A PHASE 1/11 STUDY OF INTRALESIONAL IL-2, HYPOFRACTIONATED RADIOTHERAPY, AND PEMBROLIZUMAB IN PATIENTS REFRACTORY TO STANDARD-OF-CARE PD-1/PD-L1 CHECKPOINT BLOCKADE

Institutional Study #:	UCDCC#272, Merck OTSP #55571
Investigational Product/Modality:	Interleukin-2, Pembrolizumab
Principal Investigator(s):	Alta Monjazeb, MD, PhD Depa1tment of Radiation Oncology University of California, Davis School of Medicine 4501 X Street Sacramento, CA 95817 [redacted] Megan Daly, MD Depa1tment of Radiation Oncology University of California, Davis School of Medicine 4501 X Street Sacramento, CA 95817 [redacted]
Co-Investigators:	Emanual Maverakis, MD Depa1tment of De1matology UC Davis Comprehensive Cancer Center 4501 X Street Sacramento, CA 95817 [redacted] Robe1t J. Canter, MD Depa1tment of Surgical Oncology UC Davis Comprehensive Cancer Center 4501 X Street Sacramento, CA 95817 [redacted] Jonathan Riess, MD, MS UC Davis Comprehensive Cancer Center 4501 X Street Sacramento, CA 95817 [redacted]

Statistician:	Susan Stewart, Ph.D. Division of Biostatistics, Med Sci 1-C University of California, Davis One Shields Avenue Davis, CA 95616 [redacted]
IND #:	Exempt
Sponsor:	UC Davis Comprehensive Cancer Center
ClinicalTrials.gov ID	NCT03474497
Version:	June 11, 2024 September 7, 2022 February 26, 2020 May 20, 2019 November 2, 2018 July 10, 2018 January 12, 2018 December 7, 2017 Original / June 15, 2017

PROTOCOL SIGNATURE PAGE

Protocol Number: UCDCC#272

Protocol Title: A Phase I/II Study of Intralesional IL-2, Hypofractionated Radiotherapy, and Pembrolizumab in Patients Refractory to Standard-of-Care PD-1/PD-L1 Checkpoint Blockade

I have read this protocol and agree that it contains all necessary details for carrying out this study. I will conduct the study as outlined herein and will complete the study within the time designated, in accordance with all stipulations of the protocol and in accordance with Good Clinical Practices, local regulatory requirements, and the Declaration of Helsinki.

I will provide copies of the protocol and all pertinent information to all individuals responsible to me who assist in the conduct of this study. I will discuss this material with them to ensure that they are fully informed regarding the study agent(s) and the conduct of the study.

_____
Investigator Name (print)

_____
Investigator Signature

_____
Date

LIST OF ABBREVIATIONS AND TERMS

AE	Adverse event
AESI	Adverse event of special interest
ALK	Anaplastic lymphoma kinase
ALT	Alanine aminotransferase/serum glutamic pyruvic transaminase/SGPT
aPTT	activated partial thromboplastin time
ASCO	American Society of Clinical Oncology
AST	Aspartate aminotransferase/serum glutamic oxaloacetic transaminase/SGOT
CNS	Central nervous system
CR	Complete response
CT	Computed Tomography
CRF	Case Report/Record Form
CRP	C-reactive protein
CTCAE	Common Terminology Criteria for Adverse Events
CTV	Clinical target volume
DCR	Disease control rate
DFS	Disease-free survival
DLco	diffusing capacity of the lung for carbon monoxide
DLT	Dose limiting toxicity
DSMB	Data and Safety Monitoring Board
DVH	Dose volume histogram
ECOG	Eastern Cooperative Oncology Group
EGFR	Epidermal growth factor receptor
FACS	Fluorescence-activated cell sorting
FDA	U.S. Food and Drug Administration
FFPE	Formalin fixed paraffin embedded
FNA	Fine needle aspiration
GGO	Ground glass opacities
GTV	Gross tumor volume
HCV	Hepatitis C virus
HIV	human immunodeficiency Virus
Hr	Hour
HBsAg	Hepatitis B surface antigen
HD-IL-2	High dose interleukin-2
i.v.	Intravenous(ly)
ICH	International Conference on Harmonization
IEC	Independent Ethics Committee
IHC	Immunohistochemical
IDO	Indolamine 2,3 Dioxygenase
IL-2	Interleukin-2
IMP	Investigational medicinal product
IND	Investigational New Drug
INR	international normalized ratio
IRB	Institutional Review Board
IU	International unit
irAE	immune-related adverse event
irRECIST	Immune Related RECIST (irRECIST)

ITV	Internal target volume
LD	Longest diameter
LDH	Lactate dehydrogenase
LVEF	left ventricular ejection fraction
LFTs	Liver function tests
MeSH	Medical Subject Headings
MM	Metastatic melanoma
mRCC	Metastatic renal cell carcinoma
mHNSCC	Metastatic head and neck squamous cell carcinoma
MRI	Magnetic resonance imaging
MTD	Maximum tolerated dose
NCCN	National Comprehensive Cancer Network
NSCLC	Non small cell lung cancer
ORR	Objective response rate
OS	Overall survival
PBMC	peripheral blood mononuclear cell
PD	Progressive disease
PD-1	Programmed death-1
PD-L1	Programmed death-ligand 1
PFS	Progression-free survival
PICOS	Population, Intervention, Control, Outcome, Study Design
PR	Partial response
PTV	Planning treatment volume
QTcF	Fridericia-corrected QT
RCC	Renal cell carcinoma
REB	Research Ethics Board
RECIST	Response Evaluation Criteria in Solid Tumors
RNA	Ribonucleic acid
RT	Radiotherapy
SAE	Serious Adverse Event
SAR	Stereotactic ablative radiotherapy
SD	Stable disease
SITC	Society for Immunotherapy of Cancer
T3	Tri-iodothyronine
T4	Thyroxine
Target lesion	Lesions outside of the treatment field which are followed for response assessment
TCR	T cell receptor
TIL	Tumor-infiltrating lymphocyte
TLT	Treatment limiting toxicity
TME	Tumor Microenvironment
TNF	Tumor necrosis factor
Treatment lesion	Lesion treated with radiotherapy and intralesional IL-2 therapy
TSH	Thyroid-stimulating hormone
SOP	Standard Operating Procedure
UCDCCC	UC Davis Comprehensive Cancer Center
ULN	Upper limit of normal

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

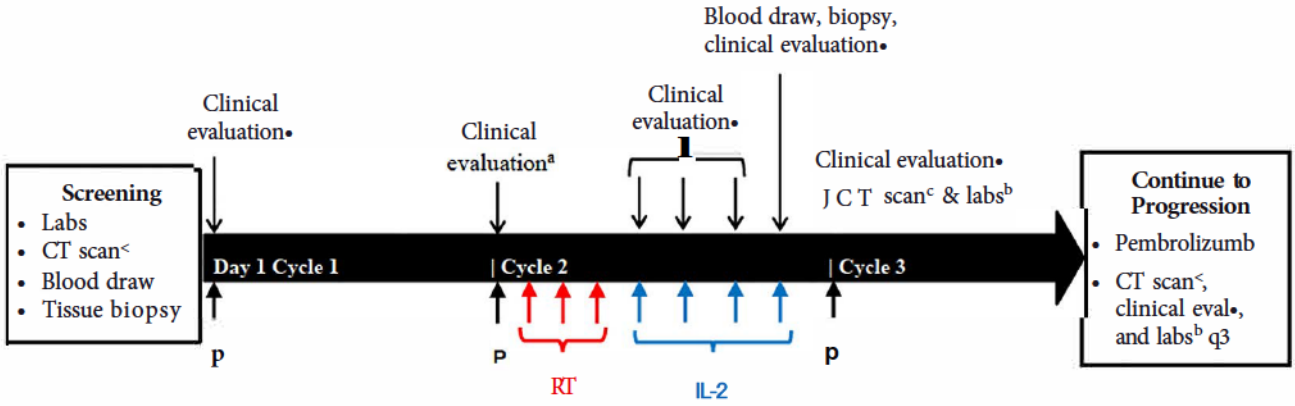
1.0 TRIAL SUMMARY

Abbreviated Title	A Phase I/II Study of Intralesional IL-2, Hypofractionated Radiotherapy, and Pembrolizumab in Patients Refractory to Standard-of-Care PD-1/PD-L1 Checkpoint Blockade
Trial Phase	I/II
Clinical Indication	Advanced solid malignancy or lymphoma. with progression on PD-1/L1 checkpoint inhibition.
Trial Type	Interventional
Route of administration	Pembrolizumab: intravenous IL-2: intratumoral injection Radiation: external beam
Trial Blinding	Unblinded, open-label
Treatment Groups	Phase I dose escalation of combination pembrolizumab, intralesional IL-2, and radiation in Advanced solid malignancy or lymphoma. Phase II dose expansion at MTD, which will incorporate a one-stage binomial design to assess efficacy and safety.
Number of trial subjects	25 maximum
Estimated enrollment period	28-36 months
Estimated duration of trial	4 years
Duration of Participation	28-36 months

2.0 TRIAL DESIGN

Patients with advanced solid malignancies or lymphoma with progression on prior PD-1/L 1 blockade will be emolled. A total maximum of 25 patients will be emolled.

Primary Objective: Abscopal response rate, ORR, DCR, PFS
Secondary Objective: Maximum tolerated dose (MTD), safety, and toxicity
Collateral Objectives: Immunophenotype serial tumor biopsies and blood samples



RT	+	Intralesional IL-2	+	Pembrolizumab
8 G y x 3 (completed in week 1 of Cycle 2)		4 doses injected into RT treatment lesion (completed in Cycle 2 after completion of RT)		200 mg q3 weeks

Phase I: IL-2 Dose Findini:
3+3 Design: (3-6 patient dose level, 6-18 patients total)

	Treatment 1 dose	Treatment 2 dose	Treatment 3 dose	Treatment4 dose
Dose Level -1	1 x 10^6 llJ	1 x 10^6 llJ	l x 1 0^6 IU	1 x 10^6 llJ
Dose Level 1	3 x 1 0^6 IU	3 x 1 0^6 IU	3 x 1 0^6 IU	3 x 10^6 llJ
Dose Level 2	3 x 1 0^6 IU	7 x 1 0^6 IU	7 x 1 0^6 IU	7 x 10^6 llJ
Dose Level 3	3 x 1 0^6 IU	7 x 1 0^6 IU	15 x 10^6 IU	15 x 10^6 IU

Phase II: IL-2 Dose Expansion

Binomial one-Stage Design (18 additional patients; analyzed with patients from MID)

- P0= 0.05, p l= 0.20, r l= 0, n l= 10, rTot=3, nTot=21, a= O.l, power=0.8
- Response will be assessed according to irRECIST and will be evaluated at the non-treatment lesions (abscopal response)

CT= computed tomography, DCR=disease control rate, MTD=maximum tolerated dose, ORR=objective response rate, PFS=progression free survival, RT=radiotherapy, P=pembrolizumab,

a. Patients will be clinically evaluated prior to each cycle of pembrolizumab for the first 3 cycles and weekly dtuing intralesional IL-2 injections, and thereafter every 3 cycles until off treatment.
b. Laboratory evaluation will occur pre-treatment at eve,y cycle.
c. For response assessment, patient will have CT imaging pre-treatment and after every three cycles of pembrolizumab.

3.0 OBJECTIVES

3.1 Primary Objective

1. To determine if this regimen converts patients with resistance to PD-1/PD-L1 checkpoint blockade into responders as determined by abscopal response rate (defined as response rate at lesions not treated with RT + IL-2) using immune related RECIST (irRECIST)
2. To determine the objective response rate (ORR), disease control rate (DCR), and progression free survival (PFS) using RECIST 1.1 (Response Evaluation Criteria for Solid Tumors)

3.2 Secondary Objectives

1. Phase 1: To determine the maximum tolerated dose (MTD) of intralesional IL-2 that can be administered with hypofractionated radiotherapy (RT) and pembrolizumab
2. Phase 2: To characterize the safety profile and toxicity of intralesional IL-2, RT, and pembrolizumab using CTCAE v4.03 (Common Toxicity Criteria for Adverse Events version 4.03).

3.3 Translational Objectives

1. To analyze serial blood samples for systemic cytokine / chemokine levels, and to quantify the number, function, T-cell diversity, and gene expression of circulating immune cell subsets.
2. To evaluate serial tumor tissue biopsies for tumor infiltrating immune cell subsets, T-cell diversity, expression of immune proteins including IDO and PD-L1, gene expression signatures, and mutational load.
3. To correlate immune parameters from translational objectives 1 and 2 with clinical outcomes to discover mechanisms of resistance and biomarkers of response.

4.0 BACKGROUND

4.1 Advanced Stage Non-Small Cell Lung Cancer

Lung cancer is the leading cause of cancer deaths worldwide because the majority of patients present with incurable, metastatic disease. In recent years, our increased understanding of the molecular biology of lung cancer has led to the development of more efficacious therapies for patients with advanced stage non-small cell lung cancer (NSCLC). As a result, overall survival has increased by several months and subsets of patients whose tumors harbor an EGFR mutation or ALK gene fusion enjoy prolonged survival beyond two years with targeted tyrosine kinase inhibitors. Nonetheless, there remains a great need to discover and develop novel agents that will improve survival for the majority of our patients.

4.2 Metastatic Melanoma

It was recognized early on that increased tumor-infiltrating lymphocytes bestowed improved survival for patients with melanoma[1]. Today it is clear that melanoma is an immunogenic malignancy with well-defined tumor antigens. The advent of immunotherapy has revolutionized the management of metastatic melanoma (mM), and immune checkpoint inhibitors such as PD-1/PD-L1 blockade have been associated with enhanced T-cell responses, increased tumor killing, and improved patient survival [2, 3]. However, despite the promising evidence for durable clinical responses, the majority of patients still fail to respond to immunotherapy. Thus, the development of multidrug treatment regiments to improve responsiveness is of paramount importance.

4.3 Metastatic Renal Cell Carcinoma

Renal cell carcinoma (RCC) often does not present until disease is locally advanced and unresectable or metastatic, and even then treatment options are often limited by the development of resistance to radiation and chemotherapy. The lack of significant efficacy with chemotherapy, with the recognition that RCC is an immunoresponsive tumor, has led research to focus on immunomodulation as a way to foster antitumor activity [4]. RCC is typically characterized by high levels of tumor infiltrating immune cells, including lymphocytes, macrophages and dendritic cells, but immune dysfunction is a clear driving factor behind RCC tumor growth [5]. The mechanisms that cause tumor escape include suppression of effector cells or immune cell dysfunction which could be mediated by immunosuppressive factors of the tumor microenvironment. Recent advances in cancer immunotherapy have benefited patients with metastatic RCC, but as with other histologies, response rates are low and resistance is common.

4.4 Metastatic Head and Neck SCC

Head and neck squamous cell carcinomas (HNSCC) are aggressive malignancies and distant metastases at presentation or recurrence is common. The mainstay of treatment for metastatic disease is platinum doublets and the EGFR inhibitor, cetuximab. The median survival for metastatic HNSCC remains less than 1 year. Immunotherapeutic approaches appear promising given patients with HNSCC have been shown to mount antibody responses to antigens that are expressed on the tumor tissue. However, trials of immunotherapeutic approaches for treatment of HNSCC have lagged significantly compared to other solid malignancies that have demonstrated advantages of combinatorial immunotherapeutic and radiation or chemo regimens.

4.5 Immune Checkpoint Inhibitors

A new treatment approach that targets the immune system has generated considerable enthusiasm. Program death-1 (PD-1) protein is a co-T-cell regulatory receptor that mediates immunosuppression by binding to the PD-L1 ligand found on tumor cells and stromal cells. Preclinical data has demonstrated that inhibition of this receptor-ligand interaction leads to an enhanced T-cell response and increased tumor killing. Pembrolizumab is a humanized antibody that targets the programmed cell death 1 (PD-1) receptor. Therapeutic blockade of PD-1 or

PD-L1 with monoclonal antibodies has been shown to lead to tumor regressions in patients with several cancer types.

In a phase I trial of anti-PD-1 blockade in 39 patients with advanced NSCLC, melanoma, RCC, colorectal cancer, or castrate-resistant prostate cancer, blocking the PD-1 immune checkpoint with intermittent antibody dosing was shown to be well-tolerated and associated with evidence of antitumor activity (sustained mean occupancy of > 70% of PD-1 molecules on circulating T cells ≥2 months following infusion) [6]. In a later phase I trial in patients with these histologies, objective responses (complete or partial responses) were observed in those with non-small-cell lung cancer, melanoma, or renal-cell cancer [7].

In nonsquamous histology the Phase III open-label, randomized CheckMate-057 trial evaluating previously treated patients was stopped early as the study met its endpoint of overall survival favoring nivolumab [8]. The median overall survival was 12.2 months in the nivolumab arm vs 9.4 months in the docetaxel arm (HR= 0.73 [96% CI: 0.59-0.89], p = 0.0015). There was not a statistically significant difference in progression-free survival in the two arms. However, an overall response rate of 19% (95% CI 15-24) was noted in the nivolumab arm (N=292) vs 12% (95% CI 9-17) in the docetaxel arm (N=290). Median duration of response in this trial was 17.2 months (range of 1.8 months to >22.6 months) in the nivolumab arm vs 5.6 months (>1.2 months to >15.2 months). In the nivolumab arm, 7% had treatment-related SAEs with 5% Grade 3-4 AEs, while in the docetaxel arm 20% treatment-related SAEs were reported with 18% Grade 3-4 AEs. Regarding treatment-related AEs of special interest, hypothyroidism occurred in 7% of patients in the nivolumab, though there were no Grade 3-4 endocrinopathies. Diarrhea was observed in 8% of patients with nivolumab, with 1 Grade 3/4 toxicity; diarrhea was noted at 23% in the docetaxel arm with 1 Grade 3/4 toxicity. Transaminitis was noted in 3% of patients in the nivolumab arm v 1% in the docetaxel arm. Pneumonitis was observed in 3% of nivolumab-treated patients, with 1% Grade 3/4 toxicity; <1% of patients in the docetaxel arm experienced pneumonitis. Skin rashes were observed with a rash in 9% of nivolumab-treated patients, pruritus in 8%, and erythema in 1%. In docetaxel-treated patients, rash was reported in 3% of patients, pruritus in 1%, erythema in 4%.

Recently, KEYNOTE-010 randomized 1034 patients with any tumor histology to pembrolizumab 2mg/kg (N=345), pembrolizumab 10 mg/kg (N=346) or docetaxel 75 mg/m2 (N=343) [9]. The median overall survival for pembrolizumab 2 mg/kg versus docetaxel was 10.4 months versus 8.5 months, respectively (HR 0.71 95% CI .58-.88; p<0.0008). For pembrolizumab 10 mg/kg the median overall survival was 12.7 months compared to 8.5 months for docetaxel (HR 0.61, 95% CI .49-.75; p<0.0001. There was no statistical difference in PFS between the groups. Objective response rates were 18% for both the pembrolizumab groups and 9% for docetaxel (p=0.0005 for pembrolizumab 2 mg/kg and p=0.0002 for pembrolizumab 10 mg/kg. Grade 3-5 adverse events occurred in 13%, 16% and 35% of patients in the pembrolizumab 2 mg/kg, 10 mg/kg and docetaxel arms. Adverse events of special interests any grade, included hypothyroidism 8%, 8% and <1%, pneumonitis 5%, 4% and 6%, and hyperthyroidism 4%, 6% and 1%, respectively. All other special interest AEs were less than 1%. Grade 3 or greater special interest AEs were only pneumonitis (2%) and skin reaction (2%) with pembrolizumab.

Based on this data that confirms the safety and efficacy of PD-1 inhibitors for the treatment of patients who have failed first line therapy these agents were FDA approved in 2015. As of 2016,

pembrolizumab is approved in patients with unresectable or metastatic melanoma; patients with metastatic NSCLC whose tumors express PD-L1 and demonstrate disease progression on or after platinum-containing chemotherapy; and patients recurrent or metastatic HNSCC with disease progression on or after platinum-containing chemotherapy. Given that approximately 20% of patients benefit from these immune checkpoint inhibitors, combination strategies to increase the number of responders must be now be pursued.

4.5.1 Checkpoint Inhibitors in Advanced NSCLC

The first report of promising efficacy for immune checkpoint inhibitors in lung cancer was seen in a phase I trial of nivolumab (a fully humanized IgG4 PD-1 blocking antibody) (2). In the subset of heavily pretreated NSCLC patients, 22 of 129 patients (17%) achieved an objective response rate (ORR). Responses were durable. The median progression free survival (PFS) for the lung cancer patients was 2.3 months with a median overall survival (OS) was 9.6 months [7, 10] was seen across all histological subtypes.

Subsequently, there has been 3 randomized trials with a PD-1 agent either nivolumab or pembrolizumab that have shown an overall survival benefit for these agents. Efficacy was higher in patients whose tumor expressed PD-L1 by immunohistochemistry but responses were seen in patients with tumors that did not expression of PD-L1. The first study, Checkmate-017, was conducted in patients with squamous cell tumor histology. A total of 227 patients received nivolumab (3 mg/kg IV every 2 weeks) or docetaxel (75 mg/m2 IV every 3 weeks) [11] . A 41% reduction in risk of death (HR: 0.59, 95% CI: 0.44-0.79) was observed with nivolumab, with the median overall survival in the nivolumab arm was 9.2 months (95% confidence interval [CI], 7.3 to 13.3) compared to 6 months (95% CI, 5.1 to 7.3) in the docetaxel arm. The confirmed objective response rate was significantly higher with nivolumab than with docetaxel (20% [95% CI, 14 to 28] vs 9% [95% CI, 5 to 15]; p=0.008).

4.5.2 Checkpoint Inhibitors in Metastatic Melanoma

Pembrolizumab was first evaluated in the large phase 1 KEYNOTE-001 study in which 411 patients with advanced melanoma were followed for 18 months and found to have a response rate of 34%, which was maintained in 81% of those patients[12]. Median OS was 25.9 months. The KEYNOTE-002 study of pembrolizumab vs. chemotherapy confirmed survival benefit in patients who had disease progression during/after ipilimumab therapy [13].

Later, in a randomized phase 3 study of pembrolizumab vs. ipilimumab (KEYNOTE-006), two regimens of pembrolizumab improved both PFS and OS in patients with advanced melanoma[14].

Recently, in an open-label phase Ib clinical trial of 655 patients with advanced melanoma, pembrolizumab was associated with an overall objective response rate of 33%, 12-month PFS rate of 35%, and median OS of 23 months. Grade 3 or 4 treatment-related AEs occurred in 14% [15].

In another recent phase Ib study, KEYNOTE-001 examined the use of pembrolizumab once every 2 weeks or once every 3 weeks, or 2 mg/kg once every 3 weeks, in 655 patients with advanced melanoma. Of 451 patients with evaluable PD-L1 expression, 76% had PD-L1-positive

tumors. PD-L1 expression in pretreatment tumor biopsy samples was correlated with response rate, and longer PFS and OS [hazard ratio, 0.76; 95% CI, 0.69 to 0.83]) [16].

4.5.3 Checkpoint Inhibitors in Metastatic Renal Cell Carcinoma

Historically, high-dose IL-2 and interferon- α2B have been viable immunotherapeutic options for subsets of patients with RCC[17]. More recently, potential for PD-1 based therapies has been shown, however evidence is still early for use of pembrolizumab in mRCC. In a Phase I study of 33 RCC patients treated with nivolumab, objective responses were seen in 24% of patients treated with 1 mg/kg and 31% of patients treated with 10 mg/kg and shown to be durable responses[7].

Pembrolizumab is currently being tested with axitinib and thus far has been shown to be well-tolerated at standard doses and exhibited substantial antitumor activity in a small pilot cohort of 11 treatment-naïve patients with advanced RCC [18]. Additional patients are being enrolled to confirm the Phase II dose and further evaluate safety and antitumor activity of this combinatorial approach.

4.5.4 Checkpoint Inhibitors in Metastatic Head and Neck Squamous Cell Carcinoma

In a phase 1b KEYNOTE 012 study of patients with recurrent or metastatic head and neck cancer, pembrolizumab was shown to be well-tolerated and demonstrated antitumor activity[19]. It achieved a response rate of 20% in patients with PD-L1 expression in the first phase of the study. The expansion cohort included 132 patients who were unselected for PD-L1 expression and received a fixed dose of pembrolizumab (200 mg/3weeks), and tumor shrinkage was reported in 57% of patients. After data from the KEYNOTE012 study showed an objective response rate (ORR) of 16% (95% CI: 11, 22), a complete response rate of 5%, with responses of six months or longer observed in 82% (n=23/28) of the responding patients, the FDA accelerated approval for this indication for pembrolizumab.

4.6 High Dose Systemic Interleukin-2

High dose IL-2 (HD-IL-2) is a cytokine produced endogenously by activated T cells and is effective in the treatment of a variety of malignancies because it has both immune-modulating and antitumor properties[20]. This therapy has proven effective in the management of both metastatic melanoma (MM) and metastatic renal cell carcinoma (mRCC) with the ability to induce durable long term responses in a minority of patients.

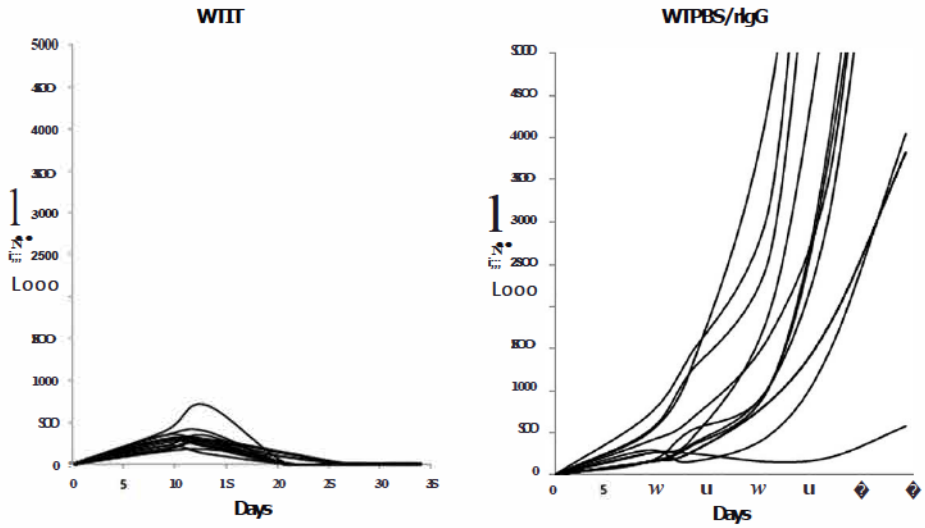
HD-IL-2 is associated with significant toxicity which has limited its use. Significant cardiopulmonary toxicity has been reported with grade 3/4 hypotension requiring vasopressor support in up to 81% of patients and resulting in subsequent end-organ dysfunction causing transient: renal failure, hepatic dysfunction, metabolic derangements and gastrointestinal toxicity [21, 22]. Related toxicities typically resolve within 2-3 days after withdrawal of IL-2 [23]. Initially, HD-IL-2 was associated with mortality rates of up to 4%, and while these rates have decreased as clinicians have gained experience and developed protocols for IL-2 use, IL-2 therapy can still cause significant dose-, delivery- and schedule- dependent morbidity. In an effort to reduce treatment toxicity with high dose systemic therapy studies have examined the

efficacy oflow-dose systemic IL-2 but found high-dose regimens to be superior [24, 25]. HD-IL-2 given as an IV bolus is more toxic than when given as a low dose bolus or subcutaneously [20, 21, 23, 25, 26]. In order to ha.mess the clinical benefits of IL-2, less toxic methods of delively needed to be developed.

4.6.1 HD-IL-2 in Advanced NSCLC

There is little clinical data testing systemic HD-IL-2 for the treatment of lung cancer but preclinical data from a murine model has shown that HD-IL-2 alone is an effective treatment for pulmonaiy metastases with the ability to achieve a durable CR in 50% oftest specimens[27]. Our group has demonstrated that HD-IL-2 used in a combinatorial approach can cause complete regression of mouse 3LL lung cancers (Figure 1).

Figure 1. IL-2 based immunotherapy induces complete regression of murine 3LL lung cancers



4.6.2 HD-IL-2 in Metastatic Melanoma

In a HD-IL-2 trial of 684 patients with mM the overall response rate was 13% with survival beyond 4 yeai·s [28]. Importantly, many of the patients responding to this therapy remain disease free for prolonged periods and are apparently cured. Promisingly, a recent retrospective analysis of 170 patients with mM and 192 patients with mRCC treated with HD IL-2 in between the years of 2005 - 2012 repo1ted an improved median overall survival compai·ed to the historical experience. The mOS for mRCC was 41 months and for mM 19.6 months [29].

4.6.3 HD-IL-2 in Metastatic Renal Cell Carcinoma

As in melanoma, high-dose IL-2 has demonstrated an ability to induce durable responses in select patients with RCC. A phase II clinical trial assessing HD-IL-2 in 255 patients with mRCC and showed an overall objective response rate of 14%, with 5% CR, 9% PR, and an estimated OS of 16.5 months [30]. However, treatment was associated with severe acute toxicities, and 4%

of patients died of adverse events judged to be probably treatment-related. More recently, in a relatively large cohort of HD-IL-2 patients, an overall objective response rate of 15.9% was shown, with a median OS of 35.5 months (22.2-38.6 months), corroborating historical reports [31].

Nonetheless its use is limited by the significant side effect profile that has led to restrictions in adoption of its widespread usage, high degree of expertise required in patient selection and HD-IL-2 administration (intensive care type of setting).

4.6.4 HD-IL-2 in Metastatic Head and Neck Squamous Cell Carcinoma

IL-2 and interferon were the first cytokines used in treating HNSCC[32]. The use of IL-2 in HNSCC has been limited to local-regional or intralesional injection. Local IL-2 based therapies have been associated with favorable local and systemic activation of the immune system. See Section 4.8.4 below.

4.7 HD-IL-2 and Radiotherapy

Traditionally, ionizing radiation therapy (RT) was believed to act as an immunosuppressant, exerting its cytotoxic mitotic effects on present tumor cells through DNA damage [33, 34]. However, a growing body of evidence demonstrates that radiation induces immunomodulatory effects, changing the tumor microenvironment and upregulating the inflammatory cascade. Tumors that are exposed to RT release antigens which subsequently activate the innate and adaptive host immune response [33-35]. To this end, radiation combined with specific cytokines enhances the anti-tumor effects of radiation [36]. Xian et al. showed a combination of IL-2 and IL-12 gene therapy and radiation generates potent anti-tumor immune responses against head and neck cancer in an orthotopic murine model [36].

In humans, promising early evidence exists for combining HD-IL-2 and RT in mM, RCC and other solid tumors [37-40]. A recent phase 1 trial testing the combination of HD-IL-2 with hypofractionated RT demonstrated a 60% response rate compared to roughly 15% in historical controls of HD-IL-2 alone. Importantly, no unexpected or additive adverse effects of combining high dose IL-2 with RT were reported.

4.8 Intralesional IL-2

In an attempt to take advantage of the robust immune activating effects of IL-2 but avoid the toxicity of high dose systemic IL-2 we and others have investigated the use of intralesional IL-2 injections.

4.8.1 Intralesional IL-2 in Advanced NSCLC

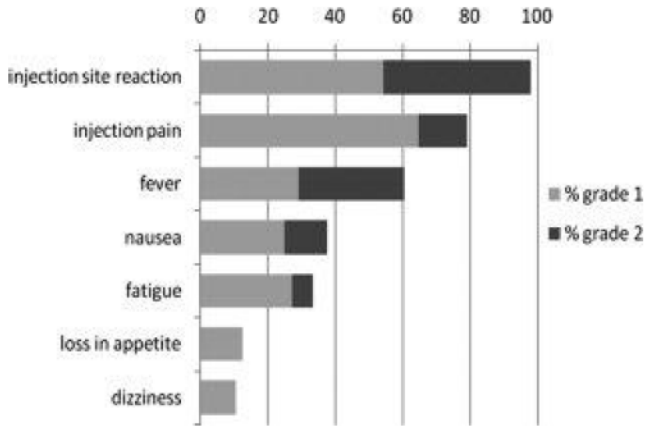
A number of studies have evaluated local IL-2 administration for primary or metastatic malignant lung lesions. Lissoni et al examined intracavitary IL-2 treatment for malignant pleural effusions and demonstrated a 72% response rate and the only toxicity was fevers in 6% of patients [41]. A study from Italy has tested systemic HD-IL-2 in combination with intralesional IL-2 for NSCLC [42]. This trial reported a 25% response rate and a 62.5% disease control rate.

No unexpected toxicities were observed from the intralesional injection of IL-2 although one third of patients experienced pneumothorax from the repeated transparietal injections. Overall intralesional toxicity rates from this trial are difficult to determine given the use of systemic high dose IL-2. Due to the risk of pnuemothorax from our transparietal lesions, our trial does not allow for the treatment of lung lesions.

4.8.2 Intralesional IL-2 in Metastatic Melanoma

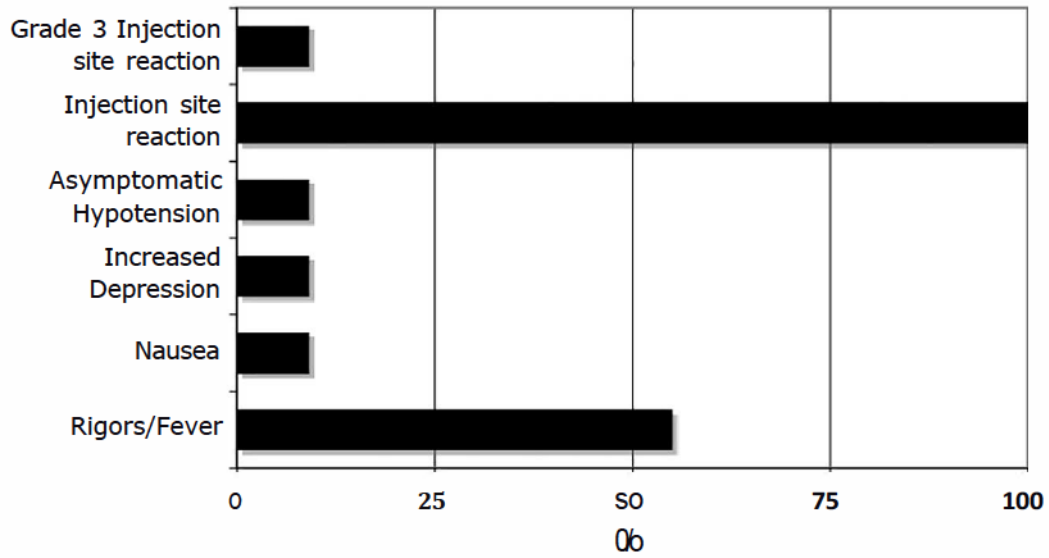
One of the largest reports, which included 48 patients with metastatic melanoma, indicates that this approach is highly effective with a 70% response rate of injected lesions and limited grade 1-2 toxicity [43]. This study incorporated an initial dose of 3 million IU which was escalated within each patient as tolerated. Treatments occurred 3x weekly for a median duration of 6 weeks and the dose was escalated up to a maximum of 16 million IU. The most common toxicities included injection site reaction in nearly all patients, fever in 58% of patients, fatigue in 36% of patients, and nausea in 34% of patients. The fevers were responsive to acetaminophen. The toxicities are outlined in Figure 2.

Figure 2. Limited Grade 1-2 Toxicity Associated with Intralesional IL-2



Researchers at UC Davis have reported a 100% response rate using intralesional IL-2 with topical immune agents for in-transit melanoma [44, 45] and have recently published guidelines for the use of intralesional IL-2 that have been adopted by NCCN [46]. Importantly, it has been demonstrated that intralesional IL-2 treatment is associated with minimal toxicity and can be administered on an outpatient basis. In the recent report, 6 of 11 patients developed rigors within 3 to 6 hours post injection which were self-limited and lasted 15-30 minutes. One patient developed asymptomatic hypotension necessitating holding antihypertensive medications on subsequent treatment days. Intralesional IL-2 therapy was discontinued in one patient after several months because of the development of local urticaria at treatment sites. One patient with interstitial lung disease developed shortness of breath 4 hours after her seventh injection (7 million IU). Although she was found to have a normal oxygen-saturation and chest x-ray was unchanged from baseline, IL-2 was discontinued as a precaution. This same patient required surgical debridement of necrotic tissue resulting from intralesional therapy. Adverse events in the UC Davis experience are summarized below with all toxicities classified as grade 1 / 2 with the exception of grade 3 injection site reactions.

Figure 3. UC Davis Experience: Intralesional IL-2 Toxicity



Impo11antly, intralesional IL-2 has also demonstrated safety and efficacy in a number of other disease sites includes oral squamous cell carcinoma [47], liver cancer [48] and lung [42].

4.8.3 Intralesional IL-2 in Metastatic Renal Cell Carcinoma

Intralesional IL-2 has been tested in patients with progressive metastatic renal cell carcinoma (RCC) in a phase I trial in combination with granulocyte macrophage colony-stimulating factor (GM-CSF) and IFN-alpha[49]. The results were promising: three of eight patients with progressive metastatic RCC after nephrectomy achieved complete remission. Huland et al. introduced IL-2 inhalation therapy for 116 patients with pulmonaiy and mediastinal metastases from RCC who could not tolerate HD-IL-2 [50]. Progressive pulmonaiy metastases responded dramatically in 15% of the patients for a median of 15.5 months and were stabilized in 55% of patients for a median of 6.6 months. Toxicity associated with IL-2 inhalation was local and consisted mainly of cough and patients were able to be treated on an outpatient basis.

4.8.4 Intralesional IL-2 in Metastatic Head and Neck Squamous Cell Carcinoma

Timai· et al. [47] repo11ed a 42 % response in the treatment of oral squamous cell cai·cinoma and repo11 the complete absence of adverse effects with this regimen although it should be noted that the dose of intralesional IL-2 they incmporated was significantly lower than that repo11ed for melanoma lesions. A phase III trial showed that patients with resectable SCCs of the oral cavity and oropha1ynx treated with perilymphatic intralesional administration of low-dose IL-2 had longer disease free and overall survival [51].

Taken together these data demonstrate cleai· efficacy and safety for the use of intralesional IL-2 for the treatment of mali$_{gn}$ant lesions at dennal, subcutaneous, and non-pulmonaiy visceral sites. A summaiy of relevant toxicities from intralesional IL-2 can be found in Table 1. It is impo11ant to note that most of these trials employed concomitant therapies including concomitant

immunotherapy. Critically, of 187 patients treated across these trials only two (1.1%) experienced grade 3 toxicity.

Table 1. Common Adverse Events with Intralesional IL-2 in ≥ 10% of Patients

	N	Site	Grade 1/2 Injection Site	Grade 1/2 Injection Pain	Grade 1/2 Fever / Chills / Flu-like Symptoms	Grade 1/2 Fatigue	Grade 1/2 Nausea	Grade 1/2 Local Infection	Grade 1/2 Stomach Pain	Grade 1/2 Diarrhea	Grade 1/2 Headache
Radny 2003	24	Dermal, SQ	100%	--	58%	46%	41%	--	17%	12%	12% (4% Gr 3)
Green 2007	13	Dermal, SQ	100%	--	Several patients	--	15%	15%	--	--	--
Weide 2010	52	Dermal, SQ	98%	78%	58%	36%	34%	--	--	--	--
Boyd 2011	39	Dermal, SQ	--	100%	85%	--	--	--	--	--	--
Shi 2015	11	Dermal, SQ	100% (9% Gr3)	--	55%	--	--	--	--	--	--
Timar 2005	39	Oral mucosa	--	--	--	--	--	--	--	--	--
Ferlazzo 1997	9	Liver	--	--	Several Patients	--	--	--	--	--	--

4.8.5 IL-2 Dosing Rationale

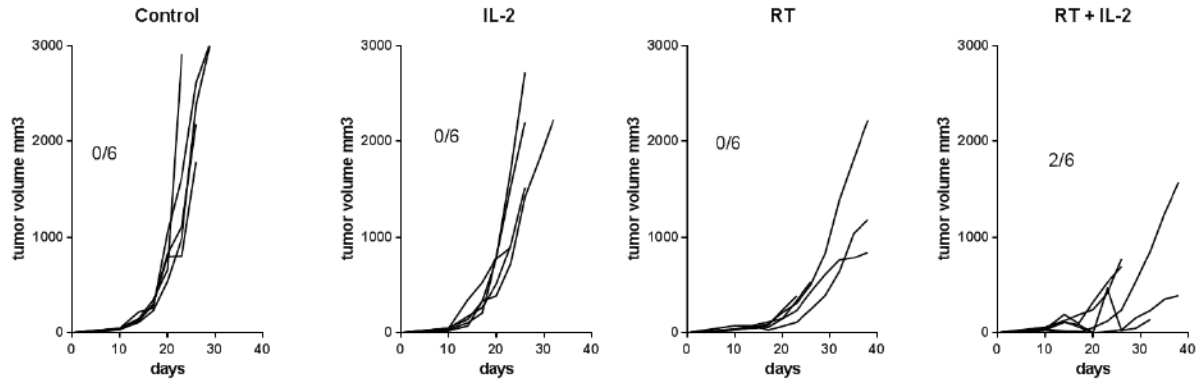
Four doses of intralesional IL-2 will be evaluated: -1) 1 x 10^6 IU; 1) 3 x 10^6 IU; 2) 7 x 10^6; 3) 15 x 10^6 IU. These dose levels were selected based on the clinical data described above and our clinical experience with intralesional IL-2. One of the largest reports, which included 48 patients with metastatic melanoma, indicates that this approach is highly effective with a 70% response rate of injected lesions and limited grade 1-2 toxicity [43]. This study incorporated an initial dose of 3 million IU which was escalated within each patient as tolerated. Treatments occurred 3x weekly for a median duration of 6 weeks and the dose was escalated up to a maximum of 16 million IU. Additionally, the selected dose levels are institutional practice at UC Davis have been reported in several clinical series [44, 45] and we have recently published these guidelines for the use of intralesional IL-2 that have been adopted by NCCN [46].

We will initiate the trial at Dose level 3 and de-escalate to lower dose levels as needed to establish the MTD. We have chosen this approach for four reasons: 1) Two patients have already been treated using this same treatment protocol at dose level 3 on our pilot trial UCDCC 269 with no evidence of dose limiting toxicities. 2) This allows patients on trial to begin treatment at the dose believed to be effective and previously shown to be safe and therefor provides the greatest potential benefit for study participants. 3) Even at dose level 3 the first dose given to patients is 3 x 10^6 IU, thus the intrapatient dose escalation which is the standard approach for intralesional IL-2 administration provides an additional safety assessment.4) Dose level 3 is our institutional practice which has been administered to numerous patients off trial with limited toxicities.

4.9 Intralesional Interleukin-2 in Combination with Radiotherapy or Checkpoint Blockade Therapy.

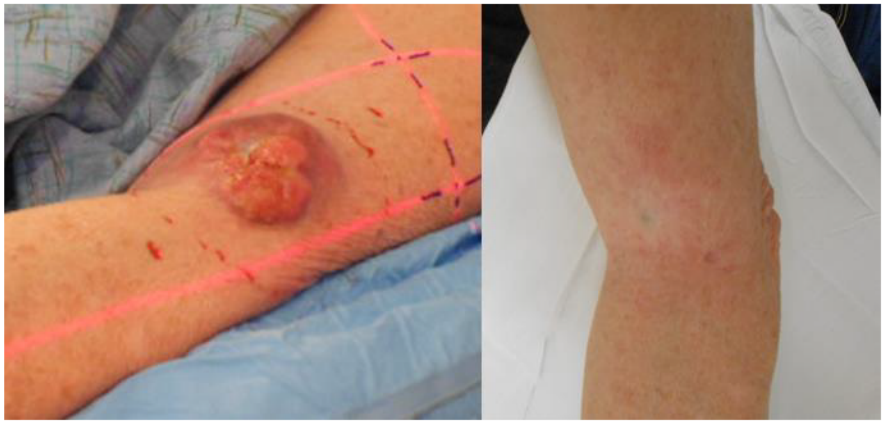
Although HD-IL-2 has been combined with RT there is limited published data examining intralesional IL-2 in combination with RT. Preliminary mouse model data generated in our lab demonstrates a synergistic anti-tumor effect to this combination similar to systemic HD-IL-2 combinations.

Figure 4. Radiotherapy + Intralesional IL-2 in a murine melanoma model



Furthermore, in two patients with metastatic melanoma who received intralesional IL-2 and palliative radiotherapy we observed complete regression of irradiated / injected lesions and long term progression free survival without any evidence of unexpected or additive toxicity. Taken together these preclinical studies and clinical observations suggest synergy between radiotherapy and intralesional IL-2.

Figure 5. Complete regression observed in irradiated/injected metastatic melanoma lesions



A recent report at ASCO 2015 has also demonstrated the safety and efficacy of intralesional IL-2 in combination with intralesional CTLA-4 checkpoint blockade. Twelve patients were treated with no dose limiting toxicities and a 75% response rate at untreated lesions.

4.10 Intralesional IL-2 + RT in patients refractory to PD-1/PD-L1 checkpoint blockade

The advent of checkpoint blockade immunotherapy has revolutionized the management of metastatic cancer. Despite the promising evidence for deep and durable responses with these agents the majority of patients fail to respond. It is hypothesized that a novel strategy combining radiotherapy and intralesional interleukin-2 (IL-2), a signaling molecule and member of the cytokine family involved in the activation of leukocytes and lymphocytes, may overcome resistance to checkpoint blockade therapy and offer significant clinical benefit to patients who fail to respond to checkpoint blockade alone. We are proposing a bold combinatorial immunotherapy strategy consisting of radiotherapy (RT), intralesional IL-2, and check-point blockade for patients with metastatic NSCLC, melanoma, RCC, and HNSCC who have progressed after checkpoint inhibition. IL-2 can upregulate PD-1 expression and activate T-cells. There is data supporting combination therapies with IL-2 and checkpoint blockade, IL-2 and radiotherapy, and checkpoint blockade and radiotherapy but clinical data is limited and the triple combination has never been tested. IL-2 + checkpoint blockade was recently tested in a small clinical trial and showed promising results but RT was not included in this trial. As outlined above RT has been demonstrated to increase the efficacy of both IL-2 and checkpoint blockade. We believe that the triple combination of radiotherapy + IL-2 + checkpoint inhibition will be highly effective as RT + IL-2 can serve highly activate the immune system and checkpoint blockade can reverse the immune suppressive pathways induced by tumor and therapy.

We hypothesize that the triple combination of radiotherapy + intralesional IL-2 + Pembrolizumab will be highly effective and convert patients with primary or secondary resistance to checkpoint blockade into responders. We hypothesize that the combination of intralesional IL-2 with radiotherapy will act as an "in-situ" vaccine inducing an anti-tumor immune response and increasing tumor infiltrating lymphocytes thereby overcoming a major mechanism of checkpoint blockade resistance while the continuation of PD-1 inhibition will be critical for overcoming tumor and therapy induced immune suppression. This trial will incorporate robust correlative assays to provide insights into mechanisms of primary and secondary resistance to checkpoint blockade and how this therapy may overcome that resistance. This trial, although small, has the potential to drastically advance both our understanding and treatment of PD-1/PD-L1 checkpoint blockade resistant cancer. We reviewed the National Institutes of Health Clinical Research Studies (https://clinicaltrials.gov/), which are open and available for patient enrollment, and there are no open trials looking at the effects of intralesional IL-2 combined with radiation and checkpoint inhibition. Collectively, this provides the basis of our proposal which is extensively detailed below.

4.11 Biomarkers

One of the major shortcomings of immunotherapy trials has been the lack of in-depth correlative studies to help identify the mechanism of action, identify biomarkers of response, and explain the mechanisms governing treatment response or failure. To address this, we plan to perform in depth immunological analysis of patient blood and tumor samples. This study incorporates serial tissues samples pre- treatment, during therapy, and at progression. The design of this study allows us to compare immune mechanisms pre- to post-therapy.

Tumor PD-L1 expression has been linked to response rates of PD-1 checkpoint blockade[7]. Pre-clinical data suggests that radiotherapy may upregulate intratumoral PD-L1 expression[52] but little is known about the dose response of these effects. Additionally, little is known about how the pattern of expression (i.e., tumor cells vs. infiltrating immune cells) affects response. Data presented at ASCO and SITC indicate that durvalumab monotherapy response rates in NSCLC are 27% in PD-L1 positive tumors and 5% in PD-L1 negative tumors. TILs and a T-cell inflamed phenotype have been linked with response to CTLA-4 checkpoint blockade in melanoma [53, 54]. Exclusion of T-cells in the tumor microenvironment has also been linked with lack of response to checkpoint inhibition [55] with down-regulation of CCL4 mediated dendritic cell recruitment as one potential mechanism [55]. RT has been demonstrated to induce TILs[56] and may potentially overcome this mechanism of resistance to checkpoint blockade. Our unpublished data suggests that RT can induce CCL4 expression and increase TILs. Likewise, IL-2 may expand intratumoral T-cells helping to circumvent the mechanisms of checkpoint blockade resistance.

Mutational burden and antigenic load are thought to represent a surrogate for tumor antigenicity and have been linked with response to checkpoint blockade in NSCLC [57]. Whether mutational load is a true indicator of tumor antigenicity and how this correlates with response to this combinatorial approach is unknown.

5.0 METHODOLOGY

5.1 Entry Criteria

5.1.1 Diagnosis/Condition for Entry into the Trial

Advanced solid malignancy or lymphoma with progression on PD-1/PD-L1 checkpoint blockade.

5.1.2 Subject Inclusion Criteria

1. Adults ≥18 years of age with histologically proven, advanced solid malignancy or lymphoma.
2. Clinical and/or radiographic progression on checkpoint blockade therapy (either after prior response or primary refractory).
3. ECOG (Eastern Cooperative Oncology Group) performance status score of 0 – 1 (Appendix 1)
4. Presence of a candidate treatment lesion (subcutaneous, muscular, nodal, or other soft tissue lesions, and other lesions will also be considered with PI approval) accessible and safe for radiotherapy and serial intralesional injections.
5. Presence of at least one target lesion (distinct from treatment lesion and outside of treatment lesion radiation field) evaluable for response by irRECIST
6. Life expectancy ≥ 6 months
7. Demonstrate adequate organ function as defined in Table 2, all screening labs should be performed within 10 days of treatment initiation.

Table 2. Adequate Organ Function Laboratory Values

System	Laboratory Value
Hematological	
Absolute neutrophil count (ANC)	≥1,500 /mcL
Platelets	≥100,000 / mcL
Hemoglobin	≥9 g/dL or ≥5.6 mmol/L without transfusion or EPO dependency (within 7 days of assessment)
Renal	
Serum creatinine **OR** Measured or calculated[a] creatinine clearance (GFR can also be used in place of creatinine or CrCl)	≤1.5 X upper limit of normal (ULN) **OR** ≥60 mL/min for subject with creatinine levels > 1.5 X institutional ULN
Hepatic	
Serum total bilirubin	≤ 1.5 X ULN **OR** Direct bilirubin ≤ ULN for subjects with total bilirubin levels > 1.5 ULN
AST (SGOT) and ALT (SGPT)	≤ 2.5 X ULN **OR** ≤ 5 X ULN for subjects with liver metastases
Albumin	≥2.5 mg/dL

Coagulation	
International Normalized Ratio (INR) or Prothrombin Time (PT)	≤1.5 X ULN unless subject is receiving anticoagulant therapy as long as PT or PTT is within therapeutic range of intended use of anticoagulants
Activated Partial Thromboplastin Time (aPTT)	≤1.5 X ULN unless subject is receiving anticoagulant therapy as long as PT or PTT is within therapeutic range of intended use of anticoagulants
[a]Creatinine clearance should be calculated per institutional standard.	

8. Deleted as of Amendment 09/07/2022.
9. Female subject of childbearing potential should have a negative urine or serum pregnancy within 4 days prior to receiving the first dose of study medication. If the urine test is positive or cannot be confirmed as negative, a serum pregnancy test will be required.
10. Female subjects of childbearing potential (Section 5.7.2) must be willing to use an adequate method of contraception as outlined in Section 5.7.2 – Contraception, for the course of the study through 120 days after the last dose of study medication.
    Note: Abstinence is acceptable if this is the usual lifestyle and preferred contraception for the subject.
11. Male subjects of childbearing potential (Section 5.7.2) must agree to use an adequate method of contraception as outlined in Section 5.7.2 - Contraception, starting with the first dose of study therapy through 120 days after the last dose of study therapy.
    Note: Abstinence is acceptable if this is the usual lifestyle and preferred contraception for the subject.
12. Signed informed consent.
13. Ability to comply with the protocol.
14. Systolic blood pressure ≥80.
15. Deleted as of Amendment 09/07/2022.
16. Deleted as of Amendment 09/07/2022.

5.1.3 Subject Exclusion Criteria

1. Uncontrolled concomitant disease.
2. Is currently participating and receiving study therapy or has participated in a study of an investigational agent and received study therapy or used an investigational device within 4 weeks of the first dose of treatment.
3. Has a diagnosis of immunodeficiency or is receiving systemic steroid therapy or any other form of immunosuppressive therapy within 7 days prior to the first dose of trial treatment. Use of inhaled or topical steroids or systemic corticosteroids < 10 mg/ day and prednisone equivalent ≤10mg/d is permitted.
4. Has a known history of active TB (Bacillus Tuberculosis)

5. Hypersensitivity to pembrolizumab or any of its excipients.
6. Has had a prior anti-cancer monoclonal antibody (mAb) (excluding PD-1/PD-L1 inhibitor) within 4 weeks prior to study Day 1 or who has not recovered (i.e., ≤ Grade 1 or at baseline) from adverse events due to agents administered more than 4 weeks earlier.
7. Has had prior chemotherapy, targeted small molecule therapy, or radiation therapy within 2 weeks prior to study Day 1 or who has not recovered (i.e., ≤ Grade 1 or at baseline) from clinically significant adverse events due to a previously administered agent.
   Note: Subjects with ≤ Grade 2 neuropathy and ≤ Grade 2 adrenal insufficiency are an exception to this criterion and may qualify for the study.
   Note: If subject received major surgery, they must have recovered adequately from the toxicity and/or complications from the intervention prior to starting therapy.
8. Has a known additional malignancy that is progressing or requires active treatment. Exceptions include basal cell carcinoma of the skin or squamous cell carcinoma of the skin that has undergone potentially curative therapy or in situ cervical cancer.
9. Has known active central nervous system (CNS) metastases and/or carcinomatous meningitis. Subjects with previously treated brain metastases may participate provided they are stable (without evidence of progression by imaging for at least four weeks prior to the first dose of trial treatment and any neurologic symptoms have returned to baseline), have no evidence of new or enlarging brain metastases, and are not using steroids for at least 7 days prior to trial treatment. This exception does not include carcinomatous meningitis which is excluded regardless of clinical stability.
10. Has active autoimmune disease that has required systemic treatment in the past 2 years (i.e., with use of disease modifying agents, corticosteroids or immunosuppressive drugs). Replacement therapy (e.g., thyroxine, insulin, or physiologic corticosteroid replacement therapy for adrenal or pituitary insufficiency, etc.) is not considered a form of systemic treatment.
11. History of severe autoimmune disease.
12. Treatment with systemic immunostimulatory agents within 4 weeks or five half-lives of the drug, whichever is shorter, prior to enrollment (with the exception of checkpoint blockade therapy).
13. Treatment with systemic corticosteroids or other systemic immunosuppressive medications within past 4 weeks or 5 half-lives whichever is shorter. Use of inhaled or topical steroids or systemic corticosteroids < 10 mg/day and prednisone equivalent ≤10mg/d is permitted.
14. Has a history of (non-infectious) pneumonitis that required steroids or current pneumonitis.
15. Has an active infection requiring systemic therapy.
16. Has a history or current evidence of any condition, therapy, or laboratory abnormality that might confound the results of the trial, interfere with the subject's participation for the full duration of the trial, or is not in the best interest of the subject to participate, in the opinion of the treating investigator.

17. Has known psychiatric or substance abuse disorders that would interfere with cooperation with the requirements of the trial.
18. Is pregnant or breastfeeding, or expecting to conceive or father children within the projected duration of the trial, starting with the pre-screening or screening visit through 120 days after the last dose of trial treatment.
19. Has a known history of Human Immunodeficiency Virus (HIV) (HIV 1/2 antibodies).
20. Has known active Hepatitis B (e.g., HBsAg reactive) or known Hepatitis C (e.g., HCV RNA [qualitative]).
21. Has received a live vaccine within 30 days of planned start of study therapy.

    Note: Seasonal influenza vaccines for injection are generally inactivated flu vaccines and are allowed; however intranasal influenza vaccines (e.g., Flu-Mist®) are live attenuated vaccines, and are not allowed.
22. Patients unable to tolerate checkpoint inhibitor therapy.
23. Unresolved Grade 3 or any grade 4 non-hematological, treatment-related AEs attributed to prior PD-1/ PD-L1 checkpoint blockade. A minimum of 2 weeks from prior PD-1/PD-L1 checkpoint blockade to initiating study treatment.

5.2 Trial Treatments

This is a phase I/II study that will evaluate the safety and toxicity of this combinatorial approach. Eligible patients ≥18 years of age with histologically proven advanced solid malignancy or lymphoma who have failed PD-1 / PD-L1 checkpoint blockade therapy will be enrolled. Patients must have a candidate treatment lesion accessible and safe for radiotherapy and serial intralesional injections as specified by the protocol. They must also have at least one target lesion (distinct from treatment lesion and outside of treatment lesion radiation field) evaluable for response by RECIST.

This study will consist of a phase I dose escalation / de-escalation using a standard 3+3 design to determine safety and MTD of intralesional IL-2 which will be dose escalated in conjunction with standard fixed doses of RT and Pembrolizumab. The study will begin at dose level 3 and the dose will be de-escalated if needed to determine the MTD. At the MTD there will be a phase II dose expansion which will incorporate a simon-two stage design to assess efficacy and safety.

Patients will receive pembrolizumab and intralesional IL-2 in combination with hypofractionated radiotherapy.

- Radiotherapy will be delivered to the treatment lesion during the second cycle of therapy using an 8 Gy x 3 fractions palliative regimen. Fractions may be delivered on consecutive days or every other day but must be completed during weeks 1-2 of cycle 2 and will not be repeated in future cycles.
- Pembrolizumab will be delivered at 200 mg in three-week cycles .

- A total of four interleukin-2 treatments will be delivered into the treatment lesion by intralesional injection biweekly (at least 48 hours apart) starting 24-96 hours after the completion of radiotherapy and to be completed during the second on-trial cycle of Pembrolizumab. Intralesional injections will be performed by direct visualization and/or palpation of the lesion or under ultrasound or CT guidance as indicated. Intralesional IL-2 injections will follow guidelines which we have previously published [46]. IL-2 will be administered at three dose levels: 1) 3 x 10^6 IU, 2) 7 x 10^6, 3) 15 x 10^6 IU. The study will begin at dose level 3 and the dose will be de-escalated as needed to establish the MTD.

    At dose escalation levels 2 and 3 and in the phase II portion each patient will receive a test dose of 3 x 10^6 IU for their first injection prior to escalation to higher doses according to our published guidelines. For example, at dose level 3 each patient will receive an initial test dose of 3 x 10^6 IU of IL-2, which will be escalated to 7 x 10^6 for the second treatment and then 15 x 10^6 IU for the final two treatments as tolerated. If a dose level is not tolerated the treatment will be de-escalated to previous dose levels for subsequent treatments. If 3 million IU IL-2 is not tolerated the dose can be de-escalated to 1 million IU IL-2. If 1 million IU IL-2 is not tolerated the treatment will be deemed intolerable and patient removed from study (see Figure 6).

Patients will be assessed by a physician once during the first week of radiotherapy, weekly during intralesional IL-2 injections, and with every cycle of pembrolizumab. Routine laboratory evaluation will occur pre-treatment. For response assessment patient will have imaging pre-treatment and after every three cycles of Pembrolizumab. For collateral studies patients will undergo mandatory treatment lesion biopsies and blood draws pre-treatment and during the needle placement for the final IL-2 treatment.

Preliminary efficacy as determined by abscopal response rate, ORR, DCR and PFS will be assessed every 3 cycles. Patients on active treatment at 12 months may continue to receive pembrolizumab but will revert to standard of care (SOC) management and be labeled in “follow up”. At this time only PFS and long-term toxicity data will be collected every 3 months.

The primary endpoint is to determine if this regimen converts patients with resistance to PD-1/PD-L1 checkpoint blockade into responders as determined by abscopal response rate (defined as response rate at lesions not treated with RT + IL-2) using irRECIST as well as ORR, DCR, and PFS using RECIST 1.1. The secondary endpoint is tolerability, safety, and toxicity using CTCAE v4.03. Correlative studies include immunophenotyping serial tumor biopsies and blood samples.

5.2.1 Trial Procedures

Patients will be recruited from the UC Davis Comprehensive Cancer Center (UCDCCC). All patients will be registered at the UC Davis facility. Patients will be identified through the various clinics at UC Davis, at the UC Davis multidisciplinary tumor boards, and the UCDCCC Phase I clinic. Coordinated recruitment efforts specifically tailored for this trial will be created. We conservatively project a 36-month accrual period.

5.2.2 Treatment Plan

Table 3. Treatment Plan

Agent	Dose	Route	Frequency
IL-2	Dose 1: 3 x 10^6 IU Dose 2: 7 x 10^6 IU Doses 3 & 4: 15 x 10^6 IU	Intratumoral	• 4 total doses • Starting 24–72 hours after the completion of radiotherapy and • Completed during Cycle 2 of pembrolizumab • Does not repeat with future cycles
Radiation	24 Gy	External beam	• 3 fractions of 8 Gy palliatiave regimen • Delivered 12–96 hours apart • Must be completed during week 1 of Cycle 2 • Does not repeat with future cycles.
Pembrolizumab	200 mg	IV	Q 3 weeks (one cycle)

Eligible patients with advanced solid malignancies or lymphoma who fail to respond to or progress on PD1/PDL1 checkpoint blockade will be enrolled. Patients will receive pembrolizumab and intralesional IL-2 in combination with hypofractionated radiotherapy.

5.2.2.1 Radiotherapy

Radiotherapy will be delivered to the treatment lesion during the first week of Cycle 2 using an 8 Gy x 3 fractions palliative regimen. Fractions may be delivered on consecutive or every other day but must be completed during week 1-2 of Cycle 2 and will not be repeated in future cycles.

5.2.2.1.1 Dose Specifications and Treatment Delivery

Radiotherapy will be delivered as 24 Gy over 3 fractions. There should be a minimum of 12 hours and a maximum of 96 hours between treatments. All treatments must be completed within week 1 – 2 of cycle 2. If a patient cannot receive a radiation fraction it will be skipped. The dose per fraction is to be prescribed such that 95% of the planning treatment volume (PTV) is encompassed by the prescription dose.

5.2.2.1.2 Technical Factors

Only photon (x-ray) beams with photon energies 4-15 MV or electron beams with electron energies of 6-12 MeV will be allowed.

5.2.2.1.3 Localization, Simulation, and Immobilization

Patients will be positioned in a stable position capable of allowing accurate reproducibility of the target position from treatment to treatment. Patient immobilization must be reliable enough to ensure that the gross tumor volume (GTV) does not deviate beyond the confines of the PTV as with any significant probability (i.e., <5%). Patients will undergo an imaging study (electron portal imaging, conebeam computed tomography or Megavoltage computed tomography)

immediately prior to treatment to ensure proper alignment of the geometric center (i.e., isocenter) of the simulated fields.

5.2.2.1.4 Target Volumes

Image Acquisition

Computed tomography will be used for targeting and treatment planning. IV contrast is encouraged when its use will enhance target delineation but is not required. Axial acquisitions with gantry 0 degrees will be required with spacing ≤3.0 mm between scans in the region of the tumor.

Target Volume

The treatment lesion will be outlined and designated the gross tumor volume (GTV). No additional clinical target volume (CTV) margin for microscopic spread will be added. Depending on the stability and reproducibility of patient setup an additional margin of 0.3 – 2 cm will be added to the GTV to constitute the PTV.

Organs at Risk

All organs at risk within axial slices containing the PTV and expected to be within the treatment field should be contoured. All standard normal tissue constraints for 3 fraction radiotherapy per RTOG guidelines should be observed. Treatment hotspots in radiosensitive organs should be avoided and the dose can be modified to respect normal tissue tolerance as outlined in section 5.4.2. The maximum point dose to 0.1cc should not exceed 25 Gy for skin (defined as outermost 0.5 cm rind on axial slices) and major peripheral nerves (i.e., brachial plexus, sacral plexus, sciatic etc.). The maximum point dose should not exceed 18 Gy to the spinal cord.

Technique and Dose Calculations

Conformal treatment approaches including 3D conformal radiotherapy using static, preferably non-coplanar fields; intensity modulated radiotherapy (IMRT), Volume Modulated Arc therapy (VMAT), Dynamic Conformal Arc Radiation Therapy (DCART), and helical tomotherapy are acceptable. Isodose lines covering the PTV must be >= 95% of the prescribed dose. When IV and/or oral contrast are used, contrast densities should be overridden in the planning process. Tissue density heterogeneity correction is required for lung tumors. Superposition/convolution dose algorithms are preferred.

All radiotherapy plans will be centrally reviewed by the principal investigator or co-principal investigator prior to delivery of the first fraction.

5.2.2.2 Pembrolizumab

Pembrolizumab will be started during week 1 of Cycle 1 with cycles every 3 weeks.

5.2.2.2.1 Timing of Dose Administration

Pembrolizumab should be administered on Day 1 of each cycle after all procedures/assessments have been completed as detailed on the Study Calendar (Section 6.0). Pembrolizumab may be administered up to 3 days before or after the scheduled Day 1 of each cycle. Pembrolizumab will be administered on an outpatient basis. Pembrolizumab 200 mg will be administered as a

30-minute IV infusion every 3 weeks. Sites should make every effort to target infusion timing to be as close to 30 minutes as possible. However, given the variability of infusion pumps from site to site, a window of -5 minutes and +10 minutes is permitted (i.e., infusion time is 30 minutes: -5 min/+10 min).

Refer to section 10.2 for specific instructions for the preparation of the pembrolizumab infusion fluid and administration of infusion solution.

5.2.2.3 Interleukin-2

Interleukin-2 will be delivered into the treatment lesion by intralesional injections starting 24-96 hours after the completion of radiotherapy and to be completed during Cycle 2. Once needle is placed, intralesional IL-2 will be administered by qualified personnel. Intralesional IL-2 injections will follow guidelines, which we have previously published [46]. Briefly, each patient will receive an initial dose of 3×10^6 IU of IL-2, which will be escalated to 7×10^6 for the second treatment and then 15×10^6 IU for the final two treatments as tolerated. At dose escalation levels 2 and 3, each patient will receive a test of 3×10^6 IU for their first injection prior to escalation to higher doses according to our published guidelines. If a dose level is not tolerated (i.e., grade 3-4 non-hematologic toxicity) the treatment will be de-escalated to previous dose levels for subsequent treatments. If the initial dose of 3×10^6 IU of IL-2 is not tolerated the dose can be reduced to 1 million IU. Safety and toxicity will be evaluated using CTCAE v4.03. A dose limiting toxicity (DLT) will be defined as a treatment related grade 3-4 toxicity non-hematologic toxicity and will require dose de-escalation. If a DLT does not resolve within 5 days, is not responsive to management, or occurs at the lowest dose level (1 million IU IL-2) after de-escalation then that patient will be removed from trial and the therapy will be deemed intolerable. Therapy will be deemed safe and tolerable if no greater than 33% of patients find the treatment intolerable.

5.3 Monitoring

Safety will be evaluated in this study through the monitoring of all serious and non-serious AEs, defined and graded according to NCI CTCAE v4.03. Patients will be assessed for safety (including laboratory values) according to the Study Calendar. Patients will be followed for safety for 30 days following the last dose of study treatment when possible or until receipt of another anticancer therapy, whichever comes first.

Patients who have an ongoing study treatment-related AE upon study completion or at discontinuation from the study will be followed until the event has resolved to baseline grade, the event is assessed by the investigator as stable, new anticancer treatment is initiated, the patient is lost to follow-up, the patient withdraws consent, or until it has been determined that study treatment or participation is not the cause of the AE.

5.4 Dose Selection/Modification

5.4.1 Intralesional IL-2

5.4.1.1 Maximum Tolerated Dose Selection

In Phase I, four target doses of intralesional IL-2 will be evaluated:

Dose Level -1: 1×10^6 IU;

Dose Level 1: 3×10^6 IU;

Dose Level 2: 7×10^6 IU;

Dose Level 3: 15×10^6 IU (starting dose)

We will initiate the trial at Dose level 3 and de-escalate to lower dose levels as needed to establish the MTD. If dose level -1 is not tolerated the study will be halted and modified after discussion with principal investigators and study sponsors.

The MTD will be defined in phase I as the highest dose at which no more than one of six patients develops a DLT or Dose Level 3 if the MTD is not reached. A DLT is defined as one of the following which occurs during DLT period which lasts until 7 days after last injection of IL-2:

- grade ≥ 4 injection site reaction OR
- grade ≥ 3 treatment related immunologic adverse event (excluding injection site reactions) OR
- other grade ≥ 3 treatment related adverse events that do not resolve to grade ≤ 2 within 14 days OR
- AST/ALT > 3 x ULN with bilirubin $> 2 \times$ ULN, OR
- pneumonitis that requires holding treatment > 14 days

Table 4 and

Table 5 describe the standard dosing rules that will be followed. At dose levels 2 and 3 each patient will receive a test dose of 3×10^6 IU for their first injection prior to escalation to higher doses according to our published guidelines. For example, at dose level 3 each patient will receive an initial test dose of 3×10^6 IU of IL-2, which will be escalated to 7×10^6 for the second treatment and then 15×10^6 IU for the final two treatments as tolerated. If a dose level is not tolerated the treatment will be de-escalated to previous dose levels for subsequent treatments. Subjects who are not evaluable for toxicity will be replaced for DLT assessment. If subjects cannot receive 75% of intended dose of study treatment, for reasons other than toxicity and did not experience a DLT, they will be replaced for DLT assessment.

Table 4. Phase I Dose Escalation and De-Escalation Rules

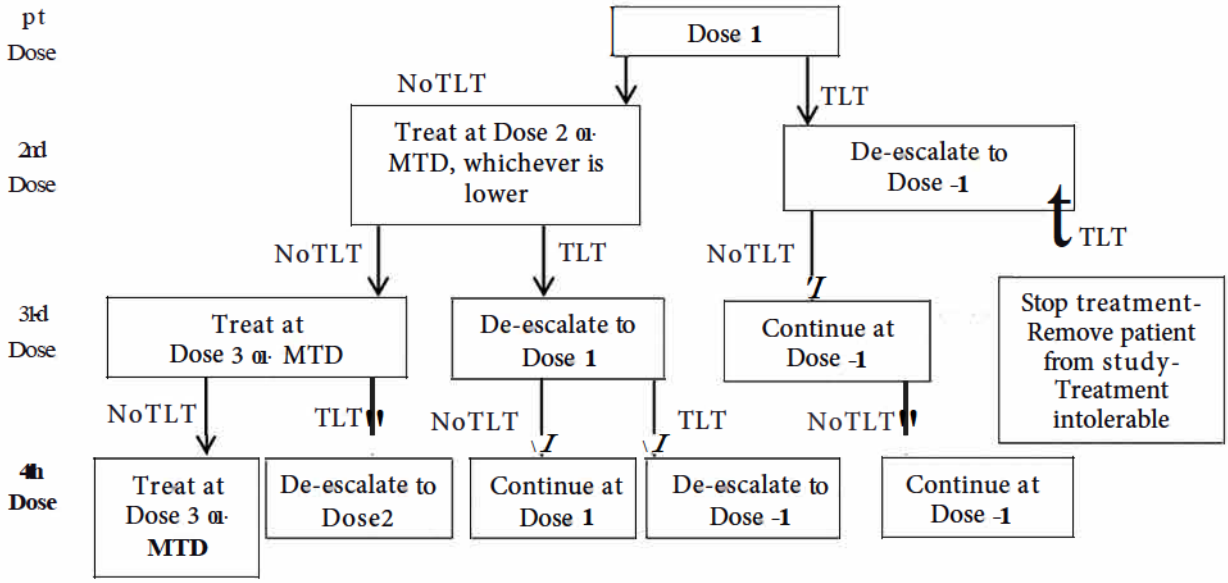
Number of Patients with DLT at a Given Dose Level	Escalation Decision Rule
0 out of 3	Enter 3 additional patients at the next dose level.
1 out of 3	Enter 3 additional patients at the dose level. If no additional DLT enter 3 additional patients at the next dose level.
2 out of 3 or 6	De-escalate the dose to the next lower level and accme 3 patients.
< 2 out of 6 at the highest dose level	This is the recommended phase 2 dose.

Table 5. Phase I Intralesional IL-2 Dosing

	Treatment 1 Dose	Treatment 2 Dose	Treatment 3 Dose	Treatment 4 Dose
Dose Level -1	1 x 10 ^{6} IU	1 x 10^6 IU	1 x 10^6 IU	1 x 10^6 IU
Dose Level 1	3 x 10^6 IU	3 x 10^6 IU	3 x 10^6 IU	3 x 10^6 IU
Dose Level 2	3 x 10^6 IU	7 x 10^6 IU	7 x 10^6 IU	7 x 10^6 IU
Dose Level 3	3 x 10^6 IU	7 x 10^6 IU	15 x 10^6 IU	15 x 10^6 IU

5.4.1.2 Phase II Dose Modification

Figure 6: Phase II Dose Modification Schema of IL-2



Any dose level that is fonnd to be intolerable or associated with grade 3-4 toxicity or intolerable grade 2 AE will be deemed a TLT. TLT = Phase II treatment limiting toxicity.

Table 6. Phase II Intralesional IL-2 dosing (intra-patient dose escalation)

P2 Dose -1	1 x 10^6 IU
Level 1 (starting dose level)	3 x 10^6 IU
Level 2 (2^{nd} dose)	7 x 10^6 IU or Phase I MTD
Level 3 (3^{rd} and 4^{th} dose)	15 x 10^6 IU or Phase I MTD

Phase II will be conducted using the MTD. For phase II, a treatment limiting toxicity (TLT) will be defined as an IL-2 treatment related grade 3-4 non-hematologic or grade 4 hematologic toxicity and will require dose de-escalation. A TLT must resolve to grade 2 or less before the next de-escalated dose is administered. Treatment intolerability will be measured in phase II to define the percentage of patients that have to come off study treatment or miss an IL-2 treatment due to inability to tolerate the full course of therapy. This is a separate measure from the MTD assessment in phase I and is only measured during phase II. Treatment intolerability will be defined as a TLT that does not resolve to grade 2 or less within 5 days, is not responsive to management, or occurs at the lowest dose level (1 million IU IL-2). In this case the patient will be removed from trial and the therapy will be deemed intolerable for that patient. If a dose of IL-2 is missed due to TLT not resolving to grade 2 or less within 5 days this falls into the definition of treatment intolerability. If a dose IL-2 is missed for other reasons this does not qualify as treatment intolerability. The TLT period begins with the administration of IL-2 and lasts up to 7 days after the last injection of IL-2. As outlined above the initial dose of intralesional IL-2 will be administered as 3 million IU test dose. If there are IL-2 related grade 3-4 non-hematologic or grade 4 hematologic treatment related adverse effects the dose will be de-escalated for the second treatment to 1 million IU. If there are grade 3-4 non-hematologic adverse effects, or grade 4 hematologic adverse effects at the 1 million IU dose the patient will be removed from study and the therapy deemed intolerable. For a given patient dose escalation will occur if there are no IL-2 related grade 3-4 non-hematologic or grade 4 hematologic treatment related toxicities. For each patient if a dose level is associated with grade 3-4 non-hematologic or grade 4 hematologic toxicity this will be deemed a TLT and the patient will be de-escalated to the lower dose for the next treatment. Once the dose is decreased there will be no further dose re-escalation allowed for an individual patient. If a planned dose cannot be delivered within the prescribed week it will be skipped.

Intratumoral IL-2 is well tolerated per Table 1 above with the majority of patients experiencing mild toxicity.

Table 7. Adverse Event Management

Injection site pain	Administer 325-650 mg acetaminophen po every 4-6 hours prn
Flu-like symptoms	Administer 325-650 mg acetaminophen po every 4-6 hours prn
Headache	Administer 325-650 mg acetaminophen po every 4-6 hours prn
Nausea/vomiting	Prescribe antiemetics per treating physician's recommendation
Diarrhea	Prescribe antidiarrheal agents per treating physician's recommendation

Hypotension is a rare event with only 1 patient from the above clinical trials and case series reporting it.

Table 8. Listing of the Blood Pressure Goals

Baseline Systolic Blood Pressure	Target Systolic Blood Pressure on Days of Treatment
<100 mm Hg	>80 mm Hg
100-120 mm Hg	>85 mm Hg
>120 mm Hg	>90 mm Hg

If patient's BP is less than 80 mm Hg on the day of treatment administer 1-1.5 L of fluid to meet the blood pressure goals. If BP remains less than 80 mm Hg, the patient cannot be treated and the dose will be delayed for up to 5 days. This is not considered a DLT or treatment intolerability unless the low BP is due to IL-2 therapy.

If patients experience a clinically significant Grade 3 or greater adverse event please contact the principal investigator to discuss AE management.

5.4.2 Hypofractionated Radiotherapy

Radiotherapy is delivered as a standard of care palliative regimen and radiotherapy related toxicities will be addressed using standard institutional practices. With a palliative dose of 24 Gy delivered in 3 fractions of 8 Gy each we anticipate minimal adverse effects for the treatment of dermal, subcutaneous, nodal, or liver lesions. The dose of radiotherapy can be reduced to no lower than 5 Gy per fraction at the discretion of the treating radiation oncologist in response to clinically significant treatment related toxicity at the treatment lesion or prior to the onset of treatment to respect normal tissue tolerances as deemed necessary by the treating radiation oncologist. If a dose of radiotherapy is missed it can be delayed. Any radiotherapy fractions not delivered during the week 1-2 of cycle 2 of therapy cannot be made up and those doses will be skipped.

5.4.3 Pembrolizumab

Adverse events (both non-serious and serious) associated with pembrolizumab exposure may represent an immunologic etiology. These adverse events may occur shortly after the first dose or several months after the last dose of treatment. See Section 5.6 for supportive care guidelines, including use of corticosteroids.

Table 9. Dose Modification Guidelines for Drug-Related Adverse Events

General instructions:

1. Corticosteroid taper should be initiated upon AE improving to Grade 1 or less and continue to taper over at least 4 weeks.
2. For situations where pembrolizumab has been withheld, pembrolizumab can be resumed after AE has been reduced to Grade 1 or 0 and corticosteroid has been tapered. Pembrolizumab should be permanently discontinued if AE does not resolve within 12 weeks of last dose or corticosteroids cannot be reduced to ≤10 mg prednisone or equivalent per day within 12 weeks.
3. For severe and life-threatening irAEs, IV corticosteroid should be initiated first followed by oral steroid. Other immunosuppressive treatment should be initiated if irAEs cannot be controlled by corticosteroids.

Immune-related AEs	Toxicity grade or conditions (CTCAEv4.0)	Action taken to pembrolizumab	irAE management with corticosteroid and/or other therapies	Monitor and follow-up
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
	Grade 4	Permanently discontinue		

Immune-related AEs	Toxicity grade or conditions (CTCAEv4.0)	Action taken to pembrolizumab	irAE management with corticosteroid and/or other therapies	Monitor and follow-up
				sufficient oral fluid intake is not feasible, fluid and electrolytes should be substituted via IV infusion.
AST / 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		

Immune-related AEs	Toxicity grade or conditions (CTCAEv4.0)	Action taken to pembrolizumab	irAE management with corticosteroid and/or other therapies	Monitor and follow-up
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		
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).				

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

5.5 Concomitant Therapy

The patient must be told to notify the investigational site about any new medications he/she takes after the start of the study drug. All medications (other than study drug) and significant non-drug therapies (including blood transfusions) administered during the study should be recorded.

If there is a clinical indication for one of these or other medications or vaccinations specifically prohibited during the trial, discontinuation from trial therapy or vaccination may be required

5.5.1 Acceptable Concomitant Medications

All treatments that the investigator considers necessary for a subject's welfare may be administered at the discretion of the investigator in keeping with the community standards of medical care. All concomitant medication will be recorded in the medical record including all prescription, over-the-counter (OTC), herbal supplements, and IV medications and fluids. If changes occur during the trial period, documentation of drug dosage, frequency, route, and date may also be included in the medical record.

All concomitant medications received within 28 days before the first dose of trial treatment and 30 days after the last dose of trial treatment should be recorded. Concomitant medications administered after 30 days after the last dose of trial treatment should be recorded for SAEs and ECIs as defined in Section 7.3.3.2.

5.5.2 Prohibited Concomitant Therapy

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

- Antineoplastic systemic chemotherapy or biological therapy
- Immunotherapy not specified in this protocol
- Chemotherapy not specified in this protocol
- Investigational agents other than pembrolizumab
- Radiation therapy not specified in this protocol
- Note: Palliative radiation therapy for symptomatic but not progressing lesions may be allowed at the investigator's discretion so long as untreated target lesions remain for response assessment.
- Live vaccines within 30 days prior to the first dose of trial treatment and while participating in the trial. Examples of live vaccines include, but are not limited to, the following: measles, mumps, rubella, varicella/zoster, yellow fever, rabies, BCG, and typhoid vaccine.
- Systemic glucocorticoids for any purpose other than to modulate symptoms from an event of clinical interest of suspected immunologic etiology. The use of physiologic doses of corticosteroids may be approved after consultation with the Sponsor.

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

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

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

In addition, all patients (including those who discontinue the study early) should not receive other immunostimulatory agents (e.g., cytokines) for 10 weeks after the last dose of IL-2 with the exception of checkpoint blockade.

5.6 Rescue Medication & Supportive Care

5.6.1 Supportive Care for Radiotherapy

Radiotherapy is delivered as a standard of care palliative regimen and radiotherapy related toxicities will be addressed using standard institutional practices. With palliative doses delivered in this trial we anticipate minimal adverse effects for the treatment of dermal, subcutaneous, nodal, or visceral lesions.

Table 10 below shows treatment guidelines for subjects who experience an infusion reaction associated with administration of pembrolizumab (MK-3475).

Table 10. Infusion Reaction Treatment Guidelines

NCI CTCAE Grade	Treatment	Premedication at subsequent dosing
Grade 1 Mild reaction; infusion interruption not indicated; intervention not indicated	Increase monitoring of vital signs as medically indicated until the subject is deemed medically stable in the opinion of the investigator.	None
Grade 2 Requires infusion interruption but responds promptly to symptomatic treatment (e.g., antihistamines, NSAIDS, narcotics, IV fluids); prophylactic medications indicated for ≤24 hrs	Stop Infusion and monitor symptoms. Additional appropriate medical therapy may include but is not limited to: IV fluids Antihistamines NSAIDS Acetaminophen Narcotics Increase monitoring of vital signs as medically indicated until the subject is deemed medically stable in the opinion of the investigator. If symptoms resolve within one hour of stopping drug infusion, the infusion may be restarted at 50% of the original infusion rate (e.g., from 100 mL/hr to 50 mL/hr). Otherwise, dosing will be held until symptoms resolve and the subject should be premedicated for the next scheduled dose. **Subjects who develop Grade 2 toxicity despite adequate premedication should be permanently discontinued from further trial treatment administration.**	Subject may be premedicated 1.5h (± 30 minutes) prior to infusion of pembrolizumab (MK-3475) with: Diphenhydramine 50 mg po (or equivalent dose of antihistamine). Acetaminophen 500-1000 mg po (or equivalent dose of antipyretic).
Grades 3 or 4 Grade 3: Prolonged (i.e., not rapidly responsive to symptomatic medication and/or brief interruption of infusion); recurrence of symptoms following initial improvement; hospitalization indicated for other clinical sequelae (e.g., renal impairment, pulmonary infiltrates) Grade 4: Life-threatening; pressor or ventilatory support indicated	Stop Infusion. Additional appropriate medical therapy may include but is not limited to: IV fluids Antihistamines NSAIDS Acetaminophen Narcotics Oxygen Pressors Corticosteroids Epinephrine Increase monitoring of vital signs as medically indicated until the subject is deemed medically stable in the opinion of the investigator. Hospitalization may be indicated. **Subject is permanently discontinued from further trial treatment administration.**	No subsequent dosing
Appropriate resuscitation equipment should be available in the room and a physician readily available during the period of drug administration.		

5.7 Diet/Activity

5.7.1 Diet

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

5.7.2 Contraception

Pembrolizumab may have adverse effects on a fetus in utero. Furthermore, it is not known if pembrolizumab has transient adverse effects on the composition of sperm.

For this trial, male subjects 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).

Female subjects will be considered of non-reproductive potential if they are either:

(1) Post-menopausal (defined as at least 12 months with no menses without an alternative medical cause; in women < 45 years of age a high follicle stimulating hormone (FSH) level in the postmenopausal range may be used to confirm a post-menopausal state in women not using hormonal contraception or hormonal replacement therapy. In the absence of 12 months of amenorrhea, a single FSH measurement is insufficient.);
OR
(2) have had a hysterectomy and/or bilateral oophorectomy, bilateral salpingectomy or bilateral tubal ligation/occlusion, at least 6 weeks prior to screening;
OR
(3) has a congenital or acquired condition that prevents childbearing.

Female and male subjects of reproductive potential must agree to avoid becoming pregnant or impregnating a partner, respectively, while receiving study drug and for 120 days after the last dose of study drug by complying with one of the following:

(1) use (or have their partner use) acceptable contraception during heterosexual activity.

Acceptable methods of contraception are‡:

Single method (one of the following is acceptable):

- intrauterine device (IUD)
- vasectomy of a female subject's male partner
- contraceptive rod implanted into the skin

Combination method (requires use of two of the following):

- diaphragm with spermicide (cannot be used in conjunction with cervical cap/spermicide)
- cervical cap with spermicide (nulliparous women only)
- contraceptive sponge (nulliparous women only)

- male condom or female condom (cannot be used together)
- hormonal contraceptive: oral contraceptive pill (estrogen/progestin pill or progestin-only pill), contraceptive skin patch, vaginal contraceptive ring, or subcutaneous contraceptive injection

Periodic abstinence (e.g., calendar, ovulation, sympto-thermal, post-ovulation methods, etc.) and withdrawal are not acceptable methods of contraception.

‡If a contraceptive method listed above is restricted by local regulations/guidelines, then it does not qualify as an acceptable method of contraception for subjects participating at sites in this country/region.

Subjects should be informed that taking the study medication may involve unknown risks to the fetus (unborn baby) if pregnancy were to occur during the study. In order to participate in the study subjects of childbearing potential must adhere to the contraception requirement (described above) from the day of study medication initiation (or 14 days prior to the initiation of study medication for oral contraception) throughout the study period up to 120 days after the last dose of trial therapy. If there is any question that a subject of childbearing potential will not reliably comply with the requirements for contraception, that subject should not be entered into the study.

5.7.3 Use in Pregnancy

If a subject inadvertently becomes pregnant while on treatment with pembrolizumab, the subject will immediately be removed from the study. The site will contact the subject at least monthly and document the subject's status until the pregnancy has been completed or terminated. The outcome of the pregnancy will be reported to the Sponsor and to Merck without delay and within 24 hours to the Sponsor and within 2 working days to Merck if the outcome is a serious adverse experience (e.g., death, abortion, congenital anomaly, or other disabling or life-threatening complication to the mother or newborn).

The study investigator will make every effort to obtain permission to follow the outcome of the pregnancy and report the condition of the fetus or newborn to the Sponsor. If a male subject impregnates his female partner the study personnel at the site must be informed immediately and the pregnancy reported to the Sponsor and to Merck and followed as described above and in Section 7.3.2.

5.7.4 Use in Nursing Women

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

5.8 Subject Withdrawal/Discontinuation Criteria

Subjects may withdraw consent at any time for any reason or be dropped from the trial at the discretion of the investigator should any untoward effect occur. In addition, a subject may be withdrawn by the investigator for any reason including the following: if enrollment into the trial

is inappropriate, the trial plan is violated, or for administrative and/or other safety reasons. Specific details regarding discontinuation or withdrawal are provided in Section 7.2.4 Other Procedures.

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

- The subject or legal representative (such as a parent or legal guardian) withdraws consent.
- Confirmed radiographic disease progression per irRECIST
  Note: For unconfirmed radiographic disease progression, please see Section 5.8.1
  Note: A subject may be granted an exception to continue on treatment with confirmed radiographic progression if clinically stable or clinically improved, please see Section 5.8.1
- Intercurrent illness that prevents further administration of treatment
- Unacceptable adverse events as described in Section 5.4.3
- Investigator's decision to withdraw the subject
- The subject has confirmed positive serum pregnancy test
- Completed 12 months of study therapy
- Administrative reasons

The End of Treatment and Follow-up visit procedures are listed in Section 6.0 (Protocol Flow Chart) and Section 7.2.5 (Visit Requirements). After the end of treatment, each subject will be followed for 30 days for adverse event monitoring (serious adverse events will be collected for 90 days after the end of treatment as described in Section 7.3.3.1). Subjects who discontinue for reasons other than progressive disease will have post-treatment follow-up for disease status until disease progression, initiating a non-study cancer treatment, withdrawing consent or becoming lost to follow-up. After documented disease progression each subject will be followed by telephone for overall survival until death, withdrawal of consent, or the end of the study, whichever occurs first.

5.8.1 Discontinuation of Study Therapy Due to Disease Progression

Immunotherapeutic agents such as pembrolizumab and IL-2 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 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.

Immune related RECIST will be used for assessment of tumor response for the purposes of managing subjects on Protocol treatment and decision making for discontinuation of study therapy due to disease progression. PD should be confirmed no earlier than 4 weeks later according to the criteria outlined in Table 11 below. Subjects who are deemed clinically unstable or who have biopsy proven new metastatic lesions are not required to have repeat imaging for confirmation. This decision will be based on clinical judgment of a subject's overall clinical

condition, including performance status, clinical symptoms, and laboratory data. At a minimum, subjects must meet the following criteria for continued treatment on study after disease progression is identified at a tumor assessment:

When feasible, subjects should not be discontinued until progression is confirmed; however, the decision to continue study treatment after the first evidence of disease progression is at the investigator's discretion based on the clinical status of the subject.

Subjects may receive study treatment while waiting for confirmation of PD if they are clinically stable as defined by the following criteria:

- No decline in ECOG performance status.
- Absence of new or worsening symptoms.
- Absence of rapid progression of disease.
- Absence of progressive tumor at critical anatomical sites (e.g., spinal cord compression) requiring urgent alternative medical intervention.

Table 11. Imaging and Treatment after First Radiologic Evidence of Progressive Disease

	Clinically Stable		Clinically Unstable	
	Imaging	**Treatment**	**Imaging**	**Treatment**
First radiologic evidence of PD	Repeat imaging at ≥ 4 weeks to confirm PD	May continue study treatment at the investigator's discretion while awaiting confirmatory scan	Repeat imaging at ≥ 4 weeks to confirm PD if possible	Discontinue treatment
Repeat scan confirms PD	No additional imaging required	Discontinue treatment	No additional imaging required	N/A
Repeat scan shows SD, PR, or CR[i]	Continue regularly scheduled imaging assessments every 6 weeks x 3 then resume imaging every 12 weeks thereafter.	Continue study treatment at the investigator's discretion	Continue regularly scheduled imaging assessments every 6 weeks x 3 then resume imaging every 12 weeks thereafter.	May restart study treatment if condition has improved and/or clinically stable per investigator's discretion

[i] SD, PR, CR is based on new baseline from first evidence of PD.

5.8.2 Discontinuation of Study Therapy after CR

Discontinuation of treatment may be considered for subjects who have attained a confirmed CR that have been treated for at least 24 weeks with pembrolizumab and had at least two treatments with pembrolizumab beyond the date when the initial CR was declared. Subjects who then experience radiographic disease progression may be eligible for up to one year of additional treatment with pembrolizumab via the Second Course Phase at the discretion of the investigator if no cancer treatment was administered since the last dose of pembrolizumab, the subject meets the safety parameters listed in the Inclusion/Exclusion criteria, and the trial is open. Subjects will resume therapy at the same dose and schedule at the time of initial discontinuation. Additional details are provided in Section 5.4.

5.9 Subject Replacement Strategy

For the phase I component, any patient who is unevaluable for DLT will be replaced. For the phase II component, patients must have completed at least 3 of the planned 4 study injections to be considered evaluable.

5.10 Clinical Criteria for Early Trial Termination

Early trial termination will be the result of principal investigator discretion. The following criteria specified below:

1. Quality or quantity of data recording is inaccurate or incomplete
2. Poor adherence to protocol and regulatory requirements
3. Incidence or severity of adverse drug reaction in this or other studies indicates a potential health hazard to subjects
4. Plans to modify or discontinue the development of the study drug

In the event that no patients are able to tolerate the lowest dose of IL-2 (3×10^6 IU), the study will be terminated.

In the event the DSMC decides the study is not safe for patients, the study will be terminated.

5.11 Duration of Follow Up

All patients will be followed for 30 days after the last dose of study treatment or until all treatment-related, clinically significant toxicities resolve to baseline or grade ≤ 1. Adverse events with attribution of possible, probable, or definite will be reported following guidelines for adverse event reporting, and all SAEs will be reported for 30 days after the last dose of study treatment.

Patients on active treatment at 12 months may continue to receive pembrolizumab but will revert to SOC management and be labeled in “follow-up”. At this time only PFS and long-term toxicity data will be collected every 3 months as part of routine SOC management.

All patients will be followed every 3 months per routine SOC management until documented progression, death, loss to follow up, consent withdrawal, or study termination. Patients who discontinue study treatment early will be followed for progression free survival and late toxicity assessment every 3 months as part of their routine SOC management.

6.0 STUDY ASSESSMENTS AND MONITORING

The study calendar lists all the study assessments and their scheduled times. All data obtained from these assessments will be recorded in the study specific case report forms that will be generated prior to study activation. See Appendix 2 for data submission schedule.

6.1 Study Calendar

Assessment Window (Days)	Screening	Treatment Cycles						End of Treatment	Post-Treatment
	Days -28 to -1	Cycle 1 (±7days)	Cycle 2			Cycle 3[j] (±7 days)	Subsequent Cycles (±7 days)	≤30 Days after last dose	Follow-up[j]
			Wk 1 (±7days)	Wk 2	Wk 3				
Administrative Procedures									
Inclusion/exclusion criteria	X								
Demographics and Medical History	X								
Prior and concomitant medication review	X	X				X	X		
Survival status							X	X[c]	X
Clinical Procedures/Assessments									
Physical examination [a]	X	X	X	X	X	X	X		X
ECOG performance status	X	X	X			X	X		
Vital signs and weight	X	X	X			X	X		
CBC with differential [b]	X	X	X			X	X[b]		
Serum chemistry[b]	X	X	X			X	X[b]		
TSH, free T3, free T4	X	X	X			X	X[b]		
Coagulation panel (PT/INR and aPTT)	X								
Urine pregnancy test	X (within 4d of cycle 1)								
Stool Sample		X[l]							
Review adverse events	X	X	X	X	X	X	X		
Investigational Treatment									
Pembrolizumab [d]		X	X			X	X		X
Intralesional IL-2 [e]				X	X				
Radiation Therapy [f]			X	X					
Efficacy measurements									
Tumor imaging [g]	X					X	X[g]		
Efficacy [c]							X		

Correlatives/ Biopsy									
Blood samples for immune assays[h]		X[k]	X[k]		X	X	X[k]		
Archival/screening FFPE tumor tissue specimen or 10 unstained slides[h]		X							
Biopsy[i]		X			X				

Abbreviations: ECOG=Eastern Cooperative Oncology Group; TSH=Thyroid Stimulating Hormone; T3=Triiodothyronine; T4=Thyroxine; FFPE=Formalin-fixed, paraffin-embedded; CT=Computerized tomography; MRI=magnetic resonance imaging; PFS=progression free survival; SOC=standard of care.

a. Patients will be clinically evaluated prior to each cycle of pembrolizumab for the first 3 cycles and with each intralesional IL-2 injection, and thereafter every 3 cycles until off treatment.

b. CBC and serum chemistry, TSH, free T3, free T4 will be obtained 4 days prior to every cycle of pembrolizumab.

c. ORR, DCR, and PFS will be assessed every 3 cycles using RECIST 1.1 and irRECIST. Patients who complete study treatment, or discontinue study treatment early, will be followed for PFS every 3 months.

d. Pembrolizumab: q 3 weeks +/- 3 days (one cycle) until intolerable toxicity or progression.

e. Intralesional IL-2: 4 injections (2 injections per week during Cycle 2 with each injection ≥48 hours apart), starting 24-96 hours after completion of radiotherapy.

f. Radiotherapy: 3 fractions of 8 Gy delivered 12-96 hours apart with Cycle 2 of pembrolizumab.

g. CT or MRI at pre-treatment and q 3 cycles until progression (e.g., at the end of cycle 3, ,6, 9, etc.).

h. See the correlative study section for detailed information (Section 8.0)

i. Biopsy: Tumor biopsies will be obtained prior to cycle 1 treatment and during the final intralesional needle placement for IL-2 delivery (Cycle 2 Week 3).

j. Follow-up: patients on active treatment at 12 months may continue to receive pembrolizumab, but will revert to SOC management and be labeled in "follow-up". At this time only PFS and long-term toxicity data will be collected every 3 months as part of routine SOC management.

k. Research blood to be drawn prior to starting therapy for cycle 1 and 2. After Cycle 3 research blood to be drawn at cycle 6 then every 6 cycles thereafter.

l. Stool sample will be collected from patients before initiation of treatment

.

7.0 TRIAL PROCEDURES

7.1 Response Assessment

7.1.1 Efficacy

Response will be evaluated in this study using the new international criteria proposed by the revised Response Evaluation Criteria in Solid Tumors (RECIST) guideline (version 1.1) [58] as well as irRECIST criteria. Progression will be evaluated using irRECIST criteria. Additionally, imaging will be reviewed by a radiologist in conjunction with a radiation oncologist to help distinguish tumor progression from post-SAR scarring. 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.

7.1.2 Disease Parameters

7.1.2.1 Measurable Disease

The presence of at least one measurable lesion.

7.1.2.2 Measurable Lesions

Lesions that can be accurately measured in at least one dimension with longest diameter (LD) 20mm using conventional techniques or 10mm with spiral CT scan.

7.1.2.3 Non-Measurable Lesions

All other lesions, including small lesions (longest diameter <20 mm with conventional techniques or <10 mm with spiral CT scan), i.e., bone lesions, leptomeningeal disease, ascites, pleural/pericardial effusion, inflammatory breast disease, lymphangitis cutis/pulmonis, cystic lesions, and also abdominal masses that are not confirmed and followed by imaging techniques.

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

Clinical lesions will only be considered measurable when they are superficial (e.g., skin nodules and palpable lymph nodes). For the case of skin lesions, documentation by color photography, including a ruler to estimate the size of the lesion, is recommended.

7.1.3 Methods of Measurement

CT and MRI are the best currently available and reproducible methods to measure target lesions selected for response assessment. Conventional CT and MRI should be performed with cuts of 10 mm or less in slice thickness contiguously. Spiral CT should be performed using a 5 mm

contiguous reconstruction algorithm. This applies to tumors of the chest, abdomen and pelvis. Head and neck tumors and those of extremities usually require specific protocols.

Lesions on chest X-ray are acceptable as measurable lesions when they are clearly defined and surrounded by aerated lung. However, CT is preferable.

When the primary endpoint of the study is objective response evaluation, ultrasound (US) should not be used to measure tumor lesions. It is, however, a possible alternative to clinical measurements of superficial palpable lymph nodes, subcutaneous lesions and thyroid nodules. US might also be useful to confirm the complete disappearance of superficial lesions usually assessed by clinical examination.

The utilization of endoscopy and laparoscopy for objective tumor evaluation has not yet been fully and widely validated. Their uses in this specific context require sophisticated equipment and a high level of expertise that may only be available in some centers. Therefore, the utilization of such techniques for objective tumor response should be restricted to validation purposes in specialized centers. However, such techniques can be useful in confirming complete pathological response when biopsies are obtained.

Cytology and histology can be used to differentiate between PR and CR in rare cases (e.g., after treatment to differentiate between residual benign lesions and residual malignant lesions in tumor types such as germ cell tumors).

7.1.4 Response Criteria

7.1.4.1 Evaluation of Target Lesions

- **Complete Response (CR):** Disappearance of the target lesion
- **Partial Response (PR):** At least a 30% decrease in the sum of the LD of target lesions, taking as reference the baseline sum LD
- **Progressive Disease (PD):** At least a 20% increase in the sum of the LD of target lesion, or the appearance of one or more new lesions
- **Stable Disease (SD):** Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum LD since the treatment started

7.1.4.2 Immune Related Response Criteria (irRECIST)

A growing body of literature indicates that radiographic responses to immunotherapy may have different patterns and kinetics than what would be expected with traditional cytotoxic therapies. To account for these differences we will also characterize radiographic outcomes using the immune related response criteria outlined by Wolchok and colleagues [59]. See Appendix 4.

7.1.4.3 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 PD the smallest measurements recorded

since the treatment started). In general, the patient's best response assignment will depend on the achievement of both measurement and confirmation criteria.

Table 12. Best Overall Response Evaluation

Target Lesion	New Lesion	Overall Response
CR	No	CR
PR	No	PR
SD	No	SD
PD	Yes or No	PD

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

In some circumstances it may be difficult to distinguish residual disease from normal tissue.

When the evaluation of complete response depends on this determination, it is recommended that the residual lesion be investigated (fine needle aspirate/biopsy) to confirm the complete response status.

Duration of Overall Response

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

Duration of Stable Disease

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

The clinical relevance of the duration of SD varies for different tumor types and grades.

Therefore, it is highly recommended that the protocol specify the minimal time interval required between two measurements for determination of SD. This time interval should take into account the expected clinical benefit that such a status may bring to the population under study.

Disease Control Rate (DCR)

DCR is defined as the percentage of patients that achieve an objective tumor response or stable disease to therapy.

Progression-Free Survival (PFS)

PFS is defined as the duration of time from start of treatment to time of progression or death, whichever occurs first.

Abscopal Response Rate (ARR)

ARR is defined as the percentage of patients that achieve an objective tumor response at unirradiated lesions as defined by irRECIST criteria.

Reporting of Results

All patients 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 patient 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).

All of the patients who met the eligibility criteria should be included in the main analysis of the response rate. Patients in response categories 4-9 should be considered as failing to respond to treatment (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 patients. Subanalyses may then be performed on the basis of a subset of patients, 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 subanalyses may not serve as the basis for drawing conclusions concerning treatment efficacy, and the reasons for excluding patients from the analysis should be clearly reported.

The 95% confidence intervals should be provided.

7.2 Trial Procedures

The Study Calendar - Section 6.0 summarizes the trial procedures to be performed at each visit. Individual trial procedures are described in detail below. It may be necessary to perform these procedures at unscheduled time points if deemed clinically necessary by the investigator.

Furthermore, additional evaluations/testing may be deemed necessary by the Sponsor and/or Merck for reasons related to subject safety. In some cases, such evaluation/testing may be potentially sensitive in nature (e.g., HIV, Hepatitis C, etc.), and thus local regulations may require that additional informed consent be obtained from the subject. In these cases, such evaluations/testing will be performed in accordance with those regulations.

7.2.1 Administrative Procedures

7.2.1.1 Informed Consent

The Investigator must obtain documented consent from each potential subject prior to participating in a clinical trial.

7.2.1.2 General Informed Consent

Consent must be documented by the subject's dated signature or by the subject's legally acceptable representative's dated signature on a consent form along with the dated signature of the person conducting the consent discussion.

A copy of the signed and dated consent form should be given to the subject before participation in the trial.

The initial informed consent form, any subsequent revised written informed consent form and any written information provided to the subject must receive the IRB/ERC's approval/favorable opinion in advance of use. The subject or his/her legally acceptable representative should be informed in a timely manner if new information becomes available that may be relevant to the subject's willingness to continue participation in the trial. The communication of this information will be provided and documented via a revised consent form or addendum to the original consent form that captures the subject's dated signature or by the subject's legally acceptable representative's dated signature.

Specifics about a trial and the trial population will be added to the consent form template at the protocol level.

The informed consent will adhere to IRB/ERC requirements, applicable laws and regulations and Sponsor requirements.

7.2.1.3 Inclusion/Exclusion Criteria

All inclusion and exclusion criteria will be reviewed by the investigator or qualified designee to ensure that the subject qualifies for the trial.

7.2.1.4 Medical History

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

7.2.1.5 Prior and Concomitant Medications Review

7.2.1.5.1 Prior Medications

The investigator or qualified designee will review prior medication use, including any protocol-specified washout requirement, and record prior medication taken by the subject within 28 days before starting the trial. Treatment for the disease for which the subject has enrolled in this study will be recorded separately and not listed as a prior medication.

7.2.1.5.2 Concomitant Medications

The investigator or qualified designee will record medication, if any, taken by the subject during the trial. All medications related to reportable SAEs and ECIs should be recorded as defined in Section 7.3.

7.2.1.6 Prior Treatment Details

The investigator or qualified designee will review all prior cancer treatments including systemic treatments, radiation, and surgeries.

7.2.1.6.1 Subsequent Anti-Cancer Therapy Status

The investigator or qualified designee will review all new anti-neoplastic therapy initiated after the last dose of trial treatment. If a subject initiates a new anti-cancer therapy within 30 days after the last dose of trial treatment, the 30-day Safety Follow-up visit must occur before the first dose of the new therapy. Once new anti-cancer therapy has been initiated the subject will move into survival follow-up.

7.2.2 Clinical Procedures/Assessments

7.2.2.1 Adverse Event (AE) Monitoring

The investigator or qualified designee will assess each subject to evaluate for potential new or worsening AEs as specified in the Trial Flow Chart and more frequently if clinically indicated. Adverse experiences will be graded and recorded throughout the study and during the follow-up period according to NCI CTCAE Version 4.0 (see Appendix 5). Toxicities will be characterized in terms regarding seriousness, causality, toxicity grading, and action taken with regard to trial treatment.

Please refer to section 7.3 for detailed information regarding the assessment and recording of AEs.

7.2.2.2 Full Physical Exam

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

7.2.2.3 Directed Physical Exam

For cycles that do not require a full physical exam per the Trial Flow Chart, the investigator or qualified designee will perform a directed physical exam as clinically indicated prior to trial treatment administration.

7.2.2.4 Vital Signs

The investigator or qualified designee will take vital signs at screening, prior to the administration of each dose of trial treatment and at treatment discontinuation as specified in the Trial Flow Chart (Section 6.0). Vital signs should include temperature, pulse, respiratory rate, weight, and blood pressure. Height will be measured at screening only.

7.2.2.5 Eastern Cooperative Oncology Group (ECOG) Performance Scale

The investigator or qualified designee will assess ECOG status (see Appendix 1) at screening, prior to the administration of each dose of trial treatment and discontinuation of trial treatment as specified in the Trial Flow Chart.

7.2.3 Laboratory Procedures/Assessments

Details regarding specific laboratory procedures/assessments to be performed in this trial are provided below. Laboratory tests for hematology, chemistry, urinalysis, and others are specified in Table 13.

Table 13. Laboratory Tests

Hematology	Chemistry	Urinalysis	Other
Hematocrit	Albumin		Serum β-human chorionic gonadotropin†
Hemoglobin	Alkaline phosphatase		(β-hCG)†
Platelet count	Alanine aminotransferase (ALT)		PT (INR)
WBC (total and differential)	Aspartate aminotransferase (AST)		aPTT
Red Blood Cell Count	Lactate dehydrogenase (LDH)		Total thriiodothyronine (T3)
Absolute Neutrophil Count	Carbon Dioxide ‡		Free tyroxine (T4)
Absolute Lymphocyte Count	*(CO_2 or biocarbonate)*	Urine pregnancy test †	Thyroid stimulating hormone (TSH)
	Uric Acid		
	Calcium		
	Chloride		Blood for correlative studies
	Glucose		
	Phosphorus		
	Potassium		
	Sodium		
	Magnesium		
	Total Bilirubin		
	Direct Bilirubin *(If total bilirubin is elevated above the upper limit of normal)*		
	Total protein		
	Blood Urea Nitrogen		

† Perform on women of childbearing potential only. If urine pregnancy results cannot be confirmed as negative, a serum pregnancy test will be required.
‡ If considered standard of care in your region.

Laboratory tests for screening or entry into the Second Course Phase should be performed within 10 days prior to the first dose of treatment. After Cycle 1, pre-dose laboratory procedures can be conducted up to 4 days prior to dosing. Results must be reviewed by the investigator or qualified designee and found to be acceptable prior to each dose of trial treatment.

7.2.4 Other Procedures

7.2.4.1 Withdrawal/Discontinuation

When a subject discontinues/withdraws prior to trial completion, all applicable activities scheduled for the final trial visit should be performed at the time of discontinuation. Any adverse events that are present at the time of discontinuation/withdrawal should be followed in accordance with the safety requirements outlined in Section 7.3 - Assessing and Recording Adverse Events. Subjects who a) attain a CR or b) complete 12 months of treatment with pembrolizumab may discontinue treatment with the option of restarting treatment if they meet the criteria specified in Section 5.8. After discontinuing treatment following assessment of CR, these subjects should return to the site for a Safety Follow-up Visit (described in Section 5.11) and then proceed to the Follow-Up Period of the study (described in Section 5.11).

7.2.5 Visit Requirements

Visit requirements are outlined in Section 6.0. Specific procedure-related details are provided above.

7.2.5.1 Screening

Screening should be completed within 28 days of Day 1.

7.2.5.2 Treatment period

See study calendar for treatment period details.

7.2.5.3 Post Treatment Visits

7.2.5.3.1 Safety Follow-up Visit

The mandatory Safety Follow-Up Visit should be conducted approximately 30 days after the last dose of trial treatment or before the initiation of a new anti-cancer treatment, whichever comes first. All AEs that occur prior to the Safety Follow-Up Visit should be recorded. Subjects with a treatment related AE of Grade > 1 will be followed until the resolution of the AE to Grade 0-1 or until the beginning of a new anti-neoplastic therapy, whichever occurs first. SAEs that occur within 90 days of the end of treatment or before initiation of a new anti-cancer treatment should also be followed and recorded. Subjects who are eligible for retreatment with pembrolizumab (as described in Section 5.8) may have up to two safety follow-up visits, one after the Treatment Period and one after the Second Course Phase.

7.2.5.4 Follow-up Visits

Subjects who discontinue trial treatment for a reason other than disease progression will move into the Follow-Up Phase and should be assessed every 6 weeks by radiologic imaging to monitor disease status or per standard of care. After 1 year, the imaging time point will occur every 9 weeks or per standard of care. Every effort should be made in the follow up phase to collect information regarding disease status until the start of new anti-neoplastic therapy, disease progression, death, end of the study or if the subject begins retreatment with pembrolizumab as

detailed in Section 5.8. Information regarding post-study anti-neoplastic treatment will be collected if new treatment is initiated.

Subjects who are eligible to receive retreatment with pembrolizumab according to the criteria in Section 5.8 will move from the follow-up phase to the Second Course Phase when they experience disease progression.

7.2.5.4.1 Survival Follow-up

Once a subject experiences confirmed disease progression or starts a new anti-cancer therapy, the subject moves into the survival follow-up phase and should be followed every 12 weeks to assess for survival status until death, withdrawal of consent, or the end of the study, whichever occurs first.

7.3 Assessing and Recording Adverse Events

An adverse event is defined as any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have to have a causal relationship with this treatment. An adverse event can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding, for example), symptom, or disease temporally associated with the use of a medicinal product or protocol-specified procedure, whether or not considered related to the medicinal product or protocol-specified procedure. Any worsening (i.e., any clinically significant adverse change in frequency and/or intensity) of a preexisting condition that is temporally associated with the use of the study intervention, is also an adverse event.

Changes resulting from normal growth and development that do not vary significantly in frequency or severity from expected levels are not to be considered adverse events. Examples of this may include, but are not limited to, teething, typical crying in infants and children and onset of menses or menopause occurring at a physiologically appropriate time.

Progression of the cancer under study is not considered an adverse event.

All adverse events that occur after the consent form is signed but before treatment allocation/randomization must be reported by the investigator if they cause the subject 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.

From the time of treatment allocation through 30 days following cessation of treatment, all adverse events must be reported by the investigator. Such events will be recorded at each examination on the Adverse Event case report forms/worksheets.

7.3.1 Definition of an Overdose for this Protocol and Reporting of Overdose to the Sponsor and to Merck

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

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

If a dose of study intervention 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. (Attn: Worldwide Product Safety; FAX 215 993-1220)

7.3.2 Reporting of Pregnancy and Lactation to the Sponsor and 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 subject (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 subject 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 subject 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 (Attn: Worldwide Product Safety; FAX 215 993-1220).

7.3.3 Immediate Reporting of Adverse Events to the Sponsor and to Merck

7.3.3.1 Serious Adverse Events

A serious adverse event is any adverse event occurring at any dose or during any use of pembrolizumab 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 another important medical event

Note: In addition to the above criteria, adverse events meeting either of the below criteria, although not serious per ICH definition, are reportable to Merck in the same timeframe as SAEs to meet certain local requirements. Therefore, these events are considered serious by Merck for collection purposes.

- Is a new cancer (that is not a condition of the study);
- Is associated with an overdose.

For the time period beginning when the consent form is signed until treatment allocation/randomization, any serious adverse event, or follow up to a serious adverse event, including death due to any cause other than progression of the cancer under study that occurs to any subject must be reported within 24 hours to the Sponsor and within 2 working days to Merck Global Safety if it causes the subject 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 30 days following cessation of treatment, whichever is earlier, 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, whether or not related to the study intervention, must be reported within 24 hours to the Sponsor and within 2 working days to Merck Global Safety.

Additionally, any serious adverse event, considered by an investigator who is a qualified physician to be related to study intervention that is brought to the attention of the investigator at any time following consent through the end of the specified safety follow-up period specified in the paragraph above, or at any time outside of the time period specified in the previous paragraph also must be reported immediately to the Sponsor and to Merck Global Safety.

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

SAE reports and any other relevant safety information are to be forwarded to the Merck Global Safety facsimile number: +1-215-993-1220.

7.3.3.2 Events of Clinical Interest

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. (Attn: Worldwide Product Safety; FAX 215 993-1220).

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 subject must be reported within 24 hours to the Sponsor and within 2 working days to Merck Global Safety if it causes the subject 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 subject initiates new anticancer therapy, whichever is earlier, any ECI, or follow up to an ECI, whether or not related to study intervention, 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. An overdose of study intervention, as defined in Section 7.3.1 - 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.

7.3.3.3 Protocol-Specific Exceptions to Serious Adverse Event Reporting

Efficacy endpoints as outlined in this section will not be reported to Merck, unless there is evidence suggesting a causal relationship between the drug and the event. Any such event will be submitted to the Sponsor within 24 hours and to Merck Global Safety within 2 working days either by electronic or paper media.

Specifically, the suspected/actual events covered in this exception include any event that is disease progression of the cancer under study.

The Sponsor will monitor unblinded aggregated efficacy endpoint events and safety data to ensure the safety of the subjects in the trial. Any suspected endpoint which upon review is not progression of the cancer under study will be forwarded to Merck Global Safety as a SAE within 2 working days of determination that the event is not progression of the cancer under study

Hospitalization related to convenience (e.g., transportation issues, etc.) will not be considered a SAE.

7.3.4 Evaluating Adverse Events

An investigator who is a qualified physician will evaluate all adverse events according to the NCI Common Terminology for Adverse Events (CTCAE), version 4.0. Any adverse event which changes CTCAE grade over the course of a given episode will have each change of grade recorded on the adverse event case report forms/worksheets.

All adverse events regardless of CTCAE grade must also be evaluated for seriousness.

7.3.5 Sponsor Responsibility for Reporting Adverse Events

All Adverse Events will be reported to regulatory authorities, IRB/IECs and investigators in accordance with all applicable global laws and regulations.

7.3.5.1 Safety Reporting Requirements for IND Exempt Studies

For Investigator Sponsored IND Exempt Studies there are some reporting requirements for the FDA in accordance with the guidance set forth in 21 CFR 314.80.

7.3.5.2 Reporting to the IRB

Both serious and non-serious adverse events will be reported in accordance with UCD IRB Administration and UCD Comprehensive Cancer Center's Office of Clinical Research (OCR) policies. The UC Davis IRB can be reached at (916) 703-9151.

Participating site(s) will report adverse events per institution's IRB guidelines.

8.0 CORRELATIVE SCIENCE (Translational Research)

8.1 Rationale

One of the major shortcomings of immunotherapy trials has been the lack of in-depth correlative studies to help identify the mechanism of action and resistance, identify biomarkers of response, and provide a foundation for further improving these approaches. To address this, we plan to perform in depth immunological analysis of mandatory serial patient blood and tumor samples. Tumor biopsies will be obtained pre-treatment and during the final intralesional needle placement for IL-2 delivery. Blood samples will be obtained pre-treatment, after the final dose on intralesional IL-2 and at the first response assessment time point at 6 weeks. We will evaluate immunologic changes systemically and in the tumor microenvironment within patient's pre to post therapy and across the cohort of patients to identify predictive biomarkers and elucidate the mechanistic immunologic effects of therapy and therapy resistance. We will examine putative biomarkers of response to checkpoint immunotherapy such as PD-L1 expression, mutational load, and TILs.

8.1.1 Translational Research Hypothesis

These studies will also evaluate our two mechanistic hypotheses: 1) We hypothesize that the triple combination of radiotherapy + intralesional IL-2 + and pembrolizumab will convert patients with primary or secondary resistance to PD-1/PD-L1 blockade into responders by inducing an anti-tumor immune response and overcoming the lack of a pre-existent anti-tumor immune response (e.g., due to a poorly immunogenic tumor or T-cell exclusion from the tumor). 2) We hypothesize that the existence of other dominant modes of immune suppression will limit response to therapy. We will examine hypothesis 1 by evaluating TILs, T cell activation, TCR diversity, and cytokines/chemokines including CCL4. We will examine hypothesis 2 by evaluating immunosuppressive cell subsets, immunosuppressive molecules, and enzymes. Correlative studies will include (in order of priority):

8.1.2 Translational Research Objectives

Blood

- FACS for quantification, immunophenotyping, and functional assessment of PBMCs and immunosuppressive cell subsets
- qPCR (quantitative polymerase chain reaction) evaluation of immune gene signatures including: cytokines, T-cell activation markers, immunosuppressive enzymes and molecules (IDO [indoleamine-pyrrole 2,3-dioxygenase], arginase, CTLA-4, PD-1/PD-L1, Tim-3, LAG3), macrophage polarization, etc.
- Luminex evaluation of plasma for systemic cytokine / chemokine signatures
- T-cell receptor (TCR) deep sequencing to determine clonal expansion of T-cells in the systemic circulation

- Whole exome sequencing of baseline PBMCs as a normal tissue control for tumor whole exome sequencing
- Other studies as deemed feasible and informative by the principal investigators

Tissue

- Multi-plexed IHC / IF to determine the expression of PD-1, PD-L1, CD8, CD4, CD3, IDO, FOXP3, CD68 and others. Automated interaction analysis to determine expression patterns and cell-cell interactions in the TME.
- qPCR evaluation of the tumor microenvironment gene signatures including: cytokines, T-cell activation markers, immunosuppressive enzymes and molecules (IDO, arginase, CTLA4, PD-1/PD-L1), macrophage polarization, etc. qPCR may also be used in a directed manner to confirm results of RNAseq.
- T-cell receptor (TCR) deep sequencing to determine clonal expansion of T-cells in the TME.
- Whole exome sequencing of baseline tumor samples to determine mutational load.
- RNAseq to examine changes in gene expression profiles pre- and post-treatment. RNAseq will also be used to confirm expression of putative tumor mutations identified by whole exome sequencing.
- Other studies as deemed feasible and informative by the principal investigators

8.2 Study Design and Methodology

Details of correlative study sample collection, preparation, and storage are outlined in

Appendix 3. For translational studies tumor tissue will be obtained pre- and post- treatment and peripheral blood will be obtained pre-treatment and at two time points post-treatment from each patient. Thus, a total maximum of 92 fresh tumor biopsies and 138 blood samples will be obtained for this study. We have extensive expertise in all of the techniques described at the UC Davis Laboratory of Tumor Immunology and immune monitoring core.

8.2.1 FACS analysis of PBMCs

To examine treatment induced changes in the quantity and phenotype of circulating immune cells PBMCs will be analyzed by flow cytometry pre-treatment and at two time points post-treatment. Blood samples will be separated into PBMCs and plasma and stored at -80 for batched analysis. PBMCs will be stained for FACS analysis using well characterized antibodies against markers including CD45RA, CD3, CD4, CD8, CD62L, FOXP3, CD25, ICOS, CD127, PD1, PDL1, Tim-3, LAG3, CD56, CD14, CD15, CD16, CD1c, CD11b, CD303, and CD83. Cellular function will be assessed by staining for intracellular cytokines and proliferation using antibodies against Granzyme B, Interferon gamma, tumor necrosis factor (TNF) alpha, and Ki-67. Results will be analyzed using a BD Fortessa multi-color flow cytometer and Flowjo software.

8.2.2 Plasma cytokine / chemokine signatures

To examine treatment induced changes in systemic cytokines / chemokines plasma proteins will be examined pre-treatment and at two time points post-treatment. Markers such as IL-2, IL-6, IL-10, IL-12p70, GM-CSF (granulocyte-macrophage colony-stimulating factor), TNF alpha, IFN (interferon) gamma, CXCL10 (C-X-C motif chemokine 10), RANTES (regulated on activation, normal T cell expressed and secreted), MIP1 (Macrophage Inflammatory Protein) alpha, MIP1 beta and others will be evaluated using the Bio-Rad human 27-plex panel and TGF-beta will be evaluated using the Bio-Rad TGF-beta 3-plex panel. These multi-plexed suspension arrays will be analyzed using a Bio-Plex 200 system and Bio-Plex Manager software.

8.2.3 TCRseq

To examine treatment induced changes in T-cell diversity and clonal expansion and to understand how T-cell clones in the TME relate to circulating T-cells TCRseq will be performed on PBMCs and tumor tissue pre- and post-treatment. For TCR deep sequencing total RNA will be reverse transcribed using SMARTScribe reverse transcriptase (Clontech), a gene specific oligonucleotide, and a template switching oligo to form a universal 5'-adapter. This allows us to amplify the entire diversity of TCR-beta variable chains using just a pair of primers, specific to the template switch oligos on the 5'-end and to the constant gene segments on the 3'-end. Subsequent library amplification is done using Netera XT index kit. Sequencing is performed on the Illumina MiSeq and HiSeq platforms. CDR3 sequences are extracted from raw sequence data and each sequence read is referred to a particular V and J family employing BLAST algorithm. After clustering individual clones (sequence reads with identical V, J and CDR3), virtual CDR3 length spectratyping for each of the V and J gene families is displayed based on the relative amount of each clonal sequence which corresponds to the actual abundance of the T cell clone.

8.2.4 qPCR

Expression levels of immune genes of interest will be assayed pre-and post-treatment within PBMCs and tumor tissue. qPCR will also be used to confirm findings of interest from RNAseq. qPCR will be performed using the StepOnePlus real time PCR system (Life technologies) with primers of interest to examine immune gene signatures of chemokines, cytokines, checkpoint molecules, and others. We will examine genes including, CCL4, Type I IFNs, T-cell markers, IDO, arginase, iNOS (inducible Nitric oxide synthases), CTLA-4, PD-1, PD-L1 and others.

8.2.5 Multi-plex IHC/IF

Immune markers and TILS will be analyzed in tumor tissue pre- and post- treatment. A section of tissue will be formalin fixed and paraffin embedded for multiplex IHC or IF staining and automated analysis in collaboration with Dr. Kurt Schalper, director of the Yale translational immuno-oncology laboratory as previously described [60, 61]. We will stain for markers including cytokeratin, CD3, CD4, CD8, CD20, FoxP3, IDO, PD1, PD-L1, Ki67, Granzyme B, IFN and others.

8.2.6 Whole exome sequencing

Genomic DNA will be extracted from baseline PBMC and tumor tissue samples to determine the mutational load of tumor tissues. Whole exome sequencing will be performed at the UC Davis genomics core. Exome capture, sequencing and variant calling: Frozen matched tumor and normal tissue samples will be used to extract at least 2 µg of high quality input gDNA. A fluorometric-based approach will be used for accurate DNA quantification. DNA quality will be assessed and must have a 260/280 nm absorbance ratio of 1.8-2. Extracted DNA will be fragmented using a Covaris S2 System in a 130 µl volume (aim: 400 bp fragments, settings: duty cycle: 10%, intensity: 4, cycles per burst: 200, time: 55s). Size selection, enrichment PCR and library preparation will be performed following manufacturers protocols. Sequencing of barcoded libraries of 150 bp paired ends will be performed on a HiSeq4000 platform in the UC-Davis sequencing facility to an average coverage of 100X. A bioinformatic pipeline will be implemented to identify putative non-synonymous protein coding variants. The process starts by pre-processing sequencing reads to remove sequencing adaptors and low quality sequences using Trimmomatic software package (V 0.36) [62]. Long reads (> 36bp) will be kept and orphans from either paired ends will be pooled in a single file. High quality reads will then be aligned to the human reference genome. Duplicate reads are excluded using the MarkDuplicates module of the Picard software (v2.2.4)[20]. GATK software package allows additional steps of read realignment to minimize mapping artifacts around indels followed by base quality recalibration to adjust for possible systematic technical errors of sequencing quality scoring[63]. Variant calling will be done using four independent variant callers; GATK HaplotypeCaller (v3.5) [63], FreeBayes (v1.2) [64], SAMtools mpileup [65], and VarScan [66]. Different callers apply different algorithms for variant detection e.g., bayesian statistics and local de novo reassembly. An ensemble machine learning pipeline will be used to incorporate results from multiple models [67]. Variants will be annotated using the Variant Annotation Integrator tool of UCSC genome browser [24]. Putative neo-antigens will be cataloged by determining the non-synonymous mutations present in the tumor tissue but absent from the paired PBMCs and their presence in

predicted protein coding sequence. Additionally, indels, copy number variations and structural rearrangements will be cataloged.

8.2.7 RNA sequencing

Tumor tissue pre- and post-treatment will undergo gene expression analysis using next generation sequencing. RNA-seq analysis: mRNA, libraries will be prepared using the TruSeq Stranded mRNA Sample Prep Kit (Illumina). In brief, polyA containing mRNA molecules will be purified from 2 ug of total RNA (RIN >7) from the frozen samples using poly-T oligos attached to magnetic beads. mRNA will then be fragmented to ~300 bp during the polyA elution step. First strand cDNA will be synthesized using SuperScript II reverse transcriptase (Invitrogen) and random primers. After second strand synthesis, cDNA will undergo 3'end adenylation followed by ligation of the indexing adapters. Adapter-ligated DNA will be enriched using 15 cycles of PCR. All purification and size selection steps will be performed using AMPure XP SPRI beads (Beckman Coulter Genomics). Libraries will be validated using the Bioanalyzer High Sensitivity DNA Kit (Agilent Technologies) and quantified on the Eco Real-Time PCR Instrument (Illumina) using KAPA Illumina Library Quantification Kits (KAPA Biosciences) according to the standard manufacturer's protocols. Paired-end cluster generation and sequencing of 2 x 101 cycles will be performed for all libraries on the Illumina Hi-Seq 2000 platform. Short read sequences will be output in FASTQ format with corresponding base quality scores. The raw data will be initially filtered for reads containing ambiguous base calls, which did not meet the Illumina chastity filter based on quality measures. Quality control of the remaining sequences from each sequenced library will be assessed using FastQC to check for adapters, and distribution of base quality. The reads will then be filtered for low quality reads, contaminating 5' adapters, and trimmed for 3' adapters. The pre-processed reads will be aligned using BWA [68] to the hg19 human reference genome Cufflinks [69], then will be used for expression estimation with both bias correction and multi-read correction parameters enabled. Final QC metrics are collected with Picard's CollectRnaSeqMetrics (http://broadinstitute.github.io/picard), RSeQC [70], and internally developed scripts. RNA-seq data will be analyzed for somatic variant confirmation and for expression of somatically altered transcripts. Additionally, changes in gene expression profiles pre- and post- treatment will be characterized.

8.3 Responsibilities for Translational Research

All translational research studies described above will be performed by the investigator at the UC Davis Laboratory of cancer Immunology and Human Immune Monitoring Core or in conjunction with collaborating intramural or extramural laboratories. Dr. Daly and Dr. Monjazeb will oversee all of the studies, data analysis, and data interpretation. Statistical Analysis will be performed by Dr. Susan L. Stewart.

Merck will be responsible for funding the research as outlined in the research budget and contract. Merck will also be responsible for PD-L1 staining, outside of the multiplexed assay described above, using the IHC 22C3 pharmDx assay.

8.4 All Study Variables

The panel of study endpoints and how they will be measured are described in the below table.

Table 14. Study Variables and Measurements

Endpoint	Variable	Measurement	Comment
Primary- Phase 1	ARR	irRECIST	Objective response at unirradiated sites, the endpoint for measuring responses for the Simon two-stage phase 2 study
Primary- Phase 1	ORR	RECIST and irRECIST	
Primary- Phase 1	DCR	RECIST and irRECIST	
Primary – Phase 1	PFS	RECIST and irRECIST	
Secondary- Phase 1	MTD	CTCAE V4.03	The maximum dose at which <2 of 6 patients experienced a DLT
Secondary- Phase 1	DLT	CTCAE V4.03	Outlined in section 5.4. A DLT is defined as ≥ grade 4 injection site reaction OR ≥ grade 3 treatment related immunologic adverse event (excluding injection site reactions) OR other ≥ grade 3 treatment related adverse events that do not resolve to ≤ grade 2 within 14 days of onset during the 30 day DLT period OR Grade 3 diarrhea, AST/ALT > 3 x ULN with bilirubin > 2 X ULN, or pneumonitis that requires holding treatment > 14 days.
Secondary- Phase 2	Safety	CTCAE V4.03	
Translational	PBMC Immunophenotype	FACS	Blood
Translational	PBMC gene expression	qPCR	Blood
Translational	Systemic Cytokine Signature	Luminex	Serum
Translational	Tissue Immune Markers	IHC / IF	Tissue
Translational	Tumor gene expression	qPCR	Tissue
Translational	Mutational load	Whole exome sequencing/ RNA sequencing	Blood and Tissue
Translational	T cell receptor diversity	TCR deep sequencing	Blood and Tissue

9.0 DATA AND STATISTICAL ANALYSIS PLAN

9.1 Sample Size

This is a phase I/II study that will enroll patients with an advanced solid malignancy, or lymphoma to determine if this regimen converts patients with resistance to PD-1/PD-L1 blockade into responders as determined by abscopal response rate (defined as response rate at lesions not treated with RT + IL-2) using irRECIST as well as ORR, DCR, and PFS using RECIST 1.1.

The phase I dose escalation will employ a standard 3 + 3 design with a goal of determining the MTD through DLT assessment. With three dose escalation levels this arm will recruit up to 18 patients.

The Phase II dose expansion will employ a binomial one stage design which will allow us to evaluate if this therapy can convert those resistant to PD-1/PD-L1 blockade into responders Response will be assessed according to RECIST and will be evaluated at the non-treatment lesions (abscopal response). In order to minimize the required patient numbers, the 3 patients enrolled at the MTD will be included in the phase II efficacy evaluation. We have chosen a threshold of 5% response as not worthy of further evaluation (p0) and a threshold of 20% as worthy of further evaluation in a larger trial (p1). The trial will enroll a total of 21 patients including the three patients treated at the MTD in the phase I (ntot) and must have greater than 3 responders (rtot) for this regimen to be deemed worthy of further evaluation. This design provides a type I error rate of 0.1 and power of 0.82. This trial design will enroll a total maximum of 25 evaluable patients

9.2 Statistical Analysis Plan

Descriptive statistics will be used. All data will be summarized by dose cohort, dose expansion and overall subject population.

9.3 Safety

The adverse events observed will be summarized as frequency, proportion of patients, and exact 95% confidence interval for proportion, categorized by type (organ affected or laboratory determination), severity by CTCAE v4.03 and nadir or maximum values for the laboratory measures), time of onset (i.e., course number), duration, and reversibility or outcome. Tables will be created to summarize these toxicities by dose and course. The secondary endpoint is safety and toxicity. A treatment limiting toxicity (TLT) will be defined as a treatment related grade 3-4 non-hematologic toxicity and will require dose de-escalation. If a TLT does not resolve within 5 days, is not responsive to management, or occurs at the lowest dose level (1 million IU IL-2) after de-escalation then that patient will be removed from trial and the therapy will be deemed intolerable. Therapy will be deemed safe and tolerable if no greater than 33% of patients find the treatment intolerable.

9.4 Efficacy

As an exploratory endpoint, all responses will be reported using RECIST 1.1 definitions [58]. Because of the potential heterogeneity of the patients, all results will be considered preliminary and hypothesis generating for future studies. Response rate among will be summarized by exact binomial confidence intervals. Disease free survival will be summarized with Kaplan-Meier plots to describe the outcome of patients treated on this protocol. The median DFS time will be estimated using standard life table methods.

9.5 Subject Course

Information regarding the subject's course such as completing the study treatment, dose delays, premature discontinuation and major protocol violation will be captured in electronic CRFs.

9.6 Correlative Laboratory Markers

Correlative biomarker endpoints are exploratory and hypothesis generating. Descriptive statistics will be applied to characterize differences within a given patient pre-treatment to post-treatment and across the cohort of patients. Changes in the correlative endpoints will be evaluated using a two-tailed paired student's T-test. To provide a preliminary estimate of the predictive or prognostic value of the various correlative endpoints for DFS we will be using multiple regression analysis with ordinal category for response (generalized linear models) or time to event (proportional hazards survival analysis) as the outcome. We will compute descriptive statistics (mean, standard deviation, median, minimum, and maximum) for mutational load and the immune-related variables (e.g., TILs, Tregs, CD8/Treg ratio, PDL-1, IDO, CTLA-4, PD-1, and immune gene signatures) by tumor type and in the sample as a whole. We will assess the association between mutational load, each immune-related variable, and clinical outcomes by computing Pearson or Spearman correlation coefficients with 95% confidence intervals, as well as using graphical methods to determine whether the associations are generally linear or whether another functional form fits the data better. We will have at least 80% power to detect a moderate correlation of 0.4 at the 0.05 level (2-sided). We may also develop multiple regression models to further explore the relationship between clinical outcomes and correlative markers.

10.0 LABELING, PACKAGING, STORAGE, AND RETURN OF CLINICAL SUPPLIES

10.1 Intralesional IL-2

10.1.1 Formulation, Preparation, Packaging and Handling

Intralesional IL-2 (also referred to as Proleukin® or aldesleukin) is a sterile, white to off-white, preservative-free, lyophilized powder.IL-2 vials should be stored in a refrigerator at 2-8 °C, protected from light and stored in carton. Each vial contains 22 million international units (1.3 mg). Reconstitute each vial with 1.2 mL sterile water for injection (sWFI). Do not shake. Do not reconstitute with 0.9% NaCl or bacteriostatic water for injection. Resultant concentration is 18 million international units/mL (1.1 mg/mL). IL-2 may be further diluted in D5W for final concentration of 30-70 mcg/mL. Avoid bacteriostatic water for injection and NS for reconstitution or dilution.

10.1.2 Administration

The final diluted dose will be delivered to the treatment lesion through intralesional needle placement. Needle placement will be performed by palpation or using ultrasound or computed tomography guidance as indicated. 4 intralesional injections will be administered over a 2-week period with two treatments each week and individual treatments will be a minimum of 48 hours apart.

10.1.3 Disposal and Destruction

Drug supply will either be disposed of at the study site according to the study site's institutional standard operating procedure. Accurate records of all investigational product received at, dispensed from, returned to, and disposed of by the study site should be recorded on the drug accountability form.

10.1.4 Supplier

Intralesional IL-2 will be supplied by the study.

10.2 Pembrolizumab

The investigator shall take responsibility for and shall take all steps to maintain appropriate records and ensure appropriate supply, storage, handling, distribution and usage of investigational product in accordance with the protocol and any applicable laws and regulations. Table 15

Pembrolizumab (also referred to as Keytruda® and MK-3475) will be provided by Merck as summarized in Table 15. Table 15 Pembrolizumab will be administered using a dose of 200 mg Q3W.

Table 15. Pembrolizumab Drug Information

Product Name & Potency	Dosage Form
Pembrolizumab 100 mg/4mL (single use vial)	Solution for Injection

10.2.1 Packaging and Labeling Information

Clinical supplies will be affixed with a clinical label in accordance with regulatory requirements.

For complete details on drug preparation, administration, storage conditions, clinical pharmacology, pharmacokinetics, and known precautions and adverse reactions please see the pembrolizumab IB and or the package insert.

10.2.2 Preparation and Administration

The solution should be visually inspected for particulate matter and discoloration prior to administration. Pembrolizumab solution is clear to slightly opalescent, colorless to slightly yellow. Discard the vial if visible particles are observed. Withdraw the required volume of pembrolizumab from the vial(s) of pembrolizumab and transfer to an intravenous (IV) bag containing 0.9% Sodium Chloride Injection, USP or 5% Dextrose Injection, USP. Mix diluted solution by gentle inversion. The final concentration of the diluted solution should be between 1 mg/mL to 10 mg/mL. Discard unused portion of the vial.

Administer pembrolizumab IV infusion over 30 minutes every 3 weeks through an intravenous line containing a sterile, non-pyrogenic, low-protein binding 0.2 micron to 5 micron in-line or add-on filter. Do not infuse other medications through the same infusion line.

10.2.3 Clinical Supplies Disclosure

This trial is open-label; therefore, the subject, the trial site personnel, the Sponsor-Investigator and/or designee are not blinded to treatment. Drug identity (name, strength) is included in the label text; random code/disclosure envelopes or lists are not provided.

10.2.4 Storage and Handling Requirements

Pembrolizumab vials must be stored in the refrigerator at 2º to 8ºC (36°F to 46°F). Pembrolizumab injection solution should be stored in original carton to protect from light; do not freeze or shake vials.

The product does not contain a preservative, Pembrolizumab should be used immediately after preparation. Pembrolizumab solutions diluted for infusion in NS or D5W may be stored at room temperature for no more than 6 hours from the time of reconstitution or dilution. This includes room temperature storage of the reconstituted vials, storage of the infusion solution in the IV bag, and the duration of infusion. Alternatively, pembrolizumab infusion solutions may be stored under refrigeration at 2 to 8ºC (36 to 46ºF) for no more than 24 hours from the time reconstitution or dilution and protected from light. The infusion should not be frozen.

Clinical supplies must be stored in a secure, limited-access location under the storage conditions specified on the label. Receipt and dispensing of trial medication must be recorded by an authorized person at the trial site. Clinical supplies may not be used for any purpose other than that stated in the protocol.

10.2.5 Drug Disposal and Accountability

The investigator and the assigned pharmacist or designee are responsible for drug accountability. Drug supply will be disposed of according to institutional standard operating procedures and policies. Accurate records of all investigational product received at and dispensed from the study site should be recorded on the drug accountability record.

10.2.6 Supplier

Pembrolizumab will be supplied by the study.

11.0 ETHICAL CONSIDERATIONS AND ADMINISTRATIVE PROCEDURES

11.1 Ethics and Good Clinical Practice

This study must be carried out in compliance with the protocol and Good Clinical Practice, as described in:

1. International Council for Harmonisation (ICH) Harmonized Tripartite Guidelines for Good Clinical Practice 1996.
2. Directive 91/507/EEC, The Rules Governing Medicinal Products in the European Community.
3. US 21 Code of Federal Regulations dealing with clinical studies (including parts 50 and 56 concerning informed consent and IRB regulations).
4. Declaration of Helsinki, concerning medical research in humans (Recommendations Guiding Physicians in Biomedical Research Involving Human Subjects, Helsinki 1964, amended Tokyo 1975, Venice 1983, Hong Kong 1989, Somerset West 1996).

The investigator agrees, when signing the protocol, to adhere to the instructions and procedures described in it and thereby to adhere to the principles of Good Clinical Practice that it conforms to.

11.2 Institutional Review Board

The protocol, the proposed informed consent form, and all patient-facing documents must be reviewed and approved by a properly constituted Institutional Review Board before any research involving human subjects has been conducted. Prior to obtaining IRB approval, the protocol must be approved by the UC Davis Comprehensive Cancer Center Scientific Review Committee (SRC).

11.3 Informed Consent

The investigator must explain to each subject (or legally authorized representative) the nature of the study, its purpose, the procedures involved, the expected duration, the potential risks and benefits involved and any discomfort it may entail. Each subject must be informed that participation in the study is voluntary and that he/she may withdraw from the study at any time and that withdrawal of consent will not affect his/her subsequent medical treatment or relationship with the treating physician.

This informed consent should be given by means of a standard written statement, written in non-technical language. The subject should read and consider the statement before signing and dating it and should be given a copy of the signed document. If the subject cannot read or sign the documents, oral presentation may be made or signature given by the subject's legally appointed representative, if witnessed by a person not involved in the study, mentioning that the patient could not read or sign the documents. No patient can enter the study before his/her informed consent has been obtained.

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

11.4 Patient Confidentiality

In order to maintain patient privacy, all study reports and communications will identify the patient by initials and the assigned patient number. Medical records of patients will be maintained in strict confidence according to legal requirements. The patient's confidentiality will be maintained and will not be made publicly available to the extent permitted by the applicable laws and regulations.

11.5 Protocol Compliance and Deviations

The investigator will conduct the study in compliance with the protocol given approval/favorable opinion by the IRB and the appropriate regulatory authority(ies). Changes to the protocol will require approval from Merck and written IRB approval/favorable opinion prior to implementation, except when the modification is needed to eliminate an immediate hazard(s) to patients. The IRB may provide, if applicable regulatory authority(ies) permit, expedited review and approval/favorable opinion for minor change(s) in ongoing studies that have the approval /favorable opinion of the IRB. The investigator will submit all protocol modifications to Merck and the regulatory authority(ies) in accordance with the governing regulations.

All protocol deviations will be reported in accordance with UCD IRB Administration and UCD Cancer Center OCR policies and the participating site's IRB policies. Any departures from the protocol must be fully documented in the source documents.

11.6 Onsite Audits

Regulatory authorities or the IRB may request access to all source documents, data capture records, and other study documentation for on-site audit or inspection. Direct access to these documents must be guaranteed by the investigator, who must provide support at all times for these activities.

11.7 Premature Closure of the Study

This study may be prematurely terminated, if in the opinion of the investigator or Merck, there is sufficient reasonable cause. Written notification documenting the reason for study termination will be provided to the investigator or Merck by the terminating party.

11.8 Record Retention

The investigator will maintain all study records according to ICH-GCP and applicable regulatory requirement(s).

11.9 Quality Assurance

Quality assurance audits of select patients and source documents may be conducted by the Quality Assurance and/or Data Safety Committees as outlined in the UC Davis Cancer Center Data and Safety Monitoring plan. Quality control will be maintained by the OCR Quality Assurance team according to OCR policy.

12.0 OVERSIGHT AND MONITORING

12.1 Data and Safety Monitoring

In addition to the requirements for adverse event reporting as outlined in Section 7.3, this protocol is also subject to the UC Davis Comprehensive Cancer Center's (UCDCCC) Data and Safety Monitoring Plan. The UCDCCC is committed to pursuing high-quality patient-oriented clinical research and has established mechanisms to ensure both scientific rigor and patient safety in the conduct of clinical research studies. The UCDCCC relies on a multi-tiered committee system that reviews and monitors all cancer clinical trials and ensures the safety of its participants, in compliance with institutional and federal requirements on adverse event (AE) reporting, verification of data accuracy, and adherence to protocol eligibility requirements, treatment guidelines, and related matters. This trial has been assigned high risk level per the Data and Safety Monitoring Plan (DSMP) version July 1, 2020, Table 3 Risk Stratification of Trials, which defines the frequency at which this trial will be audited, monitored, and reviewed by the Data and Safety Monitoring Committee (DSMC) (see Appendix 6).

12.2 Investigator Monitoring Guidelines

Investigators will conduct continuous review of patient safety while on study treatment.

13.0 REFERENCES

1. Clemente, C.G., et al., *Prognostic value of tumor infiltrating lymphocytes in the vertical growth phase of primary cutaneous melanoma.* Cancer, 1996. **77**(7): p. 1303-10.
2. Fourcade, J., et al., * CD8⁺ T Cells Specific for Tumor Antigens Can Be Rendered Dysfunctional by the Tumor Microenvironment through Upregulation of the Inhibitory Receptors BTLA and PD-1 *. Cancer Research, 2012. **72**(4): p. 887-896.
3. Iwai, Y., et al., *Involvement of PD-L1 on tumor cells in the escape from host immune system and tumor immunotherapy by PD-L1 blockade.* Proc Natl Acad Sci U S A, 2002. **99**(19): p. 12293-7.
4. Coppin, C., et al., *Immunotherapy for advanced renal cell cancer.* Cochrane Database Syst Rev, 2005(1): p. Cd001425.
5. Shabtai, M., et al., *Increased expression of activation markers in renal cell carcinoma infiltrating lymphocytes.* J Urol, 2002. **168**(5): p. 2216-9.
6. Brahmer, J.R., et al., *Phase I study of single-agent anti-programmed death-1 (MDX-1106) in refractory solid tumors: safety, clinical activity, pharmacodynamics, and immunologic correlates.* J Clin Oncol, 2010. **28**(19): p. 3167-75.
7. Topalian, S.L., et al., *Safety, Activity, and Immune Correlates of Anti–PD-1 Antibody in Cancer.* N Engl J Med, 2012. **366**(26): p. 2443-54.
8. Borghaei, H., et al., *Nivolumab versus Docetaxel in Advanced Nonsquamous Non-Small-Cell Lung Cancer.* N Engl J Med, 2015. **373**(17): p. 1627-39.
9. Herbst, R.S., et al., *Pembrolizumab versus docetaxel for previously treated, PD-L1-positive, advanced non-small-cell lung cancer (KEYNOTE-010): a randomised controlled trial.* Lancet, 2016. **387**(10027): p. 1540-50.
10. Brahmer, J.R., et al., *Survival and long-term follow-up of the phase I trial of nivolumab (Anti-PD-1; BMS-936558; ONO-4538) in patients (pts) with previously treated advanced non-small cell lung cancer (NSCLC).* J Clin Oncol, 2013. **31**(15S; abstr 8030).
11. Brahmer, J., et al., *Nivolumab versus Docetaxel in Advanced Squamous-Cell Non-Small-Cell Lung Cancer.* N Engl J Med, 2015. **373**(2): p. 123-35.
12. Robert, C., et al., *Anti-programmed-death-receptor-1 treatment with pembrolizumab in ipilimumab-refractory advanced melanoma: a randomised dose-comparison cohort of a phase 1 trial.* The Lancet. **384**(9948): p. 1109-1117.
13. Ribas, A., et al., *Pembrolizumab versus investigator-choice chemotherapy for ipilimumab-refractory melanoma (KEYNOTE-002): a randomised, controlled, phase 2 trial.* The Lancet Oncology. **16**(8): p. 908-918.
14. Robert, C., et al., *Pembrolizumab versus Ipilimumab in Advanced Melanoma.* N Engl J Med, 2015. **372**(26): p. 2521-32.
15. Ribas, A., et al., *Association of pembrolizumab with tumor response and survival among patients with advanced melanoma.* JAMA, 2016. **315**(15): p. 1600-1609.

16. Daud AI, et al., *Programmed Death-Ligand 1 Expression and Response to the Anti-Programmed Death 1 Antibody Pembrolizumab in Melanoma.*

. J Clin Oncol, 2016 Dec. **34**(34): p. 4102-4109.

17. Gangadhar, T.C. and A.K.S. Salama, *Clinical applications of PD-1-based therapy: a focus on pembrolizumab (MK-3475) in the management of melanoma and other tumor types.* Onco Targets Ther, 2015. **8**: p. 929-37.

18. Atkins, M.B., et al., *Phase Ib dose-finding study of axitinib plus pembrolizumab in treatment-naïve patients with advanced renal cell carcinoma.* J Immunother Cancer, 2015. **3**(Suppl 2): p. P353. doi:10.1186/2051-1426-3-S2-P353.

19. Seiwert, T.Y., et al., *Safety and clinical activity of pembrolizumab for treatment of recurrent or metastatic squamous cell carcinoma of the head and neck (KEYNOTE-012): an open-label, multicentre, phase 1b trial.* The Lancet Oncology. **17**(7): p. 956-965.

20. Atkins, M.B., et al., *Randomized phase II trial of high-dose interleukin-2 either alone or in combination with interferon alfa-2b in advanced renal cell carcinoma.* J Clin Oncol, 1993. **11**(4): p. 661-70.

21. Kammula, U.S., D.E. White, and S.A. Rosenberg, *Trends in the safety of high dose bolus interleukin-2 administration in patients with metastatic cancer.* Cancer, 1998. **83**.

22. Schwartzentruber, D., *Interleukin-2: Clinical applications - principles of administration and management of side effects*, in *Principles and Practice of the Biologic Therapy of Cancer*. 2000, Lippincott, Williams & Wilkins: Philadelphia.

23. Yang, J.C. and S.A. Rosenberg, *An ongoing prospective randomized comparison of interleukin-2 regimens for the treatment of metastatic renal cell cancer.* Cancer J Sci Am, 1997. **3 Suppl 1**: p. S79-84.

24. Yang, J.C., et al., *Randomized study of high-dose and low-dose interleukin-2 in patients with metastatic renal cancer.* J Clin Oncol, 2003. **21**(16): p. 3127-32.

25. Atkins, M.B., 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**.

26. Fyfe, G., et al., *Results of treatment of 255 patients with metastatic renal cell carcinoma who received high-dose recombinant interleukin-2 therapy.* J Clin Oncol, 1995. **13**.

27. Rosenberg, S.A., et al., *Regression of established pulmonary metastases and subcutaneous tumor mediated by the systemic administration of high-dose recombinant interleukin 2.* J Exp Med, 1985. **161**(5): p. 1169-88.

28. Smith, F.O., et al., *Treatment of metastatic melanoma using interleukin-2 alone or in conjunction with vaccines.* Clin Cancer Res, 2008. **14**(17): p. 5610-8.

29. Alva, A., et al., *Contemporary experience with high-dose interleukin-2 therapy and impact on survival in patients with metastatic melanoma and metastatic renal cell carcinoma.* Cancer Immunology, Immunotherapy, 2016. **65**(12): p. 1533-1544.

30. Fyfe, G., et al., *Results of treatment of 255 patients with metastatic renal cell carcinoma who received high-dose recombinant interleukin-2 therapy.* J Clin Oncol, 1995. **13**(3): p. 688-96.

31. Hanzly, M., et al., *High-dose Interleukin-2 Therapy for Metastatic Renal Cell Carcinoma: A Contemporary Experience.* Urology, 2014. **83**(5): p. 1129-1134.

32. Forni, G., et al., *Perilymphatic injections of cytokines: a new tool in active cancer immunotherapy. Experimental rationale and clinical findings.* Ann Ist Super Sanita, 1990. **26**(3-4): p. 397-409.

33. Demaria, S., et al., *Combining radiotherapy and immunotherapy: a revived partnership.* Int J Radiat Oncol Biol Phys, 2005. **63**(3): p. 655-66.

34. Levy, A., et al., *Radiation therapy and immunotherapy: implications for a combined cancer treatment.* Crit Rev Oncol Hematol, 2013. **85**(3): p. 278-87.

35. Hallahan, D.E., et al., *Increased tumor necrosis factor alpha mRNA after cellular exposure to ionizing radiation.* Proc Natl Acad Sci U S A, 1989. **86**(24): p. 10104-7.

36. Xian, J., et al., *Combination nonviral murine interleukin 2 and interleukin 12 gene therapy and radiotherapy for head and neck squamous cell carcinoma.* Arch Otolaryngol Head Neck Surg, 2005. **131**(12): p. 1079-85.

37. Seung, S.K., et al., *Phase 1 study of stereotactic body radiotherapy and interleukin-2--tumor and immunological responses.* Sci Transl Med, 2012. **4**(137): p. 137ra74.

38. Safwat, A., et al., *A phase II trial of low-dose total body irradiation and subcutaneous interleukin-2 in metastatic melanoma.* Radiother Oncol, 2005. **77**(2): p. 143-7.

39. Sung, K.W., et al., *Tandem high-dose chemotherapy and autologous stem cell transplantation in patients with high-risk neuroblastoma: results of SMC NB-2004 study.* Bone Marrow Transplant, 2013. **48**(1): p. 68-73.

40. Sasaki, R., et al., *Angiosarcoma treated with radiotherapy: impact of tumor type and size on outcome.* Int J Radiat Oncol Biol Phys, 2002. **52**(4): p. 1032-40.

41. Lissoni, P., et al., *Progress report on the palliative therapy of 100 patients with neoplastic effusions by intracavitary low-dose interleukin-2.* Oncology, 2001. **60**(4): p. 308-12.

42. Scudeletti, M., et al., *Immunotherapy with intralesional and systemic interleukin-2 of patients with non-small-cell lung cancer.* Cancer Immunol Immunother, 1993. **37**(2): p. 119-24.

43. Weide, B., et al., *High response rate after intratumoral treatment with interleukin-2: results from a phase 2 study in 51 patients with metastasized melanoma.* Cancer, 2010. **116**(17): p. 4139-46.

44. Shi, V.Y., et al., *100% Complete response rate in patients with cutaneous metastatic melanoma treated with intralesional interleukin (IL)-2, imiquimod, and topical retinoid combination therapy: results of a case series.* J Am Acad Dermatol, 2015. **73**(4): p. 645-54.

45. Garcia, M.S., et al., *Complete regression of subcutaneous and cutaneous metastatic melanoma with high-dose intralesional interleukin 2 in combination with topical imiquimod and retinoid cream.* Melanoma Res, 2011. **21**(3): p. 235-43.

46. Patel, F., et al., *Detailed protocol for administration of intralesional IL-2 for the treatment of Stage IIIc and IV M1a metastatic melanoma based on current NCCN guidelines.* Dermatol Online J, 2014. **20**(11).

47. Timar, J., et al., *Neoadjuvant immunotherapy of oral squamous cell carcinoma modulates intratumoral CD4/CD8 ratio and tumor microenvironment: a multicenter phase II clinical trial.* J Clin Oncol, 2005. **23**(15): p. 3421-32.

48. Ferlazzo, G., et al., *Intralesional sonographically guided injections of lymphokine-activated killer cells and recombinant interleukin-2 for the treatment of liver tumors: a pilot study.* J Immunother, 1997. **20**(2): p. 158-63.

49. de Gast, G.C., et al., *Phase I trial of combined immunotherapy with subcutaneous granulocyte macrophage colony-stimulating factor, low-dose interleukin 2, and interferon alpha in progressive metastatic melanoma and renal cell carcinoma.* Clin Cancer Res, 2000. **6**(4): p. 1267-72.

50. Huland, E., et al., *Inhaled interleukin-2 therapy in pulmonary metastatic renal cell carcinoma: six years of experience.* Cancer J Sci Am, 1997. **3 Suppl 1**: p. S98-105.

51. De Stefani, A., et al., *Improved survival with perilymphatic interleukin 2 in patients with resectable squamous cell carcinoma of the oral cavity and oropharynx.* Cancer, 2002. **95**(1): p. 90-7.

52. Dovedi, S.J., et al., *Acquired resistance to fractionated radiotherapy can be overcome by concurrent PD-L1 blockade.* Cancer Research, 2014. **74**(19): p. 5458-68.

53. Gajewski, T.F., J. Louahed, and V.G. Brichard, *Gene signature in melanoma associated with clinical activity: a potential clue to unlock cancer immunotherapy.* Cancer J, 2010. **16**(4): p. 399-403.

54. Harlin, H., et al., *Chemokine expression in melanoma metastases associated with CD8+ T-cell recruitment.* Cancer Res, 2009. **69**(7): p. 3077-85.

55. Spranger, S., R. Bao, and T.F. Gajewski, *Melanoma-intrinsic beta-catenin signalling prevents anti-tumour immunity.* Nature, 2015. **523**(7559): p. 231-5.

56. Klug, F., et al., *Low-Dose Irradiation Programs Macrophage Differentiation to an iNOS(+)/M1 Phenotype that Orchestrates Effective T Cell Immunotherapy*, in *Cancer Cell*. 2013. p. 589-602.

57. Rizvi, N.A., et al., *Cancer immunology. Mutational landscape determines sensitivity to PD-1 blockade in non-small cell lung cancer.* Science, 2015. **348**(6230): p. 124-8.

58. Eisenhauer, E.A., et al., *New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1).* Eur J Cancer, 2009. **45**(2): p. 228-47.

59. Wolchok, J.D., et al., *Guidelines for the evaluation of immune therapy activity in solid tumors: immune-related response criteria.* Clin Cancer Res, 2009. **15**(23): p. 7412-20.

60. Schalper, K.A., et al., *Objective measurement and clinical significance of TILs in non-small cell lung cancer.* J Natl Cancer Inst, 2015. **107**(3).

61. Schalper, K.A., et al., *In situ tumor PD-L1 mRNA expression is associated with increased TILs and better outcome in breast carcinomas.* Clin Cancer Res, 2014. **20**(10): p. 2773-82.

62. Bolger, A.M., M. Lohse, and B. Usadel, *Trimmomatic: a flexible trimmer for Illumina sequence data.* Bioinformatics, 2014. **30**(15): p. 2114-20.

63. McKenna, A., et al., *The Genome Analysis Toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data.* Genome Res, 2010. **20**(9): p. 1297-303.

64. Garrison, E. and G. Marth *Haplotype-based variant detection from short-read sequencing*. ArXiv e-prints, 2012. **1207**.

65. Li, H., et al., *The Sequence Alignment/Map format and SAMtools.* Bioinformatics, 2009. **25**(16): p. 2078-9.

66. Koboldt, D.C., et al., *VarScan: variant detection in massively parallel sequencing of individual and pooled samples.* Bioinformatics, 2009. **25**(17): p. 2283-5.

67. CHAPMAN, B. *An automated ensemble method for combining and evaluating genomic variants from multiple callers*. 2013; Available from: http://bcb.io/2013/02/06/an-automated-ensemble-method-for-combining-and-evaluating-genomic-variants-from-multiple-callers/.

68. Li, H. and R. Durbin, *Fast and accurate short read alignment with Burrows-Wheeler transform.* Bioinformatics, 2009. **25**(14): p. 1754-60.

69. Trapnell, C., et al., *Differential gene and transcript expression analysis of RNA-seq experiments with TopHat and Cufflinks.* Nat Protoc, 2012. **7**(3): p. 562-78.

70. Wang, L., S. Wang, and W. Li, *RSeQC: quality control of RNA-seq experiments.* Bioinformatics, 2012. **28**(16): p. 2184-5.

14.0 APPENDICES

Appendix 1: ECOG Performance Status Scale

Karnofsky Status	Karnofsky Grade**	ECOG Grade	ECOG Status**
Normal, no complaints	100	0	Fully active, able to carry on all pre-disease performance without restriction
Able to carry on normal activities. Minor signs or symptoms of disease	90	1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light house work, office work
Normal activity with effort	80		
Care for self. Unable to carry on normal activity or to do active work	70	2	Ambulatory and capable of all selfcare but unable to carry out any work activities. Up and about more than 50% of waking hours
Requires occasional assistance, but able to care for most of his needs	60		
Requires considerable assistance and frequent medical care	50	3	Capable of only limited selfcare, confined to bed or chair more than 50% of waking hours
Disabled. Requires special care and assistance	40		
Severely disabled. Hospitalization indicated though death nonimminent	30	4	Completely disabled. Cannot carry on any selfcare. Totally confined to bed or chair
Very sick. Hospitalization necessary. Active supportive treatment necessary	20		
Moribund	10		
Dead	0	5	Dead

* KPS will be used in this study. ECOG status is listed for reference.

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

Appendix 2: Data Collection

All data will be collected using UC Davis data collection forms. Any and all source documentation shall be maintained.

Appendix 3: Molecular Correlative Sample Handling

Specimen Submission for Correlative Studies:

Patients must be offered participation in these molecular correlative studies. With the patient's consent, tissue and blood specimens will be submitted as outlined below. Samples will be de-identified and coded with a new patient ID number to protect patient's identity.

Specimen Collection, Storage, Shipping and Submission Requirements

It is required that paraffin-embedded tissue blocks or slides from time of diagnosis (or subsequent, but prior to therapy) as well as blood specimens, as outlined below, be submitted for expression of relevant molecular targets.

- **Archival tumor specimens**: If available, 1 - 2 paraffin-embedded tissue blocks containing formalin-fixed tumor or needle aspirate slides from time of diagnosis (or subsequent, but prior to therapy) should be submitted for evaluation of expression of PD-L1 and other relevant molecules. Paraffin blocks may be processed according to standard institutional protocols. If blocks are unavailable, 16 unstained slides are acceptable alternatives.
- Fresh **tumor biopsy**: **All specimens must be labeled with protocol number, patient registration number, and date of specimen collection.** Tissue portions will be used for (in order of priority):
    1. Fixed in formalin for the preparation of FFPE blocks
    2. Placed in 1 ml RNA later and snap frozen with liquid nitrogen and stored at -70 to -80°C for future RNA extraction and qPCR and immunochip analysis,
    3. Snap frozen with liquid nitrogen and stored at -70 to -80°C for future DNA extraction
- **Blood specimens**: Blood specimens (3 x 10 ml lavender top EDTA tubes) will be collected from each patient.

    Each purple-top (EDTA) tube should be delivered to the lab where it will be inverted several times, and placed on wet ice until centrifugation. The tubes should be centrifuged as soon as possible at approximately 600 x g for 10 minutes. Approximately 1.5 ml of plasma should be removed from each tube and pooled in a fresh, sterile 15 ml conical tube. Centrifuge plasma a second time at 1500-1600 x g for 5 – 10 minutes to pellet and remaining cells. After the second spin, plasma should be removed and placed in 500 ul aliquots in labeled cryotubes. Plasma will be frozen and stored at -70 to -80°C. Plasma samples will be assayed by Luminex to evaluate systemic cytokine levels.

    Blood remaining in the original EDTA tubes will be processed for PBMC collection. Briefly, blood cells will be resuspended in room temperature PBS at a 2:1 ratio. The blood will then be carefully layered over Lymphocyte Separation Media (Ficol Solution, LSM) and centrifuged at 400 x g for 30 minutes. Resulting cells will be washed twice in PBS then resuspended in RPMI to facilitate cell counting. To retain viability of cells,

cells will be frozen at about 1.0 x 107 in a freezing media consisting of 50% RPMI, 40% FBS, & 10% DMSO. Cells will be frozen at -1°C/minute, the optimal rate for cell preservation. Cryopreserved cells will be stored in a liquid nitrogen freezer.

Appendix 4: Immune-Related Response Criteria (irRC)

Increasing clinical experience indicates that traditional response criteria (e.g., Response Evaluation Criteria in Solid Tumors, Version 1.1 [RECIST v1.1] and World Health Organization [WHO]) may not be sufficient to characterize fully activity in the new era of target therapies and/or biologics. In studies with cytokines, cancer vaccines, and monoclonal antibodies, complete response, partial response, or stable disease has been shown to occur after an increase in tumor burden as characterized by progressive disease by traditional response criteria. Therefore, conventional response criteria may not adequately assess the activity of immunotherapeutic agents because progressive disease (by initial radiographic evaluation) does not necessarily reflect therapeutic failure. Long term effect on the target disease must also be captured. The immune related response criteria [60] (irRC) are criteria that attempt to do that by enhancing characterization of new response patterns that have been observed with immunotherapeutic agents (i.e., ipilimumab). (Note: The irRC only index and measurable new lesions are taken into account.)

GLOSSARY

Term	Definition
SPD	sum of the products of the two largest perpendicular diameters
Tumor burden	$SPD_{\text{index lesions}} + SPD_{\text{new, measurable lesions}}$
Nadir	minimally recorded tumor burden
irCR	immune-related complete response
irPD	immune-related progressive disease
irPR	immune-related partial response
irSD	immune-related stable disease
irBOR	immune-related best overall response

BASELINE ASSESSMENT USING irRC

Step 1. Identify the index lesions (five lesions per organ, up to ten visceral lesions and five cutaneous lesions).

Step 2. Calculate the SPD of all of these index lesions:

$$SPD = \sum_{i} \text{(Largest diameter of lesion i)} \times \text{(Second largest diameter of lesion i)}.$$

POST-BASELINE ASSESSMENTS USING irRC

Step 1. Calculate the SPD of the index lesions.

Step 2. Identify new, measurable lesions (≥ 5 × 5 mm; up to five new lesions per organ: five new cutaneous lesions and ten visceral lesions).

Step 3. Calculate the SPD of the new, measurable lesions.

Step 4. Calculate the tumor burden:

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

Step 5. Calculate the change in tumor burden relative to baseline and the change in tumor burden relative to nadir.

Step 6. Derive the overall response using the table below.

Overall Response	Criterion
irCR	Complete disappearance of all lesions (whether measurable or not, and no new lesions) confirmed by a repeat, consecutive assessment ≥ 4 weeks from the date first documented
irPR	Decrease in tumor burden ≥ 50% relative to baseline confirmed by a consecutive assessment ≥ 4 weeks from the date first documented
irSD	Criteria for irCR, irPR, and irPD are not met; does not require confirmation
irPD	Increase in tumor burden ≥ 25% relative to nadir confirmed by a consecutive assessment ≥ 4 weeks from the date first documented

irCR = immune-related complete response; irPD = immune-related progressive disease; irPR = immune-related partial response; irSD = immune-related stable disease.

DETERMINATION OF irBOR

Once a patient has completed all tumor assessments, his/her irBOR may be determined:

Condition	irBOR
At least one irCR	irCR
At least one irPR and no irCR	irPR
At least one irSD and no irCR and no irPR	irSD
At least one irPD and no irCR, no irPR, and no irSD	irPD

irBOR = immune-related best overall response; irCR = immune-related complete response; irPD = immune-related progressive disease; irPR = immune-related partial response; irSD = immune-related stable disease.

Appendix 5: Common Terminology Criteria for Adverse Events V4.0 (CTCAE)

The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for adverse event reporting. (http://ctep.cancer.gov/reporting/ctc.html)

Appendix 6. Risk Stratification of Trials

Trial Risk Level	Oversight Level	Monitoring Frequency	Auditing Frequency
High Risk - There is a high probability of the occurrence of a serious adverse event and/or study monitoring and reporting requirements of the trial are such that events or event trends may not be immediately recognized. • Investigator-initiated IND or IDE trials • All Phase I trials • Trials in gene therapy or other high-risk areas as designated by the NIH	**High Intensity Oversight** – The PI must prospectively register participants and submit adverse event reports to the DSMC. **Every two months**, the DSMC reviews these trial-specific documents including all audits and monitoring reports, and adverse events. The PI is expected to review adverse events with the sub-investigators on the trial and the study team. For phase I trials, continuous monitoring of patient safety and dose limiting toxicities occurs in weekly meeting facilitated by the Phase I Program Directors. Cohort expansion meetings are led by the PI of the trial and the meeting minutes are forwarded to the DSMC for review. For an Institutional phase III trial, the SRC, in collaboration with the PI, is responsible for establishing an independent DSMB.	• Within 2 weeks of 1st patient cycle 1 completion • Within 2 weeks of 1st response assessment completion • 100% monitoring quarterly thereafter	Every 6 months
Moderate Risk – There is a probability of a moderate-severity event occurring but there is adequate safety monitoring in the trial to identify events promptly and to minimize their effects. • All other trials involving therapeutic interventions (Phase II/III trials with IND exemptions)	**Moderate Intensity Oversight** – The PI must prospectively register participants and submit adverse event reports to the DSMC. **Quarterly**, the DSMC reviews these trial-specific documents including all audits and monitoring reports, and adverse events. The PI is expected to review all adverse events with the sub-investigators and the study team.	• Within 2 weeks of 1st patient cycle 1 completion • Within 2 weeks of 1st response assessment completion • 100% monitoring eligibility/consent quarterly thereafter • 10% complete monitoring review quarterly thereafter	Every year
Minimal Risk – The probability and magnitude of harm or discomfort anticipated in the research are not greater than those ordinarily encountered in daily life or during the performance of routine physical and psychological examinations or tests and where confidentiality is adequately protected. • Trials involving non-therapeutic interventions (Cancer Prevention & Control, Diagnostic, Correlative, Behavioral, or Supportive Care) that are no greater than minimal risk ----------OR---------- Non-interventional trials	**Minimal Intensity Oversight** – This level applies to those studies that include a physical intervention with a participant. An example of this type of trial is a dietary intervention or exercise study aimed at symptom management. **Every six months**, the DSMC reviews these trial-specific documents including all audits reports and adverse events. ---------- **Low Intensity Oversight** – This level applies to those studies that do not include a physical intervention with a participant. Examples of this type of trial is a computer or internet-based strategy aimed at increasing awareness of cancer issues, observational trials, or epidemiological studies. Oversight by the DSMC is not required for these trials.	No monitoring requirement No monitoring requirement	Every year No auditing requirement

Source: UC Davis Comprehensive Cancer Center Data and Safety Monitoring Plan, Revised: July 2020, Table 3.