined as any one of the following (Table 4 and 5):

Lab Toxicity

AE	Patient Population	Dose Limiting Toxicity
Anemia (Hemoglobin)	All	<6.5 g/dl
Platelet count decreased	All	Grade 4 (<25,000mm3)
Febrile neutropenia	All	≥ Grade 3
Acute kidney injury	Does not resolve within 7 days	≥ Grade 3 (>3 x above baseline)
Proteinuria	Does note resolve within 7 days	≥ Grade 3
Liver function abnormalities (transaminitis/ hyperbilirubinemia)	If grade 2 (>1.5 - 3.0 x ULN) at baseline, than any ≥50% increase from baseline that does not resolve within 7 days	>5 x ULN

Table 4. Summary of Lab DLTs.

Non-Lab Toxicity

AE	Patient Population	Dose Limiting Toxicity
Rash (acneiform or macro-papular)	All	≥ Grade 3
Palmar-plantar erythrodysesthesia syndrome	All	Grade 3
Hypertension	Uncontrollable to less than 150/90 with maximal medical management within 7 days	≥ Grade 3
Bronchopulmonary hemorrhage (hemoptysis)	All	≥ Grade 3
Mucositis oral (Stomatitis)	Non-responsive to maximal supportive medication within 7 days	≥ Grade 3
Other Non-Lab Toxicity	All	≥ Grade 3 with the EXCEPTION of the following: • nausea, vomiting, diarrhea, constipation or edema controllable with medication within 7 days • grade 3 fever in the absence of infection or neutropenia • tumor flare defined as local pain, irritation or rash localized at sites of known or suspected tumor • transient grade 3 infusion reaction • grade 3 thromboembolic event IF patient is asymptomatic and initiated on anticoagulation – as there is a high prevalence in sarcomas, and this is difficult to attribute to study drug.

Table 5. Summary of Non-Lab DLTs.

8.0 **TREATMENT PLAN**

A cycle of treatment will consist of concurrent axitinib and pembrolizumab therapy for 6 weeks (Figure 3, Table 6), except for cycle 1 which will be 7 weeks as detailed above. Administration of axitinib shall occur continuously with pembrolizumab administered on day 8 and day 29 of the first cycle, and days 1 and 22 of the following cycles. The first five patients will not be dose-escalated above 5 mg PO twice daily axitinib. If these patients exhibit acceptable rates of DLT, starting with patient 6, dose-escalation of axitinib will be permitted. No dose escalations will be permitted during cycle 1. On day 1 of cycle 2 and the subsequent cycles, dose escalation of axitinib is permitted if toxicity is within the specified criteria. No dose-escalation will be conducted in the middle of any cycle. Dose reduction or interruption is permitted in the case of toxicity at any time as per section 10.0.

Trial treatment should be administered after all procedures/assessments have been completed as detailed in Section 12.0. Trial treatment may be administered up to 3 days before or after the scheduled Day 1 of each cycle due to administrative (e.g. scheduling) reasons.

Cycle(s)	**1**							**2**						Imaging prior to every subsequent odd cycle (i.e. 3, 5, 7, 9, etc.)	**3, 4, 5, etc. until progression**					
Week(s)	1	2	3	4	5	6	7	1	2	3	4	5	6		1	2	3	4	5	6
Day(s)	1	8	15	22	29	36	43	1	8	15	22	29	36		1	8	15	22	29	36
Axitinib oral, BID continuously (uptitration permitted starting from cycle 2)	X	X	X	X	X	X	X	X	X	X	X	X	X		X	X	X	X	X	X
Pembrolizumab IV q21 days		X			X			X			X				X			X		

Table 6. Treatment Schema: Axitinib and Pembrolizumab Concurrent Therapy

Once patients have completed two years of combination treatment with axitinib/pembrolizumab, pembrolizumab treatment will be stopped and patients will be treated with axitinib alone until progression. Each treatment cycle is 84 days (12 weeks) in length. Imaging will be performed every 12 weeks (±7 days).

Cycle(s)	**1, 2, 3, etc. until progression**												Imaging every 12 weeks (+/- 7 days)
Week(s)	1	2	3	4	5	6	7	8	9	10	11	12	
Day(s)	1	8	15	22	29	36	43	50	57	64	71	78	
Axitinib oral, BID continuously	X	X	X	X	X	X	X	X	X	X	X	X	

Table 7. **Treatment Schema: Axitinib Monotherapy**

For patients who progress while on axitinib monotherapy, pembrolizumab can be re-introduced and patients can be treated with the axitinib/pembrolizumab combination treatment for up to 17 additional cycles (approximately 1 year). A cycle of treatment will consist of axitinib/ pembrolizumab combination therapy for 6 weeks (Table 8). Administration of axitinib shall occur continuously with pembrolizumab administered on days 1 and 22.

Once participants complete the additional year of axitinib/pembrolizumab treatment, they will continue with axitinib monotherapy indefinitely until they experience toxicity, withdrawal or consent, or as per the treating physician's discretion. The indefinite axitinib monotherapy treatment schema will be the same as for the axitinib monotherapy (**Table 7**).

Cycle(s)	**1, 2, 3, etc. up to 17 cycles (approximately 1 year)**						Imaging prior to every subsequent odd cycle (i.e. 3, 5, 7, 9, etc.)
Week(s)	1	2	3	4	5	6	
Day(s)	1	8	15	22	29	36	
Axitinib oral, BID continuously	X	X	X	X	X	X	
Pembrolizumab IV q21 days	X			X			

Table 8. Treatment Schema: Axitinib/Pembrolizumab Combination Treatment for Patients who Progress while on Axitinib Monotherapy

				First 2 years of combination Axitinib/Pembrolizumab treatment		Axitinib Monotherapy (until progression) ***		Combination Axitinib/Pembrolizumab treatment (Additional Year)	
Agent	**Premedications & Precautions**	**Dose**	**Route**	**Schedule**	**Cycle Length* ***	**Schedule**	**Cycle Length**	**Schedule**	**Cycle Length**
Axitinib	Premedicate with ondansetron 8 mg orally if needed for nausea Take daily doses 12 hours apart and swallow whole with or without food with a glass of water	* 5mg	PO BID	** Days 1-42 (week 1-6)	42 days (6 weeks) **	Days 1-84 (week 1-12)	84 days (12 weeks)	** Days 1-42 (week 1-6)	42 days (6 weeks) **
Pembrolizumab	Monitor for infusion reactions with hypersensitivity kit at bedside. Grade 2 infusion reactions will be premedicated with diphenhydramine 50 mg IV and acetaminophen 1000 mg PO prior to subsequent infusions. Diet: Subjects should maintain a normal diet unless modifications are required to manage an AE such as diarrhea, nausea or vomiting.	200 mg	IV infusion over 30 minutes (-5min/ +10min)	Cycle 1: Day 8, 29 Subsequent cycles: Day 1, 22		NA	NA	Each Cycle: Day 1, 22	

*Intrapatient dose escalation permitted after 5-patient safety lead-in as per protocol.
** Cycle 1 is 7 weeks with the first week consisting of axitinib alone.
*** Axitinib indefinite treatment will follow the same schedule as axitinib monotherapy

Table 9. Axitinib and Pembrolizumab Regimen Description

8.1 **Second Course Axitinib (Inlyta®)**

All participants who complete the first two years of axitinib/pembrolizumab combination treatment may be eligible for axitinib monotherapy treatment until progression.

For participants who progress while on the axitinib monotherapy treatment, pembrolizumab can be re-introduced and participants will be treated with the axitinib/pembrolizumab combination treatment for up to 17 additional cycles (approximately 1 year). Once participants complete the additional year of axitinib/pembrolizumab treatment, they will continue with axitinib monotherapy indefinitely until they experience toxicity, withdrawal or consent, or as per the treating physician's discretion.

This retreatment is termed the Second Course Phase of this study and is only available if the study remains open and the participant is receiving clinical benefit from treatment.

8.2 **Second Course Pembrolizumab**

All participants who complete the first two years of axitinib/pembrolizumab combination treatment may be eligible for pembrolizumab retreatment if they progress while on axitinib monotherapy treatment. Pembrolizumab will be given as an axitinib/pembrolizumab combination treatment for up to 17 additional cycles (approximately 1 year).

Pembrolizumab re-introduction will be given for participants who progress while on axtinib monotherapy. This retreatment is termed the Second Course Phase of this study and is only available if the study remains open and the participant is receiving clinical benefit from treatment.

8.3 **Axitinib (Inlyta®)**

Axitinib shall be administered in the outpatient setting as either 5mg or multiple 1 mg tablets, orally (PO) twice a day (BID) continuously, with dose titration as per section 10.0.

Patients are to be advised that daily doses of axitinib shall be taken 12 hours apart with or without food and swallowed whole with a glass of water.

For additional information on Axitinib including mechanism of action, drug metabolism, pharmacokinetics & toxicology, known side effects, composition, and storage recommendations, see [Section 9.1].

8.4 **Pembrolizumab (Keytruda®)**

Pembrolizumab 200 mg flat dose shall be administered in the outpatient setting as a 30 minute intravenous (IV) infusion, once every 21 days. The intravenous line should contain a sterile, non-pyrogenic, low-protein binding 0.2 micron to 5 micron in-line or

add-on filter. Do not co-administer other drugs through the same infusion line. Every effort to target infusion timing to be as close to 30 minutes as possible, should be made. However, given the variability of infusion pumps from site to site, a window of -5 minutes and +10 minutes is permitted (i.e. infusion time is 30 minutes: -5 min/+10min).

For additional information on pembrolizumab including mechanism of action, drug metabolism, pharmacokinetics & toxicology, known side effects, composition, and storage recommendations, see [Section 9.2].

8.5 Treatment Dispensation, Compliance and Accountability

Concurrent administration of axitinib/pembrolizumab therapy will continue for each cycle until criteria for treatment discontinuation is/are met (See section 11.0). Once patients have completed two years of combination treatment with axitinib/pembrolizumab, pembrolizumab treatment will be stopped and patients will be treated with axitinib alone. For patients who progress while being on treatment with axitinib monotherapy, pembrolizumab can be re-introduced with the axitinib treatment, and patients can be treated with the axitinib/pembrolizumab treatment for up to an additional 17 cycles (approximately 1 year).

8.5.1 Axitinib

During the first two years of axibitinib/pembrolizumab combination treatment, eligible patients will be dispensed the appropriate number of axitinib tablets required for at least 21 days of dosing plus an additional 3-day supply to account for visit scheduling.

Once participants have completed the first two years of axitinib/pembrolizumab combination treatment, eligible patients will be treated with axitinib monotherapy until progression. During this time, eligible patients will be dispensed the appropriate number of axitinib tablets required for at least 84 days of dosing plus an additional 3-day supply to account for visit scheduling.

Pembrolizumab will be re-introduced for participants who progress while being treated with axitinib monotherapy. Eligible patients will be dispensed the appropriate number of axitinib tablets required for at least 21 days of dosing plus an additional 3-day supply to account for visit scheduling.

Once participants complete the additional year of axitinib/pembrolizumab treatment, they will continue with axitinib monotherapy indefinitely until they experience toxicity, withdrawal or consent, or as per the treating physician's discretion. During this time, eligible patients will be dispensed the appropriate number of axitinib tablets required for at least 84 days of dosing plus an additional 3-day supply to account for visit scheduling

Treatment compliance will be assessed based on return of unused axitinib tablets and a dosing diary. Eligible patients will be given a dosing diary for each treatment cycle. Date, time, and dose should be recorded at each dosing time point to confirm that each BID dose of axitinib was taken for each day.

The medication diary will be reviewed weekly during each week of axitinib administration (at a minimum during the first 3 weeks of each cycle, or otherwise noted) and returned to clinic staff at the end of each cycle. See Appendix I for the dosing diary. During the first cycle of axitinib, or for the first three weeks after any dose escalation or reduction of axitinib, patients will also be asked to record their blood pressure at least once each day before their evening dose of axitinib. See also Appendix I for the blood pressure diary.

If a dose of axitinib is missed due to patient error/oversight, then he/she should take the axitinib dose within 2 hours of the missed dose. If more than 2 hours have elapsed, that missed dose should be omitted, the patient should resume treatment at the next scheduled dosing time point, and the reason for missed dose reported on the dosing diary. If the patient vomits, an additional dose should not be taken. The next prescribed dose should be taken at the usual time.

If a dose of axitinib is held due to AE, SAE, or at the investigator's discretion, then he/she should resume treatment at the investigators discretion taking into account all of the dose modification/discontinuation guidelines provided in Section 10.0 Treatment/Dose Modifications.

8.5.2 Pembrolizumab

Eligible subjects shall be treated with the investigational supply of pembrolizumab. Clinical supplies may not be used for any purpose other than that stated in the protocol.

Clinical supplies will be affixed with a clinical label in accordance with regulatory requirements. Drug identity (name, strength) is included in the label text. Clinical supplies must be stored in a secure, limited-access location under the storage conditions specified on the label.

Receipt and dispensing of investigational product must be recorded by authorized person(s) at the trial site. The Investigator is responsible for maintaining accurate records of the clinical supplies received from Merck, the amount dispensed to subjects and the amount remaining at the conclusion of the trial.

Upon completion or termination of the study, all unused and/or partially used investigational product will be destroyed at the site per institutional policy. It is the Investigator's responsibility to arrange for the disposal of all empty containers, provided that procedures for proper disposal have been established according to applicable federal, state, local and institutional guidelines and procedures, and provided that appropriate records of disposal are kept.

8.6 **Supportive Care Guidelines**

Subjects should receive appropriate supportive care measures as deemed necessary by the treating Investigator. Suggested supportive care measures for the management of adverse events (AEs) with potential immunologic etiology are outlined below. Where appropriate, these guidelines include the use of oral or intravenous treatment with

corticosteroids as well as additional anti-inflammatory agents if symptoms do not improve with administration of corticosteroids.

Note that several courses of steroid tapering may be necessary as symptoms may worsen when the steroid dose is decreased. For each disorder, attempts should be made to rule out other causes such as metastatic disease or bacterial or viral infection, which might require additional supportive care.

It may also become necessary to perform conditional procedures such as bronchoscopy, endoscopy, or skin photography as part of evaluation of the event. Suggested conditional procedures for Events of Clinical Interest (ECI) associated with pembrolizumab, can be found in Appendix H. As appropriate, supportive care can be found in Section 8.0 for Axitinib.

Diet: Subjects should maintain a normal diet unless modifications are required to manage an AE such as diarrhea, nausea or vomiting.

The treatment guidelines are intended to be applied when the Investigator(s) determine the events to be related (possible, probable or definite) to trial treatment. Note: if after an evaluation the event is determined not to be related to the trial treatment, the Investigator is instructed to follow the reporting guidance in Appendix A (and Section 15.0). Refer to Section 10.0 for dose modification(s).

8.4.1 Concurrent Medications

Because there is a potential for interaction of axitinib with other concomitantly administered drugs through the cytochrome P450 system, the case report form must capture the concurrent use of all other drugs, over-the-counter medications, or alternative therapies. The PI should be alerted if the patient is taking any agent know to affect or with the potential to affect selected CYP450 isoenzymes. Appendix E presents guidelines for identifying medications/substances that could potentially interact with the study agent, axitinib. CYP3A4/5 substrates are allowed as concomitant medication at physician discretion and will not be exclusionary for study participation.

Strong CYP3A4/5 Inhibitors: The concomitant use of strong CYP3A4/5 inhibitors should be avoided (e.g., ketoconazole, itraconazole, clarithromycin, atazanavir, indinavir, nefazodone, nelfinavir, ritonavir, saquinavir, telithromycin, and voriconazole). Selection of an alternate concomitant medication with no or minimal CYP3A4/5 inhibition potential is recommended. Although axitinib (INLYTA®) dose adjustment has not been studied in patients receiving strong CYP3A4/5 inhibitors, **if a strong CYP3A4/5 inhibitor must be co-administered, a dose decrease of INLYTA by approximately half is recommended**, as this dose reduction is predicted to adjust the axitinib area under the plasma concentration vs time curve (AUC) to the range observed without inhibitors. The subsequent doses can be increased or decreased based on individual safety and tolerability. If co-administration of the strong inhibitor is discontinued, the INLYTA dose should be returned (after 3 – 5 half-lives of the inhibitor) to that used prior to initiation of the strong CYP3A4/5 inhibitor.

Strong CYP3A4/5 Inducers: The concomitant use of strong CYP3A4/5 inducers should also be avoided (e.g. rifampin, dexamethasone, phenytoin, carbamazepine, rifabutin, rifapentin, phenobarbital and St. John's wort). Selection of concomitant medication with no or minimal CYP3A4/5 induction potential is recommended. Moderate CYP3A4/5 inducers (e.g. bosentan, efavirenz, etravirine, modafinil and nafcillin) should also be avoided if possible.

Appendix J provides a list of common CYP3A4 substrates, inhibitors and inducers.

8.7 Concomitant Medications/ Vaccinations

Medications or vaccinations specifically prohibited in the exclusion criteria are not allowed during the ongoing trial. If there is a clinical indication for one of these or other medications or vaccinations specifically prohibited during the trial, discontinuation from trial therapy or vaccination may be required. The Investigator should discuss any questions regarding this with the Principal Investigator (PI). The final decision on any supportive therapy or vaccination rests with these Investigator(s) and/or the subject's primary physician.

8.5.1 Acceptable Concomitant Medications

All treatments that the Investigator considers necessary for a subject's welfare may be administered at the discretion of the Investigator

8.5.2 Prohibited Concomitant Medications

Subjects are prohibited from receiving the following therapies during the Screening and Treatment phase (including retreatment for post-complete response relapse) of this trial:

- Antineoplastic systemic chemotherapy or biological therapy
- Immunotherapy not specified in this protocol
- Chemotherapy not specified in this protocol
- Investigational agents other than pembrolizumab and axitinib
- Radiation therapy
    - **Note**: Radiation therapy to a symptomatic solitary lesion or to the brain may be allowed at the Investigator's discretion
- Live vaccines within 30 days prior to the first dose of trial treatment and while participating in the trial. Examples of live vaccines include, but are not limited to, the following: measles, mumps, rubella, varicella/zoster, yellow fever, rabies, BCG, and typhoid vaccine.
- Systemic glucocorticoids for any purpose other than to modulate symptoms from an event of clinical interest of suspected immunologic etiology. The use of physiologic doses of corticosteroids may be approved after consultation with the sponsor and PI.

Subjects who, in the assessment by the investigator, require the use of any of the aforementioned treatments for clinical management should be removed from the

trial. Subjects may receive other medications that the investigator deems to be medically necessary.

The Exclusion Criteria describes other medications which are prohibited in this trial.

There are no prohibited therapies during the Post-Treatment Follow-up phase.

8.8 Duration of Treatment

Trial therapy will last until withdrawal of consent, disease progression and/or unacceptable toxicity, whichever occurs first.

8.9 Duration of Follow-Up

All subjects will be followed at approximately 30-days (+5 days) after the last dose of trial treatment or before the initiation of a new anti-cancer treatment, whichever occurs first.

Subjects who discontinue trial treatment for a reason other than disease progression will be assessed at least every 12 weeks (±7 days) to monitor disease status.

Every effort should be made to collect information regarding disease status until the start of new anti-neoplastic therapy, disease progression, death, end of the study (EoS) or if new anti-neoplastic treatment is initiated.

Following confirmed disease progression or initiation of new anti-cancer therapy, survival will be assessed (at a minimum) by telephone contact every 12 weeks (±7 days).

See Section 12.5 for further details on Follow-Up assessments.

8.10 Telehealth

Patients unable to attend in-person study visits may conduct study visits via telehealth. Additionally, study assessments may be conducted locally and provided to treating physician for review.

8.11 Shipping of Investigational Product

Axitinib may be shipped to participants as per University of Miami or Pfizer policies.

9.0 AGENTS (DRUG FORMULATION AND PROCUREMENT)

9.1 Axitinib

[Refer to the FDA-approved package insert for Axitinib (Inlyta®) for more information.]

9.1.1 Other name(s)

Inlyta ®

9.1.2 Mechanism of Action

INLYTA (axitinib) is a kinase inhibitor. Axitinib has the chemical name *N*-methyl-2-[3-((*E*)2-pyridin-2-yl-vinyl)-1*H*-indazol-6-ylsulfanyl]-benzamide. The molecular formula is C22H18N4OS and the molecular weight is 386.47 Daltons.

Axitinib has been shown to inhibit receptor tyrosine kinases including vascular endothelial growth factor receptors (VEGFR)-1, VEGFR-2, and VEGFR-3 at therapeutic plasma concentrations. These receptors are implicated in pathologic angiogenesis, tumor growth, and cancer progression. VEGF-mediated endothelial cell proliferation and survival were inhibited by axitinib *in vitro* and in mouse models. Axitinib was shown to inhibit tumor growth and phosphorylation of VEGFR-2 in tumor xenograft mouse models.

9.1.3 Drug Metabolism, Pharmacokinetics and Toxicology

The population pharmacokinetic analysis pooled data from 17 trials in healthy subjects and patients with cancer. A two-compartment disposition model with first-order absorption and lag-time adequately describes the axitinib concentration-time profile.

Absorption and Distribution: Following single oral 5-mg dose administration, the median Tmax ranged from 2.5 to 4.1 hours. Based on the plasma half-life, steady state is expected within 2 to 3 days of dosing. Dosing of axitinib at 5 mg twice daily resulted in approximately 1.4-fold accumulation compared to administration of a single dose. At steady state, axitinib exhibits approximately linear pharmacokinetics within the 1-mg to 20-mg dose range. The mean absolute bioavailability of axitinib after an oral 5 mg dose is 58%.

Compared to overnight fasting, administration of INLYTA with a moderate fat meal resulted in 10% lower AUC and a high fat, high-calorie meal resulted in 19% higher AUC. INLYTA can be administered with or without food *[see Dosage and Administration (2.1)]*.

Axitinib is highly bound (>99%) to human plasma proteins with preferential binding to albumin and moderate binding to α1-acid glycoprotein. In patients with advanced RCC (n=20), at the 5 mg twice daily dose in the fed state, the geometric mean (CV%) Cmax and AUC0-24 were 27.8 (79%) ng/mL and 265 (77%) ng.h/mL, respectively. The geometric mean (CV%) clearance and apparent volume of distribution were 38 (80%) L/h and 160 (105%) L, respectively.

Metabolism and Elimination: The plasma half life of INLYTA ranges from 2.5 to 6.1 hours. Axitinib is metabolized primarily in the liver by CYP3A4/5 and to a lesser extent by CYP1A2, CYP2C19, and UGT1A1. Following oral administration of a 5-mg radioactive dose of axitinib, approximately 41% of the radioactivity was recovered in feces and approximately 23% was recovered in urine. Unchanged axitinib, accounting for 12% of the dose, was the major component identified in feces. Unchanged axitinib was not detected in urine; the carboxylic acid and sulfoxide metabolites accounted for the majority of radioactivity in urine. In plasma, the N-glucuronide metabolite represented the predominant radioactive component (50% of circulating radioactivity) and unchanged axitinib and the sulfoxide metabolite each accounted for approximately 20% of the circulating radioactivity.

The sulfoxide and N-glucuronide metabolites show approximately ≥400-fold less *in vitro* potency against VEGFR-2 compared to axitinib.

9.1.4 Management of Agent-Specific Adverse Events

Hypertension and Hypertensive Crisis

In a controlled clinical study with INLYTA for the treatment of patients with RCC, hypertension was reported in 145/359 patients (40%) receiving INLYTA and 103/355 patients (29%) receiving sorafenib. Grade 3/4 hypertension was observed in 56/359 patients (16%) receiving INLYTA and 39/355 patients (11%) receiving sorafenib. Hypertensive crisis was reported in 2/359 patients (<1%) receiving INLYTA and none of the patients receiving sorafenib. The median onset time for hypertension (systolic blood pressure >150 mmHg or diastolic blood pressure >100 mmHg) was within the first month of the start of INLYTA treatment and blood pressure increases have been observed as early as 4 days after starting INLYTA. Hypertension was managed with standard antihypertensive therapy. Discontinuation of INLYTA treatment due to hypertension occurred in 1/359 patients (<1%) receiving INLYTA and none of the patients receiving sorafenib.

Blood pressure should be well-controlled prior to initiating INLYTA. Patients should be monitored for hypertension and treated as needed with standard anti-hypertensive therapy. In the case of persistent hypertension despite use of anti-hypertensive medications, reduce the INLYTA dose. Discontinue INLYTA if hypertension is severe and persistent despite anti-hypertensive therapy and dose reduction of INLYTA, and discontinuation should be considered if there is evidence of hypertensive crisis. If INLYTA is interrupted, patients receiving antihypertensive medications should be monitored for hypotension.

Aneurysms and artery dissections

The use of Vascular Endothelial Growth Factor (VEGF) pathway inhibitors in patients with or without hypertension may promote the formation of aneurysms and/or artery dissections. Before initiating INLYTA, this risk should be carefully considered in patients with risk factors such as hypertension or history of aneurysm.

Thyroid Dysfunction

In a controlled clinical study with INLYTA for the treatment of patients with RCC, hypothyroidism was reported in 69/359 patients (19%) receiving INLYTA and 29/355 patients (8%) receiving sorafenib. Hyperthyroidism was reported in 4/359 patients (1%) receiving INLYTA and 4/355 patients (1%) receiving sorafenib. In patients who had thyroid stimulating hormone (TSH) <5 µU/mL before treatment, elevations of TSH to ≥10 µU/mL occurred in 79/245 patients (32%) receiving INLYTA and 25/232 patients (11%) receiving sorafenib.

Monitor thyroid function before initiation of, and periodically throughout, treatment with INLYTA. Treat hypothyroidism and hyperthyroidism according to standard medical practice to maintain euthyroid state.

Arterial Thromboembolic Events

In Phase 3 study A4061032 with INLYTA for the treatment of patients with RCC, Grade 3/4 arterial thromboembolic events were reported in 4/359 patients (1%) receiving INLYTA. The most frequent arterial thromboembolic event was transient ischemic attack (1%). Fatal cerebrovascular accident was reported in 1/359 patients (<1%).

In pooled clinical studies with INLYTA for the treatment of patients with RCC, arterial thromboembolic events were reported in 19/672 patients (3%) receiving INLYTA. Grade 3 arterial thromboembolic events were reported in 8/672 patients (1%). Grade 4 arterial thromboembolic events were reported in 9/672 patients (1%). Fatal arterial thromboembolic events were reported in 2 patients (<1%) receiving INLYTA.

In monotherapy studies with INLYTA, arterial thromboembolic events (including transient ischemic attack, cerebrovascular accident, myocardial infarction, cerebral infarction, arterial embolism, lacunar infarction, and retinal artery occlusion) were reported in 16/699 subjects (2%).

INLYTA should be used with caution in patients who are at risk for, or who have a history of, these events. INLYTA has not been studied in patients who had an arterial thromboembolic event within the previous 12 months.

Venous Thromboembolic Events

In Phase 3 study A4061032 with INLYTA for the treatment of patients with RCC, venous thromboembolic events were reported in 11/359 patients (3%) receiving INLYTA. Grade 3/4 venous thromboembolic events (including pulmonary embolism, deep vein thrombosis, and retinal-vein occlusion/thrombosis) were reported in 9/359 patients (3%). Fatal pulmonary embolism was reported in 1/359 patients (<1%).

In pooled clinical studies with INLYTA for the treatment of patients with RCC, venous thromboembolic events were reported in 19/672 patients (3%) receiving INLYTA. Grade 3 venous thromboembolic events were reported in 6/672 patients (1%). Grade 4 venous thromboembolic events were reported in 8/672 patients (1%). Fatal venous thromboembolic events were reported in 1/672 patients (<1%) receiving INLYTA.

INLYTA should be used with caution in patients who are at risk for, or who have a history of, these events. INLYTA has not been studied in patients who had a venous thromboembolic event within the previous 6 months.

Elevation of Hemoglobin or Hematocrit

Increases in hemoglobin or hematocrit, reflective of increases in red blood cell mass, may occur during treatment with INLYTA. An increase in red blood cell mass may increase the risk of thromboembolic events.

In Phase 3 study A4061032 with INLYTA for treatment of patients with RCC, elevated hemoglobin above the upper limit of normal (ULN) was observed in 31/320 patients (10%) receiving INLYTA.

Monitor hemoglobin or hematocrit before initiation of, and periodically throughout, treatment with INLYTA. If hemoglobin or hematocrit becomes elevated above the normal level, patients should be treated according to standard medical practice to decrease hemoglobin or
hematocrit to an acceptable level.

Hemorrhage

In Phase 3 study A4061032 with INLYTA for the treatment of patients with RCC, hemorrhagic events were reported in 58/359 patients (16%) receiving INLYTA. The most common hemorrhagic events in patients treated with INLYTA were epistaxis (6%), hematuria (3%), hemoptysis (2%), and rectal hemorrhage (2%). Grade 3/4 hemorrhagic events (including cerebral hemorrhage, haematuria, hemoptysis, lower gastrointestinal hemorrhage, and melaena) were reported in 5/359 (1%) patients. Fatal hemorrhage (gastric hemorrhage) was reported in 1/359 patients (<1%) receiving INLYTA.

In pooled clinical studies with INLYTA for the treatment of patients with RCC, hemorrhagic events were reported in 173/672 patients (26%) receiving INLYTA. Grade 3 hemorrhagic events were reported in 20/672 patients (3%). Grade 4 hemorrhagic events were reported in 7/672 patients (1%) and fatal hemorrhagic events were reported in 3/672 patients (<1%) receiving INLYTA.

INLYTA has not been studied in patients who have evidence of untreated brain metastasis or recent active gastrointestinal bleeding and should not be used in those patients. If any
bleeding requires medical intervention, temporarily interrupt the INLYTA dose.

Gastrointestinal Perforation and Fistula Formation

In Phase 3 study A4061032 with INLYTA for the treatment of patients with RCC, gastrointestinal perforation was reported in 1/359 patients (<1%) receiving

INLYTA. In addition to cases of gastrointestinal perforation, fistulas were reported in 2/359 patients (1%). In pooled clinical studies with INLYTA for the treatment of patients with RCC, gastrointestinal perforation and fistula were reported in 13/672 patients (2%) receiving INLYTA. In monotherapy studies with INLYTA (N = 699), fatal gastrointestinal perforation was reported in 1/699 patient (<1%).

Monitor for symptoms of gastrointestinal perforation periodically throughout treatment with INLYTA.

Wound Healing Complications

No formal studies of the effect of INLYTA on wound healing have been conducted. In Phase 3 study A4061032 with INLYTA for the treatment of patients with RCC, all-causality adverse events suggestive of wound healing complications were reported in 4/359 patients (1%).

Treatment with INLYTA should be stopped at least 24 hours prior to scheduled surgery. The decision to resume INLYTA therapy after surgery should be based on clinical judgment of
adequate wound healing.

Reversible Posterior Leukoencephalopathy Syndrome/Posterior Reversible Encephalopathy Syndrome

In Phase 3 study A4061032 with INLYTA for the treatment of patients with RCC, reversible posterior leukoencephalopathy syndrome (RPLS)/posterior reversible encephalopathy syndrome (PRES) was reported in 1/359 patients (<1%).

In pooled clinical studies with INLYTA for the treatment of patients with RCC, RPLS was reported in 2/672 patients (<1%) receiving INLYTA.

RPLS/PRES is a neurological disorder which can present with headache, seizure, lethargy, confusion, blindness and other visual and neurologic disturbances. Mild to severe hypertension may be present. Magnetic resonance imaging is necessary to confirm the diagnosis of RPLS/PRES. In patients with signs/symptoms of RPLS/PRES, temporarily interrupt or permanently discontinue INLYTA. The safety of reinitiating INLYTA therapy in patients previously experiencing RPLS/PRES is not known.

Proteinuria

In a controlled clinical study with INLYTA for the treatment of patients with RCC, proteinuria was reported in 39/359 patients (11%) receiving INLYTA and 26/355 patients (7%) receiving sorafenib. Grade 3 proteinuria was reported in 11/359 patients (3%) receiving INLYTA and 6/355 patients (2%) receiving sorafenib.

Monitoring for proteinuria before initiation of, and periodically throughout, treatment with INLYTA is recommended. For patients who develop moderate to severe proteinuria, reduce the dose or temporarily interrupt INLYTA treatment.

Elevation of Liver Enzymes

In a controlled clinical study with INLYTA for the treatment of patients with RCC, alanine aminotransferase (ALT) elevations of all grades occurred in 22% of patients on both arms, with Grade 3/4 events in <1% of patients on the INLYTA arm and 2% of patients on the sorafenib arm.

Monitor ALT, aspartate aminotransferase (AST) and bilirubin before initiation of and periodically throughout treatment with INLYTA.

Hepatic Impairment

In clinical studies with INLYTA, the systemic exposure to INLYTA was approximately 2-fold higher in subjects with moderate hepatic impairment (Child-Pugh class B) compared to subjects with normal hepatic function. A dose decrease is recommended when administering INLYTA to patients with moderate hepatic impairment (Child-Pugh class B). INLYTA has not been studied in patients with severe hepatic impairment (Child-Pugh class C).

9.1.5 Composition

Axitinib is a white to light-yellow powder with a pKa of 4.8. The solubility of axitinib in aqueous media over the range pH 1.1 to pH 7.8 is in excess of 0.2 µg/mL. The partition coefficient (noctanol/water) is 3.5.

INLYTA is supplied as red, film-coated tablets containing either 1 mg or 5 mg of axitinib together with microcrystalline cellulose, lactose monohydrate, croscarmellose sodium, magnesium stearate, and Opadry® II red 32K15441 as inactive ingredients. The Opadry II red 32K15441 film coating contains lactose monohydrate, HPMC 2910/Hypromellose 15cP, titanium dioxide, triacetin (glycerol triacetate), and red iron oxide.

9.1.6 Storage Recommendations and Dosage Forms

INLYTA tablets are supplied as follows:

1 mg tablets are red film-coated, oval tablets debossed with “Pfizer” on one side and “1 XNB” on the other; available in bottles of 180: NDC 0069-0145-01.

5 mg tablets are red film-coated, triangular tablets debossed with “Pfizer” on one side and “5 XNB” on the other; available in bottles of 60: NDC 0069-0151-11.

Store at 20°C to 25°C (68°F to 77°F); excursions permitted to 15°C to 30°C (59°F to 86°F) [see USP Controlled Room Temperature].

9.1.7 Dispensation and Accountability

For this study, axitinib (Inlyta®) will be provided by Pfizer as 1 mg and/or 5 mg tablets. (See also Section 8.5.1 Treatment Dispensation, Compliance and Accountability for Axitinib.)

9.2 **Pembrolizumab**

[Refer to the FDA-approved package insert for pembrolizumab (Keytruda®) for more information.]

9.2.1 Other name(s)

MK-3475, Keytruda ®

9.2.2 Mechanism of Action

MK-3475 is a potent and highly selective humanized mAb designed to block the interaction between PD-1 and its ligands, PD-L1 and PD-L2. MK-3475 potently blocks binding to both ligands with half maximal inhibitory concentration (IC50) values below 1 nM. MK-3475 enhances T cell responses in human donor blood cell cultures with an EC50 of ~0.1 to 0.3 nM. MK-3475 binds to cynomolgus PD-1 with similar affinity, blocking activity, and demonstrates equivalent enhancement of cynomolgus T cell responses. It does not cross-react with rodent PD-1. MK-3475 strongly enhances T lymphocyte immune responses in cultured blood cells from healthy human donors, cancer patients, and primates. The antibody potentiates existing immune responses only in the presence of antigen-receptor stimulation and does not nonspecifically activate all T cells. Using an anti-mouse PD-1 analog antibody, PD-1 blockade is demonstrated to significantly inhibit tumor growth in a variety of syngeneic murine tumor models. In experiments in mice, anti-PD-1 therapy is synergistic with chemotherapeutic agents such as gemcitabine and 5-FU and combination therapy results in increased efficacy and increased complete regression rates in vivo.

Pembrolizumab is generally well tolerated and demonstrates a favorable safety profile in comparison to chemotherapy. Pembrolizumab is an immunomodulatory agent, and based on this mechanism of action, immune mediated adverse events are of primary concern.

9.2.3 Drug Metabolism, Pharmacokinetics and Toxicology

No traditional metabolism studies were conducted with pembrolizumab per current ICH S6 (R1) guidance on preclinical safety evaluation of biotechnology-derived pharmaceuticals. However, in vivo studies were conducted in C.B17 SCID mice to demonstrate the lack of Fab-arm or half molecule exchange for pembrolizumab. IgG4 wild type molecules can undergo in vitro and in vivo molecular rearrangement called Fab-arm (or half molecule) exchange by swapping their half molecule with other IgG4 half molecules, thereby generating bispecific or hybrid antibodies. A point mutation (S228P) in the core hinge region in IgG4 has been shown to be sufficient to prevent the Fab-arm exchange. The results supported that

pembrolizumab, which has a hinge mutation from S to P at position 228, did not form detectable hybrid antibodies with co-administered wild type IgG4 molecules in vivo in SCID mice. This observation is consistent with the results of extensive in vitro characterization of pembrolizumab and indicates that pembrolizumab is not likely to engage in Fab-arm exchange in humans.

The pharmacokinetics of pembrolizumab was studied in 479 patients who received doses of 1 to 10 mg/kg every 2 weeks or 2 to 10 mg/kg every 3 weeks. Based on a population pharmacokinetic analysis, the mean [% coefficient of variation (CV%)] clearance (CL) is 0.22 L/day (28%) and the mean (CV%) elimination half-life (t1/2) is 26 days (24%). Steady-state concentrations of pembrolizumab were reached by 18 weeks of repeated dosing with an every 3-week regimen and the systemic accumulation was 2.1-fold. The peak concentration (Cmax), trough concentration (Cmin), and area under the plasma concentration versus time curve at steady state (AUCss) of pembrolizumab increased dose proportionally in the dose range of 2 to 10 mg/kg every 3 weeks. In animal models, inhibition of PD-1 signaling resulted in an increased incidence of infections and enhanced inflammatory responses. M. tuberculosis-infected PD-1 knockout mice exhibit markedly decreased survival compared with wild-type controls, which correlated with increased bacterial proliferation and inflammatory responses in these animals. PD-1 knockout mice have also shown decreased survival following infection with lymphocytic choriomeningitis virus (LCMV). Administration of pembrolizumab in chimpanzees with naturally occurring chronic hepatitis B infection resulted in two out of four animals with significantly increased levels of serum ALT, AST, and GGT, which persisted for at least 1 month after discontinuation of pembrolizumab.

The preclinical toxicology program for pembrolizumab is compliant with preclinical safety assessment guidelines for biotechnology products. Preclinical toxicity studies consisted of a 1-month repeat-dose study with a 4-month recovery period in Cynomolgus monkeys and a 6-month repeat-dose study with a 4-month recovery period in Cynomolgus monkeys. Additional studies consist of tissue cross-reactivity studies in both normal human and Cynomolgus monkey tissues. In conclusion, pembrolizumab administered once every other week over 6-month duration to Cynomolgus monkeys was well tolerated, with systemic exposure (AUC) up to approximately 67,500 µg.day/mL, and the no observed adverse effect level (NOAEL) was ≥200mg/kg/dose (the highest dose tested).

9.2.4 Management of Agent-Specific Adverse Events

Important identified risks for pembrolizumab are of an immune mediate nature, including: pneumonitis, colitis, thyroid disorders (hypothryoidism/hyperthyroidism), hepatitis, hypophysitis, Type I diabetes mellitis, uveitis, and nephritis. After a recent review of data, events newly characterized as identified risks also include pancreatitis, myositis, and severe skin reaction; these are included in the reference safety information below. The majority of immune-mediated adverse events were mild to moderate in severity, were

manageable with appropriate care, and rarely required discontinuation of therapy. Further details around frequency, reporting, and management of immune-related adverse events (irAEs) are described below. In addition to the previously noted identified risks, infusion-related reactions are a risk but are not considered immune mediated; these are further described below. (An irAE is defined as a clinically significant AE of any organ that is associated with study drug exposure, is of unknown etiology, and is consistent with an immune-related mechanism.)

Clinical Trials Experience

The safety of KEYTRUDA was investigated in two controlled, randomized studies (KEYNOTE-002 and KEYNOTE-006) for the treatment of unresectable or metastatic melanoma and in an uncontrolled, open-label study (KEYNOTE-001) for the treatment of unresectable or metastatic melanoma and metastatic non-small cell lung carcinoma (NSCLC). Overall, 1567 subjects with melanoma (699 previously treated with ipilimumab and 868 naïve to ipilimumab) and 550 subjects with NSCLC were treated. Safety is described for the pooled population of 2117 subjects (studied across three doses; 2 mg/kg every 3 weeks and 10 mg/kg every 2 or 3 weeks). The median treatment duration was 4.6 months (range 1 day to 28.3 months) including 906 subjects treated for greater than or equal to 6 months and 203 subjects treated for greater than or equal to one year.

KEYTRUDA was discontinued for treatment-related adverse reactions in 4% of subjects. Treatment-related serious adverse events (SAEs) reported up to 90 days after the last dose occurred in 9% of subjects receiving KEYTRUDA. Of these treatment-related SAEs, those occurring in more than five subjects (out of 2117) were pneumonitis (n=24), colitis (n=19), diarrhea (n=16), pyrexia (n=8), adrenal insufficiency (n=6) and autoimmune hepatitis (n=6).

Immune-Related Adverse Events (irAEs)

An irAE is defined as a clinically significant AE of any organ that is associated with study drug exposure, is of unknown etiology, and is consistent with an immune-related mechanism. AEs of Special Interest (AEOSI) data for melanoma and lung subjects demonstrates that irAEs were reported in 16.1% of subjects (251 of 1562) overall; AEOSI were considered by the Investigators to be drug related in 14.3% of subjects (223 of 1562). The majority of AEOSI were Grade 1 or 2 in severity. Overall, serious AEOSI occurred in 4.2% of subjects at 2 mg/kg Q3W, 3.7% of subjects at 10 mg/kg Q3W, and 3.9% of subjects at 10 mg/kg Q2W. There was one AEOSI (pneumonitis) related to death in 10 mg/kg Q3W arm, in a subject with NSCLC.

The rate of discontinuation due to AEOSI was low (2.6%). The most commonly reported immune-related adverse events across the dose schedules are hypothyroidism (7.2%), pneumonitis (2.9%), hyperthyroidism (2.2%), colitis (1.3%) and skin AEOSI (1.3% including all terms). Based on the mechanism of

action of MK-3475 and similar immunomodulatory agents, the Sponsor is interested in potential irAEs, and encourages appropriate investigation of signs and symptoms suggestive of these.

Consultation with the appropriate medical specialist should be considered when investigating a possible irAE. These events can occur after the first dose to several months after the last dose of treatment. Mild irAEs are usually treated symptomatically and do not require dosing delays or discontinuation. Higher grade and persistent lower grade irAEs typically necessitate withholding or discontinuing treatment and administration of systemic steroids or other immunosuppressive agents (such as tumor necrosis factor blockers), when systemic steroids are not effective. Early recognition of irAEs and initiation of treatment are critical to reduce the risk of complications, since the majority of irAEs are reversible with the use of steroids and other immune suppressants.

Identification and Treatment of irAEs
If an irAE is suspected, a thorough evaluation should be conducted in an effort to possibly rule out neoplastic, infectious, metabolic, toxin or other etiologic causes prior to diagnosing an irAE. Serological, immunological, and histological (biopsy) data should be considered to support the diagnosis of an immune-related toxicity.

A separate document entitled Pembrolizumab Program (MK-3475) Event of Clinical Interest Guidance Document is maintained and distributed to investigational sites along with this IB and study protocols. Please refer to this guidance document in conjunction with this IB when assessing irAEs. Updates and modifications to the irAE guidance document will be made and distributed to Investigators to provide the most current information, and therefore will be independent of the IB update cycle.

It is possible that irAEs other than those listed in the guidance document may be observed in subjects receiving pembrolizumab; therefore, all AEs of unknown etiology associated with drug exposure should be evaluated to determine if it is possibly immune related. This is meant to be a general guidance; therefore, recommendations in the current document might not be all inclusive. As such Investigators are encouraged to contact a Merck Clinical Monitor as needed to discuss cases that warrant separate discussion outside of the scope of current guidelines. Permanent discontinuation of pembrolizumab due to irAE may be subject of discussion between the SPONSOR and treating Investigator. The general approach to handling irAEs can be found in Appendix I.

All AEs are to be graded according to National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE), version 4.0 (http://ctep.cancer.gov).

If an irAE does not resolve or improve to ≤ Grade 1 within 12 weeks after last administration of pembrolizumab, study therapy discontinuation should be considered after discussion with a Merck Clinical Director.

Immune-Mediated Pneumonitis
Pneumonitis occurred in 12 (2.9%) of 411 melanoma patients, including Grade 2 or 3 cases in 8 (1.9%) and 1 (0.2%) patients, respectively, receiving KEYTRUDA in Trial 1. The median time to development of pneumonitis was 5 months (range 0.3 weeks-9.9 months). The median duration was 4.9 months (range 1 week-14.4 months). Five of eight patients with Grade 2 and the one patient with Grade 3 pneumonitis required initial treatment with high-dose systemic corticosteroids (greater than or equal to 40 mg prednisone or equivalent per day) followed by a corticosteroid taper. The median initial dose of high-dose corticosteroid treatment was 63.4 mg/day of prednisone or equivalent with a median duration of treatment of 3 days (range 1-34) followed by a corticosteroid taper. Pneumonitis led to discontinuation of KEYTRUDA in 3 (0.7%) patients. Pneumonitis completely resolved in seven of the nine patients with Grade 2-3 pneumonitis. Pneumonitis (including fatal cases) has been reported in subjects receiving KEYTRUDA. Monitor patients for signs and symptoms of pneumonitis. Evaluate patients with suspected pneumonitis with radiographic imaging and administer corticosteroids for Grade 2 or greater pneumonitis (initial dose of 1-2 mg/kg/day prednisone or equivalent followed by a taper). Withhold KEYTRUDA for moderate (Grade 2) pneumonitis, and permanently discontinue KEYTRUDA for severe (Grade 3), life-threatening (Grade 4) or recurrent moderate (Grade 2) pneumonitis. (See Immune-mediated adverse reactions below.)

Immune-Mediated Colitis
Colitis (including microscopic colitis) occurred in 4 (1%) of 411 patients, including Grade 2 or 3 cases in 1 (0.2%) and 2 (0.5%) patients, respectively, receiving KEYTRUDA in Trial 1. The median time to onset of colitis was 6.5 months (range 2.3-9.8). The median duration was 2.6 months (range 0.6 weeks-3.6 months). All three patients with Grade 2 or 3 colitis were treated with high-dose corticosteroids (greater than or equal to 40 mg prednisone or quivalent per day) with a median initial dose of 70 mg/day of prednisone or equivalent; the median duration of initial treatment was 7 days (range 4-41), followed by a corticosteroid taper. One patient (0.2%) required permanent discontinuation of KEYTRUDA due to colitis. All four patients with colitis experienced complete resolution of the event. Monitor patients for signs and symptoms of colitis. Administer corticosteroids for Grade 2 or greater colitis. Withhold KEYTRUDA for moderate (Grade 2) or severe (Grade 3) colitis, and permanently discontinue KEYTRUDA for life-threatening (Grade 4) colitis.

Immune-Mediated Hepatitis
Hepatitis (including autoimmune hepatitis) occurred in 2 (0.5%) of 411 patients, including a Grade 4 case in 1 (0.2%) patient, receiving KEYTRUDA in Trial 1. The time to onset was 22 days for the case of Grade 4 hepatitis which lasted 1.1 months. The patient with Grade 4 hepatitis permanently discontinued KEYTRUDA and was

treated with high-dose (greater than or equal to 40 mg prednisone or equivalent per day) systemic corticosteroids followed by a corticosteroid taper. Both patients with hepatitis experienced complete resolution of the event. Monitor patients for changes in liver function. Administer corticosteroids for Grade 2 or greater hepatitis and, based on severity of liver enzyme elevations, withhold or discontinue KEYTRUDA.

Immune-Mediated Hypophysitis
Hypophysitis occurred in 2 (0.5%) of 411 patients, consisting of one Grade 2 and one Grade 4 case (0.2% each), in patients receiving KEYTRUDA in Trial 1. The time to onset was 1.7 months for the patient with Grade 4 hypophysitis and 1.3 months for the patient with Grade 2 hypophysitis. Both patients were treated with high-dose (greater than or equal to 40 mg prednisone or equivalent per day) corticosteroids followed by a corticosteroid taper and remained on a physiologic replacement dose. Monitor for signs and symptoms of hypophysitis. Administer corticosteroids for Grade 2 or greater hypophysitis. Withhold KEYTRUDA for moderate (Grade 2) hypophysitis, withhold or discontinue KEYTRUDA for severe (Grade 3) hypophysitis, and permanently discontinue KEYTRUDA for lifethreatening (Grade 4) hypophysitis.

Renal Failure and Immune-Mediated Nephritis
Nephritis occurred in 3 (0.7%) patients, consisting of one case of Grade 2 autoimmune nephritis (0.2%) and two cases of interstitial nephritis with renal failure (0.5%), one Grade 3 and one Grade 4. The time to onset of autoimmune nephritis was 11.6 months after the first dose of KEYTRUDA (5 months after the last dose) and lasted 3.2 months; this patient did not have a biopsy. Acute interstitial nephritis was confirmed by renal biopsy in two patients with Grades 3-4 renal failure. All three patients fully recovered renal function with treatment with high-dose corticosteroids (greater than or equal to 40 mg prednisone or equivalent per day) followed by a corticosteroid taper.
Monitor patients for changes in renal function. Administer corticosteroids for Grade 2 or greater nephritis. Withhold KEYTRUDA for moderate (Grade 2) nephritis, and permanently discontinue KEYTRUDA for severe (Grade 3), or life-threatening (Grade 4) nephritis.

Immune-Mediated Hyperthyroidism and Hypothyroidism
Hyperthyroidism occurred in 5 (1.2%) of 411 patients, including Grade 2 or 3 cases in 2 (0.5%) and 1 (0.2%) patients, respectively, receiving KEYTRUDA in Trial 1. The median time to onset was 1.5 months (range 0.5-2.1). The median duration was 2.8 months (range 0.9 to 6.1). One of two patients with Grade 2 and the one patient with Grade 3 hyperthyroidism required initial treatment with high-dose corticosteroids (greater than or equal to 40 mg prednisone or equivalent per day) followed by a corticosteroid taper. One patient (0.2%) required permanent discontinuation of KEYTRUDA due to hyperthyroidism. All five patients with hyperthyroidism experienced complete resolution of the event. Hypothyroidism occurred in 34 (8.3%) of 411 patients, including a Grade 3 case in 1 (0.2%) patient,

receiving KEYTRUDA in Trial 1. The median time to onset of hypothyroidism was 3.5 months (range 0.7 weeks-19 months). All but two of the patients with hypothyroidism were treated with long-term thyroid hormone replacement therapy. The other two patients only required short-term thyroid hormone replacement therapy. No patient received corticosteroids or discontinued KEYTRUDA for management of hypothyroidism. Thyroid disorders can occur at any time during treatment. Monitor patients for changes in thyroid function (at the start of treatment, periodically during treatment, and as indicated based on clinical evaluation) and for clinical signs and symptoms of thyroid disorders. Administer corticosteroids for Grade 3 or greater hyperthyroidism, withhold KEYTRUDA for severe (Grade 3) hyperthyroidism, and permanently discontinue KEYTRUDA for life-threatening (Grade 4) hyperthyroidism. Isolated hypothyroidism may be managed with replacement therapy without treatment interruption and without corticosteroids.

Other Immune-Mediated Adverse Reactions
Other clinically important immune-mediated adverse reactions can occur. The following clinically significant, immune-mediated adverse reactions occurred in less than 1% of patients treated with KEYTRUDA in Trial 1: exfoliative dermatitis, uveitis, arthritis, myositis, pancreatitis, hemolytic anemia, partial seizures arising in a patient with inflammatory foci in brain parenchyma, Guillain-Barré syndrome, vitiligo and adrenal insufficiency. Across clinical studies with KEYTRUDA in approximately 2000 patients, the following additional clinically significant, immune-mediated adverse reactions were reported in less than 1% of patients: myasthenic syndrome, optic neuritis, and rhabdomyolysis. For suspected immune-mediated adverse reactions, ensure adequate evaluation to confirm etiology or exclude other causes. Based on the severity of the adverse reaction, withhold KEYTRUDA and administer corticosteroids. Upon improvement to Grade 1 or less, initiate corticosteroid taper and continue to taper over at least 1 month. Based on limited data from clinical studies in subjects whose immune-related adverse reactions could not be controlled with corticosteroid use, administration of other systemic immunosuppressants can be considered. Restart KEYTRUDA if the adverse reaction remains at Grade 1 or less. Permanently discontinue KEYTRUDA for any severe or Grade 3 immune-mediated adverse reaction that recurs and for any life-threatening immune-mediated adverse reaction

Infusion Reactions
Infusion reactions have been reported with pembrolizumab at a rate of 2.5%; these were generally Grade 1 and 2 and the majority were considered related by the Investigator. One event of Grade 4 anaphylaxis has been reported. Infusion reactions may present as allergic reaction, serum sickness, infusion reaction, cytokine release syndrome, or anaphylaxis. Mild infusion reactions can generally be treated with interruption of the infusion and medical intervention including IV fluids, antihistamines, nonsteroidal antiinflammatory drugs, acetaminophen, and narcotics as needed. More severe or life thereatening reactions may require pressors, corticosteroids, and epinephrine. Pembrolizumab therapy should not be redosed in these more severe cases.

Stevens-Johnson Syndrome (SJS) and Toxic Epidermal Necrolysis (TEN)
One fatal case of SJS in a clinical trial and one fatal case of TEN in the post-marketing setting have been reported in patients treated with Pembrolizumab. In total 8 cases of SJS and 2 cases of TEN all of which were serious. The risk of SJS and TEN is reported at approximately 0.4 – 7 cases per million patient years in the general adult population. Independent risk factors include certain medications such as anticolvulsants, sulfonamines, aminopenicillins, allopurinol and NSAIDs. Non-medication triggers include infection, contrast media, and vaccinations. Malignancy is associated with an increased mortality rate in patients with SJS and TEN.
Monitor patients for signs and symptoms of SJS and/or TEN, withhold Pembrolizumab and refer patient for specialized care and treatment. Administer corticosteroids for SJS grade 3 or 4 or TEN grade 4 (initial dose of 1-2 mg/kg/day prednisone or equivalent followed by a taper). Permanently discontinue Pembrolizumab for any grade of SJS or TEN.

Immune-Mediated Myocarditis
A total of 6 cases of myocarditis have been reported in patients treated with Pembrolizumab in clinical trials or in expanded access program. One fatal case was reported from a clinical trial. Immune-mediated myocarditis should be suspected if other causes of myocarditis, such as infection or prior radiation therapy, have been excluded. Risk factors include certain medications and treatment modalities such as radiation, anthracycline, alkylating agents and most recently checkpoint inhibitors.

Monitor patients for signs and symptoms of Immune-Mediated Myocarditis and ensure adequate evaluation to exclude the other ethiologies. Administer corticosteroids for grade 2 immune-mediated myocarditis (initial dose of 1-2 mg/kg/day prednisone or equivalent followed by a taper). Permanently discontinue Pembrolizumab for grade 3-4 myocarditis or if toxicity does not resolve within 12 weeks of start of grade 2 toxicity or inability to reduce corticosteroid to 10 mg or less of prednisone or equivalent per day within 12 weeks.

9.2.5 Composition

For injection: 50 mg, lyophilized powder in single-use vial for reconstitution

9.2.6 Storage Recommendations and Dosage Forms

Two Drug Product (DP) dosage forms are available for MK-3475: a white to off-white lyophilized powder, 50 mg/vial, and a liquid, DP 100 mg/vial, both in Type I glass vials intended for single use only.

- MK-3475) Pembrolizumab Powder for Solution for Infusion, 50 mg/vial (manufactured using the partially formulated DS), is reconstituted with sterile water for injection prior to use. MK-3475 DP is formulated with L-histidine as

buffering agent, polysorbate 80 as surfactant, sucrose as stabilizer/tonicity modifier, and hydrochloric acid (HCl) and/or sodium hydroxide (NaOH) for pH adjustment (if necessary).

- MK-3475) Pembrolizumab Solution for Infusion 100 mg/vial is a liquid DP (manufactured using the fully formulated DS with L-histidine as buffering agent, polysorbate 80 as surfactant, and sucrose as stabilizer/tonicity modifier), and has the identical formulation as that of the reconstituted lyophilized vial.

  Both drug product dosage forms are stored under refrigerated conditions (2°C - 8°C). The product after reconstitution with sterile water for injection and the liquid drug product are a clear to opalescent solution which may contain proteinaceous and extraneous particulates. The reconstituted lyophilized product and the liquid product are intended for IV administration. The reconstituted DP solution or the liquid DP can be further diluted with normal saline or 5% dextrose in the concentration range of 1 to 10 mg/mL in IV containers made of polyvinyl chloride (PVC) or non-PVC material. Reconstituted vials should be immediately used to prepare the infusion solution in the IV bag and the infusion solution should be immediately administered. If not used immediately, vials and/or IV bags may be stored at 2-8 °C for up to a cumulative time of 20 hours. If refrigerated, the vials and/or IV bags should be allowed to equilibrate to room temperature prior to subsequent use. MK-3475 solutions may be stored at room temperature for a cumulative time of up to 4 hours. This includes room temperature storage of reconstituted or liquid DP solution in vials, room temperature storage of infusion solution in the IV bag and the duration of infusion. This recommendation is based on up to 24 hours of room temperature and up to 24 hours of refrigerated stability data of diluted pembrolizumab solutions in the IV bags.

9.2.7 Dispensation and Accountability

For this study, pembrolizumab (Keytruda®) will be provided by Merck as Pembrolizumab 50 mg (lyophilized powder for injection) and/or pembrolizumab 100 mg/ 4mL (solution for injection). Please see Appendix K for Preparation and Administration/ Reconstitution of Pembrolizumab (Keytruda®) for Injection (lyophilized powder).

(See also Section 8.5.2 Treatment Dispensation, Compliance and Accountability for Pembrolizumab.)

10.0 TREATMENT/ DOSE MODIFICATIONS

10.1 Unacceptable Toxicity

Causality for all adverse events should be assessed and if deemed attributable, attempts should be made to attribute to either axitinib, pembrolizumab, or potentially both agents.

In the case of toxicities refractory to routine medical management which are assessed as possibly attributable to both drugs (i.e. liver enzyme elevation, renal dysfunction, thyroid dysfunction, diarrhea/colitis) that may be autoimmune in origin, dose interruption of axitinib should be attempted first. Generally prompt resolution of the toxicity is observed if attributable to axitinib. If the toxicity is persistent, treatment should be administered as per the pembrolizumab treatment guidelines below, including corticosteroid therapy where advised.

Drug-related grade 4 toxicities will generally require subject to discontinue all study treatment. Exceptions include the following: 1) clear evidence that the toxicity was attributable to axitinib alone, resolved upon cessation of axitinib, and there is rationale to continuing pembrolizumab monotherapy 2) Grade 4 lab abnormalities that are not immune-related, where the patient is asymptomatic, and the abnormalities can be reversed with medical treatment. Example: grade 4 hypertriglyceridemia that responds to antilipid drugs. Intolerable grade 2 and grade 3 toxicities should be managed as per dose modification guidelines listed in the tables below. For grade 2 and grade 3 toxicities attributable to axitinib that do not resolve to ≤ grade 1 within 3 weeks of holding study drug, axitinib should be permanently discontinued. Pembrolizumab therapy may be continued providing the residual adverse events are within permissible ranges for pembrolizumab dosing.

10.2 Dose Titration Guidelines for Axitinib

All patients will begin therapy at the starting dose level of 5 mg PO twice daily. Patients will be evaluated closely during cycle 1 for adverse events, laboratory studies, and review of study diaries and blood pressure logs. Additionally, patients will be instructed to call with any adverse events between study visits. **If there is no grade 2 or greater toxicity on cycle 2 day 1, the patient will be eligible for dose escalation of axitinib as per Table 8. Dose escalation DURING a cycle is not permitted. Consideration of escalation should only be done on <u>day 1</u> of all subsequent cycles. If a patient is dose-escalated on day 1, they should resume blood pressure monitoring daily during the first three weeks of that cycle until evaluated during week 4 (i.e. day 22) visit of that same cycle.**

Dose reductions for grade 2 or greater toxicities may be done at any time during the cycles as per the following guidelines, and Tables 8 and 9.

- For grade 2 or greater fatigue, hand-foot syndrome, mucositis, or diarrhea, attempt medical supportive care first: (topical creams, anti-diarrheal, magic mouthwash or similar).
- If no improvement within 3 days, dose reduce axitinib as per Table 8.
- Axitinib may be held for 3-5 days prior to dose reduction in the judgment of the treating investigator, particularly for grade 3 toxicities

<u>Management of Axitinib-related Hypertension:</u>

- Patients will be provided with blood pressure cuffs and monitoring sheets. They will be instructed to monitor their blood pressure once daily before the evening dose of axitinib. They are to notify the study team for BP ≥ 150/100 mm Hg or for BP ≥ 120/80 mm Hg with any symptoms perceived to be related to BP (headache, chest pressure, shortness of breath, dizziness, blurry vision).

- Medications are to be added and titrated aggressively. Preferred sequence as follows:

    - Calcium channel blockers: (amlodipine, nifedipine, diltiazem, etc.)
    - ACE inhibitors or ARB (lisinopril, valsartan, losartan, etc.)
    - Diuretics (hydrochlorothiazide, furosemide). Patient should be prescribed concurrent potassium and magnesium supplementation and scheduled for potassium and magnesium laboratory tests within three days of starting.
    - Avoid beta-blockers, clonidine, digoxin.
    - If BP still ≥ 150/100 mm Hg despite maximal doses of three anti-hypertensive medications, axitinib should be reduced by one dose level.

Dose Level	**Dose**	**If grade 2 or greater toxicity**	**If no grade 2 or greater toxicity after 21 days [a]**
Dose reduction level -3	2 mg BID	Discontinue axitinib	Stay on 2mg BID[c]
Dose reduction level -2	3 mg BID	Dose reduce to 2mg BID[b] (can hold axitinib prior to restarting per MD discretion)	Stay on 3mg BID[c]
Dose reduction level -1	4 mg BID	Dose reduce to 3mg BID[b] (can hold axitinib prior to restarting per MD discretion)	Stay on 4mg BID[c]
Starting dose level	**5 mg BID**	**Dose reduce to 4mg BID**[b] (can hold axitinib prior to restarting per MD discretion)	**Dose escalate to 6 mg BID**
Dose escalation level 1	6 mg BID	Dose reduce to 5mg BID[b] (can hold axitinib prior to restarting per MD discretion)	Dose escalate to 7 mg BID
Dose escalation level 2	7 mg BID	Dose reduce to 6mg BID[b] (can hold axitinib prior to restarting per MD discretion)	Dose escalate to 8 mg BID

Dose escalation level 3	8 mg BID	Dose reduce to 7mg BID[b] (can hold axitinib prior to restarting per MD discretion)	Dose escalate to 10 mg BID
Dose escalation level 4	10 mg BID	Dose reduce to 8mg BID[b] (can hold axitinib prior to restarting per MD discretion)	Maximal dose.

Table 10. Dose Titration of Axitinib

[a] Dosing modifications based only on the following grade 2 toxicities: oral mucositis, diarrhea, hand-foot syndrome, fatigue. Dose modifications for other drug-related AEs as per table below and as per study principal investigator. CTC v4 definitions of grade 2 toxicities:

Oral mucositis: Moderate pain; not interfering with oral intake; modified diet indicated
Diarrhea: Increase of 4 - 6 stools per day over baseline
HFS: Skin changes (e.g., peeling, blisters, bleeding, edema, or hyperkeratosis) with pain; limiting instrumental ADL
Fatigue: Fatigue not relieved by rest; limiting instrumental ADL

[b] After dose reduction, re-escalation as tolerated may be considered per provider discretion

Toxicity	**Grade:**	**Treatment**	**Dose reduction**
Hypertension/ Hypertensive crisis (Defined as ≥ 150/100 or symptoms such as headache, chest pressure, blurry vision)	2	Add appropriate anti-hypertensive medications	No dose reduction required
	3	Add appropriate anti-hypertensive medications	If persistent despite 3 or more medications and associated with persistent symptoms, dose reduce one level
	4	Discontinue axitinib	Discontinue axitinib
Thrombotic events (arterial)	2-4	Discontinue axitinib	Discontinue axitinib
Thrombotic events (venous) – uncomplicated DVT/PE	3	Provide anticoagulation	No dose adjustment required
Other venous thromboembolic disease (ie retinal vein occlusion)	3	Consider discontinuation of axitinib	Consider discontinuation of axitinib
Hemorrhage	2	Hold axitinib until resolution to ≤ grade 1	Dose reduce one dose level

	3	Hold axitinib until resolution to ≤ grade 1	Dose reduce one dose level. Discontinue if recurs with restarting medication.
	4	Discontinue axitinib.	Discontinue axitinib.
Thyroid dysfunction	2	Treat with replacement/suppression	No dose change
	3	Treat with replacement/suppression. Hold axitinib until resolution to ≤ grade 1. *refer to pembrolizumab dose modifications for additional treatment	Dose reduce one dose level
	4	Discontinue axitinib	Discontinue axitinib
Reversible posterior Leukoencephalopathy Syndrome	2-4	Discontinue axitinib	Discontinue axitinib
Proteinuria	2	Continue treatment if asymptomatic and not associated with creatinine rise. If accompanied by renal insufficiency or evidence of nephritis, refer to pembrolizumab guidelines for treatment and hold axitinib until ≤ grade 1.	If isolated proteinuria, no dose reduction. If associated with AKI/nephritis, dose reduce one dose level after resolution to ≤ grade 1.
	3	Hold axitinib until ≤ grade 1. Refer to pembrolizumab guidelines as per grade 2.	Dose reduce one dose level
Elevation of liver enzymes or bilirubin	2/3	Hold axitinib until ≤ grade 1. *	Dose reduce one dose level.
	4	Discontinue axitinib	Discontinue axitinib.
Other toxicity	2	Intolerable grade 2 toxicity, may hold axitinib or dose-reduce at physician discretion.	May hold axitinib or dose-reduce at physician discretion.
	3	Hold until resolution to ≤ grade 1	Dose reduce one dose level.
	4	Discontinue axitinib	Discontinue axitinib.

Table 11. Additional Dose Modification Guidelines for Axitinib.

***NOTE**: if patient starts with grade 2 LFT elevation (liver metastases), any increase of >/= to 50% of baseline that does not resolve within 7 days is considered grade 3 toxicity and patients should be dose reduced as above.

10.3 Dose Modification Guidelines for Pembrolizumab

Adverse events (AEs) both serious and non-serious associated with pembrolizumab exposure may represent an immunologic etiology. These AEs may occur shortly after the first dose or several months after the last dose of treatment. Pembrolizumab must be withheld for drug-related toxicities and severe or life-threatening AEs as per Table 10 below. See also Section 8.4 for supportive care guidelines and Appendix H.

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

Table 12. Dose Modification and Toxicity Management Guidelines for Immune-Related AEs Associated with Pembrolizumab.

General instructions: **1.** Corticosteroid taper should be initiated upon AE improving to Grade 1 or less and continue to taper over at least 4 weeks. **2.** For situations where pembrolizumab has been withheld, pembrolizumab can be resumed after AE has been reduced to Grade 1 or 0 and corticosteroid has been tapered. Pembrolizumab should be permanently discontinued if AE does not resolve within 12 weeks of last dose or corticosteroids cannot be reduced to ≤10 mg prednisone or equivalent per day within 12 weeks. **3.** For severe and life-threatening irAEs, IV corticosteroid should be initiated first followed by oral steroid. Other immunosuppressive treatment should be initiated if irAEs cannot be controlled by corticosteroids.				
Immune-related AEs	Toxicity grade or conditions (CTCAEv4.0)	Action taken to pembrolizumab	irAE management with corticosteroid and/or other therapies	Monitor and follow-up
Steven-Johnson Syndrome (SJS)	Any	Permanently discontinue	Refer patient for specialized care for assessment and treatment.	If SJS confirmed, permanently discontinue pembrolizumab
Toxic Epidermal Necrolysis (TEN)	Any	Permanently discontinue	Refer patient for specialized care for assessment and treatment.	If TEN confirmed, permanently discontinue pembrolizumab
Pneumonitis	Grade 2	Withhold	Administer corticosteroids (initial dose of 1-2 mg/kg prednisone or equivalent) followed by taper	Monitor participants for signs and symptoms of pneumonitis Evaluate participants with suspected pneumonitis with radiographic imaging and initiate corticosteroid treatment Add prophylactic antibiotics for opportunistic infections
	Grade 3 or 4, or recurrent Grade 2	Permanently discontinue		
Diarrhea / Colitis	Grade 2 or 3	Withhold	Administer corticosteroids (initial dose of 1-2 mg/kg prednisone or equivalent) followed by taper	• Monitor participants for signs and symptoms of enterocolitis (ie, diarrhea, abdominal pain, blood or mucus in stool with or without fever) and of bowel perforation (ie, peritoneal signs and ileus). • Participants with ≥ Grade 2 diarrhea suspecting colitis should consider GI consultation and performing endoscopy to rule out colitis. • Participants with diarrhea/colitis should be advised to drink liberal quantities of clear fluids. If sufficient oral fluid intake is not feasible, fluid and electrolytes should be substituted via IV

	Grade 4	Permanently discontinue		infusion.
AST / ALT elevation	3	Withhold	Administer corticosteroids (initial dose of 0.5- 1 mg/kg prednisone or equivalent) followed by taper	Monitor with liver function tests (consider weekly or more frequently until liver enzyme value returned to baseline or is stable
	4	Permanently discontinue --see exception below[2]	Administer corticosteroids (initial dose of 1-2 mg/kg prednisone or equivalent) followed by taper	
Increased bilirubin	Grade 2	Withhold	Administer corticosteroids (initial dose of 0.5- 1 mg/kg prednisone or equivalent) followed by taper	Monitor with liver function tests (consider weekly or more frequently until liver enzyme value returned to baseline or is stable
	Grade 3 or 4	Permanently discontinue	Administer corticosteroids (initial dose of 1-2 mg/kg prednisone or equivalent) followed by taper	
Type 1 diabetes mellitus (T1DM) or Hyperglycemia	Newly onset T1DM or Grade 3 or 4 hyperglycemia associated with evidence of β-cell failure	Withhold	Initiate insulin replacement therapy for participants with T1DM Administer anti-hyperglycemic in participants with hyperglycemia	Monitor participants for hyperglycemia or other signs and symptoms of diabetes.
Hypophysitis	Grade 2	Withhold	Administer corticosteroids and initiate hormonal replacements as clinically indicated.	Monitor for signs and symptoms of hypophysitis (including hypopituitarism and adrenal insufficiency)
	Grade 3 or 4	Withhold or permanently discontinue[1]		

Hyperthyroidism	Grade 2	Continue	Treat with non-selective beta-blockers (eg, propranolol) or thionamides as appropriate	Monitor for signs and symptoms of thyroid disorders.
	Grade 3 or 4	Withhold or permanently discontinue[1]		
Hypothyroidism	Grade 2-4	Continue	Initiate thyroid replacement hormones (eg, levothyroxine or liothyroinine) per standard of care	Monitor for signs and symptoms of thyroid disorders.
Nephritis and Renal dysfunction	Grade 2	Withhold	Administer corticosteroids (prednisone 1-2 mg/kg or equivalent) followed by taper.	Monitor changes of renal function
	Grade 3 or 4	Permanently discontinue		
Myocarditis	Grade 1 or 2	Withhold	Based on severity of AE administer corticosteroids	Ensure adequate evaluation to confirm etiology and/or exclude other causes
	Grade 3 or 4	Permanently discontinue		
All other immune-related AEs[3]	Intolerable/ persistent Grade 2	Withhold	Based on type and severity of AE administer corticosteroids	Ensure adequate evaluation to confirm etiology and/or exclude other causes
	Grade 3	Withhold or discontinue based on the type of event. Events that require discontinuation include and not limited to: Gullain-Barre Syndrome, encephalitis		
	Grade 4 or recurrent Grade 3	Permanently discontinue		

1. Withhold or permanently discontinue pembrolizumab is at the discretion of the investigator or treating physician.
2. For grade 3 liver toxicity with elevated AST and/or ALT. Since HCC patients may have grade 1-2 elevation of AST/ALT at baseline, if these patients have Grade 3 elevation of AST/ALT, hold therapy and resume treatment when AST/ALT return to baseline or Grade ≤1. The AST/ALT should return to baseline or < 5 x ULN within 12 weeks from start of grade 3, otherwise patient should be discontinued from study.
3. Patients with intolerable or persistent Grade 2 drug-related AE may hold study medication at physician discretion. Permanently discontinue study drug for persistent Grade 2 adverse reactions for which treatment with study drug has been held, that do not recover to Grade 0-1 within 12 weeks of the last dose.

NOTE: For participants with Grade 3 or 4 immune-related endocrinopathy where withhold of pembrolizumab is required, pembrolizumab may be resumed when AE resolves to ≤ Grade 2 and is controlled with hormonal replacement therapy or achieved metabolic control (in case of T1DM).

11.0 TREATMENT DISCONTINUATION

Treatment may be discontinued for any of the following reasons:

- The patient experiences a DLT
- The patient demonstrates radiologic progression by RECIST 1.1. **(See exception below).**
- The patient withdraws consent from the study
- The patient has not received study treatment for >21 days due to a medical/surgical events or logistical reasons not related to study therapy (e.g. elective surgery, unrelated medical events, patient vacation, and/or holidays).
- The patient has completed 24 months of uninterrupted treatment with pembrolizumab or 35 administrations of study medication, whichever is later.
- The patient experiences an adverse event that in the opinion of the Investigator makes continued study treatment an unacceptable risk
- The patient becomes pregnant (also see Section 15.6 and Appendix A for Expedited Adverse Event Reporting Requirements)
- The patient requires continuous treatment with a prohibited concomitant drug(s) for which no safe alternatives can be substituted
- The patient is significantly noncompliant with the requirements of the protocol

Exception: Patients who exhibit radiologic progression by RECIST 1.1 may remain on the study if in the opinion of the Investigator, he/she is deriving clinical benefit from study treatment, particularly if response assessment by immune-related RECIST criteria, Choi criteria, or MRI volumetrics would not require discontinuation. A follow up confirmatory scan should be scheduled in 6 weeks. For later analysis of the primary endpoint, assuming that the confirmatory scan ALSO shows RECIST progression, the initial scan will be recorded as the first date of progression.

Should discontinuation of study therapy occur, all efforts should be made to execute/ report End-of-Treatment and Follow-up Evaluations as completely as possible and to determine/ document the reason for discontinuation (unless the patient withdraws consent for follow-up).

If a patient wishes to withdraw consent from the study, the PI must be notified. The information regarding withdrawal (i.e. subject identifiers and date of withdrawal) should be documented in the subject's record and updated within any other research database(s).

12.0 SCHEDULE OF CLINICAL & LABORATORY EVALUATIONS

Prior to performing any study-specific procedures or evaluations, written informed consent and authorization for the use of protected health information (HIPAA) must be obtained in accordance with all applicable policies, regulations and laws.

Correlative evaluations must also be performed at specified visits. Please refer to Section 13.0 Correlative Studies for specific details.

12.1 Pre-Treatment Evaluation (Screening)

The following must be collected/ performed within ≤28 days prior to Cycle 1, day 1 of treatment. Clinical and laboratory evaluations performed as part of routine standard of care do not need to be repeated if performed within the appropriate window.

- Demographics: The age, race, ethnicity and gender of each patient
- Medical history: Relevant medical history including background and progress of the malignancy and description of all prior therapies
- Physical examination (PE): A complete PE will include assessments of general appearance, skin, head (eyes, ear, nose, and throat), neck, lungs, heart, abdomen, back, lymph nodes, extremities, and neurological exam
- ECOG Performance Status (ECOG PS) will be assessed as specified in Appendix C
- Height
- Weight
- Vital signs (V/S): Vital signs measurements include: diastolic blood pressure (BP) and systolic BP, heart rate (HR), respiratory rate (RR), oral temperature (oral temp) and oxygen saturation (O_2)
- Complete Blood Count (CBC): CBC evaluations with differential and platelets
- Comprehensive Metabolic Panel (CMP): CMP evaluations to include magnesium, phosphate, and direct bilirubin
- Coagulation studies: PT/ (INR), aPTT
- Thyroid Function Tests (TFTs): Thyroid stimulating hormone (TSH), thyroxine (free T4), and triiodothyronine (T3)
- Urine pregnancy test: A pregnancy test will be performed for women of childbearing potential (WOCBP) at screening and throughout the study.
- Urinalysis: A urinalysis with microscopic analysis (UA micro) will be performed at Screening. Any patient with 1+ or greater protein screen without evidence of infection or contaminated sample must have urine microalbumin and/or 24 hour urine creatinine clearance to document proteinuria.
- 12-lead Electrocardiogram (ECG)
- 2D Echocardiogram (ECHO)
- Imaging studies for tumor assessment (CT and/or MRI of all known sites of disease): the same method of assessment and technique should be used at baseline and during treatment and follow-up
- Correlative studies:
    - PET/CT

  - Tumor biopsy: Patients will require pre-treatment biopsy for tumor-infiltrating lymphocyte extraction and profiling as part of correlative studies. In the event that the pre-treatment biopsy is not adequate for immunohistochemistry testing, archival tissue may be substituted if deemed adequate by pathology for baseline staining. If the archival tissue dates prior to extensive chemotherapy/TKI therapy, or is not representative of the current tumor (i.e. primary lesion when the current disease is metastatic) then repeat biopsy should be attempted if feasible.
- Concomitant medications and procedures: Concomitant medications and procedures will be recorded beginning at the time of Screening
- Pretreatment events: baseline events, findings and conditions prior to study treatment initiation should be documented and continually reassessed throughout the study

12.2 Evaluations on Treatment

Collection of Concomitant Medications should occur throughout trial treatment until 30-days (±5 days) post-treatment discontinuation.

The collection of Adverse Events (AEs) should occur throughout trial treatment and continue during Follow-up. Any SAE, or follow up to a SAE, including death due to any cause other than progression of the cancer under study that occurs to any subject from the time the consent is signed through **90 days** following end of treatment, or the initiation of new anti-cancer therapy, whichever is earlier, whether or not related to the Merck product, must be reported within 24 hours to the Sponsor and within 2 working days to Merck Global Safety. All subjects with SAEs must be followed up for outcome. (See Section 15.0 and Appendix A for details).

Imaging studies should be done at the beginning of every odd cycle or 12 weeks (every 84 days ±7 days) from the date of Registration/Enrollment, timed to coincide with the end of the prior treatment cycle; assessing all known sites of disease using the same type(s) of scan(s) that was/were performed at baseline.

The appropriate axitinib dosing diary, blood pressure (BP) log monitoring forms and directions' sheet should be given to the patient before each treatment cycle (Appendix I). Afterwards, the dosing diary completed for that (previous) treatment cycle should be collected from the patient. This should be repeated for each cycle.

12.2.1 Cycle 1, day 1 (±3 days) – CTU visit

The following assessments should be performed on Cycle 1, day 1:

- PE
- ECOG PS
- V/S
- Weight
- CBC with differential

- CMP, Mg, Phosphate, direct bilirubin
- UA
- Urine pregnancy test (repeat only if not done within 7-days prior)
- Imaging studies (repeat only if screening imaging scans are deemed inadequate as per Radiology, i.e. missing fat suppression, contrast-enhancement, otherwise inadequate views, or performed outside of the 28 day window)
- Correlative studies: peripheral blood collection – see Section 13.0

12.2.2 Cycle 1, day 8 (±3 days) – CTU visit (Pembrolizumab administration)

The following assessments should be performed on Cycle 1, day 8:

- PE
- ECOG PS
- V/S
- Weight
- Patient dosing diary review and BP logs
- CBC with differential
- CMP, Mg, Phosphate, direct bilirubin
- Urine pregnancy test
- 12 lead EKG
- **Correlative studies: peripheral blood collection – see Section 13.0 (additional sample for expansion cohort)**

12.2.3 Cycle 1, day 15 (±3 days) – Clinic visit

The following assessments should be performed on Cycle 1, day 15:

- PE
- ECOG PS
- V/S
- Weight
- Patient dosing diary review and BP logs
- CBC with differential
- CMP, Mg, Phosphate, direct bilirubin

12.2.4 Cycle 1, day 22 (±3 days) – Labs only – may be done locally.

The following assessments should be performed on Cycle 1, day 22:

- Patient dosing diary and BP log review
- PE
- ECOG PS
- V/S
- Weight

- CBC with differential
- CMP, Mg, Phosphate, direct bilirubin

12.2.5 Cycle 1, day 29 (±3 days) – CTU visit – Pembrolizumab administration

The following assessments should be performed on Cycle 1, day 29:

- PE
- ECOG PS
- V/S
- Weight
- Patient dosing diary and BP log review
- CBC with differential
- CMP, Mg, Phosphate, direct bilirubin
- Urine pregnancy test
- 12 lead EKG

12.2.6 Cycle 1, day 36 (±3 days) – Labs only, locally permitted.

The following assessments should be performed on Cycle 1, day 36:

- PE
- ECOG PS
- V/S
- Weight
- Patient dosing diary and BP log review
- CBC with differential
- CMP, Mg, Phosphate, direct bilirubin

12.2.7 Cycle 1, day 43 (±3 days) – Labs only, locally permitted.

The following assessments should be performed on Cycle 1, day 43:

- Patient dosing diary and BP log review
- PE
- ECOG PS
- V/S
- Weight
- CBC with differential
- CMP, Mg, Phosphate, direct bilirubin

12.2.8 Cycle 2, day 1: CTU visit, Pembrolizumab administration

The following assessments should be performed on Cycle 2, day 1:

- Collection of previous cycle's patient dosing diary and BP log
- PE
- ECOG PS

- V/S
- Weight
- CBC with differential
- CMP, Mg, Phosphate, direct bilirubin
- Coagulation factors
- TFTs (T3, T4 and TSH)
- UA (if protein 2+ or greater (grade 2) with no evidence of infection or contaminated sample, obtain urine microalbumin and urine creatinine clearance)
- Urine pregnancy test
- Consider axitinib dose escalation (see Section 10.2)

12.2.9 Cycle 2, day 8 (±3 days) – Labs locally

The following assessments should be performed on Cycle 2, day 8:

- Patient dosing diary and BP log review (BP log only if escalation occurred this cycle)
- PE
- ECOG PS
- V/S
- Weight
- CBC with differential
- CMP, Mg, Phosphate, direct bilirubin

12.2.10 Cycle 2, day 15 (±3 days)

The following assessments should be performed on Cycle 2, day 15:

- Patient dosing diary and BP log review (BP log only if escalation occurred this cycle)
- PE
- ECOG PS
- V/S
- Weight
- CBC with differential
- CMP, Mg, Phosphate, direct bilirubin

12.2.11 Cycle 2, day 22 (±3 days) – CTU visit, Pembrolizumab administration

The following assessments should be performed on Cycle 2, day 22:

- PE
- ECOG PS
- V/S
- Weight

- Patient dosing diary review
- CBC with differential
- CMP, Mg, Phosphate, direct bilirubin
- Urine pregnancy test
- 12 lead EKG, ONLY if axitinib dose escalation performed at this cycle

12.2.12 Cycle 2, day 29 (±3 days) – Labs only – locally permitted

The following assessments should be performed on Cycle 2, day 29:

- PE
- ECOG PS
- V/S
- Weight
- Patient dosing diary review
- CBC with differential
- CMP, Mg, Phosphate, direct bilirubin

12.2.13 Cycle 2, day 36 (±3 days) – Labs only – locally permitted

The following assessments should be performed on Cycle 2, day 36:

- PE
- ECOG PS
- V/S
- Weight
- Patient dosing diary review
- CBC with differential
- CMP, Mg, Phosphate, direct bilirubin

12.2.14 Cycle 3, day 1 (±3 days)

The following assessments should be performed on Cycle 3, day 1:

- Note: Imaging Studies (PET/CT, CT and/or MRI) should be repeated prior to treatment every 12 weeks (**±7** days) (i.e. every 84 days or subsequent odd cycle). If there are treatment delays leading to the patient being off schedule, scans should coincide with Day 1 of the next cycle as long as within 3 weeks. If delays greater than 3 weeks then scans should be continued every 12 weeks as per schedule.
- Collection/review of previous cycle's patient dosing diary
- PE
- ECOG PS
- V/S
- Weight

- CBC with differential
- CMP, Mg, Phosphate, direct bilirubin
- Coagulation factors
- TFTs
- U/A – (if protein 2+ or greater (grade 2) with no evidence of infection or contaminated sample, obtain urine microalbumin and urine creatinine clearance)
- Urine pregnancy test
- Correlative studies: Tumor biopsy – see Section 13.0
- Correlative studies: PET/CT, Peripheral blood collection – see Section 13.0
- Consideration for axitinib dose escalation; see Section 10.2; **for all subsequent cycles, if a patient is dose-escalated on day 1, they should keep BP log for the first three weeks of the cycle.**

12.2.15 Cycle 3, day 22 (±3 days) – CTU visit, pembrolizumab administration

The following assessments should be performed on Cycle 3, day 22:

- Dosing diary review, BP log reviewed if axitinib escalated on day 1
- CBC with differential
- CMP, Mg, Phosphate, direct bilirubin
- Urine pregnancy test
- If Axitinib dose escalated on (day 1) of this cycle:
    - 12 lead EKG (ONLY if axitinib dose escalation performed)

12.2.16 Subsequent Cycles, day 1 (±3 days)

The following assessments should be performed on subsequent Cycles, day 1:

- Collection/review of previous cycle's patient dosing diary
- PE
- ECOG PS
- V/S
- Weight
- CBC with differential
- CMP, Mg, Phosphate, direct bilirubin
- Coagulation factors (PT/INR and aPTT)
- TFTs (T3, T4 and TSH)
- U/A (if protein 2+ or greater (grade 2) with no evidence of infection or contaminated sample, obtain urine microalbumin and urine creatinine clearance)
- Urine pregnancy test

- (Imaging studies (CT and/or MRI) should be performed prior to treatment on every subsequent odd cycle or every 12 weeks (±7 days) timed to coincide with the end of the prior treatment cycle; assessing all known sites of disease using the same type(s) of scans as at baseline.) If there are treatment delays leading to the patient being off schedule, scans should coincide with Day 1 of the next cycle as long as within 3 weeks. If delays greater than 3 weeks then scans should be continued every 3 months as per schedule.
- Consider axitinib dose escalation (see Section 10.2); **for all subsequent cycles, if a patient is dose-escalated on day 1, they should monitor blood pressure for the first three weeks of the cycle.**

12.2.17 Subsequent Cycles, day 22 (±3 days)

The following assessments should be performed on subsequent Cycles, day 22:

- Dosing diary review, BP log review if escalated on day 1 of that cycle
- CBC with differential
- CMP, Mg, Phosphate, direct bilirubin
- Urine pregnancy test
- **IF axitinib dose escalation on day 1:**
  - 12-lead EKG (ONLY if axitinib dose escalation performed)

12.2.18 Axitinib Monotherapy and axitinib indefinite treatment, all cycles, day 1 (±3 days)

The following assessments should be performed on day 1 (±3 days) of each cycle during axitinib monotherapy and during the indefinite axitinib treatment:

- Collection/review of previous cycle's patient dosing diary
- PE
- ECOG PS
- V/S
- Weight
- CBC with differential
- CMP, Mg, Phosphate, direct bilirubin
- Coagulation factors (PT/INR and aPTT)
- TFTs (T3, T4 and TSH)
- U/A (if protein 2+ or greater (grade 2) with no evidence of infection or contaminated sample, obtain urine microalbumin and urine creatinine clearance)
- Urine pregnancy test
- (Imaging studies (CT and/or MRI) should be performed prior to treatment on every subsequent odd cycle or every 12 weeks (±7 days) timed to coincide with the end of the prior treatment cycle; assessing all known sites of disease using the same type(s) of scans as at baseline.) If

there are treatment delays leading to the patient being off schedule, scans should coincide with Day 1 of the next cycle as long as within 3 weeks. If delays greater than 3 weeks then scans should be continued every 3 months as per schedule.

Patients unable to attend in-person study visits may conduct study visits via telehealth. Additionally, study assessments may be conducted locally and provided to treating physician for review. Axitinib may be shipped to participants as per University of Miami and Pfizer policies.

12.2.19 Pembrolizumab Re-introduction, all cycles, day 1 (±3 days)

The following assessments should be performed on day 1 (±3 days) of each cycle during pembrolizumab-reintroduction:

- Collection/review of previous cycle's patient dosing diary
- PE
- ECOG PS
- V/S
- Weight
- CBC with differential
- CMP, Mg, Phosphate, direct bilirubin
- Coagulation factors (PT/INR and aPTT)
- TFTs (T3, T4 and TSH)
- U/A (if protein 2+ or greater (grade 2) with no evidence of infection or contaminated sample, obtain urine microalbumin and urine creatinine clearance)
- Urine pregnancy test
- (Imaging studies (CT and/or MRI) should be performed prior to treatment on every subsequent odd cycle or every 12 weeks (±7 days) timed to coincide with the end of the prior treatment cycle; assessing all known sites of disease using the same type(s) of scans as at baseline.) If there are treatment delays leading to the patient being off schedule, scans should coincide with Day 1 of the next cycle as long as within 3 weeks. If delays greater than 3 weeks then scans should be continued every 3 months as per schedule.

12.2.20 Pembrolizumab Re-introduction, all cycles, day 22 (±3 days)

The following assessments should be performed on day 22 (±3 days) of each cycle during pembrolizumab-reintroduction:

- Dosing diary review, BP log review if escalated on day 1 of that cycle
- CBC with differential
- CMP, Mg, Phosphate, direct bilirubin
- Urine pregnancy test

12.3 Off-Treatment Evaluations

The following assessments must be performed at the off treatment visit (± 7 days):

- Adverse events/concurrent medications
- PE
- ECOG PS
- V/S
- Weight
- Patient dosing diary and blood pressure (bp) log
- CBC with differential
- CMP, Mg, Phosphate, direct bilirubin
- Coagulation factors
- TFTs
- U/A (if protein 2+ or greater (grade 2) with no evidence of infection or contaminated sample, obtain urine microalbumin and urine creatinine clearance)
- Urine pregnancy test
- 12-Lead ECG
- 2D ECHO
- Imaging studies (CT, and/or MRI) (not required if reason *off study* was radiographic progression)
- Correlative studies: Tumor biopsy
- Correlative studies: PET/CT, Peripheral blood collection – see Section 13.0

12.4 End of Treatment (EOT) Safety Evaluations

The following assessments must be performed at 30-days (± 5 days) after the last dose of study treatment.

- PE
- ECOG PS
- V/S
- Weight
- CBC with differential
- CMP, Mg, Phosphate, direct bilirubin
- Coagulation factors
- TFTs
- U/A (if protein 2+ or greater (grade 2) with no evidence of infection or contaminated sample, obtain urine microalbumin and urine creatinine clearance)
- Urine pregnancy test

- *Adverse Events: (Any SAE, or follow up to a SAE, including death due to any cause other than progression of the cancer under study that occurs to any subject from the time the consent is signed through **90 days** following end of treatment, or the initiation of new anti-cancer therapy, whichever is earlier, whether or not related to the Merck product, must be reported within 24 hours to the Sponsor and within 2 working days to Merck Global Safety. All subjects with SAEs must be followed up for outcome. (See Section 15.0 and Appendix A for details).)*

12.5 **Follow-up Evaluations**

After treatment completion the following assessments should be performed at least **every 3 months (or every 12 weeks)** (± 7 days) or as clinically indicated, as per standard of care.

Patients without documented evidence of objective disease progression will continue to have the same **imaging studies** as used at Baseline and during Treatment, performed at a 12-week interval during Follow-up, until disease progression.

Follow-up Visit: The following assessment(s) must be performed during Follow-up.

• Adverse events: *(Any SAE, or follow up to a SAE, including death due to any cause other than progression of the cancer under study that occurs to any subject from the time the consent is signed through **90 days** following end of treatment, or the initiation of new anti-cancer therapy, whichever is earlier, whether or not related to the Merck product, must be reported within 24 hours to the Sponsor and within 2 working days to Merck Global Safety. All subjects with SAEs must be followed up for outcome. (See Section 15.0 and Appendix A for details).)*
• PE
• ECOG PS
• Weight
• V/S (BP, HR, RR, oral temp and O_2 saturation)
• CBC (with differential and platelets)
• CMP, Mg, Phosphate, direct bilirubin

- Coagulation factors (PT/INR, aPTT)
- TFTs (T3, T4 and TSH)
- Urine Pregnancy Test
- U/A (if protein 2+ or greater (grade 2) with no evidence of infection or contaminated sample, obtain urine microalbumin and urine creatinine clearance)

• 2D ECHO: only if clinically indicated
• 12-Lead ECG, only if clinically indicated

12.6 **Survival Assessment Evaluations**

After treatment completion or if the patient starts an alternative treatment regimen, each patient will be followed for survival every 12 weeks (±7 days) until death or withdrawal of consent. A telephone call to the patient and/or the patient's family will be made to evaluate the patient's status.

12.7 **Calendar of Clinical and Laboratory Evaluations** (continued on following page)

Cycle	**Screen-ing**	**1**							**2**						**3**		**Subsequent Cycles**	**Off Treatment (treatment discontin-uation)**	**EoT Safety Evaluation**	**Follow-up[A]**	**Survival**
Week (per Cycle)		1	2	3	4	5	6	7	1	2	3	4	5	6	1	4[F]	Weeks 1 and 4[F,I,J]	(EOT) ±7 days	30 days (±5 days) *after* EOT	Q12 Weeks (± 7 days)	Q12 Weeks (± 7 days)
Day (±3d per Cycle)	-28 to 0	1	8	15	22	29	36	43	1	8	15	22	29	36	1	22[F]	Days 1 and 22[F]				
ICF	X																				
Eligibility	X																				
Demograph-ics	X																				
Medical history	X																				
PE	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X (Day 1)	X	X	X	
ECOG PS	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X (Day 1)	X	X	X	
Height	X																				
Weight	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X (Day 1)	X	X	X	
V/S (BP, HR, RR, oral temp, and O_2 sat)	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X (Day 1)	X	X	X	
CBC, diff	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X (Days 1, 22)	X	X	X	

[A] For those patients without documented evidence of objective disease progression, the same imaging studies used at Baseline/Screening; not required at Off-Treatment, if reason for 'off study' is radiographic progression.

Cycle	Screen-ing	1							2						3		Subsequent Cycles	Off Treatment (treatment discontin-uation)	EoT Safety Evaluation	Follow-up[A]	Survival
Week (per Cycle)		1	2	3	4	5	6	7	1	2	3	4	5	6	1	4[F]	Weeks 1 and 4[F,I,J]	(EOT) ±7 days	30 days (±5 days) *after* EOT	Q12 Weeks (± 7 days)	Q12 Weeks (± 7 days)
Day (±3d per Cycle)	-28 to 0	1	8	15	22	29	36	43	1	8	15	22	29	36	1	22[F]	Days 1 and 22[F]				
CMP, Mg, PO4, direct bilirubin	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X (Days 1, 22)	X	X	X	
PT/INR, aPTT	X								X						X		X (Day 1)	X	X	X	
TFTs (T3, T4, TSH)	X								X						X		X (Day 1	X	X	X	
Urine pregnancy	X	X[B]	X			X			X			X			X	X	X (Days 1 and 22)	X	X	X	
U/A (micro-albumin and Cr Clearance, if > 2+ protein without other cause)	X	X							X						X		X (Day 1)	X	X	X	
12-Lead ECG	X		X			X						X[C]				(X)[C]	X[C] (Day 22[C])	X			
2D ECHO	X																	X			

[B] Repeat only if not done within previous 7 days.
[C] Perform only if axitinib dose escalation performed on day 1 of current cycle.

Cycle	**Screen-ing**	**1**							**2**						**3**		**Subsequent Cycles**	**Off Treatment (treatment discontin-uation)**	**EoT Safety Evaluation**	**Follow-up[A]**	**Survival**
Week (per Cycle)		1	2	3	4	5	6	7	1	2	3	4	5	6	1	4[F]	Weeks 1 and 4[F,I,J]	(EOT) ±7 days	30 days (±5 days) *after* EOT	Q12 Weeks (± 7 days)	Q12 Weeks (± 7 days)
Day (±3d per Cycle)	-28 to 0	1	8	15	22	29	36	43	1	8	15	22	29	36	1	22[F]	Days 1 and 22[F]				
Imaging scans (CT, MRI)	X	X[D]													X[E]		X[E] (Cycles 3,5,7,etc)	X[A]			
Correlatives: PET/CT	X														X			X			
Axitinib (oral)		X																			
Evaluate for Axitinib dose escalation									X						X		X (Day 1)				
Pembrolizu-mab (IV)			X			X			X			X			X	X	X (Days 1, 22)				

[D] Repeat Imaging scans (for C1D1) only if Screening/Baseline scans are deemed inadequate as per Radiology.
[E] After cycle 3, repeat imaging every 12 weeks or at every subsequent odd cycle (ie Cycle 5, 7, 9, etc.).
[F] If a patient is dose-escalated during the (specified) cycle they should be evaluated at least once on the Week 4 visit (beginning on Day 22) as well, or as clinically indicated.

[G] After Follow-up during "Survival" period, a telephone call to the patient and/or the patient's family will be made to evaluate the patient's status.

Cycle	**Screen-ing**	**1**							**2**						**3**		**Subsequent Cycles**	**Off Treatment (treatment discontin-uation)**	**EoT Safety Evaluation**	**Follow-up[A]**	**Survival**
Week (per Cycle)		1	2	3	4	5	6	7	1	2	3	4	5	6	1	4[F]	Weeks 1 and 4[F,I,J]	(EOT) ±7 days	30 days (±5 days) *after* EOT	Q12 Weeks (± 7 days)	Q12 Weeks (± 7 days)
Day (±3d per Cycle)	-28 to 0	1	8	15	22	29	36	43	1	8	15	22	29	36	1	22[F]	Days 1 and 22[F]				
Patient Dosing Diary Review			X	X	X	X	X	X	X	X	X	X	X[Bplo]		X	X	X (Days 1, 22)	X			
BP log review[H]		X	X	X	X	X	X	X	X[H]	X[H]	X[H]				X[H]	X[H]	X[H]				
Correlatives : Tumor biopsy	X														X			X			
Peripheral blood for correlative studies		X	X												X			X			
Conmeds	X																				
Pretreat-ment events	X																				
AEs		X (through 90-days following end of treatment)																			
Telephone call																					X[G]

H - BP logs are required during first cycle. For second and further cycles, if dose-escalated on day 1, patients should keep BP logs until day 22. If no change in dosing and three weeks of stable BP, logs are not required.
I-Refer to section 12.2.18 for a list of the assessments that will be performed on day 1 (±3 days) of each cycle during axitinib monotherapy.
J-Refer to section 12.2.19 and 12.2.20 for a list of the assessments that will be performed on each cycle, day 1 (±3 days) and day 22 (±3 days) of pembrolizumab re-introduction.

13.0 SCHEDULE OF CORRELATIVE EVALUATIONS

All tissue and blood samples will be labeled and referred to only by subject number. The scientific investigators performing correlative analysis will be blinded to the patient identification data and clinical outcomes. Additional information regarding protection of patient data and complete details of laboratory procedures can be found in **Appendix F**.

13.1 Pre-Treatment Specimen Collection

13.1.1 Screening

All eligible patients will undergo **mandatory core needle biopsy of the tumor** prior to study therapy. If the tumor lesions are deemed unamenable to biopsy attempt, due to safety or technical concerns by the interventional radiologist, the patients will be considered *screen failures* and not enrolled on the study.

For patients that are deemed to have a tumor lesion amenable to biopsy in the judgement of the radiologist, inadequate tissue sampling despite reasonable attempts at the time of biopsy will not preclude enrollment on the clinical trial and the patient will continue with other correlative studies. Future biopsies will be foregone. In this event, archival tumor tissue will be used for baseline immunologic markers via immunohistochemistry.

All samples will be deidentified at the time of collection and labeled with a unique study identifier. Only the PI and research staff will have access to the patient identifying information and the study key linking identity with the research samples.

- 5 core biopsies (minimum of 2), 18-22 gauge in diameter and at least 1 cm in length, will be obtained. The interventional approach used to obtain the sample (ultrasound or CT-guided biopsy) will be selected by the medical personnel performing the biopsy.
  - One needle core biopsy will be transported in formalin to Pathology for immunohistochemistry evaluation of immunologic markers. This analysis will be performed in collaboration with Wistuba Ignacio, MD at MD Anderson Cancer Center. Samples will be deidentified and sent to his laboratory for multiplex immunohistochemistry.
  - Unstained slides from the formalin fixed biopsies will be sent to Nanostring for RNA expression analysis using the Universal Immunooncology Expression Panel under a collaborative research agreement to identify potential biomarkers of response.
  - The remaining cores (if any) will be collected in cell culture media and transported to the Komanduri lab for preparation of a single cell suspension, isolation of lymphocytes, and immediate analysis by flow cytometry. For expansion patients, additional cores will be collected for RNA analysis and

performance of T cell receptor sequencing and clonality assays using the Archer DX platform.

13.1.2 Cycle 1, Day 1 (PRE-DOSE)

- Immediately prior to treatment initiation on Cycle 1 Day 1, patients will also undergo phlebotomy for analysis of circulating mononuclear cells with banking of serum fractions for later analysis.

    - (6) 10 cc green top tubes and (1) 10 cc purple top tube will be collected for each time point. Samples will be separated using a Ficoll gradient. Mononuclear cells will be removed, washed, treated with RBC lysis process, then either stained immediately for flow cytometric analysis or frozen for later use. Aliquots of plasma will also be frozen. One tube of PBMCs will be preserved in RNA later for T cell clonality assays using the ArcherDX platform.
    - Banked PBMCs will be sent out to Simpatica Medicine for RNA expression analysis to identify potential biomarkers of response under a collaborative research agreement.
    - Banked plasma samples will be analyzed for plasma angiogenic activity in collaboration with Dr. Jaime Merchan at University of Miami. Samples will be exposed to human umbilical vein endothelial cells and angiogenic activity measured.

- Additional peripheral blood will be obtained for circulating tumor cell analysis.

    - Two 10 cc Cell-Saver tubes will be drawn and transported immediately to the laboratory of Dr. Ram Datar for analysis. Blood will be filtered and one slide stained for H&E to detect CTCs. The other slide will be reserved for additional analysis, including genomic analysis, FISH, or immunocytochemistry depending on the specific histology. The number of CTCs will be recorded for each patient.

13.1.3 Cycle 1, Day 8 (PRE-PEMBROLIZUMAB (expansion cohort only)

- 6) 10 cc green top tubes and (1) 10 cc purple top tube will be collected for each time point. Samples will be separated using a Ficoll gradient. Mononuclear cells will be removed, washed, treated with RBC lysis process, then either stained immediately for flow cytometric analysis or frozen for later use. Aliquots of plasma will also be frozen.
- Banked PBMCs will be sent out to Simpatica Medicine for RNA expression analysis to identify potential biomarkers of response under a collaborative research agreement.
- Banked plasma samples will be analyzed for plasma angiogenic activity in collaboration with Dr. Jaime Merchan at University of Miami.

Samples will be exposed to human umbilical vein endothelial cells and angiogenic activity measured.

13.2 On Treatment Specimen Collection

13.2.1 Cycle 3, Day 1 (PRE-DOSE)

For patients who have successfully completed two cycles of axitinib/pembrolizumab therapy, the following on-treatment correlative studies will also be performed:

- 5 core biopsies (minimum of 2), 18-22 gauge in diameter and at least 1 cm in length, will be obtained. The interventional approach used to obtain the sample (ultrasound or CT guided biopsy) will be selected by the medical personnel performing the biopsy If possible, the biopsy site should be in the same tumor initially biopsied prior to therapy.
  - One needle core biopsy will be transported in formalin to pathology for immunohistochemistry evaluation of immunologic markers. This analysis will be performed in collaboration with Wistuba Ignacio, MD at MD Anderson Cancer Center. Samples will be deidentified and sent to his laboratory for multiplex immunohistochemistry.
  - Unstained slides from the formalin fixed biopsies will be sent to Nanostring for RNA expression analysis using the Universal Immunooncology Expression Panel under a collaborative research agreement to identify potential biomarkers of response.
  - The remaining cores (if any) will be collected in cell culture media and transported to the Komanduri lab for preparation of a single cell suspension, isolation of lymphocytes, and immediate analysis by flow cytometry. For expansion patients, additional cores will be collected for RNA analysis and performance of T cell receptor sequencing and clonality assays using the Archer DX platform.

- Patients will also undergo phlebotomy for analysis of circulating mononuclear cells with banking of serum fractions for later analysis.
  - (6) 10 cc green top tubes and (1) 10 cc purple top tube will be collected for each time point. Samples will be separated using a Ficoll gradient. Mononuclear cells will be removed, washed, treated with RBC lysis process, then either stained immediately for flow cytometric analysis or frozen for later use. Aliquots of plasma will also be frozen. One tube of PBMCs will be preserved in RNA later for T cell clonality assays using the ArcherDX platform.
  - Banked PBMCs will be sent out to Simpatica Medicine for RNA expression analysis to identify potential biomarkers of response under a collaborative research agreement.

- Banked plasma samples will be analyzed for plasma angiogenic activity in collaboration with Dr. Jaime Merchan at University of Miami. Samples will be exposed to human umbilical vein endothelial cells and angiogenic activity measured.

- Additional peripheral blood will be obtained for circulating tumor cell analysis.
  - Two 10 cc Cell-Saver tubes will be drawn and transported immediately to the Datar lab for analysis. Blood will be filtered and one slide stained for H&E to detect CTCs. The other slide will be reserved for additional analysis, including genomic analysis, FISH, or immunocytochemistry depending on the specific histology. The number of CTCs will be recorded for each patient.

13.3 **Off-Treatment Specimen Collection (End of Treatment or EOT visit)**

The following must be performed at the EOT visit and should occur 30-days (± 7 days) after the last dose of study treatment.

- 5 core biopsies (minimum of 2), 18-22 gauge in diameter and at least 1 cm in length, will be obtained. The interventional approach used to obtain the sample (ultrasound or CT guided biopsy) will be selected by the medical personnel performing the biopsy If possible, the biopsy site should be in the same tumor initially biopsied prior to therapy.
  - One needle core biopsy will be transported in formalin to pathology for immunohistochemistry evaluation of immunologic markers. This analysis will be performed in collaboration with Wistuba Ignacio, MD at MD Anderson Cancer Center. Samples will be deidentified and sent to his laboratory for multiplex immunohistochemistry.
  - Unstained slides from the formalin fixed biopsies will be sent to Nanostring for RNA expression analysis using the Universal Immunooncology Expression Panel under a collaborative research agreement to identify potential biomarkers of response.
  - The remaining cores (if any) will be collected in cell culture media and transported to the Komanduri lab for preparation of a single cell suspension, isolation of lymphocytes, and immediate analysis by flow cytometry. For expansion patients, additional cores will be collected for RNA analysis and performance of T cell receptor sequencing and clonality assays using the Archer DX platform.

- Patients will also undergo phlebotomy for analysis of circulating mononuclear cells with banking of serum fractions for later analysis.
  - (6) 10 cc green top tubes and (1) 10 cc purple top tube will be collected for each time point. Samples will be separated using a Ficoll gradient. Mononuclear cells will be removed, washed, treated with RBC lysis

process, then either stained immediately for flow cytometric analysis or frozen for later use. Aliquots of plasma will also be frozen. One tube of PBMCs will be preserved in RNA later for T cell clonality assays using the ArcherDX platform.
    - Banked PBMCs will be sent out to Simpatica Medicine for RNA expression analysis to identify potential biomarkers of response under a collaborative research agreement.
    - Banked plasma samples will be analyzed for plasma angiogenic activity in collaboration with Dr. Jaime Merchan at University of Miami. Samples will be exposed to human umbilical vein endothelial cells and angiogenic activity measured.

- Additional peripheral blood will be obtained for circulating tumor cell (CTC) analysis.
    - Two 10 cc Cell-Saver tubes will be drawn and transported immediately to the laboratory of Dr. Ram Datar lab for analysis. Blood will be filtered and one slide stained for H&E to detect CTCs. The other slide will be reserved for additional analysis, including genomic analysis, FISH, or immunocytochemistry depending on the specific histology. The number of CTCs will be recorded for each patient.

13.4 **Radiology correlative studies**

Patients who undergo CT imaging for tumor response assessment will have their images analyzed by a designated sarcoma radiologist who will be blinded to patient clinical outcome and phase of treatment according to Choi criteria.

Patients who undergo MRI evaluation for tumor response assessment will have dynamic contrast enhancement (DCE) protocol added to the standard sequences obtained. The patient may experience a slightly longer duration in the scanner for these purposes. The outcomes based on DCE imaging will also be analyzed by the designated sarcoma radiologist who will be blinded to patient clinical outcome and phase of treatment. Tumor volume will be calculated using MRI volumetrics in a blinded fashion and analyzed for correlation with RECIST response.

PET/CT scans will be obtained at Screening, Cycle 3 (day 1) and End-of-Treatment visits, to assess metabolic response which may precede radiographic response. If the patient does not have a positive PET/CT at baseline, subsequent scans will be cancelled.

13.5 Calendar of Specimen Collection for Correlative Studies

Timepoints	Tumor biopsies	Tumor/TIL analysis (IHC/flow cytometry)	Circulating Tumor Cells (CTCs)	Circulating lymphocyte analysis (flow cytometry)	PET/CT
Screening	X	X			X
Cycle 1, day 1 (predose)			X	X	
Cycle 1, day 8				X	
Cycle 3, day 1 (predose)	X	X	X	X	X
End of Treatment	X	X	X	X	X

Table 13. Correlative Studies' Timepoints

13.6 Banking of Additional Specimens

In the event that study treatment results in a clinical response to the degree of permitting surgical resection, we will obtain any surgical specimens from patients who opt to withdraw from the study and undergo surgery.

Tumor tissue will be collected and analyzed as follows:
1. Immunohistochemistry for immunomodulatory markers after formalin fixation,
2. Fresh tissue will be transported to the Komanduri lab for tumor-infiltrating lymphocyte extraction and flow cytometry. TILs will be banked in RNA later for RNA expression analysis using Archer DX platform to determine T cell clonality.
3. Excess tumor tissue will be placed in RNA and frozen for later use.

14.0 MEASUREMENT OF EFFECT

14.1 Antitumor Effect in Solid Tumors

For the purposes of this study, patients should be re-evaluated for response every 12 weeks (84 days, ±7 days) from date of Enrollment, (while on treatment and until there is documented evidence of disease progression for those patients who come off treatment). Imaging studies should be timed to coincide with the end of the prior treatment cycle, assessing all known sites of disease using the same type(s) of scan(s) that was/were performed at baseline. Response assessments during follow-up after disease progression should follow the same frequency (every 12 weeks, ±7 days).

Response and progression will be evaluated in this study using the international criteria proposed by the revised Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1 guideline(s). Changes in the largest diameter (unidimensional measurement) of the

tumor lesions and the shortest diameter in the case of malignant lymph nodes are used in the RECIST criteria.

The following general principles must be followed:
1. To assess objective response, it is necessary to estimate the overall tumor burden at baseline to which subsequent measurements will be compared. All baseline evaluations should be performed as closely as possible to the beginning of treatment and never more than four weeks before registration.

2. Measurable disease is defined by the presence of at least one measurable lesion.

3. All measurements should be recorded in metric notation by use of a ruler or calipers.

4. The same method of assessment and the same technique must be used to characterize each identified lesion at baseline and during follow-up.

14.1.1 Definitions

Evaluable for Objective Response

Only those eligible patients who have measurable disease present at baseline, have received at least one cycle of therapy, and have had their disease re-evaluated will be considered evaluable for response. These patients will have their response classified according to the definitions stated below. (Note: Patients who exhibit objective disease progression prior to the end of cycle 1 will also be considered evaluable.)

Evaluable for Toxicity

All eligible patients who receive at least 80% of scheduled doses of axitinib during weeks 1-4, and receive pembrolizumab during week 2. Patients who withdraw from the study prior to week 2 for any reason other than DLT or other significant toxicity will not be considered evaluable for toxicity

14.1.2 Disease Parameters

Measurable Disease

Measurable lesions are defined as those that can be accurately measured in at least one dimension (longest diameter to be recorded) as ≥ 20 mm by chest x-ray, as ≥ 10 mm with CT scan, or ≥ 10 mm with calipers by clinical exam. All tumor measurements must be recorded in millimeters (or decimal fractions of centimeters).

NOTE: Tumor lesions that are situated in a previously irradiated area may be considered measurable if they have grown subsequent to previous radiation.

Malignant Lymph Nodes

To be considered pathologically enlarged and measurable, a lymph node must be ≥ 15 mm in **short** axis when assessed by CT scan (CT scan slice thickness

recommended to be no greater than 5 mm). At baseline and in follow-up, only the **short** axis will be measured and followed.

Non-measurable Disease

All other lesions (or sites of disease), including small lesions (longest diameter < 10 mm or pathological lymph nodes with ≥10 to < 15 mm short axis), are considered non-measurable disease. Bone lesions, leptomeningeal disease, ascites, pleural/pericardial effusions, lymphangitis cutis/pulmonitis, inflammatory breast disease, and abdominal masses (not followed by CT or MRI), are considered as non-measurable. Non-measurable also includes lesions that are < 20 mm by chest x-ray.

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

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

Target Lesions

All measurable lesions up to a maximum of 2 lesions per organ and 5 lesions in total, representative of all involved organs, should be identified as target lesions and recorded and measured at baseline. Target lesions should be selected on the basis of their size (lesions with the longest diameter), be representative of all involved organs, but in addition should be those that lend themselves to reproducible repeated measurements. It may be the case that, on occasion, the largest lesion does not lend itself to reproducible measurement in which circumstance the next largest lesion which can be measured reproducibly should be selected.

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

Non-target Lesions

All other lesions (or sites of disease) including any measurable lesions over and above the 5 target lesions should be identified as **non-target lesions** and should also be recorded at baseline. Measurements of these lesions are not required, but the presence or absence of unequivocal progression of each should be noted throughout follow-up.

14.1.3 Methods for Evaluation of Disease

All measurements should be taken and recorded in metric notation using a ruler or calipers. All baseline evaluations should be performed as closely as possible to the beginning of treatment and never more than 4 weeks (28 days) before enrollment.

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

Clinical Lesions

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

Chest X-ray

Lesions on chest x-ray are acceptable as measurable lesions when they are clearly defined and surrounded by aerated lung. However, CT is preferable since it is considered more sensitive.

Conventional CT and MRI

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

Use of MRI remains a complex issue. MRI has excellent contrast, spatial, and temporal resolution; however, there are many image acquisition variables involved in MRI which greatly impact image quality, lesion conspicuity, and measurement. Furthermore, the availability of MRI is variable globally. As with CT, if an MRI is performed, the technical specifications of the scanning sequences used should be optimized for the evaluation of the type and site of disease. Furthermore, as with CT, the modality used at follow-up must be the same as was used at baseline and the lesions should be measured/assessed on the same pulse sequence. It is beyond the scope of the RECIST guidelines to prescribe specific MRI pulse sequence parameters for all scanners, body parts, and diseases. Ideally, the same type of scanner should be used and the image acquisition protocol should be followed as closely as possible to prior scans. Body scans should be performed with breath-hold scanning techniques, if possible.

PET-CT

At present, the low dose or attenuation correction CT portion of a combined PET-CT is not always of optimal diagnostic CT quality for use with RECIST measurements. However, if the site can document that the CT performed as part of a PET-CT is of identical diagnostic quality to a diagnostic CT (slice thickness of 5

mm or less and generally with IV and sometimes with oral contrast), then the CT portion of the PET-CT can be used for RECIST measurements and can be used interchangeably with conventional CT in accurately measuring cancer lesions over time. Note, however, that the PET portion of the CT introduces additional data which may bias an investigator if it is not routinely or serially performed.

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

14.1.4 Response Criteria

Evaluation of Target Lesions

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

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

Progressive Disease (PD)
At least a 20% increase in the sum of the diameters of target lesions, taking as reference the smallest sum on study (this includes the baseline sum if that is the smallest on study). In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5 mm. (NOTE: the appearance of one or more new lesions is also considered progression).

Stable Disease (SD)
Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum diameters while on study. (Note: a change of 20% or more that does not increase the sum of the diameters by 5 mm or more is coded as stable disease).

To be assigned a status of stable disease, measurements must have met the stable disease criteria at least once after study entry at a minimum interval of 12 weeks (±7 days) after the date of Enrollment.

Evaluation of Non-Target Lesions

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

Non-CR/Non-PD
Persistence of one or more non-target lesion(s).

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

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

When the patient only has non-measurable disease, the increase in overall disease burden should be comparable in magnitude to the increase that would be required to declare PD for measurable disease: i.e., an increase in tumor burden from "trace" to "large", an increase in nodal disease from "localized" to "widespread", or an increase sufficient to require a change in therapy.

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

Evaluation of New Lesions

The appearance of new lesions constitutes Progressive Disease (PD).

A growing lymph node that did not meet the criteria for reporting as a measurable or non-measurable lymph node at baseline should only be reported as a new lesion (and therefore progressive disease) if it:

a) increases in size to ≥15 mm in the short axis or;

b) there is new pathological confirmation that it is disease (regardless of size).

Evaluation of Best Overall Response

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

Target Lesions	Non-Target Lesions	New Lesions*	Best Overall Response	Remarks
CR	CR	No	CR	
CR	Non-CR/Non-PD***	No	PR	
CR	Not evaluated	No	PR	
PR	Non-PD***/not	No	PR	
SD	Non-PD***/not evaluated	No	SD	Documented at least once ≥ 12 wks. from study entry
PD	Any	Yes or No	PD	No prior SD, PR or CR
Any	PD**	Yes or No	PD***	
Any	Any	Yes	PD	

* See RECIST 1.1 manuscript for further details on what is evidence of a new lesion.

** In exceptional circumstances, unequivocal progression in non-target lesions may be accepted as disease progression.

*** PD in non-target lesions should not normally trump target lesion status. It must be representative of overall disease status change, not a single lesion increase. Please refer to the Evaluation of Non-Target Lesions – Progressive Disease section for further explanation.

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

Table 14. RECIST 1.1 For Patients with Measurable Disease (i.e. Target Disease)

Duration of Response

Duration of Overall Response

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

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

Duration of Stable Disease

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

To be assigned a status of stable disease, measurements must have met the stable disease criteria at least once after study entry at a minimum interval of 12 weeks (84 days, ±7 days) after the date of Enrollment.

14.2 Progression-Free Survival (PFS)

Patients will be evaluated during treatment and by follow-up assessments post-treatment at: 30-days (+5 days). PFS is defined as the time from treatment initiation until documented disease progression or death (by any cause, in the absence of progression). In progression-free patients, PFS will be censored at the last evaluable tumor assessment (RECIST v1.1).

14.3 Overall Survival (OS)

OS is defined as the elapsed time from start of treatment to death or date of censoring. Patients alive or those lost to follow-up will be censored at the last date of contact (or last date known to be alive).

15.0 ADVERSE EVENTS

The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.03 will be utilized for adverse event reporting.

15.1 Purpose

Adverse event data collection and reporting, which are required as part of every clinical trial, are done to ensure the safety of patients enrolled in the studies, as well as those who will enroll in future studies using similar agents. Adverse events are reported in a routine manner at scheduled times during a trial. Additionally, certain adverse events must be reported in an expedited manner for timelier monitoring of patient safety and care.

15.2 Adverse Event

Adverse Event (AE): Can be any unfavorable and unintended sign (e.g., an abnormal laboratory finding), symptom, or disease temporally associated with the use of a drug, medical treatment, or procedure without judgment about causality. An adverse event can arise from any use and from any route of administration, formulation, or dose including an overdose. This includes any newly occurring event or a previous condition that has increased in severity or frequency since initiation of a drug, medical treatment, or procedure.

Abnormal Findings

In any clinical assessment, a value outside the normal or reference range (such as a clinical laboratory, vital sign, or ECG) will not be reported or assessed as an AE unless that value is considered to be of clinical significance by the investigator. A value of clinical significance is one that leads to discontinuation or delay in protocol treatment, dose modification, therapeutic intervention*, or is considered to be a clinically significant new finding or change from baseline by the investigator.

*Transfusion support administered to offset clinical symptoms of anemia or thrombocytopenia will not be considered therapeutic intervention.

Signs and Symptoms

Signs/symptoms resulting from an underlying clinical diagnosis should be documented as one comprehensive AE. If no underlying clinical diagnosis can be identified, each sign/symptom should be reported as a separate independent event. (A new or worsening event resulting from an underlying clinical diagnosis or a reaction to concurrent medications should be documented as a separate independent AE unless it is within the normal range of fluctuation for that patient.)

Grade Changes/Fluctuations

AEs will be reported at the maximum grade/severity experienced for the duration of the event. Should one particular event warrant further investigation, additional details may be collected at the discretion of the Principal Investigator.

Progression of Disease

Progression of disease, if documented in accordance to standard of care, should not be reported as an AE.

Tests and Procedures

Tests and procedures should not be reported as AEs. The underlying clinical diagnosis (or sign/symptom in the event an underlying clinical diagnosis is not known) requiring testing or a procedure, should be reported as an adverse event if it meets criteria for reporting.

15.3 Serious Adverse Events (see also Appendix A)

Serious AE (SAE) means any untoward medical occurrence that occurs at any dose:

1. Results in death.
2. Is life-threatening.
   The term "life-threatening" in the definition of "serious" refers to an event in which the patient 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).
3. Requires inpatient hospitalization or prolongation of present hospitalization.
   Elective hospitalization to simplify protocol treatment/evaluations or to treat a baseline condition that did not worsen from baseline will not be considered an SAE.

4. Results in persistent or significant disability/incapacity.
   Disability is defined as a substantial disruption of a person's ability to conduct normal life functions.
5. Is a congenital anomaly/birth defect.
6. Is a medically important event.
   A medically important event may not be immediately life threatening or result in death or hospitalization but may jeopardize the patient or may require intervention to prevent one of the other outcomes listed in the definition above, or involves suspected transmission via a medicinal product of an infectious agent. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse; any organism, virus, or infectious particle (e.g., prion protein transmitting Transmissible Spongiform Encephalopathy), pathogenic or nonpathogenic, is considered an infectious agent.

Additional SAE classification(s) for AEs occurring at any dose or during any use of Merck product(s):

6. Is a new cancer (that is not a condition of the study);
7. Is associated with an overdose (whether accidental or intentional): Any adverse event associated with an overdose is considered a serious adverse event. An overdose that is not associated with an adverse event is considered a non-serious event of clinical interest and must be reported within 24 hours.
8. Is another important medical event

Clarification should be made between the terms *serious* and *severe* because they ARE NOT synonymous. The term *severe* is often used to describe the intensity (severity) of a specific event (as in mild, moderate, or severe myocardial infarction); the event itself, however, may be of relatively minor medical significance (such as a severe headache). This is NOT the same as *serious*, which is based on patient/event outcome or action criteria described above and is usually associated with events that pose a threat to a patient's life or functioning. A severe AE does not necessarily need to be considered serious. For example, persistent nausea of several hours duration may be considered severe nausea but not an SAE. On the other hand, a stroke resulting in only a minor degree of disability may be considered mild but would be defined as an SAE based on the above noted criteria. Seriousness (not severity) serves as a guide for defining regulatory reporting obligations.

15.4 Adverse Event Collection Period

In this protocol, adverse events include only treatment-emergent adverse events. A treatment-emergent adverse event (TEAE) is defined as any event that begins or worsens after the start of protocol treatment. All baseline-emergent adverse events, any event that begins or worsens after completion of the informed consent protocol but prior to the start of protocol treatment, should be reported as a Baseline/Comorbid Condition.

All adverse events that occur within ≤ 30 days of the last dose of study therapy will be reported and followed until resolution. Resolution is defined as a return to baseline status or the stabilization of an event with the expectation that it will remain chronic. (Exception: If a patient begins an alternative therapy that confounds accurate assessment of AEs within ≤ 30 days of the last dose of study therapy, all adverse event collection will stop and any ongoing events will be left open.)

15.5 Adverse Event Reporting Requirements

The information to be reported in AEs will be assessed by and assigned severity using the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE), Version 4.03. The NCI CTCAE provides descriptive terminology and a grading scale for each adverse event listed. A copy of the NCI CTCAE v4.03 can be downloaded from the CTEP home page (http://evs.nci.nih.gov/ftp1/CTCAE/About.html).

Information to be reported in the description of each adverse event may be included, but is not limited to:

1. Clinical Diagnosis of the event as determined by NCI CTCAE, Version 4.03 descriptive terminology. If no clinical diagnosis can be identified, each sign/symptom should be reported as a separate independent event.
2. Date of onset of the AE (start date).
3. Date of resolution of the AE (end date).
4. Severity of the event determined by NCI CTCAE, Version 4.03 grading scale.
5. Relationship of the AE to study therapy. Categorized as follows:

Definite	The adverse event is clearly related to the investigational agent(s)
Probable	The adverse event is likely related to the investigational agent(s)
Possible	The adverse event may be related to the investigational agent(s)
Unlikely	The adverse event is doubtfully related to the investigational agent(s)
Unrelated	The adverse event is clearly not related to the investigational agent(s)

6. Whether or not the AE is Serious or Not Serious as defined in Section 15.3 Serious Adverse Events.

7. Whether the AE is Suspected and/or Unexpected.

Suspected	Any AE for which there is a reasonable possibility that the drug caused the AE. For the purposes of expedited safety reporting, 'reasonable possibility' means there is evidence to suggest a causal relationship between the drug and the AE.
Unexpected	Any AE for which the nature or severity of the event is not consistent with the applicable product information, e.g., the Investigator's Brochure or Package Insert.

8. Action taken as a result of the AE.
9. Outcome.

15.6 Expedited Adverse Event Reporting Requirements

All AEs, regardless if serious or not, will be described in the source documents, reported on the applicable AE page of the CRFs, and entered into *Velos*. However, certain adverse events must also be reported in an expedited manner for more timely monitoring of patient safety and care. Appendix A provides information about these expedited reporting requirements.

16.0 STATISTICAL CONSIDERATIONS

16.1 Overview

This single-arm phase II study will assess the progression-free rate at 3 months after concurrent axitinib and pembrolizumab therapy in advanced soft tissue sarcomas not amenable to traditional chemotherapy or that have failed standard of care treatment. (See Section 7.0, Study Design). Sample size justification of 30 patients is provided in Section 16.4 based on single-arm survival design.

16.2 Definitions and Endpoints

Analysis set

Analysis set: The analysis set for evaluation of treatment response consists of all study-eligible patients who receive at least 80% of scheduled axitinib doses and two infusions of pembrolizumab, have measurable disease at baseline and at least one post-baseline disease assessment. The analysis set for the second endpoint of toxicity will include all study-eligible patients who receive at least one dose of both drugs.

Exclusions

Patients who are enrolled on study but do not complete the first cycle and drop out due to any reason except dose limiting toxicity (DLT) or clinical progression will be excluded from analysis of efficacy endpoints. Reasons for such exclusions will be characterized such as consent withdrawn or eligibility subsequently not confirmed.

Endpoints

The primary endpoint will be the progression-free survival by RECIST 1.1 in evaluable patients treated with combination axitinib/pembrolizumab.

The secondary endpoints include:

- The objective response rate (ORR) is defined as the proportion of patients whose response by RECIST 1.1 is complete response (CR) or partial response (PR). The clinical benefit rate (CBR) is the proportion of patients whose response by RECIST 1.1 is complete response (CR), partial response (PR), or stable disease (SD). The time to progression (TTP) is defined as time from treatment initiation to first occurrence of progression by RECIST v1.1 or death of sarcoma patients treated with concurrent axitinib/pembrolizumab therapy. Overall survival (OS) is defined as the elapsed time from treatment initiation to death from any cause, whichever is earlier. Patients alive or those lost to follow-up will be censored at the last date of contact (or last date known to be alive).

- Adverse events, including dose-limiting toxicity and grade 3 or grade 4 serious adverse events

    - DLT includes treatment-related (possible, probably, or definite) grade 3 or higher toxicities as detailed in section 7.2. Toxicity will be reported by short name, type, grade, and attribution as per CTCAE v4.0.3.

The exploratory endpoints include:

- The quantity of CD3+ T-cells in peripheral blood and in tumor biopsies at each timepoint (baseline, cycle 3, and off-study).

- The expression category in tumor tissue (none (0%), low (<5%), intermediate (5-50%), or high (>50%) of various markers including PD-1, PD-L1, PD-L2, CTLA-4, TIM-3, LAG-3 at each timepoint (baseline, cycle 3, and off-study).

- The following T cell subsets will be studied in peripheral blood and tumor tissue: (CD4, CD8, T-reg, CTLA4, TIM3, LAG3, memory, naïve, PD-1, Ki67). For each marker, the absolute change in the marker(s) value will be calculated:
    - Cycle 3 marker value minus Baseline marker value
    - Progression marker value minus Cycle 3 marker value
    - Progression marker value minus Baseline marker value

- For each of the alternative (non-RECIST) radiological measurements we will categorize tumor response as CR/PR/SD vs PD. CT and/or MRI with dynamic contrast enhanced sequences will be collected throughout the study at every disease evaluation and analyzed using Choi criteria, MRI volumetrics, and

immune-related response criteria. PET/CT will be obtained at baseline, Cycle 3, and off-study and tumor response analyzed by PERCIST 1.0.

- The quantity of circulating tumor cells (CTCs) in peripheral blood will be measured at three timepoints: baseline, cycle 3, and off-study.

- RNA expression of a panel of angiogenesis and immune related genes performed using Nanostring Universal Immunooncology platform on tumor biopsies. RNA expression of immune related genes will be performed on banked peripheral blood samples by Simpatica Medicine. Expression profiles will be obtained at baseline and at Cycle 3 Day 1 on treatment.

T cell clonality indices of tumor biopsies and peripheral blood at baseline and on treatment at Cycle 3 Day 1.

Plasma angiogenic activity at baseline, Cycle 1 Day 8, and Cycle 3 Day 1.

16.3 Statistical Analysis

Baseline clinical and demographic characteristics of patients will be summarized. Categorical data will be summarized by the number and percent of patients in each category. Continuous variables will be summarized by descriptive statistics using mean, standard deviation, median, minimum and maximum.

Analysis for Primary Endpoint

The primary endpoint is progression-free survival (PFS) by RECIST 1.1 in evaluable patients treated with combination axitinib/pembrolizumab. PFS will be analyzed by Kaplan-Meier (KM) methods, and point estimates and 2-sided 95% confidence intervals for PFS will be reported for selected times such as 3, 6 and 12 months from treatment start using Greenwood's variance and the log-log transform method. Median PFS time, if attained, will also be reported.

Analysis for Secondary Endpoints

Objective response (CR or PR) and best response (CR, PR, or SD) by RECIST 1.1 to axitinib/pembrolizumab combination therapy will be tabulated, summarized and reported with 95% confidence intervals. Overall survival will be analyzed by Kaplan-Meier (KM) curves with standard error according to Greenwood's variance and the log-log transform method. Median OS will be reported if attained.

Toxicity will be summarized by reporting the number of patients treated, the number who experience DLT, SAEs and grade 3 or higher AE, the proportion of patients treated at various axitinib dose titration levels, the number of patients who discontinue therapy, and the reasons for discontinuation. Comprehensive safety data on all grade 3 and 4 toxicities will be tabulated by type, grade, duration, attribution to treatment, and dose level received.

Analysis for Exploratory Endpoints

Although a large number of factors are being tested, we will not be making adjustments for multiple testing because these analyses are exploratory and hypothesis-generating. Findings will be validated in future larger studies.

Tumor infiltrating T-cells will be quantified by immunohistochemistry using a semiquantitative scoring system as detailed in Appendix F, Section C. We will test for association of clinical benefit status (CR/PR/SD vs PD) vs. T-cell quantity using a Wilcoxon rank sum test. We will test for association with progression-free status at 3 months (progression vs. no progression) vs. T-cell quantity using Wilcoxon rank sum test.

The expression of immune markers such as PD-1, PD-L1, PD-L2, CTLA4, TIM3, and LAG3 by immunohistochemistry will be categorized as negative (0%), low (<5%), intermediate (5-50%), or high (>50%) as detailed in Appendix F, Section C. We will test for association of clinical benefit status (CR/PR/SD vs PD) and expression category using a Chi-square test. We will test for association of expression category vs. PFS using a log-rank test.

To test for the association of the absolute change of each subset (CD4+, CD8+, T-reg, CTLA4+, TIM3+, LAG3+, memory, naïve, PD-1, Ki67) by flow cytometry in blood and tumor tissue between three timepoints (Cycle 3 marker value minus Baseline marker value, Progression marker value minus Cycle 3 marker value, Progression marker value minus Baseline marker value)) with clinical benefit status (CR/PR/SD vs. PD), we will use a Wilcoxon rank sum test. Absolute changes across timepoints will be graphically presented for each marker using boxplots.

To test for the association of the absolute change between three timepoints (Cycle 3 marker value minus Baseline marker value, Progression marker value minus Cycle 3 marker value, Progression marker value minus Baseline marker value)) of each subset (CD4+, CD8+, T-reg, CTLA4+, TIM3+, LAG3+, memory, naïve, PD-1, Ki67) with progression-free status at 3 months (progression vs. no progression), we will use a Wilcoxon rank sum test.

We will describe the relationship between the assessment of tumor response according to RECIST 1.1 and tumor response according to alternative radiologic methods (CT-based Choi criteria, immune related response criteria, MRI volumetrics, and PERCIST 1.0) using a 2 x 2 table to estimate the concordance between the two approaches. Results will be reported with 95% confidence interval on the proportion concordant. We are not attempting to demonstrate superiority of the alternative approaches, thus we will not use McNemar's test. We simply will describe the relationships between the approaches.

To test for the association of absolute change in CTC counts at two time points (cycle 3 and off-study) compared to baseline with clinical benefit status (CR/PR/SD vs. PD), we will use a Student t-test and Wilcoxon rank sum test for a specific time point and analysis of variance (ANOVA) and Kruskal-Wallis test for comparing all two time points. Repeated measure data analysis method will be used to analyze CTC count profiles by clinical benefit status.

To test for the association of absolute change in CTC counts at two time points (cycle 3 and off-study) compared to baseline with progression-free status at 3 months (progression vs. no progression), we will use a Student t-test and Wilcoxon rank sum test for a specific time point and analysis of variance (ANOVA) and Kruskal-Wallis test for comparing all two time points. Repeated measure data analysis method will be used to analyze CTC count profiles by progression-free status at 3 months.

Developing candidate biomarkers: We will be obtaining a large number of variables and looking for associations with response. In parallel to the above analysis, we will also utilize penalized logistic regression with bootstrapping to identify variables associated with response. We will adjust for multiple testing using Bonferroni adjustment and false discovery rate (FDR) due to multiplicity.

Genetic expression analysis: All statistical analysis will be performed by Nanostring, initially blinded to patient outcomes. Gene expression data will be log base 2-transformed and normalized using housekeeping genes selected using the nSolver 2.6 package. To identify signatures, an unsupervised cluster analysis will be performed requiring >15 genes and a correlation coefficient (r) among the genes >0.8. Genetic signature expression will be associated with the binary outcome of progression-free status at 3 months. These signatures will be analyzed using a penalized logistic regression analysis since these signatures are high-dimensional and highly correlated with each other. Although LASSO method is used in this analysis, elastic next approach may be considered as well.

16.4 Patient Enrollment and Sample Size

The study will be conducted at Sylvester Comprehensive Cancer Center. Our expectation is that proposed treatment will improve PFS at 3 months from 19% to 40%. Assuming 15 months for enrollment and 12 months minimum follow-up, 40 evaluable patients (including expansion) will provide 97.5% power to reject 3 month PFS rate of 19% in favor of 3 month PFS rate of 40% using a one-sample survival test with one-sided 5% significance level using STPLAN (MD Anderson Biostatistics software) [6]. With estimated accrual of 1 patient every 2 months in the expansion cohort, we anticipate 18-24 months for patient enrollment followed by an additional year of follow up time. Expected time to study completion is 3 years from date open to enrollment.

For the secondary objectives utilizing patient samples, we anticipate that we will be able to obtain complete sets or measurements for all patients (pre-treatment, on-treatment, and post-treatment) to permit analysis of intrapatient pharmacodynamic effect of therapy. However, a critical component of this study is the analysis for exploratory endpoints, which are hypothesis-generating in regards to potential biomarkers for response in sarcoma patients treated with concurrent axitinib/pembrolizumab. Thus, if our primary endpoint is met by the conclusion of enrollment and the combination appears active with potential benefit to patients, yet collection of tumor biopsies and peripheral blood sample collection was incomplete, we would consider extending enrollment to an additional 10 patients in an effort to obtain complete paired samples at the baseline, cycle 3, and off-study timepoints in responders and non-responders.

Power considerations for correlative studies: If we estimate 75% collection of complete, interpretable biopsies and blood samples from 40 patients, our analysis set of responders vs. non-responders will include approximately 10 responding patients and 20 non-responding patients. A sample size of 10 in the responder group and 20 in the non-responder group will provide 89.4% statistical power to detect a difference in mean change of 2.5 with a standard deviation of 1 at both time points, and a correlation between two measurement pairs of 0.2. The significance level is 0.00083 using Bonferroni adjustment (=0.05/60).

17.0 DATA AND SAFETY MONITORING (Interim monitoring)

The Research Team will continuously monitor study accruals, toxicities, and response to treatment. The UM/Sylvester Comprehensive Cancer Center's Data and Safety Monitoring Committee (DSMC) will monitor this protocol according to the Cancer Center's DSM Plan. In its oversight capacity, the DSMC bears responsibility for suspending or terminating this study. DSMC oversight of the conduct of this trial includes ongoing review of accrual and adverse event data, and periodic review of response. The DSMC also reviews reports from internal audits of protocol compliance and data integrity conducted by the University of Miami, Office of Research Compliance Assessment. The guidelines appearing in this section are offered for DSMC consideration in assessing adverse events and response to study treatment.

Early stopping guidelines

We propose the following guidelines for the DSMC in its review of accumulating data on toxicity and response. The proposed guidelines were developed using Bayesian methods, which can be applied at any stage of enrollment without advance specification of the number of interim analyses to be performed, or the number of patients evaluable for toxicity, or response, at the time such assessments are made (45, 46).

Under the Bayesian method, we assign a prior probability (level of belief at the start of the trial) to a range of possible values for the true toxicity rate, and likewise for the true response rate. As data on treated patients become available, each of these probability distributions is revised and the resulting posterior probability becomes the basis for recommending either early termination or continuation of the study.

Early stopping rule is applied to 30 patients who receive concurrent axitinib with pembrolizumab. Details are given in the remainder of this section.

Early stopping due to safety

If a treatment-related (possible, probable, or definite) death occurs, enrollment will be suspended and continuation of the study will be reassessed by the DSMC.

If four out of first five treated patients experiences DLT then the study will be suspended and reviewed with the Data Safety Monitoring Committee for consideration of modification or

termination. It is based on Bayesian stopping rule that DLT rate should not exceed 40% with a posterior probability of 90% or higher.

For purposes of safety monitoring, we define an unacceptable toxicity to be any treatment-related DLT, where acute refers to the period of time from start of study treatment to one month following completion or discontinuation of treatment. Unacceptable toxicity is expected to occur in no more than 30% of study patients. Early stopping (suspension and possibly termination) will be considered if there is evidence that the proportion of patients experiencing unacceptable toxicity exceeds 40%. Specifically, we suggest as a guideline for early termination a posterior probability of 90% or higher that the rate of unacceptable toxicity exceeds 40%.

The table below shows specific instances where this guideline is met, thus suggesting early termination due to evidence of excessive toxicity.

Number of patients with DLT among 30 patients	Total patients evaluated	Observed toxicity rate
4	4 to 5	≥ 80%
5	6 to 7	≥ 71%
6	8 to 9	≥ 67%
7	10 to 11	≥ 64%
8	12 to 13	≥ 62%
9	14 to 15	≥ 60%
10	16 to 17	≥ 58%
11	18 to 19	≥ 57%
12	20 to 21	≥ 54%
13	22 to 24	≥ 54%
14	25 to 26	≥ 54%
15	27 to 28	≥ 53%
16	29	≥ 55%

Table 13. Stopping Rule for Safety

For example, if 7 patients have been assessed for toxicity, the second row of the above table indicates that early stopping should be considered if 5 (71%) of these patients have experienced DLT.

Posterior probabilities used to derive the preceding table are calculated under a prior beta distribution with parameters $\beta 1 = 0.6$ and $\beta 2 = 1.4$, which corresponds to an expected rate of 30% based on very limited information, roughly equivalent to having studied two patients. This prior distribution implies also an a priori chance of only 32% that the rate of unacceptable toxicity is 40% or greater.

<u>Early stopping due to lack of efficacy</u>

We do not propose early stopping of this trial based on interim assessment of efficacy. We need to gather preliminary evidence of efficacy of the treatment for this study population.

18.0 DATA REPORTING

Data must be submitted according to the protocol requirements for all patients registered. Patients for whom documentation is inadequate to determine eligibility will generally be deemed ineligible.

19.0 STUDY MONITORING

This study will be monitored and/or audited (as applicable) according to the University of Miami requirements. See also http://uresearch.miami.edu/regulatory-compliance-services/rcqa and http://research.med.miami.edu/clinical-research/crors.

20.0 INVESTIGATOR RESPONSIBILITIES

20.1 Investigator Responsibility/Performance

The investigator will ensure that this study is conducted in accordance with all regulations governing the protection of human subjects. The investigator will ensure that all work and services described in or associated with this protocol will be conducted in accordance with the investigational plan, applicable regulations, and the highest standards of medical and clinical research practice.

20.2 Confidentiality

The investigator must ensure that each subject's anonymity will be maintained and each subject's identity will be protected from unauthorized parties. A number will be assigned to each subject upon study entry and the number and the subject's initials will be used to identify the subject for the duration of the study. The investigator will maintain all documents related to this study in strict confidence.

20.3 Informed Consent and Permission to Use Protected Health Information

It is the responsibility of the investigator to obtain written informed consent from each subject participating in this study after adequate explanation, in lay language, of the methods, objectives, anticipated benefits, and potential hazards of the study. The investigator must also explain that the subject is completely free to refuse to enter the study or to discontinue participation at any time (for any reason) and receive alternative conventional therapy as indicated. Prior to study participation, each subject will sign an IRB approved informed consent form and receive a copy of same (and information leaflet, if appropriate). For subjects not qualified or able to give legal consent, consent must be obtained from a parent, legal guardian, or custodian. The investigator or designee **must** explain to the subject before enrollment into the study that for evaluation of study results, the subject's protected health information obtained during

the study may be shared with the study sponsor, regulatory agencies, and the IRB. It is the investigator's (or designee's) responsibility to obtain permission to use protected health information per HIPAA from each subject, or if appropriate, the subjects' parent or legal guardian.

20.4 Source Documentation and Investigator Files

The investigator must maintain adequate and accurate records to fully document the conduct of the study and to ensure that study data can be subsequently verified. These documents should be classified into two separate categories: (1) investigator study file and (2) subject clinical source documents that corroborate data collected on the CRF's. Subject clinical source documents may include hospital/clinic patient records; physician's and nurse's notes; appointment book; original laboratory, ECG, EEG, radiology, pathology, and special assessment reports; pharmacy dispensing records; subject diaries; signed informed consent forms; and consultant letters. When the CRF or any form is used as the source document, this must be clearly stated in the investigator study file. Minimally, the following be documented in source documents:

- Medical history/physical condition and diagnosis of the subject before involvement in the study sufficient to verify protocol entry criteria
- Study number, assigned subject number, and verification that written informed consent was obtained (each recorded in dated and signed notes on the day of entry into the study)
- Progress notes for each subject visit
- Documentation of treatment
- Laboratory test results
- Adverse events (action taken and resolution)
- Condition and response of subject upon completion of or early termination from the study

20.5 Recording and Processing of Data

If using hard copies of CRF's, study center personnel will complete individual CRF's in black ink. All corrections to entered data will be made by drawing a single line through the information to be corrected without obscuring it. All corrections will be initialed, dated and explained, if necessary. The use of "white-out" or obscuring correction tape will be prohibited. A CRF is required for every patient who received any amount of study treatment. The investigator will ensure that the CRF's are accurate, complete, legible and timely. Separate source records are required to support all CRF entries except those for which use of the CRF as source document is clearly allowed per note in the investigator study file.

Data must be submitted according to the protocol requirements for ALL patients registered. Patients for whom documentation is inadequate to determine eligibility will generally be deemed ineligible.

A list of forms to be submitted, as well as expectation dates may be found in Appendix B.

20.6 **Non-Protocol Research**

No investigative procedures other than those described in this protocol will be undertaken on the enrolled subjects without the agreement of the IRB.

20.7 **Ethics**

The investigator agrees to conduct the study in compliance with the protocol, current good clinical practices, and all applicable (local, FDA) regulatory guidelines and standard of ethics.

UM Ethics Programs' Research Ethics Consultation Service (RECS) is a free resource for UM Researchers. See the website for further information: http://www.miami.edu/index.php/ethics/projects/recs/

20.8 **Essential documents for the conduct of a clinical trial**

Essential documents are those documents with individually and collectively permit evaluation of the conduct of a trial and the quality of the data produced. The following documents should be on file: 1) 1572 or investigator's agreement (for studies involving IND drugs or devices, respectively); 2) CV's and license of all Investigators; 3) IRB documentation/correspondance and 4) Documentation of IRB certification

REFERENCES

1. WB Coley. Contribution to the Knowledge of Sarcoma. Ann Surg 1891; 14: 199-220.
2. Trucco BA, Goldberg JM. Immunotherapy in sarcoma: a new frontier. Discov Med, 2014. 17(94): p. 201-6.
3. Merchant M, et al. Genetically engineered NY-ESO-1 specific (C259) T Cells in HLA-A2+ Patients with Synovial Sarcoma. 2014 Connective Tissue Oncology Society Annual Meeting, Berlin, Germany. Paper 026.
4. Movva S, et al. Predictive biomarker profiling of >1900 sarcomas: Identification of potential novel treatment modalities. J Clin Oncol 2014; 32: abstr 10509.
5. D'Angelo SP, et al. Prevalence of tumor-infiltrating lymphocytes and PD-L1 expression in the soft tissue sarcoma microenvironment. Hum Pathol 2014; 46: 357-65.
6. Shen JK, et al. Programmed cell death ligant 1 expression in osteosarcoma. Cancer Immunol Res 2014; 2: 690-8.
7. Finke J, et al. Sunitinib reverses type-1 immune suppression and decreases T-regulatory cells in renal cell carcinoma patients. Clin Cancer Res, 2008. 14(20): p. 6674-82.
8. Gabrilovich D, et al. Production of vascular endothelial growth factor by human tumors inhibits the functional maturation of dendritic cells. Nat Med, 1996. 2(10): p. 1096-103.
9. Xin H, et al. Sunitinib inhibition of Stat3 induces renal cell carcinoma tumor cell apoptosis and reduces immunosuppressive cells. Cancer Res, 2009. 69(6): p. 2506-13.
10. Harshman L, et al. PD-1 blockade in renal cell carcinoma: To equilibrium and beyond. . Immunol Res, 2014. 2(12): p. 1132-41.
11. Hodi FS, et al. Bevacizumab plus Ipilimumab in Patients with Metastatic Melanoma. Cancer Immunol Res, 2014. 2(7): p. 632-42
12. Lazar A, et al., Angiogenesis-promoting gene patterns in alveolar soft part sarcoma. Clin Cancer Res, 2007. 13(7314-21).
13. Kummar S, et al. Cediranib for metastatic alveolar soft part sarcoma. J Clin Oncol, 2013. 31: p. 2296-302.
14. Stacchiotti S, et al. Response to sunitinib malate in advanced alveolar soft part sarcoma. Clin Cancer Res, 2009. 15: p. 1096-104.
15. Goldberg JM, et al. Biologic Activity of Autologous, Granulocyte-Macrophage Colony Stimulating Factor Secreting Alveolar Soft Parts Sarcoma and Clear Cell Sarcoma Vaccines. Clin Cancer Res, 2015. 21(3178-86).
16. Klumpen HJ, et al. Moving towards dose individualization of tyrosine kinase inhibitors. Cancer Treat Rev, 2011. 37(251:60).
17. Rini BI, et al. Axitinib in metastatic renal cell carcinoma: Results of a pharmacokinetic and pharmacodynamic analysis. J Clin Pharmacol, 2013. 53(491-504).
18. Rini BI, et al. Comparative effectiveness of axitinib versus sorafenib in advanced renal cell carcinoma (AXIS): A randomised phase 3 trial. Lancet, 2011. 378(1931-1939).
19. Herbst RS, et al. Predictive correlates of response to the anti-PD-L1 antibody MPDL3280A in cancer patients. Nature, 2014. 515: 563-567
20. Taube JM, et al. Association of PD-1, PD-1 Ligands, and Other Features of the Tumor Immune Microenvironment with Response to Anti-PD-1 Therapy. Clin Canc Res, 2014. 20: 5064-74

21. Nirschl, et al. Molecular Pathways: Coexpression of Immune Checkpoint Molecules: Signaling Pathways and Implications for Cancer Immunotherapy. Clin Canc Res , 2013. 19:4917-24.
22. Canter RJ, et al. Radiographic and histologic response to neoadjuvant radiotherapy in patients with soft tissue sarcoma. Ann. Surg. Oncol. 2010;17:2578–2584
23. Trucco, BA et al. Pazopanib in sarcoma: expanding the PALETTE. Curr Opin Oncol, 2013. 25(4): 373-8
24. Choi H, et al., Correlation of computed tomography and positron emission tomography in patients with metastatic gastrointestinal stromal tumor treated at a single institution with imatinib mesylate: proposal of new computed tomography response criteria. J Clin Oncol, 2007. 25(13): p. 1753-9.
25. Stacchiotti S, et al. High-grade soft-tissue sarcomas: tumor response assessment--pilot study to assess the correlation between radiologic and pathologic response by using RECIST and Choi criteria. Radiology 2009;251:447–456.
26. Kumar R, et al. Role of PET/PET-CT in the amanagement of sarcomas. Expert Rev Anticancer Ther, 2006. 6(8): 1241-50.
27. Wahl R, et al. From RECIST to PERCIST: Evolving Considerations for PET response criteria in solid tumors. J Nucl Med, 2009. 50 Suppl 1: 122S-50S).
28. Fendler WP, et al. PET response criteria in solid tumors predicts progression-free survival and time to local or distant progression after chemotherapy with regional hyperthermia for soft-tissue sarcoma. J Nucl Med, 2015. 56(530-7).
29. Subhawong, T et al. Value Added: Functional MR Imaging in Management of Bone and Soft Tissue Sarcomas. Curr Opin Oncol, in press.
30. Wang CS, et al. Noninvasive assessment of response to neoadjuvant chemotherapy in osteosarcoma of long bones with diffusion-weighted imaging: an initial in vivo study. PloS One 2013;8:e72679.
31. Byun BH, et al. Combination of 18F-FDG PET/CT and diffusion-weighted MR imaging as a predictor of histologic response to neoadjuvant chemotherapy: preliminary results in osteosarcoma. J. Nucl. Med. Off. Publ. Soc. Nucl. Med. 2013, 54:1053–1059.
32. Glade Bender JL, et al. Phase I pharmacokinetic and pharmacodynamic study of pazopanib in children with soft tissue sarcoma and other refractory solid tumors: a children's oncology group phase I consortium report. J. Clin. Oncol. Off. J. Am. Soc. Clin. Oncol. 2013;31:3034–3043.
33. Meyer JM, et al. Phase I Trial of Preoperative Chemoradiation plus Sorafenib for High-Risk Extremity Soft Tissue Sarcomas with Dynamic Contrast-Enhanced MRI Correlates. Clin. Cancer Res. Off. J. Am. Assoc. Cancer Res. 2013;19:6902–6911.
34. Alic L, et al. Heterogeneity in DCE-MRI parametric maps: a biomarker for treatment response? Phys. Med. Biol. 2011;56:1601–1616.
35. Alic L, et al. Regional heterogeneity changes in DCE-MRI as response to isolated limb perfusion in experimental soft-tissue sarcomas. Contrast Media Mol. Imaging 2013;8:340–349.
36. Wolchok JD, et al. Guidelines for the evaluation of immune therapy activity in solid tumors: immune-related response criteria. Clin Cancer Res, 2009. 15(23): 7412-20.
37. Balic M, et al. Circulating Tumor Cells: From Bench to Bedside. Annu Rev Med. 2013;64):31-44.

38. Goldkorn A. et al. Circulating tumor cell counts are prognostic of overall survival in SWOG S0421: a phase III trial of docetaxel with or without atrasentan for metastatic castration-resistant prostate cancer. J Clin Oncol, 2014. 32(11): 1136-4216
39. Movva S, et al. Predictive biomarker profiling of >1900 sarcomas: Identification of potential novel treatment modalities. J Clin Oncol 2014; 32: abstr 10509.
40. Dubois S, et al. Flow cytometric detection of Ewing sarcoma cells in peripheral blood and bone marrow. Pediatr Blood Cancer. 2010;54:13-8.
41. Satelli A, et al. Universal Marker and Detection Tool for Human Sarcoma Circulating Tumor Cells. Cancer Res. 2014.
42. Zheng S, et al. 3D microfilter device for viable circulating tumor cell (CTC) enrichment from blood. Biomed Microdevices, 2011. 13(203-13).
43. Van Glabbeke M, et al. Progression-free rate as the principal endpoint for phase II trials in soft-tissue sarcomas. Eur J Cancer, 2002. 38(534-9).
44. Robert C, et al. Anti-programmed-death-receptor-1 treatment with pembrolizumab in ipilimumab-refractory advanced melanoma: a randomised dose-comparison cohort of a phase 1 trial. Lancet, 2014. 384(1109).
45. Spiegelhalter DJ, et al. Bayesian approaches to randomized trials. J.R. Statist Soc. A. 1994; 157:357-416.
46. Fayers PM, et al. Tutorial in biostatistics Bayesian data monitoring in clinical trials. Stat Med, 1997. 16(1413-30).

APPENDIX A: EXPEDITED ADVERSE EVENT (AE) REPORTING REQUIREMENTS

For all AEs that meet criteria for expedited reporting, the Principal Investigator (PI) is obligated to pursue and provide follow-up reporting information until the event has resolved or until an acceptable medical endpoint has been reached (i.e. for the duration specified in the protocol), or the patient is lost to follow-up.

The PI and all applicable research study team members should become familiar with the safety profile of the investigational agent(s) and/or intervention at the start of the study and for the duration of the research, e.g. by reviewing the Investigator's Brochure (IB) and any Safety Reports released, by the Sponsor as applicable.

I. FDA Expedited Reporting

a. Sponsor-Investigators i.e. IND Holders, have additional reporting requirements to the FDA and other committees, and should consult the applicable regulations and agency guidelines for these requirements.

b. Since this protocol involves the use of FDA IND agent(s), completion of the FDA MedWatch 3500A Reporting Form is required for Sponsor-Investigators. The Form can be obtained electronically at: http://www.fda.gov/downloads/AboutFDA/ReportsManualsForms/Forms/UCM048334.pdf

   i. All serious, unexpected (unanticipated) and suspected adverse events must be directly reported to the FDA within 15 calendar days of being made known to the Principal Investigator (PI).

   ii. All fatal or life-threatening AEs must be directly reported to the FDA within 7 calendar days of being made known to the PI.

c. For more information regarding reporting to the FDA, please refer to the FDA website for REPORTING GUIDELINES: http://www.fda.gov/Safety/MedWatch/HowToReport/default.htm

II. IRB Expedited Reporting

a. All Investigators should also be aware of local Institutional requirements for AE reporting. For more information regarding the IRB policy, please refer to the UM HSRO's Investigator Manual: http://hsro.med.miami.edu/documents/HRP-103_-_INVESTIGATOR_MANUAL_4.11.2014.docx and the UM HSRO SOP on New Information (HRP-024) https://eprost.med.miami.edu/eProst/Doc/0/HLJ5OTJVQEH419E0I6QPT3B199/HRP-024%20-%20SOP%20-%20New%20Information.docx

b. All AEs that are serious, unanticipated and (at least) possibly related will be reported to the IRB within ten (10) working days of being made known to the PI.

c. Events that are more frequent than anticipated or more severe than expected must be reported to the IRB within ten (10) working days of being made known to the PI.

d. All unanticipated deaths must be reported to the IRB within 24 hours of being made known to the PI.

III. Merck Expedited Reporting (Events of Clinical Interest or ECIs)

a. In addition to the mandatory MedWatch 3500A Form, the PI is also required to comply with all reporting requirements as supplied by the Investigational Drug Sponsor: Merck.

b. Selected non-serious and serious adverse experiences must be reported to Merck within 24 hours (and within 2 working days to Merck Global Safety) regardless of attribution to study treatment. The AEs listed and any event that meets the criteria for reporting (as noted) in the protocol **Appendix H** (event term and Grade) must be reported regardless of physician-determined causality with study medication and whether or not considered immune-related by the physician (unless otherwise specified). Physicians/study coordinators/designated site personnel are required to record these experiences as events of clinical interest (ECIs) on the Adverse Events/Experience Forms and to provide supplemental information (such as medical history, concomitant medications, investigations, etc.) about the event.

c. Additional Sponsor Requirements:

i. Any other Grade 3 or higher event that the investigator/physician considers to be immune-related should be reported as an ECI regardless of whether the specific event term is in **Appendix H (Table 13)** and reported to Merck within 24 hours from the time the Investigator/physician is aware of such an occurrence. Adverse events that are both an SAE and an ECI should be reported one time as an SAE only, however the event must be appropriately identified as an ECI as well in the database (i.e. *Velos*).

ii. Any serious adverse event, or follow up to a serious adverse event, including death due to any cause other than progression of the cancer under study that occurs to any subject from the time the consent is signed through 90 days following cessation of treatment, or the initiation of new anti-cancer therapy, whichever is earlier, whether or not related to Merck product, must be reported within 24 hours to the Sponsor and within 2 working days to Merck Global Safety.

iii. Any other serious adverse event, considered by an investigator who is a qualified physician to be related to a Merck product that is brought to the attention of the investigator at any time outside of the time period specified in the previous paragraph also must be reported immediately to Merck. All subjects with serious adverse events must be followed up for outcome.

iv. A copy of all 15 Day Reports and Annual Progress Reports is submitted as required by FDA, European Union (EU), Pharmaceutical and Medical Devices

agency (PMDA) or other local regulators. Investigators will cross reference this submission according to local regulations to the Merck Investigational Compound Number (IND, CSA, etc.) at the time of submission. Additionally investigators will submit a copy of these reports to Merck & Co., Inc. (Attn: Worldwide Product Safety; FAX 215 993-1220) at the time of submission to FDA.

v. **SAE reports and any other relevant safety information are to be forwarded to the** Merck Global (ATTN: Worldwide Product) Safety Fax Number: +1-215-993-1220

IV. Pfizer Expedited Reporting Requirements

a. For complete details always refer to the *Safety Reporting Reference Manual Investigator-Initiated Research (IIR) Studies With Pfizer Products*

b. Complete the *Pfizer-provided Investigator-Initiated Research Serious Adverse Event (IIR SAE) Form* (**or other agreed reporting method as specified by and discussed with the Principal Investigator**), and submit it to Pfizer immediately for a death or life-threatening event, and within 24 hours for all other reportable SAEs.

c. Submit the *Pfizer-provided Reportable Events Fax Cover Sheet* with each submission of an SAE to Pfizer.

d. Pfizer-specified "Points to Note: SAE Reporting"

i. IIR SAE forms (**or other agreed reporting method as specified by an discussed with the Principal Investigator)** must be submitted for all SAEs in subjects receiving a Pfizer product or blinded therapy occurring in the specified reporting period as defined in your agreement with Pfizer.

ii. Even those that are not study-drug-related (e.g., those associated with concomitant medications) must be reported.

iii. Non-serious AEs observed in the clinical trial are NOT submitted.

iv. Specifically excluded from the reporting requirements for SAEs under this provision is any SAE identified in the protocol as anticipated to occur in the study population at some frequency independent of drug exposure, unless the Principal Investigator assesses such an event as related to the Pfizer Product.

v.

APPENDIX B: DATA SUBMISSION SCHEDULE

CASE REPORT FORM(S)	TIMEPOINT TO BE COMPLETED
Pre-Treatment	
ICF, including HIPAA signed/dated	Prior to registration
Eligibility Checklist	
SCCC Protocol Enrollment Form	
On-study Form	e.g. Within 30 days of registration
On Treatment	
Treatment Form Cycle X, Day Y	e.g. Due every cycle for phase II studies
End of Treatment	
Off Treatment Form	Within 14 days of discontinuation/completion of protocol therapy
Follow-Up (for studies with long term follow-up)	
Follow-up Form	Every 3 months if < 2 years from study entry; Every 6 months is 2-5 years from study entry; Every 12 months if more than 5 years from study entry
Progression/Relapse	Within 4 weeks of knowledge of progression/relapse
Notice of Death Form	Within 4 weeks of knowledge of death
Subsequent Malignancy	Within 4 weeks of knowledge of another malignancy

APPENDIX C: PERFORMANCE STATUS SCALES

PERFORMANCE STATUS CRITERIA					
ECOG (Zubrod)		**Karnofsky**		**Lansky**	
Score	Description	Score	Description	Score	Description
0	Fully active, able to carry on all pre-disease performance without restriction.	100	Normal, no complaints, no evidence of disease.	100	Fully active, normal.
		90	Able to carry on normal activity, minor signs or symptoms of disease.	90	Minor restrictions in physically strenuous activity.
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light housework, office work.	80	Normal activity with effort, some signs or symptoms of disease.	80	Active, but tires more quickly.
		70	Cares for self, unable to carry on normal activity or do active work.	70	Both greater restriction of, and less time spent in, play activity.
2	Ambulatory and capable of all selfcare but unable to carry out any work activities. Up and about more than 50% of waking hours.	60	Requires occasional assistance, but is able to care for most of his/her needs.	60	Up and around, but minimal active play; keeps busy with quieter activities.
		50	Requires considerable assistance and frequent medical care.	50	Gets dressed, but lies around much of the day; no active play; able to participate in all quiet play and activities.
3	Capable of only limited selfcare, confined to bed or chair more than 50% of waking hours.	40	Disabled, requires special care and assistance.	40	Mostly in bed, participates in quiet activities.
		30	Severely disabled, hospitalization indicated. Death not imminent.	30	In bed, needs assistance even for quiet play.
4	Completely disabled. Cannot carry on any selfcare. Totally confined to a bed or chair.	20	Very sick, hospitalization indicated. Death not imminent.	20	Often sleeping, play entirely limited to very passive activities.
		10	Moribund, fatal processes progressing rapidly.	10	No play, does not get out of bed.
5	Dead	0	Dead	0	Dead

As published in Am J Clin Oncol: Oken MM, Creech RH, Tormey DC, Horton J, Davis TE, McFadden ET, Carbone PP. Toxicity and Response Criteria of the Eastern Cooperative Oncology Group. Am J Clin Oncol 1982;5:649-655. The Eastern Cooperative Oncology Group, Robert Comis, MD, Group Chair.

APPENDIX D: NYHA CLASSIFICATION OF HEART DISEASE

New York Heart Association (NYHA) classification of heart disease

NYHA Class	Symptoms
I	No symptoms and no limitation in ordinary physical activity, e.g. shortness of breath when walking, climbing stairs etc.
II	Mild symptoms (mild shortness of breath and/or angina) and slight limitation during ordinary activity.
III	Marked limitation in activity due to symptoms, even during less-than-ordinary activity, e.g. walking short distances (20–100 m). Comfortable only at rest.
IV	Severe limitations. Experiences symptoms even while **at rest**. Mostly bedbound patients.

APPENDIX E: INFORMATION ON POSSIBLE DRUG INTERACTIONS [AXITINIB]

Information on Possible Interactions with Other Agents for Patients and Their Caregivers and Non-Study Healthcare Team*

*[Note to Investigators: This **suggested** appendix consists of an "information sheet" to be handed to the patient at the time of enrollment. Use or modify the text as appropriate for the study agent, so that the patient is aware of the risks and can communicate with their regular prescriber(s) and pharmacist. A convenient wallet-sized information card is also included for the patient to clip out and retain at all times. **This document must be IRB-reviewed and approved prior to patient distribution**.]*

The patient _____ is enrolled on a clinical trial using the experimental agent **axitinib**. *This form is addressed to the patient, but includes important information for others who care for this patient.*

Axitinib interacts with many drugs that are processed by your liver. Because of this, it is very important to tell your study doctors about all of your medicine before you start this study. It is also very important to tell them if you stop taking any regular medicine, or if you start taking a new medicine while you take part in this study. When you talk about your medicine with your study doctor, include medicine you buy without a prescription at the drug store (over-the-counter remedy), or herbal supplements such as St. John's wort.

Many health care prescribers can write prescriptions. You must also tell your other prescribers (doctors, physicians' assistants or nurse practitioners) that you are taking part in a clinical trial. **Bring this paper with you and keep the attached information card in your wallet**. These are the things that you and they need to know:

Axitinib interacts with (a) certain specific enzyme(s) in your liver.

- The enzyme(s) in question is/are cytochrome P450 (CYP) 3A4/5 and, to a lesser extent (<10 % each), by CYP1A2, CYP2C19, and uridine diphosphate glucuronosyltransferase (UGT) 1A1
- Axitinib must be used very carefully with other medicines that need these liver enzymes to be effective or to be cleared from your system.
- Other medicines may also affect the activity of the enzyme.
    - Substances that increase the enzyme's activity ("inducers") could reduce the effectiveness of the drug, while substances that decrease the enzyme's activity ("inhibitors") could result in high levels of the active drug, increasing the chance of harmful side effects.
    - Substances that increase the enzyme's activity ("inducers") could result in high levels of the active drug, increasing the chance of harmful side effects, while substances that decrease the enzyme's activity ("inhibitors") could reduce the effectiveness of the drug.
    - Axitinib is considered a(n) "[inducer/inhibitor]"of the enzyme, meaning that it can affect the levels of other drugs that are processed by that enzyme. This can lead to harmful side effects and/or reduce the effectiveness of those medications.

- You and healthcare providers who prescribe drugs for you must be careful about adding or removing any drug in this category.
- Before you start the study, your study doctor will work with your regular prescriber to switch any medicines that are considered "strong inducers/inhibitors or substrates of cytochrome P450 (CYP) 3A4/5, CYP1A2, CYP2C19, and UGT 1A1 ."
- Your prescribers should look at this web site: http://medicine.iupui.edu/clinpharm/ddis/table.aspx or consult a medical reference to see if any medicine they want to prescribe is on a list of drugs to avoid.
- Please be very careful! Over-the-counter drugs have a brand name on the label—it's usually big and catches your eye. They also have a generic name—it's usually small and located above or below the brand name, and printed in the ingredient list. Find the generic name and determine, with the pharmacist's help, whether there could be an adverse interaction.
- Be careful:
    - If you take acetaminophen regularly: You should not take more than 4 grams a day if you are an adult or 2.4 grams a day if you are older than 65 years of age. Read labels carefully! Acetaminophen is an ingredient in many medicines for pain, flu, and cold.
    - If you drink grapefruit juice or eat grapefruit: Avoid these until the study is over.
    - If you take herbal medicine regularly: You should not take St. John's wort while you are taking *axitinib.*

Other medicines can be a problem with your study drugs.

- You should check with your doctor or pharmacist whenever you need to use an over-the-counter medicine or herbal supplement.
- Your regular prescriber should check a medical reference or call your study doctor before prescribing any new medicine for you. Your study doctor's name is **Jonathan C. Trent, MD, PhD** and he can be contacted at **305-243-1287**..

INFORMATION ON POSSIBLE DRUG INTERACTIONS	
You are enrolled on a clinical trial using the experimental agent _____. _____ interacts with drugs that are processed by your liver. Because of this, it is very important to: • Tell your doctors if you stop taking regular medicine or if you start taking a new medicine. • Tell all of your prescribers (doctor, physicians' assistant, nurse practitioner, pharmacist) that you are taking part in a clinical trial. • Check with your doctor or pharmacist whenever you need to use an over-the-counter medicine or herbal supplement.	_____ interacts with a specific liver enzyme called **CYP**_____, and must be used very carefully with other medicines that interact with this enzyme. • Before you start the study, your study doctor will work with your regular prescriber to switch any medicines that are considered "strong inducers/inhibitors or substrates of **CYP**_____." • Before prescribing new medicines, your regular prescribers should go to http://medicine.iupui.edu/clinpharm/ddis/table.aspx for a list of drugs to avoid, or contact your study doctor. • Your study doctor's name is _____ and can be contacted at _____.

***Reference**: *National Cancer Institute (NCI) CTEP Generic Protocol Template for Cancer Treatment Trial, Version Date: May 15, 2013.*

APPENDIX F: BIOMARKER, CORRELATIVE AND SPECIAL STUDIES

A. Exploratory Objective 1: Characterization of immunomodulatory factors in patients treated with concurrent axitinib/pembrolizumab

Rationale and Preliminary Studies:

Although the immune system is suspected to play a role in sarcoma development and recurrence, the specific mediators of immune surveillance and suppression of tumor growth are largely unknown. Additionally, we have learned from analysis of patients treated thus far with PD-1-directed therapy that biomarkers of response to immunotherapy are extremely complex. Although PD-L1 expression in tumor cells and the presence of lymphocyte infiltration with PD-1/PD-L1 expression seem to be the best predictors of response, 10-15% of patients without tumor PD-L1 expression have responded to therapy, suggesting that other immunomodulatory factors must be playing a role. In addition to CTLA4 expression, other negative regulatory receptors have also been implicated, including LAG-3 and TIM-3, with pre-clinical blockade of some of these pathways resulting in improved anti-tumor response. Finally, the presence of myeloid-derived suppressor cells (MDSCs) and regulatory T-cells have been shown to negatively impact response to therapy in many cancers, though these are also understudied in sarcomas.

Thus, a key correlative component of our study will be to profile sarcoma tumors and infiltrating tumor lymphocytes using pre- and post-treatment tumor biopsies, and analysis of circulating peripheral blood mononuclear cells to better understand the immune environment in sarcoma patients. **We aim to define changes in intra-tumoral and peripheral blood lymphocyte phenotypes, activation and differentiation markers, and checkpoint ligand expression after treatment with axitinib/pembrolizumab, and correlate with radiographic response to therapy.**

As discussed in Section 2.6, our preliminary studies demonstrated PD-L1 expression in 9 of 10 sarcoma patients, TIL infiltration in all patients, and evidence of increased TIL infiltration and PD-L1 expression in tumors refractory to prior therapy. Using dispase/collagenase digestion as detailed below, a single cell suspension was prepared from freshly harvested sarcoma tissue from a patient undergoing surgery. One million dissociated tumor cells were stained with live/dead dye, CD3, CD4, CD8, CD45, and CD27 and analyzed using FACS. From $1x10^6$ dissociated tumor cells, 15,000 $CD3^+$ lymphocytes were detected, including 10,600 $CD4^+$ and 4200 $CD8^+$ (Figure F1). Most of the cells in the tumor were central memory (T_{cm}) or functional effector memory (T_{em}) phenotypes, with largely depleted naïve fractions. This demonstrated feasibility of isolating a sufficient number of lymphocytes from fresh sarcoma tissue for analysis using this technique.

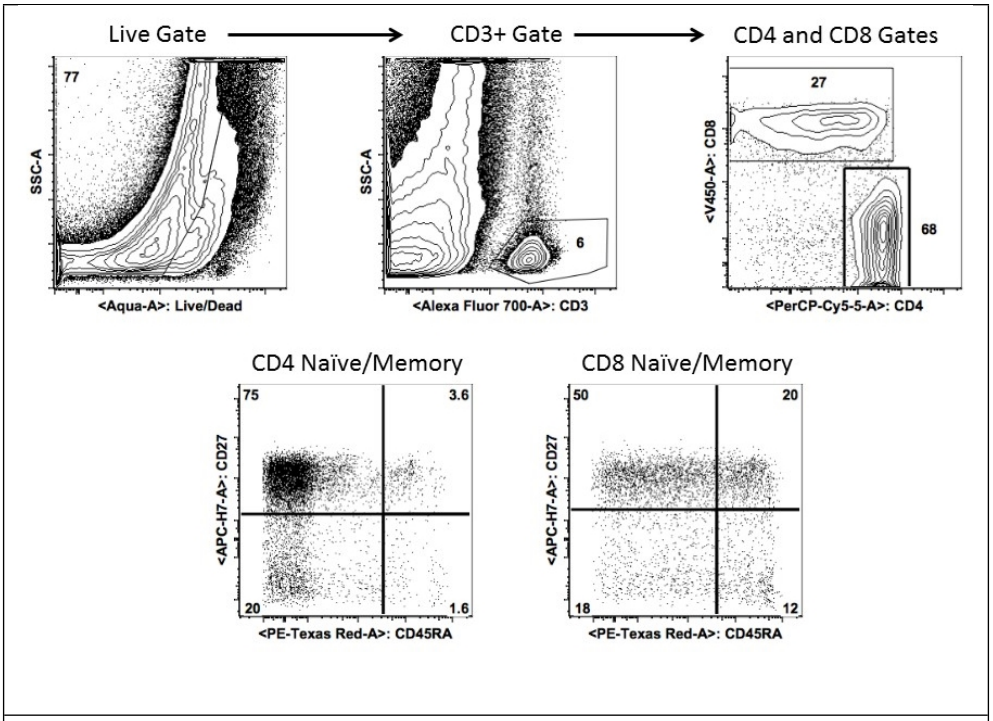


Figure F1. TIL phenotype determined by flow cytometry. After excluding doublets by height/width gating, the live cells were gated by live/dead dye exclusion. From 1 x 10^6 dissociated tumor cells, we were able to detect 15,000 CD3+ cells. Of those 10,600 were CD4+ and 4,200 were CD8+ cells. We were able to determine the memory phenotype and found most of the cells in the tumor were Tcm (CD45RA-/CD27+) or Tem (CD45RA-/CD27-) cells. In particular, the CD4 compartment were largely depleted of their naïve fraction (CD45RA+CD27+). (Courtesy of Eric Wieder/Krishna Komanduri, University of Miami, unpublished data.)

Approach:

1. Analysis of tumor biopsies by immunohistochemistry (Andrew Rosenberg, Department of Pathology

    a. A single core biopsy specimen will be collected in formalin and transported to Andrew Rosenberg in the Department of Pathology (detailed in Section 14). Due to the inefficiencies in traditional immunohistochemistry which require a large number of slides, we have opted to partner with Ignacio Wistuba at MD Anderson Cancer Center who have been performing multiplex IHC on multiple sarcoma trials utilizing immunotherapy. IHC will be performed on FFPE samples using validated antibodies and standard immunohistochemistry procedures for the following markers:

Panel 1	Panel 2	Panel 3
PD-L1	CD3	LAG-3
CD68	CD8	TIM-3
PD-1	Granzyme B	ICOS
CD8	CD45RO	OX-40
CD3	FOXP3	CD3
AE1/AE3	AE1/AE3	AE1/AE3
Co-localizations Report:	Co-localizations Report:	
• Percentage of Malignant cells PD-L1+ (AE1/AE3+PD-L1+) • Total T-cell lymphocytes (CD3+) • T-cells antigen-experienced (CD3+PD-1+) • Cytotoxic T-cells (CD3+CD8+) • Cytotoxic T-cells antigen-experienced (CD3+CD8+PD-1+) • Total macrophages (CD68+) • Macrophages PD-L1+ (CD68+PD-L1+)	• Total T-cell lymphocytes (CD3+) • Cytotoxic T cells activated (CD3+CD8+Granzyme B+) • Effector/memory cytotoxic T cells (CD3+CD8+CD45RO+) • Regulatory T cells [(CD3+FOXP3+)-(CD3+CD8+FOXP3+)]	

Figure F2 – Multiplex immunohistochemistry panels to be performed on tumor biopsies in collaboration with Dr. Wistuba at MD Anderson.

b. The immune cell infiltrate in the tumor will be assessed according to the intensity and geographic distribution within the tumor. The percentage of the immune infiltrate subset (leukocytes - CD45+, T-lymphocytes - CD3+, macrophages - CD68+) is calculated by dividing the total number of each cell type by the total number of cells present in each 20X microscopic power field. A semiquantitative scoring system for the distribution of the cells is 0-3; 0 = no infiltrate; 1 = focal infiltrate, <5%, mostly perivascular in the tumor with some intratumoral extension; 2 = moderate infiltrate, 5-50%, prominent intratumoral and intermingling with neoplastic cells; 3 = extensive infiltrate, >50%, obscuring tumor cells.

c. PD-L1 expression (cytoplasmic and cell membrane) by tumor cells and tumor infiltrating immune cells are independently scored by devising a percentage which is calculated by dividing the number of PD-L1 positive specific cells by the total number of each respective cell type. The positivity is scored as negative (0%), low (<5%), intermediate (5-50%) and high (>50%).

d. PD-1 expression (cytoplasmic and cell membrane) is evaluated in CD3+ T cells using percentage calculated by dividing PD1 positive cells by total number of CD3+ T cells. The positivity is scored as negative (0%), low (<5%), intermediate (5-50%) and high (>50%).

e. Statistical analysis and outcomes are detailed in section 16.3.

2. Analysis of tumor biopsies for immunomodulatory markers (Flow cytometry)

a. 2-4 needle core biopsy specimens will be collected in RPMI media and immediately transferred to Komanduri laboratory.

b. A single cell suspension will be made from the biopsies by digesting tissue with dispase/collagenase (Roche Liberase DH) in media with addition of DNAse at 37 degrees, and single cells isolated using a Ficoll gradient.

c. Cells will be stained with antibodies as per Table F1, followed by FACS analysis to profile tumor infiltrating lymphocytes. This panel allows for the determination of memory/naïve T cell markers, Treg, and markers associated with negative regulation of T cells.

d. Statistical analysis and outcomes as per Section 16.3.

Target	**Clone**	**Fluorochrome**	**Purpose**
CD3	UCHT1	BV570	T cells
CD4	RPA-T4	BV650	T helper subset
CD8	RPA-T8	PerCP-Cy5.5	T cytotoxic subset
CD25	M-A251	PE-Cy7	Treg/Activation
CD27	M-T271	APC-H7	Naïve/Memory
CD45RA	HL100	BV711	Naïve/Memory
CD127	HIL-7R-M21	BB-515	Treg gating
CD152 (CTLA4)	BNI3	BV421	Checkpoint
CD223 (Lag-3)	3DS223H	PE	Checkpoint
CD279 (PD-1)	EH12.1	BV786	Checkpoint
Tim-3	F38-2E2	APC	Checkpoint

Live/Dead	Invitrogen kit	Aqua	Gate on live cells
Dump CD14/16/19/56	Various	PE-CF594	Exclude non-T cells

Table F1. Antibody panel for flow cytometry evaluation of tumor-infiltrating lymphocytes.

3. Analysis of circulating peripheral blood mononuclear cells for immunomodulatory markers and activation (Flow cytometry)

   i. Peripheral blood samples will be collected by phlebotomy
      a. 60 cc blood heparinized (green top tubes) and 10 cc EDTA-anticoagulated (purple top tubes) for plasma and immediately transferred to Dr. Komanduri's laboratory.

   ii. Blood samples will be processed by Ficoll gradient to isolate serum, peripheral blood mononuclear cells, and plasma.

   iii. PBMC will be stained with antibodies as per Table F2. Due to the higher numbers of cells, we will repeat the same antibodies used in the TIL panel, but with additional markers to identify T-regulatory cells and more thoroughly assess activation status.

   iv. Residual PBMC pellets and serum will be cryopreserved for future potential studies.

   v. Statistical analysis and outcomes as per section 16.3.

Target	**Clone**	**Fluorochrome**	**Purpose**
CD3	UCHT1	BV570	T cells
CD4	RPA-T4	BV650	T helper subset
CD8	RPA-T8	PerCP-Cy5.5	T cytotoxic subset
CD25	M-A251	PE-Cy7	Treg/activation
CD27	M-T271	APC-H7	Naïve/Memory
CD45RA	HL100	BV711	Naïve/Memory
CD152 (CTLA4)	BNI3	BV421	Checkpoint
CD223 (Lag-3)	3DS223H	PE	Checkpoint
CD279 (PD-1)	EH12.1	BV786	Checkpoint

Fox-P3	259D/C7	Alexa 647	Treg
Ki-67	B56	Alexa 488	Proliferation
Live/Dead	Invitrogen kit	Aqua	Gate on live cells
Dump CD14/16/19/56	Various	PE-CF594	Exclude non-T cells

Table F2. Antibody panel for flow cytometry evaluation of circulating lymphocytes.

4. Analysis of tumor biopsies and circulating lymphocytes for RNA expression analysis of key angiogenic and immune-related genes

In collaboration with Nanostring, we will utilize a novel, 770-gene Universal Immunooncology panel to analyze pre-treatment tumor biopsies to identify immune signatures that correlate with PFR at 3 months. Additionally, we will compare immune signatures of the on-treatment biopsies to baseline which may reveal additional biomarkers of response, but also clarify mechanism of activity by including analysis of angiogenesis genes. In parallel, we will partner with Simpatica Medicine to perform similar analysis on RNA expression data from sequenced PBMC samples pre- and post-treatment.

5. Analysis of functional VEGF activity from peripheral blood

In collaboration with Jaime Merchan, we will quantify circulating endothelial cells (CEC) and endothelial progenitor cells (CPC), and determine plasma angiogenic activity as previously described. In the ASPS expansion patients, we will also collect PBMC/plasma at Cycle 1 Day 8 prior to pembrolizumab to assess effects from axitinib alone. We will use flow cytometry to measure the concentrations of CEC and CPC in mononuclear cells. Angiogenic activity of patient plasma samples at baseline and after treatment with axitinib or axitinib/pembrolizumab will be measured by exposing plasma samples to human umbilical vein endothelial cells in vitro (HUVEC cell line, Lonza). The resulting proliferation of endothelial cells will be measured using a colorimetric assay. Changes in angiogenic activity will be correlated with patient responses and the maximum dose of axitinib achieved for each patient.

6. Identify clonal populations and the associated tumor-specific antigens of tumor-infiltrating and circulating T cells in alveolar soft part sarcoma patients with clinical responses to axitinib/pembrolizumab therapy

After collecting TIL and PBMC from the expansion ASPS cohort as previously described, we will extract RNA using a microRNAeasy kit (Qiagen). In collaboration with our Oncogenomics core facility, quality and quantity of RNA will be assayed followed by library prep to identify T cell receptors corresponding to unique clones (PCR using the ArcherDX Immunoverse kit). The

libraries will be quantified and sequenced on the Illumina NextSeq sequencer, and analyzed using the ArcherDX immune repertoire analysis software platform. Once we have quantified T cell clones, we will compare baseline to on-treatment frequencies, as well as clonality of TIL vs. peripheral T cells for each patient. We will also compare frequencies of T cell clones across ASPS patients. We will perform confirmatory FISH for the ASPS1-TFE3 fusion as well as whole exome sequencing on baseline tumor samples and utilize existing databases to generate a list of candidate epitopes. In all steps, we will collaborate with Bioinformatics and Oncogenomics core facilities at SCCC.

B. <u>Exploratory Objective 2:</u> Assessment of tumor response using alternative radiologic criteria

<u>Rationale:</u>

Sarcomas often display atypical radiographic changes in response to treatment. Traditional size-based criteria, such as RECIST and WHO criteria, in musculoskeletal tumors have been shown to be relatively insensitive to biological response as measured by other markers, such as histologic necrosis. This is probably due the tendency of sarcomas to undergo internal hemorrhage and necrosis, and as a result enlarge in size (pseudoprogression) despite the fact that the tumor is actually exhibiting positive treatment response. Comparable findings have been reported in other large STS treated with pazopanib. With current advances in radiographic imaging, tumor responses or progression can be detected much earlier, and eliminate some of the clinical problems with RECIST. Additionally, tumor response to immunotherapy often shows initial increase in size, followed by stabilization and shrinkage.

While the official endpoint for this study is still RECIST 1.1, we are including alternative radiologic criteria and techniques to aid in developing the best imaging modality for subsequent trials utilizing immunotherapy for sarcomas.

<u>Approach:</u>

A. Analysis of CT imaging using Choi Criteria and Immune-Related Response Criteria

Subjects enrolled on this study will undergo CT imaging for tumor imaging as per the guidelines of RECIST 1.1 and as detailed in the protocol. In addition to standard tumor measurements performed by the investigators, a blinded radiologist will analyze tumor response by published Choi criteria and immune-related response criteria.

Modified CT Response Evaluation Criteria (Choi criteria)
Tumor size is measured in the longest cross-sectional dimension for each lesion at each time point. As in RECIST criteria, the sum of the longest dimensions of selected target lesions are measured, and the absolute and percent changes of the sum from the pretreatment evaluation to subsequent evaluations are calculated.

Response	Definition
CR	• Disappearance of all lesions • No new lesions
PR	• A decrease in size of ≥ 10% or a decrease in tumor density (Hounsfield units) ≥ 15% on CT • No new lesions • No obvious progression of nonmeasurable disease
SD	• Does not meet the criteria for CR, PR, or PD • No symptomatic deterioration attributed to tumor progression
PD	• An increase in tumor size of ≥ 10% and does not meet criteria of PR by tumor density (Hounsfield units) on CT • New lesions • New intratumoral nodules or increase in the size of the existing intratumoral nodules

Table F3: Modified CT Response Criteria (Choi criteria). Taken from Choi et al. J Clin Oncol, 2007. 25(13): 1753-1759

Immune-related Response Criteria

At the baseline tumor assessment, the sum of the products of the two largest perpendicular diameters (SPD) of all index lesions (up to 5 lesions per organ, up to 10 visceral lesions) is calculated. At each subsequent assessment, the SPD of the index lesions and of new, measurable lesions (≥ 5x5 mm, up to 5 new lesions per organ, up to 10 visceral lesions) are added together to provide the total tumor burden:

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

Response	Definition
irCR	• Complete disappearance of all lesions (whether measurable or not) • No new lesions • Confirmation by a repeat, consecutive assessment no less than 4 weeks from the date first documented
irPR	• Decrease in tumor burden by ≥50% relative to baseline confirmed by a consecutive assessment at least 4 weeks after first documentation
irSD	• Not meeting criteria for irCR or irPR in absence of irPD
irPD	• Increase in tumor burden ≥25% relative to nadir (minimum recorded tumor burden) • Confirmation by a repeat, consecutive assessment no less than 4 weeks from the date first documented

Table F4: Immune related response criteria. Adapted from Wolchok et al, Clin Cancer Res 2009, 15(23): 7412-20

B. Dynamic Contrast Enhanced Magnetic Resonance Imaging (DCE-MRI)

Subjects enrolled in this study will undergo an MRI scan as per the research protocol. All imaging will be performed on a 3T Skyra magnet (Siemens, Erlangen, Germany). A multiparametric MRI protocol containing conventional MR sequences will be obtained (including non-fat-suppressed T1 weighted, T2 weighted with fat saturation, and fat- saturated pre- and post-contrast T1 weighted images). Additional functional MRI techniques will be acquired, including diffusion-weighted imaging (DWI), magnetic resonance elastography (MRE), and dynamic contrast-enhanced MRI (DCE). The DWI sequences require approximately 5 minutes for acquisition; the MRE requires approximately 10 minutes for acquisition; the DCE sequences require approximately 5 minutes for acquisition. Formal reports for these exams will be generated by the co-investigators, and images will be stored in PACs. The MRI lasts approximately 45-60 minutes.

Images will be analyzed using third party software (iNtuition, TeraRecon, Foster City, California). Changes in index tumor size will be assessed using conventional size-based RECIST 1.1 criteria; in addition, semi-automated tumor segmentation will be performed for volumetric analysis of changes in tumor size. Apparent diffusion coefficient (ADC) maps will be constructed using the scanner's in-line software (Syngo MapIt, Siemens, Erlangen, Germany) to quantify restricted diffusion. Regions of interest will be constructed on ADC maps for comparison of ADC values; tumor volumetric segmentation on ADC map will also be performed for analysis of whole-tumor composition.

Enhancement characteristics will be analyzed according to modified Choi criteria applied to subtracted post-contrast sequences, with partial response (PR) defined as a decrease $\geq$10% in tumor size or a decrease $\geq$15% in tumor contrast enhancement; progression (PD) as new lesions, an increase $\geq$10% in tumor size without meeting any criteria for a PR according to tumor contrast enhancement, or an increase $\geq$15% in tumor contrast enhancement. Complete response (CR) will be defined as complete disappearance of all lesions and no new lesions; stable disease will be defined as tumor not meeting criteria for CR, PR, or PD.

DCE-MRI perfusion kinetics will be calculated for the index tumor. Tumor firmness will be assessed based on the shear modulus derived from MRE. Comparisons of pre- and post-treatment changes in tumor size, enhancement characteristics, ADC values, and tumor elasticity will be performed using paired t-tests and McNemar's test as appropriate.

C. Determination of tumor response by FDG-PET/CT using PERCIST 1.0

Subjects in this research study will undergo ^{18}F-FDG-PET/CT at baseline, cycle 3, and off-study as per the research protocol. Images will be analyzed by nuclear medicine radiologist and metabolic response determined by PERCIST 1.0 (Wahl et al, J Nucl Med 2009).

<u>Selection of target and non-target lesions:</u>

Measurable target lesion is the hottest single tumor lesion with maximal ROI of 1.2 cm in diameter (SUL peak). SUL peak should be at least 1.5 times higher than liver SUL mean + 2 SD. Up to five target lesions may be selected with no more than 2 per organ. These lesions will be compared in subsequent scans.

Response criteria:

Response category	Description
CMR	Complete resolution of FDG uptake within target lesion to indistinguishable levels compared to background blood-pool and liver. Disappearance of all metabolic lesions. No new lesions in the pattern of cancer.
PMR	Reduction of target lesion SUL peak by ≥30%
SMD	Not CMR, PMR, or PMD
PMD	>30% increase in target lesion SUL peak. OR increase in TLG volume with no change in SUL. OR appearance of new lesions.

Table F5. PERCIST 1.0 for determination of metabolic response. (Adapted from Wahl et al, J Nucl Med 2009).

C. Exploratory Objective 3: Measurement of circulating tumor cells pre- and post-treatment, and correlation with tumor response and time to progression

Rationale:

Circulating tumor cells (CTC) have been shown to be prognostic of metastatic disease and correlate with response to therapy in solid tumors such as breast, colorectal and prostate cancers. However, assessment of CTCs is challenging in sarcoma due to the lack of universal markers. In collaboration with Dr. Ram Datar, we have demonstrated in a pilot study (Eprost 20140062) that sarcoma circulating tumor cells are large and can be isolated using a unique, microfabricated parylene membrane-based device (Figure F2). The hypothesis for this pilot study is that CTC could serve as a marker of early micrometastatic disease in sarcomas, and may portend worse prognosis as seen in other tumor types.

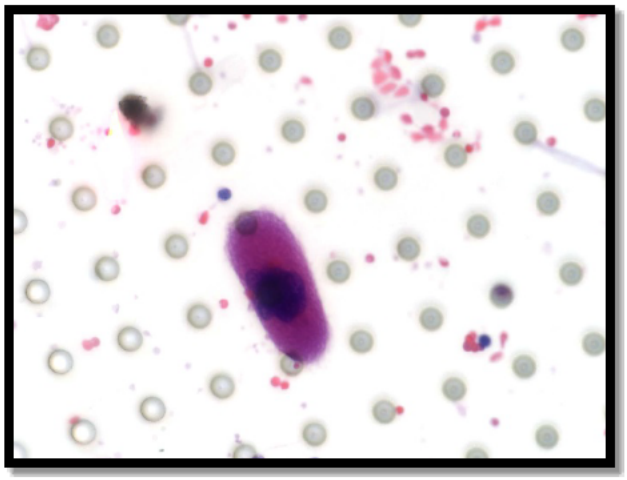


Figure F3. Circulating tumor cell identified from a patient with metastatic dedifferentiated chondrosarcoma. (Courtesy of Andrew Rosenberg, unpublished data)

We will collect data for circulating tumor cells as part of this axitinib/pembrolizumab study, in hopes that a more effective CD8+ anti-tumor effect could decrease the presence of circulating tumor cells and decrease development of new metastatic sites. Thus, peripheral blood samples will be drawn at selected timepoints and filtered to detect the numbers of circulating tumor cells using this technology. The presence or absence of CTC will be correlated with tumor response.

Approach:

a. Peripheral blood will be collected into CellSave tubes as detailed in the protocol and immediately transferred to Dr. Ram Datar's laboratory.

b. Samples will be diluted 1:1 in PBS and briefly fixed in 1% formalin at room temperature for ten minutes.

c. Following fixation, the blood samples will be passed through the microfilter device at constant low flow rate using a syringe pump.

d. The microfilter will be disengaged from its housing cassette and placed onto a glass microscope slides for analysis.

e. Samples will be stained for H&E analysis and reviewed with an expert sarcoma pathologist to identify and quantify circulating tumor cells. Where appropriate, additional immunocytochemistry will be performed using cell surface vimentin or other marker based on specific histology.

D. Data and specimen banking for later correlative analyses

a. Any additional samples, such as an extra core biopsy, or any patients that have surgery at any time along the protocol will have tissue samples banked. If possible, tissue will be frozen viably, for RNA in RNA later, and flash frozen for protein analysis. Additionally blood samples will be banked in the Komanduri lab as part of the PBMC protocol as detailed above.

b. Upon collection of all samples from the patients, a bar code system will be used to code the samples, assigning a subject number and bar code to each specimen. The actual physical samples will be tied to patient identifying information only at the time of sample collection. The patient's name will be written on the specimen collection tubes. Once the samples are coded, immediately following sample collection, the patient's name will be blacked out on the original sample tubes and the original tubes will be disposed of in the biohazardous waste, which gets sent for incineration.

c. The database linking subject identification and specimen barcodes will be kept on a password protected database housed on the University of Miami server.

d. The only investigators who will have access to samples and linking data to subjects are the principal investigator and his research staff named on the protocol. The tissue bank database will be the only location where patient's names and medical record numbers will be tied to their barcode numbers. The only information that will be stored with the actual samples in the freezers is the barcode identifier used to identify each sample. Data and specimens will be maintained indefinitely. If at some point in time, a subject decides they wish to remove their specimens and data from the tissue bank, any remaining samples belonging to that patient will be destroyed and their data removed from the database.

APPENDIX G: PROTOCOL APPROVED METHODS OF CONTRACEPTION

Pembrolizumab

Pembrolizumab may have adverse effects on a fetus in utero. Furthermore, it is not known if pembrolizumab has transient adverse effects on the composition of sperm. Non-pregnant, non-breast-feeding women may be enrolled if they are willing to use 2 methods of birth control or are considered highly unlikely to conceive. Highly unlikely to conceive is defined as 1) surgically sterilized, or 2) postmenopausal (a woman who is ≥45 years of age and has not had menses for greater than 1 year will be considered postmenopausal), or 3) not heterosexually active for the duration of the study. The two birth control methods can be either two barrier methods or a barrier method plus a hormonal method to prevent pregnancy. Subjects should start using birth control from study Visit 1 throughout the study period up to 120 days after the last dose of study therapy.

The following are considered adequate barrier methods of contraception: diaphragm, condom (by the partner), copper intrauterine device, sponge, or spermicide. Appropriate hormonal contraceptives will include any registered and marketed contraceptive agent that contains an estrogen and/or a progestational agent (including oral, subcutaneous, intrauterine, or intramuscular agents).

Subjects should be informed that taking the study medication may involve unknown risks to the fetus (unborn baby) if pregnancy were to occur during the study. In order to participate in the study they must adhere to the contraception requirement (described above) for the duration of the study and within 120 days of completing the trial, or 30 days following cessation of treatment if the subject initiates new anticancer therapy, whichever is earlier. If there is any question that a subject will not reliably comply with the requirements for contraception, that subject should not be entered into the study.

Use in Pregnancy

If a subject inadvertently becomes pregnant while on treatment with pembrolizumab, the subject will immediately be removed from the study. The site will contact the subject at least monthly and document the subject's status until the pregnancy has been completed or terminated. The outcome of the pregnancy will be reported to the Sponsor and to Merck without delay and within 24 hours to the Sponsor and within 2 working days to Merck if the outcome is a serious adverse experience (e.g., death, abortion, congenital anomaly, or other disabling or life-threatening complication to the mother or newborn). The study investigator will make every effort to obtain permission to follow the outcome of the pregnancy and report the condition of the fetus or newborn to the Sponsor. If a male subject impregnates his female partner the study personnel at the site must be informed immediately and the pregnancy reported to the Sponsor and to Merck and followed as described above.

Use in Nursing Women

It is unknown whether pembrolizumab is excreted in human milk. Since many drugs are excreted in human milk, and because of the potential for serious adverse reactions in the nursing infant, subjects who are breast-feeding are not eligible for enrollment.

APPENDIX H: EVENTS OF CLINICAL INTEREST & SUPPORTIVE CARE GUIDELINES FOR PEMBROLIZUMAB

Suggested supportive care measures for the management of AEs with potential immunologic etiology. Where appropriate, these guidelines include the use of oral or intravenous treatment with corticosteroids as well as additional anti-inflammatory agents if symptoms do not improve with administration of corticosteroids. These guidelines are intended to be applied when the Investigator determines the events to be related to pembrolizumab.

- **Pneumonitis**:
    - For **Grade 2 events**, treat with systemic corticosteroids. When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks.
    - For **Grade 3-4 events**, immediately treat with intravenous steroids. Administer additional anti-inflammatory measures, as needed.
    - Add prophylactic antibiotics for opportunistic infections in the case of prolonged steroid administration.

- **Diarrhea/Colitis**:
  Subjects should be carefully monitored for signs and symptoms of enterocolitis (such as diarrhea, abdominal pain, blood or mucus in stool, with or without fever) and of bowel perforation (such as peritoneal signs and ileus).

    - All subjects who experience diarrhea/colitis should be advised to drink liberal quantities of clear fluids. If sufficient oral fluid intake is not feasible, fluid and electrolytes should be substituted via IV infusion. For Grade 2 or higher diarrhea, consider GI consultation and endoscopy to confirm or rule out colitis.
    - For **Grade 2 diarrhea/colitis** that persists greater than 3 days, administer oral corticosteroids.
    - For **Grade 3 or 4 diarrhea/colitis** that persists > 1 week, treat with intravenous steroids followed by high dose oral steroids.
    - When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks.

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

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

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

- **Hypophysitis**:

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

- **Hyperthyroidism or Hypothyroidism**:
  Thyroid disorders can occur at any time during treatment. Monitor patients for changes in thyroid function (at the start of treatment, periodically during treatment, and as indicated based on clinical evaluation) and for clinical signs and symptoms of thyroid disorders.

  - **Grade 2** hyperthyroidism events (and **Grade 2-4** hypothyroidism):
    - In hyperthyroidism, non-selective beta-blockers (e.g. propranolol) are suggested as initial therapy.
    - In hypothyroidism, thyroid hormone replacement therapy, with levothyroxine or liothyroinine, is indicated per standard of care.
  - **Grade 3-4** hyperthyroidism
    - Treat with an initial dose of IV corticosteroid followed by oral corticosteroids. When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks. Replacement of appropriate hormones may be required as the steroid dose is tapered.

- **Hepatic**:
  - For **Grade 2** events, monitor liver function tests more frequently until returned to baseline values (consider weekly).
    - Treat with IV or oral corticosteroids
  - For **Grade 3-4** events, treat with intravenous corticosteroids for 24 to 48 hours.
  - When symptoms improve to Grade 1 or less, a steroid taper should be started and continued over no less than 4 weeks.
  - An elevated AST or ALT lab value that is ≥ 3X the upper limit of normal (ULN) **and** an elevated total bilirubin lab value that is ≥ 2X the ULN and, at the same time, an alkaline phosphatase lab value that is < 2X the ULN, as determined by way of protocol-specified laboratory testing or unscheduled laboratory testing.*****

*Note: These criteria are based upon available regulatory guidance documents. The purpose of the criteria is to specify a threshold of abnormal hepatic tests that may require an additional evaluation for an underlying etiology. The trial site guidance for assessment and follow up of these criteria can be found in the Investigator Trial File Binder (or equivalent).

- **Renal Failure or Nephritis**:
  - For **Grade 2** events, treat with corticosteroids.
  - For **Grade 3-4** events, treat with systemic corticosteroids.
  - When symptoms improve to Grade 1 or less, steroid taper should be started and continued over no less than 4 weeks.
- **Management of Infusion Reactions**: Signs and symptoms usually develop during or shortly after drug infusion and generally resolve completely within 24 hours of completion of infusion.

Table H1 below shows treatment guidelines for subjects who experience an infusion reaction associated with administration of pembrolizumab (MK-3475).

NCI CTCAE Grade	Treatment	Premedication at subsequent dosing
Grade 1 Mild reaction; infusion interruption not indicated; intervention not indicated	Increase monitoring of vital signs as medically indicated until the subject is deemed medically stable in the opinion of the investigator.	None
Grade 2 Requires infusion interruption but responds promptly to symptomatic treatment (e.g., antihistamines, NSAIDS, narcotics, IV fluids); prophylactic medications indicated for < =24 hrs	**Stop Infusion and monitor symptoms.** Additional appropriate medical therapy may include but is not limited to: IV fluids Antihistamines NSAIDS Acetaminophen Narcotics Increase monitoring of vital signs as medically indicated until the subject is deemed medically stable in the opinion of the investigator.	Subject may be premedicated 1.5h (± 30 minutes) prior to infusion of pembrolizumab (MK-3475) with: Diphenhydramine 50 mg po (or equivalent dose of antihistamine). Acetaminophen 500-1000 mg po (or equivalent dose of antipyretic).

NCI CTCAE Grade	Treatment	Premedication at subsequent dosing
	If symptoms resolve within one hour of stopping drug infusion, the infusion may be restarted at 50% of the original infusion rate (e.g., from 100 mL/hr to 50 mL/hr). Otherwise dosing will be held until symptoms resolve and the subject should be premedicated for the next scheduled dose. **Subjects who develop Grade 2 toxicity despite adequate premedication should be permanently discontinued from further trial treatment administration.**	
<u>Grades 3 or 4</u> Grade 3: Prolonged (i.e., not rapidly responsive to symptomatic medication and/or brief interruption of infusion); recurrence of symptoms following initial improvement; hospitalization indicated for other clinical sequelae (e.g., renal impairment, pulmonary infiltrates) Grade 4: Life-threatening; pressor or ventilatory support indicated	**Stop Infusion.** Additional appropriate medical therapy may include but is not limited to: IV fluids Antihistamines NSAIDS Acetaminophen Narcotics Oxygen Pressors Corticosteroids Epinephrine Increase monitoring of vital signs as medically indicated until the subject is deemed medically stable in the opinion of the investigator. Hospitalization may be indicated. **Subject is permanently discontinued from further trial treatment administration.**	No subsequent dosing

NCI CTCAE Grade	Treatment	Premedication at subsequent dosing
Appropriate resuscitation equipment should be available in the room and a physician readily available during the period of drug administration.		

Table H1. Infusion Reaction Treatment Guidelines for Pembrolizumab

- **Overdose**: An overdose that is not associated with clinical symptoms or abnormal laboratory results.

  For purposes of this trial, an overdose of pembrolizumab will be defined as any dose of 1,000 mg or greater (≥5 times the indicated dose). No specific information is available on the treatment of overdose of pembrolizumab. Appropriate supportive treatment should be provided if clinically indicated. In the event of overdose, the subject should be observed closely for signs of toxicity. Appropriate supportive treatment should be provided if clinically indicated.

  If an adverse event(s) is *associated with* ("results from") the overdose of a Merck product, the adverse event(s) is reported as a serious adverse event, even if no other seriousness criteria are met.

  If a dose of Merck's product meeting the protocol definition of overdose is taken *without* any associated clinical symptoms or abnormal laboratory results, the overdose is reported as a non-serious Event of Clinical Interest (ECI), using the terminology "accidental or intentional overdose without adverse effect."

REFERENCE: Merck. *Pembrolizumab Program (MK-3475) Event of Clinical Interest Guidance Document*. Version 5.0. 18-Dec-2014.

APPENDIX I: PATIENT DOSING DIARY AND BLOOD PRESSURE LOG FOR AXITINIB

[Note to Investigators: This appendix consists of a "directions sheet and diary" to be handed to the patient prior to each cycle. *These sheets MUST be approved by the Institutional Review Board (IRB) before it may be given to patients.**]***

Diary Directions
Begin taking axitinib twice a day, (as your doctor has told you to during the study):

1. On the days that you are supposed to take axitinib – axitinib should be taken by mouth, twice a day on an empty stomach, approximately 12 hours apart with a glass of water.
2. You may take each dose with or without food. Do not chew or break tablets.
3. If a dose is missed you may take the missed dose if it has been less than 2 hours from your scheduled dose. Do not make up missed doses if it has been more than 2 hours from your scheduled dose.
4. If you vomit after a dose, do not take another dose. Take your next dose of the day at the same time you would normally take it.
5. Avoid taking grapefruit juice and St. John's Wort.
6. Avoid smoking if you are taking axitinib because it may decrease the amount of the drug in your body.
7. Taking over-the-counter drugs to block stomach acid may interfere with the absorption of the study drug. Please avoid taking medications like omeprazole (Prilosec OTC) or cimetidine (Tagamet) unless you first discuss with your doctor.
8. Please bring the empty bottle or any leftover tablets and this diary to your next clinic visit.

Patient Dosing Diary **Axitinib** Dose Level: _____ mg twice a day
Patient Initials (L, F, M): Patient ID: _____ **Cycle: _____**

This is a diary on which you are to **record the date, time and number of tablets you take each day on the days that you are supposed to take Axitinib**. You should take your scheduled dose of each tablet. If you forget to take a dose, please write "0", but remember to take your prescribed dose at the next regularly scheduled time.

Day	Date(s)	# of Tablets	Time Taken *(Circle AM or PM)*	# of Tablets	Time Taken *(Circle AM or PM)*
1			AM PM		AM PM
2			AM PM		AM PM
3			AM PM		AM PM
4			AM PM		AM PM
5			AM PM		AM PM
6			AM PM		AM PM
7			AM PM		AM PM
8			AM PM		AM PM
9			AM PM		AM PM
10			AM PM		AM PM
11			AM PM		AM PM
12			AM PM		AM PM
13			AM PM		AM PM
14			AM PM		AM PM
15			AM PM		AM PM
16			AM PM		AM PM
17			AM PM		AM PM
18			AM PM		AM PM
19			AM		AM

			PM		PM
20			AM PM		AM PM
21			AM PM		AM PM
22			AM PM		AM PM
23			AM PM		AM PM
24			AM PM		AM PM
25			AM PM		AM PM
26			AM PM		AM PM
27			AM PM		AM PM
28			AM PM		AM PM
29			AM PM		AM PM
30			AM PM		AM PM
31			AM PM		AM PM
32			AM PM		AM PM
33			AM PM		AM PM
34			AM PM		AM PM
35			AM PM		AM PM
36			AM PM		AM PM
37			AM PM		AM PM
38			AM PM		AM PM
39			AM PM		AM PM
40			AM PM		AM PM
41			AM PM		AM PM

Days 42 to 49 below are ONLY to be completed for Cycle 1 of axitinib; After Cycle 1: Do NOT take axitinib nor complete Days 42 to 49.					
42			AM PM		AM PM
43			AM PM		AM PM
44			AM PM		AM PM
45			AM PM		AM PM
46			AM PM		AM PM
47			AM PM		AM PM
48			AM PM		AM PM
49			AM PM		AM PM

Patient Signature: _____ **Date: _____**

Patient Blood Pressure Log **Axitinib** Dose Level: _____ mg twice a day
Patient Initials (L, F, M): Patient ID: ______________________ **Cycle: _______**

1. **While on axitinib, measure and record your blood pressure at least once a day, before the evening dose of axitinib. Measure as needed for any unusual symptoms as listed below or as otherwise directed by your doctor. If axitinib is increased, please continue to measure and record your blood pressure at least once a day until you are seen in clinic (three weeks).**
2. Please bring this completed diary with you to each clinic visit.

*If your blood pressure is 150/100 or greater OR if your blood pressure is 120/80 or greater with headache, chest pressure, shortness of breath, dizziness and/or blurry vision, notify your study doctor(s) at (305)-243-1000 immediately.

Blood Pressure Measurements						**Repeat Blood Pressure Measurements (if applicable)**		
Date	Time	Systolic (top number)	Diastolic (bottom number)	Was your blood pressure ≥150/100 or ≥120/80 with symptoms? ***If yes, see above.**		Time	Systolic (top number)	Diastolic (bottom number)
	am			□ Yes	□ No	am		
	pm			□ Yes	□ No	pm		
	am			□ Yes	□ No	am		
	pm			□ Yes	□ No	pm		
	am			□ Yes	□ No	am		
	pm			□ Yes	□ No	pm		
	am			□ Yes	□ No	am		
	pm			□ Yes	□ No	pm		
	am			□ Yes	□ No	am		
	pm			□ Yes	□ No	pm		
	am			□ Yes	□ No	am		
	pm			□ Yes	□ No	pm		
	am			□ Yes	□ No	am		
	pm			□ Yes	□ No	pm		
	am			□ Yes	□ No	am		
	pm			□ Yes	□ No	pm		
	am			□ Yes	□ No	am		
	pm			□ Yes	□ No	pm		
	am			□ Yes	□ No	am		
	pm			□ Yes	□ No	pm		

	am			□ Yes	□ No	am		
	pm			□ Yes	□ No	pm		
	am			□ Yes	□ No	am		
	pm			□ Yes	□ No	pm		
	am			□ Yes	□ No	am		
	pm			□ Yes	□ No	pm		
	am			□ Yes	□ No	am		
	pm			□ Yes	□ No	pm		
	am			□ Yes	□ No	am		
	pm			□ Yes	□ No	pm		
	am			□ Yes	□ No	am		
	pm			□ Yes	□ No	pm		
	am			□ Yes	□ No	am		
	pm			□ Yes	□ No	pm		
	am			□ Yes	□ No	am		
	pm			□ Yes	□ No	pm		
	am			□ Yes	□ No	am		
	pm			□ Yes	□ No	pm		
	am			□ Yes	□ No	am		
	pm			□ Yes	□ No	pm		
	am			□ Yes	□ No	am		
	pm			□ Yes	□ No	pm		

Patient Signature: ________________________________ **Date:** __________________

APPENDIX J: CYP1A2, CYP2C8, CYP2C19, CYP3A4 SUBSTRATES, INHIBITORS & INDUCERS *

SUBSTRATE(S)				
1A2	*2C8*	*2C19*	*3A4*	
amitriptyline caffeine clomipramine clozapine cyclobenzaprine duloxetine estradiol fluvoxamine haloperidol imipramine N-DeMe mexiletine naproxen olanzapine ondansetron phenacetin→ acetaminophen→NAP QI propranolol riluzole ropivacaine tacrine theophylline tizanidine triamterene verapamil (R)warfarin zileuton zolmitriptan	[paclitaxel] torsemide amodiaquine cerivastatin repaglinide sorafinib	<u>Proton Pump Inhibitors (PPIs)</u>: esomeprazole lansoprazole omeprazole pantoprazole rabeprazole <u>Anti-epileptics</u>: diazepam→Nor phenytoin(O) S-mephenytoin phenobarbitone amitriptyline carisoprodol citalopram chloramphenicol clomipramine chlopidogrel cyclophosphamide hexobarbital imipramine N-DeME indomethacin labetalol R-mephobarbital moclobemide nelfinavir nilutamide primidone progesterone proguanil propranolol teniposide R-warfarin→8-OH voriconazole	<u>Macrolide antibiotics</u>: clarithromycin erythromycin (not 3A5) *NOT azithromycin* telithromycin <u>Anti-arrhythmics</u>: quinidine→3OH (not 3A5) <u>Benzodiazepines</u>: alprazolam diazepam→3OH midazolam triazolam <u>Immune Modulators</u>: cyclosporine tacrolimus (FK506) <u>HIV Antivirals</u>: indinavir nelfinavir ritonavir saquinavir <u>Prokinetic</u>: cisapride <u>Antihistamines</u>: astemizole chlorpheniramine terfenadine <u>Calcium Channel Blockers</u>: amlodipine diltiazem felodipine lercanidipine nifedipine nisoldipine nitrendipine verapamil <u>HMG CoA Reductase Inhibitors</u>: atorvastatin cerivastatin	<u>Steroid 6beta-OH</u>: estradiol hydrocortisone progesterone testosterone <u>Miscellaneous</u>: alfentanyl aprepitant aripiprazole boceprevir buspirone carbamazepine cafergot caffeine→TMU cilostazol cocaine codeine-N-demethylation dapsone dexamethasone dextromethorphan docetaxel domperidone eplerenone fentanyl finasteride gleevec haloperidol irinotecan LAAM lidocaine methadone nateglinide nevirapine ondansetron pimozide propranolol quetiapine quinine risperidone romidepsin NOT rosuvastatin salmeterol sildenafil sirolimus sorafenib

SUBSTRATE(S)				
1A2	*2C8*	*2C19*	*3A4*	
			lovastatin NOT pravastatin NOT rosuvastatin simvastatin	tamoxifen taxol telaprevir terfenadine torisel trazodone vemurafenib vincristine zaleplon ziprasidone zolpidem

INHIBITORS			
1A2	*2C8*	*2C19*	*3A4*
fluvoxamine ciprofloxacin cimetidine amiodarone efavirenz fluoroquinolones fluvoxamine furafylline interferon methoxsalen mibefradil ticlopidine	gemfibrozil trimethoprim glitazones montelukast quercetin	PPIs: Esomeprazole lansoprazole omeprazole pantoprazole Other: rabeprazole chloramphenicol cimetidine felbamate fluoxetine fluvoxamine indomethacin ketoconazole modafinil oral contraceptives oxcarbazepine probenicid ticlopidine topiramate voriconazole	HIV Antivirals: indinavir nelfinavir ritonavir clarithromycin itraconazole ketoconazole nefazodone saquinavir telithromycin aprepitant erythromycin fluconazole grapefruit juice verapamil diltiazem cimetidine amiodarone NOT azithromycin Chloramphenicol boceprevir ciprofloxacin delaviridine diethyldithiocarbamate fluvoxamine gestodene imatinib mibefradil mifepristone norfloxacin norfluoxetine star fruit telaprevir voriconazole

INDUCERS			
1A2	*2C8*	*2C19*	*3A4*
broccoli brussel sprouts carbanazepine char-grilled meat insulin methylcholanthrene modafinil nafcillin beta-naphthoflavone omeprazole rifampin tobacco	rifampin	carbamazepine nevirapine phenobarbital rifampin secobarbital St. John's Wort	HIV Antivirals: efavirenz nevirapine barbiturates carbamazepine glucocorticoids modafinil oxcarbazepine phenobarbital phenytoin pioglitazone rifabutin rifampin St. John's wort troglitazone

*Reference: http://medicine.iupui.edu/clinpharm/ddis/main-table/

APPENDIX K: GUIDELINES FOR PREPARATION AND ADMINISTRATION/ KEYTRUDA FOR INJECTION (LYOPHILIZED POWDER)

Reconstitution of Keytruda for Injection (Lyophilized Powder)

- Add 2.3 mL of Sterile Water for Injection, USP by injecting the water along the walls of the vial and not directly on the lyophilized powder (resulting concentration 25 mg/mL).

- Slowly swirl the vial. Allow up to 5 minutes for the bubbles to clear. Do not shake the vial.

Preparation for Intravenous Infusion

- Visually inspect the solution for particulate matter and discoloration prior to administration. The solution is clear to slightly opalescent, colorless to slightly yellow. Discard the vial if visible particles are observed.

- Dilute KEYTRUDA injection (solution) or reconstituted lyophilized powder prior to intravenous administration.

- Withdraw the required volume from the vial(s) of KEYTRUDA and transfer into an intravenous (IV) bag containing 0.9% Sodium Chloride Injection, USP or 5% Dextrose Injection, USP. Mix diluted solution by gentle inversion. The final concentration of the diluted solution should be between 1 mg/mL to 10 mg/mL.

- Discard any unused portion left in the vial.

Storage of Reconstituted and Diluted Solutions

The product does not contain a preservative.

Store the reconstituted and diluted solution from the KEYTRUDA 50 mg vial either:

- At room temperature for no more than 6 hours from the time of reconstitution. This includes room temperature storage of reconstituted vials, storage of the infusion solution in the IV bag, and the duration of infusion.

- Under refrigeration at 2°C to 8°C (36°F to 46°F) for no more than 24 hours from the time of reconstitution. If refrigerated, allow the diluted solution to come to room temperature prior to administration.

Store the diluted solution from the KEYTRUDA 100 mg/4 mL vial either:

- At room temperature for no more than 6 hours from the time of dilution. This includes room temperature storage of the infusion solution in the IV bag, and the duration of infusion.

- Under refrigeration at 2°C to 8°C (36°F to 46°F) for no more than 24 hours from the time of dilution. If refrigerated, allow the diluted solution to come to room temperature prior to administration. **Do not freeze**.

1. Lazar AJ, Das P, Tuvin D, Korchin B, Zhu Q, Jin Z, et al. Angiogenesis-promoting gene patterns in alveolar soft part sarcoma. *Clin Cancer Res* 2007; **13**.
2. Kummar S, Allen D, Monks A, Polley E.C., Hose C.D., Ivy S.P., et al. Cediranib for metastatic alveolar soft part sarcoma. *J Clin Oncol* 2013; **31**: 2296-302.
3. Stacchiotti S, Tamborini E, Marrari A, Brich S, Rota S.A., Orsenigo M, et al. Response to sunitinib malate in advanced alveolar soft part sarcoma. *Clin Cancer Res* 2009; **15**: 1096-104.
4. Robert C, Ribas A, Wolchok JD, Hodi FS, Hamid O, Kefford R, et al. Anti-programmed-death-receptor-1 treatment with pembrolizumab in ipilimumab-refractory advanced melanoma: a randomised dose-comparison cohort of a phase 1 trial. *Lancet* 2014; **384**: 1109-17.
5. Rini BI, Escudier B, Tomczak P, Kaprin A, Szczylik C, Hutson TE, et al. Comparative effectiveness of axitinib versus sorafenib in advanced renal cell carcinoma (AXIS): a randomised phase 3 trial. *Lancet* 2011; **378**: 1931-9.
6. Wu J. Sample size calculation for the one-sample log-rank test. *Pharm Stat* 2015; **14**: 26-33.
7. Montero A.J., Kwon D, Flores A, Kovacs K, Trent J.C., Benedetto P, et al. A Phase I Clinical, Pharmacokinetic, and Pharmacodynamic Study of Weekly or Every Three Week Ixabepilone and Daily Sunitinib in Patients with Advanced Solid Tumors. *Clin Cancer Res* 2016; **22**: 3209-17.
8. Gubin M.M., Artyomov M.N., Mardis E.R., and Schreiber R.D. Tumor neoantigens: building a framework for personalized cancer immunotherapy. *J Clin Invest* 2015; **125**: 3413-21.