CLINICAL PROTOCOL

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

PROTOCOL NUMBER:	C-144-01
SPONSOR:	Lion Biotechnologies, Inc. 21900 Burbank Blvd., Third Floor Woodland Hills, CA 91367
PROTOCOL VERSION:	Version 3.0
PROTOCOL DATE:	July 16, 2015

SPONSOR PROTOCOL SIGNATURE PAGE

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Approved by:

PPD

Signature July 16, 2015
Date

PPD

Signature July 16, 2015
Date

INVESTIGATOR PROTOCOL SIGNATURE PAGE

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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, Feasibility, and Efficacy of Cell Transfer Therapy Using Autologous Tumor Infiltrating Lymphocytes (LN-144) Followed by IL-2 for Treatment of 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 assess the safety and toxicities associated with the treatment regimen. • To assess the feasibility of TIL production, defined as the percentage of patients with tumor resected from which LN-144 is successfully produced (manufacture of more than 1.5 billion viable cells). **Secondary Objectives** • To assess the feasibility of LN-144 administration followed by IL-2 (defined as the percentage of patients with tumor resected with LN-144 subsequently infused). • To evaluate the anti-tumor activity defined by best overall response rate by RECIST 1.1 in patients who receive LN-144 followed by IL-2. **Exploratory Objectives** • To evaluate additional measures of efficacy for up to 24 months, including: progression-free survival (PFS), overall survival (OS), duration of response, and time to response. • To explore 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.
Rationale for Dose and Treatment Schedule:	LN-144 is a cell transfer therapy and is based on current TIL therapy regimens originally developed by the NCI then further studied and modified at institutions in the United States and Europe. The cell transfer therapy used in this study involves patients receiving a lymphocyte depleting preparative regimen, followed by infusion of between $1.5x10^9$ and $150 x10^9$ autologous TIL followed by the administration of a regimen of IL-2 at 600,000 IU/kg 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:	Approximately 18 weeks (see Schedule of Events for details)
Follow-up Period:	Patients who experience stable disease, a partial response, or a complete response, or have unresolved toxicities at the 12-week post-treatment visit, 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:	Up to five 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. The lesion must be of at least 1.5 cm in diameter 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 progressive disease 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. 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

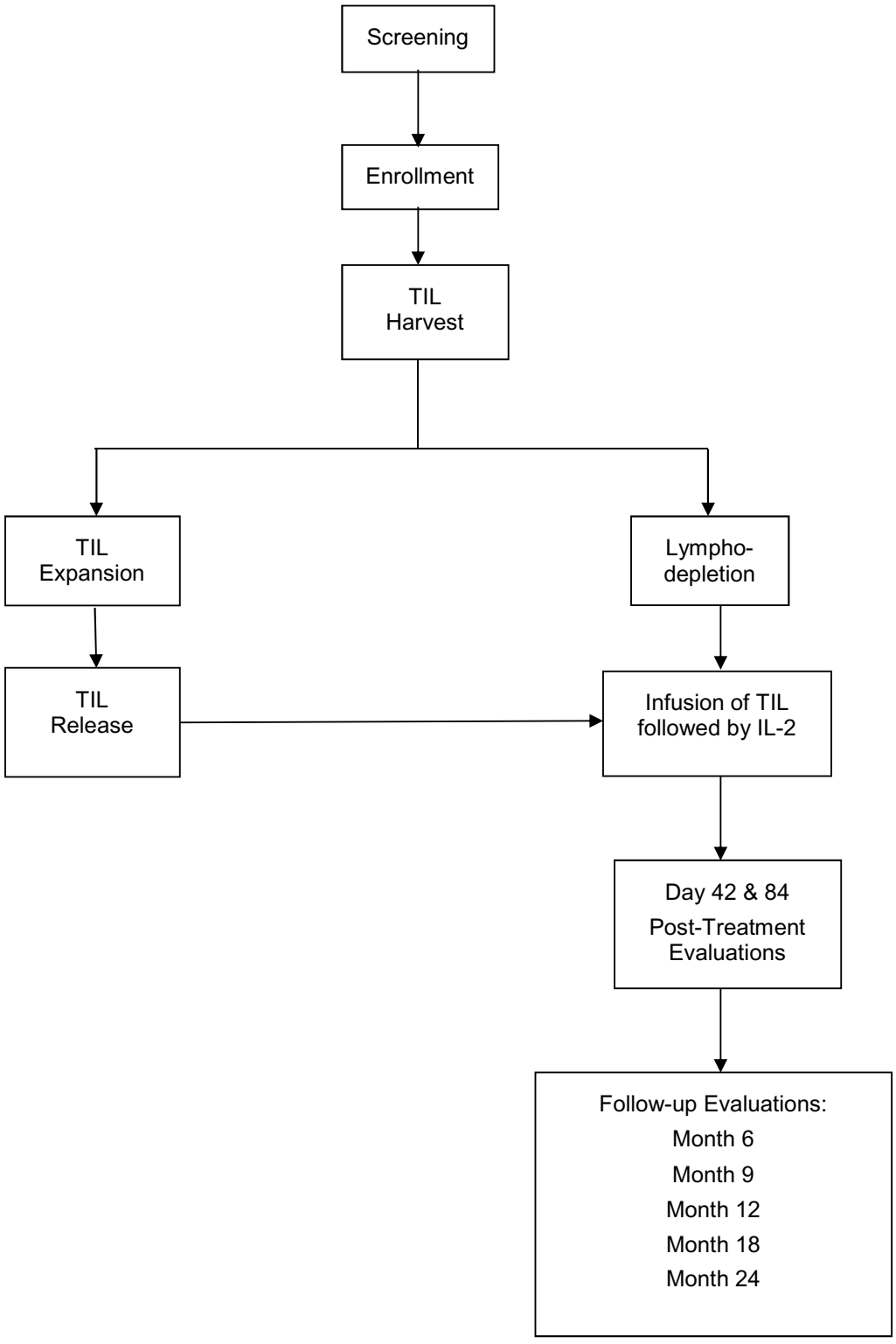
	clearance of greater than 50 mL/min (>50 mL/min), and a total bilirubin less than or equal to 2 mg/dL (≤ 2 mg/dL). Patients with Gilbert's Syndrome must have a total bilirubin less than 3 mg/dL (<3 mg/dL). j. Patients must be seronegative for the HIV antibody, hepatitis B antigen, and hepatitis C antibody or antigen. k. Patients must be EBV viral capsid antigen (VCA) IgG n positive, Epstein Barr nuclear antigen (EBNA) IgG positive, and D early antigen (EA-D) negative l. Patients must not be receiving any radiation therapy, systemic anti-cancer chemo or immunotherapy for 2 weeks (targeted therapies) and 4 weeks (all other treatment) at the time of enrollment, and there must be no intention of receiving any non-protocol systemic anti-cancer chemo or immuno- therapy 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 . 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.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 had a normal colonoscopy post treatment, including biopsy specimens. m. 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. n. 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 who have received prior cell transfer therapy which included a non-myeloablative or myeloablative chemotherapy regimen. b. Patients who have more than three brain metastases. **Note**: Patients with fewer 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 metastases larger than 2 cm are exclusionary. c. Patients who are pregnant or breastfeeding. d. 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. e. 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.

	f. Patients who have any form of primary immunodeficiency (such as Severe Combined Immunodeficiency Disease and AIDS). g. Patients who have a history of severe immediate hypersensitivity reaction to cyclophosphamide, fludarabine, or IL-2. h. Patients who have a history of coronary revascularization or ischemic symptoms. i. Patients who have a history of a positive HIV test or active Hepatitis B or C. 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 history of cigarette smoking of at least 20 packs/year within the past two years that 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.
Discontinuation of Treatment:	Criteria for early termination from study (removal from treatment) (CTCAE v4.03): • 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 • Disease Progression prior to receipt of study drug (TIL followed by IL-2) • Death • Study Terminated by sponsor • Pregnancy
Efficacy Assessment:	The descriptive summary of the clinical overall response rate and progression-free survival will be used to determine the potential efficacy of this treatment and 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. The feasibility endpoints are binomial proportions with the numerators defined as the number of patients successfully meeting pre-specified criteria of feasibility specific to each endpoint and the denominators comprised of all resected patients. These endpoints will be analyzed separately and will be summarized using both a point estimate and its two-sided, exact 95% confidence limits. Patients meeting RECIST 1.1 criteria for a complete (CR) or partial (PR) best overall response will be classified as responders in the analysis of best overall response (BOR) rate. The BOR rate is derived as the number of complete and partial responders divided by the number of patients infused with LN-144 and IL-2. 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 sample size is driven by the need to obtain 20 patients who complete treatment. Complete treatment is defined as successful infusion with LN-144 followed by IL-2.This response parameter is assumed to be a binomial proportion, derived as the number of eligible patients divided by the number of resected patients x 100%. This percentage is expected to equal 60%. To achieve this target with 20 patients who complete treatment, roughly 33 patients will need to be resected. A study accruing 20 patients who complete treatment allows adequate sensitivity to detect one or more Grade 3 or 4 toxicities. This sample of 20 patients who complete treatment also provides a denominator number of patients for the anti-tumor efficacy measurements.
Interim Analysis:	The DSMB will perform safety evaluation of data analyzed when 3 patients have completed study. A limited analysis will also be conducted reviewing all available data from these patients as specified in the DSMB charter. An additional interim analysis will be conducted after 10 patients have been treated to evaluate the feasibility of administering LN-144, including success of harvest of lymphocytes, propagation of TIL, and administration of TIL following preparative therapy.
Study Discontinuation (study or site termination):	Conditions may arise during the study that could prompt the study to be stopped or a study site to be closed. Conditions that may prompt such considerations include, but are not limited to, the following: • 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; • Study conduct at a study site may warrant termination under conditions that include the following: o Failure of the Investigator(s) to enroll eligible subjects into the

	study o Failure of the Investigator(s) to comply with FDA or country-specific regulations o Submission of false information from the research facility to the Sponsor, the Clinical Monitor, or a regulatory authority o Insufficient adherence to protocol requirements o A conflict of interest on the part of the Investigator, his/her institution, or site personnel that would negatively impact the integrity of the clinical trial o Institution or IRB under investigation for cause by a federal agency

STUDY FLOWCHART



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LIST OF ABBREVIATIONS

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
CI_{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
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
OS	Overall survival
PBMC	Peripheral Blood Mononuclear Cells
PD	Progressive disease

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
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, and 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 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 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 have been shown to be 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 different 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 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 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 as achieved relatively similar response rates.[51, 53] In this trial TIL cultures will be selected on the basis of better growth and a higher proportion of CD8+ TIL. TIL initially expanded from the tumor fragments then 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]

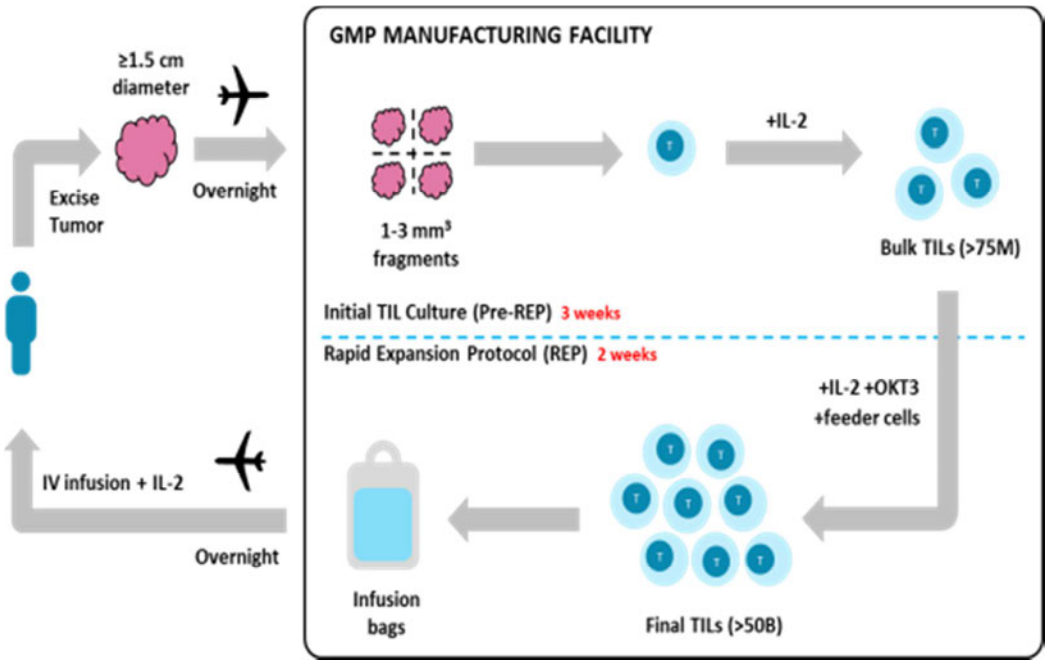

Figure 1. LN-144 Manufacturing Process

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 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 feasibility of a centralized mechanism for harvest, production, expansion, and infusion of TIL in metastatic melanoma, 2) the efficacy of TIL therapy, and 3) the safety of pre-treatment and TIL therapy regimens.

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.

A range of between 1.5 to 150 x 10^9 total 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 1% 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 >75 x 10^9.

The cell concentration range is 5.5 x 10^6 to 300 x 10^6/mL in the final product, with a dose range of 1.5 to 150 x 10^9 total viable cells.

A number of factors indicate that this is a viable range of cells for TIL therapy for metastatic melanoma, including: 1) previous experience in published melanoma TIL therapy clinical trials where the total number of infused cells have been in this range, 2) demonstrated safety when infusing cells within this range, and 3) demonstrated clinical responses in patients infused with TIL within this range of cell numbers.[21, 47-50, 52, 53, 55-59]

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. The lower limit of the range (1.5 x 10^9 viable cells) is set based on the minimum number of cells needed on day 7 of the 14-day REP) in order to make a decision to lymphodeplete the patient using the CY plus FLUD chemotherapy regimen. Once we have begun lymphodepletion based on this minimal attained cell number, we are committed to treating the patient with whatever 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 between 1.5 x 10^9 and 150 x 10^9 LN-144 followed by the administration of a regimen of IL-2 at 600,000 IU/kg 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 who experience stable disease, a partial response, or a complete response (per RECIST version 1.1) or have unresolved toxicities at the 12-week post-treatment visit, 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 assess the safety and toxicities associated with the treatment regimen.
- To assess the feasibility of TIL production, defined as the percentage of patients with tumor resected from which TIL are successfully produced (manufacture of more than 1.5 billion viable cells).

3.1.2 Secondary Objectives

- To assess the feasibility of TIL administration (defined as the percentage of patients with tumor resected with TIL subsequently infused).
- To evaluate the anti-tumor activity defined by response rate by RECIST 1.1 in patients who receive LN-144 followed by IL-2.

3.1.3 Exploratory Objectives

- To evaluate additional measures of efficacy for up to 24 months including: progression-free survival (PFS), overall survival (OS), duration of response, and time to response.
- To explore potential immune correlates of response, outcome, and toxicity of the treatment.

3.2 Study Endpoints

3.2.1 Primary Endpoints:

- Incidence and nature of AEs and SAEs related to TIL therapy followed by IL-2 as defined by treatment-emergent adverse events, SAEs, therapy-related AEs, AEs of interest, AEs leading to early discontinuation from the study, and AEs resulting in deaths on study.
- The proportion of resected patients from whom LN-144 is successfully produced (manufacture of more than 1.5 billion viable cells).

3.2.2 Secondary Endpoints:

- Percentage of resected patients who are infused with LN-144 followed by IL-2.
- Efficacy of TIL therapy as defined by best overall response (BOR) rate over the study period in patients receiving TIL followed by IL-2 treatment.

3.2.3 Exploratory Endpoints:

- Evaluation of progression-free survival (PFS), overall survival (OS), duration of response, and time to response in patients receiving TIL followed by IL-2 treatment up to 24 months after treatment.
- Evaluation of immune correlates with respect to response, outcome, and/or toxicity of the treatment.

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.

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. The lesion must be of at least 1.5 cm in diameter 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 progressive disease 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.

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 age or 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/mm3, hemoglobin greater than 9.0 g/dL, and platelet count greater than 100,000/mm3.

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 n positive, Epstein Barr nuclear antigen (EBNA) IgG positive, and D early antigen (EA-D) negative

l. Patients must not have received systemic therapy for melanoma for a minimum of two weeks (targeted therapy) and four weeks (all other therapy) prior to the point of enrollment. Prior therapy-related toxicities must have recovered to Grade 1 or less (except for alopecia or vitiligo) according to CTCAE v4.03.

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 had a normal colonoscopy, including normal biopsy specimens.

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

b. Patients who have more than three brain metastases. Note: Patients with fewer 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 metastases larger than 2 cm are exclusionary.

c. Patients who are pregnant or breastfeeding.

d. 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.

e. 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.

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

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

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

i. Patients who have a history of a positive HIV test or active Hepatitis B or C.

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 history of cigarette smoking of at least 20 packs/year within the past two years that 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. 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 TIL followed by IL-2 administration. Enrollment 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.

4.3.2 Patient Cohorts

All patients who are resected for LN-144 production will be included in the intent-to-treat cohort.

The DSMB will perform a safety evaluation of data analyzed when 3 patients have completed study. A limited analysis will also be conducted reviewing all data available from these patients as specified in the DSMB charter.

An additional interim analysis will be conducted after 10 patients have been treated to evaluate the feasibility of administering LN-144 , including success of harvest of lymphocytes, propagation of TIL, and administration of TIL following preparative therapy.

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

The final analysis will occur at the point at which all patients have either withdrawn from follow-up, died, or have been followed 24 months after the final patient is treated with LN-144 followed by IL-2.

5 PRIOR TREATMENTS, CONCOMITANT MEDICATIONS AND NON-DRUG THERAPIES

5.1 Prior Treatment

Use of all medications taken by the patient 30 days prior to enrollment will be recorded in the site's source documentation and the patient's electronic case report form (eCRF). Any changes in concomitant medications also will be recorded in the site's source documentation and the patient's eCRF until completion of the 12-week post treatment evaluation (Day 84) period.

5.2 Prohibited and Permitted Medications during Study Treatment

5.2.1 Prohibited Treatment

Patients will enter a washout period prior to enrollment. 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
- 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:

- Medical history including current medications
- Physical Exam including Height, Weight
- Vital signs - Pulse, Respirations, Blood Pressure and Temperature
- Evaluation and measurement of all skin and palpable lesions
- Slit Lamp Eye exam (noting in detail, the exact size and location of any lesions)
    - 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 (ie: patients with a prolonged history of smoking (20 packs/year))
- CT Exam
    - Chest (include neck 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 C02,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 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
- ECOG performance status evaluation
- Colonoscopy, with biopsy, (required in patients who previously have had documented Grade 2 or greater colitis or diarrhea due to prior receipt of ipilimumab, tremelimumab, anti-PD1 or anti PD-L1 antibodies)
- No symptoms and negative biopsies post treatment are required for patient to continue if patient had experienced immune-related colitis as above.

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 trial.

If enrolled, tumor resection will take place. The date of tumor resection is expected to occur approximately 44 days prior to the TIL infusion (Day 0) and is dependent on the rate of cell growth at the central TIL 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.

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 TIL 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 (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 TIL 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 Biomarker Collection and Processing

A total of 50 mL of blood will be collected from the patient for biomarker analysis utilizing vacutainer blood collection vials. Refer to the study Lab Manual for the complete procedure details.

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, Respirations, Blood Pressure and Temperature
- Evaluation and measurement of all skin and palpable lesions
- Verification of all ongoing medications
- 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 (ie: patients with a prolonged history of smoking (20 pack/yr)
  - 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 C02,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

6.4 Day -7

Prior to the start of lymphodepletion, verification of successful TIL production and expansion will be confirmed. Successful production and expansion will be evidenced by expansion to at least 50 fold over original cells.

- Physical Exam including Weight (calculate BSA using DuBois formula and BMI)
- Verification of all ongoing medications

- ECOG performance status evaluation
- Vital signs - Pulse, Respirations, Blood Pressure and Temperature
- Blood and Urine Tests (to be drawn prior to cyclophosphamide administration)
    - Hematology - CBC with Differential
    - Chemistry - Sodium, Potassium, Chloride, Total $C0_2$,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 – infuse at a rate of 15 mg/kg over 2hr. (If the patient is obese (BMI > 35) drug dosage will be calculated using practical weight as described in Appendix 3.)
    - Mesna - Begin mesna infusion at a rate of 3 mg/kg/hour intravenously diluted in a suitable diluent (see pharmaceutical section) over 23 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.

6.5 Day -6

The following procedures should be performed:

- Physical Exam including Weight (calculate BSA using DuBois formula and BMI)
- Verification of all ongoing medications
- Vital signs - Pulse, Respirations, Blood Pressure and Temperature
- Blood and Urine Tests (to be drawn prior to cyclophosphamide administration)
    - Hematology - CBC with Differential
    - Chemistry - Sodium, Potassium, Chloride, Total $C0_2$,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 – infuse at a rate of 15 mg/kg over 2hr. (If the patient is obese (BMI > 35) drug dosage will be calculated using practical weight as described in Appendix 3.)
    - Mesna - Begin mesna infusion at a rate of 3 mg/kg/hour intravenously diluted in a suitable diluent (see pharmaceutical section) over 23 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.

6.6 Day -5 to Day -1

The following procedures should be performed:

- Physical Exam including Weight (calculate BSA using DuBois formula and BMI)
- Verification of all ongoing medications
- Vital signs - Pulse, Respirations, Blood Pressure and Temperature
- Blood and Urine Tests (to be drawn prior to fludarabine administration)
    - Hematology - CBC with Differential
    - Chemistry - Sodium, Potassium, Chloride, Total C02,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 -4 and -1(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.

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 (calculate BSA using DuBois formula and BMI)
- Verification of all ongoing medications
- ECOG performance status evaluation
- Vital signs- Pulse, Respirations, Blood Pressure and Temperature
- Vital signs to be measured every 30 minutes during infusion and hourly for up to 4 hours post LN-144 infusion.
- Following the completion of 4 hour post infusion assessments, routine vital signs monitoring (every 4-6 hours for up to approximately 24 hours post TIL infusion) unless a higher frequency is clinically 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: Between 1.5 x 10^9 and 150 X 10^9 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 >75 x 10^9 LN-144 . Further details of the administration procedure will be provided in the Pharmacy Manual.

6.8 Days 1 – 4

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

- Physical Exam including weight (calculate BSA using DuBois formula and BMI)
- Verification of all ongoing medications
- Vital signs- Pulse, Respirations, 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 C02,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)
- Conduct Toxicity Assessment
- 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. 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.

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

The following procedures will be performed:

- Physical Exam including weight
- ECOG performance status evaluation ***Day 14 only***
- Verification of all ongoing medications
- Vital signs- Pulse, Respirations, Blood Pressure and Temperature

- Blood and Urine Tests
    - Hematology- CBC with Differential
    - Chemistry- Sodium, Potassium, Chloride, Total C02,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)
- Conduct Toxicity Assessment
- 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. 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.

6.10 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, Respirations, Blood Pressure and Temperature

- Conduct Toxicity Assessment
- Blood and Urine Tests
    - Hematology- CBC with Differential
    - Chemistry- Sodium, Potassium, Chloride, Total C02,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
- 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 (+/- 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, Respirations, Blood Pressure and Temperature
- Conduct Toxicity Assessment
- Slit lamp eye exam (noting in detail, the exact size and location of any lesions)
- Calculate Creatinine Clearance using Cockcroft-Gault formula
- Blood and Urine Tests
    - Hematology- CBC with Differential
    - Chemistry- Sodium, Potassium, Chloride, Total C02,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
- 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.

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

Patients who experience stable disease, a partial response, or complete response, or those with unresolved toxicities noted at day 84 visit will be evaluated. The following procedures will be performed during these visits:

- Physical Exam including Weight
- ECOG performance status evaluation
- Vital signs- Pulse, Respirations, Blood Pressure and Temperature
- Evaluation and measurement of all skin and palpable lesions
- Blood and Urine Tests
    - Hematology- CBC with Differential
    - Chemistry- Sodium, Potassium, Chloride, Total $C0_2$,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

- Obtain blood for Immune monitoring (50 mL of blood to be obtained.. Refer to lab manual) ***Month 6 only***
- CT Exam
    - Chest (include neck if there is prior or suspected neck disease)
    - Abdomen
    - Pelvis

6.13 Discontinued Patients

Patients who discontinue during the Day 84 visit due to progressive disease, or unwillingness or inability to return for follow up evaluations will be followed via phone or email contact. The following information will be requested from the patient:

- Summary of treatment received, including adverse events, since the previous contact
- Estimation of performance status
- Any additional imaging studies, physical exam or laboratory reports which the patient can provide
- Survival status

Patients who develop progressive disease during the follow up period, or are unwilling to return for follow up evaluations will be followed via phone or email contact. The following information will be requested from the patient:

- Summary of treatment received, since the previous contact
- Estimation of performance status
- Survival status

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. Additionally, Sections 6.5 and 6.6 provide some guidance on management of common adverse effects.. Treatment will be given as per investigator discretion. 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℃ once or two temperatures of 38.0℃ 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℃ once or two temperatures of 38.0℃ 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 Study Completion

Patients will be considered to have completed the study if they complete the tumor harvest, receive chemotherapy, LN-144 and IL-2 and complete the post treatment Day 14, 28, 42 & 84 Visits.

7.2 Criteria for Early Termination from Study (Removal from Treatment)

The Investigator will document on the appropriate eCRF page the reasons/circumstances for discontinuation. A subject may be removed from further study drug administration for the following medical or administrative reasons:

- Withdrawal of consent (subjects may voluntarily withdraw at any time during the course of the study. Subject's "withdrawing consent" should be carefully assessed to confirm the reason for withdrawal is not an AE)
- 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
- Disease Progression prior to receipt of study drug (TIL followed by IL-2)
- Death
- Study Terminated by sponsor
- Pregnancy
- Subject has become ineligible for study after enrollment and prior to TIL or IL2 administration

Efforts will be made to follow all subject who discontinue study drug for any reason (except those who withdraw consent). Such follow-up will include all relevant evaluations for safety including clinical assessments and collection of laboratory study results as set out in this protocol. Subjects can only be considered to have a final

disposition “lost to follow-up” after 3 documented attempts to contact the subject.

7.3 Criteria for Removal from Follow-Up

Patients will be removed from follow-up in the following situations:

- The patient voluntarily withdraws (the reason patient withdraws consent will be collected).
- There is significant noncompliance with protocol required activities.
- General or specific changes in the patient’s condition render continued participation the patient unacceptable for further participation in the judgment of the Investigator.
- Death.
- Lost to Follow-Up
- Study Terminated by Sponsor

7.4 Study Discontinuation (Study or Site Termination)

Conditions may arise during the study that could prompt the study to be stopped or a study site to be closed. Conditions that may prompt such considerations include, but are not limited to, the following:

- 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;
- Study conduct at a study site may warrant termination under conditions that include the following:
- Failure of the Investigator(s) to enroll eligible subjects into the study
- Failure of the Investigator(s) to comply with FDA or country-specific regulations
- Submission of false information from the research facility to the Sponsor, the Clinical Monitor, or a regulatory authority

- Insufficient adherence to protocol requirements
- A conflict of interest on the part of the Investigator, his/her institution, or site personnel that would negatively impact the integrity of the clinical trial
- Institution or IRB under investigation for cause by a federal agency

8 STUDY DRUG INFORMATION

Product Name: LN-144

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

Dosage Form: Live cell suspension

Quantitative Composition: Refer to Table 1

Table 1. Composition of LN-144

Ingredient	Unit and/or Percentage Formula		Function
	% v/v	Per mL	
Tumor Infiltrating Lymphocytes	1.5 x 10^9 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 1% HSA (human serum albumin) and 300 IU/mL of IL2. The suspension volume will be between 250 to 500 mL. Only one TIL cell dose is given intravenously after lymphodepletion chemotherapy followed by high dose IL-2 therapy 12-24 hours after infusion. Each dose contains a range between 1.5 to 150 x 10^9 total viable lymphocytes. 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 >75 x 10^9 LN-144.

Manufacturing Process: The overall process of tumor shipping, TIL manufacturing, and TIL product shipping, and infusion was shown above in Figure 1. The TIL 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 >75 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 TIL 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. Receipt is defined as the moment the TIL 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 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 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.

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[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.

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

(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
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 PD, changes in tumor measurements must be confirmed by repeat studies 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-eight 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 recurrent or progressive disease is objectively documented (taking as reference for progressive disease the smallest measurements recorded since the treatment started).

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

9.1.1.4.3 Duration of Stable Disease

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

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 mentioned otherwise. For cases that inferential statistics will be calculated (e.g., p-values), they will be used in a descriptive manner. All data will be listed.

An overview of the main analysis strategy is provided in the following sections. A more detailed description of the analyses and reporting plan of the data will be provided in the Statistical Analysis Plan (SAP).

10.2 Analysis Populations

Three analysis populations will be defined to summarize the data. The Intent-to-Treat population (ITT) will consist of all resected patients. The Safety Population is based on all resected patients who have been successfully infused with LN-144 followed by IL-2 (at least one dose). A subset of the Per Protocol dataset will be used to summarize the duration of overall response and is limited to those patients achieving a complete or partial overall response. The primary Safety dataset used for analysis is based on the number of patients infused with LN-144. 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 dataset.

10.3 Endpoints

10.3.1 Primary

Two endpoints are of primary interest. The first 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. The second primary endpoint is a feasibility measure defined as the number of patients from which LN-144 is successfully produced (manufacture of > 1.5×10^9 viable cells) divided by the number of resected patients x 100%. Patients not meeting numerator criteria for this definition of feasibility will be classified as failures. This is an ITT based analysis.

10.3.2 Secondary

Several secondary and exploratory endpoints are of interest. The first secondary endpoint is an additional feasibility measure and is defined as the number of patients successfully infused with LN-144 followed by IL-2 divided by the number of resected patients x 100%. Patients not meeting numerator criteria for feasibility will be classified as failures. This is an ITT based analysis.

The second endpoint is based on the assessment of anti-tumor activity by measurement of the best overall response and subsequent estimation of the best overall response (BOR) rate using RECIST 1.1 criteria. The BOR rate is derived as the number of patients with a complete response (CR) or partial response (PR) divided by the number of patients successfully infused with LN-144 followed by IL-2 (at least one dose) x 100%. Patients failing to achieve a CR or PR among the denominator patient population (i.e., the PP dataset) will be classified as non-responders.

10.3.3 Exploratory

The remaining endpoints are exploratory. The first set of such endpoints is used to evaluate additional measures of efficacy up to 24 months, including: progression-free survival (PFS), overall survival (OS), duration of response (overall, CR, and stable disease), and time to response. The definition of each of these endpoints follows.

PFS is defined as the time (in days) from the start of IL-2 therapy to PD, relapse, or death due to any cause, whichever event is earlier. Patients not experiencing PD or having expired at the end of the follow-up period will have their event times censored on the last date that a valid assessment of tumor status is made. One day will be added to each patient's derived event time.

OS is defined as the time (in days) from the start of IL-2 therapy to death due to any cause. Patients not having expired at the end of the follow-up period will have their event times censored on the last date of their known survival status. One day will be added to each patient's derived event time.

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 recurrent or progressive disease (PD) occurs. The time is measured in days. Patients not experiencing recurrent or PD prior to the end of the follow-up period will have their event times censored on the last date that a valid assessment of tumor status is made. One day will be added to each patient's derived event time. The duration of overall complete response is measured from the time measurement criteria are first met for CR until the first date that recurrent disease or PD is objectively documented. The duration of stable disease is measured from the start of the treatment until the

criteria for progression are first met, taking as reference the smallest measurements recorded since the treatment started.

Time to response will be defined as the time (in days) from the start of IL-2 therapy until achievement of a CR or PR. The analysis will be limited to just those patients with a CR or PR; no observations will be censored in the analysis. One day will be added to each patient's derived event time.

The second set of exploratory endpoints includes measures of 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 is driven by the need to obtain 20 patients 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-2This response parameter is assumed to be a binomial proportion, with the percentage (proportion x 100%) derived as the number of eligible patients divided by the number of resected patients x 100%. This percentage is expected to equal 60%. Based on this percentage value, roughly 33 patients will need to be resected in order to yield a sample size of 20 patients who complete treatment. Two-sided 95% confidence limits estimated on a binomial proportion of 60% and 33 resected patients are associated with a maximum half width value of 16.7%: the lower confidence limit bound not to be less than 43.3%. Estimating the sample size in this manner yields a small oversampling for the primary feasibility objective since the number of patients who complete treatment is a subset of the number of patients that LN-144 is successfully produced (manufacture of more than 1.5 billion viable cells).. It is expected that at least 80% of the resected patients will achieve the primary study endpoint. As such, a trial accruing 33 resected patients to net 20 eligible patients will be associated with two-sided 95% confidence limits with bounds of ± 13.6% for an 80% rate: the lower bound value no less than 66.3%. It is assumed that percentage values < 50% for the primary feasibility endpoint are not desirable, and hence, should the observed rate equal or exceed 80%, a sampling of 33 resected patients will have adequate sensitivity for the confidence bounds to exclude this undesired target. A smaller number of resected patients will likewise net

sufficient confidence limit bounds to exclude values ≤50%.These values appear reasonable for a study whereby an objective is to meet a pre-stated threshold in the number of manufactured TIL before receiving 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.

The 20 patient sample size also provides a denominator number of patients for the anti-tumor efficacy measurements. Since these assessments are of a secondary or exploratory nature, such a sample size seems reasonable.

The upper sampling limit of patients in this study is 20 patients who completed treatment as previously defined. While it is estimated that about 33 resected patients are needed to achieve this target, an additional limit of 40 resected patients is placed on patient sampling.

10.4.1 Baseline Demographic and Clinical Characteristics

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

10.4.2 Efficacy Analysis, Primary Endpoint

The primary efficacy 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 first (feasibility as a function of successful infusion with LN-144) and third secondary efficacy (BOR rate) variables are binomial proportions and will be summarized using both a point estimate and its two-sided, exact 95% confidence limits. A separate analysis of the BOR rate will be performed for just those patients achieving a CR as their best overall response.

PFS, OS, durations of overall and complete response, and time to response are time-to-event (i.e., survival analysis) variables subjected to right censoring (except for time to response). 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 IL-2: 6 months, 9 months, 18, months, and 24 months duration. The landmark analyses will be applied to just the PFS and OS data. For duration of response, a supportive analysis will include death as an event for patients not having recurrent disease or PD at the time of death. Separate analyses of duration of response will be performed for the durations of CR and stable disease, if warranted.

Analysis methods employing graphical techniques and simple statistics (e.g., Spearman's rho) will be used to explore the potential immune correlates of response, outcome, and toxicity of treatment. More complex methods based may be used, if warranted by the data. These methods will be detailed in the SAP.

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. 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 be performed, their details will be described in the SAP and Clinical Study Report, wherever applicable.

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 TIL 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. Safety will be assessed in all patients after enrollment in the study through the day 84 assessment.

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 from enrollment through the day 84 assessment 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 enrollment in the study and either observed by the Investigator or reported by the patient (whether or not attributed to the use of IL-2 or TIL treatment), will be reported on the eCRF. Monitoring and reporting AEs will be conducted through the last on-study visit (i.e., the 12-Week Post Treatment Evaluation 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, TIL, 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 during the trial or within 12 weeks of completing or stopping study treatment, 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 either of these occurs, the patient must undergo an end-of-treatment visit and be given appropriate care under medical supervision until symptoms cease or the condition becomes stable and returns for a safety Follow-up visit. 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 beginning after informed consent until six weeks after completion or discontinuation of treatment. 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 course of the investigation 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

Neither the Investigator nor Sponsor will modify this protocol without obtaining the concurrence of the other. 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 eCRF and 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 Patient Identification Register

The Investigator agrees to complete a patient identification register, which will be used for the purpose of long-term follow-up, if needed. This form will be treated as confidential, and will be filed by the Investigator in the Trial Center File. Otherwise, all reports and communications relating to the study will identify patients by initials and assigned number only.

13.4 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.

13.5 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 and direct transmission of clinical laboratory data from a central laboratory into the database. 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 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.

13.6 Data Handling and Recordkeeping

13.6.1 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.

13.6.2 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 trial.

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 trial.

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 to determine their acceptability. If necessary, Data Correction Requests will be generated for resolution by the study site.

13.7 Study Completion/Termination

13.7.1 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.

13.7.2 Study Termination

An initiative for center closure or trial termination can be taken at any time either by the Sponsor or by the Investigator, provided there is reasonable cause and sufficient notice is given in advance of the intended termination. Reasons for such action taken by the Sponsor include, but are not limited to:

- Successful completion of the trial at the center
- The required number of patients for the trial has been recruited
- Failure of the Investigator to comply with the protocol, the Sponsor's procedures or GCP guidelines
- Safety concerns
- Sufficient data suggesting lack of efficacy
- Inadequate recruitment of patients by the Investigator

13.8 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, 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 Final Report

The Investigator must complete a report notifying the IRB/IEC of the conclusion of the clinical study. This report should be made within 3 months of completion or termination of the study.

14.8 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.

14.9 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.

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APPENDIX 1: SCHEDULE OF EVENTS

Assessment	Screening & Pre-Study 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) / EOS	Month 6 (+/- 1 wk)	Month 9 (+/- 1 wk)	Month 12 (+/- 1)wk	Month 18 (+/- 3wks)	Month 24 (+/- 3wks)
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					
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		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					
ß-HCG Pregnancy Test[12]	X		X																					

Assessment	Screening & Pre-Study 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) / EOS	Month 6 (+/- 1 wk)	Month 9 (+/- 1 wk)	Month 12 (+/- 1)wk	Month 18 (+/- 3wks)	Month 24 (+/- 3wks)
ECOG performance status	X	X	X	X							X					X			X	X	X	X	X	X
HIV Titer	X																							
Hb_5AG	X																							
Anti-HCV	X																							
HLA Typing[12]	X																							
Anti CMV antibody titer	X																							
HSV serology	X																							
EBV panel	X																							
PFT[14]	X		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					
TMP/SMX DS, or appropriate Abx[19]				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					
Valacyclovir/Acyclovir[21]												X	X	X	X	X	X	X	X					
Toxicity Assessment		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X					
Immune Monitoring[22]		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 TIL 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 heart rate, respiratory rate, blood pressure, and temperature. On Day 0 (TIL 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 TIL 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 and Pre-Study, then imaging as clinically indicated. If screening image shows abnormalities, obtain at day -14. Include neck 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/EOS and during Follow-up.
9. Complete blood count with differential
10. With culture, if indicated
11. Calculate creatinine clearance using Cockcroft-Gault calculation
12. HLA typing to be sent to central laboratory.
13. Serum pregnancy test
14. Pulmonary evaluation for patients with a prolonged history of cigarette smoking (ie: 20 packs/year of smoking in the last two years)
15. Only required for documented Grades 2 or greater diarrhea or colitis as a result of previously received ipilimumab, tremelimumab, or anti-PD1 or anti-PD-L1 antibodies. As per standard clinical age-appropriate screening
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)

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
Elevated	3 or 4	Observation	For Grade 4 without liver	If changes have not improved to

Expected toxicity	**Expected grade**	**Supportive Measures suggested**	**Stop Cycle***	**Stop Treatment ****
transaminases			metastases	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

HIGHLIGHTS OF PRESCRIBING INFORMATION

These highlights do not include all the information needed to use CYCLOPHOSPHAMIDE safely and effectively. See full prescribing information for CYCLOPHOSPHAMIDE.

CYCLOPHOSPHAMIDE injection, for intravenous use
CYCLOPHOSPHAMIDE tablets, for oral use
Initial U.S. Approval: 1959

----------INDICATIONS AND USAGE----------

Cyclophosphamide is an alkylating drug indicated for treatment of:

- **Malignant Diseases**: malignant lymphomas: Hodgkin's disease, lymphocytic lymphoma, mixed-cell type lymphoma, histiocytic lymphoma, Burkitt's lymphoma; multiple myeloma, leukemias, mycosis fungoides, neuroblastoma, adenocarcinoma of ovary, retinoblastoma, breast carcinoma (1.1)
- **Minimal Change Nephrotic Syndrome in Pediatric Patients**: biopsy proven minimal change nephrotic syndrome patients who failed to adequately respond to or are unable to tolerate adrenocorticosteroid therapy (1.2)

Limitations of Use:

The safety and effectiveness for the treatment of nephrotic syndrome in adults or other renal disease has not been established.

----------DOSAGE AND ADMINISTRATION----------

Malignant Diseases: Adult and Pediatric Patients (2.1)

- Intravenous: Initial course for patients with no hematologic deficiency: 40 mg per kg to 50 mg per kg in divided doses over 2 to 5 days. Other regimens include 10 mg per kg to 15 mg per kg given every 7 to 10 days or 3 mg per kg to 5 mg per kg twice weekly.
- Oral: Usually 1 mg per kg per day to 5 mg per kg per day for both initial and maintenance dosing.

Minimal Change Nephrotic Syndrome in Pediatric Patients (2.2)

- Recommended oral dose: 2 mg per kg daily for 8 to 12 weeks (maximum cumulative dose 168 mg per kg). Treatment beyond 90 days increases the probability of sterility in males.

----------DOSAGE FORMS AND STRENGTHS----------

- Injection, lyophilized powder: 500 mg, 1 g, and 2 g (3)
- Tablet: 25 mg and 50 mg (3)

----------CONTRAINDICATIONS----------

- Hypersensitivity to cyclophosphamide (4)
- Urinary outflow obstruction (4)

----------WARNINGS AND PRECAUTIONS----------

- Myelosuppression, Immunosuppression, Bone Marrow Failure and Infections – Severe immunosuppression may lead to serious and sometimes fatal infections. Close hematological monitoring is required. (5.1)
- Urinary Tract and Renal Toxicity – Hemorrhagic cystitis, pyelitis, ureteritis, and hematuria can occur. Exclude or correct any urinary tract obstructions prior to treatment. (5.2)
- Cardiotoxicity – Myocarditis, myopericarditis, pericardial effusion, arrythmias and congestive heart failure, which may be fatal, have been reported. Monitor patients, especially those with risk factors for cardiotoxicity or pre-existing cardiac disease. (5.3)
- Pulmonary Toxicity – Pneumonitis, pulmonary fibrosis and pulmonary veno-occlusive disease leading to respiratory failure may occur. Monitor patients for signs and symptoms of pulmonary toxicity. (5.4)
- Secondary malignancies (5.5)
- Veno-occlusive Liver Disease - Fatal outcome can occur. (5.6)
- Embryo-Fetal Toxicity - Can cause fetal harm. Advise female patients of reproductive potential to avoid pregnancy. (5.7, 8.1, 8.6)

----------ADVERSE REACTIONS----------

Adverse reactions reported most often include neutropenia, febrile neutropenia, fever, alopecia, nausea, vomiting, and diarrhea. (6.1)

To report SUSPECTED ADVERSE REACTIONS, contact Baxter Healthcare at 1-866-888-2472 or FDA at 1-800-FDA-1088 or www.fda.gov/medwatch.

----------USE IN SPECIFIC POPULATIONS----------

- Nursing Mothers: Discontinue drug or nursing. (8.3)
- Females and males of reproductive potential: Counsel patients on pregnancy prevention and planning. (8.6)
- Renal Patients: Monitor for toxicity in patients with moderate and severe renal impairment. (8.7, 12.3)

See 17 for PATIENT COUNSELING INFORMATION

Revised: 05/2013

FULL PRESCRIBING INFORMATION: CONTENTS*

*** Sections or subsections omitted from the full prescribing information are not listed**

Reference ID: 3304966

FULL PRESCRIBING INFORMATION

1. INDICATIONS AND USAGE

1.1 Malignant Diseases

Cyclophosphamide is indicated for the treatment of:

- malignant lymphomas (Stages III and IV of the Ann Arbor staging system), Hodgkin's disease, lymphocytic lymphoma (nodular or diffuse), mixed-cell type lymphoma, histiocytic lymphoma, Burkitt's lymphoma
- multiple myeloma
- leukemias: chronic lymphocytic leukemia, chronic granulocytic leukemia (it is usually ineffective in acute blastic crisis), acute myelogenous and monocytic leukemia, acute lymphoblastic (stem-cell) leukemia (cyclophosphamide given during remission is effective in prolonging its duration)
- mycosis fungoides (advanced disease)
- neuroblastoma (disseminated disease)
- adenocarcinoma of the ovary
- retinoblastoma
- carcinoma of the breast

Cyclophosphamide, although effective alone in susceptible malignancies, is more frequently used concurrently or sequentially with other antineoplastic drugs.

1.2 Minimal Change Nephrotic Syndrome in Pediatric Patients:

Cyclophosphamide is indicated for the treatment of biopsy proven minimal change nephrotic syndrome in pediatrics patients who failed to adequately respond to or are unable to tolerate adrenocorticosteroid therapy.

Limitations of Use:
The safety and effectiveness for the treatment of nephrotic syndrome in adults or other renal disease has not been established.

2. DOSAGE AND ADMINISTRATION

During or immediately after the administration, adequate amounts of fluid should be ingested or infused to force diuresis in order to reduce the risk of urinary tract toxicity. Therefore, cyclophosphamide should be administered in the morning.

2.1 Dosing for Malignant Diseases

Adults and Pediatric Patients
Intravenous
When used as the only oncolytic drug therapy, the initial course of cyclophosphamide for patients with no hematologic deficiency usually consists of 40 mg per kg to 50 mg per kg given intravenously in divided doses over a period of 2 to 5 days. Other intravenous regimens include 10 mg per kg to 15 mg per kg given every 7 to 10 days or 3 mg per kg to 5 mg per kg twice weekly.

Oral
Oral cyclophosphamide dosing is usually in the range of 1 mg per kg per day to 5 mg per kg per day for both initial and maintenance dosing.

Reference ID: 3304966

Many other regimens of intravenous and oral cyclophosphamide have been reported. Dosages must be adjusted in accord with evidence of antitumor activity and/or leukopenia. The total leukocyte count is a good, objective guide for regulating dosage.

When cyclophosphamide is included in combined cytotoxic regimens, it may be necessary to reduce the dose of cyclophosphamide as well as that of the other drugs.

2.2 Dosing for Minimal Change Nephrotic Syndrome in Pediatric Patients

An oral dose of 2 mg per kg daily for 8 to 12 weeks (maximum cumulative dose 168 mg per kg) is recommended. Treatment beyond 90 days increases the probability of sterility in males *[see Use in Specific Populations (8.4)]*.

2.3 Preparation, Handling and Administration

Handle and dispose of cyclophosphamide in a manner consistent with other cytotoxic drugs.[1] Caution should be exercised when handling and preparing Cyclophosphamide for Injection, USP (lyophilized powder), or bottles containing cyclophosphamide tablets. To minimize the risk of dermal exposure, always wear gloves when handling vials containing Cyclophosphamide for Injection, USP (lyophilized powder), or bottles containing cyclophosphamide tablets. The coating of the cyclophosphamide tablets prevents direct contact of persons handling the tablets with the active substance. However, to prevent inadvertent exposure to the active substance, the cyclophosphamide tablets should not be cut, chewed, or crushed. Personnel should avoid exposure to broken tablets. If contact with broken tablets occurs, wash hands immediately and thoroughly.

Cyclophosphamide for Injection, USP

Intravenous Administration

Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration, whenever solution and container permit. Do not use cyclophosphamide vials if there are signs of melting. Melted cyclophosphamide is a clear or yellowish viscous liquid usually found as a connected phase or in droplets in the affected vials.

Cyclophosphamide does not contain any antimicrobial preservative and thus care must be taken to assure the sterility of prepared solutions. Use aseptic technique.

For Direct Intravenous Injection

Reconstitute Cyclophosphamide with 0.9% Sodium Chloride Injection, USP only, using the volumes listed below in Table 1. Gently swirl the vial to dissolve the drug completely. Do not use Sterile Water for Injection, USP because it results in a hypotonic solution and should not be injected directly.

Table 1: Reconstitution for Direct Intravenous Injection

Strength	Volume of 0.9% Sodium Chloride	Cyclophosphamide Concentration
500 mg	25 mL	20 mg per mL
1 g	50 mL	
2 g	100 mL	

For Intravenous Infusion

Reconstitution of Cyclophosphamide:
Reconstitute Cyclophosphamide using 0.9% Sodium Chloride Injection, USP or Sterile Water for Injection, USP with the volume of diluent listed below in Table 2. Add the diluent to the vial and gently swirl to dissolve the drug completely.

Table 2: Reconstitution in preparation for Intravenous Infusion

Strength	Volume of Diluent	Cyclophosphamide Concentration
500 mg	25 mL	20 mg per mL
1 g	50 mL	
2 g	100 mL	

Dilution of Reconstituted Cyclophosphamide:
Further dilute the reconstituted Cyclophosphamide solution to a minimum concentration of 2 mg per mL with any of the following diluents:

- 5% Dextrose Injection, USP
- 5% Dextrose and 0.9% Sodium Chloride Injection, USP
- 0.45% Sodium Chloride Injection, USP

To reduce the likelihood of adverse reactions that appear to be administration rate-dependent (e.g., facial swelling, headache, nasal congestion, scalp burning), cyclophosphamide should be injected or infused very slowly. Duration of the infusion also should be appropriate for the volume and type of carrier fluid to be infused.

Storage of Reconstituted and Diluted Cyclophosphamide Solution:

If not used immediately, for microbiological integrity, cyclophosphamide solutions should be stored as described in Table 3:

Table 3: Storage of Cyclophosphamide Solutions

Diluent	Storage	
	Room Temperature	Refrigerated
Reconstituted Solution (Without Further Dilution)		
0.9% Sodium Chloride Injection, USP	up to 24 hrs	Up to 6 days
Sterile Water for Injection, USP	Do not store; use immediately	
Diluted Solutions[1]		
0.45% Sodium Chloride Injection, USP	up to 24 hrs	up to 6 days
5% Dextrose Injection, USP	up to 24 hrs	up to 36 hrs
5% Dextrose and 0.9% Sodium Chloride Injection, USP	up to 24 hrs	up to 36 hrs

[1] Storage time is the total time cyclophosphamide is in solution including the time it is reconstituted in 0.9% Sterile Sodium Chloride Injection, USP or Sterile Water for Injection, USP.

Use of Reconstituted Solution for Oral Administration

Liquid preparations of cyclophosphamide for oral administration may be prepared by dissolving cyclophosphamide for injection in Aromatic Elixir, National Formulary (NF) Such preparations should be stored under refrigeration in glass containers and used within 14 days.

3. DOSAGE FORMS AND STRENGTHS

Cyclophosphamide for Injection, USP (lyophilized powder) is a sterile white cake available in

- 500 mg
- 1 g
- 2 g

Cyclophosphamide Tablets, USP are white tablets with blue flecks available in

- 25 mg
- 50 mg

4. CONTRAINDICATIONS

- Hypersensitivity

  Cyclophosphamide is contraindicated in patients who have a history of severe hypersensitivity reactions to it, any of its metabolites, or to other components of the product. Anaphylactic reactions including death have been reported with cyclophosphamide. Possible cross-sensitivity with other alkylating agents can occur.

- Urinary Outflow Obstruction

  Cyclophosphamide is contraindicated in patients with urinary outflow obstruction *[see Warnings and Precautions (5.2)]*.

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5. WARNINGS AND PRECAUTIONS

5.1 Myelosuppression, Immunosuppression, Bone Marrow Failure and Infections

Cyclophosphamide can cause myelosuppression (leukopenia, neutropenia, thrombocytopenia and anemia), bone marrow failure, and severe immunosuppression which may lead to serious and sometimes fatal infections, including sepsis and septic shock. Latent infections can be reactivated *[see Adverse Reactions (6.2)]*.

Antimicrobial prophylaxis may be indicated in certain cases of neutropenia at the discretion of the managing physician. In case of neutropenic fever, antibiotic therapy is indicated. Antimycotics and/or antivirals may also be indicated.

Monitoring of complete blood counts is essential during cyclophosphamide treatment so that the dose can be adjusted, if needed. Cyclophosphamide should not be administered to patients with neutrophils $\leq 1{,}500/mm^3$ and platelets $< 50{,}000/mm^3$. Cyclophosphamide treatment may not be indicated, or should be interrupted, or the dose reduced, in patients who have or who develop a serious infection. G-CSF may be administered to reduce the risks of neutropenia complications associated with cyclophosphamide use. Primary and secondary prophylaxis with G-CSF should be considered in all patients considered to be at increased risk for neutropenia complications. The nadirs of the reduction in leukocyte count and thrombocyte count are usually reached in weeks 1 and 2 of treatment. Peripheral blood cell counts are expected to normalize after approximately 20 days. Bone marrow failure has been reported. Severe myelosuppression may be expected particularly in patients pretreated with and/or receiving concomitant chemotherapy and/or radiation therapy.

5.2 Urinary Tract and Renal Toxicity

Hemorrhagic cystitis, pyelitis, ureteritis, and hematuria have been reported with cyclophosphamide. Medical and/or surgical supportive treatment may be required to treat protracted cases of severe hemorrhagic cystitis. Discontinue cyclophosphamide therapy in case of severe hemorrhagic cystitis. Urotoxicity (bladder ulceration, necrosis, fibrosis, contracture and secondary cancer) may require interruption of cyclophosphamide treatment or cystectomy. Urotoxicity can be fatal. Urotoxicity can occur with short-term or long-term use of cyclophosphamide.

Before starting treatment, exclude or correct any urinary tract obstructions *[see Contraindications (4)]*. Urinary sediment should be checked regularly for the presence of erythrocytes and other signs of urotoxicity and/or nephrotoxicity. Cyclophosphamide should be used with caution, if at all, in patients with active urinary tract infections. Aggressive hydration with forced diuresis and frequent bladder emptying can reduce the frequency and severity of bladder toxicity. Mesna has been used to prevent severe bladder toxicity.

5.3 Cardiotoxicity

Myocarditis, myopericarditis, pericardial effusion including cardiac tamponade, and congestive heart failure, which may be fatal, have been reported with cyclophosphamide therapy

Supraventricular arrhythmias (including atrial fibrillation and flutter) and ventricular arrhythmias (including severe QT prolongation associated with ventricular tachyarrhythmia) have been reported after treatment with regimens that included cyclophosphamide.

The risk of cardiotoxicity may be increased with high doses of cyclophosphamide, in patients with advanced age, and in patients with previous radiation treatment to the cardiac region and/or previous or concomitant treatment with other cardiotoxic agents.

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Particular caution is necessary in patients with risk factors for cardiotoxicity and in patients with pre existing cardiac disease.

Monitor patients with risk factors for cardiotoxicity and with pre-existing cardiac disease.

5.4 Pulmonary Toxicity

Pneumonitis, pulmonary fibrosis, pulmonary veno-occlusive disease and other forms of pulmonary toxicity leading to respiratory failure have been reported during and following treatment with cyclophosphamide. Late onset pneumonitis (greater than 6 months after start of cyclophosphamide) appears to be associated with increased mortality. Pneumonitis may develop years after treatment with cyclophosphamide.

Monitor patients for signs and symptoms of pulmonary toxicity.

5.5 Secondary Malignancies

Cyclophosphamide is genotoxic *[see Nonclinical Toxicology (13.1)]*. Secondary malignancies (urinary tract cancer, myelodysplasia, acute leukemias, lymphomas, thyroid cancer, and sarcomas) have been reported in patients treated with cyclophosphamide-containing regimens. The risk of bladder cancer may be reduced by prevention of hemorrhagic cystitis.

5.6 Veno-occlusive Liver Disease

Veno-occlusive liver disease (VOD) including fatal outcome has been reported in patients receiving cyclophosphamide-containing regimens. A cytoreductive regimen in preparation for bone marrow transplantation that consists of cyclophosphamide in combination with whole-body irradiation, busulfan, or other agents has been identified as a major risk factor. VOD has also been reported to develop gradually in patients receiving long-term low-dose immunosuppressive doses of cyclophosphamide. Other risk factors predisposing to the development of VOD include preexisting disturbances of hepatic function, previous radiation therapy of the abdomen, and a low performance status.

5.7 Embryo-Fetal Toxicity

Cyclophosphamide can cause fetal harm when administered to a pregnant woman *[see Use in Specific Populations (8.1)]*. Exposure to cyclophosphamide during pregnancy may cause birth defects, miscarriage, fetal growth retardation, and fetotoxic effects in the newborn. Cyclophosphamide is teratogenic and embryo-fetal toxic in mice, rats, rabbits and monkeys.

Advise female patients of reproductive potential to avoid becoming pregnant and to use highly effective contraception during treatment and for up to 1 year after completion of therapy *[see Use in Specific Populations (8.6)]*.

5.8 Infertility

Male and female reproductive function and fertility may be impaired in patients being treated with cyclophosphamide. Cyclophosphamide interferes with oogenesis and spermatogenesis. It may cause sterility in both sexes. Development of sterility appears to depend on the dose of cyclophosphamide, duration of therapy, and the state of gonadal function at the time of treatment. Cyclophosphamide-induced sterility may be irreversible in some patients. Advise patients on the potential risks for infertility *[see Use in Specific Populations (8.4 and 8.6)]*.

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5.9 Impairment of Wound Healing

Cyclophosphamide may interfere with normal wound healing.

5.10 Hyponatremia

Hyponatremia associated with increased total body water, acute water intoxication, and a syndrome resembling SIADH (syndrome of inappropriate secretion of antidiuretic hormone), which may be fatal, has been reported.

6. ADVERSE REACTIONS

The following adverse reactions are discussed in more detail in other sections of the labeling.

- Hypersensitivity *[see Contraindications (4)]*
- Myelosuppression, Immunosuppression, Bone Marrow Failure, and Infections *[see Warnings and Precautions (5.1)]*
- Urinary Tract and Renal Toxicity *[see Warnings and Precautions (5.2)]*
- Cardiotoxicity *[see Warnings and Precautions (5.3)]*
- Pulmonary Toxicity *[see Warnings and Precautions (5.4)]*
- Secondary Malignancies *[see Warnings and Precautions (5.5)]*
- Veno-occlusive Liver Disease *[see Warnings and Precautions (5.6)]*
- Embryo-Fetal Toxicity *[see Warnings and Precautions (5.7)]*
- Reproductive System Toxicity *[see Warnings and Precautions (5.8) and Use in Specific Populations (8.4 and 8.6)]*
- Impaired Wound Healing *[see Warnings and Precautions (5.9)]*
- Hyponatremia *[see Warnings and Precautions (5.10)]*

6.1 Common Adverse Reactions

Hematopoietic system:
Neutropenia occurs in patients treated with cyclophosphamide. The degree of neutropenia is particularly important because it correlates with a reduction in resistance to infections. Fever without documented infection has been reported in neutropenic patients.

Gastrointestinal system:
Nausea and vomiting occur with cyclophosphamide therapy. Anorexia and, less frequently, abdominal discomfort or pain and diarrhea may occur. There are isolated reports of hemorrhagic colitis, oral mucosal ulceration and jaundice occurring during therapy.

Skin and its structures:
Alopecia occurs in patients treated with cyclophosphamide. Skin rash occurs occasionally in patients receiving the drug. Pigmentation of the skin and changes in nails can occur.

6.2 Postmarketing Experience

The following adverse reactions have been identified from clinical trials or post-marketing surveillance. Because they are reported from a population from unknown size, precise estimates of frequency cannot be made.

Cardiac: cardiac arrest, ventricular fibrillation, ventricular tachycardia, cardiogenic shock, pericardial effusion (progressing to cardiac tamponade), myocardial hemorrhage, myocardial infarction, cardiac failure (including fatal outcomes), cardiomyopathy, myocarditis, pericarditis, carditis, atrial fibrillation,

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supraventricular arrhythmia, ventricular arrhythmia, bradycardia, tachycardia, palpitations, QT prolongation.

Congenital, Familial and Genetic: intra-uterine death, fetal malformation, fetal growth retardation, fetal toxicity (including myelosuppression, gastroenteritis).

Ear and Labyrinth: deafness, hearing impaired, tinnitus.

Endocrine: water intoxication.

Eye: visual impairment, conjunctivitis, lacrimation.

Gastrointestinal: gastrointestinal hemorrhage, acute pancreatitis, colitis, enteritis, cecitis, stomatitis, constipation, parotid gland inflammation.

General Disorders and Administrative Site Conditions: multiorgan failure, general physical deterioration, influenza-like illness, injection/infusion site reactions (thrombosis, necrosis, phlebitis, inflammation, pain, swelling, erythema), pyrexia, edema, chest pain, mucosal inflammation, asthenia, pain, chills, fatigue, malaise, headache.

Hematologic: myelosuppression, bone marrow failure, disseminated intravascular coagulation and hemolytic uremic syndrome (with thrombotic microangiopathy).

Hepatic: veno-occlusive liver disease, cholestatic hepatitis, cytolytic hepatitis, hepatitis, cholestasis; hepatotoxicity with hepatic failure, hepatic encephalopathy, ascites, hepatomegaly, blood bilirubin increased, hepatic function abnormal, hepatic enzymes increased.

Immune: immunosuppression, anaphylactic shock and hypersensitivity reaction.

Infections: The following manifestations have been associated with myelosuppression and immunosuppression caused by cyclophosphamide: increased risk for and severity of pneumonias (including fatal outcomes), other bacterial, fungal, viral, protozoal and, parasitic infections; reactivation of latent infections, (including viral hepatitis, tuberculosis), *Pneumocystis jiroveci*, herpes zoster, *Strongyloides*, sepsis and septic shock.

Investigations: blood lactate dehydrogenase increased, C-reactive protein increased.

Metabolism and Nutrition: hyponatremia, fluid retention, blood glucose increased, blood glucose decreased.

Musculoskeletal and Connective Tissue: rhabdomyolysis, scleroderma, muscle spasms, myalgia, arthralgia.

Neoplasms: acute leukemia, myelodysplastic syndrome, lymphoma, sarcomas, renal cell carcinoma, renal pelvis cancer, bladder cancer, ureteric cancer, thyroid cancer.

Nervous System: encephalopathy, convulsion, dizziness, neurotoxicity has been reported and manifested as reversible posterior leukoencephalopathy syndrome, myelopathy, peripheral neuropathy, polyneuropathy, neuralgia, dysesthesia, hypoesthesia, paresthesia, tremor, dysgeusia, hypogeusia, parosmia.

Pregnancy: premature labor.

Psychiatric: confusional state.

Renal and Urinary: renal failure, renal tubular disorder, renal impairment, nephropathy toxic, hemorrhagic cystitis, bladder necrosis, cystitis ulcerative, bladder contracture, hematuria, nephrogenic diabetes insipidus, atypical urinary bladder epithelial cells.

Reproductive System: infertility, ovarian failure, ovarian disorder, amenorrhea, oligomenorrhea, testicular atrophy, azoospermia, oligospermia.

Respiratory: pulmonary veno-occlusive disease, acute respiratory distress syndrome, interstitial lung disease as manifested by respiratory failure (including fatal outcomes), obliterative bronchiolitis, organizing pneumonia, alveolitis allergic, pneumonitis, pulmonary hemorrhage; respiratory distress, pulmonary hypertension, pulmonary edema, pleural effusion, bronchospasm, dyspnea, hypoxia, cough, nasal congestion, nasal discomfort, oropharyngeal pain, rhinorrhea.

Skin and Subcutaneous Tissue: toxic epidermal necrolysis, Stevens-Johnson syndrome, erythema multiforme, palmar-plantar erythrodysesthesia syndrome, radiation recall dermatitis, toxic skin eruption, urticaria, dermatitis, blister, pruritus, erythema, nail disorder, facial swelling, hyperhidrosis.

Tumor lysis syndrome: like other cytotoxic drugs, cyclophosphamide may induce tumor-lysis syndrome and hyperuricemia in patients with rapidly growing tumors.

Vascular: pulmonary embolism, venous thrombosis, vasculitis, peripheral ischemia, hypertension, hypotension, flushing, hot flush.

7. DRUG INTERACTIONS

Cyclophosphamide is a pro-drug that is activated by cytochrome P450s *[see Clinical Pharmacology (12.3)]*.

An increase of the concentration of cytotoxic metabolites may occur with:

- Protease inhibitors: Concomitant use of protease inhibitors may increase the concentration of cytotoxic metabolites. Use of protease inhibitor-based regimens was found to be associated with a higher Incidence of infections and neutropenia in patients receiving cyclophosphamide, doxorubicin, and etoposide (CDE) than use of a Non-Nucleoside Reverse Transcriptase Inhibitor-based regimen.

Combined or sequential use of cyclophosphamide and other agents with similar toxicities can potentiate toxicities.

- Increased hematotoxicity and/or immunosuppression may result from a combined effect of cyclophosphamide and, for example:
  - ACE inhibitors: ACE inhibitors can cause leukopenia.
  - Natalizumab
  - Paclitaxel: Increased hematotoxicity has been reported when cyclophosphamide was administered after paclitaxel infusion.
  - Thiazide diuretics
  - Zidovudine

- Increased cardiotoxicity may result from a combined effect of cyclophosphamide and, for example:
  - Anthracyclines
  - Cytarabine
  - Pentostatin
  - Radiation therapy of the cardiac region
  - Trastuzumab
- Increased pulmonary toxicity may result from a combined effect of cyclophosphamide and, for example:
  - Amiodarone
  - G-CSF, GM-CSF (granulocyte colony-stimulating factor, granulocyte macrophage colony-stimulating factor): Reports suggest an increased risk of pulmonary toxicity in patients treated with cytotoxic chemotherapy that includes cyclophosphamide and G-CSF or GMCSF.
- Increased nephrotoxicity may result from a combined effect of cyclophosphamide and, for example:
  - Amphotericin B
  - Indomethacin: Acute water intoxication has been reported with concomitant use of indomethacin
- Increase in other toxicities:
  - Azathioprine: Increased risk of hepatotoxicity (liver necrosis)
  - Busulfan: Increased incidence of hepatic veno-occlusive disease and mucositis has been reported.
  - Protease inhibitors: Increased incidence of mucositis
- Increased risk of hemorrhagic cystitis may result from a combined effect of cyclophosphamide and past or concomitant radiation treatment.

Etanercept: In patients with Wegener's granulomatosis, the addition of etanercept to standard treatment, including cyclophosphamide, was associated with a higher incidence of non-cutaneous malignant solid tumors.

Metronidazole: Acute encephalopathy has been reported in a patient receiving cyclophosphamide and metronidazole. Causal association is unclear. In an animal study, the combination of cyclophosphamide with metronidazole was associated with increased cyclophosphamide toxicity.

Tamoxifen: Concomitant use of tamoxifen and chemotherapy may increase the risk of thromboembolic complications.

Coumarins: Both increased and decreased warfarin effect have been reported in patients receiving warfarin and cyclophosphamide.

Cyclosporine: Lower serum concentrations of cyclosporine have been observed in patients receiving a combination of cyclophosphamide and cyclosporine than in patients receiving only cyclosporine. This interaction may result in an increased incidence of graft-versus-host disease.

Depolarizing muscle relaxants: Cyclophosphamide treatment causes a marked and persistent inhibition of cholinesterase activity. Prolonged apnea may occur with concurrent depolarizing muscle relaxants (e.g., succinylcholine). If a patient has been treated with cyclophosphamide within 10 days of general anesthesia, alert the anesthesiologist.

8. USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

Pregnancy Category D

Risk Summary
Cyclophosphamide can cause fetal harm when administered to a pregnant woman based on its mechanism of action and published reports of effects in pregnant patients or animals. Exposure to cyclophosphamide during pregnancy may cause fetal malformations, miscarriage, fetal growth retardation, and toxic effects in the newborn. Cyclophosphamide is teratogenic and embryo-fetal toxic in mice, rats, rabbits and monkeys. If this drug is used during pregnancy, or if the patient becomes pregnant while taking this drug, apprise the patient of the potential hazard to a fetus.

Human Data
Malformations of the skeleton, palate, limbs and eyes as well as miscarriage have been reported after exposure to cyclophosphamide in the first trimester. Fetal growth retardation and toxic effects manifesting in the newborn, including leukopenia, anemia, pancytopenia, severe bone marrow hypoplasia, and gastroenteritis have been reported after exposure to cyclophosphamide.

Animal Data
Administration of cyclophosphamide to pregnant mice, rats, rabbits and monkeys during the period of organogenesis at doses at or below the dose in patients based on body surface area resulted in various malformations, which included neural tube defects, limb and digit defects and other skeletal anomalies, cleft lip and palate, and reduced skeletal ossification.

8.3 Nursing Mothers

Cyclophosphamide is present in breast milk. Neutropenia, thrombocytopenia, low hemoglobin, and diarrhea have been reported in infants breast fed by women treated with cyclophosphamide. Because of the potential for serious adverse reactions in nursing infants from cyclophosphamide, a decision should be made whether to discontinue nursing or to discontinue the drug, taking into account the importance of the drug to the mother.

8.4 Pediatric Use

Pre-pubescent girls treated with cyclophosphamide generally develop secondary sexual characteristics normally and have regular menses. Ovarian fibrosis with apparently complete loss of germ cells after prolonged cyclophosphamide treatment in late pre-pubescence has been reported. Girls treated with cyclophosphamide who have retained ovarian function after completing treatment are at increased risk of developing premature menopause.

Pre-pubescent boys treated with cyclophosphamide develop secondary sexual characteristics normally, but may have oligospermia or azoospermia and increased gonadotropin secretion. Some degree of testicular atrophy may occur. Cyclophosphamide-induced azoospermia is reversible in some patients, though the reversibility may not occur for several years after cessation of therapy.

8.5 Geriatric Use

There is insufficient data from clinical studies of cyclophosphamide available for patients 65 years of age and older to determine whether they respond differently than younger patients. In general, dose selection for an elderly patient should be cautious, usually starting at the low end of the dosing range, reflecting the

greater frequency of decreased hepatic, renal, or cardiac functioning, and of concomitant disease or other drug therapy.

8.6 Females and Males of Reproductive Potential

Contraception
Pregnancy should be avoided during treatment with cyclophosphamide because of the risk of fetal harm *[see Use in Specific Populations (8.1)]*.

Female patients of reproductive potential should use highly effective contraception during and for up to 1 year after completion of treatment.

Male patients who are sexually active with female partners who are or may become pregnant should use a condom during and for at least 4 months after treatment.

Infertility
Females
Amenorrhea, transient or permanent, associated with decreased estrogen and increased gonadotropin secretion develops in a proportion of women treated with cyclophosphamide. Affected patients generally resume regular menses within a few months after cessation of therapy. The risk of premature menopause with cyclophosphamide increases with age. Oligomenorrhea has also been reported in association with cyclophosphamide treatment.

Animal data suggest an increased risk of failed pregnancy and malformations may persist after discontinuation of cyclophosphamide as long as oocytes/follicles exist that were exposed to cyclophosphamide during any of their maturation phases. The exact duration of follicular development in humans is not known, but may be longer than 12 months *[see Nonclinical Toxicology (13.1)]*.

Males
Men treated with cyclophosphamide may develop oligospermia or azoospermia which are normally associated with increased gonadotropin but normal testosterone secretion.

8.7 Use in Patients with Renal Impairment

In patients with severe renal impairment, decreased renal excretion may result in increased plasma levels of cyclophosphamide and its metabolites. This may result in increased toxicity *[see Clinical Pharmacology (12.3)]*. Monitor patients with severe renal impairment (CrCl =10 mL/min to 24 mL/min) for signs and symptoms of toxicity.

Cyclophosphamide and its metabolites are dialyzable although there are probably quantitative differences depending upon the dialysis system being used. In patients requiring dialysis, use of a consistent interval between cyclophosphamide administration and dialysis should be considered.

8.8 Use in Patients with Hepatic Impairment

Patients with severe hepatic impairment have reduced conversion of cyclophosphamide to the active 4□ hydroxyl metabolite, potentially reducing efficacy *[see Clinical Pharmacology (12.3)]*.

10. OVERDOSAGE

No specific antidote for cyclophosphamide is known.

Overdosage should be managed with supportive measures, including appropriate treatment for any concurrent infection, myelosuppression, or cardiac toxicity should it occur.

Serious consequences of overdosage include manifestations of dose dependent toxicities such as myelosuppression, urotoxicity, cardiotoxicity (including cardiac failure), veno-occlusive hepatic disease, and stomatitis *[see Warnings and Precautions (5.1, 5.2, 5.3, and 5.6)]*.

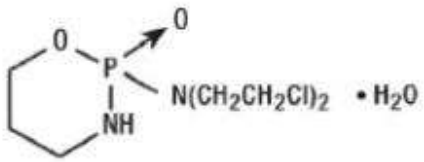
Patients who received an overdose should be closely monitored for the development of toxicities, and hematologic toxicity in particular.

Cyclophosphamide and its metabolites are dialyzable. Therefore, rapid hemodialysis is indicated when treating any suicidal or accidental overdose or intoxication.

Cystitis prophylaxis with mesna may be helpful in preventing or limiting urotoxic effects with cyclophosphamide overdose.

11. DESCRIPTION

Cyclophosphamide is a synthetic antineoplastic drug chemically related to the nitrogen mustards. The chemical name for cyclophosphamide is 2-[bis(2-chloroethyl)amino]tetrahydro-2H-1,3,2-oxazaphosphorine 2-oxide monohydrate, and has the following structural formula:



Cyclophosphamide has a molecular formula of $C_7H_{15}Cl_2N_2O_2P \cdot H_2O$ and a molecular weight of 279.1. Cyclophosphamide is soluble in water, saline, or ethanol.

Cyclophosphamide for Injection, USP is a sterile white cake available as 500 mg, 1 g, and 2 g strength vials.

- 500 mg vial contains 534.5 mg cyclophosphamide monohydrate equivalent to 500 mg cyclophosphamide and 375 mg mannitol
- 1 g vial contains 1069.0 mg cyclophosphamide monohydrate equivalent to 1 g cyclophosphamide and 750 mg mannitol
- 2 g vial contains 2138.0 mg cyclophosphamide monohydrate equivalent to 2 g cyclophosphamide and 1500 mg mannitol

Cyclophosphamide Tablets, USP are for oral use and contain 25 mg or 50 mg cyclophosphamide (anhydrous). Inactive ingredients in Cyclophosphamide Tablets are: acacia, FD&C Blue No. 1, D&C Yellow No. 10 Aluminum Lake, lactose, magnesium stearate, starch, stearic acid and talc.

12. CLINICAL PHARMACOLOGY

12.1 Mechanism of Action

The mechanism of action is thought to involve cross-linking of tumor cell DNA.

12.2 Pharmacodynamics

Cyclophosphamide is biotransformed principally in the liver to active alkylating metabolites by a mixed function microsomal oxidase system. These metabolites interfere with the growth of susceptible rapidly proliferating malignant cells.

12.3 Pharmacokinetics

Following IV administration, elimination half-life ($t_{1/2}$) ranges from 3 to 12 hours with total body clearance (CL) values of 4 to 5.6 L/h. Pharmacokinetics are linear over the dose range used clinically. When cyclophosphamide was administered at 4.0 g/m^2 over a 90 minutes infusion, saturable elimination in parallel with first-order renal elimination describe the kinetics of the drug.

Absorption
After oral administration, peak concentrations of cyclophosphamide occurred at one hour. Area under the curve ratio for the drug after oral and IV administration (AUC_{po} : AUC_{iv}) ranged from 0.87 to 0.96.

Distribution
Approximately 20% of cyclophosphamide is protein bound, with no dose dependent changes. Some metabolites are protein bound to an extent greater than 60%. Volume of distribution approximates total body water (30 to 50 L).

Metabolism
The liver is the major site of cyclophosphamide activation. Approximately 75% of the administered dose of cyclophosphamide is activated by hepatic microsomal cytochrome P450s including CYP2A6, 2B6, 3A4, 3A5, 2C9, 2C18 and 2C19, with 2B6 displaying the highest 4-hydroxylase activity. Cyclophosphamide is activated to form 4-hydroxycyclophosphamide, which is in equilibrium with its ring-open tautomer aldophosphamide. 4-hydroxycyclophosphamide and aldophosphamide can undergo oxidation by aldehyde dehydrogenases to form the inactive metabolites 4-ketocyclophosphamide and carboxyphosphamide, respectively. Aldophosphamide can undergo β-elimination to form active metabolites phosphoramide mustard and acrolein. This spontaneous conversion can be catalyzed by albumin and other proteins. Less than 5% of cyclophosphamide may be directly detoxified by side chain oxidation, leading to the formation of inactive metabolites 2-dechloroethylcyclophosphamide. At high doses, the fraction of parent compound cleared by 4-hydroxylation is reduced resulting in non-linear elimination of cyclophosphamide in patients. Cyclophosphamide appears to induce its own metabolism. Auto-induction results in an increase in the total clearance, increased formation of 4-hydroxyl metabolites and shortened t1/2 values following repeated administration at 12- to 24-hour interval.

Elimination
Cyclophosphamide is primarily excreted as metabolites. 10 to 20% is excreted unchanged in the urine and 4% is excreted in the bile following IV administration.

Special Populations

Renal Impairment

The pharmacokinetics of cyclophosphamide were determined following one-hour intravenous infusion to renally impaired patients. The results demonstrated that the systemic exposure to cyclophosphamide increased as the renal function decreased. Mean dose-corrected AUC increased by 38% in the moderate renal group, (Creatinine clearance (CrCl of 25 to 50 mL/min), by 64% in the severe renal group (CrCl of 10 to 24 mL/min) and by 23% in the hemodialysis group (CrCl of < 10mL/min) compared to the control group. The increase in exposure was significant in the severe group ($p>0.05$); thus, patients with severe renal impairment should be closely monitored for toxicity *[see Use in Specific Populations (8.7)]*.

The dialyzability of cyclophosphamide was investigated in four patients on long-term hemodialysis. Dialysis clearance calculated by arterial-venous difference and actual drug recovery in dialysate averaged 104 mL/min, which is in the range of the metabolic clearance of 95 mL/min for the drug. A mean of 37% of the administered dose of cyclophosphamide was removed during hemodialysis. The elimination half-life ($t_{1/2}$) was 3.3 hours in patients during hemodialysis, a 49% reduction of the 6.5 hours to $t_{1/2}$ reported in uremic patients. Reduction in $t_{1/2}$, larger dialysis clearance than metabolic clearance, high extraction efficiency, and significant drug removal during dialysis, suggest that cyclophosphamide is dialyzable.

Hepatic Impairment
Total body clearance (CL) of cyclophosphamide is decreased by 40% in patients with severe hepatic impairment and elimination half-life ($t_{1/2}$) is prolonged by 64%. Mean CL and $t_{1/2}$ were 45 ± 8.6 L/kg and 12.5 ± 1.0 hours respectively, in patients with severe hepatic impairment and 63 ± 7.6 L/kg and 7.6 ± 1.4 hours respectively in the control group *[see Use in Specific Populations (8.8)]*.

13. NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Cyclophosphamide administered by different routes, including intravenous, subcutaneous or intraperitoneal injection, or in drinking water, caused tumors in both mice and rats. In addition to leukemia and lymphoma, benign and malignant tumors were found at various tissue sites, including urinary bladder, mammary gland, lung, liver, and injection site *[see Warnings and Precautions (5.5)]*.

Cyclophosphamide was mutagenic and clastogenic in multiple *in vitro* and *in vivo* genetic toxicology studies.

Cyclophosphamide is genotoxic in male and female germ cells. Animal data indicate that exposure of oocytes to cyclophosphamide during follicular development may result in a decreased rate of implantations and viable pregnancies, and in an increased risk of malformations. Male mice and rats treated with cyclophosphamide show alterations in male reproductive organs (e.g., decreased weights, atrophy, changes in spermatogenesis), and decreases in reproductive potential (e.g., decreased implantations and increased post-implantation loss) and increases in fetal malformations when mated with untreated females *[see Use in Specific Populations (8.6)]*.

15. REFERENCES

1. OSHA Hazardous Drugs. *OSHA*. http://www.osha.gov/SLTC/hazardousdrugs/index.html.

16. HOW SUPPLIED/STORAGE AND HANDLING

Cyclophosphamide for Injection, USP (lyophilized powder) is a sterile white cake containing cyclophosphamide and mannitol and is supplied in vials for single dose use.

Cyclophosphamide for Injection, USP

10019-988-01	500 mg vial, carton of 1
10019-989-01	1 g vial, carton of 1
10019-990-01	2 g vial, carton of 1

Store vials at or below 25°C (77°F). During transport or storage of cyclophosphamide vials, temperature influences can lead to melting of the active ingredient, cyclophosphamide *[see Dosage and Administration (2.3)]*.

Cyclophosphamide Tablets, USP are white tablets with blue flecks containing 25 mg and 50 mg cyclophosphamide, respectively.

Cyclophosphamide Tablets, USP

10019-984-09	50 mg, bottles of 100
10019-982-09	25 mg, bottles of 100

Store tablets at or below 25°C (77°F). Tablets will withstand brief exposure to temperatures up to 30°C (86°F) but should be protected from temperatures above 30°C (86°F).

Cyclophosphamide is an antineoplastic product. Follow special handling and disposal procedures.[1]

17. PATIENT COUNSELING INFORMATION

Advise the patient of the following:

- Inform patients of the possibility of myelosuppression, immunosuppression, and infections. Explain the need for routine blood cell counts. Instruct patients to monitor their temperature frequently and immediately report any occurrence of fever *[see Warnings and Precautions (5.1)]*.
- Advise the patient to report urinary symptoms (patients should report if their urine has turned a pink or red color) and the need for increasing fluid intake and frequent voiding *[see Warnings and Precautions (5.2)]*.
- Advise patients to contact a health care professional immediately for any of the following: new onset or worsening shortness of breath, cough, swelling of the ankles/legs, palpitations, weight gain of more than 5 pounds in 24 hours, dizziness or loss of consciousness *[see Warnings and Precautions (5.3)]*.
- Warn patients of the possibility of developing non-infectious pneumonitis. Advise patients to report promptly any new or worsening respiratory symptoms *[see Warnings and Precautions (5.4)]*.
- Advise female patients of reproductive potential to use highly effective contraception during treatment and for up to 1 year after completion of therapy. There is a potential for harm to a fetus if a patient becomes pregnant during this period. Patients should immediately contact their healthcare provider if they become pregnant or if pregnancy is suspected during this period *[see Warnings and Precautions (5.7)* and *Use in Specific Populations (8.1)]*.
- Advise male patients who are sexually active with a female partner who is or may become pregnant to use condoms during treatment and for up to 4 months after completion of therapy. There is a potential for harm to a fetus if a patient fathers a child during this period. Patients should immediately contact their healthcare provider if their female partner becomes pregnant or if pregnancy is suspected during this period *[see Warnings and Precautions (5.7)* and *Use in Specific Populations (8.1)]*.

- Advise nursing mothers treated with cyclophosphamide to discontinue nursing or discontinue cyclophosphamide, taking into account the importance of the drug to the mother *[see Use in Specific Populations (8.3)]*.

- Explain to patients that side effects such as nausea, vomiting, stomatitis, impaired wound healing, amenorrhea, premature menopause, sterility and hair loss may be associated with cyclophosphamide administration. Other undesirable effects (including, e.g., dizziness, blurred vision, visual impairment) could affect the ability to drive or use machines *[see Adverse Reactions (6.1 and 6.2)]*.

- Instruct the patient to swallow cyclophosphamide tablets whole. Do not cut, chew, or crush tablets *[see Dosage and Administration (2.3)]*.

- Advise caregivers to wear gloves when handling bottles containing cyclophosphamide tablets and avoid exposure to broken tablets. If contact with broken tablets occurs, wash hands immediately and thoroughly *[see Dosage and Administration (2.3)]*.

Baxter

Vials Manufactured by:
Tablets Manufactured for:
Baxter Healthcare Corporation
Deerfield, IL 60015 USA

Made in Germany

Baxter is a trademark of Baxter International Inc.

Reference ID: 3304966

APPENDIX 8: FLUDARABINE PACKAGE INSERT

Fludara®

(fludarabine phosphate)

FOR INJECTION
FOR INTRAVENOUS USE ONLY

Rx Only

WARNING: FLUDARA FOR INJECTION should be administered under the supervision of a qualified physician experienced in the use of antineoplastic therapy. FLUDARA FOR INJECTION can severely suppress bone marrow function. When used at high doses in dose-ranging studies in patients with acute leukemia, FLUDARA FOR INJECTION was associated with severe neurologic effects, including blindness, coma, and death. This severe central nervous system toxicity occurred in 36% of patients treated with doses approximately four times greater (96 mg/m^2/day for 5-7 days) than the recommended dose. Similar severe central nervous system toxicity, including coma, seizures, agitation and confusion, has been reported in patients treated at doses in the range of the dose recommended for chronic lymphocytic leukemia.

Instances of life-threatening and sometimes fatal autoimmune phenomena such as hemolytic anemia, autoimmune thrombocytopenia/thrombocytopenic purpura (ITP), Evan's syndrome, and acquired hemophilia have been reported to occur after one or more cycles of treatment with FLUDARA FOR INJECTION. Patients undergoing treatment with FLUDARA FOR INJECTION should be evaluated and closely monitored for hemolysis.

In a clinical investigation using FLUDARA FOR INJECTION in combination with pentostatin (deoxycoformycin) for the treatment of refractory chronic lymphocytic leukemia (CLL), there was an unacceptably high incidence of fatal pulmonary toxicity. Therefore, the use of FLUDARA FOR INJECTION in combination with pentostatin is not recommended.

DESCRIPTION

FLUDARA FOR INJECTION contains fludarabine phosphate, a fluorinated nucleotide analog of the antiviral agent vidarabine, 9-β-D-arabinofuranosyladenine (ara-A) that is relatively resistant to deamination by adenosine deaminase. Each vial of sterile lyophilized solid cake contains 50 mg of the active ingredient fludarabine phosphate, 50 mg of mannitol, and sodium hydroxide to adjust pH to 7.7. The pH range for the final product is 7.2-8.2. Reconstitution with 2 mL of Sterile Water for Injection USP results in a solution containing 25 mg/mL of fludarabine phosphate intended for intravenous administration.

The chemical name for fludarabine phosphate is 9H-Purin-6-amine, 2-fluoro-9-(5-O-phosphono-β-D-arabino-furanosyl) (2-fluoro-ara-AMP). The molecular formula of fludarabine phosphate is $C_{10}H_{13}FN_5O_7P$ (MW 365.2) and the structure is:

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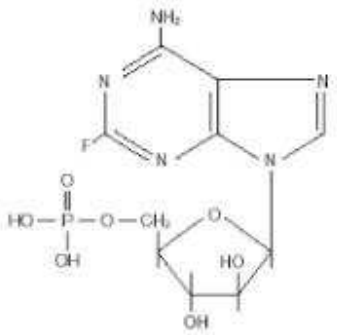

CLINICAL PHARMACOLOGY

Fludarabine phosphate is rapidly dephosphorylated to 2-fluoro-ara-A and then phosphorylated intracellularly by deoxycytidine kinase to the active triphosphate, 2-fluoro-ara-ATP. This metabolite appears to act by inhibiting DNA polymerase alpha, ribonucleotide reductase and DNA primase, thus inhibiting DNA synthesis. The mechanism of action of this antimetabolite is not completely characterized and may be multi-faceted.

Phase I studies in humans have demonstrated that fludarabine phosphate is rapidly converted to the active metabolite, 2-fluoro-ara-A, within minutes after intravenous infusion. Consequently, clinical pharmacology studies have focused on 2-fluoro-ara-A pharmacokinetics. After the five daily doses of 25 mg 2-fluoro-ara-AMP/m^2 to cancer patients infused over 30 minutes, 2-fluoro-ara-A concentrations show a moderate accumulation. During a 5-day treatment schedule, 2-fluoro-ara-A plasma trough levels increased by a factor of about 2. The terminal half-life of 2-fluoro-ara-A was estimated as approximately 20 hours. *In vitro*, plasma protein binding of fludarabine ranged between 19% and 29%.

A correlation was noted between the degree of absolute granulocyte count nadir and increased area under the concentration x time curve (AUC).

Special Populations

Pediatric Patients

Limited pharmacokinetic data for FLUDARA FOR INJECTION are available from a published study of children (ages 1-21 years) with refractory acute leukemias or solid tumors (Children's Cancer Group Study 097[1]). When FLUDARA FOR INJECTION was administered as a loading dose over 10 minutes immediately followed by a 5-day continuous infusion, steady-state conditions were reached early.

Patients with Renal Impairment

The total body clearance of the principal metabolite 2-fluoro-ara-A correlated with the creatinine clearance, indicating the importance of the renal excretion pathway for the elimination of the drug. Renal clearance represents approximately 40% of the total body clearance. Patients with moderate renal impairment (17 - 41 mL/min/m^2) receiving 20% reduced Fludara dose had a

similar exposure (AUC; 21 versus 20 nM • h/mL) compared to patients with normal renal function receiving the recommended dose. The mean total body clearance was 172 mL/min for normal and 124 mL/min for patients with moderately impaired renal function.

CLINICAL STUDIES

Two single-arm open-label studies of FLUDARA FOR INJECTION have been conducted in adult patients with CLL refractory to at least one prior standard alkylating-agent containing regimen. In a study conducted by M.D. Anderson Cancer Center (MDAH), 48 patients were treated with a dose of 22-40 mg/m^2 daily for 5 days every 28 days. Another study conducted by the Southwest Oncology Group (SWOG) involved 31 patients treated with a dose of 15-25 mg/m^2 daily for 5 days every 28 days. The overall objective response rates were 48% and 32% in the MDAH and SWOG studies, respectively. The complete response rate in both studies was 13%; the partial response rate was 35% in the MDAH study and 19% in the SWOG study. These response rates were obtained using standardized response criteria developed by the National Cancer Institute CLL Working Group[3] and were achieved in heavily pre-treated patients. The ability of FLUDARA FOR INJECTION to induce a significant rate of response in refractory patients suggests minimal cross-resistance with commonly used anti-CLL agents.

The median time to response in the MDAH and SWOG studies was 7 weeks (range of 1 to 68 weeks) and 21 weeks (range of 1 to 53 weeks) respectively. The median duration of disease control was 91 weeks (MDAH) and 65 weeks (SWOG). The median survival of all refractory CLL patients treated with FLUDARA FOR INJECTION was 43 weeks and 52 weeks in the MDAH and SWOG studies, respectively.

Rai stage improved to Stage II or better in 7 of 12 MDAH responders (58%) and in 5 of 7 SWOG responders (71%) who were Stage III or IV at baseline. In the combined studies, mean hemoglobin concentration improved from 9.0 g/dL at baseline to 11.8 g/dL at the time of response in a subgroup of anemic patients. Similarly, average platelet count improved from 63,500/mm^3 to 103,300/mm^3 at the time of response in a subgroup of patients who were thrombocytopenic at baseline.

INDICATIONS AND USAGE

FLUDARA FOR INJECTION is indicated for the treatment of adult patients with B-cell chronic lymphocytic leukemia (CLL) who have not responded to or whose disease has progressed during treatment with at least one standard alkylating-agent containing regimen. The safety and effectiveness of FLUDARA FOR INJECTION in previously untreated or non-refractory patients with CLL have not been established.

CONTRAINDICATIONS

FLUDARA FOR INJECTION is contraindicated in those patients who are hypersensitive to this drug or its components.

WARNINGS

(See **BOXED WARNINGS**)

There are clear dose dependent toxic effects seen with FLUDARA FOR INJECTION. Dose levels approximately 4 times greater (96 mg/m^2/day for 5 to 7 days) than that recommended for CLL (25 mg/m^2/day for 5 days) were associated with a syndrome characterized by delayed blindness, coma and death. Symptoms appeared from 21 to 60 days following the last dose. Thirteen of 36 patients (36%) who received FLUDARA FOR INJECTION at high doses (96 mg/m^2/day for 5 to 7 days) developed this severe neurotoxicity. Similar severe central nervous

system toxicity, including coma, seizures, agitation and confusion, has been reported in patients treated at doses in the range of the dose recommended for chronic lymphocytic leukemia.

The effect of chronic administration of FLUDARA FOR INJECTION on the central nervous system is unknown, however, patients have received the recommended dose for up to 15 courses of therapy.

Severe bone marrow suppression, notably anemia, thrombocytopenia and neutropenia, has been reported in patients treated with FLUDARA FOR INJECTION. In a Phase I study in adult solid tumor patients, the median time to nadir counts was 13 days (range, 3-25 days) for granulocytes and 16 days (range, 2-32) for platelets. Most patients had hematologic impairment at baseline either as a result of disease or as a result of prior myelosuppressive therapy. Cumulative myelosuppression may be seen. While chemotherapy-induced myelosuppression is often reversible, administration of FLUDARA FOR INJECTION requires careful hematologic monitoring.

Several instances of trilineage bone marrow hypoplasia or aplasia resulting in pancytopenia, sometimes resulting in death, have been reported in adult patients. The duration of clinically significant cytopenia in the reported cases has ranged from approximately 2 months to approximately 1 year. These episodes have occurred both in previously treated or untreated patients.

Instances of life-threatening and sometimes fatal autoimmune phenomena such as hemolytic anemia, autoimmune thrombocytopenia/thrombocytopenic purpura (ITP), Evan's syndrome, and acquired hemophilia have been reported to occur after one or more cycles of treatment with FLUDARA FOR INJECTION in patients with or without a previous history of autoimmune hemolytic anemia or a positive Coombs' test and who may or may not be in remission from their disease. Steroids may or may not be effective in controlling these hemolytic episodes. The majority of patients rechallenged with FLUDARA FOR INJECTION developed a recurrence in the hemolytic process. The mechanism(s) which predispose patients to the development of this complication has not been identified. Patients undergoing treatment with FLUDARA FOR INJECTION should be evaluated and closely monitored for hemolysis. Discontinuation of therapy with Fludara is recommended in case of hemolysis.

Transfusion-associated graft-versus-host disease has been observed after transfusion of non-irradiated blood in FLUDARA FOR INJECTION treated patients. Fatal outcome as a consequence of this disease has been reported. Therefore, to minimize the risk of transfusion-associated graft-versus-host disease, patients who require blood transfusion and who are undergoing, or who have received, treatment with FLUDARA FOR INJECTION should receive irradiated blood only.

In a clinical investigation using FLUDARA FOR INJECTION in combination with pentostatin (deoxycoformycin) for the treatment of refractory chronic lymphocytic leukemia (CLL) in adults, there was an unacceptably high incidence of fatal pulmonary toxicity. Therefore, the use of FLUDARA FOR INJECTION in combination with pentostatin is not recommended.

Of the 133 adult CLL patients in the two trials, there were 29 fatalities during study. Approximately 50% of the fatalities were due to infection and 25% due to progressive disease.

Pregnancy Category D

Based on its mechanism of action, fludarabine phosphate can cause fetal harm when administered to a pregnant woman. There are no adequate and well-controlled studies of Fludara in pregnant women. Fludarabine phosphate was embryolethal and teratogenic in both rats and rabbits. If FLUDARA FOR INJECTION is used during pregnancy, or if the patient

becomes pregnant while taking this drug, the patient should be apprised of the potential hazard to the fetus. Women of childbearing potential should be advised to avoid becoming pregnant. Women of childbearing potential and fertile males must take contraceptive measures during and at least for six months after cessation of treatment with FLUDARA FOR INJECTION.

Fludarabine phosphate was embryolethal and teratogenic in rats and rabbits. Fludarabine phosphate was administered at doses of 0, 1, 10 or 30 mg/kg/day (0.24, 2.4 times and 7.2 times the recommended human dose on a mg/m2 basis, respectively)to pregnant rats on days 6 to 15 of gestation. At 10 and 30 mg/kg/day administered during organogenesis, there was a dose-related increase in various skeletal variations and a decrease in mean fetal body weights. Maternal toxicity was not apparent at 10 mg/kg/day, and was limited to slight body weight decreases at 30 mg/kg/day. In a dose finding study malformations, such as limb and tail defects, were induced at 40 mg/kg/day (9.6 times the recommended human dose on a mg/m2 basis). In a reproduction toxicity study on rabbits Fludarabine phosphate was administered intravenously at doses of 0, 1, 5 or 8 mg/kg/day (approximately 0.5, 2.4, and 3.8 times the recommended human dose on a mg/m2 basis) on days 6 to 18 of gestation. A dose of 8 mg/kg/day administered during organogenesis increased embryo and fetal lethality as indicated by a higher number of resorptions and a decrease in live fetuses. Compound-related teratogenic effects manifested by external deformities and skeletal malformations were observed at 8 mg/kg/day. The most frequent external malformations observed in rabbits were cleft palate, adactyly, brachydactyly and syndactyly along with skeletal malformations such as fused metatarsals, phalanges, sternebrae and limb bones and some soft tissue malformations (diaphragmatic hernias). Fetal body weights were decreased in rabbits given 8 mg/kg/day."

PRECAUTIONS

General

FLUDARA FOR INJECTION is a potent antineoplastic agent with potentially significant toxic side effects. Patients undergoing therapy should be closely observed for signs of hematologic and nonhematologic toxicity. Periodic assessment of peripheral blood counts is recommended to detect the development of anemia, neutropenia and thrombocytopenia.

Tumor lysis syndrome associated with FLUDARA FOR INJECTION treatment has been reported in CLL patients with large tumor burdens. Since FLUDARA FOR INJECTION can induce a response as early as the first week of treatment, precautions should be taken in those patients at risk of developing this complication.

In patients with impaired state of health, FLUDARA FOR INJECTION should be given with caution and after careful risk/benefit consideration. This applies especially for patients with severe impairment of bone marrow function (thrombocytopenia, anemia, and/or granulocytopenia), immunodeficiency or with a history of opportunistic infection. Prophylactic treatment should be considered in patients at increased risk of developing opportunistic infections.

There are inadequate data on dosing of patients with renal insufficiency. FLUDARA FOR INJECTION must be administered cautiously in patients with renal insufficiency. The total body clearance of 2-fluoro-ara-A has been shown to be directly correlated with creatinine clearance. Patients with moderate impairment of renal function (creatinine clearance 30-70 mL/min/1.73 m^2) should have their Fludara dose reduced by 20% and be monitored closely. FLUDARA FOR INJECTION is not recommended for patients with severely impaired renal function (creatinine clearance less than 30 mL/min/1.73 m^2).

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Fludara may reduce the ability to drive or use machines, since fatigue, weakness, visual disturbances, confusion, agitation and seizures have been observed.

Laboratory Tests
During treatment, the patient's hematologic profile (particularly neutrophils and platelets) should be monitored regularly to determine the degree of hematopoietic suppression.

Drug Interactions
The use of FLUDARA FOR INJECTION in combination with pentostatin is not recommended due to the risk of severe pulmonary toxicity (see WARNINGS section).

Carcinogenesis
No animal carcinogenicity studies with FLUDARA FOR INJECTION have been conducted.

Mutagenesis
Fludarabine phosphate was not mutagenic to bacteria (Ames test) or mammalian cells (HGRPT assay in Chinese hamster ovary cells) either in the presence or absence of metabolic activation. Fludarabine phosphate was clastogenic *in vitro* to Chinese hamster ovary cells (chromosome aberrations in the presence of metabolic activation) and induced sister chromatid exchanges both with and without metabolic activation. In addition, fludarabine phosphate was clastogenic *in vivo* (mouse micronucleus assay) but was not mutagenic to germ cells (dominant lethal test in male mice).

Impairment of Fertility
Studies in mice, rats and dogs have demonstrated dose-related adverse effects on the male reproductive system. Observations consisted of a decrease in mean testicular weights in mice and rats with a trend toward decreased testicular weights in dogs and degeneration and necrosis of spermatogenic epithelium of the testes in mice, rats and dogs. The possible adverse effects on fertility in humans have not been adequately evaluated.

Pregnancy
Pregnancy Category D: (see WARNINGS section).

Nursing Mothers
It is not known whether fludarabine phosphate is excreted in human milk. Because many drugs are excreted in human milk and because of the potential for serious adverse reactions including tumorgenicity in nursing infants, a decision should be made to discontinue nursing or discontinue the drug, taking into account the importance of the drug to the mother.

Pediatric Use
Data submitted to the FDA was insufficient to establish efficacy in any childhood malignancy. Fludarabine was evaluated in 62 pediatric patients (median age 10, range 1-21) with refractory acute leukemia (45 patients) or solid tumors (17 patients). The fludarabine regimen tested for pediatric acute lymphocytic leukemia (ALL) patients was a loading bolus of 10.5 mg/m^2/day followed by a continuous infusion of 30.5 mg/m^2/day for 5 days. In 12 pediatric patients with solid tumors, dose-limiting myelosuppression was observed with a loading dose of 8 mg/m^2/day followed by a continuous infusion of 23.5 mg/m^2/day for 5 days. The maximum tolerated dose was a loading dose of 7 mg/m^2/day followed by a continuous infusion of 20 mg/m^2/day for 5 days. Treatment toxicity included bone marrow suppression. Platelet counts appeared to be more sensitive to the effects of fludarabine than hemoglobin and white blood cell counts. Other adverse events included fever, chills, asthenia, rash, nausea, vomiting, diarrhea, and infection.

There were no reported occurrences of peripheral neuropathy or pulmonary hypersensitivity reaction.

Vaccination
During and after treatment with FLUDARA FOR INJECTION, vaccination with live vaccines should be avoided.

Disease Progression
Disease progression and transformation (e.g. Richter's syndrome) have been reported in CLL patients.

ADVERSE REACTIONS
The most common adverse events include myelosuppression (neutropenia, thrombocytopenia and anemia), fever and chills, infection, and nausea and vomiting. Other commonly reported events include malaise, fatigue, anorexia, and weakness. Serious opportunistic infections have occurred in CLL patients treated with FLUDARA FOR INJECTION. Adverse events, and those reactions which are more clearly related to the drug are arranged below according to body system.

Hematopoietic Systems Hematologic events (neutropenia, thrombocytopenia, and/or anemia) were reported in the majority of CLL patients treated with FLUDARA FOR INJECTION. During FLUDARA FOR INJECTION treatment of 133 patients with CLL, the absolute neutrophil count decreased to less than 500/mm^3 in 59% of patients, hemoglobin decreased from pretreatment values by at least 2 grams percent in 60%, and platelet count decreased from pretreatment values by at least 50% in 55%. Myelosuppression may be severe, cumulative, and may affect multiple cell lines. Bone marrow fibrosis occurred in one CLL patient treated with FLUDARA FOR INJECTION.

Several instances of trilineage bone marrow hypoplasia or aplasia resulting in pancytopenia, sometimes resulting in death, have been reported in postmarketing surveillance. The duration of clinically significant cytopenia in the reported cases has ranged from approximately 2 months to approximately 1 year. These episodes have occurred both in previously treated or untreated patients.

Life-threatening and sometimes fatal autoimmune phenomena such as hemolytic anemia, autoimmune thrombocytopenia/thrombocytopenic purpura (ITP), Evan's syndrome, and acquired hemophilia have been reported to occur in patients receiving FLUDARA FOR INJECTION (see WARNINGS section). The majority of patients rechallenged with FLUDARA FOR INJECTION developed a recurrence in the hemolytic process.

In post-marketing experience, cases of myelodysplastic syndrome and acute myeloid leukemia, mainly associated with prior, concomitant or subsequent treatment with alkylating agents, topoisomerase inhibitors, or irradiation have been reported.

Infections Serious, and sometimes fatal infections, including opportunistic infections and reactivations of latent viral infections such as VZV (Herpes zoster), Epstein-Barr virus and JC virus (progressive multifocal leukoencephalopathy)) have been reported in patients treated with FLUDARA FOR INJECTION.

Rare cases of Epstein Barr Virus (EBV) associated lymphoproliferative disorders have been reported in patients treated with FLUDARA FOR INJECTION.

Metabolic Tumor lysis syndrome has been reported in CLL patients treated with FLUDARA FOR INJECTION. This complication may include hyperuricemia, hyperphosphatemia, hypocalcemia, metabolic acidosis, hyperkalemia, hematuria, urate crystalluria, and renal failure. The onset of this syndrome may be heralded by flank pain and hematuria.

Nervous System (See WARNINGS section) Objective weakness, agitation, confusion, seizures, [visual disturbances, optic neuritis, optic neuropathy, blindness and coma have occurred in CLL patients treated with FLUDARA FOR INJECTION at the recommended dose. Peripheral neuropathy has been observed in patients treated with FLUDARA FOR INJECTION and one case of wrist-drop was reported.

In post-marketing experience, cases of progressive multifocal leukoencephalopathy have been reported. Most cases had a fatal outcome. Many of these cases were confounded by prior and/or concurrent chemotherapy. The time to onset has ranged from a few weeks to approximately one year after initiating treatment.

Pulmonary System Pneumonia, a frequent manifestation of infection in CLL patients, occurred in 16%, and 22% of those treated with FLUDARA FOR INJECTION in the MDAH and SWOG studies, respectively. Pulmonary hypersensitivity reactions to FLUDARA FOR INJECTION characterized by dyspnea, cough and interstitial pulmonary infiltrate have been observed.

In post-marketing experience, cases of severe pulmonary toxicity have been observed with Fludara use which resulted in ARDS, respiratory distress, pulmonary hemorrhage, pulmonary fibrosis, and respiratory failure. After an infectious origin has been excluded, some patients experienced symptom improvement with corticosteroids.

Gastrointestinal System Gastrointestinal disturbances such as nausea and vomiting, anorexia, diarrhea, stomatitis and gastrointestinal bleeding have been reported in patients treated with FLUDARA FOR INJECTION.

Cardiovascular Edema has been frequently reported. One patient developed a pericardial effusion possibly related to treatment with FLUDARA FOR INJECTION. No other severe cardiovascular events were considered to be drug related.

Genitourinary System Rare cases of hemorrhagic cystitis have been reported in patients treated with FLUDARA FOR INJECTION.

Skin Skin toxicity, consisting primarily of skin rashes, has been reported in patients treated with FLUDARA FOR INJECTION.

Erythema multiforme, Stevens-Johnson syndrome, toxic epidermal necrolysis, and pemphigus have been reported, with fatal outcomes in some cases.

Worsening or flare up of pre-existing skin cancer lesions, as well as new onset of skin cancer, has been reported in patients during or after treatment with FLUDARA FOR INJECTION.

Data in the following table are derived from the 133 patients with CLL who received FLUDARA FOR INJECTION in the MDAH and SWOG studies.

PERCENT OF CLL PATIENTS REPORTING NON-HEMATOLOGIC ADVERSE EVENTS

ADVERSE EVENTS	MDAH (N=101)	SWOG (N=32)
ANY ADVERSE EVENT	88%	91%
BODY AS A WHOLE	72	84
FEVER	60	69
CHILLS	11	19
FATIGUE	10	38

PERCENT OF CLL PATIENTS REPORTING NON-HEMATOLOGIC ADVERSE EVENTS

ADVERSE EVENTS	MDAH (N=101)	SWOG (N=32)
INFECTION	33	44
PAIN	20	22
MALAISE	8	6
DIAPHORESIS	1	13
ALOPECIA	0	3
ANAPHYLAXIS	1	0
HEMORRHAGE	1	0
HYPERGLYCEMIA	1	6
DEHYDRATION	1	0
NEUROLOGICAL	21	69
WEAKNESS	9	65
PARESTHESIA	4	12
HEADACHE	3	0
VISUAL DISTURBANCE	3	15
HEARING LOSS	2	6
SLEEP DISORDER	1	3
DEPRESSION	1	0
CEREBELLAR SYNDROME	1	0
IMPAIRED MENTATION	1	0
PULMONARY	35	69
COUGH	10	44
PNEUMONIA	16	22
DYSPNEA	9	22
SINUSITIS	5	0
PHARYNGITIS	0	9
UPPER RESPIRATORY INFECTION	2	16
ALLERGIC PNEUMONITIS	0	6
EPISTAXIS	1	0
HEMOPTYSIS	1	6
BRONCHITIS	1	0
HYPOXIA	1	0
GASTROINTESTINAL	46	63
NAUSEA/VOMITING	36	31
DIARRHEA	15	13
ANOREXIA	7	34
STOMATITIS	9	0
GI BLEEDING	3	13
ESOPHAGITIS	3	0
MUCOSITIS	2	0
LIVER FAILURE	1	0
ABNORMAL LIVER FUNCTION TEST	1	3
CHOLELITHIASIS	0	3
CONSTIPATION	1	3

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PERCENT OF CLL PATIENTS REPORTING NON-HEMATOLOGIC ADVERSE EVENTS

ADVERSE EVENTS	MDAH (N=101)	SWOG (N=32)
DYSPHAGIA	1	0
CUTANEOUS	17	18
RASH	15	15
PRURITUS	1	3
SEBORRHEA	1	0
GENITOURINARY	12	22
DYSURIA	4	3
URINARY INFECTION	2	15
HEMATURIA	2	3
RENAL FAILURE	1	0
ABNORMAL RENAL FUNCTION TEST	1	0
PROTEINURIA	1	0
HESITANCY	0	3
CARDIOVASCULAR	12	38
EDEMA	8	19
ANGINA	0	6
CONGESTIVE HEART FAILURE	0	3
ARRHYTHMIA	0	3
SUPRAVENTRICULAR TACHYCARDIA	0	3
MYOCARDIAL INFARCTION	0	3
DEEP VENOUS THROMBOSIS	1	3
PHLEBITIS	1	3
TRANSIENT ISCHEMIC ATTACK	1	0
ANEURYSM	1	0
CEREBROVASCULAR ACCIDENT	0	3
MUSCULOSKELETAL	7	16
MYALGIA	4	16
OSTEOPOROSIS	2	0
ARTHRALGIA	1	0
TUMOR LYSIS SYNDROME	1	0

More than 3000 adult patients received FLUDARA FOR INJECTION in studies of other leukemias, lymphomas, and other solid tumors. The spectrum of adverse effects reported in these studies was consistent with the data presented above.

OVERDOSAGE

High doses of FLUDARA FOR INJECTION (see WARNINGS section) have been associated with an irreversible central nervous system toxicity characterized by delayed blindness, coma and death. High doses are also associated with severe thrombocytopenia and neutropenia due

to bone marrow suppression. There is no known specific antidote for FLUDARA FOR INJECTION overdosage. Treatment consists of drug discontinuation and supportive therapy.

DOSAGE AND ADMINISTRATION

Usual Dose

The recommended adult dose of FLUDARA FOR INJECTION is 25 mg/m^2 administered intravenously over a period of approximately 30 minutes daily for five consecutive days. Each 5 day course of treatment should commence every 28 days. Dosage may be decreased or delayed based on evidence of hematologic or nonhematologic toxicity. Physicians should consider delaying or discontinuing the drug if neurotoxicity occurs.

A number of clinical settings may predispose to increased toxicity from FLUDARA FOR INJECTION. These include advanced age, renal insufficiency, and bone marrow impairment. Such patients should be monitored closely for excessive toxicity and the dose modified accordingly.

The optimal duration of treatment has not been clearly established. It is recommended that three additional cycles of FLUDARA FOR INJECTION be administered following the achievement of a maximal response and then the drug should be discontinued.

Renal Insufficiency

Adult patients with moderate impairment of renal function (creatinine clearance 30-70 mL/min/1.73 m^2) should have a 20% dose reduction of FLUDARA FOR INJECTION. FLUDARA FOR INJECTION should not be administered to patients with severely impaired renal function (creatinine clearance less than 30 mL/min/1.73 m^2).

Preparation of Solutions

FLUDARA FOR INJECTION should be prepared for parenteral use by aseptically adding Sterile Water for Injection USP. When reconstituted with 2mL of Sterile Water for Injection, USP, the solid cake should fully dissolve in 15 seconds or less; each mL of the resulting solution will contain 25 mg of fludarabine phosphate, 25 mg of mannitol, and sodium hydroxide to adjust the pH to 7.7. The pH range for the final product is 7.2-8.2. In clinical studies, the product has been diluted in 100 cc or 125 cc of 5% Dextrose Injection USP or 0.9% Sodium Chloride USP.

Reconstituted FLUDARA FOR INJECTION contains no antimicrobial preservative and thus should be used within 8 hours of reconstitution. Care must be taken to assure the sterility of prepared solutions. Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration.

FLUDARA FOR INJECTION should not be mixed with other drugs.

Handling and Disposal

Procedures for proper handling and disposal should be considered. Consideration should be given to handling and disposal according to guidelines issued for cytotoxic drugs. Several guidelines on this subject have been published. [1-4]

Caution should be exercised in the handling and preparation of FLUDARA FOR INJECTION solution. The use of latex gloves and safety glasses is recommended to avoid exposure in case of breakage of the vial or other accidental spillage. If the solution contacts the skin or mucous membranes, wash thoroughly with soap and water; rinse eyes thoroughly with plain water. Avoid exposure by inhalation or by direct contact of the skin or mucous membranes.

HOW SUPPLIED

FLUDARA FOR INJECTION is supplied as a white, lyophilized solid cake. Each vial contains 50 mg of fludarabine phosphate, 50 mg of mannitol, and sodium hydroxide to adjust pH to 7.7. The pH range for the final product is 7.2-8.2. Store under refrigeration, between 2°-8°C (36°-46°F).

FLUDARA FOR INJECTION is supplied in a clear glass single dose vial (6mL capacity) and packaged in a single dose vial carton in a shelf pack of five.

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Manufactured for: Bayer HealthCare Pharmaceuticals Inc., Wayne, NJ 07470

U.S. Patent Number: 4,357,324

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4. Polvich, M., White, J.M., & Kelleher, L.O. (eds.) 2005. Chemotherapy and biotherapy guidelines and recommendations for practice (2nd ed.). Pittsburgh, PA: Oncology Nursing Society