Title: A Phase II Study of Eribulin and Pembrolizumab in Soft Tissue Sarcomas

NCT Number: NCT03899805

IRB Approval Date: 05JUN2024

DF/HCC Protocol #: 19-082

TITLE: A Phase II Study of Eribulin and Pembrolizumab in Soft Tissue Sarcomas

Coordinating Center: Dana-Farber / Harvard Cancer Center

Principal Investigator (PI): *Suzanne George, MD*
Dana-Farber Cancer Institute

Study Agent(s): Eribulin supplied by Eisai and Pembrolizumab supplied by Merck

IND #: NA

IND Sponsor: NA

Sponsor-Investigator: *Suzanne George, MD*

Protocol Type / Version # / Version Date: *Version #10 / 15MAY2024*

SCHEMA

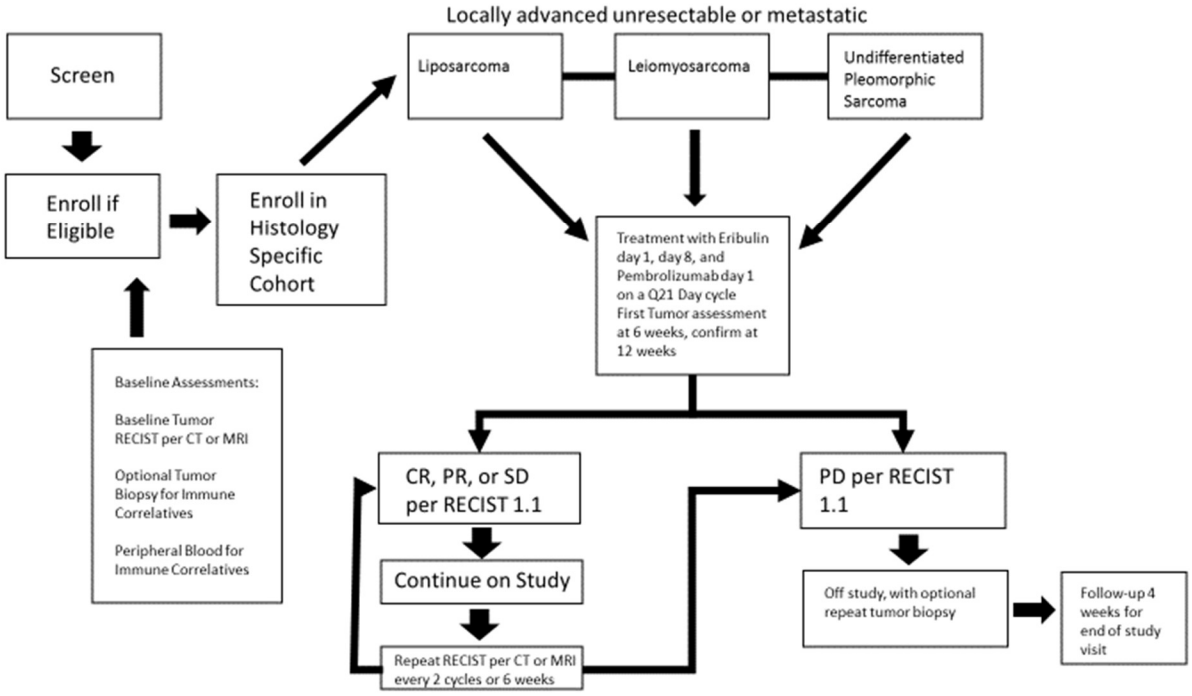

Locally advanced unresectable or metastatic
Screen
Enroll if Eligible
Enroll in Histology Specific Cohort
Liposarcoma
Leiomyosarcoma
Undifferentiated Pleomorphic Sarcoma
Baseline Assessments:
Baseline Tumor RECIST per CT or MRI
Optional Tumor Biopsy for Immune Correlatives
Peripheral Blood for Immune Correlatives
Treatment with Eribulin day 1, day 8, and Pembrolizumab day 1 on a Q21 Day cycle
First Tumor assessment at 6 weeks, confirm at 12 weeks
CR, PR, or SD per RECIST 1.1
Continue on Study
Repeat RECIST per CT or MRI every 2 cycles or 6 weeks
PD per RECIST 1.1
Off study, with optional repeat tumor biopsy
Follow-up 4 weeks for end of study visit

TABLE OF CONTENTS

LIST of TABLES

LIST of FIGURES

LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

The following abbreviations and special terms are used in this study Clinical Study Protocol.

Abbreviation and special term	**Explanation**
AE	Adverse event
CRF	Case Report Form (electronic/paper)
CSA	Clinical Study Agreement
CSR	Clinical Study Report
CTCAE	Common Terminology Criteria for Adverse Event
DAE	Discontinuation of Investigational Product due to Adverse Event
DNA	Deoxyribonucleic acid
RNA	Ribonucleic acid
EC	Ethics Committee, synonymous to Institutional Review Board (IRB) and Independent Ethics Committee (IEC)
GCP	Good Clinical Practice
ICH	International Conference on Harmonization
IP	Investigational Product
IVRS	Interactive Voice Response System
IWRS	Interactive Web Response System
LSLV	Last Participant Last Visit
OAE	Other Significant Adverse Event
PGx	Pharmacogenetic research
PI	Principal Investigator
SAE	Serious adverse event
WBDC	Web Based Data Capture
STS	Soft Tissue Sarcoma
LPS	Liposarcoma
LMS	Leiomyosarcoma
UPS	Undifferentiated Pleomorphic Sarcoma
PD-1	Programmed Death 1
PD-L1	Programmed Death Ligand 1

Abbreviation and special term	**Explanation**
PD-L2	Programmed Death Ligand 2
TILs	Tumor Infiltrating Lymphocytes
NKs	Natural Killer cells
ECIs	Events of Clinical Interest
MTD	Maximum tolerated dose
PK	Pharmacokinetics
DLT	Dose limiting toxicity
RP2D	Recommend phase 2 dose
MSI-high/MMR	Microsatellite instability/Mismatch Repair Deficient
mTNBC	Metastatic triple negative breast cancer
PFR	Progression-free rate
PFS	Progression-free survival
OS	Overall Survival
IDO	Indoleamine 2,3-dioxygenase
MDSC's	myeloid derived suppressors cells
Treg	T regulatory cells

Trial Summary

Abbreviated Title	Eribulin and Pembrolizumab in Soft Tissue Sarcomas
Trial Phase	A Phase II Study of Eribulin and Pembrolizumab in Soft Tissue Sarcomas
Clinical Indication	Locally advanced or metastatic Soft Tissue Sarcomas
Trial Type	Phase II
Type of control	Single Arm
Route of administration	Intravenous
Trial Blinding	None
Treatment Groups	Arm A: Leiomyosarcoma, Arm B: Liposarcoma, and Arm C: Undifferentiated Pleomorphic Sarcoma/Other Sarcomas
Number of trial participants	57
Estimated enrollment period	24 months
Estimated duration of trial	36 months
Duration of Participation	3 months
Estimated average length of treatment per participant	4 Cycles

1. OBJECTIVES

Hypotheses:

Eribulin in combination with PD-1 blockade with pembrolizumab will result in objective responses and durable disease control in participants with metastatic/recurrent or locally advanced liposarcomas, leiomyosarcomas, and undifferentiated pleomorphic sarcomas/other sarcomas.

1.1 Study Design

This is a phase II, parallel cohort, non-randomized, three-arm, open-label trial of eribulin in combination with pembrolizumab (EP) in participants with liposarcomas, leiomyosarcomas, and undifferentiated pleomorphic sarcomas/other sarcomas. The purpose of this study is to assess the efficacy and safety of this combination in this population, as well as to explore the baseline immunologic tumor characteristics in the enrolled cohorts.

Each sarcoma subtype cohort will be analyzed separately.

Participants will receive eribulin 1.4 mg/m2 intravenously on Day 1 and Day 8 with pembrolizumab 200mg intravenously Day 1, on a 21-day cycle until disease progression, study discontinuation, unacceptable toxicity, or two years of treatment.

All participants will undergo a history and physical examination and laboratory evaluation (CBC with Diff, Lytes, BUN, Cr, LFT's, and TSH) during screening for trial enrollment. All participants will have baseline imaging for disease assessment. Archival tissue will be submitted. If archival tissue is not available, a new tumor biopsy will be performed if feasible. Tumor assessment per RECIST 1.1 will be performed after each 2 cycles.[1] Participants will remain on treatment unless there is progressive disease per RECIST 1.1, or unacceptable toxicity, or completion of two years of therapy.

All participants will undergo a history and physical examination and laboratory evaluation (CBC with Diff, Lytes, BUN, Cr, LFT's) prior to each drug(s) administration, to assess for the development of adverse events, including immune related adverse events (irAEs). TSH will be monitored every 6 weeks. Monitoring for irAEs will continue in participants who come off study for progression or other reasons at a follow-up period of 90 days. Participants who experience grade 3 or higher irAEs will receive treatment with systemic corticosteroids as per protocol and standard guidelines [2, 3]. Discontinuation of drug, duration of corticosteroid treatment, and further participation in the trial will be at the discretion of the Principal Investigator (PI).

1.2 Primary Objective

1.2.1 To assess the 12-week progression-free survival for eribulin in combination with pembrolizumab in participants with metastatic or locally advanced liposarcomas, leiomyosarcoma, and undifferentiated pleomorphic sarcoma/other sarcomas.

1.3 Secondary Objectives

1.3.1 To assess the objective response rate (ORR) based on RECIST 1.1, clinical benefit rate (complete response (CR) + partial response (PR) + stable disease (SD) at 12 weeks), for eribulin in combination with pembrolizumab in participants with metastatic or locally advanced liposarcomas, leiomyosarcoma, and undifferentiated pleomorphic sarcoma/other sarcomas.
1.3.2 To assess tolerability and toxicities of eribulin in combination with pembrolizumab in this participant population.
1.3.3 To assess overall survival of participants with metastatic/locally advanced liposarcomas, leiomyosarcoma, and undifferentiated pleomorphic sarcomas/other sarcomas receiving eribulin in combination with pembrolizumab.

Correlative objectives
1.3.4 Determine the expression of biomarkers (including but not limited to PD-1, PD-L1, MHC class I expression, IDO), and quantification of tumor infiltrating lymphocytes (TILs) (CD8+, CD4+, Tregs), tumor infiltrating macrophages, and myeloid derived suppressors cells (MDSC's) in archival tissue or pre-treatment biopsies, and optional post-treatment biopsies via immunohistochemistry and immunofluorescence.
1.3.5 Targeted genomic analysis (Oncopanel) of archival tissue or pre-treatment biopsies, and RNA sequencing of fresh frozen tumor biopsies to evaluate for markers of response to immunotherapy, such as MMR def or high mutation load.
1.3.6 Evaluation of the microbiome in participants with soft tissue sarcoma (Liposarcoma, Leiomyosarcoma, Undifferentiated pleomorphic sarcoma, utilizing a pre-treatment stool sample
1.3.7 Identification/quantification of immunologic changes (including but not limited to cytokines, CD4+, CD8+, Teff and Treg cells, tumor infiltrating macrophages (particularly in the leiomyosarcoma cohort)) in peripheral blood.
1.3.8 Quantification of circulating Tumor DNA (ctDNA) in peripheral blood.

2. BACKGROUND

2.1 Study Diseases: Liposarcoma, Leiomyosarcoma, Undifferentiated Pleomorphic Sarcoma/Other Sarcomas

Sarcomas are rare cancers in adults, representing about 1% of all adult malignancies.[4] In adults, there are approximately 13,000 cases of soft tissue sarcomas[5]. Sarcomas are not one disease, but rather a group of multiple genetically distinct diseases, with the current WHO classification listing over 50 different histologic subtypes[6]. The primary management of these tumors is wide local surgical excision with adjuvant or neoadjuvant radiation therapy[7] and +/- adjuvant or neoadjuvant chemotherapy with an anthracycline and ifosfamide based regimen.[8-13] Prognosis and outcomes vary by histologic subtype, anatomic location, size, grade, depth of invasion, and patient age[14-16]. The five-year survival of patients with Stage III soft tissue sarcomas is still only approximately fifty percent.[17] Palliative chemotherapy in the metastatic setting can provide a modest increase in survival, but does not result in a cure, and the median overall survival for patients with metastatic soft tissue sarcoma remains less than 2 years. New therapeutic strategies

are therefore needed in this patient population. Additionally, due to the differences in prognosis, outcomes, and disease natural history by histologic subtype, histology-specific clinical trials are required to evaluate novel therapies in soft tissue sarcomas.

2.2 Immunotherapy in Sarcomas

A rapidly evolving treatment strategy in oncology is immunotherapy — manipulating a patient's immune system to create a successful anti-tumor response. This treatment actually revives an old strategy that was first described by Dr. Coley in a sarcoma patient[18, 19].Recent dramatic responses in checkpoint inhibition with PD1 and PDL1 inhibitors have revolutionized the management of melanoma, lung cancer, urothelial cancers, and MSI high solid tumors and Hodgkin lymphoma and led to multiple new drug approvals and changes in standards of care.

Efficacy of this approach in sarcomas is quite limited however. SARC028 evaluated pembrolizumab in multiple subtypes of bone and soft tissue sarcomas. Of the multiple subtypes evaluated, only modest activity was seen in liposarcoma and unclassified pleomorphic sarcoma (UPS). Although an exceptional response to pembrolizumab in uterine LMS has been reported, a single center trial evaluating single agent nivolumab in uterine LMS, demonstrated no responses and a median PFS of less than 2 months. Similarly, a multicenter randomized phase II trial evaluating the combination of nivolumab/ipilumumab and single agent nivolumab demonstrated minimal activity in soft tissue sarcomas, with isolated responses in LMS, LPS, alveolar soft parts sarcoma and UPS.

Check point inhibition alone has limited activity in soft tissue sarcomas, therefore, we now propose to combine check point inhibition with chemotherapy.

2.3 Rationale for eribulin

A Phase II study of eribulin in soft tissue sarcomas suggested a benefit in leiomyosarcomas and liposarcomas with a three-month progression-free survival of 31.6% for leiomyosarcomas and 46.9% for liposarcomas, and a 21.1% for synovial sarcomas, and a 19.2% for other soft tissue sarcomas, including UPS[65]. A Japanese phase II trial showed similar results with a 12-week progression-free survival of 60% for liposarcomas and leiomyosarcomas[66]. More recently, eribulin was approved for the treatment of metastatic or recurrent liposarcomas[67] based on the results of a randomized phase III trial comparing eribulin to dacarbazine in leiomyosarcomas and liposarcomas[68]. This trial showed an improvement in the median overall survival of 13.5 months for participants treated with eribulin vs 11.5 months for participants treated with dacarbazine[68]. There was no difference in median progression-free survival, 2.6 months in each arm; 12-week progression-free survival was 33% in the eribulin group and 29% in the dacarbazine group[68]. A subgroup analysis in the liposarcoma cohort showed an improvement in progression-free survival of 2.9 vs 1.7 months, p=0.0015, and overall survival of 15.6 vs 8.4 months, p=0.0006[69]. Thus, the FDA approved eribulin for liposarcomas, including dedifferentiated, myxoid/round cell, and pleomorphic liposarcomas. Eribulin is currently NCCN compendium listed for leiomyosarcoma and is a standard of care in the US for this indication.

2.4 Rationale for combination of eribulin and pembrolizumab

The combination of cytotoxic chemotherapy with immunotherapy is a rational approach to increase the response to immunotherapy. Cytotoxic chemotherapy may lead to cell death and the release of neoantigens that can be utilized in an immune response. Additionally, eribulin has non-mitotic effects which increase vascular perfusion and may impact the tumor stromal microenvironment. This may allow an increase in tumor infiltration by immune cells and potentiate the effects of immune checkpoint inhibitors[71]. Recently, the combination of eribulin and pembrolizumab has been shown to be safe and effective in patients with triple negative breast cancer. Because of the activity of eribulin in LPS and LMS; together with the reported activity of pembrolizumab, nivolumab, or nivolumab + ipilimumab in a subset of patients with LPS, LMS and UPS/Other sarcomas; and the potential for synergy; we now propose a parallel-cohort three-arm -uncontrolled phase II study evaluating the combination of eribulin and pembrolizumab. The dosing of eribulin will be 1.4mg/m^2 on Day 1 and Day 8, and the dosing for pembrolizumab will be 200mg every 3 weeks. This is the standard dosing for each drug and has been shown to be active and safe in the eribulin and pembrolizumab metastatic triple negative breast cancer trial.

The standard end-point for phase II soft tissue sarcoma trials is the progression-free rate (PFR) at 12 weeks,[72] with an active regimen defined as having a PFR of > 40% at 12 weeks and an inactive regimen defined as having a PFR of < 20% at 12 weeks. The activity of eribulin in the phase II soft tissue sarcoma trial was a PFS of 32% for leiomyosarcomas, and 47% for liposarcomas at 3 months. Also, a 3-month PFS seen in the SARC028 trial was 55%, and a 3-month PFS seen in the nivolumab alone arm of the Alliance A091401 was ~ 40%. Thus, for this trial, it was determined that a 40% PFS is inadequate to determine if the eribulin/pembrolizumab combination is effective, as 40% PFS at 3 months may be due to the single agents alone. For the purposes of this trial, we will define an active regimen as having a PFS of >60% at 12 weeks, indicating activity of the combination, and an inactive regimen as having a PFS of <30% at 12 weeks.

Recent advances in immunotherapy has shown significant benefit for patients with advanced lung cancer, metastatic renal cell carcinomas,[23-25] metastatic melanoma,[26-29] colon cancer or other tumors with microsatellite instability[30, 31], or Hodgkin's lymphoma[32, 33].
One potential predictor for the response to immunotherapy in solid tumors is tumor-infiltrating lymphocytes (TILs).[34] TILs have been noted in a many solid tumors[35] including colon cancer[36], non-small cell lung cancer,[37] melanoma,[38] and most importantly in many soft tissue sarcomas and gastrointestinal stromal tumors.[39, 40] In fact TILs have been reported as a possible prognostic factor in soft tissue sarcomas[41] and bone sarcomas.[42, 43] Additionally PD-1 and PD-L1 expression have been noted in soft tissue sarcomas and potentially correlate with prognosis.[39, 44, 45] This evidence suggests a potential therapeutic responsiveness of sarcomas to newer immunotherapy agents, such as immune checkpoint inhibitors. Though whether the presence of TILs, PD-1, PD-L1, and PD-L2 expression are predicative markers has yet to be determined. The PD-1/PD-L1 pathway leads to inhibition of T-cell activation and immune escape by tumor cells. Blocking this pathway can lead to immune system activation and tumor suppression. Targeting the PD-1 pathway can be accomplished by targeting PD-1, or its ligands PD-L1, PD-L2.

This rational has already lead to several phase II trials of immune checkpoint inhibitors in patients with advanced sarcomas: Single agent pembrolizumab in patients with advanced sarcoma, SARC028; Nivolumab in Uterine Leiomyosarcomas; and the Alliance trial of Nivolumab +/- Ipilimumab in soft tissue sarcomas, A091401. Chemotherapy may potentiate the immune response in soft tissue sarcomas[46]. In fact, immunotherapy (immune checkpoint inhibitor) trials in combination with chemotherapy (doxorubicin, gemcitabine, or trabectedin) are already planned or enrolling in soft tissue sarcomas.

The SARC028 trial suggested immunotherapy responses in dedifferentiated and pleomorphic liposarcoma and in undifferentiated pleomorphic sarcomas. The Alliance trial confirmed rare responses in these histologic subtypes but, also, response in leiomyosarcoma, angiosarcoma, and Alveolar soft part sarcoma, and myxofibrosarcoma[74]. The responses of alveolar soft part sarcoma have now been confirmed in two other reports, a trial of axitinib and pembrolizumab[75], and retrospective review of phase I trials of immunotherapy in soft tissue sarcomas[76]. Additionally, 7/9 cutaneous angiosarcomas were shown to benefit from immunotherapy in a retrospective series[77]. Furthermore, mismatch repair deficiency is a known prognostic factor and this has been identified to rarely occur in soft tissue sarcomas[78]. Thus there is a rational for expanding the undifferentiated pleomorphic sarcoma cohort into a undifferentiated pleomorphic sarcoma/other sarcoma cohort to include a wider spectrum of sarcomas patients that may respond to immunotherapy.

2.5 IND Agents

2.5.1 *Pembrolizumab Background*

Pembrolizumab, MK-3475, Keytruda, is potent an anti-PD-1 IgG4 humanized monoclonal anti-body with high specificity of binding to the programmed cell death 1 (PD-1) receptor, thus inhibiting its interaction with programmed cell death ligand 1 (PD-L1) and programmed cell death ligand 2 (PD-L2). Based on preclinical in vitro data, pembrolizumab has high affinity and potent receptor blocking activity for PD-1. Pembrolizumab has an acceptable preclinical safety profile and is in clinical development as an intravenous (IV) immunotherapy for advanced malignancies. Keytruda® (pembrolizumab) is indicated for the treatment of patients across a number of indications because of its mechanism of action to bind the PD-1 receptor on the T cell.

The amino acid sequence of pembrolizumab includes 4 polypeptide chains, which include 2 identical heavy chains and 2 identical light chains. The molecular weight of Pembrolizumab is 148.9-149.5 KDa. Pembrolizumab targets the programmed death–1 (PD-1, cluster of differentiation 279 [CD279]) cell surface membrane receptor. PD-1 is a negative regulatory receptor expressed by activated T and B lymphocytes. Binding of PD-1 to its ligands, programmed death–ligand 1 (PD-L1) and 2 (PD-L2), results in the down-regulation of lymphocyte activation. Pembrolizumab inhibits the binding of PD-1 to PD-L1 and PD-L2. Inhibition of the interaction between PD-1 and its ligands promotes immune responses and antigen-specific T-cell responses to both foreign antigens as well as self-antigens.[48]

2.5.1.1 Clinical Development of Pembrolizumab

The safety of Pembrolizumab was studied in a phase I trial, NCT01295827. This was a standard 3+3 dose escalation study in participants with solid tumors to examine tolerability, pharmacokinetics, pharmacodynamics, and safety, and to determine the maximum tolerated dose (MTD) as well as the preliminary recommended phase 2 dose (RP2D). The dosing studied was 1, 3, and 10 mg/kg every 2 weeks, or 2, 10 mg/kg every 3 weeks. All dose levels were well tolerated and no dose-limiting toxicity (DLT) were observed. No MTD was determined. The RP2D determined by the sponsor (MERCK) based on the safety, pharmacokinetic and pharmacodynamic data, as well as the strength of the anti-tumor activity observed was 2mg/kg Q3 weeks.[48]

Pembrolizumab has shown efficacy in the treatment of metastatic melanoma, metastatic non-small cell lung cancer, metastatic head and neck cancer, renal cell cancer, colon cancer, classical Hodgkin lymphoma, and most recently microsatellite instable tumors. Pembrolizumab has now received FDA approval for the treatment of metastatic melanoma, metastatic non-small cell lung cancer, metastatic head and neck cancer, renal cell cancer, classical Hodgkin lymphoma, and most recently microsatellite instable tumors (MSI-high) particularly colon and endometrial carcinomas.

2.5.1.2 Pharmacokinetics

The pharmacokinetics (PK) of pembrolizumab were characterized using a population PK analysis with concentration data collected from 2841 patients with various cancers who received pembrolizumab doses of 1 to 10 mg/kg every 2 weeks or 2 to 10 mg/kg every 3 weeks. The half-life ($t_{1/2}$) of pembrolizumab is approximately 4 weeks. There was no indication of dose dependency or changes in half-life between the three dose groups (1, 3, and 10 mg/kg) on the phase I study. The long $t_{1/2}$ pembrolizumab supports using a dosing interval of every 2 or 3 weeks.[48] Pembrolizumab clearance (CV%) is approximately 21% lower [geometric mean, 196 mL/day (41%)] at steady state than after the first dose [249 mL/day (38%)]; this decrease in clearance with time is not considered clinically important. The geometric mean value (CV%) for volume of distribution at steady state is 6.0 L (21%) and for terminal half-life (t1/2) is 22 days (32%).[48]

Steady-state concentrations of pembrolizumab were reached by 16 weeks of repeated dosing with an every 3-week regimen and the systemic accumulation was 2.2-fold. The peak concentration (Cmax), trough concentration (Cmin), and area under the plasma concentration versus time curve at steady state (AUCs) of pembrolizumab increased dose proportionally in the dose range of 2 to 10 mg/kg every 3 weeks.[48]

Pembrolizumab has been found to have a wide therapeutic range based on the melanoma studies. The population PK data revealed that there was no significant impact of tumor burden on exposure to pembrolizumab. Additionally, the exposure was similar between the NSCLC and melanoma studies. Therefore, there are no anticipated changes in exposure between different tumors.[48]

The choice of the 200 mg Q3 week dosing was an appropriate dose for the switch to fixed dosing. It is based on simulations performed using the population PK model of pembrolizumab showing that the fixed dose of 200 mg every 3 weeks will provide exposures that are consistent with those obtained with the 2 mg/kg dose every 3 weeks. This dose will maintain individual patient exposures in the exposure range established in melanoma studies, which were associated with maximal efficacy response and were well tolerated and safe.[48]

Furthermore, a fixed dose regimen will simplify the dosing regimen, which can reduce potential for dosing errors, reduce wastage, and reduce the complexity in the logistics chain.[48]

Anti-Drug Antibodies (ADA) Data

The occurrence of ADA has been observed in less than 1% of the patients screened, indicating a low potential of pembrolizumab to elicit the formation of ADA. No impact of ADA on pembrolizumab exposure has been observed[48].

2.5.1.3 Justification for Pembrolizumab Dose

The planned dose of pembrolizumab for this study is 200 mg every 3 weeks (Q3W), standard dosing based on the Investigational drug brochure. Based on the totality of data generated in the Keytruda development program, 200 mg Q3W is the appropriate dose of pembrolizumab for adults across all indications and regardless of tumor type. As outlined below, this dose is justified by:

- Clinical data from 8 randomized studies demonstrating flat dose- and exposure-efficacy relationships from 2 mg/kg Q3W to 10 mg/kg every 2 weeks (Q2W),
- Clinical data showing meaningful improvement in benefit-risk including overall survival at 200 mg Q3W across multiple indications, and
- Pharmacology data showing full target saturation in both systemic circulation (inferred from pharmacokinetic [PK] data) and tumor (inferred from physiologically-based PK [PBPK] analysis) at 200 mg Q3W

2.5.1.4 Safety

A maximum tolerated dose (MTD) of pembrolizumab has not been defined. Per the MERCK pembrolizumab label, serious adverse events (SAEs) include: pneumonitis, vitiligo, colitis, hepatitis, hypophysitis, and thyroiditis. Renal failure, pancreatitis and diabetes, neurologic events, and vasculitis have also been reported. The most frequent treatment-related adverse events (AEs) were fatigue, nausea, cough, pruritus, diarrhea, and rash. Most AEs were not considered serious. The most commonly-reported immune-related AEs were rash, pruritus, vitiligo, hypothyroidism, arthralgia, diarrhea, and pneumonitis. Important identified risks include: pneumonitis, thyroid disorders (hypothyroidism and hyperthyroidism), colitis, diarrhea, hepatitis, nephritis, uveitis, rash/pruritus, and neuropathy.[48]

2.5.1.5 Pharmacodynamics/Biomarkers

PD-1, PD-L1 expression may correlate with response in metastatic malignant melanoma. Tumor-cell expression (melanoma) of PD-L1 was characterized in combination with ipilimumab with the use of IHC staining and pharmacodynamic changes in the peripheral-blood absolute lymphocyte count.[52] With PD-L1 positivity defined as expression in at least 5% of tumor cells, biopsy specimens from 21 of 56 patients (38%) were PD-L1–positive. Among patients treated with the concurrent regimen of nivolumab and ipilimumab, ORs were observed in patients with either PDL1–positive tumor samples (6 of 13 patients) or PD-L1–negative tumor samples (9 of 22). In the sequenced regimen cohorts, a higher number of overall responses was seen among patients with PD-L1–positive tumor samples (4 of 8 patients) than among patients with PD-L1–negative tumor samples (1 of 13) suggesting the possibility that these tumors have higher response rates to the combination. The relationship between PDL-1 expression and responses may not be present in patients treated with the combination.

Additionally, PD-L1 is being investigated as a predictive biomarker for nivolumab and pembrolizumab treatment in lung cancer. In fact, the FDA approved nivolumab for patients with adenocarcinoma of the lung and squamous cell carcinoma of the lung after progression on platinum-based therapy. In contrast, pembrolizumab was approved by the FDA for metastatic non-small cell lung cancer, which has progressed on prior treatment, but only with PD-L1 positivity by IHC. This was based on the KEYNOTE-001 study[53]. Positive PD-L1 staining was defined as ≥ 50%. Patients with positive PD-L1 staining had an ORR of 45% compared to 17% in patients with 1% to 49% staining by IHC for PD-L1, and 11% in patients with <1% IHC staining for PD-L1. [53]

In the SARC028 trial, PD-L1 expression correlated with response in UPS, though this analysis included a total of 10 participants.[49] In the metastatic triple negative breast cancer eribulin and pembrolizumab trial, PD-L1 expression status did not correlate with response.

Additionally, tissue expression of PDL-2, interferon-γ (IFN- γ), indoleamine 2,3-dioxygenase (IDO), T cell infiltrates with CD8+ cells, CD4+ cells, T-suppressor Foxp3, or tumor infiltrating macrophages are of investigational interest as potential biomarkers. Tumor mutational load and microsatellite instability are also of interest.

Until more reliable data based on standardized procedures for tissue collection and assays are available, PD-L1 status cannot be used to select patients for treatment at this time in soft tissue sarcomas, but it is a biomarker worth investigating.

2.5.2 *Eribulin*

2.5.2.1 Nonclinical development of Eribulin: Mechanism of action

Eribulin mesylate (eribulin) is a microtubule inhibitor, derived from the marine sponge, Halichondria okadai[54, 55]. The natural compound Halichondrin B (Hal B), of which Eribulin is an analog, is a large polyether macrolide, naturally occurring in Halichondria okadai[54]. This compound has anti-cancer activity based a on a microtubule destabilizing anti-mitotic mechanism of action[56]. Other anti-cancer drugs, such as vinca alkaloids or taxanes, also inhibit microtubule activity[57]. However, the interaction between Hal B and tubulin is unique[58]. Eribulin sequesters tubulin into nonproductive aggregates, leading to tubulin polymerization inhibition, and resulting in a mitotic block.[59]

Summary of mechanism of action

In vitro studies have shown that analogues of Hal B inhibit cell growth at sub-Nano molar concentrations[60]. This occurs in a wide variety of cancer cell types, such as breast, ovary, colon, melanoma, and sarcomas[60]. Eribulin exerts its effects by binding to the positive end of microtubules, this leads to suppression of microtubule growth[61]. Eribulin sequesters tubulin into nonproductive tubulin aggregates. This leads to inhibition of tubulin polymerization and microtubule dynamics. The result is an interference with normal mitotic spindle formation and the blockage of the pro-metaphase portion of mitosis[61]. The end effect is the induction of irreversible cell cycle block at G2/M and cell death via apoptosis after this prolonged mitotic block.[62]

Eribulin is a substrate for the P-glycoprotein drug efflux pump. Eribulin has reduced potency against cells expressing high levels of this pump. Additionally, upregulation of this pump is a possible resistant mechanism to eribulin. Taxane-resistant cell lines have β-tubulin mutations. However, *in vitro*, these cell lines are sensitive to eribulin. *In vivo,* eribulin has led to tumor regression and even eradication against several human cancer xenograft models, including sarcomas.[54, 63]

2.5.2.2 Pharmacokinetics pre-clinical development of Eribulin

In animal models (mice, rat and dogs), the pharmacokinetics of eribulin after intravenous administration is characterized by a rapid distribution phase, a large volume of distribution and a prolonged elimination phase (t1⁄2: 3.6 -6.9 hours in

mice, 15.9- 27.9 hours in rats, 21.9-28.2 hours in dogs). Eribulin also has low penetration in brain, likely related to its role as a substrate of the P-glycoprotein drug efflux pump. It also has high penetration in tissues such as the lung, bladder, renal cortex and medulla, liver, spleen, thyroid, stomach, and salivary gland.

Unchanged eribulin is the major circulating compound in plasma following its administration. Metabolism is a minor component of eribulin clearance, with minor metabolic changes occurring through cytochrome P450 3A4 (CYP3A4). Eribulin is eliminated primarily unchanged in feces.[64]

2.5.2.3 Pharmacokinetics clinical development of Eribulin

In clinical studies, eribulin 's PK is characterized by a rapid distribution phase, with a prolonged elimination phase after intravenous infusion. The disposition of eribulin follows linear kinetics over the dose range studied (0.25mg/m^2- 4.0mg/m^2). It has low plasma clearance, with a mean clearance=1.16-2.42 L/hr/m^2, a large mean volume of distribution at steady state (43-114 L/m2) and a half-life of elimination of 40 hours. The human plasma protein binding of eribulin occurs at concentrations of 100ng/mL to 1.000ng/mL and ranges from 49% to 65%. Eribulin exposure after multiple doses is comparable to a single dose, with no accumulation of eribulin with weekly administration. As expected based on work in pre-clinical models, metabolism is a minor component of eribulin clearance. Metabolites represent less than 0.6% of parent compound in plasma. Renal elimination is also a minor route of eribulin excretion, with less than 10% of drug excreted unchanged in urine. Most of excretion of eribulin is fecal and unchanged. It is unknown if eribulin is excreted into human milk.[64]

Eribulin does not induce or inhibit hepatic CYP3A4 activity at clinically relevant concentrations. Concomitant administration of ketoconazole, a CYP3A4 inhibitor, or rifampicin, a CYP3A4 inducer, had no effect on exposure to eribulin. Eribulin does not induce or inhibit CYP1A, CYP2C9, CYP2C19, CYP2D6 activity at clinically relevant concentrations.[64]

Hepatic impairment may decrease the clearance of eribulin and prolong the elimination half-life, resulting in increased exposure to eribulin. As such, it is proposed that the eribulin dose in patients with moderate hepatic impairment should be adjusted. No studies for patients with severe hepatic impairment have been performed.[64]

Moderate renal impairment (CrCL 30-50 mL/min) may also impact the clearance of eribulin, resulting in increased exposure. As such, it is proposed that eribulin dose in patients with moderate renal impairment should be adjusted. No studies for patients with severe renal impairment have been performed.[64]

Population PK analyses based on Phase 1 and 2 studies showed that eribulin meslyate's clearance is affected by body weight, serum albumin, alkaline phosphatase

and bilirubin. The effects of age, gender, race and concomitant medications (CYP3A4 inhibitors and inducers) on clearance were not found to be significant.[64]

2.5.2.4 Efficacy

A Phase II study of eribulin in soft tissue sarcomas suggested a benefit in leiomyosarcomas and liposarcomas with a three-month progression-free survival of 31.6% for leiomyosarcomas and 46.9% for liposarcomas, and a 21.1% for synovial sarcomas, and a 19.2% for other soft tissue sarcomas[65]. A Japanese phase II trial showed similar results with a 12-week progression-free survival of 60% for liposarcomas and leiomyosarcomas[66]. More recently, eribulin was approved for the treatment of metastatic or recurrent liposarcomas[67] based on the results of a randomized phase III trial comparing eribulin to dacarbazine in leiomyosarcomas and liposarcomas[68]. This trial showed an improvement in the median overall survival of 13.5 months for participants treated with eribulin vs 11.5 months for participants treated with dacarbazine[68]. There was no difference in median progression-free survival, 2.6 months in each arm; 12-week progression-free survival was 33% in the eribulin group and 29% in the dacarbazine group[68]. A subgroup analysis in the liposarcoma cohort showed an improvement in progression-free survival of 2.9 vs 1.7 months, p=0.0015, and overall survival of 15.6 vs 8.4 months, p=0.0006[69]. Thus, the FDA approved eribulin for liposarcomas, including dedifferentiated, myxoid/round cell, and pleomorphic liposarcomas.

2.5.2.5 Toxicology and Safety

A variety of nonclinical toxicology studies have been conducted to support the use of eribulin in humans. The findings from these studies are summarized below. No significant serious adverse events (AE) were observed in any preclinical safety studies with regard to central nervous, respiratory, or cardiovascular systems. Eribulin induced no significant reduction in nerve conduction velocity or peak nerve amplitude in caudal and digital nerves, in contrast to paclitaxel. The morphological changes in sciatic nerve and dorsal root ganglia were less severe in eribulin than those observed with paclitaxel. In animal models, eribulin induced markedly less neuropathy than paclitaxel.[64]

Bone marrow toxicity appeared to be dose-limiting in both rats and dogs. Intestinal toxicity was also present in dogs. Other toxicities that were considered to be drug-related occurred in the lymphoid tissue, testes, and skeletal muscle. All observed toxicities (except testicular toxicity) were reversible in both dogs and rats. In repeated-dose toxicity studies in rats, testicular toxicity, thymic atrophy, bone marrow toxicity, and fiber degeneration of sciatic nerve were found.[64]

Although the changes in testes and sciatic nerve were still present after a 14-day recovery period, other toxicities were reversible. Repeated-dose toxicity in dogs produced leukopenia, which was fully reversible in 14 days with compensatory extramedullary hematopoiesis. The chronic toxicity studies in rats and dogs were conducted over 6 months. In rats, bone marrow and testicular toxicity were the most

important effects observed. Hypocellularity of bone marrow and a reduction in the weight of testes (correlating with hypocellularity of seminiferous epithelium with associated hypospermia/aspermia of the epididymides) were found. Increases in alanine aminotransaminase (ALT), aspartate aminotransaminase (AST), and cholesterol were also observed. In dogs, bone marrow toxicity mainly represented by leukopenia was observed. Regarding findings in the testes, this finding was limited to high doses of drugs and included decrease of testes weights and microscopic changes with mild to moderate hypocellularity of testis with hypospermia/aspermia of the epididymides. In addition, hyper-cellularity of bone marrow, lymphoid depletion in mesenteric lymph nodes and Peyer's patches, and thymic atrophy were noted. These studies suggest that male fertility may be compromised by eribulin treatment. Carcinogenicity studies have not been conducted with eribulin. Genotoxicity was evaluated both *in vitro* and *in vivo*. *In vitro,* results indicated that the effect was due primarily to chromosome segregation interference rather than chromosome breakage because eribulin was negative in the Ames test with or without S9 and was weakly positive in the L5178Y tk+/- mouse lymphoma mutagenesis assay. *In vivo,* in rats, dose-related increases of micro-nucleated- polychromatic erythrocytes (MN-PCEs) at either sampling time and dose- related decreases in the proportion of PCEs were observed, which may indicate some degree of bone marrow suppression. In the intravenous embryo and fetal developmental studies in rats, eribulin was given to rats at doses of 0.01, 0.03, 0.10, and 0.15 mg/kg/day on gestational days 8, 10, and 12. External and/or soft tissue anomalies were noted at 0.15 mg/kg/day, indicating that eribulin has teratogenic potential.[64]

Summary of safety data for eribulin

In the phase III EMBRACE trial, AEs occurred in 99% of participants receiving eribulin, however serious AEs occurred in 25% and AEs leading to therapy discontinuation occurred in 13% of participants on study. The primary toxicities with eribulin were asthenia or fatigue (54%) and neutropenia (52%). Grade 4 neutropenia lasting more than 1 week occurred in 12% of participants and F+N occurred at low incidence (5%). Participants with elevated ALT/AST or bilirubin experienced a higher frequency of grade 4 neutropenia or F+N. Peripheral neuropathy was the most common AE leading to discontinuation of therapy, occurring in 5% of participants. Neuropathy lasting more than one year occurred in 5% of the participants. Alopecia occurred in 45% of participants. Nausea occurred in 35% of participants, but it was grade 3 just in 1% of participants. Diarrhea occurred in 18% of cases; in all cases was grade 1 or 2. Dose interruptions, delays and reductions were undertaken in 6%, 49% and 29% in the eribulin group, respectively.[70]

Overall Adverse events and serious adverse events

In clinical studies, the most common treatment-related toxicities were hematological toxicity, including neutropenia; asthenia or fatigue; alopecia; nausea and peripheral neuropathy. Most adverse events were grade 1 or grade 2 in severity.

Peripheral neuropathy was an important adverse event leading to discontinuation of

therapy. The most frequently reported eribulin-related adverse events were asthenia/fatigue (65%), alopecia (60%), neutropenia (60%), nausea (44%), anemia (28%), pyrexia (23%), leucopenia (22%), anorexia (21%), constipation (19%), vomiting (18%), and peripheral neuropathy (5.5%; only grade 3). Grade 4 neutropenia occurred in 32% of participants, and fever plus neutropenia occurred in 5.5% of participants. The frequency of all other grade 3/4 AEs was less than 3%.[64]

2.5.2.6 Pharmacodynamics / Biomarkers

No known biomarker exists for predicting response to Eribulin.

2.6 Summary of Pembrolizumab and Eribulin combination Data.

The safety and efficacy of eribulin and pembrolizumab was studied in a Phase I/II study of patients with metastatic triple-negative breast cancer (mTNBC), NCT02513472. In the combination arm, the dosing of pembrolizumab was 200mg IV Q 3 weeks and the dosing of eribulin was 1.4mg/m2 IV day 1 and day 8 of a 21-day cycle. The overall response rate (ORR) was 26.4% (95% CI 18.3-35.9) in 106 evaluable participants. The CR rate was 2.8%, and PR was 23.6%. The clinical benefit rate (CR+PR+SD for ≥ 24 weeks) was 36.8%. Median duration of response was 8.3 months (95% CI 6.5-12.9) for the 28 responders in the participant population. Response lasted >6 months in 53.6% of responders. Median PFS was 4.2 months and median OS was 17.7 months, suggesting activity in mTNBC. PD-L1 staining by IHC was not predictive of response in this cohort on interim analysis. The ORR was 29.4% (95% CI 11.1-51.1) in the PD-L1 positive participants and 33.3% (95% CI 14.1-54.6) in the PD-L1 negative participants. Most importantly, this combination was well tolerated. There were no dose-limiting toxicities observed in the phase Ib portion of this study. The recommend phase II dose (RP2D) was 1.4mg/m^2 of Eribulin, day 1, day 8, and 200mg IV of Pembrolizumab day 1 on a Q21 day schedule. The most common treatment-related adverse events (all grades) were fatigue (73.8%), peripheral neuropathy (62.6%), nausea (61.7%), alopecia (42.1%), neutropenia (41.1%), anemia (29.9%) and constipation (39.3%). Grade 3 or 4 treatment-related adverse events were seen in 47.7% and 18.7% of participants, respectively, the most common grade 3 or 4 adverse events being neutropenia (30.8%), peripheral neuropathy (9.3%), anemia (5.6%), fatigue (5.6%), and hypokalemia (5.6%). On this study, 24 participants (22.4%) had treatment emergent adverse events (TEAEs) leading to study drug withdrawal (2 participants in phase 1b, 22 participants in phase 2). On this study 34 Participants (31.8%) had TEAEs leading to eribulin dose reduction (all in phase 2). Seven participants on this study had grade 5 events, all of which were related to disease progression: Respiratory failure, n = 1; respiratory failure/sepsis, n = 1; pleural effusion, n = 1; unknown, n = 1; multiple organ dysfunction syndrome, n = 1; cardiac arrest, n = 1; and malignant neoplasm progression, n = 1. Other adverse events (all grades) that occurred in >15% of participants were decreased appetite (36.4%), pyrexia (35.5%), cough (30.8%), diarrhea (29.9%), arthralgia (29.9%), vomiting (25.2%), headache (24.3%), weight decrease, (24.3%), rash (20.6%), dyspnea (19.6%), stomatitis (19.6%), dehydration (18.7%), hypothyroidism (18.7%), dry mouth (16.8%), AST increase (16.8%), ALT increase (16.8%), UTI (15.9%), dysgeusia (15.0%), and musculoskeletal pain (15.0%). Possible immune-related events (all grades) occurred in 82.2% of participants. These included in > 4% of participants: Anemia (29.9%), diarrhea (29.9%), rash (20.6%), hypothyroidism (18.7%), pruritus (14.0%), maculopapular rash (12.1%), pneumonitis (11.2%), hyperglycemia (9.3%), and hyperthyroidism

(8.4%). Possible immune related events (all grades) that occurred in <4% of participants were infusion-related reaction, colitis, generalized rash, pruritic rash, adrenal insufficiency, pancreatitis, renal failure, photosensitivity reaction, type I DM, cheilitis, dermatitis, contact dermatitis, exfoliative rash, hypophysitis, miliaria, and uveitis. Grade 3 or 4 immune-related adverse events occurred in only 18.7% of participants. The most common grade 3 or 4 immune-related side effects included anemia (5.6%), diarrhea (4.7%), rash (1.9%), pneumonitis (0.9%), hyperglycemia (1.9%), adrenal insufficiency (0.9%), pancreatitis (1.9%), renal failure (0.9%), and type I DM (1.9%). These data indicate that the combination of eribulin and pembrolizumab is well tolerated with non-overlapping side effect profiles.

2.7 Rationale

The combination of cytotoxic chemotherapy with immunotherapy is a rational approach to increase the response to immunotherapy. Cytotoxic chemotherapy may lead to cell death and the release of neoantigens that can be utilized in an immune response. Additionally, eribulin has non-mitotic effects which increase vascular perfusion and may impact the tumor stromal microenvironment. This may allow an increase in tumor infiltration by immune cells and potentiate the effects of immune checkpoint inhibitors[71]. Recently, the combination of eribulin and pembrolizumab has been shown to be safe and effective in patients with triple negative breast cancer. Because of the activity of eribulin in LPS and LMS; together with the reported activity of pembrolizumab, nivolumab, or nivolumab + ipilimumab in a subset of patients with LPS, LMS and UPS/Other sarcomas; and the potential for synergy; we now propose a parallel-cohort three-arm -uncontrolled phase II study evaluating the combination of eribulin and pembrolizumab. The dosing of eribulin will be 1.4mg/m^2 on Day 1 and Day 8, and the dosing for pembrolizumab will be 200mg every 3 weeks. This is the standard dosing for each drug and has been shown to be active and safe in the eribulin and pembrolizumab metastatic triple negative breast cancer trial.

The standard end-point for phase II soft tissue sarcoma trials is the progression-free rate (PFR) at 12 weeks,[72] with an active regimen defined as having a PFR of > 40% at 12 weeks and an inactive regimen defined as having a PFR of < 20% at 12 weeks. The activity of eribulin in the phase II soft tissue sarcoma trial was a PFS of 32% for leiomyosarcomas, and 47% for liposarcomas at 3 months. Also, a 3-month PFS seen in the SARC028 trial was 55%, and a 3-month PFS seen in the nivolumab alone arm of Alliance A091401was ~ 40%. Thus, for this trial, it was determined that a 40% PFS is inadequate to determine if the eribulin/pembrolizumab combination is effective, as 40% PFS at 3 months may be due to the single agents alone. For the purposes of this trial, we will define an active regimen as having a PFS of >60% at 12 weeks, indicating activity of the combination, and an inactive regimen as having a PFS of <30% at 12 weeks.

2.8 Correlative Studies Background

There is currently no biomarker for predicting response to eribulin. There are multiple possible biomarkers for predicting response to immunotherapy. These include multiple factors influencing the tumor microenvironment, such as PD-1, PD-L1, and PD-L2 expression. The expression of PD-1, PD-L1, and PD-L2 vary in soft tissue sarcomas. Additionally, the presence

and composition of TILs or tumor infiltrating macrophages may be important for predicting response to immunotherapy. Both TILs and tumor infiltrating macrophages have been noted in soft tissue sarcomas. Tumors with genomic instability, such as microsatellite unstable tumors, or tumors with a high mutational load may be more sensitive to immunotherapy as well. It will be critically important to investigate potential biomarkers for response to eribulin in combination with pembrolizumab in soft tissue sarcomas. Current results from the SARC 028 trial with pembrolizumab suggest PD-L1 expression as a potential predictor of response. Though in the eribulin and pembrolizumab metastatic triple negative breast cancer trial, PD-L1 expression was not predictive of response. As a correlative study to this trial, we will characterize the immune profile of available pre-treatment tissue biopsies, with particular regard to PD-1, PD-L1, and PD-L2 expression and influence on response to therapy and progression-free survival. Tumor infiltrating lymphocytes (CD3, CD4, CD8, FOX-P3), tumor infiltrating macrophages (CD68, CD63), and tumor genomics will be investigated as well. We hypothesize that the expression of these markers, either alone or in combination, may lead to the identification of a subset of patients with liposarcoma, leiomyosarcoma, or undifferentiated pleomorphic sarcoma/other sarcomas patients who are more likely to respond to the combination of eribulin and pembrolizumab. Examination of peripheral blood (pre-treatment, on-treatment, and post-treatment blood draws) for immunologic markers and cytokines will be performed as well.

This evaluation will be performed on fresh tumor pre-treatment biopsy samples, or on archival tumor material. Any archival tissue is acceptable, however, if there are multiple archival samples, it is preferable to use the archival tissue that was obtained within 6 months and was obtained prior to any new systemic therapy. When possible, pre-treatment tumor samples will be compared to optional post-treatment tumor biopsies.

This work will be performed under the direction of Scott Rodig, MD, PhD at the Center for Immuno-oncology Pathology Core at Dana-Farber/Harvard Cancer Center (DF/HCC). The immuno-oncology core at DF/HCC has optimized IHC for these markers.[73] Additionally, the immuno-oncology core lab at DF/HCC has experience evaluating the gene expression profiling of tumor samples and peripheral blood with the aim of identifying an immune gene signature. This effort may result in identifying patients more likely to respond to therapy with immune checkpoint inhibitors. Further details can be found in Section 9.

3. PARTICIPANT SELECTION

3.1 Eligibility Criteria

3.1.1 Histologically confirmed liposarcoma, leiomyosarcoma, or undifferentiated/unclassified pleomorphic sarcoma, or other sarcomas (that have been reported to respond to immunotherapy such as, but not limited to, alveolar soft part sarcoma, cutaneous angiosarcoma, mismatch repair deficiency sarcomas, after review by the principal investigator and treating physician) by a Dana-Farber Cancer Institute, Brigham and Women's Hospital or Massachusetts General Hospital pathologist.

Alternative terms for undifferentiated/unclassified pleomorphic sarcomas meeting inclusion criteria include but are not limited to the following:

pleomorphic undifferentiated sarcoma
unclassified spindle cell sarcoma

spindle cell sarcoma not otherwise specified

pleomorphic spindle cell sarcoma

pleomorphic fibroblastic sarcoma

undifferentiated high-grade pleomorphic sarcoma

pleomorphic sarcoma with prominent inflammation

pleomorphic sarcoma with giant cells

malignant fibrous histiocytoma (including storiform-pleomorphic and inflammatory subtypes)

fibrosarcoma

myxofibrosarcoma
extraskeletal osteosasrcoma (soft tisse sarcoma with osteoid differentiation)

3.1.2 Participants must have measurable disease, defined as at least one lesion that can be accurately measured in at least one dimension (longest diameter to be recorded for non-nodal lesions and short axis for nodal lesions) as ≥20 mm with conventional techniques or as ≥10 mm with spiral CT scan, MRI, or calipers by clinical exam. See Section 11 for the evaluation of measurable disease.

3.1.3 Participants must have received at least one prior line of chemotherapy. No limit on prior lines of therapy.

3.1.4 Age ≥ 18 years.

3.1.5 ECOG performance status of 0 or 1 (see Appendix A).

3.1.6 Participants must have normal organ and marrow function as defined below:

- leukocytes ≥3,000/mcL
- absolute neutrophil count ≥1,500/mcL
- platelets ≥100,000/mcL
- Hemoglobin ≥ 8 g/dL within the first 2 weeks prior to the first dose of study drugs, transfusion is allowed.
- total bilirubin ≤1.5× institutional upper limit of normal (ULN)
  (except participants with Gilbert Syndrome, who can have total bilirubin <3.0 mg/dL)
- AST(SGOT)/ALT(SGPT)<2.5 x ULN in a participant with no documented liver metastases; ALT and AST <5.0 x ULN in a participant with documented liver metastases
-
- creatinine ≤1.5× ULN
  OR

- creatinine clearance — ≥50 mL/min/1.73 m^2 for participants with creatinine levels above institutional normal (using the Cockcroft-Gault Formula below):

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

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

3.1.7 The effects of eribulin and pembrolizumab on the developing human fetus are unknown. For this reason, women of child-bearing potential (WOCBP) and men must agree to use adequate contraception (hormonal or barrier method of birth control; abstinence) prior to study entry and for the duration of study participation. A male participant must agree to use a contraception as detailed in Appendix G of this protocol during the treatment period and for at least 20 weeks, corresponding to the time needed to eliminate any study treatments, plus an additional 120 days (a spermatogenesis cycle) after the last dose of study treatment. A female participant is eligible to participate if she is not pregnant (see Appendix G), not breastfeeding, and at least one of the following conditions applies:

a.) Not a woman of childbearing potential (WOCBP) as defined in Appendix G
OR
b.) A WOCBP who agrees to follow the contraceptive guidance in Appendix G during the treatment period and for at least 20 weeks plus an additional 30 days (a menstruation cycle) after the last dose of study treatment.

WOCBP should use an adequate method to avoid pregnancy for at least 20 weeks plus an additional 30 days after the last dose of investigational drug. Women of childbearing potential must have a negative serum pregnancy test within 72 hours prior to the start of Eribulin and Pembrolizumab. Women must not be breastfeeding. Women who are not of childbearing potential (*i.e.*, who are postmenopausal or surgically sterile) do not require contraception. Women of childbearing potential (WOCBP) is defined as any female who has experienced menarche and who has not undergone surgical sterilization (hysterectomy or bilateral oophorectomy) or who is not postmenopausal. Menopause is defined clinically as 12 months of amenorrhea in a woman over 45 in the absence of other biological or physiological causes. In addition, women under the age of 55 must have a documented serum follicle stimulating hormone (FSH) level less than 40 mIU/mL.

3.1.8 Ability to understand and the willingness to sign a written informed consent document.

3.2 Exclusion Criteria

3.2.1 Participants who have had standard chemotherapy or radiotherapy within 3 weeks prior to entering the study.

3.2.2 Participants who have not recovered from adverse events (grade 2 or higher toxicities) due to agents administered, radiotherapy, or surgery, with the exception of alopecia and anemia.

3.2.3 Previous treatment with eribulin or any anti-PD-1, PD-L1, or PD-L2 agent, or with an agent directed to another stimulatory or co-inhibitory T-cell receptor (eg, CTLA-4, OX-40, CD137).

3.2.4 Participants who are currently participating in or have participated in a study of an investigational agent or have used an investigational device within 3 weeks prior to the first dose of study treatment.
Note: Participants who have entered the follow-up phase of an investigational study may participate as long as it has been 3 weeks after the last dose of the previous investigational agent.

3.2.5 Known brain metastases that are untreated, symptomatic or require therapy to control symptoms. Participants with previously diagnosed brain metastases are eligible if they have completed treatment at least 4 weeks prior to registration, are neurologically stable and have not experienced any new neurologic symptoms for the last 4 weeks prior to study entry, and have recovered from the effects of radiotherapy or surgery. There must also be no requirement for immunosuppressive doses of systemic corticosteroids (>10 mg/day prednisone equivalents) for at least 2 weeks prior to study drug administration. Treatment for brain metastases may have included whole brain radiotherapy, radiosurgery, surgery, or a combination as deemed appropriate by the treating physician.

3.2.6 Have received a live vaccine within 30 days prior to the first dose of study drug. Examples of live vaccines include, but are not limited to, the following: measles, mumps, rubella, varicella/zoster (chicken pox), yellow fever, rabies, Bacillus Calmette–Guérin (BCG), and typhoid vaccine. Seasonal influenza vaccines for injection are generally killed virus vaccines and are allowed; however, intranasal influenza vaccines (eg, FluMist®) are live attenuated vaccines and are not allowed.

3.2.7 Inability to comply with study and/or follow-up procedures.

3.2.8 History of severe hypersensitivity reaction (≥Grade 3) to any monoclonal antibody.

3.2.9 History of allergic reactions attributed to compounds of similar chemical or biologic composition to Eribulin or Pembrolizumab.

3.2.10 Uncontrolled intercurrent illness including, but not limited to, ongoing or active infection, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situations which in the PI's opinion makes it undesirable for the participant to participate in the trial or which would jeopardize compliance with the trial and study requirements.

3.2.11 Pregnant women (WOCBP who had a positive serum pregnancy test on screening or 72 hours prior to initiation of study protocol) are excluded from this study because the effects of Eribulin and Pembrolizumab on the developing fetus are unknown. There is the potential for teratogenic or abortifacient effects. Because there is an unknown but potential risk for adverse events in nursing infants secondary to treatment of the mother with Eribulin and Pembrolizumab, breastfeeding should be discontinued if the mother is treated with Eribulin and Pembrolizumab.

3.2.12 Because the effects of pembrolizumab on chronic viral infection are not well known, participants should be excluded if they have known history of testing positive for human immunodeficiency virus (HIV) (true positive) or known acquired immunodeficiency syndrome (AIDS) or if they have a positive test for hepatitis B virus surface antigen (HBV sAg) or hepatitis C virus ribonucleic acid (HCV antibody) indicating acute or chronic infection.

3.2.13 Participants with active autoimmune disease or history of autoimmune disease that might recur, which may affect vital organ function or require immune suppressive treatment including systemic corticosteroids, are excluded. These include but are not limited to participants with a history of immune related neurologic disease such as multiple sclerosis, autoimmune (demyelinating) neuropathy, Guillain-Barre syndrome, or myasthenia gravis; participants with a history of systemic autoimmune disease such as SLE, connective tissue diseases, scleroderma, inflammatory bowel disease (IBD), Crohn's, ulcerative colitis, or hepatitis; and participants with a history of toxic epidermal necrolysis (TEN), Stevens-Johnson syndrome, or phospholipid syndrome. These participants should be excluded because of the risk of recurrence or exacerbation of disease. Participants with vitiligo or endocrine deficiencies, including thyroiditis managed with replacement hormones such as physiologic corticosteroids, are eligible. Participants with rheumatoid arthritis or other arthropathies; Sjögren's syndrome; psoriasis controlled with topical medication; or participants with positive serology, such as antinuclear antibodies (ANA) or anti-thyroid antibodies, should be evaluated for the presence of target organ involvement and potential need for systemic treatment but should otherwise be eligible.

3.2.14 Participants are permitted to enroll if they have vitiligo, type I diabetes mellitus, residual hypothyroidism due to autoimmune condition only requiring hormone replacement, psoriasis not requiring systemic treatment, or conditions not expected to recur in the absence of an external trigger (precipitating event).

3.2.15 Participants should be excluded if they have a condition requiring systemic treatment with either corticosteroids (>10 mg daily prednisone equivalents) or other immunosuppressive medications within 14 days of study drug administration. Inhaled or topical steroids and adrenal replacement doses <10 mg daily prednisone equivalents are permitted in the absence of active autoimmune disease. Participants are permitted to use topical, ocular, intraarticular, intranasal, and inhalational corticosteroids (with minimal systemic absorption). Physiologic replacement doses of systemic corticosteroids are permitted, even if <10 mg/day prednisone equivalents. A brief course of corticosteroids for prophylaxis (*e.g.*, contrast dye allergy) or for treatment of non-autoimmune conditions (*e.g.*, delayed-type hypersensitivity reaction caused by contact allergen) is permitted.

3.2.16 Participants with a history of pneumonitis or interstitial lung disease.

3.2.17 History of primary immunodeficiency or solid organ transplantation.

3.2.18 Participants who have had evidence of active or acute diverticulitis, intra-abdominal abscess, GI obstruction, or fistula or abdominal carcinomatosis (which are known risk factors for bowel perforation) should be evaluated for the potential need for additional treatment before coming on study.

3.3 Inclusion of Women and Minorities

Both men and women of all races and ethnic groups are eligible for this trial. Every attempt will be made to enter all eligible participants into this protocol in this rare set of diseases.

4. REGISTRATION PROCEDURES

4.1 General Guidelines for DF/HCC Institutions

Institutions will register eligible participants in the Clinical Trials Management System (CTMS) OnCore. Registrations must occur prior to the initiation of protocol therapy. Any participant not registered to the protocol before protocol therapy begins will be considered ineligible and registration will be denied.

An investigator will confirm eligibility criteria and a member of the study team will complete the protocol-specific eligibility checklist.

Following registration, participants may begin protocol therapy. Issues that would cause treatment delays should be discussed with the Overall PI. If a participant does not receive protocol therapy following registration, the participant's registration on the study must be canceled. Registration cancellations must be made in OnCore as soon as possible.

4.2 Registration Process for DF/HCC Institutions

DF/HCC Standard Operating Procedure for Human Subject Research Titled *Subject Protocol Registration* (SOP #: REGIST-101) must be followed.

5. TREATMENT PLAN

5.1 Treatment Regimen

This is a non-randomized phase 2 study of eribulin in combination with pembrolizumab for the treatment of liposarcomas, leiomyosarcoma, and undifferentiated pleomorphic sarcomas. Eligible participants with liposarcoma, leiomyosarcoma, or undifferentiated pleomorphic sarcoma/other sarcomas will be divided into three separate cohorts by histologic subtype. Arm A: Leiomyosarcoma, Arm B: Liposarcoma, and Arm C: Undifferentiated Pleomorphic Sarcoma/other sarcomas. A treatment cycle will be defined as 21 consecutive days. Treatment will be eribulin 1.4 mg/m2 administered on Day 1 and on Day 8 of each 21-day cycle, plus pembrolizumab 200mg administered on Day 1, of each 21-day cycle. If treatment is delayed, protocol required restaging assessments will remain on-schedule. Eribulin and pembrolizumab will be administered on an outpatient basis. Reportable adverse events and potential risks are described in Section 7. Appropriate dose modifications are described in Section 6. No investigational or commercial agents or therapies other than those described below may be administered with the intent to treat the participant's malignancy.

Treatment regimen is described in Table l.

Table 1 Treatment Regimen.

Regimen Description					
Agent	***Premedication; Precautions***	***Dose***	***Route****	***Schedule***	***Cycle Length***
Eribulin	No routine premedication required, addition of 4 to 8mg of dexamethasone and/or 8 to 16mg of Zofran allowed for nausea as per institutional standard[+]	*1.4mg/m^2*	*IV, infuse over 2 to 5 min, may be administered per institutional standard*	*Day 1 and Day 8*	*21 days (3 weeks)*
Pembrolizumab	Not routinely necessary unless prior infusion reaction.[+]	*200mg*	*IV infuse over ~ 30 minas per institutional standard, infuse prior to starting eribulin infusion, per institutional standard*	*Day 1*	
* Dose reductions may be made as per Tables 3.1-4.9. Further details about dose reductions can					

be found in Section 6. +Pre-medications can be administered prior to pembrolizumab. Pre-medications for pembrolizumab and eribulin may be administered per institutional standards.

5.2 Pre-Treatment Criteria

5.2.1 Cycle 1, Day 1

Criteria to treat on Cycle 1 Day 1 (Patient does not need to re-meet full eligibility criteria):
-Absolute neutrophil count >1500/ mcL
-Platelets >100,000/ mcL
-ALT and AST <2.5 x ULN in a participant with no documented liver metastases; ALT and AST <5.0 x ULN in a participant with documented liver metastases
-Total bilirubin <1.5 x ULN (2.0 x ULN in a participant with well documented Gilbert's syndrome)

5.2.2 Cycle 1, Day 8 and Day 8 of subsequent cycles

Criteria to treat on Cycle 1 Day 8 and Day 8 of subsequent cycles:
-Absolute neutrophil count >1000/ mcL
-Platelets >100,000/ mcL
-ALT and AST <2.5 x ULN in a participant with no documented liver metastases; ALT and AST <5.0 x ULN in a participant with documented liver metastases
-Total bilirubin <1.5 x ULN (2.0 x ULN in a participant with well documented Gilbert's syndrome)

5.2.3 Day 1 of subsequent cycles

Criteria to treat on Day 1 of subsequent cycles:
-Absolute neutrophil count >1000/ mcL
-Platelets >100,000/ mcL
-ALT and AST <2.5 x ULN in a participant with no documented liver metastases; ALT and AST <5.0 x ULN in a participant with documented liver metastases
-Total bilirubin <1.5 x ULN (2.0 x ULN in a participant with well documented Gilbert's syndrome)

Any non-hematologic toxicities must return to baseline or to < Grade 2 beginning with Day 1of each cycle.

If dose is held or missed on Day 1 of a cycle, that cycle will not be considered to start until the day the dose is actually administered to the patient (ie, D1-D8-rest, X-D1-D8-rest, etc.)

5.3 Agent Administration

5.3.1 *Eribulin*

Eribulin will be administered by trained medical personnel at the investigational site. Treatment compliance will be monitored through documentation of study treatment administration in the participant's medical record.

Please refer to the FDA-approved package insert for eribulin for product information and a comprehensive list of adverse events.

Eribulin will be administered on Days 1 and 8 of each 21-day cycle. Eribulin should be prepared and administered per institutional standard. Institutional standard procedures include BSA calculations, body weight assessments and dose calculations/recalculations.

5.3.2 *Pembrolizumab*

Pembrolizumab will be administered by trained medical personnel at the investigational site. Treatment compliance will be monitored through documentation of study treatment administration in the participant's medical record.

Pembrolizumab will be administered in the outpatient clinic on Day 1 of each cycle. Pembrolizumab should be prepared and administered as an approximately 30-minute IV infusion per institutional standard, infuse prior to Eribulin

Please refer to the FDA-approved package insert for pembrolizumab for product information and a comprehensive list of adverse events. Pembrolizumab should be prepared and administered per institutional standard. Institutional standard procedures include BSA calculations, body weight assessments and dose calculations/recalculations. Infusion times may be prolonged in participants who experience an infusion reaction as per section 5.9.

Pembrolizumab should be administered prior to eribulin administration. There should be no overlap in timing of the two administrations.

The standard dose of pembrolizumab is 200mg IV.

5.3.3 *Discontinuation of eribulin*

If eribulin is stopped for toxicity, participants are permitted to continue protocol therapy with pembrolizumab alone.

5.3.4 *Discontinuation of pembrolizumab*

If pembrolizumab is stopped for toxicity, participants are permitted to continue protocol therapy with eribulin alone.

5.4 General Concomitant Medication and Supportive Care Guidelines

5.4.1 General Concomitant Medication Guidelines

Pembrolizumab is a human monoclonal antibody and as such is not expected to be metabolized by the cytochrome P450 (CYP) enzymes or other typical drug metabolizing enzymes. Thus, it is not expected to have any effect on the CYP or other drug metabolizing enzymes in terms of inhibition or induction, and is, therefore, not expected to induce this type of PK-based drug interaction. However, with eribulin there is a potential for interaction with other concomitantly administered drugs through the cytochrome P450 system. Concurrent use of all other drugs, over-the-counter medications, or alternative therapies will be reviewed at each visit. The Overall PI should be alerted if the participant is taking any agent known to affect or with the potential to affect selected CYP450 isoenzymes. Appendix C presents guidelines for identifying medications/substances that could potentially interact with the study agent(s).

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 may be required. The investigator should discuss any questions regarding this with the overall PI who will communicate with the Merck Clinical team and Eisai Clinical Team. The final decision on any supportive therapy or vaccination rests with the PI and/or the participant's primary physician.

Acceptable Concomitant Medications

All treatments that the investigator considers necessary for a participant’s welfare may be administered at the discretion of the investigator in keeping with the community standards of medical care with the exception of those prohibited medications noted below. Concomitant medications will be reviewed as noted in table 7.

Prohibited Concomitant Medications

Participants are prohibited from receiving the following therapies during the Screening and Treatment Phase of this trial:

- Antineoplastic systemic chemotherapy or biological therapy
- Immunotherapy not specified in this protocol
- Chemotherapy not specified in this protocol
- Biologic or targeted agents not specified in this protocol
- Investigational agents other than pembrolizumab and eribulin
- Live vaccines within 28 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 (10 mg prednisone by mouth daily or equivalent) is allowed.
- Care should be taken with concomitant use of strong CYP3A4 inhibitors/inducers (e.g. ketoconazole and itraconazole; see Appendix C) and eribulin. An alternate medication with no or minimal potential to inhibit CYP3A4 should be considered. If a strong CYP3A4 inhibitor is co-administered with eribulin, participants should be closely monitored for adverse reactions.
- Participants 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. Participants may receive other medications that the investigator deems to be medically necessary.
- There are no prohibited therapies during the Post-Treatment Follow-up Phase.

5.4.2 Supportive Care Guidelines – General Medications

The following treatments are permitted throughout the duration of the study treatment phase and during follow-up:

- Standard therapies for pre-existing medical conditions unless listed as prohibited therapy below. Any medication intended solely for supportive care (e.g., analgesics, antidiarrheal, anti-depressants) may be used at the investigator's discretion.
- Anti-diarrheal medications should not be administered prophylactically before initial treatment with study drugs. At the discretion of the investigator, prophylactic antiemetic and anti-diarrheal medication(s) may be used as per standard clinical practice before doses of study drugs.
- Hematopoietic growth factors (e.g., G-CSF, granulocyte macrophage colony stimulating factor) may be used at investigator's discretion for the primary prophylaxis and/or management of treatment-emergent neutropenia and/or for secondary prophylaxis as per NCCN/European Society for Medical Oncology guidelines [47, 48] or local standard practice.
- Bisphosphonate or denosumab therapy to be used in accordance with the approved labeled indication and/or nationally recognized treatment guidelines. Participants already receiving bisphosphonate/denosumab at the time of study entry can continue the treatment.
- Anticoagulants - Anticoagulation with heparin, heparin derivatives, and/or warfarin, and/or NOAC's may be given at the discretion of the treating physician.
- Pain medications administered per standard clinical practice are acceptable while the participant is enrolled in the study.
- Participants who experience toxicities should be treated symptomatically as clinically indicated. Medications that are considered necessary for the participant's welfare and that are not expected to interfere with the evaluation of study treatment or be restricted may be given at the discretion of the investigator. Ancillary treatments will be given as medically indicated.

5.5 Duration of Therapy

Duration of therapy will depend on individual response, evidence of disease progression and tolerance. In the absence of treatment delays due to adverse event(s), treatment may continue for 2 years or 35 cycles of therapy or until one of the following criteria applies:

- Disease progression in any participant who showed first radiologic evidence of progressive disease (PD) by RECIST 1.1 (see Section 11.1.4) and is deemed clinically stable, it is at the discretion of the investigator to continue treating the participant until progression is confirmed at least 4 weeks from the date of the first radiologic evidence of PD. Further details are described below.
- Intercurrent illness that prevents further administration of treatment
- Unacceptable adverse event(s) which include the following (see also section):
    - Any Grade 2 drug-related uveitis or eye pain or blurred vision that does not respond to topical therapy and does not improve to Grade 1 severity within the re-treatment period OR requires systemic treatment
    - Participants requiring > two dose delays for the same type of event should be removed from protocol
    - Any adverse event, laboratory abnormality, or intercurrent illness which, in the judgment of the investigator, presents a substantial clinical risk to the participant with continued study drug dosing
    - Any dosing interruption lasting >5 weeks, with the following exceptions:
        - Participants being tapered after high dose corticosteroids over one month followed by a two-week observation period will be allowed an additional two weeks to restart treatment (a maximum eight-week interruption).
        - Dosing interruptions >5 weeks that occur for non-drug-related reasons may be allowed if approved by the Investigator. Prior to re-initiating treatment in a participant with a dosing interruption lasting >5 weeks, the PI must be consulted.
    - Grade 2 pneumonitis requiring steroids.
    - Grade 3 or Grade 4 drug-related autoimmune or inflammatory events including uveitis, pneumonitis, diarrhea, colitis, neurologic adverse events, hypersensitivity reaction, or infusion reaction of any duration requires discontinuation except as noted below:
        - Any other grade 3 non-skin, drug-related AE lasting >7 days including fatigue.
        - Any grade 3 or 4 drug-related laboratory abnormality or electrolyte abnormality, not associated with underlying organ pathology, that does not require treatment except for electrolyte replacements **does not** require treatment discontinuation.
        - Grade 3 amylase or lipase abnormalities that are not associated with diabetes mellitus (DM), associated liver or gall bladder inflammation clinical manifestations of pancreatitis and which decrease to < Grade 2

within 1 week of onset **may** resume study treatment when resolved.
- Any grade 4 events except as noted above.

- Any participants who require additional immune suppressive treatment beyond steroids should go off study treatment
- Participant demonstrates an inability or unwillingness to comply with the medication regimen and/or documentation requirements
- Participant decides to withdraw from the protocol therapy
- General or specific changes in the participant's condition render the participant unacceptable for further treatment in the judgment of the treating investigator

Participants will be removed from the protocol therapy when any of these criteria apply. The reason for removal from protocol therapy, and the date the participant was removed, must be documented in the case report form (CRF). Alternative care options will be discussed with the participant.

In the event of unusual or life-threatening complications, treating investigators must immediately notify the Overall PI, Dr. Suzanne George, MD.

Confirmation of Progressive Disease

Pembrolizumab, like other immunotherapeutic agents, may produce antitumor effects by potentiating endogenous cancer-specific immune responses. The response patterns seen with such an approach may extend beyond the typical time course of image responses seen with cytotoxic agents, and can manifest as a clinical response after an initial increase in tumor burden or even the appearance of new lesions.

For any participant who shows first radiologic evidence of progressive disease (PD) by RECIST1.1 (see Section 11.1.4) and is deemed clinically stable, it is at the discretion of the investigator to continue treating the participant until progression is confirmed at least 4 weeks from the date of the first radiologic evidence of PD. If progression is confirmed, the participant will be discontinued from study treatment. If progression is not confirmed on the subsequent scan, the participant should continue to receive study treatment and have radiographic scans performed every 6 weeks if the participant has been on study for less than 24 weeks, or every 9 weeks for participants who have been on study greater than 24 weeks to monitor disease status. Any participant who had initial radiologic progression and is deemed clinically unstable should be discontinued from both study drugs and no subsequent scan for confirmation is required. Exceptions may be considered to continue treatment in the presence of clinically stable or improved condition only after consultation with the PI.

For purposes of PFS assessment on this trial, in addition to radiographic assessment of tumor response or progression, the investigator should take into account the clinical condition/stability of participants. Clinically stable is defined by the following criteria:

- Absence of signs and symptoms (including worsening of laboratory values) indicating disease progression

- No decline in ECOG performance status
- Absence of rapid progression of disease
- Absence of progressive tumor(s) at critical anatomical sites (eg, cord compression) requiring urgent alternative medical intervention

5.6 Duration of Follow-Up

Participants will follow-up at approximately 4 weeks for an end of study clinic visit after the last dose of study drug whenever possible. If an in-person visit is not possible, the participant will be contacted by phone. Immune mediated event surveillance will be conducted approximately 100 days after the last dose of study treatment via telephone. Otherwise, ongoing long-term follow-up will be accomplished by phone for all participants. Participants will be followed approximately every three months until death.

In addition to the follow-up schedule above, participants removed from protocol therapy for unacceptable adverse event(s) will be followed until resolution or stabilization of the adverse event.

In participants who come off trial for a reason other than progressive disease, if possible during the follow-up period, scans should be performed every 8 weeks or 2 months to evaluate for disease progression, until the participant has disease progression or dies or starts a new systemic therapy.

NOTE: With Protocol Version 15MAY2024, further long-term follow-up is being terminated.

5.7 Criteria for Taking a Participant Off-Study

Participants will be removed from study when any of the following criteria apply:

- Lost to follow-up
- Withdrawal of consent for data submission
- Death
- Inability to follow study protocol as listed in section 5.6.

The reason for taking a participant off study and the date the participant was removed must be documented in the CRF.

For Decentralized Participant Registrations, the research team updates the relevant Off Treatment/Off Study information in OnCore.

5.8 Criteria to Resume Treatment after a pembrolizumab related iAE

Management of adverse events is summarized in section 6 and may include holding of pembrolizumab, and/or the use of steroids for the management of immune related AEs. If pembrolizumab is held for toxicity, participants are permitted to continue protocol therapy with eribulin. Eribulin can be continued during corticosteroid treatment.

If drug administration is held until the next scheduled time point, please continue to count weeks/doses. If participants must be delayed due to toxicity, all assessments can be obtained with the modified treatment dose schedule as noted in this section.

Pembrolizumab may be interrupted for situations other than treatment-related AEs such as medical / surgical events or logistical reasons not related to study therapy. Participants should be placed back on study therapy within 8 weeks of the scheduled interruption, unless otherwise discussed with the Sponsor. The reason for interruption should be documented in the participant's study record.

Restarting pembrolizumab may be considered in participants who experience grade 2 events and some grade 3 events (skin rash and thyroiditis). Because of this, stopping treatment and starting steroids earlier, as per section 6, to obtain resolution with the possibility for restarting rather than waiting for higher grade events is encouraged.

For non-autoimmune or non-inflammatory events, participants may resume treatment with study drug when the drug-related AE(s) resolves to Grade ≤1 or the baseline value, with the following exceptions:

- Evaluation to exclude any additional immune mediated events (endocrine, GI, and liver / pancreas function) as clinically indicated must be made prior to restarting.
- Non-drug-related toxicity including hepatic, pulmonary toxicity, diarrhea, or colitis, must have resolved to baseline before treatment is resumed. If the criteria to resume treatment are met, the participant should restart treatment no sooner than the next scheduled time point per protocol. However, if the treatment is delayed past the next scheduled time point per protocol, the treatment should resume at the earliest convenient point that is within the five-week delay period.

For participants treated with corticosteroids:

For participants treated with high dose steroids with pembrolizumab, toxicity must resolve to baseline within 8 weeks of treatment.

Participants should be off steroids for at least 2 weeks with no recurrence or new events. New immune related events or exacerbation of existing events during steroid treatment or taper suggest the presence of ongoing immune activation and should require permanent discontinuation of pembrolizumab.

Grade 2 events must resolve to grade < 1 or baseline before considering retreatment.

All participants treated with steroids for grade ≥2 events should have pembrolizumab held until resolution to grade < 1 for at least 2 weeks following complete removal from steroid treatment except for maintenance replacement doses for adrenal insufficiency (preferably no greater than 10mg prednisone equivalent daily).

All participants treated with steroids for grade ≥3 events should have pembrolizumab discontinued. Participants with grade 3 thyroiditis and skin rash may continue therapy as for grade 2 events with resolution and stable replacement treatment.

Participants with hepatitis, pancreatitis, pneumonitis, and colitis are at risk for exacerbation with retreatment if there is residual inflammation and should resolve to Grade 0 or baseline before retreatment. Baseline can mean the initial grade *i.e.* grade ≤1 where permitted on study.

Participants with thyroiditis or hypopituitarism who are stable as above may be restarted with replacement hormones including thyroid hormone and physiologic doses only of corticosteroids. Please note that hypophysitis with symptoms of headache, visual or neurologic changes or radiologic evidence of pituitary enlargement and other CNS events such as aseptic meningitis or encephalitis should be considered grade 3 events.

Prior to starting corticosteroids or hormone replacement for any reason, appropriate endocrine testing including cortisol, ACTH, TSH and T4 must be drawn if clinically feasible to document baseline function and distinguish the pituitary from peripheral organ dysfunction and later from steroid (or thyroid) treatment associated ACTH (or TSH) suppression. Steroids should be started prior to obtaining results based on clinical indications.

5.9 Treatment of Pembrolizumab Related Infusion Reactions

Since pembrolizumab contains only human immunoglobulin protein sequences, it is unlikely to be immunogenic and induce infusion or hypersensitivity reactions. However, in rare cases, Pembrolizumab may cause severe or life-threatening infusion-reactions including severe hypersensitivity or anaphylaxis. Signs and symptoms usually develop during or shortly after drug infusion and generally resolve completely within 24 hours of completion of infusion. However, if such a reaction were to occur, it might manifest with fever, chills, rigors, headache, rash, urticaria, angioedema, pruritus, arthralgias, hypo- or hypertension, bronchospasm, or other symptoms.

All Grade 3 or 4 infusion reactions should be reported as an SAE if criteria are met. Infusion reactions should be graded according to NCI CTCAE version 5.0 guidelines.

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

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

Remain at bedside and monitor participant until recovery from symptoms. Infusion rate may be slowed. If the infusion is interrupted, then restart the infusion at 50% of the original infusion rate when symptoms resolve; if no further complications ensue after 30 minutes, the rate may be increased to 100% of the original infusion rate. Monitor participant closely.

The following prophylactic pre-medications are recommended for future infusions:

diphenhydramine 50 mg (or equivalent) and/or paracetamol 325 to 1000 mg (acetaminophen) at least 30 minutes before additional pembrolizumab administrations, slowing infusion rate as above.

For Grade 2 symptoms: (Moderate reaction requires therapy or infusion interruption but responds promptly to symptomatic treatment [*e.g.*, antihistamines, non-steroidal anti-inflammatory drugs, narcotics, corticosteroids, bronchodilators, IV fluids]; close observation for recurrence and treatment medications may need to be continued for 24-48 hours).

Stop the pembrolizumab infusion, begin an IV infusion of normal saline, and treat the participant with diphenhydramine 50 mg IV (or equivalent) and/or paracetamol 325 to 1000 mg (acetaminophen); remain at bedside and monitor participant until resolution of symptoms. Corticosteroid or bronchodilator therapy may also be administered as appropriate. If symptoms resolve within 1 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 participant should be premedicated for the next scheduled dose. If the infusion was interrupted, then restart the infusion at 50% of the original infusion rate when symptoms resolve; if no further complications ensue after 30 minutes, the rate may be increased to 100% of the original infusion rate. Monitor participant closely. If symptoms recur, then no further pembrolizumab will be administered at that visit. Administer diphenhydramine 50 mg IV, and remain at bedside and monitor the participant until resolution of symptoms. The amount of study drug infused must be recorded on the electronic case report form (eCRF). The following prophylactic pre-medications are recommended for future infusions: diphenhydramine 50 mg (or equivalent) and (acetaminophen) (or paracetamol) 325 to 1000 mg should be administered at least 30 minutes before additional pembrolizumab administrations. If necessary, corticosteroids (recommended dose: up to 25 mg of IV hydrocortisone or equivalent) may be used.

For Grade 3 or Grade 4 symptoms:
Grade 3 symptoms: (Severe reaction; 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 symptoms: (life threatening; pressor or ventilatory support indicated).

Immediately discontinue infusion of pembrolizumab. Begin an IV infusion of normal saline, and treat the participant as follows: Recommend bronchodilators, epinephrine 0.2 to 1 mg of a 1:1,000 solution for subcutaneous administration or 0.1 to 0.25 mg of a 1:10,000 solution injected slowly for IV administration, and/or diphenhydramine 50 mg IV with methylprednisolone 100 mg IV (or equivalent), as needed. Participant should be monitored until the investigator is comfortable that the symptoms will not recur. Pembrolizumab will be permanently discontinued. Investigators should follow their institutional guidelines for the treatment of anaphylaxis. Remain at bedside and monitor participant until recovery from symptoms. In the case of late-occurring hypersensitivity symptoms (*e.g.*, appearance of a localized or generalized pruritus within 1 week after treatment), symptomatic treatment may be given (*e.g.*, oral antihistamine, or corticosteroids).

Please note that late occurring events including isolated fever and fatigue may represent the presentation of systemic inflammation. Please evaluate accordingly.

5.10 Treatment Beyond Progression

A minority of participants treated with immunotherapy may derive clinical benefit as either delayed responses, stable disease, or increased overall survival despite initial evidence of progressive disease (PD) with pembrolizumab and eribulin.

Participants may be permitted to continue treatment beyond initial RECIST 1.1-defined PD that occurs during the initial (24 weeks) of treatment as long as they meet the following criteria:

- No more than 4 new lesions, total sum of the longest diameter (SHORT diameter for LN) cannot exceed 40% of the initial sum including new lesions
- Participants must be clinically stable with no change in performance status due to disease progression
- No indication for immediate alternative treatment, however, palliative radiation to isolated symptomatic lesions is allowed
- Participant [assessed by the investigator] is showing clinical benefit and tolerates study drug. The assessment of clinical benefit should take into account whether the participant is clinically stable or deteriorating and likely or unlikely to receive further benefit from continued treatment.
- The time of progression is noted from the first assessment that exceeds standard criteria

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

5.11 Lifestyle Restrictions

5.11.1 Meals and Dietary Restrictions

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

5.11.2 Contraception

Pembrolizumab or Eribulin may have adverse effects on a fetus in utero. Refer to Appendix G for approved methods of contraception.

For this study, male participants will be considered to be of non-reproductive potential if they have azoospermia (whether due to having had a vasectomy or due to an underlying medical condition).

5.11.3 Pregnancy

If a participant inadvertently becomes pregnant while on treatment with pembrolizumab or Eribulin, the participant will be immediately discontinued from study treatment. The site will contact the participant at least monthly and document the participant's status until the pregnancy has been completed or terminated. The outcome of the pregnancy will be reported to Merck within 2 working days if the outcome is a serious adverse experience (eg, 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 Merck and Eisai. If a male participant impregnates his female partner, the study personnel at the site must be informed immediately and the pregnancy must be reported to Merck and followed as described in Section 7.6.

5.11.4 Use in Nursing Women

It is unknown whether pembrolizumab is excreted in human milk. Eribulin may be 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, participants who are breast-feeding are not eligible for enrollment.

6. DOSING DELAYS/DOSE MODIFICATIONS

Dose delays and modifications will be made as indicated in the following table(s). The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 will be utilized for dose delays and dose modifications. A copy of the CTCAE version 5.0 can be downloaded from the CTEP website http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

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, participant vacation, and/or holidays). Participants should be placed back on study therapy within 5 weeks of the scheduled interruption, unless otherwise discussed with the Sponsor. The reason for interruption should be documented in the participant's study record.

Dose modifications and delays for eribulin will be allowed and are described below. No re-escalation will be allowed for eribulin. Dose delays for pembrolizumab for adverse events are described below. There are no dose reductions of pembrolizumab allowed. See Section 5.9 for guidelines regarding resumption of pembrolizumab following dose delays.

For toxicities in this Section which are attributable to pembrolizumab alone, only pembrolizumab should be held as directed. It is permissible to continue eribulin despite discontinuation of pembrolizumab in these select cases. This will be left to the treating physician's discretion after discussion and approval of the study PI.

For toxicities in this Section which are attributable to eribulin alone, only eribulin should be held

as directed. It is permissible to continue pembrolizumab despite discontinuation of eribulin in these select cases. This will be left to the treating physician's discretion after discussion and approval by the study PI.

6.1 Eribulin dosing delays/dose modifications

Participants should be carefully monitored for toxicity. If a treatment delay is required for eribulin toxicity or if treatment with eribulin is withheld permanently, treatment with pembrolizumab is allowed. Dosing levels for eribulin are shown in Table 2. Eribulin toxicities should be managed according to the guidelines in Tables 3.1 through 3.5. Once reduced, the dose of eribulin will not be re-escalated.

Clinical judgement should be used to determine appropriate management of the patient during any adverse event. Temporary interruption, dose reduction or permanent discontinuation of Eribulin should be considered if clinically indicated.

Table 2. Eribulin dosing levels

Dose Level	*Eribulin* Dose
Starting dose	*1.4mg/m^2 (1 cycle = 21 days)*
1st dose reduction	*1.1 mg/m^2*
2nd dose reduction	*0.7 mg/m^2*

- Toxicities graded in accordance with National Cancer Institute Common Toxicity Criteria for AEs, version 5.0
- Discontinuation of eribulin is required if a dose reduction to less than 0.7 mg/m^2 is required.
- A minimum of 6 days between Day 1 and Day 8 of eribulin administration will be required. Day 8 administration of Eribulin must not be given prior to Day 8. A minimum of 13 days between Day 8 and Day 1 of next cycle will be required.
- If a participant does not meet criteria to receive Day 8 of eribulin, the Day 8 dose should be skipped (i.e., it will not be made up on Day 15) for that cycle.
- Once a dose is reduced, it should not be re-escalated.
- Treatment with eribulin will be discontinued if participants require a delay greater than 5 weeks.
- QTc prolongation has been observed in patients receiving eribulin. Cardiac monitoring, including EKG, will be performed per institutional standard of care.

Table 3.1 Eribulin Diarrhea Toxicity Dose Modification

Diarrhea	**Management/Next Dose for Eribulin**
≤ Grade 2	No dose modification

Diarrhea	**Management/Next Dose for Eribulin**
Grade 3, first occurrence	Hold eribulin until recovery to grade < 2. Maximize supportive care measures. If symptom recovery occurs within 1 week, then eribulin may be resumed at the same dose if deemed appropriate by the investigator, otherwise, reduce by 1 dose level.
Grade 3, despite maximal supportive measures	Hold eribulin until recovery to grade < 2 then reduce by 1 dose level.
Grade 4	Hold eribulin until recovery to grade < 2 then reduce by 1 dose level.

Table 3.2 Eribulin Peripheral Neuropathy Dose Modification

Peripheral Neuropathy	**Management/Next Dose for Eribulin**
≤ Grade 1	No dose modification
Grade 2	For intolerable (as determined by physician and participant) grade 2, decrease eribulin by one dose level.
Grade 3	Hold eribulin until recovery to grade < 2 then reduce by 1 dose level.
Grade 4	Permanently discontinue eribulin.

Table 3.3 Eribulin Neutropenia Dose Modification

Neutropenia	**Management/Next Dose for Eribulin**
ANC < 500 cells/mm3 lasting > 7 days with or without use of growth factors	Hold eribulin until recovery to grade < 2 and reduce by 1 dose level. Prophylactic growth factor support should be instituted for subsequent cycles.
ANC < 500 cells/mm^3 lasting ≤7 days without use of growth factors	Hold eribulin until recovery to grade < 2. Resume eribulin at the same dose. Growth factor support should be provided.
ANC < 500 cells/mm^3 lasting ≤ 7 days in despite use of growth factors	Hold eribulin until recovery to grade < 2, and reduce by 1 dose level. Continue ongoing prophylactic growth factor support for subsequent cycles.
ANC <1000 /mm^3 with fever or infection without use of growth factors	Hold eribulin until recovery to grade < 2. Resume eribulin at the same dose. Growth factor support should be provided.
ANC <1000 /mm^3 with fever or infection despite use of growth factors	Hold eribulin until recovery to grade < 2, and reduce by 1 dose level. Growth factor support should be provided.
ANC <1000 /mm3 without fever or infection	First occurrence: Hold eribulin until recovery to grade < 2 then resume at the same dose. Growth factor support should be provided. If uncomplicated neutropenia (<1000 /mm3 without fever or infection) occurs/recurs despite growth factor support, then hold eribulin until recovery to grade < 2. Eribulin may be then be resumed at the same dose with growth factor support, or it may be reduced by one dose level. If a participant misses two consecutive Day 8 eribulin doses due to uncomplicated neutropenia, then hold eribulin until

Neutropenia	**Management/Next Dose for Eribulin**
	recovery to grade < 2, reduce by 1 dose level, and consider prophylactic growth factor support for subsequent cycles.
ANC = absolute neutrophil count	

Table 3.4 Eribulin Thrombocytopenia Dose Modification

Thrombocytopenia	**Management/Next Dose for Eribulin**
≤ Grade 1	No dose modification
Grade 2	Hold eribulin until recovery to grade < 2.
Grade 3	Hold eribulin until recovery to grade < 2 then reduce by 1 dose level.
Grade 4	Hold eribulin until recovery to grade < 2 then reduce by 1 dose level.

Table 3.5 Eribulin Anemia Dose Modification

Anemia	**Management/Next Dose for Eribulin**
≤ Grade 1	No dose modification
Grade 2	No dose modification
Grade 3	No dose modification
Grade 4	Hold eribulin until recovery to grade < 2 then reduce by 1 dose level. PRBC transfusions are permitted to treat anemia on protocol.

6.2 Pembrolizumab dosing delays

Participants should be carefully monitored for toxicity. If a treatment delay is required for pembrolizumab, treatment with Eribulin is allowed. Guidelines for dose interruption recommendations for pembrolizumab are shown in Table 4. If treatment with pembrolizumab is withheld permanently, participants are allowed to continue to receive eribulin therapy.Evaluation of possible AEs should occur early, with early withholding of drug, and appropriate treatment as indicated in the management tables and following event specific guidelines. In some cases, pembrolizumab may be resumed as per section 5.9

AEs associated with pembrolizumab exposure may represent an immunologic etiology. These immune-related AEs (irAEs) may occur shortly after the first dose or several months after the last dose of pembrolizumab treatment and may affect more than one body system simultaneously. Therefore, early recognition and initiation of treatment is critical to reduce complications. Based on existing clinical study data, most irAEs were reversible and could be managed with interruptions of pembrolizumab, administration of corticosteroids, and/or other supportive care. For suspected irAEs, ensure adequate evaluation to confirm etiology or exclude other causes. Additional procedures or tests such as bronchoscopy, endoscopy, skin biopsy may be included as part of the evaluation. Based on the severity of irAEs, withhold or permanently discontinue pembrolizumab and administer corticosteroids. Dose interruption and toxicity management guidelines for irAEs associated with pembrolizumab are provided in Table 4.1 to Table 4.9.

Table 4.1 Guidelines for dose interruption 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 (CTCAEv5.0)	Action taken to pembrolizumab	irAE management with corticosteroid and/or other therapies	Monitor and follow-up
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 infusion.
	Grade 4	Permanently discontinue		
AST and ALT elevation or 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	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. **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).				

Table 4.2 Pembrolizumab dose delay for non-immunologically mediated adverse events

ALL OTHER EVENTS*	**Management/Next Dose for Pembrolizumab**
≤ Grade 1	No change in dose
Grade 2	Hold until ≤ Grade 1 OR baseline (exceptions as noted below); Evaluate and continue at investigator discretion
Grade 3	Stop pembrolizumab (exceptions as noted below)
Grade 4	Stop pembrolizumab
*Not Pembrolizumab related, or pembrolizumab related non-immunologically mediated Recommended management: As clinically indicated	

- Any grade 2 drug-related uveitis or eye pain or blurred vision that does not respond to topical therapy and does not improve to Grade 1 severity within the re-treatment period OR requires systemic treatment should result in protocol treatment discontinuation.

- Any adverse event, laboratory abnormality, or intercurrent illness which, in the judgment of the investigator, presents a substantial clinical risk to the participant with continued study drug dosing should result in protocol treatment discontinuation.

- Tumor pain or associated tumor flare does not require permanent discontinuation.

- Participants with any grade < 2 laboratory abnormality may continue study treatment at the discretion of the investigator. Any grade 3 or 4 drug-related laboratory abnormality or electrolyte abnormality, that can be managed independently from underlying organ pathology with electrolyte replacement, hormone replacement, insulin, or that does not require treatment **does not** require discontinuation.

- Expected hematologic AEs which are related to eribulin will be managed per eribulin dose modification tables and do not require delay of pembrolizumab, see section 6.1

Table 4.3 Pembrolizumab dose delay for skin rash and oral lesions

Skin Rash and Oral Lesions	**Management/Next Dose for Pembrolizumab**
≤ Grade 1	No change in dose; * evaluate and continue at investigator discretion
Grade 2	Hold* until ≤ Grade 1 or resolved. Evaluate and continue at investigator discretion

Grade 3	Hold* until ≤ Grade 1; Evaluate and resume at investigator discretion at same dose level
Grade 4	Stop pembrolizumab.
*Participants with purpuric or bullous lesions must be evaluated for vasculitis, Steven-Johnson syndrome, TEN, and autoimmune bullous disease including oral lesions of bullous pemphigus/pemphigoid. Pruritus may occur with or without skin rash and should be treated symptomatically if there is no associated liver or GI toxicity. Note skin rash typically occurs early and may be followed by additional events particularly during steroids tapering.	

Table 4.4 Pembrolizumab dose delay for pancreatitis

Pancreatitis Amylase/Lipase	**Management/Next Dose for Pembrolizumab**
≤ Grade 1	Continue treatment at same level at investigator discretion.
Grade 2	Continue treatment at same level at investigator discretion if asymptomatic.
Grade 3	Hold until Grade< 2. Resume at same dose level if asymptomatic. Participants who develop symptomatic pancreatitis or DM should stop pembrolizumab. .
Grade 4	Hold until Grade< 2. Resume at same dose level if asymptomatic. Participants who develop symptomatic pancreatitis or DM should stop pembrolizumab. .
Participants may develop symptomatic and radiologic evidence of pancreatitis as well as DM and DKA. Lipase elevation may occur during the period of steroid withdrawal and with other immune mediated events or associated with colitis, hepatitis, and participants who have asymptomatic lipase elevation typically have self-limited course and may be retreated. For treatment management of symptomatic pancreatitis please follow the Hepatic Adverse Event Management Algorithm.	

Table 4.5 Pembrolizumab dose delay for nausea and vomiting

Other GI Nausea, Vomiting	**Management/Next Dose for Pembrolizumab**
≤ Grade 1	No change in dose.
Grade 2	Hold pending evaluation for gastritis duodenitis and other immune adverse events or other causes. Resume at investigator discretion.
Grade 3	Hold pending evaluation until ≤ Grade 2. Resume at same dose level. If symptoms do not resolve within 7 days with symptomatic treatment participants should stop pembrolizumab.
Grade 4	Stop pembrolizumab
Participants with grade 2 or 3 N-V should be evaluated for upper GI inflammation and other immune related events.	

Table 4.6 Pembrolizumab dose delay for fatigue

Fatigue	**Management/Next Dose for Pembrolizumab**
≤ Grade 1	No change in dose.
Grade 2	No change in dose.
Grade 3	Hold until ≤ Grade 2. Resume at same dose level at investigator discretion.
Grade 4	Stop pembrolizumab
Fatigue is the most common adverse event associated with immune checkpoint therapy. Grade 2 or greater fatigue should be evaluated for associated or underlying organ involvement including pituitary, thyroid, and hepatic, or muscle (CPK) inflammation	

Table 4.7 Pembrolizumab dose delay for neurological events

Neurologic events*	**Management/Next Dose for Pembrolizumab**
≤ Grade 1	Hold dose pending evaluation and observation. Resume with no change in dose when resolved to baseline at investigator discretion
Grade 2	Hold dose pending evaluation and observation. Hold until ≤ Grade 1. Stop pembrolizumab if treatment with steroids is required. Resume at same dose level for peripheral isolated n. VII (Bell's palsy)
Grade 3	Stop pembrolizumab
Grade 4	Stop Pembrolizumab
*Participants with any CNS events including aseptic meningitis, encephalitis, symptomatic hypophysitis, or myopathy, peripheral demyelinating neuropathy, cranial neuropathy (other than peripheral n. VII), GB syndrome, myasthenia gravis should be off study.	

Table 4.8 Pembrolizumab dose delay for infusion reactions

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 participant is deemed medically stable in the opinion of the investigator.	None
Grade 2 Requires therapy or infusion interruption but responds promptly to symptomatic treatment (e.g., antihistamines, NSAIDs, narcotics, IV fluids); prophylactic medications indicated for ≤24 hrs	**Stop Infusion.** 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 participant is deemed medically stable in the opinion of the investigator. If symptoms resolve within 1 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 participant should be premedicated for the next scheduled dose. **Participants who develop Grade 2 toxicity despite adequate premedication should be permanently discontinued from further study drug treatment**	Participant may be premedicated 1.5h (± 30 minutes) prior to infusion of _____ with: Diphenhydramine 50 mg po (or equivalent dose of antihistamine). Acetaminophen 500-1000 mg po (or equivalent dose of analgesic).
Grades 3 or 4 Grade 3: Prolonged (i.e., not rapidly responsive to symptomatic medication and/or brief interruption of infusion); recurrence of symptoms following initial improvement; hospitalization indicated for other clinical sequelae (e.g., renal impairment, pulmonary infiltrates) Grade 4: Life-threatening; pressor or ventilatory support indicated	**Stop Infusion.** Additional appropriate medical therapy may include but is not limited to: Epinephrine** IV fluids Antihistamines NSAIDs Acetaminophen Narcotics Oxygen Pressors Corticosteroids Increase monitoring of vital signs as medically indicated until the participant is deemed medically stable in the opinion of the investigator. Hospitalization may be indicated. **In cases of anaphylaxis, epinephrine should be used immediately. **Participant is permanently discontinued from further study drug treatment.**	No subsequent dosing
Appropriate resuscitation equipment should be available at the bedside and a physician readily available during the period of drug administration. For further information, please refer to the Common Terminology Criteria for Adverse Events v5.0 (CTCAE) at http://ctep.cancer.gov		

Table 4.9 Pembrolizumab dose delay for fever

Fever	Management/Next Dose for Pembrolizumab
≤ Grade 1	Evaluate and continue at same dose level
Grade 2	Continue at investigator discretion
Grade 3	Hold until ≤ Grade 1. Resume at same dose level at investigator discretion.
Grade 4	Stop pembrolizumab
Participants with fever should be evaluated as clinically appropriate. Participants may experience isolated fever during infusion reactions or up to several days after infusion. Evaluation over the course of 1-2 weeks should be done for other autoimmune events that may present as fever	
See section 5.10: Treatment of Pembrolizumab Infusion reactions	

If treatment is delayed >8 weeks for an adverse event, the participant must be permanently discontinued from study therapy.

Participants requiring high dose steroid treatment for autoimmune or inflammatory events should be managed as described in section 5.9, except for a short course of tapering steroids for infusion reaction, skin rash, or endocrine events.

Participants with grade 3 thyroiditis and skin rash may continue therapy as for grade 2 events with resolution and stable replacement treatment.

Participants with thyroiditis or hypopituitarism who are stable as above may be restarted with replacement hormones including thyroid hormone and physiologic doses of corticosteroids. Please note that grade for hypophysitis with symptoms of headache, visual, or neurologic changes or radiologic evidence of pituitary enlargement and other CNS events such as aseptic meningitis or encephalitis should be considered grade 3 events.

Any participant who requires additional immune suppressive treatment beyond steroids should stop pembrolizumab.

Participants requiring > two dose delays, other than for evaluation for the same event, should stop pembrolizumab. Participants may be dose-delayed for evaluation and restarted depending on results.

Prior to starting corticosteroids or hormone replacement for any reason, appropriate endocrine testing including cortisol, ACTH, TSH and T4 must be obtained to document baseline.

Please note that in some cases the treatment algorithms recommend steroids if symptoms do not resolve in 7 days. However, this recommendation is not meant to delay steroid treatment at any time it is clinically indicated.

Any participant started on corticosteroids initially who is determined to not require steroids treatment for an autoimmune adverse event may resume therapy after a 2-week observation period without further symptoms at the discretion of the PI or investigator.

7. ADVERSE EVENTS: LIST AND REPORTING REQUIREMENTS

Adverse event (AE) monitoring and reporting is a routine part of every clinical trial. The following list of reported and/or potential AEs (Section 7.1) and the characteristics of an observed AE (Section 7.2) will determine whether the event requires expedited reporting **in addition** to routine reporting.

7.1 Expected Toxicities

7.1.1 Adverse Events Lists

7.1.1.1 Adverse Event Lists for Pembrolizumab

In the pembrolizumab monotherapy trials (P001/P002, P012, P013, and P028, plus the P011 monotherapy arm), the overall incidence of AEs ranged from 83.0% (73 of 88 participants in P012) to 100% (10 of 10 participants in P011). Not all the adverse events in the following list are all-inclusive and refer to the Pembrolizumab investigator brochure for a complete list.[48] The most commonly reported AEs included fatigue, diarrhea, decreased appetite, nausea, and anemia. The incidence of drug-related AEs (DRAEs) ranged from 39.8% (35 of 88 participants in P013) to 80.0% (8 of 10 participants in P011). The most commonly reported DRAEs across all studies were nausea, fatigue, and diarrhea. The incidence of Grade 3-5 DRAEs across studies ranged from 6.8% (6 of 88 in P013) to 12.0% (187 of 1562 participants) in P001/P002. The most commonly reported Grade 3-5 DRAEs were anemia, alanine aminotransferase increased, and aspartate aminotransferase increased. Most participants who experienced an AE continued in the study, with the incidence of AEs leading to discontinuation ranging from 1.9% (8 of 430 participants in P028) to 12.3% (192 of 1562 participants in P001/P002). The majority of AEs leading to discontinuation were not considered drug-related. Discontinuations due to a DRAE were infrequent and ranged from 0% (no participants in P011) to 4.5% (4 of 88 participants in P013). The most commonly reported DRAEs leading to discontinuation were pneumonitis, alanine aminotransferase increased, and aspartate aminotransferase increased.

List of AEs considered expected:

- Endocrine disorders: Adrenal insufficiency, Hyperthyroidism, Hypophysitis, Hypopituitarism, Hypothyroidism, Secondary adrenal insufficiency, Thyroid disorder
- Eye disorders: Uveitis
- Gastrointestinal disorders: Abdominal pain, Colitis, Diarrhea, Pancreatitis
- General disorders and administration site conditions: Asthenia, Pyrexia

- Hepatobiliary disorders: Autoimmune hepatitis, Hepatitis
- Infusion related reaction
- Metabolism and nutrition disorders: Diabetic ketoacidosis, Hyponatremia, Type 1 diabetes mellitus
- Musculoskeletal and connective tissue disorders: Arthralgia, Back pain, Myositis
- Nervous system disorders: Guillain-Barré syndrome
- Renal and urinary disorders: Nephritis
- Respiratory, thoracic and mediastinal disorders: Cough, Pneumonitis
- Skin and subcutaneous tissue disorders: Pruritus, Rash, Severe skin reaction, Vitiligo

7.1.1.2 Adverse Event Lists for Eribulin

Not all the adverse events in the following list are all-inclusive and refer to the Eribulin investigator brochure for a complete list.[64] Most AEs on studies with eribulin are grade 1 or 2. Peripheral neuropathy was an important AE leading to discontinuation of therapy. The most common adverse reactions (≥25%) reported in participants receiving eribulin on the eribulin versus dacarbazine phase II trial in soft tissue sarcomas were fatigue, nausea, alopecia, constipation, peripheral neuropathy, abdominal pain, and pyrexia. The most common (≥5%) grade 3 to 4 laboratory abnormalities reported in participants receiving eribulin were neutropenia, hypokalemia, and hypocalcemia. The most common serious adverse reactions reported in participants receiving eribulin were neutropenia (4.9%) and pyrexia (4.5%). The most common adverse reactions resulting in discontinuation of eribulin were fatigue and thrombocytopenia. 26% of participants required at least one dose reduction. The most frequent adverse reactions that led to dose reduction were neutropenia (18%) and peripheral neuropathy (4%).

The most frequently reported eribulin -related AEs were:

- Asthenia/fatigue (all grade 62%)
- Alopecia (all grades 35%)
- Urinary Tract Infection (all grades 11%, grade 3 to 4 2.2%)
- Neutropenia (all grades 63%, grade 3 to 4 32%)
- Nausea (all grade 41%)
- Anemia(all grades 70%, grade 3 to 4 4.1%)
- Pyrexia(all grades 28%, grade 3 to 4 0.9%)
- Leucopenia
- Anorexia(all grade 19%)
- Constipation(all grades 32%, grade 3 to 4 0.9%)
- Abdominal Pain (all grades 29%, grade 3 to 4 1.8%)
- Stomatitis (all grades 14%, grade 3 to 4 0.9%)
- Vomiting(all grade 19%)
- Diarrhea (all grade 17%)

- Headache (all grades 18%, grade 3 to 4 0%)
- Peripheral Edema (all grade 12%)
- Cough (all grade 18%)
- Back Pain (all grade 16%)
- Arthralgia/Myalgia (all grade 16%)
- Peripheral neuropathy (all grades 29%, grade 3 to 4 3.1%)
- Increased ALT (all grades 43%, grade 3 to 2.3%)
- Increased AST (all grades 36%, grade 3 to 4 0.9%)
- Hypokalemia (all grades 30%, grade 3 to 4 5.4%)
- Hypocalcemia (all grades 28%, grade 3 to 4 5.0%)
- Hypophosphatemia (all grades 20%, grade 3 to 4 3.2%)

Less common adverse reactions (≥5% to < 10%) included thrombocytopenia, increased lacrimation, dyspepsia, hyperglycemia, muscle spasms, musculoskeletal pain, dizziness, dysgeusia, insomnia, anxiety, hypotension, and oropharyngeal pain.

7.2 Adverse Event Characteristics

- **CTCAE term (AE description) and grade:** The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 5.0. A copy of the CTCAE version 5.0 can be downloaded from the CTEP web site http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

- **For expedited reporting purposes, only:**
  - AEs for the agent(s) that are listed above should be reported only if the adverse event varies in nature, intensity or frequency from the expected toxicity information which is provided.
  - Other AEs for the protocol that do not require expedited reporting are outlined in the next section (Expedited Adverse Event Reporting) under the sub-heading of Protocol-Specific Expedited Adverse Event Reporting Exclusions.

- **Attribution** of the AE:
  - Definite – The AE *is clearly related* to the study treatment.
  - Probable – The AE *is likely related* to the study treatment.
  - Possible – The AE *may be related* to the study treatment.
  - Unlikely – The AE *is doubtfully related* to the study treatment.
  - Unrelated – The AE *is clearly NOT related* to the study treatment.

7.3 Expedited Adverse Event Reporting

7.3.1 In the event of an unanticipated problem or life-threatening complications treating investigators must immediately notify the Overall PI.

7.3.2 Investigators **must** report to the Overall PI any serious adverse event (SAE) that occurs after the initial dose of study treatment, during treatment, or within 30 days of the last dose of treatment on the local institutional SAE form.

7.3.3 DF/HCC Expedited Reporting Guidelines

Investigative sites within DF/HCC will report AEs directly to the DFCI Office for Human Research Studies (OHRS) per the DFCI IRB reporting policy.

Table. 5 DF/HCC Reportable AEs

Attribution	DF/HCC Reportable AEs				
	Gr. 2 & 3 AE Expected	**Gr. 2 & 3 AE Unexpected**	**Gr. 4 AE Expected**	**Gr. 4 AE Unexpected**	**Gr. 5 AE Expected or Unexpected**
Unrelated Unlikely	Not required	Not required	5 calendar days#	5 calendar days	24 hours*
Possible Probable Definite	Not required	5 calendar days	5 calendar days#	5 calendar days	24 hours*
# If listed in protocol as expected and not requiring expedited reporting, event does not need to be reported.					
* For participants enrolled and actively participating in the study ***or*** for AEs occurring within 30 days of the last intervention, the AE should be reported within 1 business day of learning of the event.					

7.3.4 Protocol-Specific Expedited Adverse Event Reporting Exclusions

No expedited adverse event reporting exclusions.

7.4 Expedited Reporting to the Food and Drug Administration (FDA)

The Overall PI, as study sponsor, will be responsible for all communications with the FDA. The Overall PI will report to the FDA, regardless of the site of occurrence, any serious adverse event that meets the FDA's criteria for expedited reporting following the reporting requirements and timelines set by the FDA.

7.5 Expedited Reporting to Hospital Risk Management

Participating investigators will report to their local Risk Management office any participant safety reports or sentinel events that require reporting according to institutional policy.

7.6 Expedited Reporting to MERCK

A serious adverse event (SAE) is any adverse event occurring at any dose or during any use of Merck's product that:

- Results in death;
- Is life threatening;
- Results in persistent or significant disability/incapacity;
- Results in or prolongs an existing inpatient hospitalization;
- Is a congenital anomaly/birth defect;
- Is a new cancer (that is not a condition of the study);
- Is associated with an overdose;
- Is another important medical event

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 participant 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.

Non-serious Events of Clinical Interest will be forwarded to Merck Global Safety and will be handled in the same manner as SAEs.

Additionally, any serious adverse event, considered by an investigator who is a qualified physician to be related to 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.

SAE reports and any other relevant safety information are to be forwarded to the Merck Global Safety.

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. at the time of submission to FDA.

All participants with serious adverse events must be followed up for outcome.

7.6.1 Events of Clinical Interest (ECIs)

Selected non-serious and serious adverse events are also known as Events of Clinical Interest (ECI) and must be reported within 24 hours to the Sponsor and within 2 working days to Merck Global Safety.

For the time period beginning when the consent form is signed until treatment allocation/randomization, any ECI, or follow up to an ECI, that occurs to any participant must be reported within 24 hours to the Sponsor and within 2 working days to Merck Global Safety if it causes the participant to be excluded from the trial, or is the result of a protocol-specified intervention, including but not limited to washout or discontinuation of usual therapy, diet, placebo treatment or a procedure.

For the time period beginning at treatment allocation/randomization through 90 days following cessation of treatment, or 30 days following cessation of treatment if the participant initiates new anticancer therapy, whichever is earlier, any ECI, or follow up to an ECI, whether or not related to Merck product, must be reported within 24 hours to the Sponsor and within 24 hours to Merck Global Safety.

Events of clinical interest for this trial include:

1. Overdose of Merck product, as defined in Section 7.6.2 - Definition of an Overdose for This Protocol and Reporting of Overdose to the Sponsor, that is not associated with clinical symptoms or abnormal laboratory results.

2. An elevated AST or ALT lab value that is greater than or equal to 3X the upper limit of normal and an elevated total bilirubin lab value that is greater than or equal to 2X the upper limit of normal and, at the same time, an alkaline phosphatase lab value that is less than 2X the upper limit of normal, 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.

7.6.2 Definition of an Overdose of Pembrolizumab for This Protocol and Reporting of Overdose to Merck

For purposes of this trial, an overdose of pembrolizumab will be defined as any dose of 1,000 mg or greater. 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 participant should be observed closely for signs of toxicity.

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.”

All reports of overdose with and without an adverse event must be reported within 24 hours to the Sponsor and within 2 working days' hours to Merck Global Safety.

7.6.3 Reporting of Pregnancy and Lactation to Merck

Although pregnancy and lactation are not considered adverse events, it is the responsibility of investigators or their designees to report any pregnancy or lactation in a participant (spontaneously reported to them) that occurs during the trial.

Pregnancies and lactations that occur after the consent form is signed but before treatment allocation/randomization must be reported by the investigator if they cause the participant to be excluded from the trial, or are the result of a protocol-specified intervention, including but not limited to washout or discontinuation of usual therapy, diet, placebo treatment or a procedure.

Pregnancies and lactations that occur from the time of treatment allocation/randomization through 120 days following cessation of Sponsor's product, or 30 days following cessation of treatment if the participant initiates new anticancer therapy, whichever is earlier, must be reported by the investigator. All reported pregnancies must be followed to the completion/termination of the pregnancy. Pregnancy outcomes of spontaneous abortion, missed abortion, benign hydatidiform mole, blighted ovum, fetal death, intrauterine death, miscarriage and stillbirth must be reported as serious events (Important Medical Events). If the pregnancy continues to term, the outcome (health of infant) must also be reported.

Such events must be reported within 24 hours to the Sponsor and within 2 working days to Merck Global Safety.

7.7 Expedited Reporting to Eisai

SAEs where the Overall PI considers a relationship to the eribulin therapy to be at least a reasonable possibility, will be reported to EISAI on a Medwatch 3500A form within one business day of the notification of the event. Serious adverse events (SAEs) that are not related to eribulin therapy and non-serious AEs will be provided to Eisai in the final study report and any interim reports provided.

A serious adverse event is any adverse event occurring at any dose or during any use of Eisai's product that:

- Results in death;
- Is life threatening;
- Results in persistent or significant disability/incapacity;
- Results in or prolongs an existing inpatient hospitalization;
- Is a congenital anomaly/birth defect;
- Is a new cancer (that is not a condition of the study);
- Is associated with an overdose;
- Is another important medical event

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 participant 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 Eisai's product, must be reported within 24 hours to the Sponsor.

Additionally, any serious adverse event, considered by an investigator who is a qualified physician to be related to Eisai 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 Eisai.

Events **not** considered to be serious adverse events are hospitalizations for:

- Routine treatment or monitoring of the studied indication, not associated with any deterioration in condition, or for elective procedures
- Elective or pre-planned treatment for a pre-existing condition that did not worsen
- Emergency outpatient treatment for an event not fulfilling the serious criteria outlined above and not resulting in inpatient admission
- Respite care

The reports will be sent on MedWatch 3500A form to EISAI

7.8 Routine Adverse Event Reporting

All Adverse Events **must** be reported in routine study data submissions to the Overall PI on the toxicity case report forms. **AEs reported through expedited processes (e.g., reported to the IRB, FDA, etc.) must <u>also</u> be reported in routine study data submissions.**

8. PHARMACEUTICAL INFORMATION

A list of the adverse events and potential risks associated with the investigational or other agents administered in this study can be found in Section 7.1.

8.1 *Pembrolizumab*

8.1.1 Description

Classification: Anti-PD-1Mab, Pembrolizumab is a humanized monoclonal antibody of the IgG4/kappa isotype.

Other names: MK-3475, Keytruda.

Amino Acid Sequence: 4 polypeptide chains, which include 2 identical heavy chains and 2 identical light chains.

Mode of Action: Pembrolizumab targets the programmed death–1 (PD-1, cluster of differentiation 279 [CD279]) cell surface membrane receptor. PD-1 is a negative regulatory receptor expressed by activated T and B lymphocytes. Binding of PD-1 to its ligands, programmed death–ligand 1 (PD-L1) and 2 (PD-L2), results in the down-regulation of lymphocyte activation. Pembrolizumab inhibits the binding of PD-1 to PD-L1 and PD-L2. Inhibition of the interaction between PD-1 and its ligands promotes immune responses and antigen-specific T-cell responses to both foreign antigens as well as self-antigens.

The molecular weight of Pembrolizumab is 148.9-149.5 KDa.

The pharmacokinetics (PK) of pembrolizumab was characterized using a population PK analysis with concentration data collected from 2841 participants with various cancers who received pembrolizumab doses of 1 to 10 mg/kg every 2 weeks or 2 to 10 mg/kg every 3 weeks. Pembrolizumab clearance (CV%) is approximately 21% lower [geometric mean, 196 mL/day (41%)] at steady state than that after the first dose [249 mL/day (38%)]; this decrease in clearance with time is not considered clinically important. The geometric mean value (CV%) for volume of distribution at steady state is 6.0 L (21%) and for terminal half-life (t1/2) is 22 days (32%).

Steady-state concentrations of pembrolizumab were reached by 16 weeks of repeated dosing with an every 3-week regimen and the systemic accumulation was 2.2-fold. The peak concentration (Cmax), trough concentration (Cmin), and area under the plasma concentration versus time curve at steady state (AUCs) of pembrolizumab increased dose proportionally in the dose range of 2 to 10 mg/kg every 3 weeks.

8.1.2 Form

Clinical supplies will be manufactured and provided by Merck as summarized below.

Product Name & Potency Dosage Form
Pembrolizumab 100 mg/ 4mL (25mg/ml concentration) Solution for Injection in single-dose vials. Pembrolizumab for injection is a sterile, preservative-free, clear to slightly opalescent, colorless to slightly yellow solution that requires dilution for intravenous infusion. Each vial contains 100 mg of pembrolizumab in 4 mL of solution. Each 1 mL of solution contains 25 mg of pembrolizumab and is formulated in: L-histidine (1.55 mg), polysorbate 80 (0.2 mg), sucrose (70 mg), and Water for Injection, USP.

8.1.3 Storage and Stability

Store intact vials between 2°C-8°C (36°F-46°F). Do not freeze. Protect from light by storing in the original box.

Stability testing of the intact vials is ongoing.

Administer prepared solutions immediately after preparation. If not administered

immediately, prepared solutions may be stored refrigerated for up to 24 hours. PEMBROLIZUMAB solutions may be stored at room temperature for a cumulative time of up to 6 hours. This includes room temperature storage of liquid drug product solution in vials, room temperature storage of infusion solution in the IV bag, and the duration of infusion.

8.1.4 **Compatibility**

Compatible IV bag materials: PVC plasticized with DEHP, non-PVC (polyolefin), EVA, or PE lined polyolefin.

8.1.5 **Handling**

Qualified personnel, familiar with procedures that minimize undue exposure to themselves and the environment, should undertake the preparation, handling, and safe disposal of the chemotherapeutic agent in a self-contained and protective environment.

8.1.6 **Availability**

Pembrolizumab is FDA approved and will be supplied free of charge from Merck.

8.1.7 **Preparation**

Pembrolizumab solution for infusion must be diluted prior to administration. Allow the required number of vials to equilibrate to room temperature. Do not shake the vials. Do not use if opaque or extraneous particulate matter other than translucent to white proteinaceous particles are observed. Do not use if discolored. To prepare the infusion solution add the dose volume of Pembrolizumab to an infusion bag containing 0.9% Sodium Chloride Injection, USP of 5% Dextrose Injection, USP. Gently invert the bag 10-15 times to mix the solution. The final concentration must be between **1 mg/mL to 10 mg/mL.** Discard any unused portion left in the vial.

8.1.8 **Administration**

Pembrolizumab is FDA approved and will be administer per Institutional Standard administration guidelines.

8.1.9 **Ordering**

Pembrolizumab will be obtained directly from Merck.

8.1.10 **Accountability**

The investigator, or a responsible party designated by the investigator, should maintain a careful record of the inventory and disposition of the agent using the NCI Drug

Accountability Record Form (DARF) or another comparable drug accountability form. (See the NCI Investigator's Handbook for Procedures for Drug Accountability and Storage.)

8.1.11 Destruction and Return

At the end of the study, unused or expired supplies of Pembrolizumab should be destroyed according to institutional policies. Destruction will be documented in the Drug Accountability Record Form.

8.2 *Eribulin*

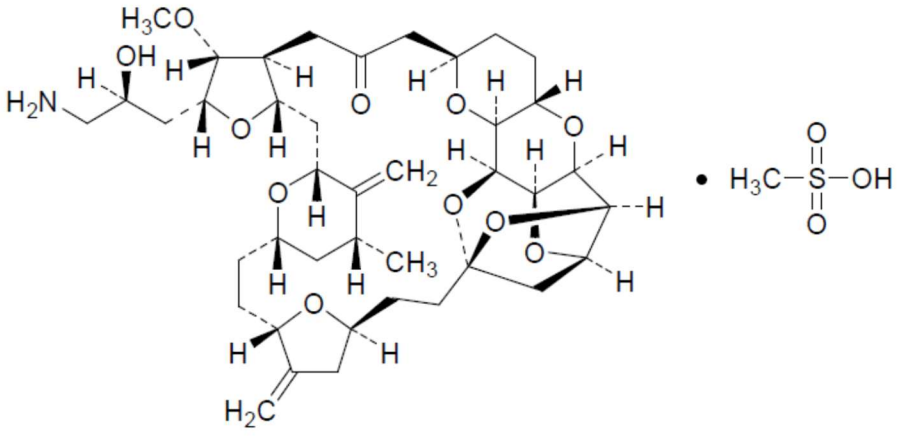
8.2.1 Description

Classification: Microtubule inhibitor

Other names: Halaven, E7389, ER-086526, US NCI designation NSC-707389

Chemical Name: The chemical name for eribulin mesylate is (2*R*,3*R*,3a*S*,7*R*,8a*S*,9*S*,10a*R*,11*S*,12*R*,13a*R*,13b*S*,15*S*,18*S*,21*S*,24*S*,26*R*,28*R*,29a*S*)-2-[(2*S*)-3-Amino-2-hydroxypropyl]-3-methoxy-26-methyl-20,27-dimethylidenehexacosahydro-11,15:18,21:24,28-triepoxy-7,9-ethano-12,15-methano-9*H*,15*H*-furo[3,2-*i*]furo[2',3':5,6]pyrano[4,3-*b*][1,4]dioxacyclopentacosin-5(4*H*)-one methanesulfonate (salt)

Chemical Structure:



Mode of Action: Eribulin is a fully synthetic macrocyclic ketone analogue of the marine natural product of halichondrin B, from the Halichondria genus of sponges, specifically *Halichondria okadai.* Eribulin inhibits the growth phase of microtubules without affecting the shortening phase and sequesters tubulin into nonproductive aggregates, by binding to a small number of high affinity sites at the plus ends of existing microtubules.

Eribulin exerts its effects via a tubulin-based antimitotic mechanism leading to G2/M cell-cycle block, disruption of mitotic spindles, and, ultimately, apoptotic cell death after prolonged mitotic blockage. Eribulin has both cytotoxic and non-cytotoxic mechanisms of action. Eribulin can cause vascular remodeling that leads to increase tumor perfusion, and phenotypic changes consistent with the reversal of the epithelial-mesenchymal transition.

Description:
The molecular weight of Eribulin is 826.00 KDa.
The molecular formula of Eribulin is $C_{41}H_{63}NO_{14}S$ ($C_{40}H_{59}NO_{11}$*CH_4O_3S)

Eribulin is a white powder. Eribulin is freely soluble in water, methanol, ethanol. Eribulin drug product is as a clear, colorless, sterile solution, packaged in a glass vial, for intravenous administration.

The pharmacokinetics (PK) of eribulin is linear with a mean elimination half-life of approximately 40 hours, a mean volume of distribution of 43 L/m2 to 114 L/m2 and mean clearance of 1.16 L/hr/m2 to 2.42 L/hr/m2 over the dose range of 0.25 mg/m2 to 4.0 mg/m2. The human plasma protein binding of eribulin at concentrations of 100 ng/mL to 1,000 ng/mL ranges from 49% to 65%. Eribulin exposure after multiple dosing is comparable to that following a single dose. No accumulation of eribulin is observed with weekly administration.

Elimination

Metabolism
Unchanged eribulin was the major circulating species in plasma following administration of 14C-eribulin to participants. Metabolite concentrations represented <0.6% of parent compound, confirming that there are no major human metabolites of eribulin. Cytochrome P450 3A4 (CYP3A4) negligibly metabolizes eribulin *in vitro*.

Excretion
Eribulin is eliminated primarily in feces unchanged. After administration of 14C-eribulin to participants, approximately 82% of the dose was eliminated in feces and 9% in urine. Unchanged eribulin accounted for approximately 88% and 91% of total eribulin in feces and urine, respectively.

Drug Interaction Studies

Effect of Strong Inhibitors or Inducers of CYP3A4 on Eribulin: The effect of a strong CYP3A4 inhibitor and a P-gp inhibitor, ketoconazole, on the PK of eribulin was studied in a crossover trial of 12 participants with advanced solid tumors. No clinically relevant PK interaction was observed when eribulin was administered with or without ketoconazole (the geometric mean ratio of the AUC: 0.97; 90% CI: 0.83, 1.12).

The effect of a CYP3A4 inducer, rifampin, on the PK of eribulin was studied in a crossover trial of 14 participants with advanced solid tumors. No clinically relevant PK interaction was observed when eribulin was administered with or without rifampin (the geometric mean ratio of the AUC: 1.10; 90 CI%: 0.91, 1.34).

Effect of Eribulin on CYP Substrates: Eribulin shows no induction potential for CYP1A, CYP2B6, CYP2C9, CYP2C19, and CYP3A in primary human hepatocytes. Eribulin inhibits CYP3A4 activity in human liver microsomes, but it is unlikely that eribulin will substantially increase the plasma levels of CYP3A4 substrates. No significant inhibition of CYP1A2, CYP2C9, CYP2C19, CYP2D6, or CYP2E1 was detected with eribulin concentrations up to 5 μM in pooled human liver microsomes. In vitro drug interaction studies indicate that eribulin does not inhibit drugs that are substrates of these enzymes and it is unlikely that eribulin will affect plasma levels of drugs that are substrates of CYP enzymes.

Effect of Transporters on Eribulin: In vitro data suggest that eribulin at clinically relevant concentrations is a substrate of P-gp, but is not a substrate of breast cancer resistance protein (BCRP), multidrug resistance proteins (MRP2, MRP4), bile salt extrusion pump (BSEP), organic anion transporting polypeptides (OATP1B1, OATP1B3), organic anion transporters (OAT1, OAT3), organic cation transporters (OCT1, OCT2), or multidrug and toxin extrusion 1 (MATE1).
Effect of Eribulin on Transporters: In vitro data suggest that eribulin at clinically relevant concentrations may inhibit P-gp, but does not inhibit BCRP, OATP1B1, OCT1, OAT1, OAT3, or MATE1.

8.2.2 **Form**

Clinical supplies will be manufactured and provided by Eisai as summarized below.

Product Name & Potency Dosage Form
Eribulin 0.5mg/ml solution in ethanol: water (5:95).

Eribulin is a sterile, ready-to-use, clear, colorless aqueous solution for i.v. administration.

Eribulin for i.v. injection will be supplied on an open-label basis by the sponsor in single-use vials. Each single-use vial contains 1 mg/2 mL (0.5mg/ml concentration) of clear, colorless solution. Each single-use vial of eribulin is primarily packaged in a 5 mL nominal volume United States Pharmacopeia (USP) Type 1 Flint glass, stoppered with a FluroTec® plug stopper and sealed with an aluminum seal and a flip-off cap. Six labeled vials of eribulin are packaged in a labeled carton. Each of the 6 eribulin vials within a given labeled carton may be assigned individually to 6 different participants or to the same participant, as required by the study site or as clinical supply inventory demands. Three to four vials of Eribulin will be required per participant for each dose on the study protocol. Exact dose determined by participant body-surface-area calculation.

8.2.3 Storage and Stability

Eribulin must be stored in accordance with labeled storage conditions. Intact and unopened vials must be stored at ambient room temperature 25ºC. **Do NOT refrigerate or freeze.** Shelf-life surveillance of the intact vials is ongoing. The 0.5 mg/mL solution has been shown to be stable in syringes at ambient temperature and ambient lighting for up to 4 hours, or under refrigeration for up to 24 hours. The drug is also stable at concentrations ranging from 0.005 ng/mL to 0.2 mg/mL when diluted in normal saline (0.9% sodium chloride [NaCl]) and kept refrigerated in syringes or i.v. bags for up to 48 hours at ambient temperature and ambient lighting, or under refrigeration. It is not compatible with solutions with dextrose. Photostability studies have demonstrated that protection from light is not necessary for the eribulin drug product.

Product vials that are opened and refrigerated between 2°C to 8°C (36°F – 46°F) must be used within 24 hours of being opened, and the remaining drug solution discarded. Temperature monitoring for eribulin is required at the storage location to ensure that the study drug is maintained within an established temperature range. The investigator is responsible for ensuring that the temperature is monitored throughout the total duration of the trial and that records are maintained; the temperature should be monitored continuously by using either an in-house validated data acquisition system, a mechanical recording device such as a calibrated chart recorder, or by manual means, such that minimum and maximum thermometric values over a specific time period can be recorded and retrieved as required.

8.2.4 Compatibility

Eribulin is not compatible with solutions with dextrose. Infused after Pembrolizumab.

8.2.5 Handling

Qualified personnel, familiar with procedures that minimize undue exposure to themselves and the environment, should undertake the preparation, handling, and safe disposal of the chemotherapeutic agent in a self-contained and protective environment.

8.2.6 Availability

Eribulin (Halaven) is FDA approved, and will be supplied free of charge from Eisai.

8.2.7 Preparation

Dosing is 1.4mg/m^2. Each single-use vial contains 1 mg/2 mL of Eribulin. Eribulin may be diluted in 100ml of normal saline.

8.2.8 **Administration**

Eribulin is FDA approved and will be administer per Institutional Standard administration guidelines.

8.2.9 **Ordering**

Eribulin will be obtained directly from Eisai.

8.2.10 **Accountability**

The investigator, or a responsible party designated by the investigator, should maintain a careful record of the inventory and disposition of the agent using the NCI Drug Accountability Record Form (DARF) or another comparable drug accountability form. (See the NCI Investigator's Handbook for Procedures for Drug Accountability and Storage.)

8.2.11 **Destruction and Return**

At the end of the study, unused or expired supplies of Eribulin should be destroyed according to institutional policies. Destruction will be documented in the Drug Accountability Record Form.

9. **BIOMARKER, CORRELATIVE, AND SPECIAL STUDIES**

All participants will be asked to provide archival tumor tissue (either paraffin blocks or 20 unstained slides, ideally 4 micron thickness) if available. If no archival tumor tissue is available, a new tumor biopsy may be performed if feasible. The feasibility of the biopsy procedure, in terms of patient safety and tumor accessibility, will be determined by the Investigator. An optional pre-treatment biopsy for fresh tumor tissue may be collected. Biopsies will be encouraged but are optional. Plan to perform optional pre-treatment biopsy for fresh tumor tissue in approximately 10 participants in each cohort (10 LPS, 10 LMS, 10 UPS), for a total of 30 participant biopsies. Archival tissue and fresh frozen tissue will be used for immune profiling assays to ascertain baseline values in participants treated with eribulin and pembrolizumab. Additionally, specimens we will be banked for planned correlative studies. Fresh tumor tissue obtained will be allocated as below:

- 1 to 2 adequate cores fixed and paraffin embedded for immunohistochemial analysis
- 1 core adequate core fixed and paraffin embedded for targeted genomic analysis via DFCI Oncopanel
- 1 core adequate core fresh frozen tissue for RNA sequencing analysis

Serial blood draws for correlative science are required on this trial as well; blood draws will be obtained prior to the first administration of study drug on Cycle 1 Day 1, then Cycle 1 Day 8, Cycle 2 Day 1, and Cycle 3 Day 1, and at the end-of-treatment visit in participants

who go off-study for progressive disease. All efforts will be made to obtain a sample at the time of progressive disease from participants who went off-study for anything other than progressive disease.

A stool sample will be obtained during the study screening period for evaluation of the tumor microbiome.

An optional research tumor biopsy at the time of progression will be collected for participants who consent and have biopsy accessible tumor. These biopsies will undergo the same characterization testing as described for baseline biopsies.

9.1 Summary table: Research tissue and blood specimen collection

Table 6. Research Tissue and Blood Specimen Collection

Research Sampling	Time Point	Contents
Blood	Cycle 1 Day 1 (pre-treatment)	5- purple top tubes (EDTA), total 30ml
	Cycle 1 Day 8	3- purple top tubes (EDTA), total 15 ml
	Cycle 2 day 1	3- purple top tubes (EDTA), total 15 ml
	Cycle 3 day 1	3- purple top tubes (EDTA), total 15 ml
	Off-Treatment (if off for PD)	3- purple top tubes (EDTA), total 15 ml
	At PD (for participants who go off-treatment for a reason other than PD)	3- purple top tubes (EDTA), total 15 ml
Stool Sample	Pre-treatment	1 Stool sample
Fresh Tissue	Optional pre-treatment biopsy	3 to 4 adequate cores
	Optional post-treatment biopsy for participants with PD	3 to 4 adequate cores
Archival Tissue	Anytime	1 block, or 20, 4 micron unstained slides

9.2 Biomarker Studies

9.2.1 Immunohistochemical Staining for PD-L1 (CD274), PD-L2 (CD273), and PD-1 (CD279)

We hypothesize that CD274, CD273 and CD279 protein expression in tumor tissues might be associated with favorable clinical response to immune checkpoint inhibition, and might serve as biomarkers for participant selection for CD279 blockade in clinical treatment. PDL1 assessment will be considered an integrated biomarker for this study because PDL1 assessment by this method has been used across multiple studies evaluating pembrolizumab and therefore will support standardization, reproducibility, and comparison across studies. Additional staining for PDL1 by the antibody method noted below will occur in parallel for exploratory comparison of these two techniques.

9.2.1.1 Collection of Tissue Specimens

Participant and specimen information

Immunohistochemical (IHC) staining of CD274 will be used as an integrated biomarker in this clinical trial, and to identify a group of participants most likely to respond to the immune checkpoint inhibition with eribulin and pembrolizumab. This information may be used in the future phase II trials as a stratification variable. IHC staining of CD273, CD279 will also be performed as exploratory biomarkers to explore a possible relationship between expression and response to eribulin and pembrolizumab in this population. Pre-treatment archival tumor material or fresh tumor biopsy should be collected for all eligible participants. In addition, because expression of these markers may change over time, immediate pre-treatment tumor biopsies will be paired with the optional post-treatment tumor biopsies when possible.

Collection and handling of biopsy specimens

At the site of origin (prior to shipment to BWH), 2 to 3 adequate cores, and any additional tissue will be fixed in 4% buffered formalin overnight at room temperature, to adequately preserve tissue morphology. Of note, one core will be saved as fresh frozen tissue for RNA sequencing analysis. Additionally, a 2nd core will be used for DFCI Oncopanel, targeted gene-expression profiling, per standard DFCI Oncopanel procedure. Then, the tissue will be transferred in 70% ethanol at room temperature for at least 48 hours. After fixation, the tissue will be processed, using an automated tissue processor following standard protocols within histology laboratories. To summarize this procedure, the tissue will be exposed to increasing concentration of ethanol (80%, 96% and 100%) and then to xylene for at least 2 hours. The tissue will be then infiltrated several times in hot paraffin and afterwards will be embedded in paraffin. Tissue section of 4-5 microns will be cut using a microtome and placed on "plus" (charged) glass slides amenable to IHC, and at 4C. A minimum of 15 unstained slides for BWH analysis will be generated per case. Batched cases (slides) will then be via overnight mail to BWH for further studies. Four-micrometer-thick sections will be cut. The paraffin blocks and unstained slides will be stored at room temperature. All IHC staining will be performed in the Center for Immuno-Oncology Pathology Core at Dana-Farber/Harvard Cancer Center.

Pre-treatment research tumor core biopsies are optional, and time of progression research core biopsies of an accessible lesion are optional. Guidelines for biopsy from various metastatic sites can be found in Appendix D. Biopsies should not be performed on Friday afternoons, as there may not be time for processing of the fresh tissue.

9.2.1.2 Site Preforming Correlative Studies

Laboratory information

The DF/HCC Specialized Histopathology Core will be a central research laboratory for this clinical trial. The IHC and immunofluorescent staining, including the Cell Signaling PD-L1 40.59All mouse ab, 29122S, will be conducted at the DFCI Center for Immuno-Oncology, Immune Assessment Laboratory, by Dr. Evisa Gjini, PhD, and Dr. Scott Rodig, MD, PhD. Peripheral blood analysis for cytokines and flow cytometry, as well as tumor microbiome, will be conducted by Mariano Severgnini, PhD at the DFCI Center for Immuno-Oncology, Immune Assessment Laboratory. Targeted genomic profiling will be conducted utilizing the standard DFCI Oncopanel. RNA sequencing will be performed under the direction of Dr. Matthew Hemming, MD, PhD.

9.3 *Tumor Tissue Exploratory/Ancillary Correlative Studies*

All of the below-mentioned analyses may be altered based on novel developments in the field of cancer immune profiling at the time of correlative science. Additional markers or alternative technologies (based on scientific developments and/or novel technologies) may also be used, to explore potential prognostic or predictive candidate markers/panels or markers related to treatment benefit and/or safety, to improve diagnostic tests, or to understand sarcoma biology.

- Tumor Infiltrating Lymphocytes and Tumor Infiltrating Macrophages and Myeloid derived suppressors cells.

  Paraffinized, hematoxylin and eosin-stained slides taken from two tissue planes will be derived from each biopsy and will be reviewed by certified pathologists. The extent of lymphocytic infiltrate in tumor tissue will be assessed, and stromal TIL percentage will be determined. Subsets of TIL will be determined, CD3+, CD4+, CD8+, FOXP3, too evaluate T regulatory cells. The percentages of tumor infiltrating macrophages will be determined as well, particularly in leiomyosarcoma participants. The percentages of tumor infiltrating myeloid derived suppressors cells will be determined as well. More detailed guidelines for the quantification of stromal TILs in sarcoma can be found in the recommendations from the International TILs Working Group 2014.

- Additional Immunohistochemistry will be performed for potential markers of immune response, such as IDO, and MHC class I expression.

- Targeted gene-expression profiling will be conducted using Oncopanel to evaluate specific mutations, mutation burden, MMR signature, and copy number variations as potential markers of immune response. One core biopsy sample from each participant biopsy may be paraffin embedded and saved for this analysis.

- RNA sequencing, as an exploratory biomarker, will be performed as well on available fresh frozen tumor samples. One core biopsy sample from each participant biopsy, fresh frozen, will be saved for this analysis.

9.4 Microbiome Exploratory Correlative Studies

Each participant will provide one stool sample, pre-treatment, obtained during screening. Participants should be instructed to collect the stool sample once eligibility has been confirmed. This will be proceeded and analyzed by the DFCI Center for Immuno-Oncology, Immune Assessment Laboratory, under the direction of Mariano Severgnini, PhD. The purpose of which is to evaluate participant's microbiome as a possible predictive marker for immunotherapy treatment.

9.5 Laboratory Exploratory Correlative Studies

9.5.1 Monitoring Peripheral Blood for Changes in Immune Function

9.5.1.1 Collection of Specimens

Serial blood/serum samples will be collected Cycle 1 Day 1 (pre-treatment), on-treatment (Cycle 1 Day 8, Cycle 2 Day 1, Cycle 3 Day 1), and off-treatment. Peripheral blood will be collected as whole blood and separated into plasma and buffy coat for circulating tumor DNA analysis, cytokine analysis and for generation of PBMCs.

A panel of cytokines and chemokines will be tested in serum using Luminex cytokine assay. Changes in cytokine production in immune cell subsets as a function of treatment will be determined by ELISA and intracellular cytokine staining. Absolute lymphocyte count (ALC) will be monitored. Research blood collection is mandatory for all participants for flow cytometry and DNA isolation. The samples will be banked in the DF/HCC Clinical Trials Core Laboratory for these and for optional future research purposes. These specimens will become the property of the DF/HCC. Blood draws should not be performed on Friday afternoons, as there may not be time for processing of the blood.

Peripheral blood mononuclear cells (PBMCs) will be collected from whole blood to assess immune cell populations. Surface staining with a panel of antibodies (CD3, CD4, CD8, CD25, FoxP3, CD11c, CD83, CD86, CD56) and intracytoplasmic cytokine staining, followed by flow cytometry will be performed in order to identify different T cell populations, their activation status, and the production of different cytokines as well as other immune cell populations as described below. Whole blood collection protocol, plasma and buffy coat collection protocol, and PBMCs collection protocols below:

Once collected in K_2EDTA tubes, peripheral blood should be placed at 4 degrees Celsius until processing. Buffy coat/plasma isolation should be performed within 4 hours of peripheral blood collection to ensure optimal results for circulating cell free DNA analyses. Samples may be stored at 4 degrees Celsius up to 24hrs prior to processing.

Whole Blood Processing

Normal DNA can be obtained through whole blood. A venous blood draw of 10 milliliters of blood is sufficient. Blood should be drawn into a K_2EDTA lavender top tube and gently rocked back and forth to mix the blood and the preservatives. Do not spin blood tube and do not freeze the blood tube at -80 degrees Celsius or through flash freezing. For long term storage samples may be stored at 4 degrees Celsius for less than 1 month. Samples that are to be stored for more than 1 month should be frozen at -20 degrees Celsius first and then transferred to a -80 degrees Celsius freezer. Do not place the tubes in Styrofoam holders for freezing.

Blood collection tube should be labeled with a unique identifier and date and time of blood draw. Tube should be wrapped in an absorbent paper towel and placed within a biohazard bag. Tube should be packed in a foam container delivered overnight at 4 degrees Celsius.

Plasma and buffy coat isolation from peripheral blood processed within 24 hours from collection

Normal DNA and circulating cell free tumor DNA can be obtained through whole blood. A venous blood draw of 20 milliliters of blood is sufficient. Blood should be drawn into a K_2EDTA lavender top tube and gently rocked back and forth to mix the blood and the preservatives.

Once collected in K_2EDTA tubes, peripheral blood should be placed at 4 degrees Celsius until processing. Buffy coat/plasma isolation should be performed within 4 hours of peripheral blood collection to ensure optimal results for circulating cell free DNA analyses. Samples may be stored at 4 degrees Celsius up to 24hrs prior to processing.

1. Use a minimum of 20cc of peripheral blood.
2. Centrifuge at 800 rcf at 4 degrees Celsius for 10 min.
3. Aspirate supernatant and transfer to new 5ml tubes.
4. Collect the buffy coat (extract from the middle whitish layer by going slightly under the layer with the tip) and aliquot in 0.5-1ml pellets in labeled 1.5ml tubes.
5. Centrifuge the supernatant at 18000 rcf for 10 min at room temperature.
6. Collect the top layer of plasma into 5ml cryo tubes
7. Store the plasma samples into -80°C

For shipment, plasma samples should be packed in cryoboxes within a foam container and shipped overnight on dry ice.

Plasma and buffy coat isolation from peripheral blood processed within 5 days from collection.

In case blood samples cannot be processed locally as per protocol above, blood samples should be collected in Streck tubes and shipped to DFCI Center for Immuno-Oncology

at room temperature within 5 days from collection (up to 3 days is preferred).

1. If a butterfly is used for venipuncture, an EDTA (or non-additive) tube should be connected first to eliminate air from the tubing before the Streck tube is connected.
2. Collect 20cc of whole blood in 2 10cc Streck tubes.
3. Immediately mix collected blood by gentle inversion 8 to 10 times.
4. Local transportation, storage and subsequent shipping of Streck tube samples should be performed at room temperature within 5 days from collection.

Protocol for Generation of PBMC's

1. Pour blood from green-cap, or purple tubes (heparin or EDTA treated tubes) into two 50 ml conical tubes (Corning, 430290).
2. Spin tubes at 1500 rpm for 10 min (Sorvall Legend XTR centrifuge).
3. Aspirate 2 ml plasma/tube and aliquot into 4 tubes mircocentrifuge tubes (Fisherbrand, 05-408-138)
4. Spin plasma at 3000 RPM for 5 minutes (Sorvall Legend Micro 21R centrifuge)
5. Aspirate plasma into Cryogenic tubes 2 ml plasma/ tube (Corning, 430488).
6. Dilute blood 1:1 with PBS. (Blood amount should not exceed 25 ml per tube.)
7. Take 2 new 50 ml conical tubes and add 12 ml ficoll-paque (Cat# 17144003; GE Healthcare) per tube.
8. Slowly and gently layer the diluted blood on top of the ficoll-paque of the tube with a maximum volume of 35 ml.
9. Centrifuge the tube at 1900 rpm for 20 min at room temperature with slow acceleration (#7) and deceleration (#7) (Sorvall Legend XTR centrifuge).
10. Remove the PBMC layer from between the upper layer (diluted plasma) and middle layer (ficoll-paque) and transfer into a 50 ml conical tube. The lower layer is composed of red blood cells.
11. Completely fill conical tube containing isolated PBMC with PBS, mixing well.
12. Count viable cells by mixing 10 µl Trypan Blue with 10µl PBMC/PBS dilution in a microcentrifuge tube. Load 10 µl of mixture onto Countess Cell Counting Chamber Slide (Invitrogen, C10283) and read with Countess Automated Cell Counter (Invitrogen).
13. Centrifuge the tubes containing PBMC/PBS mixture at 1500 rpm for 5 min at room temperature (Sorvall Legend XTR centrifuge).
14. Remove PBS, and resuspend PBMC pellet in appropriate amount of freezing solution so that there are approx 5×10^6 cells/cryo vial in 300-500 µl of Fetal Bovine Serum (heat inactivated) plus 15% DMSO.
15. Put vials in CoolCell container (Biocision Inc.) and transfer to -80C freezer overnight.
16. Transfer cells to liquid nitrogen tank.

Serum marker levels will be summarized descriptively and graphically for the participant population. The time course of expression levels will also be summarized graphically by participant, noting times of disease response and disease progression.

9.5.1.2 Handling of Specimens

All samples should be de-identified and labeled with the Participant initials, Participant Study ID number and date of collection and time point (e.g., “Baseline” or “Cycle 1” or “Progressive Disease”). Must be processed within 3-4hrs of its being drawn.

9.5.1.3 Shipping of Specimens

9.5.1.4 Site Performing Correlative Study

DFCI Center for Immuno-Oncology

DFCI Clinical Trials Core Laboratory

9.5.1.5 Blood and tissue banking

If the participant agrees, any leftover blood or tissue may be banked in the lab of Mariano Severgnini or the DF/HCC Clinical Trials Core Laboratory, respectively, as per standard lab protocol, such that it can be used for additional or future analyses as needed.

10. **STUDY CALENDAR**

Baseline evaluations are to be conducted within 2 weeks (14 days) prior to start of protocol therapy. Baseline assessments include: hematology panel, serum chemistry/LFT panel, pregnancy test, EKG, thyroid studies, performance status, HIV, Hep B, C testing, physical examination, medical history, concurrent medications evaluations, vital signs, demographics, weight, and height. Scans and x-rays should be done $\leq$2 weeks prior to the start of therapy. CBC and serum chemistries must be obtained on Cycle 1 Day 1to determine safety of study drug administration as per section 5.2.1.. In the event that the participant’s condition is deteriorating, laboratory evaluations should be repeated within 48 hours prior to initiation of the next cycle of therapy.

Assessments must be performed prior to administration of any study agent. Study assessments and agents should be administered within $\pm$ 3 days of the protocol-specified date, unless otherwise noted. Please reference section 6.1 table 2 for window restrictions due to eribulin dosing.

Table 7. Study Calendar

	Pre-Study	Cycle 1			Cycle 2			Cycle 3			Cycle 4 and beyond[j]			Off Treatment[c]
		Day 1	Day 8	Day 15	Day 1	Day 8	Day 15	Day 1	Day 8	Day 15	Day 1	Day 8	Day 15	
Eribulin		A	A		A	A		A	A		A	A		
Pembrolizumab		B			B			B			B			
Informed consent	X													
Demographics	X													
Medical history	X													
Concurrent meds	X[f]	X[e]----------											----------X[e]	
Physical exam	X[f]	X[e]	X[e]		X[e]	X[e]		X[e]	X[e]		X[e]	X[e]		X
Vital signs	X[f]	X[e]	X[e]		X[e]	X[e]		X[e]	X[e]		X[e]	X[e]		X
Height	X[f]													
Weight	X[f]	X[e]	X[e]		X[e]	X[e]		X[e]	X[e]		X[e]	X[e]		X
Performance status	X[f]	X	X		X	X		X	X		X	X		X
CBC w/diff, plts	X[f]	X[e]	X[e]		X[e]	X[e]		X[e]	X[e]		X[e]	X[e]		X
Serum chemistry[a]	X[f]	X[e]	X[e]		X[e]	X[e]		X[e]	X[e]		X[e]	X[e]		X
Thyroid studies[d]	X							X[d]						
EKG[p]	X	X	X		X	X		X	X		X	X		
Adverse event evaluation[i]		X----------											----------X	X
Tumor measurements	X[h]	Tumor measurements are repeated every <u>*2 cycles*</u> +/- 3 days. Documentation (radiologic) must be provided for participants removed from study for progressive disease.												X
Radiologic evaluation	X[h]	Radiologic measurements should be performed every <u>*2 cycles*</u> +/- 3 days.												X
Serum pregnancy (B-HCG) test[b]	X[b]													
HIV, Hep C, B Screen[n]	X													
Tumor Biopsy	X[k]													X[l]
Correlative blood biomarker studies[m]		X	X		X			X						X
Stool sample for microbiome	X[o]													

A: *Eribulin initial dosing 1.4mg/m^2 Day 1 and 8 of each 21-day cycle*

B: *Pembrolizumab dosing 200mg, Day 1 of each 21-day cycle*

a: Albumin, alkaline phosphatase, total bilirubin, bicarbonate, BUN, calcium, chloride, creatinine, glucose, LDH, phosphorus, magnesium, potassium, total protein, SGOT [AST], SGPT [ALT], sodium, lipase, amylase.

b: Serum pregnancy test in women of childbearing potential.

c: Off-treatment evaluation. Participants will return to clinic within 4 weeks after progression, for end of study visit and for AE related assessment.

Immune AE assessment will be performed at ~100 days following last dose of study drug. This may be done by phone. Participants who complete all planned protocol therapy will continue to be monitored for tumor measurements beyond completion of treatment until disease progression or the initiation of a new anticancer therapy. Scans will be performed as standard of care approximately every 2 months or 8 weeks. Participants will be followed for survival with telephone calls approximately every 3 months from off treatment visit. For participants who come off study for reasons other than progression, or completion of all planned protocol therapy, they will be monitored with standard of care scans until progression or the initiation of a new anticancer therapy.

NOTE: With Protocol Version 15MAY2024, further long-term follow-up is being terminated.

d: TSH, free T4, obtained during study screening, then Cycle 3 Day 1, and then every 6-8 weeks thereafter.

e: Assessments done prior to drug administration within 48 hours.

f: Repeat screening tests if >2 weeks of 14 days from Cycle 1 Day 1.

h: Initial imaging CT or MRI Tumor measurements should be within 2 weeks from Cycle 1 Day 1.

i: Including specific focus on immune-related adverse events.

j: Schedule post Cycle 4 will repeat as per Cycles 1 -4, with the exception of pre/post tumor biopsy and correlative studies. Participants will continue to receive the study treatment each cycle (Day 1, Day 8) until progression or discontinuation with physical exam and laboratory evaluation prior to each drug administration, and repeat imaging (same as baseline) every 6 weeks/2 cycles for up to two years of treatment, or 35 cycles. For participants beyond Cycle 8, repeat imaging may be obtained every 12 weeks/4 cycles. If patients stop eribulin for treatment related effects and are continuing pembrolizumab alone, day 8 assessments will not be required.

k: If archival tissue is available, collect approximately 20 slides as described in section 9. If archival tissue is not available, a biopsy may be collected if determined feasible by the Investigator. The biopsy would be collected after eligibility is confirmed and prior to first dose of pembrolizumab.

l: Optional Tumor Biopsy on progression for correlative biomarker assay's.

m: Peripheral blood will be drawn prior to the first administration of study drug on Cycle 1 Day 1, Cycle 1 Day 8, Cycle 2 Day 1, Cycle 3 Day 1, and at off-treatment, to assess changes in immune function as per section 9.2.

n: Testing for human immunodeficiency virus (HIV) or known acquired immunodeficiency syndrome (AIDS), hepatitis B virus surface antigen (HBV sAg) and hepatitis C virus ribonucleic acid (HCV antibody) indicating acute or chronic infection. These tests do not need to be re-drawn if a participant has to re-screen.

o: Participants should be instructed to collect a stool sample once they are deemed eligible.

p: EKG's should be collected pre-dose and do not need to be collected if dose is missed/held.

11. MEASUREMENT OF EFFECT

11.1 Antitumor Effect – Solid Tumors

For the purposes of this study, participants should be re-evaluated for response every 6 weeks (+/- 3 days). In addition to a baseline scan, confirmatory scans should also be obtained at 6 weeks (+/- 3 days) following initial documentation of objective response. For participants who are beyond Cycle 8, restaging scans may be obtained at 12 week intervals (+/- 3 days).

Response and progression will be evaluated in this study using the new international criteria proposed by the Response Evaluation Criteria in Solid Tumors (RECIST) guideline (version 1.1)[1]. 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.

All participants who receive at least one dose of eribulin and pembrolizumab will be included in the toxicity analysis. This includes participants who receive study drug and are ultimately deemed ineligible to measure response.

Pembrolizumab, like other immunotherapeutic agents, may produce antitumor effects by inducing or potentiating the endogenous cancer-specific immune response. The response to treatment with immunotherapy may differ from traditional cytotoxic agents. Reponses may be seen after the typical time course predicted with cytotoxic agents. Additionally, responses may be seen after initial tumor increase in size or burden or even the appearance of new lesions. To account for these potential response patterns, imaging data will be collected to potentially evaluate for response by irRECIST, in addition to standard RECIST 1.1 assessments. On prior sarcoma immunotherapy trials, such as SARC028, there has not been a significant difference between responses per RECIST 1.1 or irRECIST. Comparison of RECIST 1.1 and irRECIST on this trial may help to clarify if immune response patterns are seen in sarcoma participant's receiving immunotherapy. Clinical decision-making regarding progressive disease and trial discontinuation will still utilize RECIST 1.1.

For any participant who showed first radiologic evidence of progressive disease (PD) by RECIST 1.1 and is deemed clinically stable, it is at the discretion of the investigator to continue treating the participant until progression is confirmed at the next scheduled restaging (or with a confirmatory completed at least 4 weeks from the initial date of PD). If progression is not confirmed on the subsequent scan, the participant should continue to receive treatment and have radiographic scans performed according to the study calendar (every 6 weeks). If radiologic progression is confirmed, then the participant should be discontinued from all study treatment. If the treating investigator feels that the participant is clinically stable, demonstrates improved condition, or is clearly continuing to benefit from the treatment; the PI may approve the participant to continue to receive study treatment. In all participants, the date of progression will be documented as the first date progression was observed.

11.1.1 Definitions

Evaluable for Target Disease response. Only those participants 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 target disease response. These participants will have their response classified according to the definitions stated below. (Note: Participants who exhibit objective disease progression prior to the end of cycle 1 will also be considered evaluable.)

Evaluable Non-Target Disease Response. Participants who have lesions present at baseline that are evaluable but do not meet the definitions of measurable disease, have received at least one cycle of therapy, and have had their disease re-evaluated will be considered evaluable for non-target disease. The response assessment is based on the presence, absence, or unequivocal progression of the lesions.

Evaluable for toxicity. All participants will be evaluable for toxicity from the time of their first treatment with eribulin and pembrolizumab.

11.1.2 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 or ≥10 mm with CT scan, MRI, or 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 will not be considered measurable, unless there is clear evidence of progression of the lesion since completion of radiation and prior to enrollment on study.

Malignant lymph nodes. To be considered pathologically enlarged and measurable, a lymph node must be ≥15 mm (≥1.5cm) in short axis when assessed by CT scan (CT scan slice thickness recommended to be no greater than 5 mm(0.5cm). 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 (<1.0 cm) or pathological lymph nodes with ≥10 to <15 mm (≥1.0 cm to <1.5 cm) short axis), are considered non-measurable disease. Bone lesions, leptomeningeal disease, ascites, pleural/pericardial effusions, lymphangitis cutis/pneumonitis, inflammatory breast disease, abdominal masses (not followed by CT or MRI), and cystic lesions are all considered non-measurable.

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 participant, 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 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, absence, or in rare cases unequivocal progression of each should be noted throughout follow up.

11.1.3 Methods for Evaluation of Disease

All measurements should be taken and recorded in metric notation using a ruler, calipers, or a digital measurement tool. All baseline evaluations should be performed as closely as possible to the beginning of treatment and never more than 4 weeks before the beginning of the treatment.

The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during follow-up. 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 (≥1.0cm) 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.

Conventional CT and MRI are the preferred imaging modalities on this study.

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.

Conventional CT and MRI. This guideline has defined measurability of lesions on CT scan based on the assumption that CT thickness is 5mm or less. If CT scans have slice

thickness greater than 5 mm, the minimum size of 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 should 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.

11.1.4 Response Criteria

11.1.4.1 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 (<1.0 cm).

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 progressions).

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.

11.1.4.2 Evaluation of Non-Target Lesions

Complete Response (CR): Disappearance of all non-target lesions and normalization of tumor marker level. All lymph nodes must be non-pathological in size (<10 mm (<1.0cm) short axis).

Note: If tumor markers are initially above the upper normal limit, they must normalize for a participant to be considered in complete clinical response.

Non-CR/Non-PD: Persistence of one or more non-target lesion(s) and/or maintenance of tumor marker level above the normal limits.

Progressive Disease (PD): 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.

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 PI).

11.1.4.3 Evaluation of New Lesions

The finding of a new lesion should be unequivocal (i.e. not due to difference in scanning technique, imaging modality, or findings thought to represent something other than tumor (for example, some 'new' bone lesions may be simply healing or flare of pre-existing lesions). However, a lesion identified on a follow-up scan in an anatomical location that was not scanned at baseline is considered new and will indicate PD. If a new lesion is equivocal (because of small size etc.), follow-up evaluation will clarify if it truly represents new disease and if PD is confirmed, progression should be declared using the date of the initial scan on which the lesion was discovered.

11.1.4.4 Evaluation of Best Overall Response

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

For Participants with Measurable Disease (*i.e.*, Target Disease)

Table 8.1 RECIST Target Lesions

Target Lesions	Non-Target Lesions	New Lesions	Overall Response	Best Overall Response when Confirmation is Required*
CR	CR	No	CR	≥4 wks Confirmation**
CR	Non-CR/Non-PD	No	PR	≥4 wks Confirmation**
CR	Not evaluated	No	PR	
PR	Non-CR/Non-PD/not evaluated	No	PR	
SD	Non-CR/Non-	No	SD	Documented at least once ≥4

	PD/not evaluated			wks from baseline**
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.
** Only for non-randomized trials with response as primary endpoint.
*** In exceptional circumstances, unequivocal progression in non-target lesions may be accepted as disease progression.

Note: Participants 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.

For Participants with Non-Measurable Disease (*i.e.*, Non-Target Disease)

Table 8.2 RECIST Non-Target Lesions

Non-Target Lesions	New Lesions	Overall Response
CR	No	CR
Non-CR/non-PD	No	Non-CR/non-PD*
Not all evaluated	No	not evaluated
Unequivocal PD	Yes or No	PD
Any	Yes	PD

* 'Non-CR/non-PD' is preferred over 'stable disease' for non-target disease since SD is increasingly used as an endpoint for assessment of efficacy in some trials so to assign this category when no lesions can be measured is not advised

11.1.5 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, or death due to any cause. Participants without events reported are censored at the last disease evaluation).

Duration of overall complete response: 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, or death due to any cause. Participants without events reported are censored at the last disease evaluation.

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.

11.1.6 Overall Survival, Progression-Free Survival, Time-to-Progression

Overall Survival: Overall Survival (OS) is defined as the time from randomization (or registration) to death due to any cause, or censored at date last known alive.

Progression-Free Survival: Progression-Free Survival (PFS) is defined as the time from randomization (or registration) to the earlier of progression or death due to any cause. Participants alive without disease progression are censored at date of last disease evaluation.

Time to Progression: Time to Progression (TTP) is defined as the time from randomization (or registration) to progression, or censored at date of last disease evaluation for those without progression reported.

11.1.7 Response Review

At study completion, all objective responses (RECIST PR or CR) will be reviewed by the tumor imaging metrics core (TIMC) at the Dana-Farber Harvard Cancer Center. Participant files will be available to the readers for concurrent review.

11.2 Other Response Parameters: irRECIST

11.2.1 Definition of Tumor Response Using Immune-Related Response Criteria (irRECIST)

The sum of the longest diameter of lesions (SPD) at tumor assessment using the immune related response criteria (irRECIST) for progressive disease incorporate the contribution of new measurable lesions. Each net Percentage Change in Tumor Burden per assessment using irRC criteria accounts for the size and growth kinetics of both old and new lesions as they appear.

11.2.1.1 Impact of New Lesions on irRECIST

New lesions in and of themselves do not qualify as progressive disease. However, their contribution to total tumor burden is included in the SPD which in turn feeds into the irRECIST criteria for tumor response. Therefore, new non-measurable lesions will not discontinue any participant from the study.

11.2.1.2 Definition of Target Lesions Response Using irRECIST

-irRECIST Complete Response (irRECIST CR): Complete disappearance of all target lesions. This category encompasses exactly the same participants as “CR” by the WHO criteria.

-irRECIST Partial Response (irRECIST PR): Decrease, relative to baseline, or 50% or greater in the sum of the products of the two largest perpendicular diameters of all target and all new measurable target lesions (i.e., Percentage Change in Tumor Burden). Note: the appearance of new measurable lesions is factored into the overall tumor burden, but does not automatically qualify as progressive disease until the SBD increases by >25% when compared to SPD at nadir.

-irRECIST Stable Disease (irRECIST SD): Does not meet criteria for irRECIST RC or irPR, in the absence of progressive disease.

-irRECIST Progressive Disease (irRECIST PD): At least 25% increase Percentage Change in Tumor Burden (i.e. taking SPD of all target lesions and any new lesions) when compared to SPD at nadir.

11.2.1.3 Definition of Non-Target Lesions Response Using irRECIST

-irRECIST Complete Response (irRECIST CR): Complete disappearance of all non-target lesions. This category encompasses exactly the same participants as "CR" by WHO criteria.

-irRECIST Partial Response (irRECIST PR) or irRECIST Stable Disease (irRECIST SD): Non-target lesion(s) are not considered in the definition of PR; these terms do not apply.

-irRECIST Progressive Disease (irRECIST PD): Increases in number or size of non-target lesion(s) does not constitute progressive disease unless/until the Percentage Change in Tumor Burden increases by 25% (i.e. the SPD at nadir of the target lesions increases by the required amount).

11.2.1.4 Definition of Overall Response Using irRECIST

Overall response using irRECIST will be based on these criteria:

-Immune-Related Complete Response (irRECIST CR): Complete disappearance of all tumor lesions (target a non-target) together with no new measurable/unmeasurable lesions for at least 4 weeks from the date of documentation of complete response.

-Immune-Related Partial Response (irRECIST PR): The sum of the products of the two largest perpendicular diameters of all target lesions is measured and captured as the SPD baseline. At each subsequent tumor assessment, the SPD of the two largest perpendicular diameters of all target lesions and of new measurable lesions are added together to provide the Immune Response Sum of Product Diameters (irRECIST SPD). A decrease, relative to baseline, of the irRECIST SPD compared to the previously SPD baseline of 50% or greater is considered an irRECIST PR.

-**Immune-Related Stable Disease (irRECIST SD):** irRECIST SD is defined as the failure to meet criteria for immune complete response or immune partial response, in the absence of progressive disease

-**Immune-Related Progressive Disease (**irRECIST **PD):** It is recommended in difficult cases to confirm PD by serial imaging. Any of the following will constitute PD:

-At least 25% increase in the SPD of all target lesions over nadir SPD calculated for the target lesions.

-At least 25% increase in the SPD of all target lesions and new measurable lesions (irRECIST SPD) over the baseline SPD calculated for the target lesions.

11.2.1.5 Criteria for determining overall response by irRECIST are summarized as follows:

Immune-Related Best Overall Response Using irRECIST (irRECIST BOR)
irRECIST BOR is the best confirmed overall response over the study as a whole, recorded between the date of first dose until the last tumor assessment before subsequent therapy (except for local palliative radiotherapy for painful bone lesions) for the individual participant in the study. For the assessment of irBOR, all available assessments per participant are considered. irRECIST CR or irRECIST PR determinations included in the irRECIST BOR assessment must be confirmed by a second (confirmatory) evaluation meeting the criteria for response and performed no less than 4 weeks after the criteria for response are first met.

Table 9. irRECIST

Immune-Related Response Criteria Definitions

Target Lesion Definition	Non-Target Lesion Definition	New Measurable Lesions	New Unmeasurable Lesions	Percent change in tumor burden (including measurable new lesions when present)	Overall irRC Response
Complete Response	Complete Response	No	No	–100%	irCR
Partial Response	Any	Any	Any	≥ –50%	irPR
				<–50% to <+25%	irSD
				>+25%	irPD
Stable Disease	Any	Any	Any	<–50% to <+25%	irSD
				>+25%	irPD
Progressive Disease	Any	Any	Any	≥+25%	irPD

12. DATA REPORTING / REGULATORY REQUIREMENTS

Adverse event lists, guidelines, and instructions for AE reporting can be found in Section 7.0 (Adverse Events: List and Reporting Requirements).

12.1 Data Reporting

12.1.1 Method

The Office of Data Quality (ODQ) will collect, manage, and perform quality checks on the data for this study.

12.1.2 Responsibility for Data Submission

Investigative sites within DF/HCC or DF/PCC are responsible for submitting data and/or data forms to the Office of Data Quality in accordance with DF/HCC SOPs.

12.2 Data Safety Monitoring

The DF/HCC Data and Safety Monitoring Committee (DSMC) will review and monitor toxicity and accrual data from this study. The committee is composed of clinical specialists with experience in oncology and who have no direct relationship with the study. Information that raises any questions about participant safety will be addressed with the Overall PI and study team.

The DSMC will review each protocol up to four times a year or more often if required to review toxicity and accrual data. Information to be provided to the committee may include: up-to-date participant accrual; current dose level information; DLT information; all grade 2 or higher unexpected adverse events that have been reported; summary of all deaths occurring with 30 days of intervention for Phase I or II protocols; for gene therapy protocols, summary of all deaths while being treated and during active follow-up; any response information; audit results, and a summary provided by the study team. Other information (e.g. scans, laboratory values) will be provided upon request.

13. STATISTICAL CONSIDERATIONS

13.1 Study Design/Endpoints

We have designed a single stage phase II, three-arm study for the primary endpoint of progression-free rate at 12 weeks. Participants will be stratified among three-arms based upon participant diagnosis. Arm A will consist of leiomyosarcoma participants. Arm B will consist of liposarcoma participants. Arm C will consist of undifferentiated pleomorphic sarcoma/other sarcomas.

The primary endpoint is the 12-week progression-free survival. The progression-free survival is defined as the absence of disease progression at 12 weeks from study enrollment. Participants without stable disease or complete or partial response, with deaths of any cause within 12 weeks, with progression within 12 weeks, or without confirmatory scans at 12 weeks' post baseline evaluation will be counted as failures. We will enroll 19 participants in each arm following a single stage approach.

Toxicity is an important secondary endpoint. With 19 participants in each arm, there is at least 86% probability of observing one or more toxicities with a true rate as low as 10%. With 19 treated participants in each arm, the maximum width of a 90% two-sided confidence interval for any estimated adverse event proportion is ± 20%.

Progression-free survival (PFS) and overall survival (OS) of participants receiving combination therapy will be described using the method of Kaplan and Meier and 90% confidence intervals for PFS and OS will be reported.

NOTE: With Protocol Version 15MAY2024, further long-term follow-up is being terminated.

13.2 Sample Size, Accrual Rate and Study Duration

Arm A (Leiomyosarcoma)
We will assess the progression-free rate at 12 weeks (null: 30%, alternative: 60%). We will need to observe at least 9 participants absent of disease progression out of 19 participants to accept the treatment. The overall power for the progression-free rate at 12 weeks is 91% using the exact binomial distribution. The operating characteristics of this design are calculated using a one-sided exact test with 10% type I error.

Arm B (Liposarcoma)
We will assess the progression-free rate at 12 weeks (null: 30%, alternative: 60%). We will need to observe at least 9 participants absent of disease progression out of 19 participants to accept the treatment. The overall power for the progression-free rate at 12 weeks is 91% using the exact binomial distribution. The operating characteristics of this design are calculated using a one-sided exact test with 10% type I error.

*Arm C (*Undifferentiated Pleomorphic Sarcoma/Other Sarcomas*)*
We will assess the progression-free rate at 12 weeks (null: 30%, alternative: 60%). We will need to observe at least 9 participants absent of disease progression out of 19 participants to accept the treatment. The overall power for the progression-free rate at 12 weeks is 91% using the exact binomial distribution. The operating characteristics of this design are calculated using a one-sided exact test with 10% type I error. Additionally, response to individual sarcomas histologic subtypes will be reported descriptively.

Table 10. Accrual Targets

Accrual Targets					
Ethnic Category	**Sex/Gender**				
	Females		**Males**		**Total**
Hispanic or Latino	1	+	1	=	2
Not Hispanic or Latino	27	+	28	=	55
Ethnic Category: Total of all participants	28	+	29	=	57
Racial Category					
American Indian or Alaskan Native	1	+	1	=	2
Asian	1	+	1	=	2
Black or African American	1	+	1	=	2
Native Hawaiian or other Pacific Islander	1	+	1	=	2
White	24	+	25	=	49
Racial Category: Total of all participants	28	+	29	=	57

13.3 Stratification Factors

There are no planned stratification factors within each cohort of this study.

13.4 Interim Monitoring Plan

No planned interim efficacy analysis. This is a single stage study in each of three liposarcoma, leiomyosarcoma, and undifferentiated pleomorphic sarcoma cohorts/other sarcomas.

13.5 Analysis Plan

Participants' demographic characteristics including age, gender, and race will be analyzed, with categorical variables summarized in frequency tables while continuous variables will be summarized using mean (±s.d.) and median (range). The student t-test/Wilcoxon test and ANOVA/Kruskal-Wallis test [Woolson and Clarke, 2000] will be used to compare continuous variables between different participant groups. The chi-square test or the Fisher's exact test [Woolson and Calrke, 2000] will be applied to assess the association between two categorical variables.

13.6 Analysis of Primary Endpoints

For the analysis of the primary endpoint ($PFS_{12\ weeks}$), we will provide respective point estimate along with 90% confidence interval for each of the combinations. PFS is defined as the time from baseline disease evaluation to either disease progression as defined by RECIST or death from any cause, whichever occurs first. For events that have not occurred by the time of data analysis, times will be censored at the last contact at which the participant was known to be progression-free. Log-rank test (Mantel, 1966) will be performed to test the difference in survival between groups. Regression analyses will be conducted of survival data based on the Cox proportional hazards model (Cox, 1972). The proportional hazards assumption will be evaluated graphically and analytically, and regression diagnostics (e.g., martingale and Shoenfeld residuals) will be examined to ensure that the models are appropriate.

13.7 Analysis of Secondary Endpoints

OS is defined as the time from treatment onset to death. For events that have not occurred by the time of data analysis, times will be censored at the last contact at which the participant was known to be progression-free for PFS, or the last time the participant was known to be alive for OS. PFS and OS will be estimated using the Kaplan-Meier method (Kaplan,1958) and 90% confidence intervals for PFS and OS will be reported. Log-rank test (Mantel, 1966) will be performed to test the difference in survival between groups. Regression analyses of survival data based on the Cox proportional hazards model (Cox, 1972) will be conducted on PFS or OS. The proportional hazards assumption will be evaluated graphically and analytically, and regression diagnostics (e.g., martingale and Shoenfeld residuals) will be examined to ensure that the models are appropriate.

NOTE: With Protocol Version 15MAY2024, further long-term follow-up is being terminated.

13.8 Analysis of Correlative Biomarkers

Paired pre- and post-treatment biopsies will be obtained when available in consenting participants. We will explore the relationship between integrated biomarkers PDL1/PD1/PD2 status (PDL1 status, PDL1 in infiltrating lymphocytes, PD2 status in archival tumor) and the absence of disease progression to combination treatment eribulin and pembrolizumab. We will use Fisher's Exact Test to assess the relationship between each biomarker and the absence of

disease progression to combination treatment. Each is expected to have similar characteristics. For example, with this design, assuming tissue is available on 90% of the sample, the probability of concluding combination treatment eribulin and pembrolizumab response is related to the exploratory biomarker is 82%; given the unknown true response is 65% and 4% in positive marker versus negative marker participants, respectively, assuming 45% of the population is over-expressed for PDL1. The power to detect the relationship of interest increases as the prevalence of the biomarker increases. The operating characteristics of this design are calculated using a two-sided exact test with 10% type I error.

13.9 Reporting and Exclusions

All participants will be evaluable for toxicity from the time of their first treatment with eribulin and pembrolizumab. All participants who receive at least one dose of eribulin and pembrolizumab will be included in the toxicity analysis. This includes participants who receive study drug and are ultimately deemed ineligible.

13.9.1 Evaluation of Toxicity

Toxicity data will be summarized by frequency tables. For the toxicity endpoint, per-treated analysis will be used to include any participant who received the treatment regardless of the eligibility nor the duration or dose of the treatment received. Toxicity rate will be estimated with 95% credible interval.

13.9.2 Evaluation of the Primary Efficacy Endpoint

All analyses will be intent-to-treat analyses. All the analyses will be conducted separately by each cohort, leiomyosarcoma, liposarcoma, and undifferentiated pleomorphic sarcoma/other sarcomas.

13.9.3 Evaluation or Response

All eligible participants included in the study must be assessed for response to treatment, even if there are major protocol treatment deviations or if they are ineligible. Each participant will be assigned one of the following categories: 1) complete response, 2) partial response, 3) stable disease, 4) progressive disease, 5) early death from malignant disease, 6) early death from toxicity, 7) early death because of other cause, or 9) unknown (not assessable, insufficient data). [Note: By arbitrary convention, category 9 usually designates the “unknown” status of any type of data in a clinical database.] All of the participants who met the eligibility criteria (with the possible exception of those who received no study medication) should be included in the main analysis of the response rate. Participants in response categories 4-9 should be considered to have a treatment failure (disease progression). Thus, an incorrect treatment schedule or drug administration does not result in exclusion from the analysis of the response rate. Precise definitions for categories 4-9 will be protocol specific. All conclusions should be based on all eligible participants. Sub-analyses may then be performed on the basis of a subset of participants, excluding those for whom major protocol deviations have been identified (*e.g.*, early death due to other reasons, early discontinuation of treatment, major protocol violations, etc.). However, these sub-analyses may not serve as the basis for drawing conclusions concerning treatment

efficacy, and the reasons for excluding participants from the analysis should be clearly reported. The 95% confidence intervals should also be provided.

Statistical References:

1. Cox DR: Regression models and life tables (with discussion). J. R. Statistical Soc, B 34:187,220, 1972.
2. Kaplan EL, Meier P: Nonparametric estimator from incomplete observations. J. American Statistical Association 53:457-81, 1958.
3. Mantel N: Evaluation of survival data and two new rank order statistics arising in its consideration. Cancer Chemotherapy Reports 60:163-170, 1966.
4. Liang, K.Y., Zeger, S.L. Longitudinal data analysis using generalized linear models. Biometrika 73:13-22, 1986.
5. Lee, J.J., Tu, Z.N.: "A versatile one-dimensional distribution plot: the BLiP plot." The American Statistician, 51:353-358, 1997.
6. Simon R. Optimal two-stage designs for phase II clinical trials. Controlled clinical trials 1989;10(1):1-10.
7. Thall PF, Simon RM, Estey EH. New statistical strategy for monitoring safety and efficacy in single-arm clinical trials. J Clin Oncol 1996;14(1):296-303.
8. Wathen JK, Thall PF, Cook JD, Estey EH. Accounting for participant heterogeneity in phase II clinical trials. Stat Med. 2008;27(15):2802-15. doi: 10.1002/sim.3109. PubMed PMID: 17948869.
9. Woolson, RF and Calrke, WR. Statistical Methods for the Analysis of Biomedical Data. 2nd Edition, John Wiley & Sons, New York, 2000.

14. **PUBLICATION PLAN**

The results should be made public within 24 months of reaching the end of the study. The end of the study is the time point at which the last data items are to be reported, or after the outcome data are sufficiently mature for analysis, as defined in the section on Sample Size, Accrual Rate and Study Duration. If a report is planned to be published in a peer-reviewed journal, then that initial release may be an abstract that meets the requirements of the International Committee of Medical Journal Editors. A full report of the outcomes should be made public no later than three (3) years after the end of the study.

15. **REFERENCES**

1. Schwartz LH, Litiere S, de Vries E, et al. RECIST 1.1-Update and clarification: From the RECIST committee. Eur J Cancer. 2016;62: 132-137.
2. Fecher LA, Agarwala SS, Hodi FS, Weber JS. Ipilimumab and its toxicities: a multidisciplinary approach. Oncologist. 2013;18: 733-743.
3. Trinh VA, Hwu WJ. Ipilimumab in the treatment of melanoma. Expert Opin Biol Ther. 2012;12: 773-782.
4. Siegel R, Ma J, Zou Z, Jemal A. Cancer statistics, 2014. CA Cancer J Clin. 2014;64: 9-29.
5. Hayat MJ, Howlader N, Reichman ME, Edwards BK. Cancer statistics, trends, and multiple primary cancer analyses from the Surveillance, Epidemiology, and End Results (SEER) Program. Oncologist. 2007;12: 20-37.
6. Coindre JM. [New WHO classification of tumours of soft tissue and bone]. Ann Pathol. 2012;32: S115-116.
7. Davis A, Osullivan B, Turcotte R, et al. Late radiation morbidity following randomization to preoperative versus postoperative radiotherapy in extremity soft tissue sarcoma. Radiotherapy and Oncology. 2005;75: 48-53.
8. Tierney JF, Stewart LA, Parmar MKB, et al. Adjuvant chemotherapy for localised resectable soft-tissue sarcoma of adults: meta-analysis of individual data. The Lancet. 1997;350: 1647-1654.
9. Pervaiz N, Colterjohn N, Farrokhyar F, Tozer R, Figueredo A, Ghert M. A systematic meta-analysis of randomized controlled trials of adjuvant chemotherapy for localized resectable soft-tissue sarcoma. Cancer. 2008;113: 573-581.
10. Woll PJ, Reichardt P, Le Cesne A, et al. Adjuvant chemotherapy with doxorubicin, ifosfamide, and lenograstim for resected soft-tissue sarcoma (EORTC 62931): a multicentre randomised controlled trial. The Lancet Oncology. 2012;13: 1045-1054.
11. Frustaci S, Gherlinzoni F, De Paoli A, et al. Adjuvant chemotherapy for adult soft tissue sarcomas of the extremities and girdles: results of the Italian randomized cooperative trial. J Clin Oncol. 2001;19: 1238-1247.
12. Gronchi A, Stacchiotti S, Verderio P, et al. Short, full-dose adjuvant chemotherapy (CT) in high-risk adult soft tissue sarcomas (STS): long-term follow-up of a randomized clinical trial from the Italian Sarcoma Group and the Spanish Sarcoma Group. Ann Oncol. 2016;27: 2283-2288.
13. Gronchi A, Ferrari S, Quagliuolo V, et al. Histotype-tailored neoadjuvant chemotherapy versus standard chemotherapy in patients with high-risk soft-tissue sarcomas (ISG-STS 1001): an international, open-label, randomised, controlled, phase 3, multicentre trial. Lancet Oncol. 2017;18: 812-822.
14. Callegaro D, Miceli R, Gronchi A. Sarcoma nomograms: a light over the darkness. Oncoscience. 2017;4: 15-16.
15. Eilber FC, Brennan MF, Eilber FR, Dry SM, Singer S, Kattan MW. Validation of the postoperative nomogram for 12-year sarcoma-specific mortality. Cancer. 2004;101: 2270-2275.
16. Eilber FC, Brennan MF, Riedel E, Alektiar KM, Antonescu CR, Singer S. Prognostic factors for survival in patients with locally recurrent extremity soft tissue sarcomas. Ann Surg Oncol. 2005;12: 228-236.
17. Weitz J, Antonescu CR, Brennan MF. Localized extremity soft tissue sarcoma: improved knowledge with unchanged survival over time. J Clin Oncol. 2003;21: 2719-2725.

18. Coley WB. II. Contribution to the Knowledge of Sarcoma. Ann Surg. 1891;14: 199-220.
19. Starnes CO. Coley's toxins in perspective. Nature. 1992;357: 11-12.
20. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell. 2011;144: 646-674.
21. Robbins PF, Kassim SH, Tran TL, et al. A pilot trial using lymphocytes genetically engineered with an NY-ESO-1-reactive T-cell receptor: long-term follow-up and correlates with response. Clin Cancer Res. 2015;21: 1019-1027.
22. Robbins PF, Morgan RA, Feldman SA, et al. Tumor regression in patients with metastatic synovial cell sarcoma and melanoma using genetically engineered lymphocytes reactive with NY-ESO-1. J Clin Oncol. 2011;29: 917-924.
23. Rosenberg SA, Yang JC, Topalian SL, et al. Treatment of 283 consecutive patients with metastatic melanoma or renal cell cancer using high-dose bolus interleukin 2. JAMA. 1994;271: 907-913.
24. Fyfe G, Fisher RI, Rosenberg SA, Sznol M, Parkinson DR, Louie AC. Results of treatment of 255 patients with metastatic renal cell carcinoma who received high-dose recombinant interleukin-2 therapy. J Clin Oncol. 1995;13: 688-696.
25. Fyfe GA, Fisher RI, Rosenberg SA, Sznol M, Parkinson DR, Louie AC. Long-term response data for 255 patients with metastatic renal cell carcinoma treated with high-dose recombinant interleukin-2 therapy. J Clin Oncol. 1996;14: 2410-2411.
26. Atkins MB, Lotze MT, Dutcher JP, et al. High-dose recombinant interleukin 2 therapy for patients with metastatic melanoma: analysis of 270 patients treated between 1985 and 1993. J Clin Oncol. 1999;17: 2105-2116.
27. Atkins MB, Kunkel L, Sznol M, Rosenberg SA. High-dose recombinant interleukin-2 therapy in patients with metastatic melanoma: long-term survival update. Cancer J Sci Am. 2000;6 Suppl 1: S11-14.
28. Eggermont AM, Suciu S, Santinami M, et al. Adjuvant therapy with pegylated interferon alfa-2b versus observation alone in resected stage III melanoma: final results of EORTC 18991, a randomised phase III trial. Lancet. 2008;372: 117-126.
29. Eggermont AM, Suciu S, Testori A, et al. Long-term results of the randomized phase III trial EORTC 18991 of adjuvant therapy with pegylated interferon alfa-2b versus observation in resected stage III melanoma. J Clin Oncol. 2012;30: 3810-3818.
30. Le DT, Durham JN, Smith KN, et al. Mismatch-repair deficiency predicts response of solid tumors to PD-1 blockade. Science. 2017.
31. Le DT, Uram JN, Wang H, et al. PD-1 Blockade in Tumors with Mismatch-Repair Deficiency. N Engl J Med. 2015;372: 2509-2520.
32. Jelinek T, Mihalyova J, Kascak M, Duras J, Hajek R. PD-1/PD-L1 inhibitors in haematological malignancies: update 2017. Immunology. 2017.
33. Chen R, Zinzani PL, Fanale MA, et al. Phase II Study of the Efficacy and Safety of Pembrolizumab for Relapsed/Refractory Classic Hodgkin Lymphoma. J Clin Oncol. 2017;35: 2125-2132.
34. Pages F, Galon J, Dieu-Nosjean MC, Tartour E, Sautes-Fridman C, Fridman WH. Immune infiltration in human tumors: a prognostic factor that should not be ignored. Oncogene. 2010;29: 1093-1102.
35. Balch CM, Riley LB, Bae YJ, et al. Patterns of human tumor-infiltrating lymphocytes in 120 human cancers. Arch Surg. 1990;125: 200-205.
36. Galon J, Costes A, Sanchez-Cabo F, et al. Type, density, and location of immune cells within

human colorectal tumors predict clinical outcome. Science. 2006;313: 1960-1964.
37. Kilic A, Landreneau RJ, Luketich JD, Pennathur A, Schuchert MJ. Density of tumor-infiltrating lymphocytes correlates with disease recurrence and survival in patients with large non-small-cell lung cancer tumors. J Surg Res. 2011;167: 207-210.
38. Bogunovic D, O'Neill DW, Belitskaya-Levy I, et al. Immune profile and mitotic index of metastatic melanoma lesions enhance clinical staging in predicting patient survival. Proc Natl Acad Sci U S A. 2009;106: 20429-20434.
39. D'Angelo SP, Shoushtari AN, Agaram NP, et al. Prevalence of tumor-infiltrating lymphocytes and PD-L1 expression in the soft tissue sarcoma microenvironment. Hum Pathol. 2015;46: 357-365.
40. Rusakiewicz S, Semeraro M, Sarabi M, et al. Immune infiltrates are prognostic factors in localized gastrointestinal stromal tumors. Cancer Res. 2013;73: 3499-3510.
41. Sorbye SW, Kilvaer T, Valkov A, et al. Prognostic impact of lymphocytes in soft tissue sarcomas. PLoS One. 2011;6: e14611.
42. Berghuis D, Santos SJ, Baelde HJ, et al. Pro-inflammatory chemokine-chemokine receptor interactions within the Ewing sarcoma microenvironment determine CD8(+) T-lymphocyte infiltration and affect tumour progression. J Pathol. 2011;223: 347-357.
43. Brinkrolf P, Landmeier S, Altvater B, et al. A high proportion of bone marrow T cells with regulatory phenotype (CD4+CD25hiFoxP3+) in Ewing sarcoma patients is associated with metastatic disease. Int J Cancer. 2009;125: 879-886.
44. Kim JR, Moon YJ, Kwon KS, et al. Tumor infiltrating PD1-positive lymphocytes and the expression of PD-L1 predict poor prognosis of soft tissue sarcomas. PLoS One. 2013;8: e82870.
45. Vanderstraeten A, Luyten C, Verbist G, Tuyaerts S, Amant F. Mapping the immunosuppressive environment in uterine tumors: implications for immunotherapy. Cancer Immunol Immunother. 2014;63: 545-557.
46. Mattarollo SR, Loi S, Duret H, Ma Y, Zitvogel L, Smyth MJ. Pivotal role of innate and adaptive immunity in anthracycline chemotherapy of established tumors. Cancer Res. 2011;71: 4809-4820.
47. Saresella M, Rainone V, Al-Daghri NM, Clerici M, Trabattoni D. The PD-1/PD-L1 pathway in human pathology. Curr Mol Med. 2012;12: 259-267.
48. KEYTRUDA Prescribing Information. Available from URL: https://www.merck.com/product/usa/pi_circulars/k/keytruda/keytruda_pi.pdf 2017].
49. Tawbi H, Reinke DK, Reed DR, et al. Pembrolizumab (P) in patients with advanced soft tissue (STS) and Bone sarcomas (BS): Upated efficacy results of multicenter phase II study SARC028 and correlates of response. CTOS # 2570708. Lisbon Portugla, 2016.
50. Ben-Ami E, Barysauskas CM, Solomon S, et al. Immunotherapy with single agent nivolumab for advanced leiomyosarcoma of the uterus: Results of a phase 2 study. Cancer. 2017.
51. George S, Miao D, Demetri GD, et al. Loss of PTEN Is Associated with Resistance to Anti-PD-1 Checkpoint Blockade Therapy in Metastatic Uterine Leiomyosarcoma. Immunity. 2017;46: 197-204.
52. Wolchok JD, Kluger H, Callahan MK, et al. Nivolumab plus ipilimumab in advanced melanoma. N Engl J Med. 2013;369: 122-133.
53. Ramalingam S, Hui R, Gandhi L, et al. P2.39: Long-Term OS for Patients With Advanced NSCLC Enrolled in the KEYNOTE-001 Study of Pembrolizumab: Track: Immunotherapy. J Thorac Oncol. 2016;11: S241-S242.
54. Maki RG. Eribulin in soft-tissue sarcomas. Lancet Oncol. 2011;12: 988-989.

55. Dybdal-Hargreaves NF, Risinger AL, Mooberry SL. Eribulin mesylate: mechanism of action of a unique microtubule-targeting agent. Clin Cancer Res. 2015;21: 2445-2452.
56. Bai RL, Paull KD, Herald CL, Malspeis L, Pettit GR, Hamel E. Halichondrin B and homohalichondrin B, marine natural products binding in the vinca domain of tubulin. Discovery of tubulin-based mechanism of action by analysis of differential cytotoxicity data. J Biol Chem. 1991;266: 15882-15889.
57. Bai R, Cichacz ZA, Herald CL, Pettit GR, Hamel E. Spongistatin 1, a highly cytotoxic, sponge-derived, marine natural product that inhibits mitosis, microtubule assembly, and the binding of vinblastine to tubulin. Mol Pharmacol. 1993;44: 757-766.
58. Bai R, Nguyen TL, Burnett JC, et al. Interactions of halichondrin B and eribulin with tubulin. J Chem Inf Model. 2011;51: 1393-1404.
59. Luduena RF, Roach MC, Prasad V, Pettit GR. Interaction of halichondrin B and homohalichondrin B with bovine brain tubulin. Biochem Pharmacol. 1993;45: 421-427.
60. Towle MJ, Salvato KA, Budrow J, et al. In vitro and in vivo anticancer activities of synthetic macrocyclic ketone analogues of halichondrin B. Cancer Res. 2001;61: 1013-1021.
61. Jordan MA, Kamath K, Manna T, et al. The primary antimitotic mechanism of action of the synthetic halichondrin E7389 is suppression of microtubule growth. Mol Cancer Ther. 2005;4: 1086-1095.
62. Kuznetsov G, Towle MJ, Cheng H, et al. Induction of morphological and biochemical apoptosis following prolonged mitotic blockage by halichondrin B macrocyclic ketone analog E7389. Cancer Res. 2004;64: 5760-5766.
63. Eribulin Shows Promise in Advanced Sarcoma. Cancer Discov. 2015;5: OF4.
64. HALAVEN-Full-Prescribing-Information. Available from URL: http://www.halaven.com/pdfs/HALAVEN-Full-Prescribing-Information.pdf 2017].
65. Schoffski P, Ray-Coquard IL, Cioffi A, et al. Activity of eribulin mesylate in patients with soft-tissue sarcoma: a phase 2 study in four independent histological subtypes. Lancet Oncol. 2011;12: 1045-1052.
66. Kawai A, Araki N, Naito Y, et al. Phase 2 study of eribulin in patients with previously treated advanced or metastatic soft tissue sarcoma. Jpn J Clin Oncol. 2016.
67. Landhuis E. FDA Approves Eribulin for Advanced Liposarcoma. Cancer Discov. 2016;6: OF1.
68. Schoffski P, Chawla S, Maki RG, et al. Eribulin versus dacarbazine in previously treated patients with advanced liposarcoma or leiomyosarcoma: a randomised, open-label, multicentre, phase 3 trial. Lancet. 2016.
69. chawla S, Schoffski P, Grignani G, et al. Abstract 11037: Subtype-specific Activity in Liposarcoma Patients From a Phase 3, Open-label, Randomized Study of Eribulin Versus Dacarbazine in Patients With Advanced Liposarcoma or Leiomyosarcoma. Annual ASCO Meeting 2016. Chicago: J Clin Oncol, 2016.
70. Cortes J, O'Shaughnessy J, Loesch D, et al. Eribulin monotherapy versus treatment of physician's choice in patients with metastatic breast cancer (EMBRACE): a phase 3 open-label randomised study. Lancet. 2011;377: 914-923.
71. Kawano S, Asano M, Adachi Y, Matsui J. Antimitotic and Non-mitotic Effects of Eribulin Mesilate in Soft Tissue Sarcoma. Anticancer Res. 2016;36: 1553-1561.
72. Van Glabbeke M, Verweij J, Judson I, Nielsen OS, Tissue ES, Bone Sarcoma G. Progression-free rate as the principal end-point for phase II trials in soft-tissue sarcomas. Eur J Cancer. 2002;38: 543-549.

73. Chen BJ, Chapuy B, Ouyang J, et al. PD-L1 expression is characteristic of a subset of aggressive B-cell lymphomas and virus-associated malignancies. Clin Cancer Res. 2013;19: 3462-3473.
74. D'Angelo SP, Mahoney MR, Van Tine BA, et al. Nivolumab with or without ipilimumab treatment for metastatic sarcoma (Alliance A091401): two open-label, non-comparative, randomized, phase 2 trials. Lancet Oncol. 2018; 19(3):416-426.
75. Wilky BA, Trucco MM, Subhawong TK, et al. Axitinib plus pembrolizumab in patietns with advanced sarcomas including alveolar soft-part sarcoma: a single-centre, single-arm, phase 2 trial. Lancet Oncol. 2019; 20(6):837-848.
76. Groisberg R, Hong DS, Behrang A, et al. Characteristics and outcomes of patients with advanced sarcoma enrolled in early phase immunotherapy trials. J Immunother Cancer. 2017; 5(1): 100.
77. Florou V, Rosenberg AE, Wieder E, et al. Angiosarcoma patients treated with immune checkpoint inhibitors: a case series of seven patients from a single institution. J Immunother Cancer. 2019; 7(1):213.
78. Doyle LA, Nowak JA, Nathenson MJ, et al. Characteristics of mismatch repair deficiency in sarcomas. Mod Pathol. 2019; 32(7):977-987.

16. APPENDIX A: PERFORMANCE STATUS CRITERIA

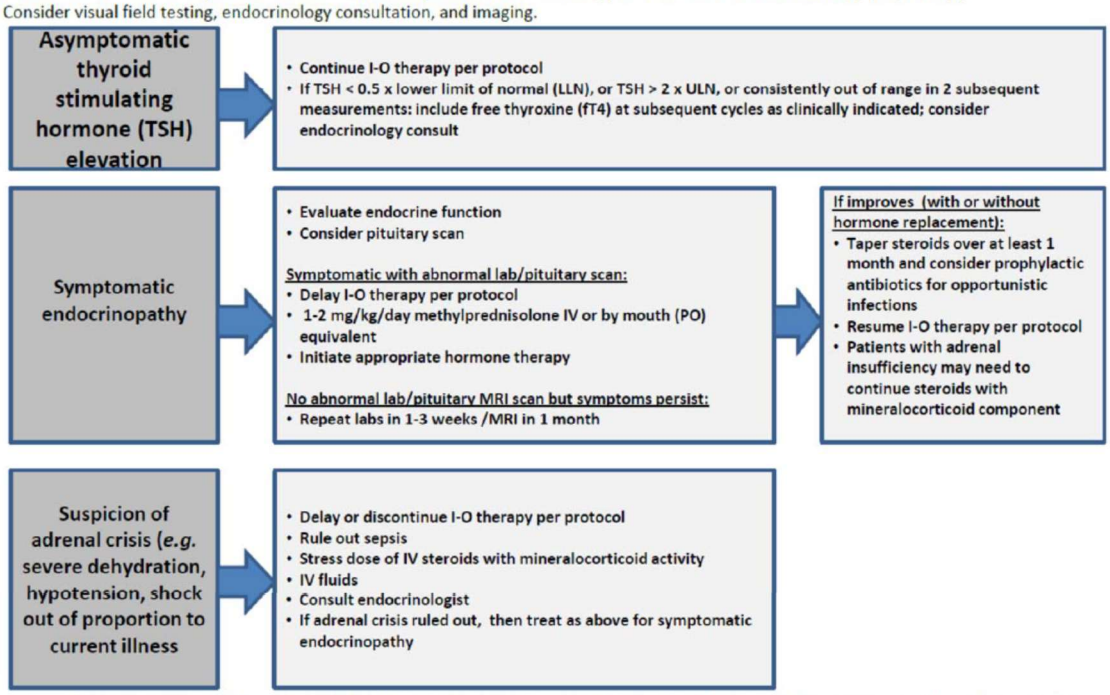
Table 11. Performance Status

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

17. APPENDIX B: MANAGEMENT ALGORITHMS FOR ENDOCRINOPATHY, GASTROINTESTINAL, HEPATIC, NEUROLOGICAL, PULMONARY, RENAL, AND SKIN ADVERSE EVENTS

Endocrinopathy Management Algorithm

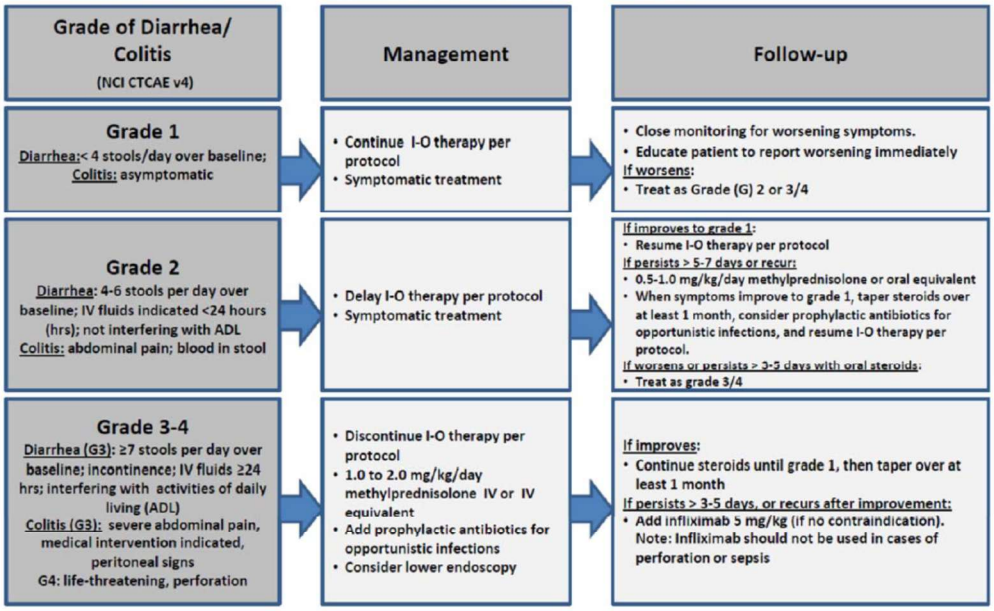
Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue immuno-oncology (I-O) therapy.
Consider visual field testing, endocrinology consultation, and imaging.



Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (e.g. prednisone) at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of oral corticosteroids.

GI Adverse Event Management Algorithm

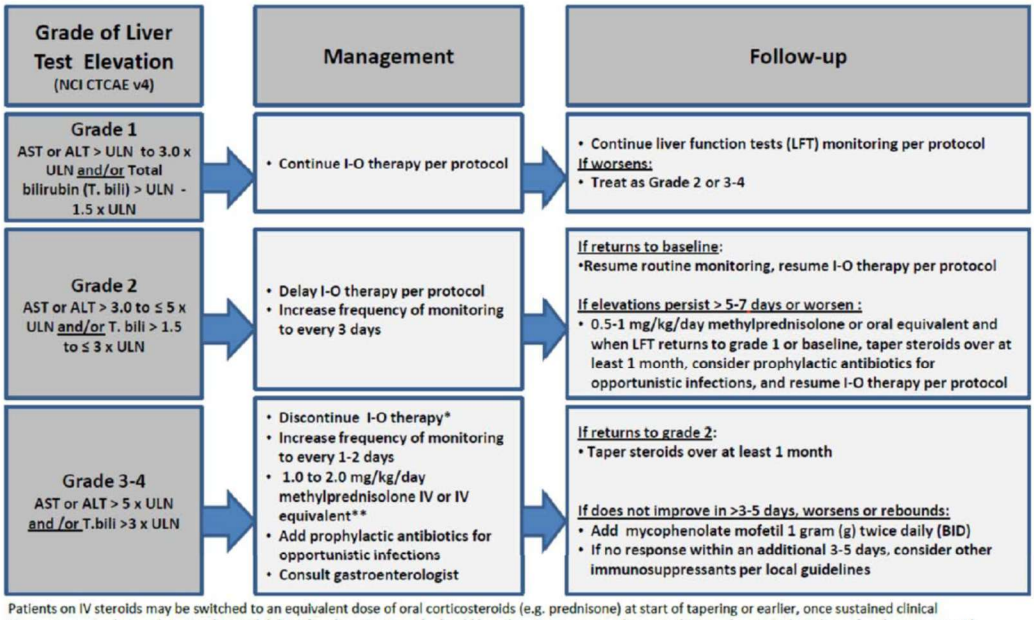
Rule out non-inflammatory causes. If non-inflammatory cause is identified, treat accordingly and continue I-O therapy. Opiates/narcotics may mask symptoms of perforation. Infliximab should not be used in cases of perforation or sepsis.



Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (e.g. prednisone) at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of oral corticosteroids.

Hepatic Adverse Event Management Algorithm

Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue I-O therapy. Consider imaging for obstruction.



Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (e.g. prednisone) at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of oral corticosteroids.

*I-O therapy may be delayed rather than discontinued if AST/ALT ≤ 8 x ULN and T.bili ≤ 5 x ULN.

**The recommended starting dose for grade 4 hepatitis is 2 mg/kg/day methylprednisolone IV.

Neurological Adverse Event Management Algorithm

Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue I-O therapy.

Grade of Neurological Toxicity (NCI CTCAE v4)	Management	Follow-up
Grade 1 Asymptomatic or mild symptoms; Intervention not indicated	• Continue I-O therapy per protocol	Continue to monitor the patient. If worsens: • Treat as Grade 2 or 3-4
Grade 2 Moderate symptoms; Limiting instrumental ADL	• Delay I-O therapy per protocol • Treat symptoms per local guidelines • Consider 0.5 to 1.0 mg/kg/day methylprednisolone IV or PO equivalent	If improves to baseline: •Resume I-O therapy per protocol when improved to baseline If worsens: • Treat as Grade 3-4
Grade 3-4 Severe symptoms; Limiting self-care ADL; Life-threatening	• Discontinue I-O therapy per protocol • Obtain neurology consult • Treat symptoms per local guidelines • 1.0-2.0 mg/kg/day IV methylprednisolone IV or IV equivalent • Add prophylactic antibiotics for opportunistic infections	If improves to Grade 2: • Taper steroids over at least 1 month If worsens or atypical presentation: • Consider IVIG or other immunosuppressive therapies per local guidelines

Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (e.g. prednisone) at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of oral corticosteroids.

Pulmonary Adverse Event Management Algorithm

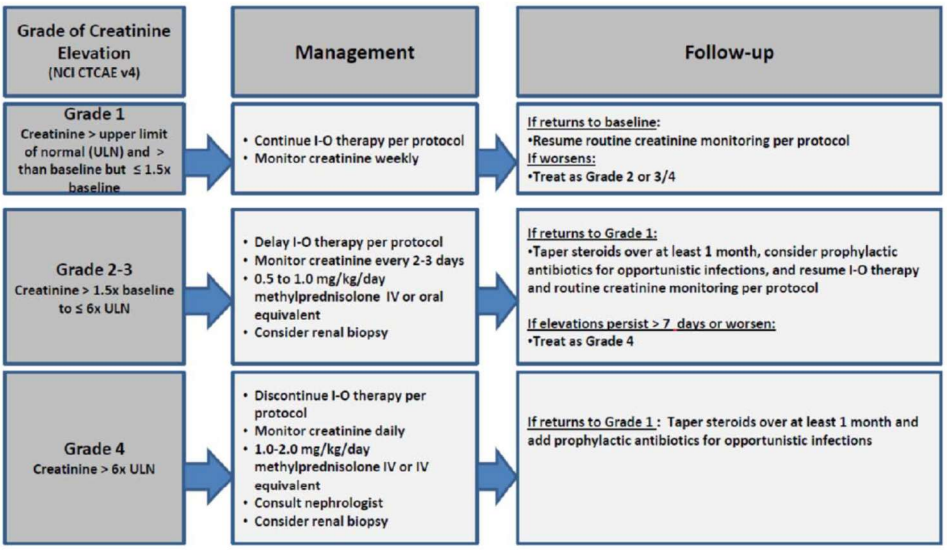
Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue I-O therapy. Evaluate with imaging and pulmonary consultation.

Grade of Pneumonitis (NCI CTCAE v4)	Management	Follow-up
Grade 1 Radiographic changes only	• Consider delay of I-O therapy • Monitor for symptoms every 2-3 days • Consider Pulmonary and Infectious Disease (ID) consults	• Re-image at least every 3 weeks If worsens: • Treat as Grade 2 or 3-4
Grade 2 Mild to moderate new symptoms	• Delay I-O therapy per protocol • Pulmonary and ID consults • Monitor symptoms daily, consider hospitalization • 1.0 mg/kg/day methyl-prednisolone IV or oral equivalent • Consider bronchoscopy , lung biopsy	• Re-image every 1-3 days If improves: • When symptoms return to near baseline, taper steroids over at least 1 month and then resume I-O therapy per protocol and consider prophylactic antibiotics If not improving after 2 weeks or worsening: • Treat as Grade 3-4
Grade 3-4 Severe new symptoms; New/worsening hypoxia; Life-threatening	• Discontinue I-O therapy per protocol • Hospitalize • Pulmonary and ID consults • 2-4 mg/kg/day methylprednisolone IV or IV equivalent • Add prophylactic antibiotics for opportunistic infections • Consider bronchoscopy, lung biopsy	If improves to baseline: • Taper steroids over at least 6 weeks If not improving after 48 hours or worsening: • Add additional immunosuppression (e.g. infliximab, cyclophosphamide, intravenous immunoglobulin (IVIG), or mycophenolate mofetil)

Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (e.g. prednisone) at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of oral corticosteroids.

Renal Adverse Event Management Algorithm

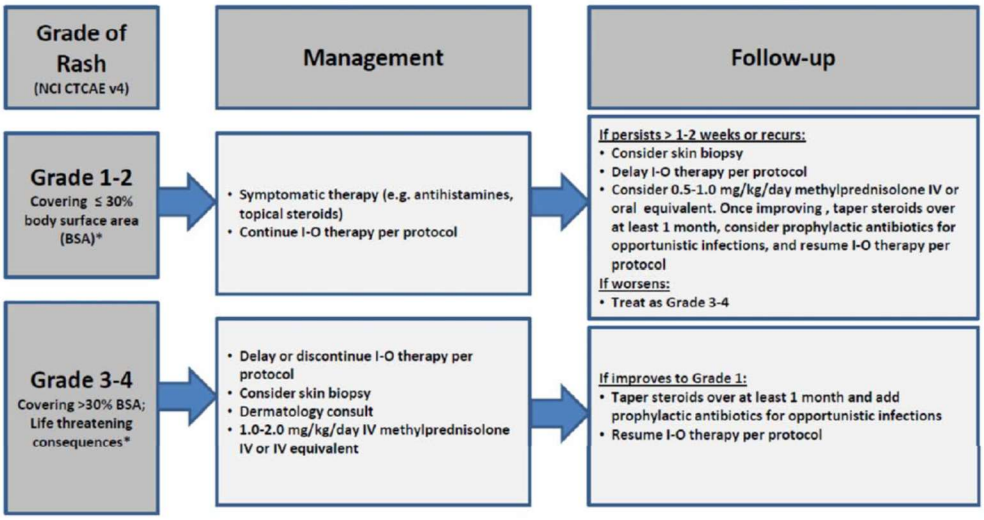
Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue I-O therapy



Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (e.g. prednisone) at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of oral corticosteroids.

Skin Adverse Event Management Algorithm

Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue I-O therapy.



Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (e.g. prednisone) at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of oral corticosteroids.
*Refer to NCI CTCAE v4 for term-specific grading criteria.

18. APPENDIX C: INFORMATION ON POSSIBLE DRUG INTERACTIONS

The list provided below is not exhaustive. For a more comprehensive, frequently updated list, please visit: http://medicine.iupui.edu/clinpharm/ddis/table.aspx.

Medications that strongly inhibit CYP3A:
Amprenavir
Atazanavir
Boceprevir
Clarithromycin
Conivaptan
Delavirdine
Diltiazem
Erythromycin
Fosamprenavir
Indinavir
Itraconazole
Ketoconazole
Lopinavir
Mibefradil
Miconazole
Nefazodone
Nelfinavir
Posaconazole
Ritonavir
Saquinavir
Telaprevir
Telithromycin
Verapamil
Voriconazole
Grapefruit, grapefruit juice, or any product containing grapefruit

Medications that strongly induce CYP3A:
Carbamazepine
Felbamate
Nevirapine
Phenobarbital
Phenytoin
Primidone
Rifabutin
Rifampin
Rifapentin
St. John's wort

19. APPENDIX D: INFORMATION ON POSSIBLE INTERACTIONS WITH OTHER AGENTS FOR PATIENTS AND THEIR CAREGIVERS AND NON-STUDY HEALTHCARE TEAM

The participant _____ is enrolled on a clinical trial using the experimental agents eribulin and pembrolizumab. This clinical trial is sponsored by the National Cancer Institute. This form is addressed to the participant, but includes important information for others who care for this participant.

Eribulin and pembrolizumab 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:

Eribulin interacts with certain specific enzymes in your liver.

- *Eribulin* 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.
- You and healthcare providers who prescribe drugs for you must be careful about adding or removing any drug in this category.
- 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 eribulin and pembrolizumab.

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

  _____

  and he or she can be contacted at

  _____

20. APPENDIX E: PARTICIPANT INFORMATION CARD

INFORMATION ON POSSIBLE DRUG INTERACTIONS

You are enrolled on a clinical trial using the experimental agent _____. This clinical trial is sponsored by the Suzanne George, MD. _____ 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, and 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 _____.

21. APPENDIX F: GUIDELINES FOR COLLECTING RESEARCH BIOPSY TISSUE

Tissue specimens will be collected from metastatic lesions using standard institutional procedures. The amount of tissue collected may follow the guidelines listed below:

Skin/chest wall: A goal of 2 to 3 5-mm punch biopsies will be obtained.

Soft Tissue or Liver: A goal of 3-4 core biopsy specimens will be obtained using an 18-gauge needle.

Lung: Because of the risk of pneumothorax associated with core needle biopsies of lung nodules, no core biopsies of lung nodules are mandated on this protocol, unless they are clinically indicated.

Bone: Because the yield of malignant tissue from bone biopsies tends to be relatively low, if a participant has another accessible site of disease (i.e. skin, lymph node, liver), that site should be biopsied preferentially. If bone is the only biopsy-accessible site, then a goal of 3-6 core biopsy specimens will be obtained using an 11-13 gauge needle.

Please note that the above are guidelines for the amount of tissue to be obtained, and are not meant to replace clinical judgment at the time the procedure is performed. Less than the goal quantity of tissue is accepted for each type of biopsy, and will be left to the clinical judgment of the physician performing the procedure.

If a participant is undergoing resection of a lesion for clinical reasons (i.e. wedge resection of a new lung lesion for confirmation of diagnosis; or, resection of a chest wall lesion; or, resection of a liver or bone lesion), then the participant may opt to have a portion of that tissue (roughly equivalent to the goal amount of tissue listed in the guidelines above, i.e. the equivalent of two 5-mm punch biopsies of the skin, or 3-6 18-gauge core biopsies) stored for research at the time of the procedure (provided that the tissue is processed as specified), in which case, the participant would not be required to undergo a separate research biopsy at baseline on this protocol.

Coded laboratory specimens will be stored in the Tumor Bank of the DFCI. These specimens will become the property of DFCI. Participants will be informed that their specimens may be used for research by investigators at DF/HCC and other approved collaborators. Shared specimens will be identified with a sample ID number; all participant identifying material will be removed.

Risks of Research Biopsy and Procedures for Minimizing Risk

Potential risks according to site are:

Skin/chest wall (punch biopsy):

- Likely: local discomfort and minor bleeding
- Less likely: moderate or major bleeding, or infection

Liver, or bone (core needle biopsy):

- Likely: local discomfort and minor bleeding
- Less likely: moderate or major bleeding, need for blood transfusion, hospitalization due to bleeding or other complications, infection, damage to adjacent organs. Additional risks may be present if intravenous conscious sedation is required

Prior to the procedure, the physician performing the procedure will discuss the risks with each study participant, answer any questions, and obtain separate procedure consent. Participants will be evaluated for comorbidities or concomitant medications that may increase the risk of potential complications. For biopsies of lesions that are not superficial and clearly palpable, imaging studies such as CT or ultrasound will be used to guide the biopsy in order to minimize the risk of damage to adjacent structures. After liver biopsies, participants will be observed a minimum of 4 hours (range 4-6 hours) after the procedure, or according to standard institutional guidelines. Less than the goal quantity of tissue is accepted for each type of biopsy, and will be left to the clinical judgment of the physician performing the procedure.

Risks of Anesthesia

Local Anesthesia

All biopsy procedures require local anesthesia using lidocaine, xylocaine, or related compounds. There is a small risk of an allergic reaction associated with these drugs. In order to minimize the risk of local anesthesia, only qualified personnel will perform the biopsy procedure. Participants will be queried if they have had previous allergic reactions to local anesthetics.

Intravenous Conscious Sedation

Certain biopsy procedures, such as liver or bone biopsies, may require intravenous conscious sedation (IVCS). IVCS is a minimally depressed level of consciousness that retains the participant's ability to maintain a patent airway independently and continuously and respond appropriately to physical stimulation and verbal commands. The risks of intravenous conscious sedation include: inhibition of the gag reflex and concomitant risk of aspiration, cardiopulmonary complications (myocardial infarction, cardiac arrhythmias, hypoxemia), and allergic reactions to the sedative or analgesic medications. These risks are small but real; for example, in a prospective study of 14,149 participants undergoing IVCS during upper gastrointestinal endoscopies, the rate of immediate cardiopulmonary events was 2 in 1000. The 30-day mortality was 1 per 2,000 cases. In this study, there was a strong association between lack of monitoring and use of high-dose benzodiazepines with adverse outcomes. There was also an association between the use of local anesthetic sprays to the oropharynx and the development of pneumonia. In order to minimize the risk of intravenous conscious sedation, only qualified personnel will be responsible for conscious sedation. A minimum of two individuals will be involved in the care of participants undergoing conscious sedation—the physician performing the biopsy procedure, and the individual (M.D. or R.N.) who monitors the participants and his/her response to both the sedation and the procedure, and who is capable of assisting with any supportive or resuscitative measures. The room where the procedure utilizing IVCS takes place

will have adequate equipment to provide supplemental oxygen, monitor vital signs, and maintain an airway should this be necessary. An emergency cart will also be immediately accessible to the room where the procedure is to take place, and emergency support services will be available on page. Participants will be screened and evaluated for their fitness to undergo conscious sedation by a trained physician. Participants with active cardiac disease are excluded from this study. No local anesthetic spray to the oropharynx will be necessary, given that endoscopy is not a planned procedure. Following the procedure, participants will be observed closely in the recovery room for a minimum of 2 hours.

General Anesthesia

Because of the higher risk of general anesthesia compared with local anesthesia or intravenous conscious sedation, biopsies that would require general anesthesia in order to be performed *are not permitted* on this protocol, unless they are being done for clinical reasons, and excess tissue that otherwise would have been discarded is then banked for the purpose of this protocol.

For Fresh Frozen Biopsies of Soft Tissue, Liver, Bone, Etc:

1. After biopsy is performed, the tissue mass is placed on a sterile gauze

2. Using forceps, separate the tumor tissue

3. Place 2 pieces (cores) of tumor tissue in each cassette (typically end up with 3 cassettes per biopsy); the last cassette will contain many small pieces of tumor tissue

4. Fill cassettes with OCT

    a. Completely cover tissue

    b. Limit the amount of bubbles

5. Place cassettes on dry ice and prepare for transport by limiting OCT leakage

6. Return samples to the lab and complete freezing of samples in OCT with dry ice (about 10 minutes freezing time)

7. Once samples are frozen, place in plastic bag; label bag with date, protocol number, participant number, and number of initials included

8. Store in –80C freezer

For Paraffin Embedded Tissue:

At the site of origin (prior to shipment to BWH), 2 to 3 adequate cores, and any additional tissue will be fixed in 4% buffered formalin overnight at room temperature, to adequately preserve tissue morphology. Of note one core will be saved as fresh frozen tissue for RNA sequencing analysis. Additionally, a 2nd core will be used for DFCI Oncopanel, targeted gene-expression profiling, per standard DFCI Oncopanel procedure. Then, the tissue will be transferred in 70%

ethanol at room temperature for at least 48 hours. After fixation, the tissue will be processed, using an automated tissue processor following standard protocols within histology laboratories. To summarize this procedure, the tissue will be exposed to increasing concentration of ethanol (80%, 96% and 100%) and then to xylene for at least 2 hours. The tissue will be then infiltrated several times in hot paraffin and afterwards will be embedded in paraffin.

22. APPENDIX G: CONTRACEPTIVE GUIDANCE AND PREGNANCY TESTING

Woman of Childbearing Potential (WOCBP)
A woman is considered fertile following menarche and until becoming post-menopausal unless permanently sterile (see below)
Women in the following categories are not considered WOCBP:

- Premenarchal
- Premenopausal female with 1 of the following:
  - Documented hysterectomy
  - Documented bilateral salpingectomy
  - Documented bilateral oophorectomy

  Note: Documentation can come from the site personnel's review of the participant's medical records, medical examination, or medical history interview.
- Postmenopausal female
  - A postmenopausal state is defined as no menses for 12 months without an alternative medical cause.
    - A high follicle stimulating hormone (FSH) level in the postmenopausal range may be used to confirm a postmenopausal state in women not using hormonal contraception or hormonal replacement therapy (HRT). However, in the absence of 12 months of amenorrhea, confirmation with two FSH measurements in the postmenopausal range is required.
  - Females on HRT and whose menopausal status is in doubt will be required to use one of the non-hormonal highly effective contraception methods if they wish to continue their HRT during the study. Otherwise, they must discontinue HRT to allow confirmation of postmenopausal status before study enrollment.

Contraception Requirements

Male Participants:
Male participants with female partners of childbearing potential are eligible to participate if they agree to one of the following during the protocol defined time frame in section 3.1.10:

- Be abstinent from penile-vaginal intercourse as their usual and preferred lifestyle (abstinent on a long term and persistent basis) and agree to remain abstinent
- Use a male condom plus partner use of a contraceptive method with a failure rate of <1% per year as described in Table 12 when having penile-vaginal intercourse with a woman of childbearing potential who is not currently pregnant.
  - Note: Men with a pregnant or breastfeeding partner must agree to remain abstinent from penile-vaginal intercourse or use a male condom during each episode of penile penetration.

Female Participants:
Female participants of childbearing potential are eligible to participate if they agree to use a highly effective method of contraception consistently and correctly as described in Table 12 during the protocol-defined time frame in Section 3.1.10.

Table 12 Highly Effective Contraception Methods

Highly Effective Contraceptive Methods That Are User Dependent [a] *Failure rate of <1% per year when used consistently and correctly.*
● Combined (estrogen- and progestogen- containing) hormonal contraception [b, c] ○ Oral ○ Intravaginal ○ Transdermal ○ Injectable
● Progestogen-only hormonal contraception [b, c] ○ Oral ○ Injectable
Highly Effective Methods That Have Low User Dependency *Failure rate of <1% per year when used consistently and correctly.*
● Progestogen- only contraceptive implant [b, c] ● Intrauterine hormone-releasing system (IUS) [b] ● Intrauterine device (IUD) ● Bilateral tubal occlusion
● **Vasectomized partner** A vasectomized partner is a highly effective contraception method provided that the partner is the sole male sexual partner of the WOCBP and the absence of sperm has been confirmed. If not, an additional highly effective method of contraception should be used.
● **Sexual abstinence** Sexual abstinence is considered a highly effective method only if defined as refraining from heterosexual intercourse during the entire period of risk associated with the study treatment. The reliability of sexual abstinence needs to be evaluated in relation to the duration of the study and the preferred and usual lifestyle of the participant.)
Notes: Use should be consistent with local regulations regarding the use of contraceptive methods for participants of clinical studies. a) Typical use failure rates are lower than perfect-use failure rates (i.e. when used consistently and correctly). b) If hormonal contraception efficacy is potentially decreased due to interaction with study treatment, condoms must be used in addition to the hormonal contraception during the treatment period and for at least 20 weeks corresponding to time needed to eliminate study treatment plus 30 days after the last dose of study treatment. c) If locally required, in accordance with Clinical Trial Facilitation Group (CTFG) guidelines, acceptable hormonal contraceptives are limited to those which inhibit ovulation.

Pregnancy Testing

WOCBP should only be included after a negative highly sensitive serum pregnancy test. Following initiation of treatment, pregnancy testing will be performed whenever an expected menstrual cycle is missed or when pregnancy is otherwise suspected; at the time points specified

in the Schedule of Activities, and as required locally.
Pregnancy testing will be performed whenever an expected menstrual cycle is missed or when pregnancy is otherwise suspected.