CLINICAL PROTOCOL

A Phase 2, Multicenter, Single-arm Study to Assess the Safety and Efficacy of Cell Transfer Therapy Using Autologous Tumor Infiltrating Lymphocytes (LN-144) Followed by IL-2 for Treatment of Patients with Metastatic Melanoma

PROTOCOL NUMBER:	C-144-01
SPONSOR:	Lion Biotechnologies, Inc. 112 W 34th Street, New York, NY 10120
PROTOCOL VERSION:	Final Version 4.0
PROTOCOL DATE:	July 18, 2016

SPONSOR PROTOCOL SIGNATURE PAGE

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Approved by:

PPD

PPD

PPD

July 18, 2016

Date

INVESTIGATOR PROTOCOL SIGNATURE PAGE

Protocol Title:	A Phase 2, Multicenter, Single-arm Study to Assess the Safety and Efficacy of Cell Transfer Therapy Using Autologous Tumor Infiltrating Lymphocytes (LN-144) Followed by IL-2 for Treatment of Patients with Metastatic Melanoma
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I agree to conduct the study as detailed in the protocol and in compliance with ICH Guidelines for Good Clinical Practice.

I acknowledge that I am responsible for overall study conduct, and I agree to personally conduct or supervise the described clinical study.

I agree to ensure that all associates, colleagues, and employees assisting in the conduct of the study are informed about their obligations. Mechanisms are in place to ensure that site staff receives the appropriate information throughout the study.

_____
Principal Investigator Printed Name

_____ _____
Principal Investigator Signature Date

PROTOCOL SYNOPSIS

Protocol Title:	A Phase 2, Multicenter, Single-arm Study to Assess the Safety and Efficacy of Cell Transfer Therapy Using Autologous Tumor Infiltrating Lymphocytes (LN-144) Followed by IL-2 for Treatment of Patients with Metastatic Melanoma
Study Type:	Phase 2
Indication:	Treatment of metastatic melanoma that has progressed following prior systemic therapy
Investigational Agent:	LN-144: Autologous Tumor Infiltrating Lymphocytes (TIL) derived from the patient's own tumor
Study Objectives:	**Primary Objectives** • To characterize the safety profile of LN-144 in patients with metastatic melanoma. **Secondary Objectives** • To evaluate the efficacy of LN-144 in patients with metastatic melanoma using the best overall response rate (ORR) and complete response rate (CR). • To evaluate efficacy parameters of LN-144 in patients with metastatic melanoma such as progression-free survival (PFS), duration of response (DOR), and overall survival (OS). **Exploratory Objectives** • To explore persistence of LN-144 and potential immune correlates of response, outcome, and toxicity of the treatment.
Study Design:	Prospective, single-arm interventional study evaluating adoptive cell therapy (ACT) with autologous TIL infusion (LN-144) followed by IL-2 after a non-myeloablative chemotherapy preparative regimen.
Dose and Treatment Schedule:	The cell transfer therapy used in this study involves patients receiving a lymphocyte depleting preparative regimen, followed by infusion of autologous TIL (LN-144) followed by the administration of a regimen of IL-2 at 600,000 IU/kg approximately every eight hours for up to a maximum of six doses starting 12-24 hours after cell infusion. Patients will be evaluated for response approximately 12 weeks following the LN-144 therapy. Patients will receive one course of treatment.
Duration of Study Participation:	Screening and tumor resection/TIL harvest: up to 6 weeks Lymphodepletion: 1 week Treatment period: 12 weeks Long term Follow-up period: up to 2 years
Follow-up Period:	Patients will be evaluated as noted below: • At six months (+/- 1 week) following LN-144 treatment • At nine months (+/- 1 week) following LN-144 treatment

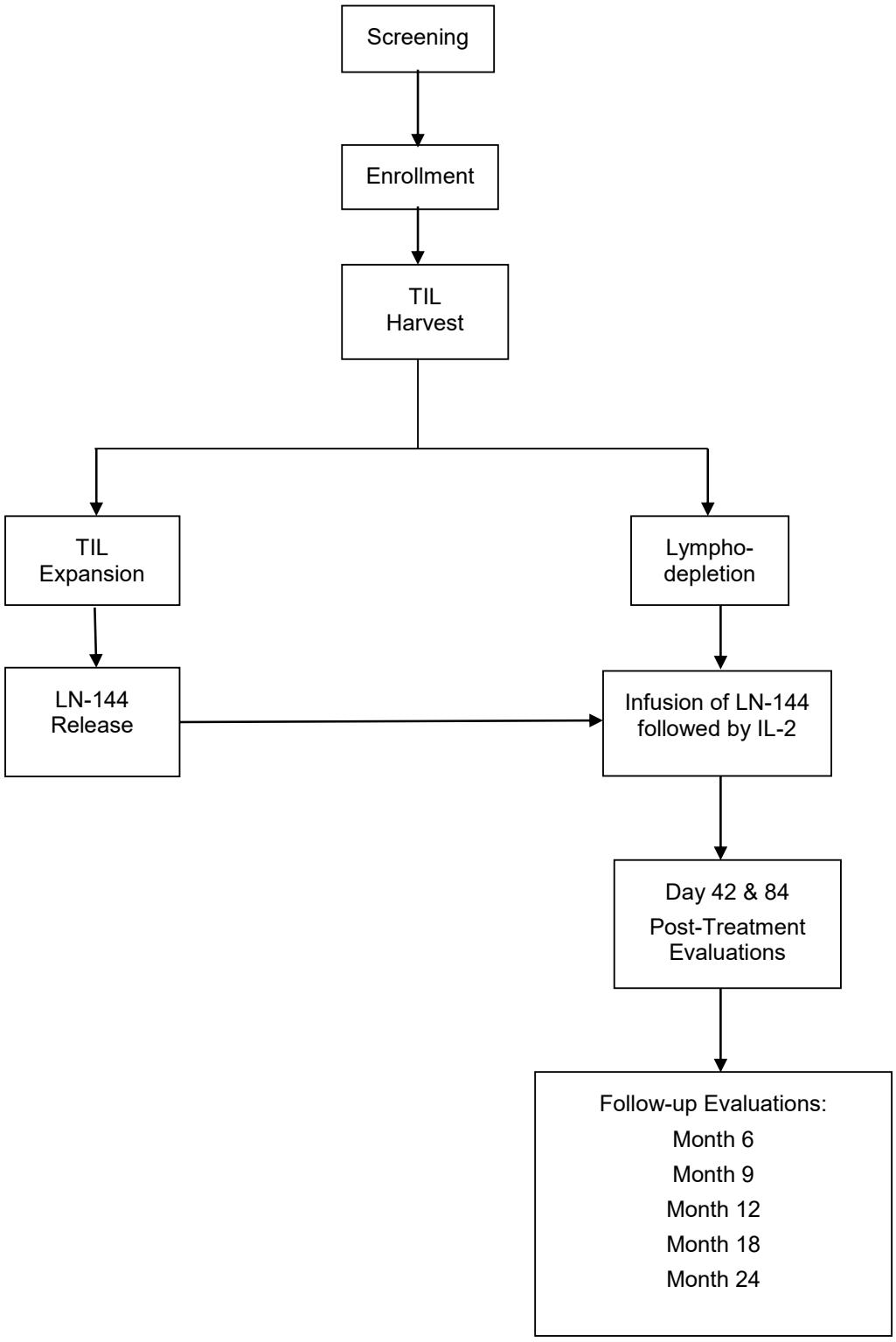
	• At 12 months (+/- 1 week) following LN-144 treatment • At 18 months (+/- 3 weeks) following LN-144 treatment • At 24 months (+/- 3 weeks) following LN-144 treatment
Number of Study Centers:	Approximately ten clinical sites
Number of Planned Patients:	Twenty patients who complete treatment. Complete treatment is defined as successful infusion with LN-144 followed by IL-2.
Study Population: **Diagnosis and Main Criteria for Inclusion:**	To be eligible for the study, patients must meet ALL of the following criteria prior to enrollment in the study: a. Patients must have measurable metastatic melanoma and at least one lesion that is resectable for TIL generation. Ideally the lesion must be at least 1.5 cm in diameter post-prosection and can be surgically removed with minimal morbidity (defined as any operation for which expected hospitalization is less than or equal to three days). b. Patients must have undergone at least one prior systemic treatment for metastatic melanoma. c. Patients must have either progressive disease or no response (i.e., no PR or CR) while receiving or after completion of most recent prior treatment. d. Patients must be greater than 18 years of age at the time of consent. Patients greater than 65 years of age may be allowed in the study after discussion between the Principle Investigator and Medical Monitor regarding the patient's ability to tolerate high dose IL-2. e. Patients must have an Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1. f. In the opinion of the Investigator, patient must be capable of participating and completing study procedures. g. Patients of childbearing potential or with partners of childbearing potential must be willing to practice birth control during treatment and for four months after receiving all protocol related therapy. h. Patients must have a serum absolute neutrophil count (ANC) greater than 1000/mm^3, hemoglobin greater than 9.0 g/dL, and platelet count greater than 100,000/mm^3. i. Patients must have a serum ALT/SGPT and AST/SGOT less than three times the upper limit of normal (<3x ULN), a calculated creatinine clearance of greater than 50 mL/min (>50 mL/min), and a total bilirubin less than or equal to 2 mg/dL (≤ 2 mg/dL). Patients with Gilbert's Syndrome must have a total bilirubin less than 3 mg/dL (<3 mg/dL). j. Patients must be seronegative for the HIV antibody, hepatitis B antigen, and hepatitis C antibody or antigen. k. Patients must be EBV viral capsid antigen (VCA) IgG positive and/or Epstein Barr nuclear antigen (EBNA) IgG positive, and have no clinical evidence of active EBV infection.

	l. Patients must not have received systemic chemotherapy or immunotherapy for 2 weeks (targeted therapies) and 4 weeks (all other anti-cancer treatment) at the time of enrollment, and there must be no intention of receiving any non-protocol systemic anti-cancer chemotherapy or immunotherapy during the trial period. Additionally, all prior therapy-related toxicities must have recovered to Grade 1 or less (CTCAE v4.03), except for alopecia or vitiligo prior to enrollment. Palliative radiation therapy is permitted between biopsy and lymphodepletion as long as it does not involve lesions being followed for response **Note:** Patients may have undergone minor surgical procedures not involving general anesthesia within three weeks prior to enrollment as long as all toxicities have recovered to Grade 1 or less or as specified in the eligibility criteria. m. Patients with documented Grade 2 or greater diarrhea or colitis as a result of previous treatment with ipilimumab, tremelimumab, anti-PD1 for anti-PD-L1 antibodies must have been asymptomatic for at least 6 months or had a normal colonoscopy post treatment, with uninflamed mucosa by visual assessment. n. Patients must have the ability to understand the requirements of the study, have provided written informed consent as evidenced by signature on an informed consent form (ICF) approved by an institutional review board (IRB), and agree to abide by the study restrictions and return to the site for the required assessments. o. Patients have provided written authorization for use and disclosure of protected health information.
Main Criteria for Exclusion:	Patients who meet ANY of the following criteria will be excluded from the study: a. Patients with melanoma of uveal/ocular origin b. Patients who have received prior cell transfer therapy which included a non-myeloablative or myeloablative chemotherapy regimen. c. Patients who have three or more active brain metastases. **Note**: Patients with one or two untreated or inadequately treated brain lesions or three or more adequately treated brain metastases may be eligible. If lesions are symptomatic or greater than or equal to 1 cm each, these lesions must have been definitively treated and stable for one month. Brain metastases with significant edema and or hemorrhage and metastases larger than 2 cm are excluded. d. Patients who are pregnant or breastfeeding. e. Patients who are on a systemic steroid therapy regimen defined as the need for chronic steroid use for at least seven

	or more days at a dose of greater than 10 mg of prednisone or equivalent per day. f. Patients who have active systemic infections, coagulation disorders or other active major medical illnesses of the cardiovascular, respiratory or immune system, as evidenced in the medical history by a positive stress thallium or comparable test, myocardial infarction, cardiac arrhythmias, obstructive or restrictive pulmonary disease. g. Patients who have any form of primary immunodeficiency (such as Severe Combined Immunodeficiency Disease and AIDS). h. Patients who have a history of severe immediate hypersensitivity reaction to cyclophosphamide, fludarabine, or IL-2. i. Patients who have a history of coronary revascularization or ischemic symptoms. j. Patients who have an estimated glomerular filtration rate (eGFR) less than 40 mL/min using the Cockcroft Gault formula at Screening or have end-stage renal disorder requiring hemodialysis. k. Patients who have an LVEF less than 45%. (Older patients [60 – 70 years] must have received an echocardiogram within the previous 60 days demonstrating LVEF ≥ 45%). l. Patients who have a documented FEV1 (forced expiratory volume in one second) of less than or equal to 60%. m. Patients who have had another primary malignancy within the previous three years (with the exception of carcinoma in situ of the breast, urothelial cancer *in situ*, and non-melanoma skin cancer that has been adequately treated).
Treatment Groups:	LN-144 (autologous TIL) followed by IL-2 after a lymphocyte-depleting preparative regimen as a single arm, open-label treatment.
Early Discontinuation from Study or Treatment:	Criteria for early discontinuation from treatment: • Grade 3 or greater autoimmunity that involves vital organs (heart, kidneys, brain, eye, liver, colon, adrenal gland, lungs) with symptoms emerging prior to first IL-2 administration. • Grade 3 or greater allergic reaction including bronchospasm or generalized urticaria that does not resolve after medical management in the opinion of the investigator. • Grade 3 or greater toxicity due to IL-2 that does not decrease to Grade 2 or less within 96 hours of management. • Determination by the PI that continued treatment is not in the best interest of the patient • Withdrawal by patient. The patient may withdraw consent to treatment but continue consent for follow-up evaluations and/or survival status • Pregnancy Criteria for early discontinuation from study: • Subject has become ineligible for study after enrollment and prior to LN-144 or IL2 administration

	• Progressive disease at 12 weeks or later • Withdrawal of consent • Death
Efficacy Assessment:	The descriptive summary of the best overall response rate (ORR), complete response rate (CR), duration of response (DOR), progression-free survival (PFS) and overall survival (OS) will be used to determine the potential efficacy of LN-144. These data will be used to support the design of future clinical trials.
Safety Assessment:	Treatment-emergent adverse events and serious adverse events will be evaluated to assess the safety of this treatment.
Overview of Statistical Plan:	The primary statistical plan of analysis is based on use of descriptive methods. Point estimates of treatment effect will be derived from maximum likelihood methods. All data will be listed. Patients meeting RECIST 1.1 criteria for a complete (CR) or partial (PR) response will be classified as responders in the analysis of the overall response rate (ORR). This rate will be summarized using both a point estimate and its two-sided exact 95% confidence limits. All time-to-event efficacy endpoints will use Kaplan-Meier survival curve methods to summarize the data. The time origin for all such analyses (except for response duration) will be the date on which patients began treatment with IL-2 after infusion with LN-144. The assessment of safety data will be descriptive and based on the summarization of treatment-emergent adverse events, serious adverse events, adverse events leading to discontinuation from the study, vital signs, physical examinations, and clinical laboratory tests.
Sample Size Determination:	The per protocol sample size is 20 patients treated with LN-144 followed by IL-2 administration, which is considered minimum to detect one or more Grade 3 or 4 toxicities.
DSMB Safety Assessments:	DSMB will evaluate cumulative safety data on the first 3 patients completing 12 weeks of assessment. Enrollment will not be halted during DSMB review. Additional evaluations of safety data may be specified in the charter.

STUDY FLOWCHART



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ACS	American Cancer Society
ACT	Adoptive Cell Therapy
AE	Adverse event
ALT	Alanine transaminase
ANC	Absolute neutrophil count
ASCO	American Society of Clinical Oncology
AST	Aspartate transaminase
BOR	Best overall response rate
BP	Blood pressure
CBC	Complete blood count
CFR	Code of Federal Regulations
CI	Confidence interval
Cl_{CR}	Calculated creatinine clearance
CMV	Cytomegalovirus
CNS	Central nervous system
CR	Complete response
CRO	Contract Research Organization
CT	Computed tomography
CTCAE v4.03	Common Terminology Criteria for Adverse Events Version 4.03
CY	Cyclophosphamide
DLT	Dose limiting toxicity
DOR	Duration of response
ECHO	Echocardiogram
ECOG	Eastern Cooperative Oncology Group
eCRF	Electronic case report form
EDC	Electronic data capture
EKG	Electrocardiogram
EOS	End of Study
FDA	Food and Drug Administration
FEV	Forced Expiratory Volume in the first second
F/U	Follow-up
GCP	Good Clinical Practice
HIPAA	Health Insurance Portability and Accountability Act of 1996
IB	Investigator's Brochure
ICH	International Conference on Harmonization
ICU	Intensive Care Unit
IL-2	Interleukin-2 (also known as "aldesleukin")
IND	Investigational New Drug (Application)
IP	Investigational product
IRB	Institutional Review Board
IV	Intravenous
LVEF	Left ventricular ejection fraction
MRI	Magnetic resonance imaging
MUGA	Multiple gated acquisition scan
NCI	National Cancer Institute
NE	In evaluable
Non-CR	Non-complete response
Non-PD	Non-progression

ORR	Overall response rate
OS	Overall survival
PBMC	Peripheral Blood Mononuclear Cells
PD	Progressive disease
PE	Physical exam
PFS	Progression-free survival
PI	Principal Investigator
PFT	Pulmonary Function Test
PO	Per Os (by mouth)
PR	Partial response
PT	Prothrombin time
PTT	Partial thromboplastin time
QD	(Taken) once daily
RECIST	Response Evaluation Criteria in Solid Tumors
RR	Response rate
SAE	Serious adverse event
SD	Stable disease
T	Body temperature
TEAE	Treatment-emergent adverse event
TIL	Tumor infiltrating lymphocyte
TSH	Thyroid stimulating hormone
ULN	Upper limit normal

1 INTRODUCTION

1.1 Background

There were an estimated 76,100 new cases of melanoma diagnosed in 2014, making it the 5th most common malignancy in men and the 7th most common malignancy in women. Unlike most malignancies, the incidence is increasing by greater than 2% per year in both sexes. While most melanoma is diagnosed early, up to 20% is regionally or distantly metastatic at the time of diagnosis.[1] In patients with advanced disease (Stage IV), prognosis is extremely poor with five-year survival of less than 5%. Numerous novel approaches, including chemotherapy, targeted therapy and immunotherapy have been developed as treatment with varying results.

Chemotherapy regimens using dacarbazine or temozolomide have been reported to result in tumor regression in 10 – 20% of patients; however, the responses have been of limited duration and rarely result in a complete response.[2] No chemotherapy regimen has, to date, demonstrated a survival benefit for patients with advanced melanoma. As such, although chemotherapy was widely used in the past, it now has a secondary role and is currently reserved for melanoma that can no longer be controlled with immunotherapy or targeted therapy.

Approved targeted therapies to date are limited to use in metastatic melanoma patients who have mutation in the V600 position of the gene encoding BRAF in the mitogen-activated protein kinase (MAPK) pathway. This mutation results in the expression of a modified BRAF protein, which directs growth of cancer cells. Two approved drugs, vemurafenib (Zelboraf) and dabrafenib (Tafinlar) directly inhibit the mutant BRAF gene.[3] Vemurafenib has demonstrated a survival benefit over chemotherapy in patients, while dabrafenib has demonstrated a notable PFS benefit, with crossover blunting the effect on overall survival.[4] Another gene product in the MAPK pathway being targeted is MEK which is downstream of BRAF. This gene product is being targeted in its wild-type or non-mutant form, as MEK mutations are not found in melanoma or are quite rare. MEK has been targeted by a kinase inhibitor called trametinib and has also demonstrated survival benefit over chemotherapy.[5]

These targeted agents prolong the time until tumor growth and extend overall survival in patients with BRAF mutant melanoma. However, disease eventually progresses

despite continuation of treatment with such therapy. More recently, it has been demonstrated that combining a BRAF inhibitor and a MEK inhibitor increases both response rates and duration of response, and improves overall survival as compared to BRAF inhibition alone, though all cases still are ultimately expected to develop resistance.[6]

While targeted therapies are noted to have high response rates but short durations of response, cancer immunotherapy tends to have fewer objective responses, but longer duration of response which may sometimes even translate to a cure, as defined when complete remission from disease has lasted for years after therapy with no evidence of recurrence after repeated follow-up. Cancer immunotherapy is categorized into three general treatment modalities: active immunization, non-specific immune stimulation, and passive immunotherapy/adoptive cell transfer. In the treatment of metastatic melanoma, active immunization with agents such as peptides, whole tumor cell vaccines, recombinant viruses encoding tumor-associated antigens, or dendritic cells have historically shown low tumor response rates of less than 5%,[7-10] though more recently tested agents such as the oncolytic virus talimogene laherparepvec have shown promising response rates of up to 26%, with 16% lasting more than 6 months.[11]

Nonspecific immune stimulation with high-dose interleukin-2 (IL-2) as a single agent or ipilimumab can lead to durable cancer regression, although the overall tumor response rates for each agent have been low. The response rate for high-dose IL-2 was reported at 16%, with only approximately half of these complete responses,[12] and the response rate to ipilimumab was only 11%.[7] Despite these low objective response rates, among complete responders to high-dose IL-2, 50% never experience disease recurrence. Among patients treated with ipilimumab, up to 22% are still alive after three years, with some patients surviving beyond 10 years.[13] A pilot trial of 36 patients with melanoma treated with ipilimumab combined with high-dose IL-2 had overall response (OR) rates of 25%, with 17% achieving a CR lasting more than eight years ongoing;[14] however, IL-2 plus ipilimumab combination has not been further tested to confirm these results. Anti-PD1 and anti-PD-L1 antibodies have recently been reported to have OR rates of up to 38%,[15, 16] and 17%,[17] respectively, in

patients with melanoma, and OR rates of up to 40% when combined with ipilimumab,[18] although the long-term durability of the responses is not yet known.

Cancer immunotherapy with adoptive transfer of tumor infiltrating lymphocytes (TIL) presents a potentially effective treatment for patients with metastatic melanoma. Adoptive Cell Transfer (ACT) with TIL involves the ex vivo numerical expansion of antitumor lymphocytes that have infiltrated into tumors. These TIL are numerically expanded in culture using T-cell growth factor, IL-2, either from small cut tumor fragments from surgically-resected lesions or from single cell suspensions isolated from resected tumors. The expanded TIL are re-infused ("transferred") back into the patient. These cells can be activated *ex vivo*, free from the potentially suppressive tumor microenvironment that may prevent them from fully living up to their antitumor potential. ACT has theoretical and practical advantages over active immunization and nonspecific immune stimulation. These include: 1) ability to numerically expand and re-infuse much higher number of tumor-reactive T cells than is possible with these other approaches, 2) the ability to numerically expand tumor-specific T cells in the absence of the effects suppressive T-regulatory cells, 3) the wider array of tumor antigens, such as mutated tumor antigens, recognized by the expanded T cells intrinsic to the TIL product and 4) the ability to further manipulate these infused T cells using immune modulators such as IL-2, T-cell checkpoint blockade agents, or other active or non-specific immune stimulating agents.[19, 20] Preparation of the host patient with lymphodepletion immediately prior to the transfer of the antitumor cells also eliminates potentially suppressive influences (such as regulatory T cells and cytokine sinks) to provide an optimal milieu for the transferred TIL to proliferate and become activated in vivo. When combined with a preparative lymphodepleting regimen pre-transfer, ACT using autologous TIL has demonstrated consistently high objective response rates, from 49% to 72%, with long-term durable and potentially curative CR rates of up to 20%.[19, 21, 22]

1.2 Overview of Adoptive Cell Transfer for Metastatic Melanoma

The partial success of IL-2 therapy in the treatment of metastatic melanoma revealed that manipulation of the immune response could alter the clinical course of the disease.[23] The induction of tumor regression by IL-2 is believed to be related to its immune regulatory effects, including the expansion of T lymphocytes following

activation by specific antigen and NK cells.[24-26] T cell recognition leading to tumor cell killing and/or the release of helper and other cytokines is due to the presence of specifically recognized antigens present on the tumor cells.[27, 28] In the case of melanoma, a number of antigens have now been identified that can be recognized by both $CD8^+$ cytotoxic T cells and $CD4^+$ T-helper cells, including MART-1, gp100, MAGE-1, tyrosinase, TRP-1, TRP-2 and NY-ESO-1.[28, 29] The presence of these antigens on melanoma tumor cells has led to immunotherapy regimens that focused on the ability of effector T cells to mediate tumor destruction specially the development of adoptive cell transfer regimens using TIL.

The identification of melanoma-specific antigens that are recognized by T cells and the ability to isolate and expand the tumor-reactive T cells population *in vitro* has led to the development of adoptive cell transfer regimens for treatment of metastatic melanoma. TIL derived from resected melanoma tumors and expanded *in vitro* are capable of specifically recognizing tumor antigens, particularly MART-1, in over two-thirds of melanoma patients.[30, 31] In addition, recent studies have shown that TIL from melanoma tumors can recognize antigens derived from mutated gene products in the cancer cells recognized as “neo-antigens” by the T cells.

The success of IL-2 therapy for metastatic melanoma and the discovery of tumor antigens recognized by TIL led to first attempts to isolate tumors, expand lymphocytes from tumor fragments, and re-infuse these expanded cells back into the patient. Some of the first clinical trials performed in individual centers in the USA and Europe, such as the NCI, used TIL expanded for a number of weeks from tumor tissue with IL-2 alone followed by re-infusion into patients. This was followed up by low-dose IL-2 infusion or subcutaneous IL-2 administration.[32-35] Although these protocols were found to be feasible, they had inconsistent and widely varying response rates ranging from 0% to 66%, with the caveat that some of these trials were only conducted on small numbers (<10) of patients (e.g., Tessier et al.).[33]

During this time, the Surgery Branch at the National Cancer Institute (NCI, Bethesda, MD) also embarked on performing TIL trials for metastatic melanoma using a similar expansion method for TIL with IL-2 alone. The NCI however included a preparative chemotherapy regimen using low-dose cyclophosphamide (CY) before TIL infusion that resulted in a partial and transient depletion of host lymphocytes. IL-2 was

administered after TIL infusion. This led to more promising response rates in small pilot clinical trials of 30-60%.[36-38] This prior CY pre-conditioning approach resulted from work on murine tumor models at the NCI showing that the host immune environment may significantly impact the efficacy of adoptive T- cell therapy. In these studies an improved persistence and anti-tumor activity of transferred TIL expanded from implanted murine tumors was found when host mice were treated with CY or non-lethally irradiated to deplete endogenous lymphocytes.[39, 40] Prior lymphodepletion with CY was later also found to remove a new subset of suppressive $CD4^+$ T-regulatory cells ($CD4^+Foxp3^+$ cells) that inhibit anti-tumor immune responses in mice. Higher T-regulatory cell frequencies in the blood are also correlated with an unfavorable prognosis in cancer patients.[41-44] Alternatively, prior depletion of lymphocytes may create 'space' for the adoptively transferred cells within the lymphocyte compartment.[45] Under this model, homeostatic lymphocyte survival may result in increased proliferation and enhanced survival of transferred T cells, perhaps through a mechanism involving increased access to endogenous cytokines like IL-7 and IL-15.[46] The success of prior lymphodepletion in animal models and the use of single agent CY preconditioning in initial TIL therapy trials, led to testing of more intensive pre-conditioning regimens yielding a complete depletion of host lymphocytes for a longer window of time than the prior CY alone regimens.

The NCI first reported a study on 35 patients including this more intense lymphodepleting conditioning regimen to adoptive cell transfer therapy in patients with metastatic melanoma.[47, 48] Patients received a lymphodepleting chemotherapy regimen consisting of high-dose cyclophosphamide and standard doses of fludarabine before administration of selected, expanded, tumor-reactive TIL and IL-2. The lymphodepletion step resulted in a transient myelosuppression and the elimination of all circulating lymphocytes for approximately one week, after which time patients recovered endogenous marrow function and reconstituted their lymphocyte compartments towards normal levels within two to three weeks.[47, 48]

Because of the immunosuppression of fludarabine, one patient who had clonal repopulation from infused TIL and a complete response of metastatic melanoma, developed Epstein-Barr virus (EBV) - associated B cell lymphoma. This patient was EBV-naïve prior to treatment. The potential source of EBV was thought to be multiple

blood products received after chemotherapy. The patient later died of complications from the treatment of the lymphoma. Another patient developed polyneuropathy consisting of vision loss and motor and sensory defects approximately 2 months after chemotherapy. The etiology of this complication is unknown, but was possibly related to fludarabine.[48]

Published clinical trials evaluating TIL therapy from several institutions using similar protocols as the NCI are reporting reproducible and promising results. Rosenberg et al.[21] reported results of clinical trials conducted at NCI that used three different pre-treatment regimens prior to TIL infusion for treatment of patients with melanoma. Objective responses were seen in 52/93 patients (56%) of which 20/93 (22%) were complete responses. The complete responses were durable (defined as "ongoing after 64 -109 months of follow-up") in 19/20 (95%) of the patients. Radvanyi et al.[49] reported the MD Anderson Cancer Center experience with ACT using selected TIL for treatment of metastatic melanoma with objective clinical response in 15/31 (48.4%) patients with two resulting in a complete response (6.5%). Progression free survival of a duration of greater than 12 months was reported in 9/15 (60%) of the patients that responded to therapy. The H. Lee Moffitt Cancer Center also reported a 38% response rate in 13 treated patients with 2/13 (15%) achieving a complete response ongoing for more than 14 and 16 months at the time of publication, respectively.[50] Outside the U.S., Itzhaki et al.[51] reported the experience from Sheba Medical Center in Israel using "young, unselected –TIL therapy." Of the 31 patients evaluated, 15 (48%) of the patients achieved a clinical response including four patients (12.9%) with complete responses. In addition, a group in Denmark[52] used decrescendo low-dose IL-2 as an adjuvant after cell infusion to reduce treatment related toxicity in a small study (6 patients). They reported objective clinical responses in 2/6 patients (33%) with ongoing complete responses for more than 10 and 30 months (respectively), 2 patients (33%) with stable disease for 4 and 5 months (respectively) and 2 patients (33%) whose disease progressed shortly after treatment.

These collective results suggest that the non-myeloablative lymphodepleting chemo-preparative regimen proposed in this current study can be tolerated and contributes to the potent efficacy of TIL for the treatment of advanced metastatic disease.

1.3 Production and Expansion of Tumor Infiltrating Lymphocytes

Generating LN-144 involves resecting a tumor deposit (generally > 1 cm, preferably 1.5 cm in diameter) and culturing tumor fragments in media containing IL-2 to expand them *in vitro* (**Figure 1**). Appropriately expanded TIL cultures should reach several million cells (combined) in two to three weeks. The cells can be screened at this stage for their capacity to kill autologous tumor cells if autologous tumor cells are available. Alternatively, the TIL can be screened using allogeneic HLA-A-matched tumor lines. Although anti-tumor reactivity assays were used to select TIL for further expansion in initial clinical trials, data from a number of studies indicates that both responding and non-responding patients have tumor-reactive TIL to similar extents (e.g., Radvanyi et al.).[49] In addition, clinical trials using young, unselected TIL have achieved relatively similar response rates.[51, 53] In this trial TIL cultures will not be selected but rather proceed when sufficient numbers are obtained to proceed. TIL isolated from the tumor fragments undergo a rapid expansion protocol (REP) using the T-cell–stimulating antibody muromonab-CD3, resulting in billions of cells for patient infusion. In a retrospective study evaluating surgical resections for TIL in 402 patients from 2002 to 2007 at the Surgery Branch of the National Cancer Institute, TIL were successfully generated in 677 (86%) of the 787 specimens from all tumor sites, although tumors from the gastrointestinal tract had a decreased rate of TIL growth (70%; P = 0.008).[54]

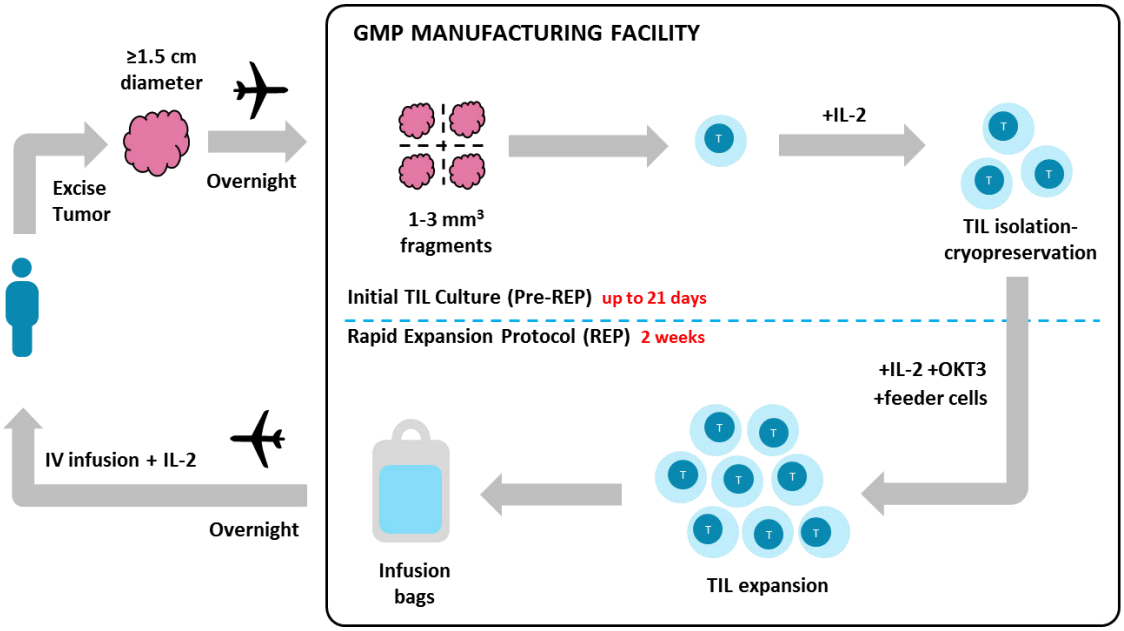

Figure 1. LN-144 Manufacturing Process

1.4 LN-144 TIL Therapy

LN-144 is an autologous, ready-to-infuse, TIL therapy and is almost identical to that developed by Dr. Steven Rosenberg and colleagues at the National Cancer Institute. TIL have demonstrated efficacy in the treatment of Stage IV melanoma, and Phase 2 clinical trials evaluating this product have shown an objective response rate of 49% or more, exceeding rates reported by other immunotherapies in melanoma. The present study is being conducted to further evaluate: 1) the safety of LN-144 therapy and 2) the efficacy of LN-144 therapy.

Several treatment regimens have been used in conjunction with TIL therapy. Lymphodepleting regimens have included cyclophosphamide/fludarabine, total body irradiation or the combination of the two. The lymphodepletion protocol used in the current study is based on the method developed and tested by the NCI and is also the most often used. It involves two days of cyclophosphamide followed by five days of fludarabine as a lymphodepleting pre-treatment. The treatment regimen includes

treatment with LN-144 (TIL therapy) followed by high-dose IL-2. Protocols for the tumor harvest and LN-144 administration are provided in separate operating manuals.

Up to 150 x 10^9 viable cells will be infused in this clinical trial. The final cell product is formulated in a minimum of 50% HypoThermosol™ in Plasma-Lyte A™ (volume/volume) and up to 0.5% HSA (compatible for human infusion) containing 300 IU/mL IL2. The final product will be available for administration in one of two volumes for infusion:

1) 250 mL (in a 300-mL capacity infusion bag) when the total TIL harvested are ≤ 75x10^9

OR

2) 500 mL (in a 600-mL capacity infusion bag) when the total TIL harvested are < 150 x 10^9

We cannot predict the total number of cells that will be generated for the final LN-144 infusion product for each patient due to patient-to-patient variation in T-cell expansion rates during the REP step. A lower limit of cells on day 7 of the 14-day REP is set based on the minimum number of cells needed in order to make a decision to lymphodeplete the patient using the cyclophosphamide plus fludarabine chemotherapy regimen. Once we have begun lymphodepletion based on this minimal attained cell number, we are committed to treating the patient with the available number of TIL we generate in the REP by day 14. The upper limit of the range for infusion (150 x 10^9 viable cells) is based on the known published upper limit safely infused where a clinical response has been attained.[49] There is no evidence that moving beyond this upper limit will have more clinical benefit.

2 STUDY DESIGN

2.1 Description of the Study

This is a prospective single-arm interventional study evaluating patients who receive adoptive cell therapy (ACT) with LN-144 (autologous TIL). Patients will receive LN-144 followed by the administration of a regimen of IL-2 at 600,000 IU/kg approximately every eight hours starting 12 to 24 hours after the LN-144 infusion and continuing for up to six doses. Patients will be evaluated for response approximately 12 weeks following LN-144 therapy.

Patients will be evaluated at six, nine, 12, 18 and 24 months following LN-144 treatment. Formal response evaluations will be per RECIST 1.1.

2.2 Description of the Study Centers

Patients may be seen at the Investigators' private offices or affiliated medical centers for evaluations prior to enrollment and during follow-up. The patients will require hospitalization during the LN-144 infusion and IL-2 treatment.

3 STUDY OBJECTIVES AND ENDPOINTS

3.1 Study Objectives

3.1.1 Primary Objectives

- To characterize the safety profile of LN-144 in patients with metastatic melanoma

3.1.2 Secondary Objectives

- To evaluate the efficacy of LN-144 in patients with metastatic melanoma using the best overall response rate (ORR) and complete response rate (CR).
- To evaluate the efficacy parameters of LN-144 in patients with metastatic melanoma such as progression-free survival (PFS), duration of response (DOR), and overall survival (OS).

3.1.3 Exploratory Objectives

- To explore the persistence of LN-144 and potential immune correlates of response, outcome, and toxicity of the treatment.

3.2 Study Endpoints

3.2.1 Primary Endpoints:

- Incidence, severity, seriousness, relationship to study treatment, and characteristics of treatment-emergent AEs (TEAEs), including AEs leading to early discontinuation from treatment or withdrawal from the study, and AEs resulting in deaths while on the study.

3.2.2 Secondary Endpoints:

- Efficacy of LN-144 therapy as defined by:
    - Best overall response rate (ORR)
    - Complete response rate (CR) as best overall
    - Duration of response (DOR)
    - Progression free survival (PFS)
    - Overall survival (OS)

3.2.3 Exploratory Endpoints:

- Evaluation of TIL persistence in the peripheral blood and immune correlates with respect to response, outcome, and/or toxicity of the treatment. These data will not be reported in the clinical study report but instead in its own report.

4 SELECTION OF PATIENT POPULATION

Patients greater than 18 years of age, with a diagnosis of metastatic melanoma who have undergone at least one prior immunotherapy or chemotherapy regimen will be selected for this study. Patients greater than 65 years of age may be allowed in the study after discussion between the Principle Investigator and Medical Monitor regarding the patient's ability to tolerate the high dose IL-2.

Details about specific benefits and risks for patients participating in this clinical trial may be found in the accompanying Investigator's Brochure and Informed Consent documents.

4.1 Inclusion Criteria

To be eligible for the study, patients must meet ALL of the following criteria prior to enrollment

a. Patients must have measurable metastatic melanoma and at least one lesion that is resectable for TIL generation. Ideally the lesion must be of at least 1.5 cm in diameter post-prosection and can be surgically removed with minimal morbidity

(defined as any operation for which expected hospitalization is less than or equal to three days).

b. Patients must have undergone at least one prior systemic treatment for metastatic melanoma.

c. Patients must have either progressive disease or no response (i.e., no PR or CR) while receiving or after completion of most recent prior treatment.

d. Patients must be greater than 18 years of age at the time of consent. Patients greater than 65 years of age may be allowed in the study after discussion between the Principle Investigator and Medical Monitor regarding the patient's ability to tolerate the high dose IL-2.

e. Patients must have an Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1 (Appendix 2).

f. In the opinion of the Investigator, patient must be capable of participating and completing study procedures.

g. Patients of child bearing potential or with partners of childbearing potential must be willing to practice birth control during treatment and for four months after receiving all protocol related therapy.

h. Patients must have a serum absolute neutrophil count (ANC) greater than 1000/mm^3, hemoglobin greater than 9.0 g/dL, and platelet count greater than 100,000/mm^3.

i. Patients must have a serum ALT/SGPT and AST/SGOT less than three times the upper limit of normal (<3x ULN), a calculated creatinine clearance of greater than 50 mL/min (>50 mL/min), and a total bilirubin less than or equal to 2 mg/dL (≤ 2 mg/dL). Patients with Gilbert's Syndrome must have a total bilirubin less than 3 mg/dL (<3 mg/dL).

j. Patients must be seronegative for the HIV antibody, hepatitis B antigen, and hepatitis C antibody or antigen.

k. Patients must be EBV viral capsid antigen (VCA) IgG positive and/or Epstein Barr nuclear antigen (EBNA) IgG positive, and have no clinical evidence of active EBV infection.

l. Patients must not have received systemic chemotherapy or immunotherapy for 2 weeks (targeted therapy) and 4 weeks (all other anti-cancer treatment) at the time of enrollment, and there must be no intention of receiving any non-protocol systemic anti-cancer chemotherapy or immunotherapy during the trial period. Additionally, all prior therapy-related toxicities must have recovered to Grade 1 or less (CTCAE v4.03), except for alopecia or vitiligo prior to enrollment. Palliative radiation therapy is permitted between biopsy and lymphodepletion for LN-144 infusion as long as it does not involve lesions being followed for response.

   Note: Patients may have undergone minor surgical procedures not involving general anesthesia within three weeks prior to enrollment as long as all toxicities have recovered to Grade 1 or less or as specified in the eligibility criteria.

m. Patients with documented Grade 2 or greater diarrhea or colitis as a result of previous treatment with ipilimumab, tremelimumab, anti-PD1 or anti-PD-L1 antibodies must have been asymptomatic for at least 6 months or had a normal colonoscopy post treatment, with uninflamed mucosa by visual assessment.

n. Patients must have the ability to understand the requirements of the study, have provided written informed consent as evidenced by signature on an informed consent form (ICF) approved by an institutional review board (IRB), and agree to abide by the study restrictions and return to the site for the required assessments.

o. Patients have provided written authorization for use and disclosure of protected health information.

4.2 Exclusion Criteria

Patients who meet ANY of the following criteria will be excluded from the study:

a. Patients with melanoma of uveal/ocular origin

b. Patients who have received prior cell transfer therapy which included a non-myeloablative or myeloablative chemotherapy regimen.

c. Patients who have three or more active brain metastases. **Note:** Patients with one or two untreated or inadequately treated brain lesions or three or more adequately treated brain metastases may be eligible. If lesions are symptomatic or greater than or equal to 1 cm each, these lesions must have been definitively treated and stable for one month. Brain metastases with significant edema and or hemorrhage and metastases larger than 2 cm are exclusionary.

d. Patients who are pregnant or breastfeeding.

e. Patients who are on a systemic steroid therapy regimen defined as the need for chronic steroid use for at least seven or more days at a dose of greater than 10 mg of prednisone or equivalent per day.

f. Patients who have active systemic infections, coagulation disorders or other active major medical illnesses of the cardiovascular, respiratory or immune system, as evidenced in the medical history by a positive stress thallium or comparable test, myocardial infarction, cardiac arrhythmias, obstructive or restrictive pulmonary disease.

g. Patients who have any form of primary immunodeficiency (such as Severe Combined Immunodeficiency Disease and AIDS).

h. Patients who have a history of severe immediate hypersensitivity reaction to IL-2, fludarabine or cyclophosphamide.

i. Patients who have a history of coronary revascularization or ischemic symptoms.

j. Patients who have an estimated glomerular filtration rate (eGFR) less than 40 mL/min using the Cockcroft-Gault formula at Screening or have end-stage renal disorder requiring hemodialysis.

k. Patients who have an LVEF less than 45%. (Older patients [60 – 70 years], must have received an echocardiogram within the previous 60 days demonstrating LVEF ≥ 45%).

l. Patients who have a documented FEV1 (forced expiratory volume in one second) of less than or equal to 60%.

m. Patients who have had another primary malignancy within the previous three years (with the exception of carcinoma in situ of the breast, urothelial cancer in situ, and non-melanoma skin cancer that has been adequately treated).

4.3 Number of Patients

Patients that meet all of the inclusion criteria and do not meet any of the exclusion criteria will be enrolled in the study.

Patients who sign an ICF and fail to meet the inclusion and/or exclusion criteria are defined as screen failures.

The screening and tumor resection/TIL harvest period is up to 6 weeks. However, it can be extended, after approval by the Medical Monitor, if there is delay in scheduling the tumor resection.

For all screen failures, the Investigator is to maintain a screening log that documents, at a minimum, the patient initials, or other identifier used by site, patient date of birth and reason(s) for screen failure. A copy of the log should be retained in the Investigator's study files. Minimum data for screen failures will be captured in the EDC database as defined in the data management plan and eCRF completion manual.

Patients will be enrolled until 20 patients have been successfully treated with LN-144 followed by IL-2 administration. Screening may halt once it becomes likely that the full accrual goal will be met.

4.3.1 Re-screening Patients

Patients who fail the initial screening process may be re-screened for eligibility. The Principal Investigator and Medical Monitor will discuss the patient prior to any rescreening procedures and agree on which screening procedures need to be redone.

4.3.2 Patient Cohorts

All patients treated with LN-144 followed by at least 1 dose of IL-2 are defined as the all-treated population, and all patients resected for harvest will be defined as the modified Intention to Treat population. Efficacy and safety analyses will be performed on both populations.

DSMB will evaluate safety data on first 3 patients completing 12 weeks of assessment. A limited analysis will also be conducted reviewing all data available from these patients as specified in the DSMB charter.

The primary efficacy and safety analyses will take place after 12 weeks following the LN-144 administration of the 20th patient treated.

5 PRIOR TREATMENTS, CONCOMITANT MEDICATIONS AND NON-DRUG THERAPIES

5.1 Prior Treatment and Concomitant Medications

Use of all medications taken by the patient 30 days prior to consent will be recorded in the site's source documentation and the patient's electronic case report form (eCRF). All medications taken by the patient, or any changes in medications will also be recorded until completion of the study.

5.2 Prohibited and Permitted Medications during Study Treatment

5.2.1 Prohibited Treatment

Patients will enter a washout period prior to enrollment. Systemic chemotherapy or immunotherapy (targeted therapy) must be stopped at least 2 weeks prior to enrollment and all other anti-cancer treatments must be stopped at least 4 weeks prior to enrollment.

The following guidelines should be used regarding concomitant medications:

- Systemic therapies intended to treat melanoma are not permitted while the patient is on study
- Use of tumor directed therapy (including radiation therapy) during the study must be discussed with the Medical Monitor on a case by case basis. Note: Palliative radiation therapy is permitted between biopsy and

lymphodepletion as long as it does not involve lesions being followed for response.

- Use of investigational drugs is not permitted

5.2.2 Permitted Medications – Use with Caution

Current medications for conditions other than their metastatic melanoma are permitted with the exception of any medications that may have an anti-tumor effect. Although prohibited for study entry, systemic steroid therapy greater than 10 mg/day prednisone or equivalent may be initiated on study per PI discretion.

6 STUDY PROCEDURES

6.1 Screening

The following procedures should be completed after completion of Informed Consent:

- Review of inclusion and exclusion criteria
- Medical history including current medications
- Physical exam including height and weight
- Vital signs – pulse rate, respiratory rate, blood pressure and temperature
- Evaluation and measurement of all skin and palpable lesions
- Slit Lamp eye exam
- EKG
- Cardiac evaluation (stress thallium) for all patients. Echocardiogram or MUGA) for patients ≥ 60 years or patients who have a history of ischemic heart disease, chest pain, or clinically significant atrial and/or ventricular arrhythmias. Stress thallium must show normal LVEF and unimpaired wall movement
- Pulmonary function tests if indicated
- CT Exam
    - Chest (include neck if there is prior or suspected neck disease)
    - Abdomen

- Pelvis

- MRI of brain
- Blood and Urine Tests
  - Hematology - CBC with differential
  - Chemistry - Sodium, Potassium, Chloride, Total CO_2 or Bicarbonate, Creatinine, Glucose, BUN, Albumin, Calcium, Magnesium, Phosphorus, Alkaline Phosphatase, ALT/SGPT, AST/SGOT, Total Bilirubin, Direct Bilirubin, LDH, Total protein, Total CK, Uric Acid, and thyroid panel (to include TSH and free T4)
  - Serum pregnancy test for all women of child bearing potential
  - HIV antibody titer, HbsAG determination (HSV-1 IgG and HSV-2 IgG), CMV antigen assay, Anti HCV, Anti CMV IgG antibody titer, HSV serology and EBV panel (VCA-IgM, VCA-IgG, EA-D IgG, EBNA, IgG) (may be within previous 3 months as of enrollment)
  - HLA typing (to be shipped to central lab. Refer to central lab manual for details)
  - Urinalysis (complete urine culture if indicated)
- Calculate creatinine clearance using Cockcroft-Gault formula

Males:		
Creatinine CL (mL/min)	=	Weight (kg) x (140 – Age) 72 x serum creatinine (mg/dL)
Females:		
Creatinine CL (mL/min)	=	Weight (kg) x (140 – Age) x 0.85 72 x serum creatinine (mg/dL)

- ECOG performance status evaluation
- Patients with documented Grade 2 or greater diarrhea or colitis as a result of previous treatment with ipilimumab, tremelimumab, anti-PD1 or

anti PD-L1 antibodies must have been asymptomatic for at least 6 months or had a normal colonoscopy post treatment, with uninflamed mucosa by visual assessment

- Assessment of AE/SAEs

6.2 Enrollment and Tumor Resection

Following confirmation of patient eligibility, the medical monitor, or designee, will either approve or not approve patient for enrollment into the clinical study.

If enrolled, tumor resection will take place. The date of tumor resection is expected to occur approximately 44 days prior to the LN-144 infusion (Day 0) and is dependent on the rate of cell growth at the central LN-144 manufacturing facility. The following procedures should be completed during this visit.

- Verification of all ongoing medications
- ECOG performance status evaluation
- Obtain blood for Immune monitoring (50 mL of blood to be obtained. Refer to lab manual)
- Tumor Harvest
- Six paraffin embedded slides created from the tumor resection for biomarker analyses
- Assessment of AE/SAEs

6.2.1 Tumor Harvest and Processing Procedure

A detailed Tumor Procurement Manual will be provided to each clinical site and training will be performed on the procedures for collecting and shipping of the tumor to the LN-144 Manufacturing Facility.

Tumors will be harvested at the investigational centers participating in the trial according to their respective institutional protocols for sterile harvest for TIL preparation.

The tumor (ideally minimum 1.5 cm in diameter) will be surgically resected from the patient. The resected tumor sample will be handled aseptically at all times. Care will be taken to keep the tumor hydrated by adding Hank's Balanced Salt Solution (HBSS) to the tissue, as needed, to keep it hydrated through drop-wise addition. Using sterile tweezers and a scalpel or other suitable sterile instruments, the tumor is trimmed to remove extraneous non-tumor tissue by a trained surgeon or pathologist or otherwise qualified personnel. Using sterile forceps the trimmed tumor is placed into a sterile, previously sealed and unopened sterile 100 mL bottle of HypoThermosol®. The lid should be screwed on tightly. The bottle containing the tumor will be placed in a plastic sealable bag secondary container containing absorbent tissue or paper towels. This secondary container is placed in an activated NanoCool™ shipper adjacent to a TempTale® 4 temperature monitor. The NanoCool™ shipper is then closed and packaged as instructed in the Tumor Procurement Manual. The package will be shipped overnight to the central LN-144 manufacturing facility). The NanoCool™ shipper will be supplied to the site with address labels affixed for shipment to the manufacturing facility as well as all appropriate labels for shipping.

Further details and additional instructions are available in the Tumor Procurement Manual.

LN-144 is an autologous product which is procured and delivered by means which have more in common with autologous blood product delivery than those of traditional drug production. It is imperative that only the patient's own (autologous) study treatment (LN-144) be administered to the same individual patient. For these reasons, the patient specimen must be procured and handled according to a strict protocol to ensure optimal quality of the specimen and minimum transport time to and from the processing facility, as well as to ensure the unique identification of the specimen at all times including injection back into the patient.

6.2.2 Immune Monitoring and Sequencing of Tumor and Lymphocyte DNA

A total of 50 mL of blood will be collected from the patient for immune monitoring (biomarker analysis) and sequencing of lymphocyte DNA utilizing vacutainer blood collection vials. Refer to the study Lab Manual for the complete procedure details. In addition, DNA from tumor tissue will be sequenced.

6.3 Day -14

The following procedures should be completed during this visit, which is approximately 2 weeks prior to the treatment date:

- Physical exam including weight
- Vital signs – pulse rate, respiratory rate, blood pressure and temperature
- Evaluation and measurement of all skin and palpable lesions
- Verification of all ongoing medications
- EKG
- CT Exam
    - Chest (include neck there is prior or suspected neck disease)
    - Abdomen
    - Pelvis
- MRI- Brain in patients who had brain abnormalities on screening exam
- Blood and urine tests
    - Hematology - CBC with differential
    - Chemistry - Sodium, Potassium, Chloride, Total CO_2 or Bicarbonate, Creatinine, Glucose, BUN, Albumin, Calcium, Magnesium, Phosphorus, Alkaline Phosphatase, ALT/SGPT, AST/SGOT, Total Bilirubin, Direct Bilirubin, LDH, Total protein, Total CK, Uric Acid, and thyroid panel (to include TSH and free T4)

- Serum pregnancy test for all women of child bearing potential
- Urinalysis (complete urine culture if indicated)

- ECOG performance status evaluation
- Assessment of AE/SAEs

6.4 Day -7

Prior to the start of lymphodepletion, verification of sufficient LN-144 expansion at this timepoint will be confirmed.

- Physical exam including weight, calculated BSA and BMI
- Verification of all ongoing medications
- ECOG performance status evaluation
- Vital signs – pulse rate, respiratory rate, blood pressure and temperature
- Blood and urine tests (to be drawn prior to cyclophosphamide administration)
  - Hematology - CBC with differential
  - Chemistry - Sodium, Potassium, Chloride, Total CO_2 or Bicarbonate, Creatinine, Glucose, BUN, Albumin, Calcium, Magnesium, Phosphorus, Alkaline Phosphatase, ALT/SGPT, AST/SGOT, Total Bilirubin, Direct Bilirubin, LDH, Total protein, Total CK, Uric Acid, and thyroid panel (to include TSH and free T4)
  - Urinalysis (complete Urine culture if indicated)
  - CMV antigen assay, as clinically indicated
- Obtain blood for immune monitoring (50 mL of blood to be obtained. Refer to lab manual)
- Administration of the following medications:
  - Cyclophosphamide 60 mg/kg IV in 250 mL D5W with mesna 15 mg/kg are infused over 2hr. (If the patient is obese (BMI > 35)

drug dosage will be calculated using practical weight as described in Appendix 3.)

- Mesna infusion will continue to be infused at a rate of 3 mg/kg/hour in a suitable diluent (see pharmaceutical section) over 22 hours after each cyclophosphamide dose
- Ondansetron (0.15 mg/kg/dose [*rounded to the nearest even mg dose between 8 mg and 16 mg based on patient weight]* IV every eight hours X 3 days) will be given for nausea. (If the patient is obese (BMI > 35) drug dosage will be calculated using practical weight as described in Appendix 3)
- Prophylactic antibiotics such as TMP/SMX DS 160 mg/800 mg may be given as clinically indicated, per standard of care
- Assessment of AE/SAEs

6.5 Day -6

The following procedures should be performed:

- Physical exam including weight
- Verification of all ongoing medications
- Vital signs – pulse rate, respiratory rate, blood pressure and temperature
- Blood and urine tests (to be drawn prior to cyclophosphamide administration)
    - Hematology - CBC with differential
    - Chemistry - Sodium, Potassium, Chloride, Total CO_2 or Bicarbonate, Creatinine, Glucose, BUN, Albumin, Calcium, Magnesium, Phosphorus, Alkaline Phosphatase, ALT/SGPT, AST/SGOT, Total Bilirubin, Direct Bilirubin, LDH, Total protein, Total CK, Uric Acid, and thyroid panel (to include TSH and free T4)
    - CMV antigen assay, as clinically indicated

- Urinalysis (complete urine culture if indicated)

- Administration of the following medications
  - Cyclophosphamide 60 mg/kg IV in 250 mL D5W with mesna15 mg/kg are infused over 2hr. (If the patient is obese (BMI > 35) drug dosage will be calculated using practical weight as described in Appendix 3.)
  - Mesna infusion will continue to be infused at a rate of 3 mg/kg/hour in a suitable diluent (see pharmaceutical section) over 22 hours after each cyclophosphamide dose
  - Ondansetron (0.15 mg/kg/dose [*rounded to the nearest even mg dose between 8 mg and 16 mg based on patient weight]* IV every eight hours X 3 days) will be given for nausea. (If the patient is obese (BMI > 35) drug dosage will be calculated using practical weight as described in Appendix 3)
  - Prophylactic antibiotics such as TMP/SMX DS 160 mg/800 mg may be given as clinically indicated, per standard of care
- Assessment of AE/SAEs

6.6 Day -5 to Day -1

The following procedures should be performed:

- Physical exam including weight
- Verification of all ongoing medications
- Vital signs – pulse rate, respiratory rate, blood pressure and temperature
- Blood and urine tests (to be drawn prior to fludarabine administration)
  - Hematology - CBC with Differential
  - Chemistry - Sodium, Potassium, Chloride, Total CO_2 or Bicarbonate, Creatinine, Glucose, BUN, Albumin, Calcium, Magnesium, Phosphorus, Alkaline Phosphatase, ALT/SGPT, AST/SGOT, Total Bilirubin, Direct Bilirubin, LDH, Total protein,

Total CK, Uric Acid, and thyroid panel on Days -5, -4 and -1 only (to include TSH and free T4)

- CMV antigen assay, as clinically indicated
- Urinalysis (complete urine culture if indicated)

- The following medication should be administered:
  - Fludarabine 25mg/ m2 to be given IV over approximately 30 minutes once daily each day
  - Prophylactic antibiotics such as TMP/SMX DS 160 mg/800 mg may be given as clinically indicated, per standard of care
- Assessment of AE/SAEs

6.7 Day 0 (+2 days)

Day 0 is the day of LN-144 infusion.

Upon completion of the manufacturing process the product will be labeled with a patient specific label. A certificate of authenticity verifying the result of the release testing and the accuracy of the labels will be issued. The product will then be released for shipment from the manufacturing facility. The product will be shipped overnight by courier to the clinical site pharmacy in a NanoCool™ shipper validated to maintain a product temperature of 2-8^{o}C. The product temperature will be continuously monitored by a TempTale 4™ which will be placed in the container in contact with the product.

The product will be received by the appropriate clinical pharmacy for the particular patient. After verification and labeling at the pharmacy, the product will be returned to the NanoCool™ shipper to maintain temperature as the product is transferred to the patient bedside. Upon receipt by the infusing physician and double verification for identity the product may be removed from the NanoCool™ shipper and prepared for infusion.

If not already hospitalized, the patient will be admitted 1-2 days prior to planned LN-144 administration and prepared for study drug administration. Patients will remain

hospitalized until the completion of the IL-2 therapy, as per institutional standards. The following procedures should be performed:

- Physical exam including weight
- Verification of all ongoing medications
- ECOG performance status evaluation
- Vital signs- pulse rate, respiratory rate, blood pressure and temperature
- Vital signs will be monitored every 30 minutes during infusion then hourly (+/-15 minutes) for four hours and then routinely (every four to six hours), unless otherwise clinically indicated, for up to approximately 24 hours post LN-144 infusion.
- Blood and urine tests (to be drawn prior to LN-144 infusion)
    - Hematology – CBC with differential
    - Chemistry - Sodium, Potassium, Chloride, Total CO_2 or Bicarbonate, Creatinine, Glucose, BUN, Albumin, Calcium, Magnesium, Phosphorus, Alkaline Phosphatase, ALT/SGPT, AST/SGOT, Total Bilirubin, Direct Bilirubin, LDH, Total protein, Total CK, Uric Acid, and thyroid panel (to include TSH and free T4)
    - Urinalysis (complete urine culture if indicated)
- The following medications will be administered:
    - Prophylactic antibiotics such as TMP/SMX DS 160 mg/800 mg may be given as clinically indicated, per standard of care
    - LN-144 Infusion: Autologous TIL (LN-144) will be administered intravenously. The product will be administered (by gravity) within 45 minutes. If interruption of infusion is required for medical reasons, the product infusion should complete within 3 hours of beginning infusion. The total volume to be infused will be approximately 250 mL for cell concentrations ≤ 75 x 10^9 LN-144 or 500 mL for cell concentrations < 150 x 10^9 LN-144. Further details

of the administration procedure will be provided in the Pharmacy Manual

- Assessment of AE/SAEs

6.8 Days 1 – 4

During these days, while patient remains hospitalized, the following procedures should be performed:

- Physical exam including weight
- Verification of all ongoing medications
- Vital signs- pulse rate, respiratory rate, blood pressure and temperature
- Blood and urine tests (to be drawn prior to the first IL-2 administration of each calendar day)
    - Hematology- CBC with differential
    - Chemistry- Sodium, Potassium, Chloride, Total CO_2 or Bicarbonate, Creatinine, Glucose, BUN, Albumin, Calcium, Magnesium, Phosphorus, Alkaline Phosphatase, ALT/SGPT, AST/SGOT, Total Bilirubin, Direct Bilirubin, LDH, Total protein, Total CK, Uric Acid, and thyroid panel (to include TSH and free T4)
    - CMV antigen assay - required only on Days 1 and 3
    - Urinalysis (complete Urine culture if indicated)
- Obtain blood for immune monitoring on Days 1 and 4 only (50 mL to blood to be obtained. Refer to lab manual)
- Assessment of AE/SAEs
- The following medications will be administered:
    - IL-2 – begin infusion on Day1: 12 - 24 hours after conclusion of the LN-144 infusion. IL-2 will be administered at a dose of 600,000 IU/kg (based on total body weight). Administer by intravenous infusion at a frequency not greater than every 8 hours as per

institutional standard of care. Continue for up to a maximum of six doses. IL-2 doses will be skipped if patient experiences a Grade 3 or 4 toxicity due to IL-2 except: reversible Grade 3 toxicities common to IL-2 such as diarrhea, nausea, vomiting, hypotension, skin changes, anorexia, mucositis, dysphagia, or constitutional symptoms and laboratory changes as detailed in Appendix 4. Toxicities will be managed as outlined in Appendix 5. If these toxicities can be easily reversed within 24 hours by supportive measures, then additional doses may be given. If greater than 2 doses of IL-2 are skipped, IL-2 administration will be stopped. In addition, dosing may be held or stopped at the discretion of the treating investigator. Refer to Appendix 5 for guidance.

- Filgrastim 5 mcg/kg/day administered by subcutaneous injection. This will be administered each day until the absolute neutrophil count reaches >1000/mm^3 for three consecutive days.
- Fluconazole 400 mg PO daily. This should be administered each day until the absolute neutrophil count reaches >1000/mm^3
- Prophylactic antibiotics such as TMP/SMX DS 160 mg/800 mg may be given as clinically indicated, per standard of care
- Herpetic treatment will be initiated in patients positive for HSV. Valacyclovir PO or acyclovir IV will be administered daily, and continued until CD4 > 200 cells/mm^3.

- Assessment of AE/SAEs

6.9 Day 14, 28 (+/- 1 day)

The following procedures will be performed:

- Physical exam including weight
- ECOG performance status evaluation - Day 14 only
- Blood tests
    - Hematology- CBC with differential

- Chemistry- Sodium, Potassium, Chloride, Total CO_2 or Bicarbonate, Creatinine, Glucose, BUN, Albumin, Calcium, Magnesium, Phosphorus, Alkaline Phosphatase, ALT/SGPT, AST/SGOT, Total Bilirubin, Direct Bilirubin, LDH, Total protein, Total CK, Uric Acid, and thyroid panel (to include TSH and free T4)
- CD4 count – Day 28 only

- Obtain blood for Immune monitoring (50 mL of blood to be obtained. Refer to lab manual) – Day 14 only
- If required, the following medications will continue to be administered
  - Filgrastim 5 mcg/kg/day administered by subcutaneous injection. This will be administered each day until the absolute neutrophil count reaches >1000/mm^3 for three consecutive days.
  - Fluconazole 400 mg PO daily. This should be administered each day until the absolute neutrophil count reaches >1000/mm^3.
  - Prophylactic antibiotics such as TMP/SMX DS 160 mg/800 mg may be given as clinically indicated, per standard of care.
  - Herpetic treatment will be initiated in patients positive for HSV. Valacyclovir PO or acyclovir IV will be administered daily, and continued until CD4 > 200 cells/mm^3.
- Assessment of AE/SAEs

6.10 Day 42 (+/- 3 days)

The following procedures will be performed:

- Physical exam including weight
- Evaluation and measurement of all skin and palpable lesions
- Verification of all ongoing medications
- Vital signs- pulse rate, respiratory rate, blood pressure and temperature
- Assessment of AE/SAEs

- Blood tests
    - Hematology- CBC with differential
    - Chemistry- Sodium, Potassium, Chloride, Total CO_2 or Bicarbonate, Creatinine, Glucose, BUN, Albumin, Calcium, Magnesium, Phosphorus, Alkaline Phosphatase, ALT/SGPT, AST/SGOT, Total Bilirubin, Direct Bilirubin, LDH, Total protein, Total CK, Uric Acid, and thyroid panel (to include TSH and free T4)
    - CMV antigen assay, as clinically indicated
    - CD4 count
- Obtain blood for immune monitoring (50 mL of blood to be obtained. Refer to lab manual)
- CT Exam
    - Chest (include neck if there is prior or suspected neck disease)
    - Abdomen
    - Pelvis
- MRI of brain
- If required, the following medications will continue to be administered
    - Filgrastim 5 mcg/kg/day administered by subcutaneous injection. This will be administered each day until the absolute neutrophil count reaches >1000/mm^3 for three consecutive days. The maximum filgrastim dose should not exceed 300 mcg per day.
    - Fluconazole 400 mg PO daily. This should be administered each day until the absolute neutrophil count reaches >1000/mm^3.
    - Prophylactic antibiotics such as TMP/SMX DS 160 mg/800 mg may be given as clinically indicated, per standard of care.
    - Herpetic treatment will be initiated in patients positive for HSV. Valacyclovir PO or acyclovir IV will be administered daily, and

continued until CD4 > 200 cells/mm^3.

6.11 Day 84/ Week 12 (+/- 3 days)

The following procedures will be performed during this post treatment evaluation visit:

- Physical exam including weight
- ECOG performance status evaluation
- Evaluation and measurement of all skin and palpable lesions
- Verification of all ongoing medications
- Vital signs- pulse rate, respiratory rate, blood pressure and temperature
- Assessment of AE/SAEs
- Slit lamp eye exam
- Blood tests
    - Hematology- CBC with Differential
    - Chemistry- Sodium, Potassium, Chloride, Total CO_2 or Bicarbonate, Creatinine, Glucose, BUN, Albumin, Calcium, Magnesium, Phosphorus, Alkaline Phosphatase, ALT/SGPT, AST/SGOT, Total Bilirubin, Direct Bilirubin, LDH, Total protein, Total CK, Uric Acid, and thyroid panel (to include TSH and free T4)
    - CMV antigen assay, as clinically indicated
    - CD4 count
- Calculate Creatinine Clearance using Cockcroft-Gault formula
- Obtain blood for immune monitoring (50 mL of blood to be obtained. Refer to lab manual)
- CT Exam
    - Chest (include neck if there is prior or suspected neck disease)
    - Abdomen

- Pelvis

- MRI of brain

- If required, the following medications will continue to be administered

    - Filgrastim 5 mcg/kg/day administered by subcutaneous injection. This will be administered each day until the absolute neutrophil count reaches >1000/mm^3 for three consecutive days.

    - Fluconazole 400 mg PO daily. This should be administered each day until the absolute neutrophil count reaches >1000/mm^3.

    - Prophylactic antibiotics such as TMP/SMX DS 160 mg/800 mg may be given as clinically indicated, per standard of care.

    - Herpetic treatment will be initiated in patients positive for HSV. Valacyclovir PO or acyclovir IV will be administered daily, and continued until CD4 > 200 cells/mm^3.

6.12 Months 6 (+/- 1 week), 9 (+/- 1 week), 12 (+/- 1 week), 18 (+/- 3 weeks), and 24 (+/- 3 weeks)

The following procedures will be performed during these visits:

- Physical exam including weight

- ECOG performance status evaluation

- Verification of all ongoing medications

- Vital signs- pulse rate, respiratory rate, blood pressure and temperature

- Evaluation and measurement of all skin and palpable lesions

- Blood tests

    - Hematology- CBC with Differential

    - Chemistry- Sodium, Potassium, Chloride, Total CO_2 or Bicarbonate, Creatinine, Glucose, BUN, Albumin, Calcium, Magnesium, Phosphorus, Alkaline Phosphatase, ALT/SGPT, AST/SGOT, Total Bilirubin, Direct Bilirubin, LDH, Total protein,

Total CK, Uric Acid, and thyroid panel (to include TSH and free T4)

- CD4 count – Month 6 only
- CMV antigen assay, as clinically indicated

- Obtain blood for immune monitoring (50 mL of blood to be obtained Refer to lab manual) - Month 6, 9 and 12 only
- CT Exam
  - Chest (include neck if there is prior or suspected neck disease)
  - Abdomen
  - Pelvis
- MRI of brain
- If required, the following medications will continue to be administered
  - Filgrastim 5 mcg/kg/day administered by subcutaneous injection. This will be administered each day until the absolute neutrophil count reaches >1000/mm^3 for three consecutive days.
  - Fluconazole 400 mg PO daily. This should be administered each day until the absolute neutrophil count reaches >1000/mm^3.
  - Prophylactic antibiotics such as TMP/SMX DS 160 mg/800 mg may be given as clinically indicated, per standard of care.
  - Herpetic treatment will be initiated in patients positive for HSV. Valacyclovir PO or acyclovir IV will be administered daily, and continued until CD4 > 200 cells/mm^3.
- Assessment of AE/SAEs

6.13 Patients Discontinued from Treatment

Patients who are discontinued from treatment should stay on the study and continue with all scheduled study visit assessments.

6.14 Expected Toxicities and Treatment Guidelines

6.14.1 LN-144

Early toxicities related specifically to the infusion of the cells (those which are seen immediately following the cell infusion and prior to IL-2 administration are generally mild and include fevers, chills, headache, and malaise. Toxicities which occur following administration of IL-2 but are thought to be related to the cells include immune mediated events such as vitiligo, transient uveitis, hearing loss and vestibular dysfunction. (IL-2 specific toxicity is discussed in 6.14.2) The use of the non-myeloablative regimen prior to cell administration increases the toxicity of this treatment as profound myelosuppression occurs in all patients.

6.14.2 IL-2

IL-2 administration has been associated with capillary leak syndrome (CLS) which is characterized by a loss of vascular tone and extravasation of plasma proteins and fluid into the extravascular space. CLS results in hypotension and reduced organ perfusion which may be severe and can result in death. CLS may be associated with cardiac arrhythmias (supraventricular and ventricular), angina, myocardial infarction, respiratory insufficiency requiring intubation, gastrointestinal bleeding or infarction, renal insufficiency, edema, and mental status changes.

IL-2 treatment is also associated with impaired neutrophil function (reduced chemotaxis) and with an increased risk of disseminated infection, including sepsis and bacterial endocarditis. Consequently, preexisting bacterial infections should be adequately treated prior to initiation of IL-2 therapy. Patients with indwelling central lines are particularly at risk for infection with gram positive microorganisms. Antibiotic prophylaxis with oxacillin, nafcillin, ciprofloxacin, or vancomycin has been associated with a reduced incidence of staphylococcal infections. IL-2 administration should be withheld in patients developing moderate to severe lethargy or somnolence; continued administration may result in coma.

The standard approach to the administration of high-dose IL-2 in all studies is to continue dosing until grade 3 or 4 events occur. The most commonly seen grade 4 events are pulmonary and renal impairment, and mental status changes. These toxicities may sometimes require intubation for protection of the patient's airway. It is

important to note that although these patients require significant supportive measures during this period, all toxicities are reversible and the overwhelming majority of patients have suffered no long term sequelae following this treatment regimen. However, fatal complications are possible and it is therefore only appropriate to carry out this experimental treatment in the context of life threatening metastatic cancer.

6.14.3 Treatment Guidelines for Toxicity Management

Concomitant medications to control side effects of therapy will be given. Meperidine (25-50 mg), or other medication per site standard of care may be given intravenously if severe chills develop. Other supportive therapy shall be given as required. Supportive therapy includes acetaminophen (650 mg q4h), indomethacin (50-75 mg q6h) and ranitidine (150 mg q12h). The investigator should use supportive therapies as per institutional standard of care. Additional antiemetic therapy will be administered for breakthrough nausea and vomiting. Patients shall receive supportive care as indicated for IL-2 toxicities as listed in Appendix 5.

Expected toxicities with cyclophosphamide and fludarabine administration are listed in the package inserts (See Appendix 7 and 8 respectively). Also included in the package inserts is information on supportive care and management of toxicities. Treatment will be given as per investigator discretion and can be given as per institutional standard of care. Additional guidelines for toxicity management are as below.

6.14.4 Empiric Antibiotics

Patients will start on broad-spectrum antibiotics, either a 3rd or 4th generation cephalosporin or a quinolone for fever – defined as 38.3°C once or two temperatures of 38.0°C or above at least one hour apart, AND an ANC <500/mm^3. Infectious disease consultation will be obtained for all patients with unexplained fever or any infectious complications.

6.14.5 Blood Product Support

Using daily CBCs as a guide, the patient will receive platelets and packed red blood cells (PRBCs) as needed. Attempts will be made to keep hemoglobin >7.5 g/dL, and platelets >10,000/mm^3. All blood products will be irradiated. Leukocyte filters will be

utilized for all blood and platelet transfusions to decrease sensitization to transfused WBCs and decrease the risk of CMV infection.

6.14.6 Renal Toxicity

Renal toxicity defined by rapid rise in creatinine levels or clinical symptoms is a risk. If patients exhibit signs or symptoms of renal toxicity, manage as per institutional standard of care.

6.15 Infection Prophylaxis

Note: Other anti-infective agents may be substituted at the discretion of the treating Investigator.

6.15.1 Pneumocystis Jiroveci Pneumonia

All patients will receive the fixed combination of trimethoprim (TMP) and sulfamethoxazole [SMX] as double strength (DS) tab (DS tabs = TMP 160 mg/tab, and SMX 800 mg/tab) (PO) daily three times a week on non-consecutive days, beginning on the first Monday, Wednesday, or Friday on or after the first dose of chemotherapy.

Pentamidine will be substituted for TMP/SMX DS in patients with sulfa allergies. It will be administered aerosolized at 300 mg per nebulizer within one week prior to receiving study treatment and continued monthly until CD4 count is above 200/mm^3 and for at least six months post chemotherapy, or as Investigator deems appropriate.

Pneumonia prophylaxis will continue for six months post chemotherapy. If the CD4 count is less than 200/mm^3 at six months post chemotherapy, or as Investigator deems appropriate, prophylaxis will continue until the CD4 count is greater than 200/mm^3.

6.15.2 Herpes Virus Prophylaxis

Patients with positive HSV serology will be given valacyclovir orally at a dose of 500 mg daily the day after chemotherapy ends, or acyclovir, 250 mg/m^2 IV every 12 hours if the patient is not able to take medication by mouth. Reversible renal insufficiency has been reported with IV but not oral acyclovir. Neurologic toxicity including delirium,

tremors, coma, acute psychiatric disturbances, and abnormal EEGs has been reported with higher doses of acyclovir. Should this occur, a dosage adjustment will be made or the drug will be discontinued. Acyclovir will not be used concomitantly with other nucleoside analogs which interfere with DNA synthesis, e.g. ganciclovir. In renal disease, the dose is adjusted as per product labeling.

Herpes prophylaxis will continue for six months post-chemotherapy, or as long as Investigator deems necessary. If the CD4 count is less than 200/mm^3 at six months post chemotherapy, prophylaxis will continue until the CD4 count is greater than 200/mm^3.

6.15.3 Fungal Prophylaxis (Fluconazole)

Patients will start fluconazole 400 mg (PO) the day after chemotherapy concludes and continue until the absolute neutrophil count is greater than 1000/mm^3. The drug may be given IV at a dose of 400 mg in 0.9% sodium chloride USP daily in patients unable to take it orally.

6.15.4 Empiric Antibiotics

Patients will start on broad-spectrum antibiotics, either a 3rd or 4th generation cephalosporin or a quinolone for fever – defined as 38.3°C once or two temperatures of 38.0°C or above at least one hour apart, AND an ANC <500/mm3. Aminoglycosides should be avoided unless there is clear evidence of sepsis. Infectious disease consultation will be obtained for all patients with unexplained fever or any infectious complications as per institutional standard of care.

6.15.5 Blood Product Support

Using CBCs as a guide, the patient will receive platelets and packed red blood cells (PRBCs) as needed. Attempts will be made to keep hemoglobin >7.5 g/dL, and platelets >10,000/mm^3. All blood products will be irradiated. Leukocyte filters will be utilized for all blood and platelet transfusions to decrease sensitization to transfused WBCs and decrease the risk of CMV infection

7 COMPLETION / DISCONTINUATION AND WITHDRAWAL OF PATIENTS

7.1 Treatment Completion

Completion of treatment is defined as successful infusion with LN-144 followed by the 12-week of IL-2.

7.2 Criteria for Early Discontinuation from Study or Treatment

Criteria for early discontinuation from treatment:

- Grade 3 or greater autoimmunity that involves vital organs (heart, kidneys, brain, eye, liver, colon, adrenal gland, lungs) with symptoms emerging prior to first IL-2 administration.
- Grade 3 or greater allergic reaction including bronchospasm or generalized urticaria that does not resolve after medical management in the opinion of the investigator.
- Grade 3 or greater toxicity due to IL-2 that does not decrease to Grade 2 or less within 96 hours of management.
- Determination by the PI that continued treatment is not in the best interest of the patient.
- Withdrawal by patient. The patient may withdraw consent to treatment but continue consent for follow-up evaluations and/or survival status
- Pregnancy

Criteria for early discontinuation from study

- Subject has become ineligible for study after enrollment and prior to TIL or IL2 administration
- Withdrawal of consent
- Death
- Lost to follow-up after 3 documented attempts to contact the subject.

8 STUDY DRUG INFORMATION

Product Name: LN-144

Active Product Components: Autologous, viable, tumor infiltrating lymphocytes (TIL)

Dosage Form: Live cell suspension

Table 1. Composition of LN-144

Ingredient	Unit and/or Percentage Formula		Function
	% v/v	Per mL	
Tumor Infiltrating Lymphocytes	Up to 150 x 10^9		Active Ingredient
Interleukin 2	Not applicable	300 IU	Lymphocyte growth factor
Human Serum Albumin	0.5	Not applicable	Stabilizer
Plasma-Lyte® A	≤50	Not applicable	Diluent
HypoThermosol™	≥50	Not applicable	Transport medium

Qualitative Composition: LN-144 is a cell product of autologous tumor-infiltrating lymphocytes (TIL) derived from the patient's own tumor. LN-144 is an autologous cell therapy for the treatment of patients with advanced melanoma. LN-144 is a live cell suspension that is formulated in HypoThermosol™ transport medium, Plasma-Lyte® A with 0.5% HSA (human serum albumin) and 300 IU/mL of IL2. The suspension volume will be between 250 to 500 mL. Only one LN-144 dose is given intravenously after lymphodepletion chemotherapy followed by high dose IL-2 therapy 12-24 hours after infusion. The total volume to be infused will be approximately 250 mL (300 mL transfer bag) for cell concentrations ≤ 75 x 10^9 LN-144 or 500 mL (600mL transfer bag) for cell concentrations < 150 x 10^9 LN-144.

Manufacturing Process: The overall process of tumor shipping, LN-144 manufacturing, and LN-144 product shipping, and infusion was shown above in **Figure 1**. The LN-144 product is manufactured ex vivo using autologous tumor as starting material. The key manufacturing steps include:

- Surgical removal of autologous metastatic tumor and shipment to manufacturing facility
- Culture of small 2-3 mm (length x width x height) fragments of autologous tumor in IL-2 for up to three weeks to expand TIL.
- Harvesting and cryopreservation of TIL for further scheduling of patient and expansion in a rapid expansion protocol (REP)
- REP culture for 14 days in the presence of IL-2, OKT3, and irradiated allogeneic MNC feeder cells
- Harvesting and formulation of REP expanded product in transport medium and overnight shipment to clinical site for infusion

Final Product Container: The live suspension of LN-144 is stored in a 300 mL blood transfer pack (Baxter) for cell concentrations ≤ 75 x 10^9 LN-144 or 600 mL blood transfer pack (Baxter) for cell concentrations < 150 x 10^9 LN-144.

Transport: Each dose of the live suspension LN-144 will be shipped/sent by courier to the clinical site from the LN-144 Manufacturing Facility the day before administration using a method that is intended to support 24-hour delivery. The live suspension product will be packaged in a protective bag containing absorbent padding then placed into an insulated container (Therapak NanoCool™ shipper), designed to maintain transit temperature between 2 - 8°C. A temperature monitoring device will be included to monitor the temperature inside the container during shipping.

Receipt at Clinical Site and Administration: The dose of LN-144 will be received at the clinical site in the pharmacy on the day of administration under quarantine. Receipt is defined as the moment the LN-144 package is signed for by site personnel and released from courier's custody. After receiving, verification, and labelling with the clinical sites specific labels at the pharmacy, the investigational product, LN-144, will be released by Lion and transferred to the patient bedside. The product is infused by gravity within 45 minutes. If interruption of infusion is required for medical reasons, the product infusion should complete within 3 hours of beginning infusion. Refer to Product Administration Manual for additional details.

9 STUDY ASSESSMENTS

9.1 Efficacy Assessments

9.1.1 Response Criteria

Clinical response will be determined using RECIST version 1.1 with a modification to require confirmation of PD. Refer to **Table 2** and **Table 3** for RECIST 1.1 response criteria definitions.

9.1.1.1 Evaluation of Target Lesions[1]

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

[1] All measurable lesions up to a maximum of two lesions per organ and five lesions in total, representative of all involved organs should be identified as **target lesions** and recorded and measured during screening. A sum of the diameters (longest for non-nodal lesions, short axis for nodal lesions) for all target lesions will be calculated and reported as the baseline sum diameters. The baseline sum diameters will be used as reference by which to characterize any objective tumor regression in the measurable dimension of the disease. If lymph nodes are to be included in the sum, only the short axis will contribute.

diameters while on study.

9.1.1.2 Evaluation of Non-target Lesions[2]

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

9.1.1.3 Evaluation of Best Overall Response

The best overall response is determined once all the data for the patient is known. The best overall response is the best response recorded from the start of treatment until disease progression/recurrence, the initiation of new anti-cancer therapy, death or 24 months whichever comes first. The patient's best response assignment will depend on the achievement of both measurement and confirmation criteria.

Table 2. Time Point Response: Patients with Target (± Non-target) Disease

Target Lesions	Non-Target Lesions	New Lesions	Overall Response
CR	CR	No	CR
CR	Non-CR/Non-PD	No	PR
CR	Not evaluated	No	PR
PR	Non-PD or not all evaluated	No	PR
SD	Non-PD or not all evaluated	No	SD
Not all evaluated	Non-PD	No	NE
PD	Any	Yes or No	PD

[2] All other lesions (or sites of disease) should be identified as **non-target lesions** and should also be recorded at baseline.

Any	PD	Yes or No	PD
Any	Any	Yes	PD

Table 3. Time Point Response: Patients with Non-target Disease Only

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

9.1.1.4 Confirmatory Measurement/Duration of Response

9.1.1.4.1 Confirmation

To be assigned a status of response, changes in tumor measurements must be confirmed by a subsequent assessment that should be performed at least 4 weeks after the criteria for response are first met. In the case of SD, follow-up measurements must have met the SD criteria at least once after study entry at a minimum interval of six weeks.

9.1.1.4.2 Duration of Overall Response

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

10 STATISTICAL AND ANALYTICAL PLANS

10.1 Introduction

The primary statistical plan of analysis is based on use of descriptive methods unless mentioned otherwise. Continuous data will be summarized as the number of patients with non-missing data (N), mean, standard deviation, median, minimum, and maximum values. Categorical data will be summarized as counts and their related percentages, where applicable. Point estimates of treatment effect will be derived

from maximum likelihood methods. Estimation of confidence limits will use two-sided, 95% criteria and implement exact methods. Missing data will not be imputed unless defined in the statistical analysis plan (SAP). If inferential statistics are calculated (e.g., p-values), they will be used in a descriptive manner.

A more detailed description of the analyses and reporting plan of the data will be provided in the SAP.

10.2 Analysis Populations

Two analysis populations will be defined to summarize the data. The modified Intention-to-Treat population (ITT) will consist of all resected patients. The All-Treated population is based on all resected patients who have been successfully treated with LN-144 followed by IL-2 (at least one dose). Responders (PR or CR) among both populations will be used to summarize the duration of overall response.

10.3 Endpoints

10.3.1 Primary

The primary endpoint is based on summarizing the safety and toxicity data. Safety and toxicity will be based on the assessment of multiple clinical evaluations and will mainly include adverse events, clinical laboratory tests, vital signs, and physical examinations.

10.3.2 Secondary

The secondary endpoints are the best overall response (ORR), complete response rate (CR) as best overall using RECIST 1.1 criteria. The ORR is derived as the number of patients with a complete response (CR) or partial response (PR) divided by the number of patients either resected (modified Intention to Treat population) and successfully infused with LN-144 followed by IL-2 (at least one dose, All-Treated population) x 100%. Patients failing to achieve a CR and PR among the denominator patient population will be classified as non-responders.

The other secondary endpoints will be progression-free survival (PFS), duration of response (DOR) and overall survival (OS). The definition of each of these endpoints follows.

PFS is defined as the time (in months) from the start of IL-2 therapy to PD or death due to any cause, whichever event is earlier. Patients not experiencing PD or death at the time of data cut or end of study (i.e., database lock) will have their event times censored on the last adequate assessment of tumor status.

Duration of overall response is measured from the first time measurement criteria are met for a CR or PR, whichever response is observed first, until the first date that progressive disease (PD) or death occurs. Patients not experiencing PD or death prior to the time of data cut or end of study will have their event times censored on the last adequate disease assessment date.

OS is defined as the time (in months) from the start of the lymphodepletion therapy to death due to any cause. Patients not having expired at the time of data cut or end of study will have their event times censored on the last date of their known survival status.

10.3.3 Exploratory

The exploratory endpoints includes measures of LN-144 persistence in the peripheral blood as well as immune response with the objective to evaluate their correlation with response, outcome, and toxicity of the treatment.

10.4 Sample Size Justification

The sample size of 20 patients is based on the All-Treated population who complete treatment; the number of resected patients is not the sampling target. Complete treatment is defined as successful infusion with LN-144 followed by IL-2.

A sample size of 20 patients who completed treatment is associated with acceptable cumulative probabilities of observing at least one Grade 3 or 4 toxicity. Assuming an underlying rate of observing a Grade 3 or 4 toxicity is 0.05, 0.10, or 0.15, the probability of observing at least 1 such toxicity in a sample of 20 patients who completed treatment is 0.642, 0.898, and 0.961, respectively.

10.4.1 Baseline Demographic and Clinical Characteristics

Baseline demographic and clinical (disease) characterized will be summarized descriptively for the All-Treated patient populations. Age will be derived as a function of the date of informed consent.

10.4.2 Safety Analysis, Primary Endpoint

The primary safety variable is a binomial proportion and will be summarized using both a point estimate and its two-sided, exact 95% confidence limits.

10.4.3 Efficacy Analysis, Secondary Endpoints

The secondary efficacy (ORR and CR) variables are binomial proportions and will be summarized using both a point estimate and its two-sided, exact 95% confidence limits.

PFS, OS, durations of overall are time-to-event variables subjected to right censoring. Kaplan-Meier probabilities and related summary statistics will be provided for the entire survival curve as well as for the following landmark times following the initial dose of lymphodepletion: 6 months, 12 months, 18, months, and 24 months duration. The landmark analyses will be applied to the PFS and OS data.

10.4.4 Safety Analysis

The assessment of safety data will be descriptive and based on the summarization of treatment-emergent adverse events, serious adverse events, adverse events leading to discontinuation from the study, vital signs, physical examinations, and clinical laboratory tests. Treatment emergent is considered to start the time of harvest surgery (modified Intention to Treat population) or lymphodepletion (All-Treated population). Adverse event summaries will be based on patient incidence counts and their related percentages; the number of events will be displayed as appropriate. In additional to an overall summary of adverse events, separate displays will be made by intensity and relationship. Certain safety data will be amenable to summary by use of toxicity grades, and all such analyses will evaluate the distribution of grades over time in addition to the worst grade observed per patient while on study. These toxicity grade summaries will be derived separately for each measure under consideration (e.g., ANCs for neutropenia; platelets for thrombocytopenia).

A limited amount of safety data is collected at the time of resection until infusion with LN-144 followed by IL-2. These data will be summarized as needed, but separately from the primary safety analyses.

10.4.5 Other Planned Analyses

No additional analyses are planned. Should analyses other than those described in the study protocol, the SAP, or the DSMB charter be performed, their details will be described in the Clinical Study Report.

11 CONTRAINDICATIONS, PRECAUTIONS AND WARNINGS

11.1 Drugs Administered during the Study

Please refer to the Information for Use package insert provided with all drugs used in this study to understand the contraindications, precautions and warning relative to a specific drug.

11.2 LN-144 Treatment

Early toxicities related specifically to the infusion of the cells (those which are seen immediately following cell infusion and prior to IL-2 administration) are generally mild and include fevers, chills, headache, and malaise. Toxicities which occur following administration of IL-2 but are thought to be related to the cells include immune mediated events such as vitiligo, transient uveitis, hearing loss and vestibular dysfunction. (IL-2 specific toxicity is discussed in 6.14.2). The use of the non-myeloablative regimen prior to cell administration increases the toxicity of this treatment as profound myelosuppression occurs in all patients.

11.3 IL-2 Administration

See section 6.14.2 for IL-2 toxicity considerations. The standard approach to the administration of high-dose IL-2 in all studies is to continue dosing without putting the patient at risk for severe or irreversible toxicities. The most commonly seen Grade 4 events are pulmonary and renal impairment, and mental status changes. It is important to note that although these patients require significant supportive measures during this period, most toxicities are reversible and the overwhelming majority of patients have suffered no long term sequelae following this treatment regimen.

However, fatal complications are possible and it is therefore only appropriate to carry out this experimental treatment in the context of life threatening metastatic cancer.

12 ADVERSE EVENTS

Toxicities will be recorded as AEs and SAEs in the patient's source documents and on the Adverse Event eCRF and must be graded using the NCI's CTCAE v4.03 dated June 14, 2010.

12.1 Definitions

Adverse Event

An AE is defined as any untoward medical occurrence that occurs during a clinical investigation regardless of causal relationship with the investigational product. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom or disease assessed in eligible patients after enrollment in the study.

Events meeting the definition of an AE include:

- Adverse event temporally associated with the use of any of the study drugs or TIL treatment whether or not considered related to the use of any of the study drugs or TIL treatment.
- Any abnormal laboratory test results (e.g. hematology, clinical chemistry, or urinalysis) or other safety assessments (e.g., EKGs, radiological scans, vital signs measurements), that worsen from baseline, and are felt to be clinically significant in the medical and scientific judgment of the Investigator.
- Exacerbation of a chronic or intermittent pre-existing condition including either an increase in frequency and/or intensity of the condition.
- New conditions detected or diagnosed after investigational product administration.
- Signs, symptoms, or the clinical sequelae of a suspected interaction with investigational product.

- Signs, symptoms, or the clinical sequelae of a suspected overdose of either investigational product or a concomitant medication.

Events that do not meet the definition of an AE include:

- Any clinically significant abnormal laboratory finding or other abnormal safety assessments that is associated with the underlying disease, unless judged by the Investigator to be more severe than expected for the patient's condition.
- Medical or surgical procedure (e.g., endoscopy, appendectomy); the condition that leads to the procedure is an AE.
- Situations where an untoward medical occurrence did not occur (social and/or convenience admission to a hospital).
- Anticipated day-to-day fluctuations of pre-existing disease(s) or condition(s) present or detected at the start of the study that do not worsen.

During clinical trials, AEs can be spontaneously reported or elicited during open-ended questioning, examination, or evaluation of a patient.

Serious Adverse Event

An AE is considered 'serious" if, in the view of either the Investigator or the Sponsor, it results in any of the following outcomes:

- Death
- Is Life Threatening
- Inpatient hospitalization or prolongation of an existing hospitalization
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- A congenital anomaly/birth defect

Important medical events that may not directly result in death, be life-threatening, or require hospitalization may be considered serious when, based on Investigator

decision, they may jeopardize the patient and may require intervention to prevent one of the above outcomes as listed in this definition.

Hospitalization including admission to a telemetry unit or ICU specifically for administration of study treatment is not considered a serious adverse event.

Any pregnancy that occurs while on the study must be reported to the Sponsor or their representative. The pregnancy must be followed up until discharge following delivery or premature termination to determine outcome and status of mother and child. Pregnancy complications and elective terminations for medical reasons must be reported as an AE or SAE. Spontaneous abortions must be reported as an SAE. Any SAE occurring in association with a pregnancy, brought to the Investigator's attention after the patient has completed the study and considered by the Investigator as possibly related to the investigational product, must be promptly reported to the Sponsor or their representative. In addition, the Investigator must attempt to collect pregnancy information on any female partners of male study patients who become pregnant while the patient is enrolled in the study. Pregnancy information must be reported to the Sponsor or their representative.

12.2 Reporting Procedures for Adverse Events

12.2.1 All Adverse Events

All AEs occurring after signature of informed consent and either observed by the Investigator or reported by the patient (whether or not attributed to the use of IL-2 or LN-144 treatment), will be reported on the eCRF. Monitoring and reporting of AEs will be conducted through the last study visit.

Medically significant AEs considered related to the investigational product by the Investigator or the Sponsor will be followed until resolved or resolved with sequelae. The Investigator shall categorize the cause of the AE as chemotherapy, LN-144, IL-2 or other and must assign the following attributes: description; dates of onset and resolution; severity; assessment of relatedness to investigational product, and action taken. The Investigator may be asked to provide follow-up information.

If any patient should die while on the study, the Investigator will inform the Sponsor as soon as possible. (Note: Death due to disease progression should not be reported as

a SAE unless it is deemed to be related to the use of study treatment.) The cause of death should be recorded in detail on the SAE Report Form.

Each site will be responsible for reporting SAEs occurring at the site to the applicable IRB per the IRB's reporting guidelines. Sites that are required to utilize a local IRB will be responsible for their own local IRB submissions.

It will be left to the Investigator's clinical judgment whether or not an AE is of sufficient severity to require the patient's removal from the study treatment. A patient may also voluntarily discontinue treatment due to what he or she perceives as an intolerable AE. This should be captured in the eCRF. If the patient was permanently removed from the study or investigational product due to an SAE, this information must be included in either the initial or follow-up SAE Report Form and in the eCRF.

12.2.2 Relationship to Study Drug

The following categories and definitions of causal relationship to study drug should be considered:

- Definite There is a known causal relationship between the study drug and the AE/SAE. The event responds to withdrawal of study drug (de-challenge), and recurs with re-challenge when clinically feasible. (>95% certainty of relatedness).
- Probable: There is reasonable causal relationship between the study drug and the AE/SAE. The event responds to de-challenge. Re-challenge is not required. (65%-95% probability of relatedness).
- Possible: There is reasonable causal relationship between the study drug and the AE/ SAE. De-challenge information is lacking or unclear. (35%-65% probability of relatedness).
- Not likely: There is temporal relationship to study drug administration, but there is not a reasonable causal relationship between the study drug and the AE/SAE. (5-35% probability of relatedness).
- Not related: There is not a temporal relationship to study drug administration (too early, or late, or study drug not taken), or there is known causal relationship between the AE/SAE and another drug,

concurrent disease, or other circumstance. (<5% chance of relatedness).

12.2.3 Severity

The severity of an event describes the degree of impact and/or the need for medical care necessary to treat an event.

AE grading will be defined by the CTCAE v 4.03. In the event the CTCAE v 4.03 does not apply, the severity descriptions below will be used.

Mild:	Asymptomatic; clinical or diagnostic observations only; intervention not indicated
Moderate:	Minimal, local, or noninvasive intervention indicated; limiting age-appropriate activities of daily life
Severe:	Medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization may be required; disabling; limiting activities of daily life
Life-threatening:	Urgent intervention is required

12.2.4 Serious Adverse Events

Investigator Reporting to Sponsor:

All SAEs, regardless of relationship to study treatment, must be collected while on the study (from signature of informed consent until last study visit). In addition, the Investigator must notify the Sponsor of any SAE that may occur after this time period which (s)he believes to be certainly, probably, or possibly related study treatment.

SAE terminology and severity grading will be based on the NCI's CTCAE v4.03 guidelines.

All SAEs that occur during the study must be reported by the Investigator to the Sponsor or designee within 24 hours of learning of the event. The initial notification should be as complete as is possible with the information available and include the Investigator's assessment of whether there is a reasonable possibility that the study drug caused the event.

SAE reports will be reported to Drug Safety Solutions, Inc. via PPD

PPD

Reporting to Regulatory Agencies and Institutional Review Boards (IRBs):

In the event of a serious adverse event, the Sponsor, or their designee, will notify the appropriate regulatory authorities and all appropriate parties as per the regulations. In addition, the Sponsor must submit expedited reports of an increased rate of occurrence of serious adverse events over that listed in the protocol or Investigational Brochure. Sponsor will notify participating sites of any serious adverse events which occur during trial.

12.2.5 Data Safety Monitoring Board

An independent DSMB will monitor patient safety during the study. The DSMB's roles, responsibilities, and conduct are described in an independent charter.

13 ADMINISTRATIVE REQUIREMENTS

13.1 Protocol Modifications

The Investigator will not modify this protocol without obtaining the concurrence of the sponsor. All protocol amendments must be issued by the Sponsor, signed and dated by the Investigator, and should not be implemented without prior IRB approval, except where necessary to eliminate immediate hazards to the patients or when the change(s) involves only logistical or administrative aspects of the trial (e.g., change in monitor[s], change of telephone number[s]). Responsibilities for reporting protocol amendments to any Regulatory Authority (if applicable) and/or IRB are further described in the Ethical Aspects section of the protocol.

In situations requiring a departure from the protocol, the Investigator or other physician in attendance will contact the site manager or other appropriate Sponsor representative by fax or telephone (see the Contact Information page). If possible, this contact will be made before implementing any departure from protocol. In all cases, contact with the Sponsor must be made as soon as possible in order to discuss the situation and agree on an appropriate course of action. The source documents will describe any departure from the protocol and the circumstances requiring it.

13.2 Regulatory Documentation

Documents that must be provided to the Sponsor prior to study drug shipment are as follows:

- Up-to-date curriculum vitae for each Investigator.
- Signed and dated Investigator Agreement.
- Applicable local regulatory documentation (e.g., FDA 1572 Form).
- A copy of the formal written notification to the Investigator regarding approval of the protocol by an IEC/IRB that is in compliance with regulatory guidelines. The written notification is to be signed by the chairman or authorized designee and must identify the specific protocol. In cases where an IEC/IRB member has a known conflict of interest, abstention of that individual from voting should be documented; an Investigator may be a member of the IEC/IRB, but may not vote on any research in which he or she is involved.
- Name and address of the IRB with a statement that it is organized and operates according to GCP and the applicable laws and regulations, and a current list of the IRB members. If accompanied by a letter of explanation from the IRB, a general statement may be substituted for this list.
- A copy of the IRB approved informed consent and other adjunctive materials (e.g., advertising) to be used in the study, including written documentation of IEC approval of these items.
- Name and address of any local laboratory conducting tests for the study, a dated copy of the laboratory reference values for tests to be performed during the study and a copy of the certification or other documentation establishing adequacy of the facility.
- Required financial agreement.

In addition to the documents required prior to the study, other documentation may be required during the course of the study.

13.3 Record Retention

In compliance with the ICH/GCP guidelines the Investigator/institution will be responsible for all information in the eCRF and will maintain the source documents that support the data collected from each patient, and all trial documents as specified in Essential Documents for the Conduct of a Clinical Trial and as specified by the applicable regulatory requirement(s). The Investigator/Institution will take measures to prevent accidental or premature destruction of these documents. Essential documents must be retained until at least 2 years after the last approval of a marketing application in an ICH region or at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational product. These documents will be retained for a longer period if required by the applicable regulatory requirements or by an agreement with the Sponsor. It is the responsibility of the Sponsor to inform the Investigator/institution as to when these documents no longer need to be retained. If the responsible Investigator retires, relocates, or for other reasons withdraws from the responsibility of keeping the study records, custody must be transferred to a person who will accept the responsibility. The Sponsor must be notified in writing of the name and address of the new custodian.

13.4 Data Quality Assurance

Steps to be taken to assure the accuracy and reliability of data include; the selection of qualified Investigators and appropriate study centers, review of protocol procedures with the Investigator and associated personnel prior to the study, periodic monitoring visits by the Sponsor/designee. Electronic CRFs will be reviewed for accuracy and completeness by Clinical Research Monitors during on- site monitoring visits and after their return from the site, and any discrepancies will be resolved with the Investigator or designees, as appropriate. The data will be verified for accuracy.

Agreements made by the Sponsor with the Investigator/Institution and any other parties involved in the clinical trial will be in writing as a separate agreement. On-Site Audits

Representatives of the Sponsor’s Clinical Quality Assurance department/designee may visit the site to carry out an audit of the study in compliance with regulatory guidelines and company policy. Such audits will require access to all study records,

including source documents, for inspection and comparison with the eCRFs. Patient privacy must, however, be respected. Sufficient prior notice will be provided to allow the Investigator to prepare properly for the audit.

Similar auditing procedures may also be conducted by agents of any regulatory body reviewing the results of this study in support of a Licensing Application. The Investigator should immediately notify the Sponsor if they have been contacted by a regulatory agency concerning an upcoming inspection.

13.5 Data Handling and Recordkeeping

13.5.1 Electronic Data

When using electronic data handling, the Sponsor or their designee will ensure that systems comply with 21CFR Part 11 requirements. Documentation regarding the electronic data systems used in this protocol is located in the study-specific plans or SOPS for that particular task.

13.5.2 Electronic Case Report Form (eCRF) Completion

Electronic data capture (EDC) will be used for this study. The site will be suitably trained on the use of the eCRF and appropriate site personnel will be provided electronic signatures. Data must be entered into the eCRF screens in English. The eCRFs are to be completed at the time of the patient's visit, with the exception of results of tests performed outside the Investigator's office, so that they always reflect the latest observations on the patients participating in the study.

Data must be recorded first on a source document that can be verified before it is entered in the EDC system. Completed eCRFs are to be signed off by the Investigator as per the data completion guidelines written for this study.

All eCRF corrections are to be made by the Investigator or other authorized study site personnel. The Investigator must authorize changes to the recorded safety and efficacy data.

Completed eCRFs will be submitted according to the Sponsor's instructions, and reviewed by the Sponsor/designee to determine their acceptability. If necessary, Data Correction Requests will be generated for resolution by the study site.

13.6 Study Completion/Termination

13.6.1 Study Completion

The Investigator will complete the study and submit all eCRFs in satisfactory compliance with the protocol after study completion. Continuation of this study beyond this time must be agreed upon by both the Investigator and Sponsor and may be implemented without amendment to the protocol.

13.6.2 Study Termination

The Sponsor reserves the right to temporarily suspend or terminate the study at any time. Reasons for such action taken by the Sponsor include, but are not limited to:

- The discovery of unexpected, serious, or unacceptable risk to subjects enrolled in the study
- A decision on the part of the Sponsor to suspend, discontinue, or shorten the study

13.7 Monitoring

On-site monitoring visits will be performed by the Sponsor as frequently as necessary. Visits are usually made at intervals of at least four to twelve weeks. The dates of the visits will be recorded by the monitor in a trial center visit log to be kept at the site. The first post-initiation visit will usually be made as soon as possible after enrollment has begun. At these visits the monitor will compare the data entered into the eCRFs with the hospital or clinic records (source documents). At a minimum, source documentation must be available to substantiate proper informed consent procedures, adherence to protocol procedures, adequate reporting and follow-up of adverse events, administration of concomitant medication, drug receipt/dispensing/return records, and study drug administration information. Specific items required as source documents will be reviewed with the Investigator prior to the study. Findings from this review of eCRFs and source documents will be discussed with the Investigator. The Sponsor expects that, during monitoring visits, the Investigator (and as appropriate the Study Coordinator) will be available, the source documentation will be available, and a suitable environment will be provided for review of study-related documents.

14 INVESTIGATOR REGULATORY OBLIGATIONS

14.1 Institutional Review Board

This trial will be undertaken only after full approval of the protocol and addenda has been obtained from an IRB and a copy of this approval has been received by the Sponsor. The IRB must be informed of all subsequent protocol amendments issued by the Sponsor. Reports on, and reviews of, the trial and its progress will be submitted to the IRB by the Investigator at intervals stipulated in their guidelines.

The IRB must meet all regulatory requirements governing IRBs (CFR, Title 21, Part 56).

14.2 Informed Consent

Each patient (or a legally authorized representative) must give written consent (and sign other locally required documents) according to local requirements after the nature of the study has been fully explained. The consent form must be signed prior to performance of any study-related activity. The consent form that is used must be approved both by the Sponsor and by the reviewing IRB. The informed consent should be in accordance with the current revision of the Declaration of Helsinki, current International Conference on Harmonization (ICH) and Good Clinical Practice (GCP) guidelines, and the Sponsor’s policies.

The Investigator must explain to potential patients or their legal representatives the aims, methods, reasonably anticipated benefits and potential hazards of the trial, and any discomfort it may entail. Patients will be informed that they are free not to participate in the trial and that they may withdraw consent to participate at any time. They will be told which alternative treatments are available if they refuse to take part and that such refusal will not prejudice future treatment. Finally, they will be told that their records may be examined by competent authorities and authorized persons but that personal information will be treated as strictly confidential and will not be publicly available. Patients must be given the opportunity to ask questions. After this explanation and before entry into the trial, consent should be appropriately recorded by means of the patient's or his/her legal representative's dated signature. If a patient and his/her legal representative are unable to read, an impartial witness must be present during the entire informed consent discussion. The signature of the impartial witness will certify the patient's consent. The patient should receive a signed and

dated copy of the informed consent. The informed consent process should be documented in the patient's medical record.

In accordance with HIPAA, the written Informed Consent Form must include a patient authorization to release medical information to the Sponsor or their representative and/or allow the Sponsor or their representative, a regulatory authority, or IRB access to patient's medical information that includes all hospital records relevant to the study, including a patient's medical history.

14.3 Declaration of Helsinki

The study will be performed in accordance with ethical principles that have their origin in the Declaration of Helsinki and are consistent with ICH GCP, applicable regulatory requirements.

14.4 Patient Data Protection

The Principal Investigator at each site and designees, employees, and agents involved with this study will comply with relevant state and federal laws relating to the confidentiality, privacy, and security of patient's personal health information (PHI). They will only create, maintain, use, or disclose any data that is generated by this study or other information disclosed to the Principal Investigator or their employees or agents during the course of the study to the Sponsor, the Sponsor's collaborators, IRB, FDA, or other authorized recipients as appropriate for the execution, analysis, review, and reporting of this study. Such information shall not be used for any other purposes and will remain confidential. Patient records are only to be identified by initials and patient ID numbers.

14.5 Adverse Event Reporting

The Investigator agrees to report all AEs to the Sponsor as described in the Adverse Events section. Furthermore, the Investigator is responsible for ensuring that any co-Investigator or sub-Investigator promptly bring AEs to the attention of the Investigator. If applicable, the Investigator also is responsible for informing the participating IRB/IEC of any SAEs.

14.6 Investigator

The Investigator will permit study-related monitoring, audits, IEC/IRB review, and regulatory inspections by providing direct access to source data and documents. The Investigator must notify the Sponsor when contacted by a regulatory authority regarding inspection of her/his study site.

All required data will be recorded in the eCRFs in a timely manner. All eCRF data must be submitted to the Sponsor throughout and at the end of the study.

If an Investigator retires, relocates, or otherwise withdraws from conducting the study, the Investigator must notify the Sponsor to agree upon an acceptable storage solution. Regulatory authorities will be notified with the appropriate documentation detailing the person to whom the responsibility has been transferred.

14.7 Confidentiality

Unless otherwise specified in the clinical study agreement, the following process shall occur: The Investigator must assure that patients' anonymity will be maintained and that their identities are protected from unauthorized parties. In the eCRFs or other documents submitted to the Sponsor, patients should not be identified by their names, but by an identification code. The Investigator should keep a site enrollment log showing codes, names, and addresses. Documents not for submission to the Sponsor (e.g., patients' written consent forms) should be maintained by the Investigator in strict confidence, in accordance with all applicable local and national regulations. All information provided to the Investigator prior to the study, as well as all data developed during the study, is confidential and remains the property of the Sponsor. The Investigator agrees that no information based on the conduct of this study (including the protocol, the data resulting from this study, or the fact that this study is/was conducted) will be released without prior written consent of the Sponsor unless this requirement is superseded by local or national regulations.

14.8 Publications

The Sponsor will be responsible for determining when the study results should be published. The Sponsor will work jointly with the Investigators to publish information.

The Investigator shall not submit a publication to journals or professional societies without the prior written approval of the Sponsor.

15 REFERENCES

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APPENDIX 1: SCHEDULE OF EVENTS

Assessment	Screening & Enrollment Procedures				Treatment															Follow-up				
	Screening	Enrollment/ Tumor Resection	Day -14	Day -7	Day -6	Day -5	Day - 4	Day -3	Day - 2	Day -1	Day 0 (+2d)	Day 1	Day 2	Day 3	Day 4	Day 14 (+/- 1 d)	Day 28 (+/- 1 d)	Day 42 (+/-3d)	Day 84 (+/- 3d) / Week 12	Month 6 (+/- 1 wk)	Month 9 (+/- 1 wk)	Month 12 (+/- 1 wk)	Month 18 (+/- wks)	Month 24 (+/- 3 wks)
Informed Consent	X																							
Inclusion/Exclusion	X																							
Physical Exam[1]	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Evaluation and measurement of skin and palpable lesions	X		X															X	X	X	X	X	X	X
Eye Exam	X																		X					
Medical History	X																							
Concomitant Meds	X	X[2]	X	X	X	X	X	X	X	X	X	X	X	X	X			X	X	X	X	X	X	X
Height	X																							
Weight	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Vital Signs[3]	X		X	X	X	X	X	X	X	X	X[3]	X	X	X	X			X	X	X	X	X	X	X
CMV Antigen Assay[4]	X			X	X	X	X	X	X	X		X		X				X	X	X	X	X	X	X
EKG	X		X																					
Stress Thallium[5]	X																							
CT Chest /Abdomen/Pelvis[6]	X		X															X	X	X	X	X	X	X
MRI – Brain[6]	X		X															X	X	X	X	X	X	X
Serum Chemistry[7]	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Thyroid Panel[8]	X		X	X	X	X	X			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Hematology[9]	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Urinalysis[10]	X		X	X	X	X	X	X	X	X	X	X	X	X	X									
Calculated Creatinine Clearance[11]	X																		X					

Assessment	Screening & Enrollment Procedures			Treatment																Follow-up				
	Screening	Enrollment/ Tumor Resection	Day -14	Day -7	Day -6	Day -5	Day - 4	Day -3	Day - 2	Day -1	Day 0 (+2d)	Day 1	Day 2	Day 3	Day 4	Day 14 (+/- 1 d)	Day 28 (+/- 1 d)	Day 42 (+/-3d)	Day 84 (+/- 3d) / Week 12	Month 6 (+/- 1 wk)	Month 9 (+/- 1 wk)	Month 12 (+/- 1 wk)	Month 18 (+/- wks)	Month 24 (+/- 3 wks)
ß-HCG Pregnancy Test[12]	X		X																					
ECOG performance status	X	X	X	X							X					X			X	X	X	X	X	X
HIV Titer	X																							
Hb_sAG	X																							
Anti-HCV	X																							
HLA Typing[13]	X																							
Anti CMV antibody titer	X																							
HSV serology	X																							
EBV panel	X																							
PFT[1]	X																							
Colonoscopy[15]	X																							
Tumor Harvest for TIL		X																						
Six paraffin embedded slides from resected tumor		X																						
Ondansetron				X	X																			
Cyclophosphamide 60 mg/kg				X	X																			
Mesna				X	X																			
Fludarabine 25 mg/m^2/day						X	X	X	X	X														
LN-144 Infusion[16]											X													
IL-2 600,000 IU/kg[17]												X	X	X	X									
Filgrastim[18]												X	X	X	X	X	X	X	X	X				
TMP/SMX DS, or appropriate Abx[19]				X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X				
Fluconazole[20]												X	X	X	X	X	X	X	X	X				

Assessment	Screening & Enrollment Procedures			Treatment																Follow-up				
	Screening	Enrollment/ Tumor Resection	Day -14	Day -7	Day -6	Day -5	Day - 4	Day -3	Day - 2	Day -1	Day 0 (+2d)	Day 1	Day 2	Day 3	Day 4	Day 14 (+/- 1 d)	Day 28 (+/- 1 d)	Day 42 (+/-3d)	Day 84 (+/- 3d) / Week 12	Month 6 (+/- 1 wk)	Month 9 (+/- 1 wk)	Month 12 (+/- 1 wk)	Month 18 (+/- wks)	Month 24 (+/- 3 wks)
Valacyclovir/Acyclovir[21]												X	X	X	X	X	X	X	X	X				
Immune Monitoring[22]		X		X								X			X	X		X	X	X	X	X		
Assessment of AE/SAEs	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

1. Physical examination (PE) will include gastrointestinal (abdomen, liver), cardiovascular, extremities, head, eyes, ears, nose, and throat, respiratory system, skin, psychiatric (mental status), general nutrition. PE conducted during follow-up will be symptom directed. Post LN-144 infusion PE should occur three times a week until discharge.
2. List only medications that are NOT part of the tumor harvest procedure.
3. Vital signs will include pulse rate, respiratory rate, blood pressure, and temperature. On Day 0 (LN-144 infusion), vital signs will be monitored every 30 minutes during infusion then hourly (+/-15 minutes) for four hours and then routinely (every four to six hours), unless otherwise clinically indicated, for up to approximately 24 hours post LN-144 infusion.
4. CMV assay if clinically indicated
5. Cardiac evaluation (stress thallium) for all patients (per current package insert for IL2). Echocardiogram or MUGA for patients ≥ 60 years or patients who have a history of ischemic heart disease, chest pain, or clinical significant atrial and/or ventricular arrhythmias. Stress thallium must show normal LVEF and unimpaired wall movement.
6. Required for Screening, then imaging as clinically indicated. If screening image shows abnormalities, obtain at day -14. Include neck if there is prior or suspected neck disease
7. Chem 20: [Sodium (Na), Potassium (K), Chloride (Cl), Total CO2 (bicarbonate), Creatinine, Glucose, Urea nitrogen (BUN), Albumin, Calcium total, Magnesium total (Mg), Phosphorus, Alkaline Phosphatase, ALT/SGPT, AST/SGOT, Total Bilirubin, Direct Bilirubin, LDH, Total Protein, Total CK, Uric Acid]
8. Thyroid panel: TSH and Free T4. Obtain only as clinically indicated beginning at Day 84/Week 12 and during Follow-up.
9. Complete blood count with differential
10. Dipstick urinalysis with culture, if indicated
11. Calculate creatinine clearance using Cockcroft-Gault calculation
12. Serum pregnancy test for women of child bearing potential
13. HLA typing to be sent to central laboratory
14. Pulmonary evaluation for all patients
15. Patients with documented Grades 2 or greater diarrhea or colitis as a result of previous treatment with ipilimumab, tremelimumab, or anti-PD1 or anti-PD-L1 antibodies must have been asymptomatic for at least 6 months or had a normal colonoscopy post treatment, with uninflamed mucosa by visual assessment.
16. One to two days after the last dose of agent in the preparative regimen
17. Initiate within approximately 12-24 hours after TIL infusion and continue every eight hours for up to six doses
18. Continue until neutrophils count > 1000/mm^3 X 3 days.
19. The TMP/SMX DS schedule should be adjusted to QD three times per week (Monday, Wednesday, Friday) and continue for at least six (6) months and until CD4 > 200/mm^3
20. Continue until ANC > 1000/mm^3
21. In patients positive for HSV continue until CD4 > 200/mm^3
22. 50 mL of blood drawn using vacutainers (refer to the Lab Manual). To be sent to central laboratory

APPENDIX 2: ECOG SCALE

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

Adapted from Oken MM, Creech RH, Tormey DC, et al. Toxicity and Response Criteria Of The Eastern Cooperative Oncology Group. Am J Clin Oncol. 1982;5:649-655.

APPENDIX 3: PRACTICAL WEIGHT

Modification of Dose Calculations* in Patients whose BMI is > 35

Unless otherwise specified in this protocol, actual body weight is used for dose calculations of treatment agents. In patients who are determined to be obese (BMI > 35), the **practical weight** (see 3 below) will be used.

1. BMI Determination:

BMI = weight (kg) / [height (m)]^2

2. Calculation of ideal body weight

Male = 50 kg + 2.3 (number of inches over 60 inches)

Example: ideal body weight of 5'10'' male

50 + 2.3 (10) = 73 kg

Female = 45.5 kg + 2.3 (number of inches over 60 inches)

Example: ideal body weight of 5'3'' female

45.5 + 2.3 (3) = 57 kg

3. Calculation of "practical weight"

Calculate the average of the actual and the ideal body weights. This is the practical weight to be used in calculating the doses of chemotherapy and associated agents designated in the protocol.

*Practical weight will NOT be used in the calculation of dose for IL-2.

APPENDIX 4: HIGH-DOSE IL-2 TOXICITIES

Adverse Events occurrence in ≥ 10% of patients treated with IL-2 (n=525)			
Body System/Events	% patients	Body System/Events	% patients
Body as a whole		*Metabolic and Nutritional Disorders*	
Chills	52	Bilirubinemia	40
Fever	29	Creatinine Increase	33
Malaise	27	Peripheral Edema	28
Asthenia	23	SGOT increase	23
Infection	13	Weight gain	16
Pain	12	Edema	15
Abdominal pain	11	Acidosis	12
Enlarged Abdomen	10	Hypomagnesemia	12
Cardiovascular System		Hypocalcemia	11
Hypotension	71	Alkaline Phosphatase Increase	10
Tachycardia	23	*Nervous System*	
Vasodilation	13	Confusion	34
Supraventricular Tachycardia	12	Somnolence	22
Cardiovascular disorder[a]	11	Anxiety	12
Arrhythmia	10	Dizziness	11
Digestive System		*Respiratory System*	
Diarrhea	67	Dyspnea	43
Vomiting	50	Lung Disorder[b]	24
Nausea	35	Respiratory Disorder[c]	11
Stomatitis	22	Cough increase	11
Anorexia	20	Rhinitis	10
Nausea and Vomiting	19	*Skin and Appendages*	
Hematologic and Lymphatic		Rash	42
Thrombocytopenia	37	Pruritus	24
Anemia	29	Exfoliative dermatitis	18
Leukopenia	16	*Urogenital System*	
		Oliguria	63

[a] Cardiovascular disorder: fluctuations in blood pressure, asymptomatic ECG changes, CHF.
[b] Lung disorder: physical findings associated with pulmonary congestion, rales, rhonchi.
[c] Respiratory disorder: ARDS, CXR infiltrates, unspecified pulmonary changes.
Source: Proleukin® Prescribing Information – June 2007

APPENDIX 5: EXPECTED IL-2 TOXICITIES AND THEIR MANAGEMENT

Expected toxicity	Expected grade	Supportive Measures suggested	Stop Cycle*	Stop Treatment **
Chills	3	IV Meperidine 25-50 mg, IV q1h, prn,	No	No
Fever	3	Acetaminophen 650 mg, po, q4h; Indomethacin 50-75 mg, po, q8h	No	No
Pruritus	3	Hydroxyzine HCl 10-20 mg po q6h, prn; Diphenhydramine HCl 25-50 mg, po, q4h, prn	No	No
Nausea/ Vomiting/ Anorexia	3	Ondansetron 10 mg, IV, q8h, prn; Granisetron 0.01 mg/kg IV daily prn; Droperidol 1 mg, IV q4-6h, prn; Prochlorperazine 25 mg q4h p.r., prn or 10 mg IV q6h prn	No	No
Diarrhea	3	Loperamide 2mg, po, q3h, prn; Diphenoxylate HCl 2.5 mg and atropine sulfate 25 µg, po, q3h, prn; codeine sulfate 30-60 mg, po, q4h, prn	If uncontrolled after 24 hours despite all supportive measures	No
Malaise	3 or 4	Bedrest interspersed with activity	If other toxicities occur simultaneously	No
Hyperbilirubinemia	3 or 4	Observation	If other toxicities occur simultaneously	No
Anemia	3 or 4	Transfusion with PRBCs	If uncontrolled despite all supportive measures	No
Thrombocytopenia	3 or 4	Transfusion with platelets	If uncontrolled despite all supportive measures	No

Expected toxicity	**Expected grade**	**Supportive Measures suggested**	**Stop Cycle***	**Stop Treatment ****
Edema/Weight gain	3	Diuretics prn	No	No
Hypotension	3	Fluid resuscitation Vasopressor support	If uncontrolled despite all supportive measures	No
Dyspnea	3 or 4	Oxygen or ventilatory support	If requires ventilatory support	No
Oliguria	3 or 4	Fluid boluses or dopamine at renal doses	If uncontrolled despite all supportive measures	No
Increased creatinine	3 or 4	Observation	Yes (Grade 4)	No
Renal failure	3 or 4	Dialysis	Yes	Yes
Pleural effusion	3	Thoracentesis	If uncontrolled despite all supportive measures	No
Bowel perforation	3	Surgical intervention	Yes	Yes
Confusion	3	Observation	Yes	No
Somnolence	3 or 4	Intubation for airway protection	Yes	Yes
Arrhythmia	3	Correction of fluid and electrolyte imbalances; chemical conversion or electrical conversion therapy	If uncontrolled despite all supportive measures	No
Elevated Troponin levels	3 or 4	Observation	Yes	If changes in LV function have not improved to baseline by next dose
Myocardial Infarction	4	Supportive care	Yes	Yes

Expected toxicity	**Expected grade**	**Supportive Measures suggested**	**Stop Cycle***	**Stop Treatment ****
Elevated transaminases	3 or 4	Observation	For Grade 4 without liver metastases	If changes have not improved to baseline by next dose
Electrolyte imbalances	3 or 4	Electrolyte replacement	If uncontrolled despite all supportive measures	No
Neutropenia	4	Observation	No	No

*Unless the toxicity is not reversed within 12 hours
** Unless the toxicity is not reversed to Grade 2 or less by next re-treatment.

APPENDIX 6: COMMON TERMINOLOGY CRITERIA FOR ADVERSE EVENTS

http://evs.nci.nih.gov/ftp1/CTCAE/CTCAE_4.03_2010-06-14_QuickReference_5x7.pdf

APPENDIX 7: CYCLOPHOSPHAMIDE PACKAGE INSERT

http://www.accessdata.fda.gov/drugsatfda_docs/label/2013/012141s090,012142s112lbl.pdf

APPENDIX 8: FLUDARABINE PACKAGE INSERT

http://www.accessdata.fda.gov/drugsatfda_docs/label/2009/020038s032lbl.pdf

APPENDIX 9: IL-2 (ALDESLEUKIN) PACKAGE INSERT

http://www.accessdata.fda.gov/drugsatfda_docs/label/2012/103293s5130lbl.pdf