CLINICAL PROTOCOL

TITLE OF STUDY:

A Phase 1B Study to Evaluate the Safety and Induction of Immune Response of CRS-207 in Combination with Pemetrexed and Cisplatin as Front-line Therapy in Adults with Malignant Pleural Mesothelioma

Protocol ID: **ADU-CL-02**

Sponsor: **Aduro Biotech, Inc.**
740 Heinz Avenue
Berkeley, CA 94710-2224
Phone: 510-848-4400

IND: **13,389**

Date of Issue: **08 March 2018, Version 8**

NCT Number: **NCT01675765**

Signatures of Approval for Protocol (Version 8)

Affiliation	Name	Signature	Date:
Sponsor:	Natalie Sacks, MD	Natalie Sacks	08 Mar 2018

This study is to be performed in accordance with Good Clinical Practice, the ethical principles that have their origin in the Declaration of Helsinki: Title 21 of the Code of Federal Regulations (CFR) Parts 50 (Protection of Human Subjects), 56 (IRBs), and 312 (INDA); and ICH E6 (Guideline for Good Clinical Practice).

Study Sponsor:	Aduro Biotech, Inc. 740 Heinz Avenue Berkeley, CA 94710 Phone: 510-848-4400 Fax: 510-848-5614
Lead Investigator:	[redacted]

STUDY SYNOPSIS

<table>
<tr><td>Name of Sponsor Company:
Aduro Biotech, Inc.</td><td rowspan="3">Individual Study Table Referring to Part of the Dossier

Volume:

Page:</td><td rowspan="3">(For National Authority Use Only)</td></tr>
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CRS-207; pemetrexed; cisplatin; cyclophosphamide</td></tr>
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<tr><td colspan="3">Primary Investigator: Raffit Hassan, M.D.</td></tr>
<tr><td colspan="3">Number of Sites: Up to 7</td></tr>
<tr><td>Phase of Development: Phase 1B</td><td>Treatment Period: 25 Weeks</td><td>Study Duration: 7 years</td></tr>
<tr><td colspan="3">Objectives:
Primary: To determine the safety of CRS-207 (with or without low-dose cyclophosphamide [Cy] given one day prior CRS-207) when administered in combination with pemetrexed and cisplatin and to evaluate the induction of immune response to mesothelin as measured by IFN-gamma ELISPOT assay prior to treatment and at time points during and after treatment.
Secondary: To evaluate objective tumor response, progression free survival, time to progression, overall survival, and the predictive value of serum mesothelin for therapeutic response for each treatment regimen.
Exploratory: i) conduct immune subset analysis (e.g. CD4, CD8, T_{reg}) and gene expression profiling of tumor tissue pre- and post-vaccination; ii) to assess induction of anti-mesothelin humoral immune response; iii) to measure tumor marker kinetics as biomarkers of tumor response; iv) evaluate the association between pulmonary function improvement and tumor response; v) evaluate the relationship of tumor response to overall survival.</td></tr>
<tr><td colspan="3">Methodology:
This is a phase 1B study to evaluate the safety and induction of immune response of CRS-207 (with or without Cy) in combination with pemetrexed and cisplatin in front-line therapy of adults with malignant pleural mesothelioma (MPM).

Up to 16 subjects were originally planned to be enrolled in this study. Up to an additional 44 subjects will be enrolled in an expansion phase of this study (for a total of up to 60 subjects) to obtain additional safety, immune and efficacy data. Subjects will be enrolled into 2 mutually exclusive cohorts described below.

Cohort 1: At least 32 subjects will receive 2 infusions (prime vaccinations; PV) of CRS-207 (1×10^9 CFU given intravenously [i.v.] over approximately 1 hour) 2 weeks apart followed 2 weeks later by up to 6 cycles of pemetrexed and cisplatin 3 weeks apart. Three weeks after completion of chemotherapy, subjects will receive an additional 2 infusions (boost vaccinations; BV) of CRS-207 3 weeks apart. Subjects enrolled in Cohort 1 include the 16 subjects originally planned plus a minimum of the first 16 subjects enrolled into the expansion phase.

Cohort 2: The remaining subjects enrolled in the expansion phase (up to 28 total) will receive CRS-207 in combination with chemotherapy at the dose and schedule described above, however, these subjects will also receive low-dose Cy (200 mg/m^2) intravenously over 30 minutes one day prior to each CRS-207 infusion (i.e. prior to each PV and BV infusion). Low-dose Cy inhibits regulatory T cells and enhances immune responses when given in context with vaccines[1-3]. Preclinical studies of low-dose Cy given prior to CRS-207 in mouse tumor models have shown improved immunogenicity and survival (Section 1.3).

All subjects will return to the clinic 4 weeks after their 2^{nd} boost vaccination for an End of Course (EOC) visit. Subjects will have follow-up visits 4 weeks after the EOC visit and every 8 weeks thereafter until treatment</td></tr>
</table>

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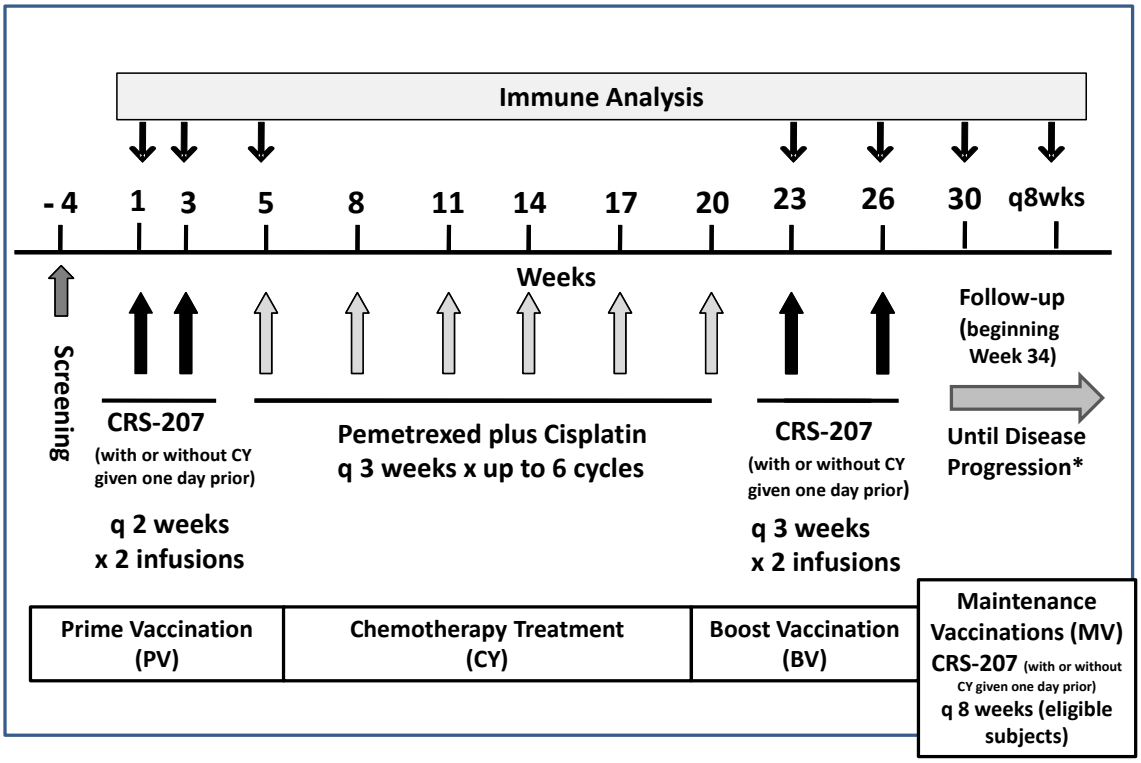
discontinuation. Subjects who continue to meet dosing eligibility will receive additional CRS-207 infusions (maintenance vaccinations; MV), with or without Cy following their original schedule, at every follow-up visit. Subjects may continue on treatment with radiographic disease progression if subject is clinically stable and investigator believes the treatment may be providing benefit.

See figure below for dosing schedule (based on full regimen of 6 chemotherapy cycles).



*Or investigator believes subject is no longer receiving benefit if treatment continued beyond radiographic progression

Safety and follow-up:
The initial safety of CRS-207 (with or without Cy), pemetrexed and cisplatin treatment will be monitored closely. Subjects will be followed for AEs and dose-limiting toxicities (Section 3.6) which may warrant either CRS-207 dose reduction or halting treatment after review by the lead investigator, sponsor and medical monitor. [redacted] All subjects will complete an End of Study (EOS) visit no more than four weeks following the final dose of study medication or prior to receipt of other cancer-related

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treatment. [redacted]

Subjects will return to the study site for an End of Course (EOC) visit approximately 4 weeks after their 2nd boost vaccination for safety, clinical response, and immune response evaluations. Thereafter, subjects will have follow-up visits 4 weeks after the EOC visit and every 8 weeks thereafter until treatment discontinuation. Subjects will continue to receive maintenance vaccinations (MV) with (Cohort 2) or without Cy (Cohort 1) following their original schedule at each follow-up visit if they are clinically stable and continue to meet dosing eligibility.

Number of subjects (planned):
Up to 16 subjects with MPM who meet all eligibility criteria were originally planned to be enrolled in the study. Up to 44 additional subjects (for a total of 60 subjects) with MPM who meet all eligibility criteria will be enrolled in an expansion phase of the study.

Subject discontinuation:
A subject may be removed from the study for the following reasons:
(1) Occurrence of an adverse event that presents an unacceptable consequence or risk to the subject
(2) Development of an illness or complication (including progressive disease) that justifies withdrawal from the study, as determined by the investigator and medical monitor
(3) Noncompliance: failure to receive clinical study medication or treatment as mandated by the protocol, or failure to comply with protocol requirements
(4) Study discontinued by the sponsor

Subjects who withdraw consent or are removed from the study before completing at least 4 cycles of chemotherapy may be replaced at the discretion of the investigator and Aduro Biotech, Inc. (Aduro). Subjects may continue on treatment with radiographic disease progression if subject is clinically stable and the investigator believes the treatment is providing benefit.

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Inclusion criteria: Subjects must meet all of the following criteria to be eligible to participate in the study:

(1) Subjects must have histologically-confirmed epithelial or biphasic pleural mesothelioma not amenable to potentially curative surgical resection at the time of study entry (subjects with biphasic tumors that have a predominantly (≥50%) sarcomatoid component will be excluded)
(2) Measurable disease, as defined by modified RECIST for MPM [4]
(3) Eastern Cooperative Oncology Group (ECOG) Performance Status of 0 or 1
(4) Normal organ and marrow function, as defined by:
- ANC ≥ 1,500/µl
- Platelet count ≥ 100,000/µl
- Bilirubin ≤ 1.5 × upper limit of normal (ULN), or bilirubin ≤ 3× ULN if due to Gilbert's disease
- AST and ALT ≤ 2.5× ULN
- Albumin ≥ 2.5 g/dL
- Creatinine clearance ≥ 50 mL/min per Cockcroft-Gault formula (Creatinine Clearance = {[(140-age) x weight in kilograms] x [0.85 if female]}/[72 x creatinine in mg/dL])
- CD4 ≥ 0.2×10^9/L
- Hemoglobin ≥9.0 g/dL

(5) Age 18 years and over
(6) Must have pulmonary function testing with documented values for VC and $FEV_1 \geq 45\%$ of predicted value and oxygen saturation ≥ 90% on room air, as measured by pulse oximeter
(7) Anticipated life expectancy greater than 6 months
(8) Female subjects of childbearing potential must consent to use a medically acceptable method of highly effective contraception (oral hormonal contraceptive, condom plus spermicide, or hormone implants) throughout the study period and for 28 days after their final study drug administration (A barrier method of contraception must be employed by all subjects (male and female), regardless of other methods)
(9) Subject provides informed consent and is willing and able to comply with all study procedures

Exclusion criteria: Subjects must not meet any of the following criteria to be eligible to participate in the study:

(1) Be a candidate for curative surgery at the time of study entry
(2) Have had surgery or pleurodesis within 2 weeks prior to dosing
(3) Have had prior radiotherapy (except palliative extra-thoracic localized radiotherapy) or biologic therapy for MPM within 3 weeks prior to dosing
(4) Have had prior systemic chemotherapy for MPM. Prior intracavitary cytotoxic drugs or immunomodulators are not permitted, unless given for the purpose of pleurodesis
(5) Have an active malignancy with the exception of any of the following:
- adequately treated basal cell carcinoma
- squamous cell carcinoma of the skin, or in situ cervical cancer
- adequately treated Stage I cancer from which the subject is currently in remission and has been in remission for ≥3 years
- Stage I prostate cancer that does not require treatment
- any other cancer from which the subject has been disease-free for ≥ 2 years

(6) Have documented and ongoing central nervous system (CNS) involvement with their malignant disease [history of CNS involvement is not an exclusion criterion but the CNS metastases should have been adequately treated

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(radiation or surgical resection) and subjects are neurologically stable for 3 months off steroids]

(7) Have any evidence of clinically significant ascites
(8) Have clinically significant pleural effusion
(9) Have clinically significant heart disease (such as uncontrolled angina, myocardial infarction within 3 months, congestive heart failure of New York Heart Association III or IV)
(10) Have valvular heart disease that requires antibiotic prophylaxis for prevention of endocarditis, consistent with American Heart Association (AHA) guidelines
(11) History of any autoimmune disease, including patients with a history of inflammatory bowel disease, (including ulcerative colitis and Crohn's Disease), patients with a history of symptomatic disease (e.g. rheumatoid arthritis, systemic progressive sclerosis [scleroderma], systemic lupus erythematosus, autoimmune vasculitis [e.g. Wegener's granulomatosis]); central nervous system or motor neuropathy considered of autoimmune origin (e.g. Guillain-Barre Syndrome, Myasthenia gravis, multiple sclerosis). Patients with Graves', Hashimoto's Disease, vitiligo, and diabetes type 1 will be allowed
(12) Have any immunodeficiency disease or immune-compromised state (e.g. use of immunosuppressive agents) within 28 days of study treatment
(13) Baseline coagulopathy > Grade 3 unless due to anticoagulant therapy
(14) Peripheral neuropathy ≥ Grade 2
(15) Use of systemically-active steroids within 14 days of study treatment
(16) Use of more than 3 grams per day of acetaminophen
(17) Use of aspirin or other nonsteroidal anti-inflammatory drugs for 2 days before, during, and for 2 days after each administration of pemetrexed disodium (5 days before, during, and 2 days after each administration of pemetrexed disodium for piroxicam, naproxen, diflunisal, or nabumetone)
(18) Have received an investigational product within 28 days of study treatment
(19) Have a known allergy to both penicillin and sulfa drugs
(20) Have known or suspected allergy or hypersensitivity to yeast or any other component of CRS-207 [redacted] Platinol or platinum-containing compounds, or pemetrexed
(21) Have received a diagnosis of HIV, hepatitis B, or hepatitis C (exception: clear evidence of natural immunity, immunity subsequent to vaccination, or successful eradication of the virus following antiviral therapy)
(22) Be a woman who is pregnant or breastfeeding
(23) Have prosthetic heart valves; major implant(s) or device(s) (e.g. artificial joints and prosthetics) placed within 12 months of study screening; or current or prior history of infection or clinically significant adverse events (AEs) associated with an exogenous implant(s) or device(s) that cannot be easily removed
(24) Have had major surgery or significant traumatic injury occurring within 28 days prior to CRS-207 administration or anticipated surgery or procedure requiring general anesthesia or deep sedation during study participation (including 28 days after last dose of CRS-207) (Minor procedures [dental work, skin biopsy, etc.] celiac plexus block, and biliary stents are allowed.)

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Exclusion criteria, continued:

(25) Have an unhealed wound which increases risk for bacterial infection
(26) Be unable to avoid intimate contact with another individual known to be at high risk of listeriosis (e.g. newborn infant, pregnant female, etc.) for at least 7 days after receiving a CRS-207 infusion
(27) Have an intercurrent illness that is either life-threatening or of clinical significance such that it might limit compliance with study requirements (including, but not limited to, ongoing or active infection, metabolic (e.g. diabetes) or neurologic disease, peripheral vascular disease, or psychiatric illness/social situations)
(28) Have insufficient peripheral venous access to permit completion of the study dosing and compliance with study phlebotomy regimen
(29) Have a history of alcohol dependence or use of illicit drugs that could potentially interfere with adherence to study procedures or requirements (e.g. opioids, cocaine, amphetamines, hallucinogens, etc.)

Dose eligibility:

Subjects must have adequate organ function as defined by the laboratory values in Table 1 before dosing of CRS-207. Laboratory tests may be done up to 3 days prior to CRS-207 dosing or 2 days prior to Cy for subjects receiving Cy. Delayed doses will be discussed with the medical monitor.

Table 1. Dosing-Eligibility Requirements

Hematologic	Renal	Hepatic
ANC ≥1,500/μL Platelets ≥100,000/μl Hemoglobin ≥9 g/dL CD4 ≥ 0.2×10^9/L prior to 1st post-chemotherapy CRS-207 boost vaccination	Creatinine ≤2.0 × ULN	AST/ALT ≤2.5× ULN Bilirubin ≤1.5× ULN or bilirubin ≤ 3× ULN if due to Gilbert's disease

ALT = alanine aminotransferase; ANC = absolute neutrophil count; AST = aspartate aminotransferase; ULN = upper limit of normal; WBC = white blood cell.
* Cockcroft-Gault formula: Creatinine Clearance = {[(140-age) x weight in kilograms] x [0.85 if female]}/[72 x creatinine in mg/dL]

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Prohibited medications:
The following therapies are not permitted during the study and may result in early termination of the subject from treatment:

- Non-study chemotherapy or immunotherapy (approved or investigational)
- Systemically active steroids for more than 3 days or use of any systemic steroids within 14 days before or after dosing, with exception of study-prescribed steroids.
- TNF pathway inhibitors or PI3 kinase inhibitors
- Any other investigational product

In addition, the following therapies should not be administered unless medically necessary, and approval must be obtained from the medical monitor for a subject to continue dosing if therapy is given concurrently with study participation:

- General anesthesia or deep sedation
- Aspirin >325 mg/d (chronic daily use of aspirin ≤325 mg/d and heparin flushes for central lines are allowed)
- More than 4 g/d of acetaminophen
- Systemic antibiotics

[redacted]

Filgrastim (Neupogen or G-CSF) or Sargramostin (Leukine or GM-CSF) should not be administered 14 days prior to or 14 days after any CRS-207 dose. Approval must be obtained from the medical monitor for a subject to continue dosing if administered within this timeframe.

Prophylactic vaccines (e.g. pneumococcal vaccine, influenza vaccine) should not be administered 28 days prior to or 28 days after any CRS-207 dose. Approval must be obtained from the medical monitor for a subject to continue dosing if a prophylactic vaccine is administered within this timeframe.

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Test product, dose, and mode of administration:
CRS-207 [Cohort 1 and Cohort 2]: CRS-207, live-attenuated *Lm* encoding human mesothelin, will be given i.v. over approximately 1 hour at 1×10^9 colony-forming units (CFU); reconstituted in approximately 100 mL 0.9% sodium chloride for 2 infusions (prime vaccinations) 2 weeks apart prior to receiving pemetrexed and cisplatin chemotherapy, and for 2 infusions (boost vaccinations) 3 weeks apart following chemotherapy. Subjects who continue to meet dosing eligibility will receive maintenance vaccinations of CRS-207 beginning at the first follow-up visit and every 8 weeks thereafter during follow-up phase.
Pemetrexed [Cohort 1 and Cohort 2]: ALIMTA®, pemetrexed disodium heptahydrate (pemetrexed), has a recommended dose of 500 mg/m^2 body surface area (BSA) in 100mL 0.9% sodium chloride administered over 10 minutes as an intravenous infusion on Day 1 of a 21-day cycle. Up to 6 cycles of pemetrexed and cisplatin will be given after the initial 2 infusions of CRS-207.
Cisplatin [Cohort 1 and Cohort 2]: Cisplatin, USP (cis-diamminedichloroplatinum), has a recommended dose of 75 mg/m^2 BSA in 1L 0.9% sodium chloride with mannitol 30g/L infused over 2 hours, beginning approximately 30 minutes after the end of pemetrexed administration on Day 1 of each 21-day cycle. Up to 6 cycles of pemetrexed and cisplatin will be given after the initial 2 infusions of CRS-207.
Cyclophosphamide [Cohort 2]: 200 mg/m^2 in 100 mL normal saline administered by i.v. infusion over 30 minutes one day prior to CRS-207.

Study duration:
The duration of one full treatment course [i.e. CRS-207 with or without Cy (2 infusions, 2 weeks apart), pemetrexed (500 mg/m^2) and cisplatin (75 mg/m^2) every 3 weeks × 6 cycles, CRS-207 with or without Cy (2 infusions, 3 weeks apart)] is 25 weeks. Subjects enrolled in Cohort 1 will receive CRS-207 and pemetrexed and cisplatin per the schedule described above. Subjects enrolled in Cohort 2 will receive Cy/CRS-207 and pemetrexed and cisplatin per the schedule described above. Clinical and immune response assessments will be performed 4 weeks after EOC. Eligible subjects will receive maintenance vaccinations of CRS-207 (with or without Cy) beginning at the first follow-up visit and every visit thereafter.

Reference therapy, dose, and mode of administration:
There is no reference therapy or placebo administered in this study.

Criteria for Evaluation:
Safety: Safety will be assessed by collection of data on AEs, vital signs, physical examination, clinical hematology, serum chemistry, deaths, and blood cultures for CRS-207 clearance.
Efficacy: Induction of immune response to mesothelin will be evaluated by IFN-gamma ELISPOT assay prior to treatment and at time points during and after treatment. Other evaluations will include immune subset analysis (e.g. CD4, CD8, T_{reg}) by immunohistochemistry and gene expression profiling of tumor tissue pre and post vaccination, evaluating induction of anti-mesothelin humoral immune response by ELISA, assessing tumor marker kinetics by measuring serum levels of mesothelin and CA-125 and plasma osteopontin. Objective tumor response, progression free survival and time to progression will be measured using modified RECIST for assessment of response in MPM (Appendix A) and immune-related response criteria (irRC; Appendix B). Pulmonary function will be measured by forced vital capacity (FVC), forced expiratory volume in 1 second (FEV1) and vital capacity (VC).

Data analysis:
The sample size of the study was originally planned to enroll up to 16 subjects in order to evaluate the induction of

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immune response to mesothelin and initial safety of CRS-207 combined with pemetrexed and cisplatin chemotherapy. Up to 44 additional subjects will be enrolled in an expansion phase to obtain additional safety, immune and preliminary efficacy data. At least 32 subjects will be enrolled in Cohort 1 and up to 28 subjects will be enrolled in Cohort 2. The expansion cohort of up to 44 additional subjects, including subjects receiving Cy one day prior to CRS-207 (Cohort 2) is intended to obtain additional safety, immune and efficacy data for future study planning. The expansion sample size is based on practical rather than statistical considerations.

These data will be summarized in tables listing the mean, standard deviation, median, minimum, maximum, and number of subjects in a group for continuous data; in tables listing count and percentage for categorical data; and median and standard error for time-to-event data. Data will be listed for each subject. Statistical analyses will be performed and data appendices will be created by using SAS. The effects of noncompliance, dropouts, and covariates will be assessed to determine the impact on the general applicability of results from this study.

Efficacy analyses will be conducted on the full analysis set and per protocol analysis set. The full analysis set will consist of all subjects who receive at least one dose of any study treatment. The per protocol analysis set will consist of all subjects with MPM who receive the initial 2 prime vaccinations with CRS-207 (with or without Cy) and at least 4 cycles of pemetrexed and cisplatin.

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LIST OF APPENDICES

LIST OF ABBREVIATIONS

ActA	*Listeria monocytogenes* protein encoded by *actA* gene
AE	adverse event
ALT	alanine aminotransferase
ANC	absolute neutrophil count
APTT	activated partial thromboplastin time
AST	aspartate aminotransferase
BP	blood pressure
BSA	body surface area
BUN	blood urea nitrogen
CBC	complete blood count
CFR	code of federal regulations
CFU	colony-forming unit
CRF	case report form
CT	computed tomography
CTCAE	common terminology criteria for AEs
CTM	clinical trial material
Cy	cyclophosphamide
DLTs	dose-limiting toxicities
DTH	delayed type hypersensitivity
ECOG	Eastern Cooperative Oncology Group
EDC	electronic data capture
ELISPOT	enzyme-linked immunosorbent spot
FDA	Food and Drug Administration
FDG-PET	fluorodeoxyglucose positron emission tomography
FEV1	forced expiratory volume in 1 second
FNA	fine needle aspiration
GM-CSF	granulocyte-macrophage colony-stimulating factor
HIV	human immunodeficiency virus
HLA	human leukocyte antigen
hMeso	human mesothelin
IB	Investigator's Brochure
IBC	institutional biosafety committee
ICF	informed consent form
ICH	international conference on harmonisation
InlB	protein encoded by *inlB* gene
IRB	institutional review board
irRC	immune-related response criteria
i.v.	intravenous
LDH	lactate dehydrogenase
Lm	*Listeria monocytogenes*
MPM	malignant pleural mesothelioma
MRI	magnetic resonance imaging
MTD	maximum tolerated dose
NCI	National Cancer Institute
NIH OBA	National Institutes of Health Office of Biotechnology Activities
PBMC	peripheral blood mononuclear cells
PHI	protected health information
p.o.	per oral
PT	prothrombin time
RECIST	response evaluation criteria in solid tumors
SAE	serious adverse event
SAP	statistical analysis plan
SOP	standard operating procedure
SPM	study procedures manual
UA	urinalysis
ULN	upper limit of normal
VC	vital capacity
WBC	white blood cell

1.0 INTRODUCTION

1.1 BACKGROUND

Malignant mesothelioma is a rare tumor that arises from mesothelial cells. Approximately 2,000 to 4,500 new cases of mesothelioma are diagnosed in the United States each year.[5,6] Pleural mesothelioma is most common, but mesothelioma can also occur in the peritoneum and pericardium. In the United States, the majority of mesotheliomas occur in men (approximately 80%). These are most often of pleural origin, and 90% or more of these tumors are caused by asbestos.[6-8]

1.1.1 Current Therapies for Malignant Mesothelioma

The best-documented, potentially curative approach to malignant pleural mesothelioma (MPM) has been extrapleural pneumonectomy, followed by chemotherapy and radiotherapy in selected patients with earlier stages of disease,[6,9] but the majority of patients present with advanced disease and are not candidates for surgical resection. For patients whose disease is unresectable or who otherwise are not candidates for curative surgery, pemetrexed in combination with cisplatin is the standard of care. In a randomized Phase 3 clinical trial treatment with pemetrexed and cisplatin led to an improvement in overall survival of 12.7 months compared to 9.3 months with cisplatin alone. The objective response rate was 41%.[10,11] It is clear that new therapies are needed, and immunotherapy offers the promise of improving outcomes in these patients by developing specific immune responses against tumor antigens. In support of this notion, the presence of high levels of CD8+ tumor-infiltrating lymphocytes is associated in better prognosis in patients undergoing extrapleural pneumonectomy for MPM.[12]

1.1.2 Mesothelin

Mesothelin is a cell surface tumor differentiation antigen present on normal mesothelial cells that is highly expressed in many human tumors including virtually all mesothelioma and pancreatic cancers, 55% to 71% of ovarian cancers and 41% to 55% of lung adenocarcinomas.[13-19] The limited distribution of mesothelin in normal human tissues and high expression in many cancers makes it an attractive candidate for antibody-targeted immunotherapy and clinical trials of anti-mesothelin antibodies are currently ongoing.[20,21] [redacted]

[redacted] have shown mesothelin to be also an attractive antigen for active cancer immunotherapy. Dr. Hassan and colleagues have previously shown that a mesothelin-specific IgG antibody response is present in 39% and 42% of patients with mesothelioma and ovarian cancer respectively, suggesting that immune tolerance to mesothelin can be overcome.[22] In addition, studies by Dr. Elizabeth Jaffee and colleagues have demonstrated that in patients with pancreatic carcinoma, positive clinical outcomes following vaccination with an irradiated allogeneic whole cell vaccine encoding granulocyte/macrophage-colony stimulating factor (GM-CSF; GVAX) correlated with induction of mesothelin-specific cellular immunity.[23,24] In a Phase 1 study, a vaccine with two allogeneic, GM-CSF-secreting pancreatic cell lines induced a dose-dependent delayed type hypersensitivity (DTH) response and CD8+ T cell response to multiple human leukocyte antigen (HLA)-A2, -A3, and –A24 restricted mesothelin epitopes exclusively in 3 of 14 patients. Patients with DTH and CD8+ T cell responses had increased disease-free survival, remaining disease-free at least 25 months after diagnosis. Additionally, Dr. Hassan in collaboration with Dr. Jeff Schlom at the National Cancer Institute (NCI) elucidated the immunogenic epitopes of mesothelin and showed that mesothelin-specific T cells cause lysis of mesothelioma tumor cells.[25] The mesothelin-targeting therapeutic cancer vaccine furthest along in clinical development is CRS-207, a live-attenuated strain of the bacterium *Listeria monocytogenes* (*Lm*) encoding human mesothelin (hMeso).[26,27]

1.1.3 *Listeria monocytogenes* (*Lm*)-Based Vaccine Therapy

Lm is an attractive platform for presentation of tumor-associated antigens and activation of immune response directed against cancer cells. *Lm* provides both a potent stimulation of innate immunity and also stimulates an adaptive immune response through recruitment and activation of CD4+ and CD8+ T-cell immunity specific for encoded heterologous antigens.[28-31] Aduro Biotech, Inc. (Aduro) developed a live-attenuated *Lm* platform strain (*Lm* Δ*actA*/Δ*inlB*), known as ANZ-100 (previously CRS-100), which has deletions of two genes, *actA* and *inlB*, that encode the virulence-determinant proteins ActA and Internalin B, respectively[27]. These two proteins facilitate cell-to-cell spread and invasion of nonphagocytic cells, and deletion of *actA* and *inlB* in ANZ-100 results in 1,000-fold attenuation of these processes in mice as compared with wild-type Lm.[32, 29] Uptake of ANZ-100 by macrophages and other phagocytic cells in the liver and spleen is still retained and results in a local inflammatory response as well as activation and recruitment of natural killer cells and T cells to the liver. ANZ-100 underwent clinical evaluation in a Phase 1 dose-escalation study in

adults with carcinoma and liver metastases and was found to be safe and well tolerated in 9 subjects given intravenous doses up to 3×10^8 colony-forming units (CFU). Additionally, no shedding of ANZ-100 in urine, stools, or sputum was observed at any dose.

ANZ-100 has additionally been engineered to express mesothelin and the resulting strain has been termed CRS-207. After uptake of CRS-207 by dendritic cells and macrophages, mesothelin is expressed and released into the cytosolic compartment and subsequently processed through the endogenous MHC Class I presentation pathway, resulting in activation of mesothelin-specific T cell-mediated immunity. Other mechanisms to activate mesothelin-specific, cell-mediated immunity may include uptake and cross-presentation of antigens by dendritic cells and other cells after infection by CRS-207 and apoptosis.

Nonclinical studies have shown that CRS-207 elicits mesothelin-specific cellular immunity in mice and nonhuman primates and demonstrates therapeutic efficacy in tumor-bearing mice (refer to the Investigator's Brochure [IB] for details). Findings in a Good Laboratory Practice repeated-dose study in cynomolgus monkeys showed that treatment with up to 3×10^{10} CFU of CRS-207 resulted in no changes related to body weight, food consumption or body temperature and in no findings related to ocular or functional cardiovascular evaluations. CRS-207 was detected in the blood at 24 hours after administration, but was undetectable at 72 hours. There were transient and dose-dependent decreases in red blood cell, platelet and white blood cell (WBC) counts. Hepatic and renal function changes were transient and generally less than two-fold from that at baseline status. A Phase 1 study (VAC07001; NCT00585845) has been completed with CRS-207 to determine the maximum tolerated dose and to explore the safety profile in subjects with advanced mesothelioma, non–small-cell lung cancer, ovarian cancer or pancreatic adenocarcinoma who had failed standard therapy.[26] CRS-207 was found to be well tolerated at doses up to 1×10^9 CFU. Adverse events (AEs) such as fevers, chills, and nausea reported as the most common, immediate, transient, mild, and temporally related to CRS-207 administration were self-correcting and were resolved by the time of the subjects' discharge. Lymphopenia was observed in all doses (1×10^8, 3×10^8, 1×10^9 and 1×10^{10} CFU), and transaminase elevations were observed at doses of 3×10^8, 1×10^9 and 1×10^{10} CFU. Both were dose-dependent, although transient and not considered clinically significant. Two CRS-207-related serious adverse events (SAEs) were reported. One SAE of moderate constipation occurred in one subject after the second dose of CRS-207 at 1×10^8 CFU. The second SAE, a significant decrease in blood pressure (BP)

after infusion, occurred in another subject after one dose of CRS-207 at 1×10^{10} CFU. This subject required aggressive fluid management and recovered to baseline status within 24 hours. This dose level was subsequently discontinued and confirmed the maximum tolerated dose (MTD) as 1×10^{9} CFU. No shedding of CRS-207 in urine or stools was observed at any dose.

While the VAC07001 study enrolled subjects with multiple disease types and was not powered to assess survival, 6 of 17 subjects survived for ≥ 15 months. Of these 6 "long-term" survivors, 3 had pancreatic cancer, 2 had non-small-cell lung cancer, and 1 had mesothelioma. All 6 subjects had prior immunotherapy or subsequent local radiation. Five of 6 subjects received all 4 doses of CRS-207 and all 5 evaluable subjects demonstrated vaccine-induced *Lm*-specific responses. In addition, 4 of the 5 evaluable subjects among the long-term survivors had stable disease by RECIST at day 91 (end of study).

As 3 of the 6 "long-term" survivors were pancreatic cancer patients had previously received GVAX pancreas vaccine in separate clinical studies, CRS-207 in combination with GVAX pancreas vaccine (with low-dose cyclophosphamide [Cy]) is currently being evaluated in a Phase 2 study in metastatic pancreatic cancer patients who have received or refused chemotherapy (Protocol ADU-CL-01; NCT01417000). Enrollment has been completed in this study and a recent interim analysis showed a significant improvement in median overall survival in subjects who received GVAX pancreas vaccine and CRS-207 compared to subjects who received GVAX pancreas vaccine alone (6.0 months vs. 3.4 months; one-sided p=0.0.0057). These interim data were reviewed by an independent data monitoring committee who recommended that the study be stopped for efficacy and subjects on the GVAX pancreas vaccine arm offered rollover treatment with GVAX pancreas vaccine and CRS-207. FDA concurred with this decision. Subjects in the study continue to receive treatment and all subjects are being followed for survival, safety and clinical and immune responses. Safety findings have been consistent with prior clinical experience. The most frequent Grade 3/4 related toxicities were fever, lymphopenia, hypophosphatemia, elevated liver enzymes and fatigue following CRS-207 in <5% of subjects. To date, there have been no treatment-related SAEs, significant laboratory abnormalities or safety signals.

Another Phase 2 study for pancreatic cancer, ADU-CL-04, was initiated in 2014. This study enrolls subjects with previously-treated metastatic pancreatic cancer for evaluation of GVAX pancreas vaccine (with low-dose Cy) and CRS-207

compared to CRS-207 alone or single-agent chemotherapy alone. As of February 2015, over 200 subjects have been enrolled and there have been three SAEs reported as unexpected and related to CRS-207: Grade 3 confusion, Grade 3 hypoxia and Grade 3 hypertension. A second Phase 2 study in previously-treated metastatic pancreatic cancer was initiated in January 2015 which is evaluating GVAX pancreas vaccine (with low-dose Cy) and CRS-207 with and without anti-PD-1 antibody (nivolumab) in 88 subjects. Six patients have been treated with no significant toxicities or SAEs reported.

1.2 RATIONALE FOR COMBINATION OF CRS-207 AND CHEMOTHERAPY

The combination of vaccines with chemotherapy has traditionally not been used due to the possibility of post-chemotherapy leukopenia eliminating antitumor directed lymphocytes. Emerging data, however, suggest that vaccines and chemotherapies may be synergistic by augmenting anti-tumor effectiveness of subsequent chemotherapies.[33,34] Proposed mechanisms of synergy for activating relatively dormant T cells include chemotherapy-induced alterations in tumor microenvironment, up-regulation of MHC class I and numerous tumor-associated antigens on the surface of tumor cells, reduction of suppressor cell populations and tumor lysis followed by cross priming.[35-37]

Synergistic activity of combinations of immunotherapy and chemotherapy is also supported by clinical studies. High chemotherapy response rates were observed in patients with small-cell lung cancer who developed an immunologic response to an Ad.p53-dendritic cell vaccine.[38] Advanced-stage cancer patients who developed immunity to cytochrome P4501B1 following administration of a plasmid/microparticle vaccine showed marked response to a subsequent chemotherapy regimen.[39] Patients with metastatic androgen independent prostate cancer who received recombinant viral vectors containing the PSA and B7.1 genes followed by docetaxel had a longer time to progression than patients who received docetaxel alone[40]. Glioblastoma patients vaccinated with autologous tumor-pulsed dendritic cell vaccine who received subsequent chemotherapy exhibited significantly longer times to tumor recurrence after chemotherapy relative to their own previous recurrence times, as well as significantly longer post-chemotherapy recurrence times and survival relative to patients receiving isolated vaccination or chemotherapy.[41,42] In mesothelioma patients, three vaccinations at two week intervals with autologous tumor lysate-pulsed dendritic

cells following chemotherapy with cisplatin/pemetrexed induced tumor specific CD8+ T lymphocyte activity in four of the six patients.[43]

CRS-207 has been shown to be safe and well-tolerated at the proposed route of administration and dose for this study.[26] Five subjects with mesothelioma were enrolled in the Phase 1 study and tolerated the treatment at doses up to 1×10^9 CFU. One subject with mesothelioma survived for 27 months post-first dose of CRS-207 in the Phase 1 study. The proposed dose level for this study (1×10^9 CFU) is the MTD as determined in the Phase 1 study and is the dose being administered in the current Phase 2 pancreatic cancer study. This dose level has shown an acceptable safety profile in the 12 patients treated thus far (in both Phase 1 and Phase 2). In addition, 3 of 4 evaluable patients at this dose level in the Phase 1 study demonstrated CRS-207-induced mesothelin-specific CD8+ T cell responses (as compared to 1/3 and 2/3 patients at lower dose levels) and 2 of 6 of the "long-term" survivors received CRS-207 at this dose level.

This study will be the first to investigate the safety and immunogenicity of CRS-207 with or without Cy when combined with standard of care chemotherapy in patients with malignant mesothelioma.

Safety data from the first 23 subjects treated on this study have shown no additive or synergistic toxicities when administering CRS-207 prior to or after pemetrexed and cisplatin. The most frequent AEs have been mild to moderate infusion reactions (e.g. nausea, chills, headache, and vomiting) as well as fatigue, anemia, decreased appetite, night sweats and lymphocyte count decreases related to CRS-207 and expected AEs related to chemotherapy dosing. No treatment-related unexpected Grade 3 or greater AEs, SAEs or deaths have occurred. Additionally, in the first 25 subjects evaluated for objective clinical responses post-study treatment, 15 had best overall response of partial response (PR), 7 had stable disease (SD), and 3 had progressive disease (PD). No subjects have discontinued treatment due to AEs related to CRS-207.

The initial safety and clinical response data warranted further evaluation in an expansion phase of this Phase 1B study and the original sample size was increased from 16 to 40. The sample size has been further increased to 60 in order to evaluate the use of low-dose Cy in this patient population and treatment regimen.

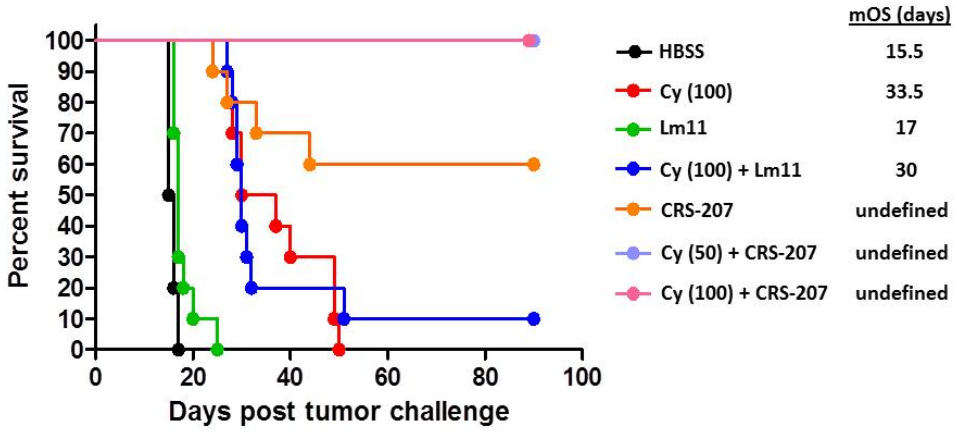
1.3 RATIONALE FOR USE OF LOW-DOSE CYCLOPHOSPHAMIDE AND CRS-207

Cyclophosphamide (Cy) is currently approved as a high-dose chemotherapy agent, however, when administered at low-doses, it has exhibited immune-modulatory effects, namely in the suppression of regulatory T cells (Tregs).[44] In the context of cancer vaccines, the preferential inhibition of Tregs may enhance vaccine-induced tumor-specific immunity, thereby improving vaccine efficacy.

Data suggest that immune-modulating doses of Cy may enhance vaccine-induced antitumor immune responses by inhibiting $CD4^+$ $CD25^+$ regulatory T cell activity. Several studies have demonstrated this concept and shown prolonged survival of patients receiving low-doses of Cy 24-72 hours prior to vaccination with minimal side effects.[1,2,45,46]

Increased vaccine-induced T cell responses and improved survival has been demonstrated in nonclinical studies of CRS-207 administered with immune-modulatory doses of Cy. In mice administered 50 mg/kg Cy (equivalent to 140 mg/m^2 human dose), immune responses measured seven days after administration of CRS-207 demonstrated that the combination of Cy and CRS-207 increased mesothelin-specific T cell responses compared to saline and CRS-207 alone. In a lung tumor model, tumor-bearing mice received 50 or 100 mg/kg Cy (corresponding to 140 to 280 mg/m^2 of the human dose equivalent, respectively) followed one day later with 2×10^6 CFU CRS-207 or ANZ-100 (the empty LADD platform strain, also named Lm11) and were monitored for survival over 90 days. The combination of low-dose Cy and CRS-207 increased the rate of survival to 100% compared to CRS-207 alone (60% survival) or Cy alone (0%, mOS = 33.5 days) (Figure 1). The combination of Cy and LADD-based vaccines has been shown to improve anti-tumor efficacy compared to LADD-vaccine alone in several models.

Figure 1. Cyclophosphamide Followed by CRS-207 Improves Vaccine Potency.



Female Balb/c mice (n=10) were implanted i.v. with 2×10^5 CT26 tumor cells expressing mesothelin. Two days later, mice were administered 50 or 100 mg/kg Cy. One day later, mice were vaccinated i.v. with 2×10^6 CFU CRS-207 or Lm11 (empty LADD platform). Mice that received only saline (HBSS) were included as controls. Animals were monitored for survival. mOS = median overall survival.

2.0 OBJECTIVES

2.1 PRIMARY OBJECTIVE

To determine the safety of CRS-207 (with or without Cy) when administered in combination with pemetrexed and cisplatin and to evaluate the induction of immune response to mesothelin as measured by IFN-γ enzyme-linked immunosorbent spot (ELISPOT) assay prior to treatment and at time points during and after treatment.

2.2 SECONDARY OBJECTIVES

To evaluate objective tumor response, progression free survival, time to progression, overall survival and the predictive value of serum mesothelin for therapeutic response for each treatment regimen.

2.3 EXPLORATORY OBJECTIVES

i) to conduct immune subset analysis (e.g. CD4, CD8, T_{reg}) and gene expression profiling of tumor tissue pre- and post-vaccination; ii) to assess induction of anti-mesothelin humoral immune response; iii) to measure tumor marker kinetics as biomarkers of tumor response; iv) evaluate the association between improvement in pulmonary function and tumor response; v) evaluate the relationship of tumor response to overall survival.

3.0 STUDY DESIGN

3.1 BASIC DESIGN CHARACTERISTICS

This is a phase 1B study to evaluate the safety and induction of immune response of CRS-207 with or without low-dose Cy in combination with pemetrexed and cisplatin in front-line therapy in subjects with MPM.

Up to 16 subjects were originally planned to be enrolled in this study. Up to an additional 44 subjects will be enrolled in an expansion phase of this study (for a total of up to 60 subjects) to obtain additional safety, immune and efficacy data. Subjects will be enrolled into 2 mutually exclusive cohorts described below.

Cohort 1: At least 32 subjects will receive 2 prime vaccinations (PV) of CRS-207 (1×10^9 CFU given i.v. over approximately 1 hour) 2 weeks apart followed 2 weeks later by up to 6 cycles of pemetrexed and cisplatin 3 weeks apart. Three weeks after completion of chemotherapy, subjects will receive an additional 2 infusions (boost vaccinations; BV) of CRS-207 3 weeks apart (Figure 1). Subjects enrolled in Cohort 1 include the 16 subjects originally planned plus a minimum of the first 16 subjects enrolled into the expansion phase.

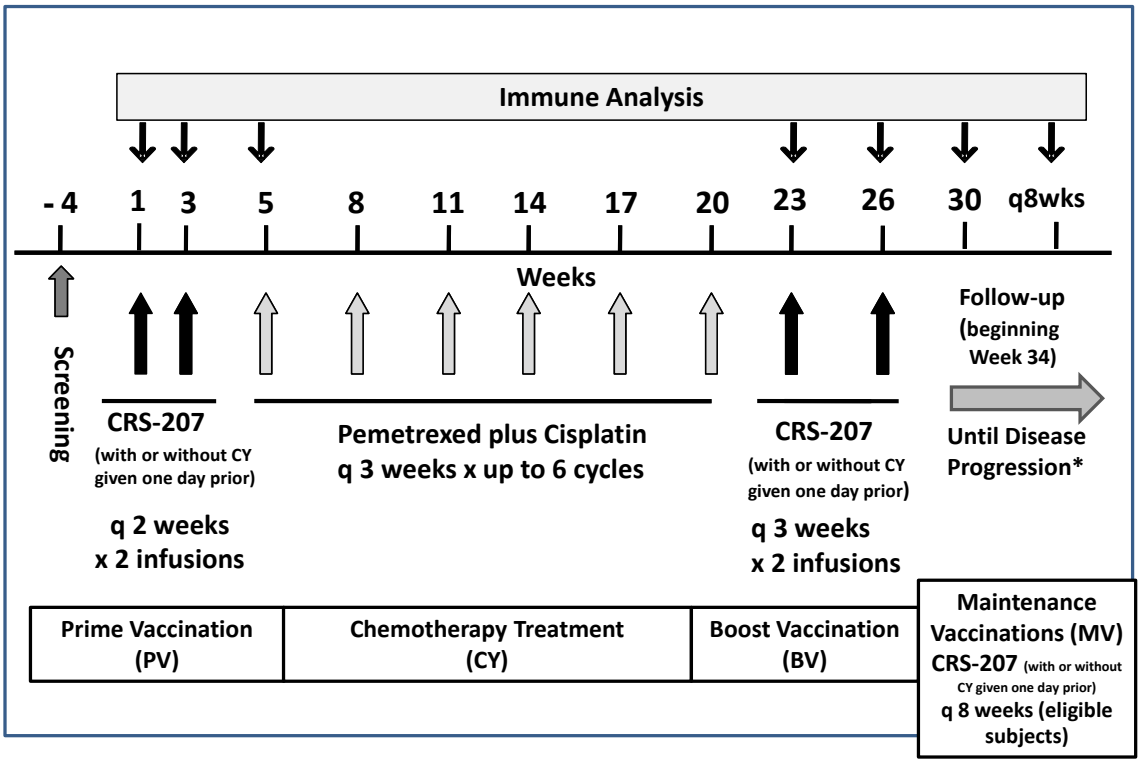
Cohort 2: The remaining subjects enrolled in the expansion phase (up to 28 total) will receive CRS-207 in combination with chemotherapy at the dose and schedule described above, however, these subjects will also receive low-dose Cy (200 mg/m^2) over 30 minutes one day prior to each CRS-207 infusion (i.e. prior to each PV and BV infusion).

All subjects will return to the clinic 4 weeks after their 2^{nd} boost vaccination for an End of Course (EOC) visit. Subjects will have follow-up visits 4 weeks after the EOC visit and every 8 weeks thereafter until treatment discontinuation. Subjects will continue to receive maintenance vaccinations (MV) with (Cohort 2) or without Cy (Cohort 1) following their original schedule at each follow-up visit if they are clinically stable and continue to meet dosing eligibility. Subjects may continue on treatment with radiographic disease progression if subject is clinically stable and investigator believes the treatment may be providing benefit.

The study will consist of a screening period (within 28 days of the first administration of study drug), followed by administration of test treatments per Figure 2. Tumor assessment by computed tomography (CT) with optional fluorodeoxyglucose positron emission tomography (FDG-PET) or magnetic

resonance imaging (MRI) will be performed every 6-8 weeks until subject comes off study. Tumor biopsies, which are optional, will be performed pre-treatment, before start of chemotherapy (i.e. after initial 2 infusions of CRS-207), after completion of chemotherapy, and after CRS-207 to evaluate tumor lymphocytic (e.g. CD4, CD8, T_{reg}) infiltrate. [redacted]

Figure 2. Study Treatment Timeline, Doses Scheduled and Follow-up



*Or investigator believes subject is no longer receiving benefit if treatment continued beyond radiographic progression

To monitor initial safety of the sequential vaccine regimen, no more than 1 subject per week will be enrolled for the first 6 subjects. Dose-limiting toxicities are defined in Section 3.6. If >33% of subjects (i.e. 1 out of 3 subjects) experience a dose-limiting toxicity during the study, the dose will be lowered to 3 × 10^8 CFU for all subsequent dosing with CRS-207. Blood will be collected at EOS to assess the clearance of CRS-207.

Safety will be assessed by collection of data on AEs, vital signs, physical examination, clinical hematology, serum chemistry, and deaths. Induction of

immune response to mesothelin will be evaluated by ELISPOT assay prior to treatment and at time points during and after treatment. Objective tumor response, progression free survival and time to progression will be measured using modified RECIST for assessment of response in MPM (Appendix A) and immune-related response criteria. [47] Other evaluations will include overall survival, immune subset analysis (e.g. CD4, CD8, T_{reg}) and gene expression profiling of tumor tissue pre and post vaccination by immunohistochemistry, evaluating induction of anti-mesothelin humoral immune response by ELISA, assessing tumor marker kinetics by measuring serum levels of mesothelin and CA-125 and plasma osteopontin.

3.2 STUDY POPULATION

3.2.1 Inclusion Criteria

To be considered eligible to participate in this study, subjects must meet the inclusion criteria listed below:

(1) Subjects must have histologically-confirmed epithelial or biphasic pleural mesothelioma not amenable to potentially curative surgical resection at the time of study entry (subjects with biphasic tumors that have a predominantly (≥50%) sarcomatoid component will be excluded)

(2) Measurable disease, as defined by modified RECIST for MPM [4]

(3) ECOG Performance Status of 0 or 1

(4) Normal organ and marrow function, as defined by:

- Absolute neutrophil count (ANC) ≥ 1,500/µl
- Platelet count ≥ 100,000/µl
- Bilirubin ≤ 1.5 × upper limit of normal (ULN), or bilirubin ≤ 3× ULN if due to Gilbert's disease
- AST and ALT ≤ 2.5× ULN
- Albumin ≥ 2.5 g/dL
- Creatinine clearance ≥ 50 mL/min per Cockcroft-Gault formula (Creatinine clearance = {[(140-age) x weight in kilograms] x [0.85 if female]}/[72 x creatinine in mg/dL])
- CD4 ≥ 0.2×10^9/L
- Hemoglobin ≥9.0 g/dL

(5) Age 18 years and over

(6) Must have pulmonary function testing with documented values for vital capacity (VC) and forced expiratory volume in 1 second (FEV_1) $\geq$ 45% of predicted value and oxygen saturation $\geq$ 90% on room air, as measured by pulse oximeter

(7) Anticipated life expectancy greater than 6 months

(8) Female subjects of childbearing potential must consent to use a medically acceptable method of highly effective contraception (oral hormonal contraceptive, condom plus spermicide, or hormone implants) throughout the study period and for 28 days after their final study drug administration (A barrier method of contraception must be employed by all subjects (male and female), regardless of other methods.)

(9) Subject provides informed consent and is willing and able to comply with all study procedures

3.2.2 Exclusion Criteria

To be eligible for entry into the study, subjects must not meet any of the exclusion criteria listed below:

(1) Be a candidate for curative surgery at the time of study entry

(2) Have had surgery or pleurodesis within 2 weeks prior to dosing

(3) Have had prior radiotherapy (except palliative extra-thoracic localized radiotherapy) or biologic therapy for MPM within 3 weeks prior to dosing

(4) Have had prior systemic chemotherapy for MPM (Prior intracavitary cytotoxic drugs or immunomodulators are not permitted, unless given for the purpose of pleurodesis.)

(5) Have an active malignancy with the exception of any of the following:

- adequately treated basal cell carcinoma
- squamous cell carcinoma of the skin, or in situ cervical cancer

- adequately treated Stage I cancer from which the subject is currently in remission and has been in remission for ≥ 3 years
- Stage I prostate cancer that does not require treatment
- any other cancer from which the subject has been disease-free for ≥ 2 years

(6) Have documented and ongoing central nervous system (CNS) involvement with their malignant disease (history of CNS involvement is not an exclusion criterion but the CNS metastases should have been adequately treated [radiation or surgical resection] and subjects are neurologically stable for 3 months off steroids)

(7) Have any evidence of clinically significant ascites

(8) Have clinically significant pleural effusion

(9) Have clinically significant heart disease (such as uncontrolled angina, myocardial infarction within 3 months, congestive heart failure of New York Heart Association III or IV)

(10) Have valvular heart disease that requires antibiotic prophylaxis for prevention of endocarditis, consistent with American Heart Association (AHA) guidelines

(11) History of any autoimmune disease, including patients with a history of inflammatory bowel disease, (including ulcerative colitis and Crohn's Disease), patients with a history of symptomatic disease (e.g. rheumatoid arthritis, systemic progressive sclerosis [scleroderma], systemic lupus erythematosus, autoimmune vasculitis [e.g. Wegener's granulomatosis]); central nervous system or motor neuropathy considered of autoimmune origin (e.g. Guillain-Barre Syndrome, Myasthenia gravis, multiple sclerosis). Patients with Graves', Hashimoto's Disease, vitiligo, and diabetes type 1 will be allowed

(12) Have any immunodeficiency disease or immune-compromised state (e.g. use of immunosuppressive agents) within 14 days prior to study enrollment

(13) Have baseline coagulopathy > Grade 3 unless due to anticoagulant therapy

(14) Have peripheral neuropathy ≥ Grade 2

(15) Use of systemically-active steroids within 14 days prior to enrollment in the study

(16) Use of more than 3 grams per day of acetaminophen

(17) Use of aspirin or other nonsteroidal anti-inflammatory drugs for 2 days before, during, and for 2 days after each administration of pemetrexed disodium (5 days before, during, and 2 days after each administration of pemetrexed disodium for piroxicam, naproxen, diflunisal, or nabumetone)

(18) Have received treatment with an investigational product within 28 days of study treatment or planned to receive within 28 days after vaccine administration.

(19) Have known allergy to both penicillin and sulfa drugs

(20) Have known or suspected allergy or hypersensitivity to yeast or any other component of CRS-207 [redacted] Platinol or platinum-containing compounds, or pemetrexed

(21) Have received a diagnosis of human immunodeficiency virus (HIV), hepatitis B, or hepatitis C (exception: clear evidence of natural immunity, immunity subsequent to vaccination, or successful eradication of the virus following antiviral therapy)

(22) Be a woman who is pregnant or breastfeeding

(23) Have prosthetic heart valves; major implant(s) or device(s) (e.g. artificial joints and prosthetics) placed within 12 months of study screening; or current or prior history of infection or clinically significant AEs associated with an exogenous implant(s) or device(s) that cannot be easily removed

(24) Have had major surgery or significant traumatic injury occurring within 28 days prior to CRS-207 administration or anticipated surgery or procedure requiring general anesthesia or deep sedation during study participation (including 28 days after last dose of CRS-207) (Minor procedures [dental work, skin biopsy, etc.], celiac plexus block, and biliary stents are allowed.)

(25) Have an unhealed wound which increases risk for bacterial infection

(26) Be unable to avoid intimate contact with another individual known to be at high risk of listeriosis (e.g. newborn infant, pregnant female, etc.) for at least 7 days after receiving a CRS-207 infusion

(27) Have an intercurrent illness that is either life-threatening or of clinical significance such that it might limit compliance with study requirements (including, but not limited to, ongoing or active infection, metabolic [e.g. diabetes] or neurologic disease, peripheral vascular disease, or psychiatric illness/social situations)

(28) Have insufficient peripheral venous access to permit completion of the study dosing and compliance with study phlebotomy regimen

(29) Have a history of alcohol dependence or use of illicit drugs that could potentially interfere with adherence to study procedures or requirements (e.g. opioids, cocaine, amphetamines, hallucinogens, etc.)

3.2.3 Dosing Eligibility

Subjects must have adequate organ function as defined by the laboratory values in Table 1 before each dose of CRS-207 in Cohort 1 or Cy in Cohort 2. Laboratory tests may be done up to 3 days prior to CRS-207 dosing (Cohort 1) or 2 days prior to Cy dosing for subjects receiving Cy (Cohort 2). Subjects who do not meet the dosing-eligibility requirements will be monitored. Delayed doses will be discussed with the medical monitor.

Table 1. Dosing-Eligibility Requirements

Hematologic	Renal	Hepatic
ANC ≥1,500/µL Platelets ≥100,000/µl Hemoglobin ≥9 g/dL CD4 ≥ 0.2×10^9/L prior to 1st post-chemotherapy CRS-207 boost vaccination	Creatinine ≤2.0 × ULN	AST/ALT ≤2.5× ULN Bilirubin ≤1.5× ULN or Bilirubin ≤3.0 × ULN if due to Gilbert's disease

ALT = alanine aminotransferase; ANC = absolute neutrophil count; AST = aspartate aminotransferase; ULN = upper limit of normal; WBC = white blood cell.
* Cockcroft-Gault formula: Creatinine Clearance = {[(140-age) x weight in kilograms] x [0.85 if female]}/[72 x creatinine in mg/dL]

3.3 ENDPOINTS

3.3.1 Primary Endpoint

The primary endpoints are as follows:

- The induction of mesothelin-specific T cell responses as measured by IFN-γ ELISPOT assay prior to treatment and at different time points during treatment. T cell responses to mesothelin will be considered positive when the frequency of specific responses are $\geq$ 1 in 10^5 $CD8^+$ peripheral blood lymphocytes (PBL) above the control sample and increased by at least 2-fold compared to baseline.

- Safety assessed by evaluation of AEs and deaths, vital signs, physical examination findings, clinical chemistry and hematology laboratory findings in subjects treated with CRS-207 (with or without Cy) combined with pemetrexed and cisplatin

3.3.2 Secondary Endpoints

The secondary endpoints are as follows:

- Objective tumor response, progression free survival and time to progression assessed by modified RECIST for assessment of response in MPM[4] and irRC[47]

- Overall survival

- Measure of serum mesothelin to assess predictive value for therapeutic response

3.3.3 Exploratory Endpoints

Exploratory endpoints are as follows:

- Immune subset analysis (e.g. CD4, CD8, T_{reg}) by immunohistochemistry and gene expression profiling of tumor tissue pre and post vaccination

- Induction of anti-mesothelin humoral immune response by ELISA

- Tumor marker kinetics measured by serum levels of mesothelin and CA-125 and plasma osteopontin; other markers may also be evaluated

- Pulmonary function tests (PFTs) measured by forced vital capacity (FVC), forced expiratory volume in 1 second (FEV1) and vital capacity (VC)

3.4 RANDOMIZATION AND BLINDING

No randomization or blinding is planned.

3.5 REPLACEMENT OF DROPOUTS

Subjects who withdraw consent or are removed from the study before completing at least 4 cycles of chemotherapy will be considered dropouts and may be replaced at the discretion of the lead investigator, sponsor and medical monitor.

3.6 DOSE-LIMITING TOXICITIES

Dose-limiting toxicities (DLTs) are defined as events that are determined by the investigator as related to CRS-207 that meet one of the following criteria:

- A fever of >40°C that lasts for greater than 24 hours and does not respond to antipyretics
- Clinically significant hypotension unresponsive to intravenous fluids (e.g. systolic BP <90 mm Hg or mean arterial pressure <55 mm Hg as measured on two separate occasions at least 10 minutes apart)
- Grade 3 or greater decreases in leukocytes, ANC, or platelets that persist for more than 4 days
- Hemoglobin ≤7.0 g/dL
- ALT, AST, or alkaline phosphatase elevations >5 times the ULN (Grade 3) that persist for more than 7 days
- [redacted]
- Unexpected Grade 3 laboratory abnormalities lasting >48 hours
- Grade 3 or greater hypophosphatemia or lymphopenia that persist for more than 7 days

- Any other Grade 3 or greater event according to National Cancer Institute's Common Terminology Criteria for AEs (CTCAE) Version 4.03

All unexpected Grade 3 events will be reviewed by the lead investigator, sponsor and medical monitor. If the event is determined to be related to CRS-207 dosing and clinically meaningful, it will be considered a dose-limiting toxicity. Grade 3 laboratory abnormalities should be repeated within 24-72 hours if clinically indicated and monitored as necessary to determine if event meets DLT criteria.

DLTs will be continuously monitored per cohort and across cohorts. If more than 33% of subjects (i.e. 1 in 3 subjects) experience a DLT at the starting dose of 1×10^9 CFU, the dose will be lowered to 3×10^8 CFU for all subsequent dosing with CRS-207. Subjects currently receiving treatment may continue to receive CRS-207 dosing at the lower dose and all subsequent subjects will receive CRS-207 at the lower dose. DLTs will continue to be monitored at the lower dose.

4.0 DRUGS AND DOSAGES

4.1 IDENTIFICATION AND DESCRIPTION OF CLINICAL TRIAL MATERIAL (CTM)

4.1.1 CTM

CRS-207 is a formulated live-attenuated strain hMeso38 of *Lm*, derived by deletion of *actA* and *inlB* coding sequences from a Streptomycin-resistant, wild-type strain and insertion of the hMeso coding sequence. The CRS-207 drug product consists of attenuated *Lm* (1×10^9 CFU total) [redacted] filled into a single-use 3-mL glass vial with a gray butyl stopper and aluminum crimp seal with a flip-off cap, and stored frozen at –60ºC or colder until intravenous administration. CRS-207 is supplied by Aduro.

ALIMTA®, pemetrexed for injection, is an antifolate antineoplastic agent that exerts its action by disrupting folate-dependent metabolic processes essential for cell replication. Pemetrexed disodium heptahydrate has the chemical name L-Glutamic acid, *N*-[4-[2-(2-amino-4,7-dihydro-4-oxo-1*H*-pyrrolo[2,3-*d*]pyrimidin-5-yl)ethyl]benzoyl]-, disodium salt, heptahydrate. It is a white to almost-white solid with a molecular formula of $C_{20}H_{19}N_5Na_2O_6 \cdot 7H_2O$ and a molecular weight of 597.49. Pemetrexed is supplied as a sterile lyophilized powder for i.v. infusion available in single-dose vials. The product is a white to either light yellow or green-yellow lyophilized solid. Each 500-mg vial contains pemetrexed disodium equivalent to 500 mg pemetrexed and 500 mg of mannitol. Pemetrexed will be supplied by the institutional pharmacy.

Cisplatin, USP (cis-diamminedichloroplatinum) is a heavy metal complex containing a central atom of platinum surrounded by two chloride atoms and two ammonia molecules in the cis position. It is a white powder with the molecular formula $PtCl_2H_6N_2$, and a molecular weight of 300.1. Cisplatin injection is a sterile aqueous solution for intravenous use, each mL containing 1 mg cisplatin and 9 mg sodium chloride. Cisplatin will be supplied by the institutional pharmacy.

Cyclophosphamide (Cy; active ingredient cyclophosphamide monohydrate) is a synthetic antineoplastic drug chemically related to the nitrogen mustards. It is commercially available in a sterile powder formulation for use in i.v.

administration. Cyclophosphamide will be supplied by the institutional pharmacy.

4.1.2 Labeling

Because the study is not blinded, the labeling will be that used on commercial vials of Cy, ALIMTA®, pemetrexed for injection, and cisplatin. The vials for CRS-207 will be labeled with the following: product name; volume; storage conditions; product lot number; sponsor name and address; fill date; and a caution statement ("Caution: New drug limited by Federal law to investigational use"). CRS-207 drug product is packaged in kit boxes that are also labeled with product name, number of vials, concentration, storage condition, a caution statement, sponsor name and address, expiration date, and kit lot number.

4.2 DOSING INSTRUCTIONS AND SCHEDULE

4.2.1 Cyclophosphamide

Cohort 2 only: Cy will be administered to subjects by i.v. infusion at the immunomodulating dose of 200 mg/m^2 in 100 mL normal saline over 30 minutes one day prior to each CRS-207 dose.

For complete product information on dosage and administration, contraindications, warnings/precautions, adverse reactions, and drug interactions, see package insert for cyclophosphamide (Appendix E).

4.2.2 CRS-207

CRS-207 will be administered by IV infusion (1×10^9 CFU in 100 mL sterile saline, USP, over approximately 1 hour). CRS-207 is prepared by thawing one vial of drug product at room temperature. A total of 1 mL of product is drawn with a syringe and inserted into 1 bag of 100 mL saline solution for IV injection. Additional details for storage and preparation of CRS-207 are provided in the study Pharmacy Manual.

CRS-207 will be administered to subjects by i.v. infusion on Day 1 of Weeks 1 and 3 for Cohort 1 or Day 2 of Weeks 1 and 3 for Cohort 2 prior to chemotherapy (prime vaccinations) and 3 and 6 weeks after the final chemotherapy cycle (boost vaccinations). Subjects who continue to meet dosing eligibility will receive

additional CRS-207 maintenance vaccinations at each follow-up visit. CRS-207 must not be administered via central venous catheters or infusion ports.

Before each CRS-207 infusion, subjects will be premedicated with 650 mg acetaminophen. Subjects will receive a minimum of 500 mL of normal saline immediately before CRS-207 infusion and at least 500 mL after infusion (1 L is recommended for patients who can tolerate it, to mitigate infusion reactions). Additional fluids may be given for persistent tachycardia, fever or hypotension based on investigator's discretion. Subjects will be observed in the clinic for at least 4 hours after each infusion. Subjects who are not stable to be released at 4 hours after infusion should continue to be monitored until stable. Hospital admissions for overnight monitoring will not be considered an SAE unless the event meets criteria for seriousness other than hospitalization.

Accumulating subject experience during and after CRS-207 infusions at 1×10^9 CFU have further demonstrated the following clinical observations which are to be expected:

- **Fevers.** Despite the acetaminophen premedication, subjects can spike fevers up to 40°C starting at the end of the CRS-207 infusion generally through the next 24 hours. Oral Ibuprofen (400 to 800 mg) and acetaminophen (650 to 1000 mg) can be used in alternate sequence every 4 hours.

- **Rigors**. Rigors (generally once or twice) have been observed starting during or at the end of the CRS-207 infusion through 24 hours. Intravenous narcotics such as morphine or meperidine can be administered per institutional policy. Oral morphine or non-steroidal anti-inflammatory drugs (NSAIDs) may be used as home treatment.

- **Blood pressure**. Decreases in blood pressure have been observed necessitating additional IV fluids during the 4 hour observation period (up to 1 or 2 L). This may be the result of fever, compartmental shifts of fluid resulting from the CRS-207 infusion, and the use of narcotics. Some subjects have also been slightly hypotensive at 24 hours. Subjects are encouraged to hydrate themselves liberally at home with oral fluids.

- **Nausea and vomiting**. Nausea and vomiting have been reported and observed within 24 hours after CRS-207 infusion. Subjects may be given anti-emetics as needed.

4.2.3 Pemetrexed

The recommended dose of pemetrexed is 500 mg/m^2 body surface area (BSA) in 100 mL 0.9% sodium chloride administered over 10 minutes as an intravenous infusion on Day 1 of a 21-day cycle. Dose reductions may be required for subjects with hepatic or renal impairment or history of hematologic or non-hematologic toxicity to pemetrexed/cisplatin (see Appendix C, prescribing information for Alimta®). Subjects may receive pre-medications (e.g. folic acid and cyanocobalamin) and pre- and post-infusion hydration per standard of care and investigator's practice.

For complete product information on dosage and administration, contraindications, warnings/precautions, adverse reactions, and drug interactions, see package insert for ALIMTA®, pemetrexed for injection (Appendix C).

4.2.4 Cisplatin

The recommended dose of cisplatin is 75 mg/m^2 BSA in 1L 0.9% sodium chloride with mannitol 30g/L infused over 2 hours, beginning approximately 30 minutes after the end of pemetrexed administration on Day 1 of each 21 day cycle.

Subjects may receive anti-emetics, e.g. dexamethasone, prior to cisplatin infusion per standard of care and investigator's practice.

Dose reductions may be required for subjects with hepatic or renal impairment or history of hematologic or non-hematologic toxicity to pemetrexed/cisplatin (see Appendix D, prescribing information for cisplatin). If a subject is determined to be intolerant to cisplatin after at least one dose, carboplatin may be substituted to be administered with pemetrexed per standard of care with the approval of the Sponsor.

For complete product information on dosage and administration, contraindications, warnings/precautions, adverse reactions, and drug interactions, see the package insert for cisplatin (Appendix D).

4.2.5 Delayed Dosing

Dosing of CRS-207 with or without Cy (prime vaccination 2 [PV2], boost vaccination 1 [BV1], boost vaccination 2 [BV2] or maintenance vaccinations [MVs]) may be delayed up to 2 weeks. The medical monitor should be contacted for further instruction on continued dosing for any delay greater than 2 weeks. The total delay time during the prime vaccination period may not exceed 2 weeks, i.e. the first cycle of chemotherapy treatment should be administered no later than Week 7, after which the medical monitor should be contacted to discuss continued dosing. For subjects enrolled in Cohort 2, if CRS-207 dosing is delayed more than 72 hours after administration of Cy for a particular dose, the dose will be delayed and Cy re-administered a minimum of 1 week after the original Cy dosing and CRS-207 administered according to schedule thereafter.

Delayed chemotherapy dosing or missed chemotherapy doses due to the subject's clinical status are at the discretion of the investigator, but the medical monitor may be contacted to discuss plans for continued dosing. The Sponsor should be notified of all modifications to the planned study schedule during the chemotherapy dosing period.

If administration of CRS-207 doses (with or without Cy) or chemotherapy cycles are delayed, the subsequent dosing schedule will be adjusted according to the delayed dose(s).

4.3 STORAGE AND HANDLING OF CLINICAL TRIAL MATERIAL

4.3.1 Cyclophosphamide

Cyclophosphamide powder should be kept at or below 25ºC. Cy reconstituted in normal saline is chemically and physically stable for 24 hours at room temperature and for 6 days when refrigerated. Guidelines outlining the procedures for proper handling and disposal of anticancer drugs should be followed when handling Cy (OSHA 1996). Protective gloves should be worn when handling Cy in both powder and reconstituted forms.

4.3.2 CRS-207

CRS-207 must be stored at –60ºC or colder until just before use. The investigational site(s), per institutional guidelines, will destroy used CRS-207 vials after formulation for administration. The formulation of CRS-207 for administration and the destruction of each used vial will be carefully documented in the study Pharmacy Manual. The principal investigator is responsible for CRS-207 accountability during on-site monitoring visits. Unused CRS-207 will be destroyed at the study site after final investigational product accountability and notification by Sponsor, unless otherwise directed by Sponsor.

Wild-type *Lm* is classified by the Centers for Disease Control and Prevention for handling in the laboratory according to Biosafety Level 2 practices. Since CRS-207 is attenuated by greater than 1,000-fold compared to wild-type *Lm*, individuals who prepare CRS 207 for injection should take appropriate precautions to avoid contamination or direct contact with the agent. Once it is prepared for infusion, CRS-207 can be handled with BSL 1 practices, including use of protective gloves, eye and face protection when handling and thorough hand washing after handling as the chance for direct exposure to CRS-207 by study personnel should be greatly diminished. Study personnel and staff should also adhere to the institutional guidelines for standard precautions.

4.3.3 Environmental Precautions

Wild-type *Lm* is a common pathogen that is widely distributed in the environment and contaminates a variety of ready-to-eat foods. Despite the presence of *Lm* in diverse locations, clinically apparent human infection is not commonly reported in immunocompetent, normal individuals. Direct human-to-human spread of *Lm* is believed to be limited mainly to vertical transmission from mother to neonate. Standard isolation precautions are usually recommended for subjects infected with wild-type *Lm*. CRS-207 is a live-attenuated construct originating from wild-type *Lm* which contains deletion of 2 virulence genes that render it greater than 1,000-times less toxic than wild-type *Lm*. Preclinical studies demonstrated that virtually all CRS-207 was cleared from the body within 4 days of infusion and clinical studies with CRS-207 and similar live-attenuated *Lm* strains showed no shedding of the bacterium in urine or feces post-infusion. While the risk of infection with CRS-207 is unknown and expected to be low or none based on these data, precautions should be exercised to avoid intimate contact between subjects and individuals who are at high risk of listeriosis (e.g. newborn infants,

pregnant women, HIV-positive individuals) for at least 7 days after each CRS-207 infusion.

4.4 PRODUCT ACCOUNTABILITY

The investigator is responsible for the control of the investigational agent under study. An investigational agent dispensing log must be kept current and should contain the following information:

- The subject number and initials of each subject to whom the investigational agent is administered
- The date(s) and quantity of the investigational agent administered to the subject
- Documentation of proper disposal of used investigational drug vials
- Documentation of proper disposal (or return, at sponsor's request) of unused investigational drug vials

All records and unused supplies of the investigational agent must be available for inspection at every monitoring visit.

4.5 PRIOR, CONCOMITANT, AND EXCLUDED THERAPY

During the course of the clinical study, subjects are anticipated to continue the use of prescribed medications identified during the screening procedures, consistent with study inclusion and exclusion criteria. Concomitant medications used in this study include:

- [redacted]
- Acetaminophen prior to each CRS-207 infusion
- Folic acid and cyanocobalamin administered with pemetrexed
- Anti-emetics (per investigator's standard practice), including dexamethasone, administered with cisplatin
- Medications to treat any treatment-emergent AEs

Medications to treat treatment-emergent AEs should not interfere with the study and can be used at the investigator's discretion. Antipyretics may be used to treat fever or to prevent recurrence of fever. The details of any concomitant medications must be recorded in the case report form (CRF). The generic name, dosage, duration, and reason for the concomitant medication should be included in this report.

The following therapies are not permitted during the study (if administered, the subject may be removed from the study):

- Non-study chemotherapy or immunotherapy (approved or investigational)
- TNF pathway inhibitors or PI3 kinase inhibitors
- Systemically active steroids for more than 3 days or use of any systemic steroids within 14 days before or after dosing, with exception of study-prescribed steroids
- Any other investigational product
- Filgrastim (Neupogen or G-CSF) or Sargramostin (Leukine or GM-CSF) should not be administered 14 days prior to or 14 days after any CRS-207 dose. Approval must be obtained from the medical monitor for a subject to continue dosing if administered within this timeframe.
- Prophylactic vaccines (e.g. pneumococcal vaccine, influenza vaccine) should not be administered 28 days prior to or 28 days after any CRS-207 dose. Approval must be obtained from the medical monitor for a subject to continue dosing if a prophylactic vaccine is administered within this timeframe.

In addition, the following therapies should not be administered during the study unless medically necessary and approval must be obtained from the medical monitor for a subject to continue dosing if therapy is given concurrently with study participation:

- General anesthesia or deep sedation
- Aspirin >325 mg/d (chronic daily use of aspirin ≤325 mg/d and heparin flushes for central lines are allowed)
- More than 4 g/d of acetaminophen

[redacted]

5.0 EXPERIMENTAL PROCEDURES

5.1 OVERVIEW: SCHEDULE OF TIME AND EVENTS

An overview of study time and events for Cohort 1 is presented in Table 2a and for Cohort 2 in Table 2b.

Table 2a. CRS-207 and Pemetrexed/Cisplatin [Cohort 1]

Nominal Weeks (Wk)[a]	**Screening**	**Wk 1**			**Wk 3**			**Wk 5**	**Wk 8**	**Wk 11**	**Wk 14**	**Wk 17**	**Wk 20**	**Wk 23**			**Wk 26**			**Wk 30**	**Follow-up: 4 wks after EOC then every 8 wks**[b]
Visit ID	**SCR**	**PV1**			**PV2**			**CY 1**	**CY 2**	**CY 3**	**CY 4**	**CY 5**	**CY 6**	**BV1**			**BV2**			**EOC**	**FU/EOS**
Cycle Study Day	**(-28)**	**1**	**2**	**8**	**1**	**2**	**8**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**2**	**8**	**1**	**2**	**8**	**1**	**1**
Visit Windows (days)		-	-	±1	-	-	±1	-	-	-	-	-	-	-	-	±1	-	-	±1	+7	±7
Informed consent	X																				
Inclusion/exclusion	X																				
Medical history, medication history	X																				
Cancer-related treatment	X																				
Baseline signs/symptoms	X																				
Virology screen[c]	X																				
Coagulation panel, UA[d]	X																				
Pulmonary Function Test[e]	X																				
Electrocardiogram, 12-lead	X																			X	
CT, tumor assess.; optional FDG-PET[f]	X							X		X		X		X						X	
Physical examination[g]	X	X			X			X	X	X	X	X	X	X			X			X	X
ECOG performance status	X	X			X			X	X	X	X	X	X	X			X			X	X
Vital signs, pulse oximetry, weight, height[h]	X	X	X		X	X		X	X	X	X	X	X	X	X		X	X		X	X
Pregnancy test[i]	X	X			X									X			X				X
CD4 count	X													X							
Creatinine clearance[j]	X																				
Clinical hematology, serum chemistry[k,l]	X	X	X		X	X		X[l]	X[l]	X[l]	X[l]	X[l]	X[l]	X	X		X	X		X	X
Tumor marker(s)[m]	X	X			X			X	X	X	X	X	X	X			X			X	
Concomitant medications, AEs[n]		X	X	X[n]	X	X	X[n]	X	X	X	X	X	X	X	X	X[n]	X	X	X[n]	X	X
PBMC[o]		X			X			X						X			X			X	
Serum for *Lm*/mesothelin immunity[p]		X	X		X	X		X						X	X		X	X		X	
HLA-typing[q]		X																			
Archival tissue		X[r]																			
Tumor biopsy (optional)[s]		X						X						X						X	
Antibiotics[t]							X														X[u]
Blood sample for CRS-207 testing																					X[v]
Study Drug Administration																					
CRS-207[w,x]		X			X									X			X				X[u]
Pemetrexed/cisplatin[y, z]								X	X	X	X	X	X								

Footnotes:

a Study schedule and timing displayed is based on the subject completing the full treatment regimen of 6 chemotherapy cycles. However, subjects may receive CRS-207 boost vaccinations if they complete between 4-6 cycles in which case the visit timing will be modified. If CRS-207 doses or chemotherapy cycles are delayed, subsequent dosing schedule will be adjusted according to the delayed dose(s).

b Follow-up visit to occur 4 weeks after EOC then every 8 weeks until treatment discontinuation. EOS Visit will occur within 4 weeks after the last dose of study drug or prior to commencing the new therapy. If the EOS visit occurs earlier than 4 weeks, a safety follow-up telephone call on Day 28 (+/- 7 days) is required; document contact in the study records.

c Virology screen will be performed if one has not been done within 14 days before screening: HIV antibody, hepatitis B surface antigen, and hepatitis C antibody (exception: clear evidence of natural immunity, immunity subsequent to vaccination, or successful eradication of the virus following antiviral therapy); additional virology may also be evaluated.

d Coagulation panel includes the following: prothrombin time (PT), international normalized ratio of prothrombin time (INR), activated partial thromboplastin time (APTT); UA includes the following: bilirubin, blood, glucose, ketones, leukocytes, nitrite, pH, protein, specific gravity.

e Pulmonary function tests (PFTs) to include VC, FVC and FEV_1.

f A spiral CT of the thorax and abdomen will be performed. If a subject cannot have a CT scan (e.g. allergy to contrast dye) an MRI should be performed. CT scans may be done within 1 week prior to or after scheduled visit. Optional FDG-PET may be done. A clinical assessment of tumor status using modified RECIST will be completed.

g Complete physical examinations will be conducted at baseline and EOS; symptom-directed physical examinations to be conducted on Day 1 of dosing weeks.

h Blood pressure, pulse, respiratory rate, temperature and pulse oximetry are required as indicated. Weight will be taken on Day 1 of dosing weeks. Height required at screening only. Pre-dose blood pressure should be performed in a supine position after the subject has been resting for at least 5 minutes

i Pregnancy tests will be administered to women of childbearing potential: serum pregnancy test is required at screening, and pregnancy tests (urine or serum) are required before doses on Day 1 of CRS-207-dosing weeks. Pregnancy tests (urine or serum) only required at follow-up visits at which maintenance vaccinations will be administered.

j Creatinine clearance calculated by Cockcroft-Gault formula = {[(140-age) x weight in kilograms] x [0.85 if female]}/[72 x creatinine in mg/dL]

k Clinical hematology: CBC with differential ANC, platelet count; serum chemistry: sodium, potassium, chloride, bicarbonate, glucose, BUN, creatinine, LDH, ALT, AST, alkaline phosphatase, bilirubin (total, direct, and indirect), total protein, albumin, calcium, magnesium, uric acid and phosphate. Blood draws may be taken up to 3 days prior to dosing with CRS-207.

l Clinical hematology and chemistry tests to be taken prior to each chemotherapy treatment.

m Tumor markers to be assessed include Mesothelin, CA-125, and plasma osteopontin; other markers may also be assessed.

n Adverse Events (AEs) and concomitant medications review. A phone call follow-up for AEs and concomitant medications will be conducted on day 2 ± 1 days after each CRS-207 infusion.

o Up to 200 mL of whole blood to be processed within 6 hours into peripheral blood mononuclear cells (PBMCs) and stored frozen in liquid nitrogen.

p Whole blood (10 mL) will be collected between 20 and 26 hours after start of dosing for assessment of *Lm* and mesothelin immunity.

q The HLA-typing should include type A and B of class I antigens, low resolution.

r Attempts to obtain surgical or biopsy archival tumor samples will be made for every subject until the sample is obtained or documentation that the sample cannot be obtained. Detailed instructions for tissue collection, processing and shipment are provided in the Laboratory Manual.

s An optional CT or an ultrasound-guided core needle biopsy should be performed at the site presented for mesothelioma for consenting subjects.

t [redacted]

u Subjects who are clinically stable and meet dosing eligibility, will continue to receive maintenance vaccinations beginning at the first follow-up visit 4 weeks after EOC and every 8 weeks thereafter. Subjects may continue on treatment with radiographic disease progression if they are clinically stable and the Investigator believes the treatment may be providing benefit. A 7-day course of antibiotics starting 7 days after vaccination will be administered following the second dose of CRS-207 (PV2) and after final dose of CRS-207.

v Blood for CRS-207 culture will be collected at EOS to assess clearance of CRS-207. At EOS, blood will be collected prior to initiation of antibiotics (if applicable, blood should also be collected from the central line port).

w CRS-207 will be administered via i.v. injection (1×10^9 CFU in approximately 100 mL 0.9% sodium chloride). Vital signs (BP, pulse, respiratory rate, temperature) will be measured every 30 minutes during the CRS-207 infusion and every hour during post-infusion follow-up. Subjects will be observed for at least 2 hours after each infusion. Subjects who are not stable enough to be released at 2 hours after infusion should continue to be monitored until subject is considered clinically stable.

x CRS-207 premedications include the following: 650 mg of acetaminophen to be administered orally prior to each dose of CRS-207. Subjects will receive a minimum of 500 mL of normal saline immediately before CRS-207 infusion and at least 500 mL after infusion. Additional fluids may be given for persistent tachycardia, fever or hypotension based on investigator's discretion.

y Subjects may receive folic acid, cyanocobalamin, and anti-emetics premedication for chemotherapy per standard of care and investigator's practice.

z The recommended dose of pemetrexed is 500 mg/m^2 BSA in 100 mL 0.9% sodium chloride administered over 10 minutes as an i.v. infusion on Day 1 of 21-day cycle. The recommended dose of cisplatin is 75 mg/m^2 BSA in 1 L 0.9% sodium chloride with mannitol 30 g/L infused over 2 hours beginning approximately 30 minutes after end of pemetrexed administration on day 1 of 21-day cycle. Dose reductions may be required for subjects with hepatic or renal impairment or history of toxicity to pemetrexed or cisplatin. If a subject is determined to be intolerant to cisplatin after at least one dose, carboplatin may be substituted to be administered with pemetrexed per standard of care with the approval of the Sponsor.

Abbreviations:
AE = adverse event; ALT = alanine aminotransferase; ANC = absolute neutrophil count; APTT = activated partial thromboplastin time; AST = aspartate aminotransferase; BUN = blood urea nitrogen; BV = boost vaccine; CBC = complete blood count; CT =computed tomography; EOS = End of Study; FDG-PET = positron emission tomography with fluorodeoxyglucose; FEV1 = forced expiratory volume in 1 second; HIV = human immunodeficiency virus; HLA = human leukocyte antigen; LDH = lactate dehydrogenase; *Lm* = *Listeria monocytogenes;* MRI = magnetic resonance imaging; PBMC = peripheral blood mononuclear cells; PD = progressive disease; i.v. = intravenous; p.o. = per oral; PV = prime vaccination; UA = urinalysis; VC = vital capacity.

Table 2b. Schedule of Procedures – Low-dose Cy/CRS-207 and Pemetrexed/Cisplatin [Cohort 2]

Nominal Weeks (Wk)[a]		**Wk 1**				**Wk 3**				**Wk 5**	**Wk 8**	**Wk 11**	**Wk 14**	**Wk 17**	**Wk 20**	**Wk 23**				**Wk 26**				**Wk 30**	**Follow-up: 4 wks after EOC then every 8 wks**
Visit ID	**SCR**	**XP1**	**PV1**			**XP2**	**PV2**			**CY1**	**CY2**	**CY3**	**CY4**	**CY5**	**CY6**	**XB1**	**BV1**			**XB2**	**BV2**			**EOC**	**FU/EOS**
Cycle Study Days	**(-28)**	**1**	**2**	**3**	**9**	**1**	**2**	**3**	**9**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**2**	**3**	**9**	**1**	**2**	**3**	**9**	**1**	**1**
Visit Windows (days)		-	-	-	±1	-	-	-	±1	-	-	-	-	-	-	-	-	-	±1	-	-	-	±1	+7	±7
Informed consent	X																								
Inclusion/exclusion	X																								
Medical history, medication history	X																								
Cancer-related treatment	X																								
Baseline signs/symptoms	X																								
Virology screen[c]	X																								
Coagulation panel, UA[d]	X																								
Pulmonary Function Test[e]	X									X		X		X		X								X	
Electrocardiogram, 12-lead	X																							X	
CT, tumor assessment; optional FDG-PET[f]	X									X		X		X		X								X	
Physical examination[g]	X	X				X				X	X	X	X	X	X	X				X				X	X
ECOG performance status	X	X				X				X	X	X	X	X	X	X				X				X	X
Vital signs, pulse oximetry, weight, and height[h]	X	X	X	X		X	X	X		X	X	X	X	X	X	X	X	X		X	X	X		X	X
Pregnancy test[i]	X	X														X				X					X
CD4 count	X															X									
Creatinine clearance[j]	X																								
Clinical hematology, serum chemistry[k,l]	X	X		X		X		X		X[l]	X[l]	X[l]	X[l]	X[l]	X[l]	X		X		X		X		X	X
Tumor marker(s)[m]	X	X				X				X	X	X	X	X	X	X				X				X	
Concomitant medications, AEs[n]		X	X	X	X[n]	X	X	X	X[n]	X	X	X	X	X	X	X	X	X	X[n]	X	X	X	X[n]	X	X
PBMC[o]		X				X				X						X				X				X	
Serum for *Lm*/mesothelin immunity[p]		X		X		X		X		X						X		X		X		X		X	
HLA-typing[q]		X																							
Archival Tissue		X[r]																							
Tumor biopsy (optional)[s]		X								X						X								X	
Antibiotics[t]									X																X
Blood for CRS-207 testing																									X[v]
Study Drug Administration																									
Cyclophosphamide[w]		X				X										X				X					X[w]
CRS-207[x, y]			X				X										X				X				X[w]
Pemetrexed/cisplatin[z, aa]										X	X	X	X	X	X										

a Study schedule and timing displayed is based on the subject completing the full treatment regimen of 6 chemotherapy cycles. However, subjects may receive Cy/CRS-207 boost vaccinations if they complete between 4-6 cycles in which case the visit timing will be modified. If CRS-207 doses or chemotherapy cycles are delayed, subsequent dosing schedule will be adjusted according to the delayed dose(s).

b Follow-up visit to occur 4 weeks after EOC then every 8 weeks until treatment discontinuation. EOS Visit will occur within 4 weeks after the last dose of study drug or prior to commencing the new therapy. If the EOT visit occurs earlier than 4 weeks, a safety follow-up telephone call on Day 28 (+/-7 days) is required; document contact in the study records.

c Virology screen will be performed if one has not been done within 14 days before screening: HIV antibody, hepatitis B surface antigen, and hepatitis C antibody (exception: clear evidence of natural immunity, immunity subsequent to vaccination, or successful eradication of the virus following antiviral therapy); additional virology may also be evaluated.

d Coagulation panel includes the following: prothrombin time (PT), international normalized ratio of prothrombin time (INR), activated partial thromboplastin time (APTT); UA includes the following: bilirubin, blood, glucose, ketones, leukocytes, nitrite, pH, protein, specific gravity.

e Pulmonary function tests to include VC, FVC and FEV_1.

f A spiral CT scan of the thorax and abdomen will be performed. If a subject cannot have a CT scan (eg, due to an allergy to contrast dye), an MRI should be performed. CT scans may be done within 1 week prior to or after scheduled visit, and must also be done prior to initiation of chemotherapy up to the start of chemotherapy. Optional FDG-PET may be done. A clinical assessment of tumor status using modified RECIST will be completed.

g Complete physical examinations will be conducted at baseline and EOC; symptom-directed physical examinations to be conducted on Day 1 of dosing weeks.

h Blood pressure, pulse, respiratory rate, temperature and pulse oximetry are required as indicated. Weight will be recorded on Day 1 of dosing weeks. Height is required at screening only. Pre-dose blood pressure should be measured in a supine position after the subject has been resting for at least 5 minutes.

i Pregnancy tests will be administered to women of childbearing potential: serum pregnancy test is required at screening, and pregnancy tests (urine or serum) are required before doses on Day 1 of CRS-207 dosing weeks.

j Creatinine clearance is to be calculated by Cockcroft-Gault formula = {[(140-age) x weight in kilograms] x [0.85 if female]}/[72 x creatinine in mg/dL]

k Clinical hematology: CBC with differential ANC, platelet count; serum chemistry: sodium, potassium, chloride, bicarbonate, glucose, BUN, creatinine, LDH, ALT, AST, alkaline phosphatase, bilirubin (total, direct, and indirect), total protein, albumin, calcium, magnesium, uric acid and phosphate. Blood draws may be taken up to 3 days prior to dosing with CRS-207 or 2 days prior to Cy. Clinical hematology and chemistry tests should be measured prior to each chemotherapy treatment.

l Clinical hematology and chemistry tests to be taken prior to each chemotherapy treatment

m Tumor markers to be assessed include mesothelin, CA-125, and plasma osteopontin; other markers may also be assessed.

n AEs and concomitant medications review. A phone call follow-up for AEs and concomitant medications will be conducted on day 3 ± 1 days after CRS-207 vaccination.

o Up to 200 mL of whole blood should be processed within 6 hours into PBMCs and stored frozen in liquid nitrogen.

p Whole blood (10 mL) will be collected between 20 and 26 hours after start of dosing for assessment of *Lm* and mesothelin immunity.

q The HLA-typing should include type A and B of class I antigens, low resolution.

r Attempts to obtain surgical or biopsy archival tumor samples will be made for every subject until the sample is obtained or documentation that the sample cannot be obtained. Detailed instructions for tissue collection, processing and shipment are provided in the Laboratory Manual.

s An optional CT or an ultrasound-guided core needle biopsy should be performed at the site presented for mesothelioma for consenting subjects.

t [redacted]

u Cy will be administered one day prior to each CRS-207 dose by i.v. infusion at the dose of 200 mg/m^2 in 100 ml normal saline over 30 minutes.

v Blood for CRS-207 culture will be collected at EOS to assess clearance of CRS-207. At EOS, blood will be collected prior to initiation of antibiotics (if applicable, blood should also be collected from the central line port).

w Subjects who are clinically stable and meet dosing eligibility will continue to receive maintenance vaccinations with Cy (dosing day 1) and CRS-207 (dosing day 2) beginning at the first follow-up visit 4 weeks after EOC and every 8 weeks thereafter. Subjects may continue on treatment with radiographic disease progression if they are clinically stable and the Investigator believes the treatment may be providing benefit.[redacted]

x CRS-207 will be administered via i.v. injection (1×10^9 CFU in 100 mL 0.9% sodium chloride). Vital signs (BP, pulse, respiratory rate, temperature) will be measured every 30 minutes during the CRS-207 infusion and every hour during post-infusion follow-up. Subjects will be observed for at least 2 hours after each infusion. Subjects who are not stable enough to be released at 2 hours after infusion should continue to be monitored until subject is considered clinically stable.

y CRS-207 premedications include the following: 650 mg of acetaminophen should be administered orally prior to each dose of CRS-207. Subjects will receive a minimum of 500 mL of normal saline immediately before CRS-207 infusion and at least 500 mL after infusion. Additional fluids may be given for persistent tachycardia, fever or hypotension based on investigator's discretion.

z Subjects may receive folic acid, cyanocobalamin, and anti-emetics premedication for chemotherapy per standard of care and investigator's practice.

aa The recommended dose of pemetrexed is 500 mg/m^2 BSA in 100 mL 0.9% sodium chloride administered over 10 minutes as an i.v. infusion on Day 1 of the 21-day cycle. The recommended dose of cisplatin is 75 mg/m^2 BSA in 1 L 0.9% sodium chloride with mannitol 30 g/L infused over 2 hours beginning approximately 30 minutes after the end of pemetrexed administration on Day 1 of the 21-day cycle. Dose

reductions may be required for subjects with hepatic or renal impairment or history of toxicity to pemetrexed or cisplatin. If a subject is determined to be intolerant to cisplatin after at least one dose, carboplatin may be substituted to be administered with pemetrexed per standard of care with the approval of the Sponsor.

Abbreviations:
AE = adverse event; ALT = alanine aminotransferase; ANC = absolute neutrophil count; APTT = activated partial thromboplastin time; AST = aspartate aminotransferase; BUN = blood urea nitrogen; BV = boost vaccine; CBC = complete blood count; CT = computed tomography; CY = cycle; EOC = end of cycle; EOS = End of Study; FDG-PET = positron emission tomography with fluorodeoxyglucose; FEV1 = forced expiratory volume in 1 second; FVC = forced vital capacity; HIV = human immunodeficiency virus; HLA = human leukocyte antigen; LDH = lactate dehydrogenase; *Lm* = *Listeria monocytogenes;* MRI = magnetic resonance imaging; PBMC = peripheral blood mononuclear cells; PD = progressive disease; PT = prothrombin time; PV = prime vaccine; INR = international normalized ratio of prothrombin time; i.v. = intravenous; p.o. = per oral; UA = urinalysis; SCR = screening; VC = vital capacity.

5.2 STUDY PROCEDURES

5.2.1 Screening Period (28 days prior to first study dose)

Before screening assessments are conducted, the subject must be given a complete explanation of the purpose and evaluations of the study. Subsequently, the subject must sign and receive a copy of an informed consent form (ICF) that was approved by the institutional review board (IRB) and an authorization for use and disclosure of protected health information (PHI) before any study-specific procedure is performed. An original signed consent form will be retained in the subject's source documentation at the site, and a copy will be provided for the subject to take home. Screening will occur within 28 days before treatment administration. Potential subjects will be evaluated for entry into the study according to the stated inclusion and exclusion criteria. Individuals who are identified during this screening as not eligible for study enrollment need not complete all screening procedures. The reason for ineligible status will be documented.

The following evaluations will be performed to assess the subject's eligibility for the study:

- Medical history, including history of carcinoma treatment
- Medication history over the past 28 days, including prescription and over-the-counter medications, herbs, vitamins, minerals, and prophylactic vaccines
- Baseline signs and symptoms
- Physical examination
- ECOG performance status
- Vital signs (BP, pulse, respiratory rate, temperature), height, weight, and pulse oxygen saturation
- Resting 12-lead electrocardiogram
- Pulmonary function tests: VC, FVC and FEV_1

- Clinical hematology: complete blood count (CBC) with differential ANC and platelet count
- Serum chemistry: sodium, potassium, chloride, bicarbonate, glucose, BUN, creatinine, lactate dehydrogenase (LDH), ALT, AST, alkaline phosphatase, bilirubin (total, direct, indirect), total protein, albumin, calcium, magnesium, uric acid, and phosphate
- Coagulation panel: prothrombin time (PT), international normalized ratio of prothrombin time (INR), and activated partial thromboplastin time (APTT)
- Virology screen if one has not been done within 14 days before screening: HIV antibody, hepatitis B surface antigen, and hepatitis C antibody
- Urinalysis (UA): bilirubin, blood, glucose, ketones, leukocytes, nitrite, pH, protein, and specific gravity
- Creatinine clearance calculated by Cockcroft-Gault formula = {[(140-age) x weight in kilograms] x [0.85 if female]}/[72 x creatinine in mg/dL]
- CD4 count
- Tumor markers: mesothelin, CA-125 and plasma osteopontin. Other markers may also be evaluated. (See Section 5.3)
- Serum pregnancy test (for women of childbearing potential only)
- Spiral CT scan of thorax and abdomen if one has not been done within 14 days before screening. If a subject cannot have a CT scan [e.g. allergy to contrast dye], a magnetic resonance imaging [MRI] should be performed.). CT with FDG-PET scan may be done (optional).
- Clinical assessment of tumor status using modified RECIST for assessment of response in MPM[4] and irRC[47].

The investigator may use clinical judgment when determining the clinical significance of laboratory parameter findings throughout the study. The medical monitor may, depending on study criteria, be consulted before enrollment about a potential subject with abnormal laboratory values that are not considered clinically significant.

5.2.2 Treatment Period

5.2.2.1 CRS-207 Prime Vaccinations (PV1, PV2) (with or without low-dose Cy)

All subjects will be administered CRS-207 during Weeks 1 and 3 (Day 1 for Cohort 1 and Day 2 for Cohort 2). Cohort 2 only will receive a low-dose Cy administration one day prior to each CRS-207 prime vaccination by i.v. infusion at the dose of 200 mg/m^2 in 100 mL normal saline over 30 minutes.

CRS-207 will be administered by i.v. infusion at 1×10^9 CFU in 100 mL 0.9% sodium chloride over approximately 1 hour. Prior to CRS-207 administration, subjects will be premedicated with 650 mg of acetaminophen before drug administration. Subjects will receive a minimum of 500 mL of normal saline immediately before CRS-207 infusion and at least 500 mL after infusion (1 L is recommended for subjects who can tolerate it, to mitigate infusion reactions). Additional fluids may be given for persistent tachycardia, fever or hypotension based on investigator's discretion. Investigators will not make dose adjustments or changes to administration schedule or rate without prior approval from sponsor. CRS-207 must not be administered via central venous catheter or infusion port.

Vital signs (BP, pulse, respiratory rate, temperature) will be obtained every 30 minutes during the CRS-207 infusion and every hour during post infusion follow-up. Subjects will be observed for at least 4 hours after each infusion. Subjects who are not stable enough to be released at 4 hours after infusion should continue to be monitored until stable. Presence of fever alone does not indicate subject is not clinically stable.

Because results for clinical hematology and serum chemistry need to be obtained before each CRS-207 administration, subjects may have blood drawn for these evaluations up to 3 days prior to receiving CRS-207 in Cohort 1 or 2 days prior to Cy for subjects in Cohort 2. Blood samples must not be collected from a central line after infusion of CRS-207 for at least 4 days.

Prior to receiving any study drug on Day 1 of Weeks 1 (PV1 for Cohort 1 or XP1 for Cohort 2) and 3 (PV2 for Cohort 1 or XP2 for Cohort 2), the following evaluations will be performed:

- Symptom-directed physical examination (May be done up to 3 days prior to CRS-207 dosing (Cohort 1) or 2 days prior to Cy for subjects receiving Cy (Cohort 2)).

- ECOG performance status
- Vital signs (BP, pulse, respiratory rate, temperature, pulse oximetry) and weight taken prior to CRS-207 dosing for on Day 1 for Cohort 1 and prior to Cy dosing on Day 1 and CRS-207 dosing on Day 2 for Cohort 2; blood pressure taken prior to CRS-207 administration (pre-dose) should be taken in supine position after subject has been resting for at least 5 minutes
- Urine or serum pregnancy test (for women of childbearing potential only)
- Blood draws for clinical hematology, serum chemistry. Blood draw may be done up to 3 days prior to CRS-207 dosing (Cohort 1) or 2 days prior to Cy for subjects receiving Cy (Cohort 2).
- Blood draw (serum and plasma) for tumor marker(s). Blood draw may be done up to 3 days prior to CRS-207 dosing (Cohort 1) or 2 days prior to Cy for subjects receiving Cy (Cohort 2). (See Section 5.3)
- AEs and concomitant medications review
- Blood draw for isolation of PBMC (up to 200 mL). Blood draw may be done up to 3 days prior to CRS-207 dosing (Cohort 1) or 2 days prior to Cy for subjects receiving Cy (Cohort 2). (See Section 5.3)
- Blood draw for *Lm*- and mesothelin-specific immunity assays (10 mL). Blood draw may be done up to 3 days prior to CRS-207 dosing (Cohort 1) or 2 days prior to Cy for subjects receiving Cy (Cohort 2). (See Section 5.3)
- Blood draw for Class I HLA-typing (A and B, low resolution); at Week 1 (PV1) only. Blood draw may be done up to 3 days prior to CRS-207 dosing (Cohort 1) or 2 days prior to Cy for subjects receiving Cy (Cohort 2). (see Section 5.3)
- Optional core needle biopsy; may be done anytime between screening and prior to dosing at Week 1 (PV1) only (see Section 5.3)

  Note: Urine pregnancy test and blood draws for hematology, chemistry and tumor markers are not required at Week 1 (PV1) if Screening Visit is conducted within 3 days prior to PV1 Visit.

5.2.2.2 CRS-207 Prime Vaccinations Follow-up

One day after CRS-207 infusion (Day 2 for Cohort 1 or Day 3 for Cohort 2), the following evaluations will be performed:

- Vital signs (BP, pulse, respiratory rate, temperature, pulse oximetry)
- Blood draws for clinical hematology, serum chemistry. Any unexpected Grade 3 laboratory abnormalities should be repeated within 24-72 hours.
- AEs and concomitant medications review
- Blood draw for *Lm*- and mesothelin-specific immunity assays (10 mL). Blood should be drawn within 20-26 hours after start of CRS-207 dosing. (See Section 5.3)

[redacted]

5.2.2.3 Chemotherapy Treatment: Pemetrexed and Cisplatin Administration (CY1 to CY6)

On Day 1 of Weeks 5 (CY1), 8 (CY2), 11 (CY3), 14 (CY4), 17 (CY5) and 20 (CY6), pemetrexed and cisplatin treatment will be administered. Pemetrexed will be administered at 500 mg/m^2 BSA in 100 mL 0.9% sodium chloride administered over 10 minutes as an intravenous infusion. Cisplatin will be administered at 75 mg/m^2 BSA in 1L 0.9% sodium chloride with mannitol 30g/L infused over 2 hours, beginning approximately 30 minutes after the end of pemetrexed administration. Subjects may receive pre-medications (e.g. folic acid, cyanocobalamin, and anti-emetics) and pre- and post-infusion hydration per standard of care and investigator's practice. Dose reductions may be required for subjects with hepatic or renal impairment or history of toxicity to pemetrexed or cisplatin.

If a subject is determined to be intolerant to cisplatin after at least one dose, carboplatin may be substituted to be administered with pemetrexed per standard of care with the approval of the Sponsor.

Subjects who complete at least 2 cycles of pemetrexed/cisplatin may go on to receive CRS-207 3 weeks after their final cycle of pemetrexed/cisplatin. Subjects

who receive less than 2 cycles will be discontinued from treatment and continued to be followed for safety and clinical and immune responses.

Prior to pemetrexed/cisplatin treatment, the following procedures will be performed:

- Symptom-directed physical examination (May be done up to 3 days prior to dosing)
- ECOG performance status
- Vital signs (BP, pulse, respiratory rate, temperature, pulse oximetry) and weight
- Blood draws for clinical hematology, serum chemistry. Blood draw may be done up to 3 days prior to dosing.
- Blood draw (serum and plasma) for tumor marker(s). Blood draw may be done up to 3 days prior to dosing. (See Section 5.3)
- AEs and concomitant medications review
- Spiral CT scan of thorax and abdomen with optional FDG-PET scan; at Weeks 5 (CY1), 11 (CY3), 17 (CY5). If a subject cannot have a CT scan [e.g. allergy to contrast dye], an MRI should be performed. CT scan may be done within ±7 days of scheduled timepoint.
- Clinical assessment of tumor status using modified RECIST for assessment of response in MPM[4] and irRC[47]; at Weeks 5 (CY1), 11 (CY3), 17 (CY5)
- Pulmonary function tests: VC, FVC and FEV_1; at Weeks 5 (CY1), 11 (CY3), 17 (CY5) for Cohort 2 only
- Blood draw for isolation of PBMC (up to 200 mL); at Week 5 (CY1) only. Blood draw may be done up to 3 days prior to dosing. (See Section 5.3)
- Blood draw for *Lm*- and mesothelin-specific immunity assays (10 mL); at Week 5 (CY1) only. Blood draw may be done up to 3 days prior to dosing. (See Section 5.3)

- Optional core needle biopsy; may be done anytime between PV2 and prior to dosing at Week 5 (CY1) only (See Section 5.3)

5.2.2.4 CRS-207 Post-chemotherapy Boost Vaccination (BV1, BV2)

During the post-chemotherapy boost vaccination (BV) period, all subjects (Cohorts 1 and 2) will be administered two infusions of CRS-207 3 weeks apart at least 3 weeks after the last chemotherapy cycle.

Cohort 2 only will receive a low-dose Cy administration one day prior to each CRS-207 boost vaccination by i.v. infusion at the dose of 200 mg/m^2 in 100 mL normal saline over 30 minutes. Subjects must receive at least 2 cycles of pemetrexed and cisplatin to continue to receive CRS-207 (with or without Cy) after chemotherapy.

CRS-207 will be administered and monitored and the same procedures conducted as on Day 1 in Section 5.2.2.1 and Day 2 in Section 5.2.2.2. One week (7 ± 1 days) after each CRS-207 infusion, subjects will be contacted by phone to assess AEs and concomitant medications.

In addition to those procedures listed in Section 5.2.2.1, prior to the first study drug dosing (Cy or CRS-207) at boost vaccination (BV1) following chemotherapy, the following procedures will be conducted:

- CD4 count. Blood draw may be done up to 3 days prior to CRS-207 dosing (Cohort 1) or 2 days prior to Cy for subjects receiving Cy (Cohort 2).
- Spiral CT scan of thorax and abdomen with optional FDG-PET scan. If a subject cannot have a CT scan [e.g. allergy to contrast dye], an MRI should be performed. CT scan may be done within ±7 days of BV2.
- Clinical assessment of tumor status using modified RECIST for assessment of response in MPM[4] and irRC[47]
- Pulmonary function tests: VC, FVC and FEV_1 for Cohort 2 only
- Optional core needle biopsy; may be done anytime between CY6 (or final chemotherapy cycle) and prior to dosing at BV1 (See Section 5.3)

[redacted]

[redacted]

5.2.2.6 End of Course (EOC) Follow-up

Subjects will return to the clinic for follow-up approximately 4 weeks after their 2nd boost vaccination. The following evaluations will be performed:

- Spiral CT scan of thorax and abdomen with optional FDG-PET scan. If a subject cannot have a CT scan [e.g. allergy to contrast dye], an MRI should be performed. CT scan may be done within ±7 days of EOC.
- Clinical assessment of tumor status using modified RECIST for assessment of response in MPM[4] and irRC[47]
- Pulmonary function tests: VC, FVC and FEV_1 for Cohort 2 only
- Physical examination
- ECOG performance status
- Resting 12-lead electrocardiogram
- Vital signs (BP, pulse, respiratory rate, temperature, pulse oximetry) and weight
- Blood draws for clinical hematology, serum chemistry
- Blood draw (serum and plasma) for tumor marker(s) (See Section 5.3)
- AEs and concomitant medications review
- Blood draw for isolation of PBMC (up to 200 mL) (See Section 5.3)
- Blood draw for *Lm*- and mesothelin-specific immunity assays (10 mL) (See Section 5.3)
- Optional core needle biopsy; may be done any time after final dose in course (see Section 5.3)

5.2.3 Study Follow-up Period/End of Study (EOS)

Subjects will return to the clinic for follow-up approximately 4 weeks after their EOC visit and every 8 weeks thereafter until disease progression or investigator determines subject is no longer receiving benefit from treatment (if subject continues treatment beyond progression). Subjects who are clinically stable and continue to meet dosing eligibility will receive maintenance vaccinations (with or without Cy) at every follow-up visit. Subjects may continue on treatment with radiographic disease progression if subject is clinically stable and investigator believes the treatment is providing benefit. All subjects will complete an EOS visit no more than four weeks following the final dose of study medication or prior to receipt of other cancer-related treatment. If the EOS visit occurs earlier than 4 weeks, a safety follow-up telephone call on Day 28 (+/- 7 days) is required; document contact in the study records.

The following evaluations will be done at each follow-up (FU) visit and at the end of study (EOS) visit:

- Symptom-directed physical examination
- ECOG performance status
- Vital signs (BP, pulse, respiratory rate, temperature, pulse oximetry) and weight (day 1 only)
- Blood draws for clinical hematology, serum chemistry
- AEs and concomitant medications review
- For eligible subjects receiving maintenance vaccinations (MVs), CRS-207 (without Cy [Cohort 1] or with Cy [Cohort 2]) will be administered at every follow-up visit. CRS-207 and Cy will be administered following the same procedures stated in Section 5.2.2.1. Subjects will be monitored for at least 2 hours after each infusion or until they are considered clinically stable. Blood draws for clinical labs may be done up to 3 days prior to CRS-207 dosing (Cohort 1) or 2 days prior to Cy for subjects receiving Cy (Cohort 2). Subjects will be contacted by phone to assess AEs and concomitant medications 1 day after CRS-207 infusion.

- Blood will be collected to assess clearance of CRS-207 at EOS Visit. For subjects with a central line port, a blood sample will also be taken through the port. This blood collection must be taken prior to administration of the EOS antibiotics.

- [redacted]

In accordance with good medical practice, any ongoing AE present at end of the study, including a clinically significant laboratory test abnormality, which is determined by the investigator as possibly or probably related to the study investigational agents, will be followed until resolved, until the event stabilizes and the overall clinical outcome has been ascertained, or until the subject is lost to follow-up.

5.3 LABORATORY EVALUATIONS

5.3.1 Immune Monitoring Assessments

Peripheral blood mononuclear cells and serum

Peripheral blood mononuclear cells (PBMCs) and serum will be collected at various time points as listed in Table 2, prior, during and post treatment to assess *Lm*- and mesothelin-specific immunity. T cell responses to mesothelin will be considered positive when the frequency of specific responses are ≥ 1 in 10^5 $CD8^+$ peripheral blood lymphocytes (PBL) above the control sample and increased by at least 2-fold compared to baseline. T cell responses to *Lm* (LLO) will be considered positive when specific T cell frequencies were ≥ 1 in 10^5 PBMCs and increased by at least 2-fold compared to baseline.

Collection

Briefly, up to 200 mL of whole blood will be collected in green top tubes (heparin sulfate) or heparinized syringes. At NCI site, please page 102-11964 (Gareth Peters or alternate technician from Dr. Figg's laboratory) for immediate pick-up. At other sites, please refer to laboratory manual for storage and shipping instructions.

Processing and Storage

- PBMCs will be processed within 6 hours post-collection using Ficoll-Hypaque centrifugation. Isolated PBMCs will be stored in liquid nitrogen. Plasma collected from the PBMC process will be aliquoted and stored at -70°C or colder.

- Serum will be processed within 6 hours post-blood draw from a 10 mL serum tube. The serum samples will be stored at -70°C or colder.

- The detailed procedures for PBMC and serum processing will be provided in a laboratory manual. Training will also be provided and only trained, qualified personnel will process samples.

- Samples will be processed and stored at each site locally. At NCI, samples will be processed and stored by the Molecular Pharmacology Program (Building 10, Room 5A01; William Figg, Pharm.D.)

Testing Sites

- Cellular mesothelin- and *Lm*-specific immunity will be assessed by IFN-γ ELISPOT analysis as previously described [26]. Additionally, serum cytokines/chemokines will be assessed 20 to 26 hours following each CRS-207 infusion. Samples for ELISPOT and cytokine/chemokine analysis will be stored locally and batch-shipped in liquid nitrogen to Aduro for testing.

- Antibody specific to mesothelin will be tested by ELISA as previously described [22]. Antibody testing will be conducted at NCI at the laboratory of Dr. Raffit Hassan, M.D (Building 37, Room 5108).

5.3.2 Tumor Markers

Tumor markers such as mesothelin, CA-125 and osteopontin will be monitored before, during and after the treatment course. Serum will be collected to determine circulating levels of mesothelin and CA-125, two tumor markers that have been demonstrated to be elevated in subjects with pleural mesothelioma. Systemic levels of osteopontin will be determined from plasma by a commercially-available ELISA.

CA-125 will be collected as per local laboratory standards.

Mesothelin and Osteopontin Sample Collection

Samples for mesothelin testing will be drawn in a 5 mL red top tube and samples for osteopontin testing will be drawn in a 5 mL green top tube before during and after treatment as indicated in the study calendar.

At NCI site, please page 102-11964 (Gareth Peters or alternate technician from Dr. Figg's laboratory) for immediate pick-up. At other sites, please refer to laboratory manual for storage and shipping instructions.

Mesothelin and Osteopontin Processing and Storage

Serum and plasma specimens will be stored by the Molecular Pharmacology Program (Building 10, Room 5A01; William Figg, Pharm.D.) at -70°C or colder.

Mesothelin and Osteopontin Testing Sites

The analyses will be performed in batch at NCI in the laboratory of Dr. Raffit Hassan, M.D. (Building 37, Room 5108).

5.3.3 Tumor Biopsy

Collection

Tissue biopsies will be encouraged but done strictly on a voluntary basis. Standard techniques will be used for pre-treatment and post-treatment percutaneous biopsies which may include CT, cone beam CT, ultrasound, or fusion guided biopsy. When clinically feasible and judged minimal risk by Interventional Radiology, the same total number biopsies will be prospectively acquired from areas of tumor that may exhibit imaging features of higher or lower cellularity, such as higher PET scan activity, or evidence solid features or viability on CT scan. This will allow biopsy specimens to be marked as "high imaging cellularity" or "low imaging cellularity", which could enhance the ability to obtain quality tissue biomarkers, immunohistochemistry or mRNA, and could facilitate the identification of reliable biomarkers for response.

Biopsies will be obtained from primary tumor sites and metastatic sites (if applicable). No more than 4 cores may be obtained per site. Half of the biopsy specimen will be used for immunohistochemistry (IHC) analysis. The other half will be used for gene expression analysis.

Archival tissue from all subjects will be obtained if possible, with exception for subjects who agree to the optional tumor biopsy at screening do will not need archival tissue obtained.

Processing and Storage

The fixed tissue samples that will be used for IHC analysis will be processed and embedded in paraffin. Tissue blocks will be made from the paraffin-embedded tissue. Tissue blocks will be stored at room temperature.

The tissue that will be used for gene expression analysis will be stored immediately after collection in at least 5 volumes of RNA*later*® solution. The samples will be stored overnight at 4°C and transferred to -70°C or colder for long-term storage until RNA isolation. Samples obtained at NCI will be stored by the Molecular Pharmacology Program (Building 10, Room 5A01; William Figg, Pharm.D)

Please consult laboratory manual for shipping instructions.

Testing Sites

IHC analysis will be performed at the Laboratory of Pathology at NCI to determine mesothelin expression within the tumor. Furthermore, collected tumor tissue will be assessed for CD4+ and CD8+ T cell infiltration as well as the presence of regulatory T cells (FoxP3+). Additional markers might be assessed. Tumor expression analysis will be performed on the isolated RNA from tumor biopsy tissue as previously described.[48]

Gene expression profiling of tumor tissue samples will be conducted by Aduro.

6.0 PROCEDURES FOR HANDLING AES AND SAES

6.1 DEFINITION OF AN ADVERSE EVENT

The following definition of an AE will be used for this study:

> Any untoward medical occurrence in a subject administered an investigational product and which does not necessarily have to have a causal relationship with this treatment. An adverse event can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not related to medicinal (investigational) product.

Examples of AEs include the following:

- Significant or unexpected worsening or exacerbation of the indication under study
- Exacerbation of a chronic or intermittent preexisting condition, including an increase in frequency or intensity of the condition
- New conditions detected or diagnosed after investigational product administration, even if they were present before the start of the study
- Signs, symptoms, or the clinical sequelae of a suspected interaction with another medical product
- Signs, symptoms, or the clinical sequelae of a suspected overdose of either investigational product or a concurrent medication

An overdose should not be reported as an AE or SAE; instead the symptoms resulting from the overdose should be reported as the AE or SAE.

Examples of AEs do not include the following:

- Medical or surgical procedures (e.g. endoscopy, appendectomy) (Instead, the medical condition that led to the procedure is an AE.)
- Situations that are unwanted but in which an untoward medical occurrence did not occur, for example social inconvenience after admission to a hospital

- Anticipated day-to-day fluctuations of a preexisting disease or condition (present or detected before enrollment) that does not worsen overall

- Expected progression of the disease being studied, including signs or symptoms of the disease being studied, unless progression is more severe than expected for the subject's condition

It is the responsibility of the investigator to perform periodic and special assessments for AEs. The investigator and clinical staff will note all AEs offered by the subject at baseline, during administration of the CTM, and at the follow-up visit. All clinical complaints volunteered by, or elicited from, the subject during the study will be recorded on the appropriate page of the CRF for the study period indicated. If any AE occurs, the subject will receive appropriate treatment and medical supervision.

All AEs judged to be clinically significant, including clinically significant laboratory abnormalities, will be followed until resolution. All AEs will be summarized in the annual report or more frequently, if requested by the regulatory agency. SAEs require special reporting in addition to documentation in the CRF as described below.

6.2 DEFINITION OF A SERIOUS ADVERSE EVENT (SAE)

In this study, the definition of an SAE is an AE that meets any of the following criteria:

- Results in death

- Is life-threatening

  Note: The term *life-threatening* in the definition of *serious* refers to an event in which the subject was at risk of death at the time of the event. It does not refer to an event that hypothetically might have caused death if it were more severe.

- Requires hospitalization or prolongation of existing hospitalization

  Note: In general, hospitalization signifies that the subject has been detained at the hospital or emergency ward for observation or treatment that would not have been appropriate in the physician's office or out-patient setting. Complications that occur during hospitalization are AEs but not necessarily

SAEs. An occurrence or complication that prolongs hospitalization is an SAE. When there is doubt as to whether hospitalization occurred or was necessary, the AE should be considered an SAE. Hospitalization for elective treatment of a preexisting condition that did not worsen from its original baseline severity is not considered an SAE.

Hospital admissions for overnight monitoring following CRS-207 infusion will not be considered an SAE unless the event meets criteria for seriousness other than hospitalization.

- A persistent or significant disability or incapacity

  Note: The term *disability* means a substantial disruption of a person's ability to conduct normal life functions. This definition is not intended to include AEs of relatively minor medical significance, such as uncomplicated headache, nausea, vomiting, diarrhea, influenza, or accidental trauma (e.g. sprained ankle), that may interfere or prevent everyday life functions, but do not constitute a substantial disruption of a person's ability to conduct normal life functions.

- A congenital anomaly or birth defect

- Other important medical event

  Note: Medical or scientific judgment should be exercised in deciding whether reporting is appropriate for other important medical events that may not result in death, be life-threatening, or require hospitalization but still may jeopardize the subject or may require medical intervention to prevent one of the outcomes listed in this definition. These events should also be considered serious.

An SAE requires additional detailed reports and follow-up. The content of these detailed reports must address the investigator's estimate of causality.

6.3 RECORDING AEs AND SAEs

All AEs will be reported from the time study treatment is first administered to the subject (PV1 for Cohort 1 or XP1 for Cohort 2) through 28 days after receipt of final dose of investigational product.

All preexisting medical conditions will be recorded on the baseline physical examination page of the CRF. Starting with the first administration of first investigational product, any new event or experience that was not present at screening, or worsening of an event present at screening, is considered to be an AE. Unchanged, chronic conditions are not AEs and should not be recorded on the AE page of the CRF.

When an AE or SAE occurs, it is the responsibility of the investigator to review all documentation (e.g. hospital progress notes, laboratory and diagnostic reports) relative to the event. The investigator is to record all relevant information about any AE (including SAEs) on the AE page of the CRF. It is not acceptable for the investigator to send photocopies of the subject's medical records in lieu of the proper completion of the appropriate AE (or SAE) CRF pages. However, there may be instances where copies of medical records for certain cases are requested. In such instances, all subject identifiers and PHI will be blinded on the copies of the medical records before submission to the appropriate authorities.

The investigator will also attempt to establish a diagnosis of the event on the basis of signs, symptoms, or other clinical information. In such cases, the diagnosis, not the individual signs and symptoms, should be documented on the appropriate CRF as the AE or SAE. In addition, SAEs need to be reported on the SAE report form provided in the study procedures manual (SPM). The SPM provides additional guidelines.

6.4 ASSESSMENT OF GRADE

The investigator will make an assessment of grade for each AE and SAE reported during the study, which will be recorded in the CRF. The assessment will be based on the National Cancer Institute's CTCAE, Version 4.03, and graded as shown below:

- Grade 1: Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated
- Grade 2: Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental activities of daily living
- Grade 3: Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care activities of daily living

- Grade 4: Life-threatening consequences; urgent intervention indicated
- Grade 5: Death related to AE

Any AE that changes in grade during its course will be recorded in the CRF at the highest level experience by the subject.

6.5 ASSESSMENT OF CAUSALITY

The investigator is obligated to estimate the relationship between the investigational products and the occurrence of each AE or SAE by using his or her best clinical judgment. Other causes, such as the history of the underlying disease, concomitant therapy, other risk factors, and the temporal relationship of the event to the investigational products will be considered and investigated. The investigator will also consult the IB or product labeling information for marketed products in the determination of the assessment.

There may be situations when an SAE has occurred and the investigator has minimal information to include in the initial report. However, it is very important that the investigator always assess causality for every event before the transmission of the SAE. The investigator may change his or her opinion of the causality in light of follow-up information, amending the SAE report. The causality assessment (Table 3) is one of the criteria used to determine regulatory reporting requirements and should not be left blank.

Table 3. Assessment of Causality/Relatedness of AEs

Term	Definition
Definitely related	The AE *is clearly related* to the investigational agent(s) or research intervention: the AE has a temporal relationship to the administration of the investigational agent(s) or research intervention, follows a known pattern of response, and no alternative cause is present.
Probably related	The AE is *likely related* to the investigational agent(s) or intervention: the AE has a temporal relationship to the administration of the investigational agent(s) or research intervention and follows a known or suspected pattern of response, but an alternative cause may be present.
Possibly related	The AE *may be related* to the investigational agent(s) or intervention: the AE has a temporal relationship to the administration of the investigational agent(s) or research intervention, follows a suspected pattern of response, but an alternative cause is present.
Unlikely to be related	The AE is *doubtfully related* to the investigational agent(s) or intervention: the AE has a temporal relationship to the administration of the investigational agent(s) or research intervention but follows no known or suspected pattern of response, and an alternative cause is present.
Unrelated (or not related)	The AE is *clearly NOT* related to the investigational agent(s) or intervention: the AE has no temporal relationship to the administration of the investigational agent(s) or research intervention and follows no known or suspected pattern of response, and an alternative cause is present.

AE = adverse event.

6.6 REPORTING OF SAEs

Reporting of SAEs will begin at the time study treatment is first administered to the subject (PV1 for Cohort 1 or XP1 for Cohort 2) and will continue through 28 days after receipt of final dose of investigational product.

SAEs will be reported to the sponsor by phone, fax, or e-mail within 24 hours of the time the investigator becomes aware of the event.

[redacted]

The Medical Monitor may also be contacted to discuss a safety event:

[redacted]

The urgency for reporting SAE(s) is fourfold:

1. To enable the safety department to fulfill the reporting requirements to the appropriate regulatory authority

2. To facilitate discussion (and implementation, if necessary) between the safety department and the investigator of appropriate follow-up measures in the event an expedited report is required

3. To facilitate Aduro's rapid dissemination of information about AEs to other investigators or sites in a multicenter study by using expediting reporting

4. To facilitate investigator reporting of unanticipated problems involving risk to human subjects to the IRB and institutional biosafety committee

In the event an SAE is observed, the SAE report will be completed as thoroughly as possible including the following:

- All available details about the event
- Signature of the investigator

The SAE report will be forwarded to the sponsor or designee within the designated time frames. If the investigator does not have all information about an SAE, the investigator will *not* wait to receive additional information before notifying the sponsor of the event and completing the form. The form will be updated when additional information is received.

Aduro will report all SAEs that are unexpected and considered possibly or probably related to the administration of the investigational agents to the Food and Drug Administration in the form of an expedited safety report within 15 calendar days after receiving information on the SAE. Aduro will also report to the United States Food and Drug Administration (FDA) and National Institutes of Health Office of Biotechnology Activities (NIH OBA) by fax or phone within 7 days of receiving the information, any unexpected life-threatening or fatal SAEs that are considered at least possibly associated with the investigational agents. Aduro, or designee, will also notify all participating investigators of expedited safety reports within 15 calendar days after receiving information. The investigators will notify their reviewing IRB and institutional biosafety committee (IBC) as required by institutional policies.

6.7 FOLLOW-UP OF AEs AND SAEs

After the initial AE or SAE report, the investigator is required to proactively follow each subject and provide further information to the safety department in regards to the subject's condition.

All AE(s) and SAE(s) will be followed until:

- Resolution
- The condition stabilizes
- The event is otherwise explained
- The subject is lost to follow-up

Once the event is resolved, the appropriate AE or SAE report page will be updated. The investigator will also ensure that the follow-up includes any supplemental information that may explain the causality of the event(s).

New or updated information will be recorded on the originally completed AE or SAE report, with all changes signed and dated by the investigator or designee. The updated AE or SAE report will then be signed by the investigator and resubmitted to the safety department.

[redacted]

[redacted]

[redacted]

[redacted]

7.0 STUDY OR STUDY SITE TERMINATION AND SUBJECT DISCONTINUATION

7.1 PREMATURE STUDY OR STUDY SITE TERMINATION

If Aduro, the investigator, the medical monitor, the study monitor, or appropriate regulatory officials discover conditions arising during the study that indicate that the study should be halted or that the study center should be terminated, this action may be taken after appropriate consultation among Aduro, the investigator, the medical monitor, and the study monitor. Conditions that may warrant termination of the study include, but are not limited to the following:

- The discovery of an unexpected, serious, or unacceptable risk to the subjects enrolled in the study
- A decision on the part of Aduro to suspend or discontinue testing, evaluation, or development of the product for any reason

A study conducted at a single study site in a multicenter study may also warrant termination under the following conditions:

- Failure of the investigator to enroll subjects into the study at an acceptable rate
- Failure of the investigator to comply with pertinent regulations of appropriate regulatory authorities
- Submission of knowingly false information from the research facility to Aduro, the study monitor, or appropriate regulatory authority
- Insufficient adherence to protocol requirements

Study termination and follow-up will be performed in compliance with the conditions set forth in the International Conference on Harmonisation (ICH) E6, Guideline for Good Clinical Practice, Sections 4.12, 4.13, 5.20, and 5.21.

7.2 SUBJECT DISCONTINUATION

Subjects will be encouraged to complete the study; however, they may voluntarily withdraw at any time. The investigator will provide a written explanation describing the reason for discontinuation in a source document, which will be

transcribed to the appropriate CRF page. Subjects who wish to withdraw from the study will be encouraged to complete the planned administration of antibiotics, complete EOS assessments, post-treatment monitoring for CRS-207, and to complete assessments scheduled during the follow-up visit.

Subjects who withdraw consent or discontinue treatment before completing at least 2 cycles of chemotherapy will be considered dropouts and may be replaced at the discretion of the lead investigator, sponsor and medical monitor. A subject may be removed from treatment for the reasons listed in Sections 7.2.1 through 7.2.4.

7.2.1 Adverse Event

If a subject suffers an AE that, in the judgment of the investigator, Aduro, or the medical monitor, presents an unacceptable consequence or risk to the subject, the subject may be discontinued from further participation in the study.

7.2.2 Intercurrent Illness

A subject may be discontinued from the study if, in the judgment of the investigator, the subject develops an intercurrent illness or complication (including progressive disease) that, in any way, justifies withdrawal from the study.

7.2.3 Noncompliance

After consultation between the investigator, the medical monitor, or study monitor, and Aduro when appropriate, a subject may be discontinued from the study for the following administrative reasons:

- Failure to receive study medication or treatment as mandated by the protocol
- Failure to comply with protocol requirements
- Unauthorized, subject-initiated changes in dosing regimen

7.2.4 Refusal of CTM Administration

If, for any reason, the subject refuses CTM administration during the study, the subject may be discontinued from the study, and the reason(s) for refusal will be documented on the appropriate CRF page. Reasonable efforts should be made to monitor the subject for AEs and to complete follow-up assessments. These efforts should be documented on the appropriate CRF page.

8.0 DATA COLLECTION AND PROCESSING AND STATISTICAL ANALYSIS

8.1 DATA COLLECTION AND PROCESSING

CRFs will be used to capture study results and data. The study coordinator or other authorized study personnel will transcribe data from source documents onto paper or electronic CRFs. All CRFs will be reviewed and source verified by the study monitor during periodic site visits, and the study monitor will ensure that all data in the CRF are correct and complete. Before or between visits, the medical monitor or study monitor may request copies of the CRFs for preliminary medical review. Once the CRFs are complete and source-verified, the investigator must sign and date all required pages, verifying the accuracy of all data contained within the CRF. Training will be provided on proper completion of CRFs.

If electronic CRFs are used, training will be provided for the electronic data capture (EDC) system. All personnel using the EDC system must have appropriate education, training, and experience. The investigator will be provided with standard operating procedures (SOPs) (contained in the SPM or a vendor-specific SOP) on the use of the EDC system. The investigator will be responsible for documenting employee education, training, and previous experience that pertains to the EDC system.

The investigator must maintain adequate security of the EDC system, including documentation that all users have been trained on the appropriate SOPs and a list of authorized users. To ensure attributability, all personnel responsible for data entry must obtain a unique electronic signature before any data can be entered in the CRFs. The system must be configured to ensure that the signer cannot readily repudiate the signed record as not genuine. Authorized study personnel will be assigned a unique password and associated electronic signature after receiving SOP training.

If EDC systems other than those provided and maintained by the sponsor are used for documentation and data capture, the investigator must ensure that the systems are validated and ensure data backup as described in Section 9.2.

8.2 STATISTICAL ANALYSIS

8.2.1 General Overview

The data will be summarized in tables listing the mean, standard deviation, median, minimum, maximum and number of subjects in a group for continuous data; in tables listing count and percentage for categorical data; and median and standard error for time-to-event data. Data will be listed for each subject. All statistical analyses will be performed and data appendices will be created by using SAS. The effects of noncompliance, dropouts, and covariates will be assessed to determine the impact on the general applicability of results from this study. Unless specified otherwise, summaries will be reported by treatment regimen (Cohort) and overall. This corresponds to Cohort 1 (CRS-207 without Cy), Cohort 2 (CRS-207 with Cy), and overall. For select summaries of disposition, baseline data, and key efficacy endpoints, summaries of Cohort 1 will be further broken out by subjects enrolled in the original and expansion phase. No formal comparisons across enrollment phase (original vs. expansion) or treatment regimen (Cohort 1 vs. Cohort 2) will be performed.

Further details of the analysis, including the handling of missing data transformations, assessing impact of the expansion cohort, impact of changes to the planned chemotherapy regimen and further modifications to populations of analysis will be provided in a separate statistical analysis plan.

8.2.2 Populations of Interest

The full analysis (FAS) population is all subjects enrolled who receive at least one dose of study treatment.

The per protocol population is defined as subjects who receive two doses of CRS-207 (with or without Cy) followed by at least 4 cycles of pemetrexed and cisplatin.

Subjects with major protocol violations will be excluded from the per protocol population. The precise reasons for excluding subjects from the per protocol set will be fully defined and documented before database lock.

8.2.3 Baseline Comparability

Demographics and baseline clinical variables for subjects will be summarized using descriptive statistics. Relationship to efficacy and safety endpoints may be

assessed via scatter plots for pairs of continuous endpoints and summary statistics within categories for categorical / continuous pairs, and contingency tables (N, percent) for pairs of categorical endpoints. Differences in baseline variables across enrollment phase (original vs. expansion) will be evaluated descriptively and may be used as covariates in sensitivity analyses of efficacy endpoints.

8.2.4 Efficacy Analysis

Efficacy analysis will be conducted on full analysis and per protocol populations. Standard adjustments to analyses for pre-specified or baseline-suggested clinical covariates will be performed; these covariate-adjusted analyses will be considered secondary analyses. The primary endpoint is the change in IFN-γ production of mesothelin-specific T cells by ELISPOT assay from baseline to (1) immediately after CRS-207 (with or without Cy), (2) after receiving chemotherapy, and (3) after receiving two subsequent doses of CRS-207 (with or without Cy) following chemotherapy. Change from baseline will be compared via paired t-test of log-transformed post/pre ELISPOT values and assessed for statistical significance via Hochberg multiplicity alpha adjustment. T cell responses to mesothelin will be considered positive when the frequency of specific responses are ≥ 1 in 10^5 $CD8^+$ peripheral blood lymphocytes (PBL) above the control sample and increased by at least 2-fold compared to baseline. T cell responses to *Lm* (LLO) will be considered positive when specific T cell frequencies were ≥ 1 in 10^5 PBMCs and increased by at least 2-fold compared to baseline. In addition, if change from baseline in the logarithmic scale is not normally distributed (that is, if $p<0.05$ by a Shapiro-Wilks test) then a Wilcoxon signed rank test will be performed instead.

Analyses of other efficacy endpoints including changes in PFTs, objective tumor response, progression free survival, time to progression and overall survival will be conducted on the full analysis and per protocol populations. Time to event data will be analyzed using Kaplan-Meier methods and will be calculated from date of first dose of study treatment. Further details on the analyses including censoring strategies will be described in a statistical analysis plan.

Exploratory analyses will be conducted to evaluate the relationship between PFT improvement and tumor response, as well as to evaluate the relationship between overall survival and tumor response.

8.2.5 Safety Analysis

AE data will be listed individually and incidence of AEs summarized by system organ class and preferred terms within a system organ class for each treatment group. When calculating the incidence of AEs, each AE (based on preferred terminology defined by Medical Dictionary for Regulatory Activities, Version 13.1, or the most current version) will be counted only once for a given subject. In analyses of grade and causality, if the same AE occurs on multiple occasions, the highest grade and strongest relationship to study drug will be assumed. If two or more AEs are reported as a unit, the individual terms will be reported as separate experiences.

Changes in vital signs, hematology, and clinical chemistry parameters from baseline to the end of the study will be examined. Treatment-emergent changes from normal to abnormal values in key laboratory parameters will be identified.

8.2.6 Pharmacokinetic Analysis

No formal pharmacokinetic or pharmacodynamic analyses are planned for the investigational agents used in this study.

8.2.7 Interim Analysis

Safety data will be reviewed on an ongoing basis by Aduro, the investigator(s), and medical monitor.

No formal interim analysis for this phase 1B study is planned.

8.2.8 Sample Size

Up to 60 subjects in total may be enrolled, based on the original planned cohort of 16 subjects and an expansion cohort that may enroll up to an additional 44 subjects. At least 32 subjects will receive CRS-207 in combination with chemotherapy (Cohort 1). Additional subjects (up to 28) will receive Cy one day prior to each CRS-207 administration combined with chemotherapy (Cohort 2).

The originally planned sample size calculation was based on the primary endpoint of change in mesothelin-specific T cell responses as measured from ELISPOT. With one primary parameter (ELISPOT) to be measured with respect to a change from baseline at three post-baseline time points, the sample size will be selected to allow each test to be performed using a 0.017 two-tailed significance level, in

order to allow the overall set of three tests to be very conservatively performed as if at an overall 0.05 level using a Bonferroni adjustment. Assuming 14 subjects with complete measurements of the main parameter at baseline and the three subsequent time points, there would be 80% power to detect a change from baseline to each time point equal to 1.0 standard deviations of the change (1.0 effect size) using a two-tailed 0.017 level paired t-test. This stringent multiplicity adjustment is for power computation only. In practice, for analysis purposes, instead of requiring that each test achieve a 0.017 level in order to be declared significant, a less overly stringent Hochberg adjustment may be used. In order to allow for a small number of inevaluable subjects, up to 16 subjects could be enrolled.

The expansion cohort of up to 44 additional subjects, including subjects receiving Cy one day prior to CRS-207 (Cohort 2) is intended to obtain additional safety, immune and efficacy data for future study planning. The expansion sample size is based on practical rather than statistical considerations.

9.0 CLINICAL STUDY ADMINISTRATION

9.1 INFORMED CONSENT AND AUTHORIZATION FOR USE AND DISCLOSURE OF PHI

Written informed consent and authorization of use and disclosure of PHI must be obtained from each subject (or the subject's legally authorized representative) before performing any study-specific screening/baseline period evaluations. One copy of the signed ICF and authorization for use and disclosure of the PHI form will be given to the subject, and the investigator will retain the original. The ICF and authorization for use and disclosure of PHI, which is prepared by the investigator or the site, must be reviewed and approved by Aduro, the study monitor (if applicable), and the site's IRB before the initiation of the study. The ICF must contain the 20 elements of informed consent described in ICH E6, Section 4.8. The authorization for use and disclosure of PHI must contain the elements required by Title 45 of the CFR, Section 164.508(b), for valid authorizations.

9.2 STUDY DOCUMENTATION

9.2.1 Investigator Information

Investigator information is included in the SPM, which is updated as needed.

9.2.2 Investigator Study Files

Documentation about the investigator and study staff, the IRB, and the institution, is required by the sponsor before study site initiation. Copies of these documents as well as supplemental information, such as the investigator's obligations, IB, clinical study protocol and amendments, safety information, CTM, biological samples, laboratory, SPM and study logs, monitoring activities, sponsor/investigator/study monitor correspondence, will be kept on-site in study site-specific binders.

Aduro will be responsible for maintaining backup of all CRF data. The investigator is responsible for maintaining backup of all electronic data systems used for primary documentation or source documentation. Backup of electronic data will be performed periodically as described in the site-specific SOPs. Backup records must be stored at a secure location on site, and backup and recovery logs must be maintained to facilitate data recovery. Finally, if an

electronic medical records system that is not supported by the sponsor (or is discontinued or decommissioned) is used, the investigator must maintain a system to retrieve these records or arrange for the transfer of these records to an alternate electronic format or to paper.

Changes to any electronic records requires an audit trail, in accordance with 21 CFR 11.10(e), and should include who made the changes and when and why the changes were made. An audit trail is defined as a secure, computer-generated, time-stamped electronic record that will allow reconstruction of the course of events relating to the creation, modification, and deletion of an electronic record. Audit trails must be created incrementally, in chronological order, and in a manner that does not allow new audit trail information to overwrite existing data. Finally, audit trails should be in a readable format and readily available at the study site and any other location where electronic study records are maintained.

Audit trails are generated automatically for electronic CRFs. The investigator is responsible for maintaining audit trails of all electronic data systems used for primary documentation or source documentation.

9.2.3 CRFs and Source Documentation

The investigator must make study data accessible to the site monitor, to other authorized representatives of Aduro, and to the appropriate regulatory authority inspectors. The original CRF for each subject will be checked against source documents at the study site by the site monitor.

9.2.4 Retention of Study Documents

According to ICH E6, Section 4.9, all CRFs, as well as supporting paper and electronic documentation and administrative records, must be retained by the investigator for a minimum of 2 years after notification that the appropriate regulatory authority has approved the product for the indication under study, notification that the entire clinical investigation will not be used in support of a marketing application, or notification that the marketing application was not approved. No study documents will be destroyed or moved to a new location without prior written approval from Aduro. If the investigator relocates, retires, or withdraws from the clinical study for any reason, all records required to be maintained for the study should be transferred to an agreed-upon designee, such as another investigator at the institution where the study was conducted.

Audit trails for electronic documents must be retained for a period at least as long as that required for the subject electronic records to which they pertain. The investigator must retain either the original or a certified copy of audit trails.

9.3 CONFIDENTIALITY

9.3.1 Data

All information about the nature of the proposed investigation provided by Aduro or the study monitor to the investigator (with the exception of information required by law or regulations to be disclosed to the IRB, the subject, or the appropriate regulatory authority) must be kept in confidence by the investigator.

9.3.2 Subject Anonymity

The anonymity of participating subjects must be maintained. Subjects will be identified by their initials and an assigned subject number on CRFs and other documents retrieved from the site or sent to the study monitor, Aduro, regulatory agencies, or central laboratories/reviewers. Documents that identify the subject (e.g. the signed ICF) must be maintained in strict confidence by the investigator, except to the extent necessary to allow auditing by the appropriate regulatory authority, the study monitor, or Aduro representatives.

9.4 PROTOCOL COMPLIANCE

Substantive changes in the protocol include changes that affect the safety of subjects or changes that alter the scope of the investigation, the scientific quality of the study, the experimental design, dosages, assessment variable(s), the number of subjects treated, or the subject selection criteria. Such changes must be prepared as a protocol amendment by Aduro only upon joint approval of the changes by Aduro and the investigator. A protocol amendment must receive IRB approval before implementation. In parallel with the IRB approval process, the protocol amendment will be submitted to the appropriate regulatory authority as an amendment to the regulatory submission under which the study is being conducted. If a protocol amendment requires changes in the ICF, the revised ICF prepared by the investigator must also be approved by Aduro, the study monitor, and the IRB before implementation.

Emergency departures from the protocol that eliminate an apparent immediate hazard to a particular subject and that are deemed crucial for the safety and

well-being of that subject may be instituted for that subject only. The investigator or the attending physician also will contact the medical monitor as soon as possible in the case of such a departure. These departures do not require preapproval by the IRB; however, the IRB and the medical monitor must be notified in writing as soon as possible after the departure has been made. In addition, the investigator will document in the subject's CRF the reasons for the protocol deviation and the ensuing events.

9.5 STUDY MONITOR FUNCTIONS AND RESPONSIBILITY

The study monitor, in accordance with Aduro's requirements, will ensure that the study is conducted and documented properly by carrying out the relevant activities outlined in ICH E6, Section 5.18.4.

9.6 GENERAL INFORMATION

The investigator should refer to the IB, product labels, and the SPM, any other information provided during the study initiation visit or by the study monitor, and the appendices of this protocol for further information about this investigational new product or details of the procedures to be followed during the course of this study.

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APPENDICES

APPENDIX A

Modified RECIST for Assessment of Response in MPM

Original article

Annals of Oncology 15: 257–260, 2004
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Modified RECIST criteria for assessment of response in malignant pleural mesothelioma

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Background: The growth pattern of malignant pleural mesothelioma makes the use of RECIST (response evaluation criteria in solid tumours) response criteria difficult. We have developed and validated Modified RECIST criteria adapted to the growth pattern of malignant pleural mesothelioma.

Patients and methods: We evaluated 73 patients from two clinical trials of cisplatin/gemcitabine chemotherapy in malignant pleural mesothelioma. Tumour thickness perpendicular to the chest wall or mediastinum was measured in two positions at three separate levels on thoracic CT scans. The sum of the six measurements defined a pleural unidimensional measure. Bidimensionally measureable lesions were measured unidimensionally as for RECIST. All measurements were added to obtain the total tumour measurement. A reduction of at least 30% on two occasions 4 weeks apart defined a partial response; an increase of 20% over the nadir measurement, progressive disease. The validity of the modified criteria was gauged by evaluating survival and pulmonary function.

Results: Response according to these criteria predicted for superior survival (15.1 versus 8.9 months; $P = 0.03$) and forced vital capacity (FVC) increase during treatment ($P < 0.0001$). A significant correlation between change in linear tumour measurement and FVC was seen ($R = 0.63$; $P = 0.0001$).

Conclusion: These Modified RECIST criteria for tumour response correlate with survival and lung function and can be used to measure outcome in pleural mesothelioma.

Key words: chemotherapy, clinical trials, mesothelioma, RECIST, response criteria, validation

Introduction

The ability to measure reproducibly tumour response to treatment is vital in the development of new drugs and therapeutic combinations, particularly for the conducting of phase II studies. Conventional response criteria have always been difficult to apply to malignant mesothelioma due to its unique pattern of growth.

Malignant mesothelioma most commonly grows as a 'rind' around the pleural surface, and on computed tomography (CT) scan may not produce spherical lesions with bidimensionally measurable diameters. The WHO criteria [1] are poorly suited to evaluating response in mesothelioma, as they were developed principally to assess bidimensionally measurable disease. When used for unidimensional measurement, these criteria require a 50% decrease in the sum of unidimensional measurements to define a partial response (PR). This equates to a 75% decrease in the sum of the products of perpendicular diameters rather than the 50% decrease required to define response in bidimensionally measurable lesions.

The recently developed RECIST (response evaluation criteria in solid tumours) criteria [2] are more suited to tumour assessment in mesothelioma, as they specify the use of unidimensional measurements, with PR defined as a 30% decrease in the sum of the longest diameter for all target lesions. However, the selection of measurement sites in mesothelioma is difficult, and without further definition of the method of measurement, the RECIST criteria could be applied differently by different investigators (Figure 1A). Early experience suggests that modification of the criteria may be required in the special case of mesothelioma [3].

We have developed a modification of the RECIST criteria specifically to address the difficulties of measurement inherent in assessing changes in tumour bulk in pleural mesothelioma. We have previously performed two phase II clinical trials of the use of cisplatin and gemcitabine in patients with measurable pleural mesothelioma; a single centre study [4] and a subsequent confirmatory multicentre study [5]. In these studies, response rates of 47.6% [4] and 33% [5] were seen. Response criteria that incorporated both unidimensional and bidimensional measurements had been developed and used to assess response in these two trials ('original response criteria'). Using Modified RECIST criteria, we have now reassessed the response to treatment for a total of 73 patients who entered these two studies. To validate these new

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criteria we have related response to survival and to serial changes in pulmonary function on treatment.

Patients and methods

Patient population

Data on the 73 patients entered in the two previous clinical trials of cisplatin and gemcitabine in malignant pleural mesothelioma were obtained. All patients had measurable disease, defined as pleural tumour thickness of at least 1.5 cm on spiral CT scan, and histologically or cytologically confirmed mesothelioma. CT scans had been performed in all patients prior to the first cycle of chemotherapy, and then before the second, fourth and sixth cycles. Further CT scans were performed after the final cycle of chemotherapy, and then twice a month until disease progression. Forced vital capacity (FVC) had been measured prior to study entry and on day 1 of each chemotherapy cycle in 52 patients entered on the second study.

The original response criteria used in both trials were as follows. Tumour measurements were performed on transverse cuts on thoracic CT scans at three separate anatomically reproducible levels on the study entry CT scan and at the same levels on subsequent scans. Where possible, bidimensional lesions were measured. If there were no bidimensionally measurable lesions, unidimensional measurements of pleural tumour thickness were performed. Bidimensionally measurable lesions were measured using the longest dimension and the length perpendicular to the longest measurement. For unidimensionally measurable lesions, thickness of pleural tumour was measured at two separate sites on each of the three levels and the six measurements summated to produce a total measurement. Palpable masses were measured clinically on day 1 of each cycle as for bidimensionally measurable lesions. A pleural effusion was not considered a measurable lesion.

Tumour response was defined as: (i) complete response (CR): disappearance of all known disease, determined by two observations not less than 4 weeks apart; (ii) PR: a ≥50% decrease in the sum of the products of perpendicular diameters of bidimensionally measured lesions on two occasions not less than 4 weeks apart, or a ≥30% decrease in the sum of linear tumour measurements on two observations not less than 4 weeks apart; (iii) no change: a decrease in bidimensional tumour area of <50% or an increase of <25%, or a decrease in the sum of unidimensional measurements of <30% or an increase of <25%, provided no new lesions have appeared; (iv) progressive disease: a ≥25% increase in the size of the tumour being measured (unidimensional or bidimensional) or the appearance of new lesions.

Where response at sites measured bidimensionally differed from that at sites measured unidimensionally, the overall patient response was assessed by an audit group. The sites with dominant tumour bulk were favoured.

The protocols for each study were approved by the Committee for Human Rights of the University of Western Australia and the Sir Charles Gairdner Hospital Clinical Drug Trials Committee. Written informed consent was obtained from each patient before entry.

Modified RECIST criteria

Modified RECIST criteria were developed. The major problems in applying the RECIST criteria to malignant pleural mesothelioma are in the interpretation of the meaning and placement of the 'longest unidimensional diameter' of the target tumour mass to be measured. The longest diameter of a tumour mass is frequently that which follows the inner curve of the chest wall. Defining the limits of such a diameter is often problematical. When the tumour regresses with treatment the line may cross an area outside the tumour margin because of the curve of the chest wall. This may produce difficulty with reproducibility of measurement. Furthermore, the longest tumour diameter may be between two fixed structures, such as the thoracic vertebrae and the carina, and measurement in these areas may not fully reflect tumour response.

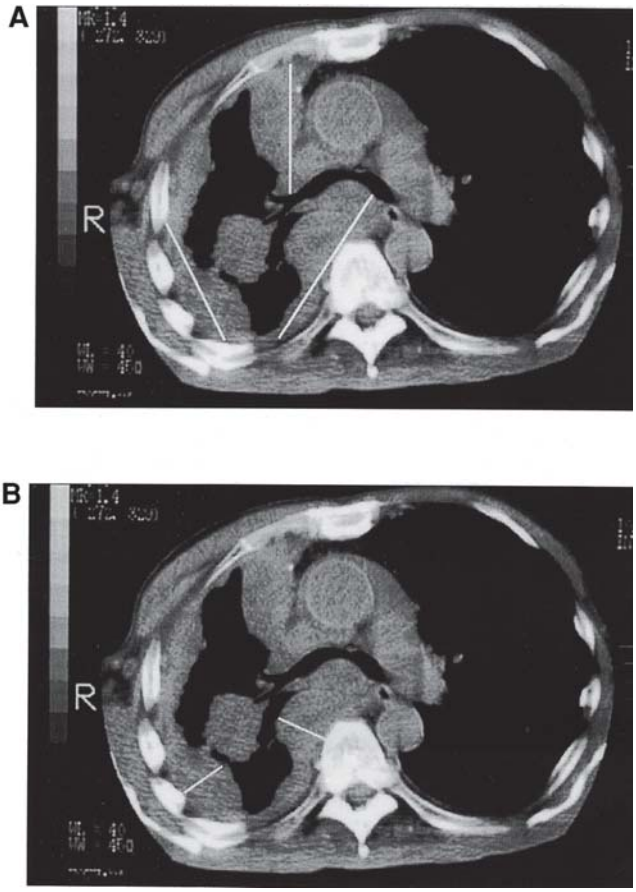

Figure 1. Example of measurement of a single computed tomography scan slice. (**A**) Lines represent possible interpretations of 'longest tumour diameter' according to current RECIST criteria. (**B**) Lines represent suggested measurement sites perpendicular to fixed structures, chest wall and vertebral column, according to Modified RECIST criteria.

The Modified RECIST criteria we have developed were as follows. Tumour thickness perpendicular to the chest wall or mediastinum was measured in two positions at three separate levels on transverse cuts of CT scan (Figure 1B). The sum of the six measurements defined a pleural unidimensional measure. Transverse cuts at least 1 cm apart and related to anatomical landmarks in the thorax were chosen to allow reproducible assessment at later time points. If measureable tumour was present, transverse cuts in the upper thorax, above the level of division of the main bronchi were preferred. At reassessment, pleural thickness was measured at the same position at the same level and by the same observer. This was not necessarily the greatest tumour thickness at that level. Nodal, subcutaneous and other bidimensionally measurable lesions were measured unidimensionally as per the RECIST criteria. Unidimensional measurements were added to obtain the total tumour measurement.

CR was defined as the disappearance of all target lesions with no evidence of tumour elsewhere, and PR was defined as at least a 30% reduction in the total tumour measurement. A confirmed response required a repeat observation on two occasions 4 weeks apart. Progressive disease (PD) was defined as an increase of at least 20% in the total tumour measurement over the nadir measurement, or the appearance of one or more new lesions. Patients with stable disease (SD) were those who fulfilled the criteria for neither PR nor PD.

Table 1. Comparison of response at individual time point observations between original response criteria and Modified RECIST criteria

Original response criteria	Modified RECIST criteria				
	CR	PR	SD	PD	Total
CR	0	0	0	0	0
PR	0	72	5	4	81
SD	0	11	93	1	105
PD	0	1	2	47	50
Total	0	84	100	52	236

RECIST, response evaluation criteria in solid tumours; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease.

Table 2. Comparison of response rates for original response criteria versus Modified RECIST criteria

	CR	PR	SD	PD	Overall response (%)
Original response criteria	0	27	40	6	37
Modified RECIST criteria	0	27	40	6	37

RECIST, response evaluation criteria in solid tumours; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease.

Validation of modified criteria

The response status of all 73 patients was re-assessed at each trial time point according to these Modified RECIST criteria. Patients were assigned to one of two groups: responding patients ('responders') and patients with SD or PD ('non-responders'). Overall survival from the start of treatment and serial changes in FVC were analysed for these two groups as a surrogate measure of patient benefit.

Results

Tumour measurements from a total of 236 CT scans from 73 patients were reassessed for response status at each time point in the trials according to the modified criteria. In general there was a close correlation between the original response criteria and the Modified RECIST. Of 81 time points originally showing PR, 72 remained classified as PR, five became SD and four became PD. Of 105 time points originally showing SD, 93 remained as SD, 11 became PR and one became PD. Of 50 time points originally showing PD, 47 remained PD, two became SD and one became PR (Table 1). The overall confirmed response rate as assessed by the two systems did not differ, as two patients classified as SD became PR and two patients classified as PR became SD (Table 2).

Median survival was plotted as a Kaplan–Meier curve for the responding and non-responding patients (Figure 2). There was a statistically significant difference in survival between the two patient groups (log rank test $P = 0.03$). Median survival was 15.1 months for responding patients and 8.9 months for non-responding patients.

FVC was plotted as a percentage of starting FVC for the responding and non-responding patients (Figure 3). FVC improved

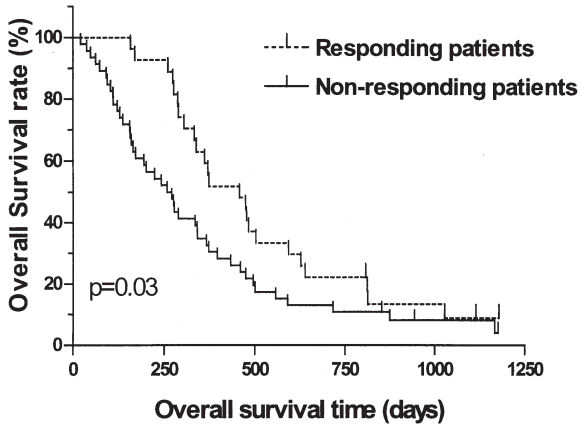

Figure 2. Kaplan–Meier survival curve of overall survival (days) from start of treatment for 73 patients treated with cisplatin and gemcitabine. Responding (dashed line) versus non-responding (solid line) patients as per Modified RECIST criteria. *P* value represents results of log rank test.

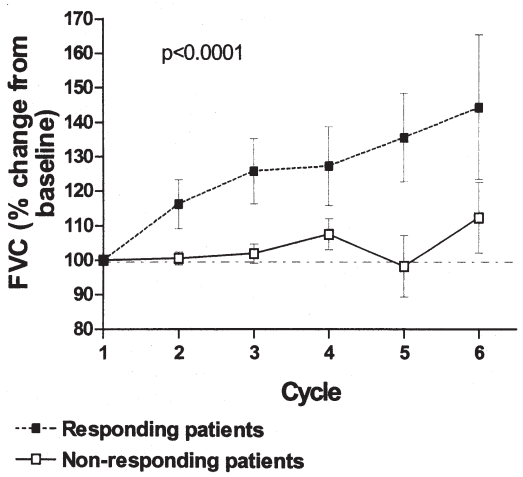

Figure 3. Forced vital capacity (FVC) as percentage of individual baseline FVC for all 73 patients treated with cisplatin and gemcitabine. Responding (filled squares) versus non-responding (open squares) patients as per Modified RECIST criteria. *P* value represents results ANOVA.

significantly over the course of treatment for responding patients as compared with non-responding patients ($P = 0.0001$).

The change in FVC against change in linear tumour measurement was plotted for each patient (Figure 4); a significant correlation was seen ($R = 0.63$, $P = 0.001$).

Discussion

The response criteria used in our previous two trials of chemotherapy in mesothelioma incorporated bidimensional measurements, as the WHO response criteria were widely used when the first of these trials began accrual. These response criteria were not altered for the second trial, which sought to broaden the applicability of our findings in the first single-centre study into a

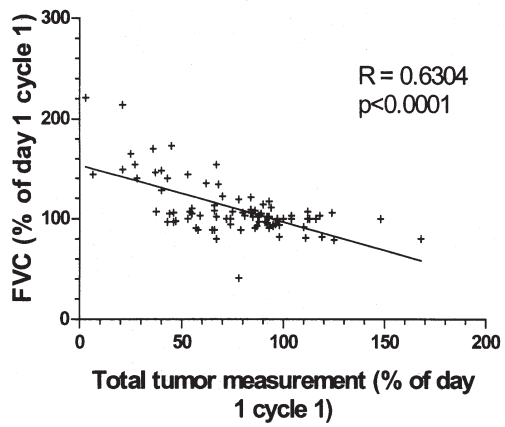

Figure 4. Forced vital capacity (FVC) versus total tumour measurement standardised as percentage of individual baseline on day 1 of cycle 1. $R = 0.6304$, the result of linear regression analysis; $P < 0.0001$, significance of deviation from 0 of the line of regression.

multicentre setting, adding prospective evaluation of quality of life and lung function end points. In order to make the response rates of the two trials directly comparable, it was important that the measurement criteria used in the two studies were the same. Subsequently, the RECIST criteria have been developed and become widely accepted and used in clinical trials. The application of RECIST criteria could, however, be variably interpreted by different investigators in mesothelioma, and this may lead to unsatisfactory results [3]. We have developed Modified RECIST criteria that are specifically designed to address the unique growth pattern of pleural malignant mesothelioma. Use of these modified response criteria did not materially alter the response rates in our two previous trials. However, the modified criteria avoided difficult and ambiguous situations that arose in response interpretation in the two previous trials; for example, how to evaluate response when discordance between unidimensional and bidimensional lesions occurred.

Whilst tumour response and progression directly reflect changes in tumour bulk, they are most clinically useful when they relate closely to other measures of a patient's condition. Response is a surrogate for patient benefit in the evaluation of new drugs and combinations. Patient benefit in pleural mesothelioma may include an improvement in survival or lung function, and improvement in symptom control or quality of life. Thus, it is important that any valid measurement of response should reflect changes in these parameters. We have demonstrated that these Modified RECIST criteria successfully distinguish between responders and non-responders for the parameters of survival and change in FVC, thus demonstrating their validity.

Further evaluation of these modified criteria should be performed before they can be incorporated routinely into future clinical trials. The development of an automated measurement format may enhance the speed and reproducibility of the measurements [6] and go some way to overcoming the potential problem of inter-observer variability. We have avoided this issue by using the same observer or an audit group to undertake the measurements. Tests of inter-observer variability are important, however, as has been demonstrated in the assessment of response in lung cancer [7]. To confirm the practicality of the criteria they should be applied to a group of clinicians who lack extensive experience in the measurement of mesothelioma in clinical trials.

Acknowledgements

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APPENDIX B

Immune-related Response Criteria (irRC) Quick Reference

Comparison between RECIST 1.1 criteria and the irRC*

	RECIST 1.1	irRC RECIST 1.1
New, measurable lesions	Always represent PD	Incorporated into tumor burden
New, non-measurable lesions	Always represent PD	Does not define progression (but precludes irCR)
Non-index lesions	Changes contribute to defining best overall response (BOR) of CR, PR, SD, and PD	Contribute to defining irCR (complete disappearance required)
CR	Disappearance of all lesions in two consecutive observations not less than 4 week apart	Disappearance of all lesions in two consecutive observations not less than 4 week apart if single arm trial and primary endpoint only
PR	≥ 30% decrease in the sum of the diameters of all index lesions compared with baseline in two observations at least 4 week apart, in absence of new lesions or unequivocal progression of non-index lesions	≥ 30% decrease in tumor burden compared with baseline in two observations at least 4 week apart if single arm trial and primary endpoint only
SD	< 30% decrease in sum of longest diameters of all index lesions compared with baseline cannot be established nor < 20% increase compared with nadir, in the absence of new lesions or unequivocal progression of non-index lesions	< 30% decrease in tumor burden compared with baseline cannot be established nor < 20% increase compared with nadir
PD	At least 20% increase in the sum of the longest diameters of index lesions and/or unequivocal progression of non-index lesions	At least 20% increase in tumor burden compared with nadir (at any single time point) in two consecutive observations at least 4 week apart
Handling of lymph nodes	Lymph nodes are considered pathologically enlarged if >10 mm in SAD. To be measurable, nodal lesions must be ≥ 15 mm in SAD. Nodal lesions with SAD >10 mm and <15 mm are non- measurable. The sum of the diameters (LD for extranodal target lesions, SAD for nodal lesions) is followed through the course of therapy	Not differentiated from other tumor measurements

- Based on Wolchok et al., 2009

**Derivation of irRC Overall Responses*
(Modified for RECIST 1.1. Criteria)**

Measurable response	**Non-measureable response**		**Overall response**
Index and new, measurable lesions (tumor burden)** %	Non-index lesions	New, non-measurable lesions	Using irRC
↓ 100	Absent	Absent	irCR/^
↓ 100	Stable	Any	irPR/^
↓ 100	Unequivocal progression	Any	irPR/^
↓ ≥ 30	Absent/ Stable	Any	irPR/^
↓ ≥ 30	Unequivocal progression	Any	irPR/^
↓ <30 to <20↑	Absent/ Stable	Any	irSD
↓ <30 to <20↑	Unequivocal progression	Any	irSD
≥ 20↑	Any	Any	irPD/^

*Based on Wolchok et al., 2009

**Decreases assessed relative to baseline, including measurable lesions only (>5 x 5 mm).

^Assuming response (irCR or irPR) and progression (irPD) are confirmed by a second consecutive assessment at least 4 weeks apart.

Defining immune-related Response Criteria by RECIST 1.1 criteria at the appropriate radiographic assessment time points (irDCR at the second consecutive radiographic assessment):

1. Any patient with stable disease or progressive disease at any time in the trial with "rapid clinical deterioration" felt to be related to disease progression is irPD
2. Any patient who meets the criteria for RECIST 1.1 CR at a radiographic assessment time point is irCR
3. Any patient who meets the criteria for RECIST 1.1 PR at a radiographic assessment time point is irPR
4. Any patient who meets the criteria for RECIST 1.1 SD at a radiographic assessment time point is irSD
5. A patient with RECIST 1.1 PD but no rapid clinical deterioration may stay on study if his/her next tumor measurement evaluation is stable disease or better.
6. If patient has first time PD by RECIST 1.1 criteria, call it unconfirmed PD for irRC RECIST 1.1.
7. A patient with unconfirmed irPD at a radiographic assessment time point whose next tumor measurement is SD or better will be considered to be included in the irDCR at the second consecutive radiographic assessment time point.
8. A patient with unconfirmed irPD at a radiographic assessment time point who fails to qualify for RECIST 1.1 SD or unconfirmed CR or PR by next tumor measurement will be considered to have RECIST 1.1 PD and irPD at the initial radiographic assessment time point.

APPENDIX C

ALIMTA Prescribing Information

HIGHLIGHTS OF PRESCRIBING INFORMATION

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

ALIMTA (pemetrexed for injection) Lyophilized Powder, for Solution for Intravenous Use

Initial U.S. Approval: 2004

---------- RECENT MAJOR CHANGES ----------

Warnings and Precautions, Third Space Fluid (5.7) --- removal 11/2011

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

ALIMTA® is a folate analog metabolic inhibitor indicated for:
- Locally Advanced or Metastatic Nonsquamous Non-Small Cell Lung Cancer:
  - Initial treatment in combination with cisplatin. (1.1)
  - Maintenance treatment of patients whose disease has not progressed after four cycles of platinum-based first-line chemotherapy. (1.2)
  - After prior chemotherapy as a single-agent. (1.3)
- Mesothelioma: in combination with cisplatin. (1.4)

Limitations of Use:
- ALIMTA is not indicated for the treatment of patients with squamous cell non-small cell lung cancer. (1.5)

---------- DOSAGE AND ADMINISTRATION ----------
- Combination use in Non-Small Cell Lung Cancer and Mesothelioma: Recommended dose of ALIMTA is 500 mg/m^2 i.v. on Day 1 of each 21-day cycle in combination with cisplatin 75 mg/m^2 i.v. beginning 30 minutes after ALIMTA administration. (2.1)
- Single-Agent use in Non-Small Cell Lung Cancer: Recommended dose of ALIMTA is 500 mg/m^2 i.v. on Day 1 of each 21-day cycle. (2.2)
- Dose Reductions: Dose reductions or discontinuation may be needed based on toxicities from the preceding cycle of therapy. (2.4)

---------- DOSAGE FORMS AND STRENGTHS ----------
- 100 mg vial for injection (3)
- 500 mg vial for injection (3)

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

History of severe hypersensitivity reaction to pemetrexed. (4)

---------- WARNINGS AND PRECAUTIONS ----------
- Premedication regimen: Instruct patients to take folic acid and vitamin B_{12}. Pretreatment with dexamethasone or equivalent reduces cutaneous reaction. (5.1)
- Bone marrow suppression: Reduce doses for subsequent cycles based on hematologic and nonhematologic toxicities. (5.2)
- Renal function: Do not administer when CrCl <45 mL/min. (2.4, 5.3)
- NSAIDs with renal insufficiency: Use caution in patients with mild to moderate renal insufficiency (CrCl 45-79 mL/min). (5.4)
- Lab monitoring: Do not begin next cycle unless ANC ≥1500 cells/mm^3, platelets ≥100,000 cells/mm^3, and CrCl ≥45 mL/min. (5.5)
- Pregnancy: Fetal harm can occur when administered to a pregnant woman. Women should be advised to use effective contraception measures to prevent pregnancy during treatment with ALIMTA. (5.6)

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

The most common adverse reactions (incidence ≥20%) with single-agent use are fatigue, nausea, and anorexia. Additional common adverse reactions when used in combination with cisplatin include vomiting, neutropenia, leukopenia, anemia, stomatitis/pharyngitis, thrombocytopenia, and constipation. (6.1)

To report SUSPECTED ADVERSE REACTIONS, contact Eli Lilly and Company at 1-800-LillyRx (1-800-545-5979) or FDA at 1-800-FDA-1088 or www.fda.gov/medwatch.

---------- DRUG INTERACTIONS ----------
- NSAIDs: Use caution with NSAIDs. (7.1)
- Nephrotoxic drugs: Concomitant use of these drugs and/or substances which are tubularly secreted may result in delayed clearance. (7.2)

See 17 for PATIENT COUNSELING INFORMATION and FDA-approved patient labeling

Revised: 12/2011

FULL PRESCRIBING INFORMATION: CONTENTS*

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

FULL PRESCRIBING INFORMATION

1 INDICATIONS AND USAGE

1.1 Nonsquamous Non-Small Cell Lung Cancer - Combination with Cisplatin

ALIMTA is indicated in combination with cisplatin therapy for the initial treatment of patients with locally advanced or metastatic nonsquamous non-small cell lung cancer.

1.2 Nonsquamous Non-Small Cell Lung Cancer - Maintenance

ALIMTA is indicated for the maintenance treatment of patients with locally advanced or metastatic nonsquamous non-small cell lung cancer whose disease has not progressed after four cycles of platinum-based first-line chemotherapy.

1.3 Nonsquamous Non-Small Cell Lung Cancer - After Prior Chemotherapy

ALIMTA is indicated as a single-agent for the treatment of patients with locally advanced or metastatic nonsquamous non-small cell lung cancer after prior chemotherapy.

1.4 Mesothelioma

ALIMTA in combination with cisplatin is indicated for the treatment of patients with malignant pleural mesothelioma whose disease is unresectable or who are otherwise not candidates for curative surgery.

1.5 Limitations of Use

ALIMTA is not indicated for the treatment of patients with squamous cell non-small cell lung cancer. *[see Clinical Studies (14.1, 14.2, 14.3)]*

2 DOSAGE AND ADMINISTRATION

2.1 Combination Use with Cisplatin

Nonsquamous Non-Small Cell Lung Cancer and Malignant Pleural Mesothelioma

The recommended dose of ALIMTA is 500 mg/m^2 administered as an intravenous infusion over 10 minutes on Day 1 of each 21-day cycle. The recommended dose of cisplatin is 75 mg/m^2 infused over 2 hours beginning approximately 30 minutes after the end of ALIMTA administration. Patients should receive appropriate hydration prior to and/or after receiving cisplatin. See cisplatin package insert for more information.

2.2 Single-Agent Use

Nonsquamous Non-Small Cell Lung Cancer

The recommended dose of ALIMTA is 500 mg/m^2 administered as an intravenous infusion over 10 minutes on Day 1 of each 21-day cycle.

2.3 Premedication Regimen

Vitamin Supplementation

To reduce toxicity, patients treated with ALIMTA must be instructed to take a low-dose oral folic acid preparation or multivitamin with folic acid on a daily basis. At least 5 daily doses of folic acid must be taken during the 7-day period preceding the first dose of ALIMTA; and dosing should continue during the full course of therapy and for 21 days after the last dose of ALIMTA. Patients must also receive one (1) intramuscular injection of vitamin B_{12} during the week preceding the first dose of ALIMTA and every 3 cycles thereafter. Subsequent vitamin B_{12} injections may be given the same day as ALIMTA. In clinical trials, the dose of folic acid studied ranged from 350 to 1000 mcg, and the dose of vitamin B_{12} was 1000 mcg. The most commonly used dose of oral folic acid in clinical trials was 400 mcg *[see Warnings and Precautions (5.1)]*.

Corticosteroid

Skin rash has been reported more frequently in patients not pretreated with a corticosteroid. Pretreatment with dexamethasone (or equivalent) reduces the incidence and severity of cutaneous reaction. In clinical trials, dexamethasone 4 mg was given by mouth twice daily the day before, the day of, and the day after ALIMTA administration *[see Warnings and Precautions (5.1)]*.

2.4 Laboratory Monitoring and Dose Reduction/Discontinuation Recommendations

Monitoring

Complete blood cell counts, including platelet counts, should be performed on all patients receiving ALIMTA. Patients should be monitored for nadir and recovery, which were tested in the clinical study before each dose and on days 8 and 15 of each cycle. Patients should not begin a new cycle of treatment unless the ANC is ≥1500 cells/mm^3, the platelet count is ≥100,000 cells/mm^3, and creatinine clearance is ≥45 mL/min. Periodic chemistry tests should be performed to evaluate renal and hepatic function *[see Warnings and Precautions (5.5)]*.

Dose Reduction Recommendations

Dose adjustments at the start of a subsequent cycle should be based on nadir hematologic counts or maximum nonhematologic toxicity from the preceding cycle of therapy. Treatment may be delayed to allow sufficient time for recovery. Upon recovery, patients should be retreated using the guidelines in Tables 1-3, which are suitable for using ALIMTA as a single-agent or in combination with cisplatin.

Table 1: Dose Reduction for ALIMTA (single-agent or in combination) and Cisplatin - Hematologic Toxicities

Nadir ANC <500/mm^3 and nadir platelets ≥50,000/mm^3.	75% of previous dose (pemetrexed and cisplatin).
Nadir platelets <50,000/mm^3 without bleeding regardless of nadir ANC.	75% of previous dose (pemetrexed and cisplatin).
Nadir platelets <50,000/mm^3 with bleeding[a], regardless of nadir ANC.	50% of previous dose (pemetrexed and cisplatin).

[a] These criteria meet the CTC version 2.0 (NCI 1998) definition of ≥CTC Grade 2 bleeding.

If patients develop nonhematologic toxicities (excluding neurotoxicity) ≥Grade 3, treatment should be withheld until resolution to less than or equal to the patient's pre-therapy value. Treatment should be resumed according to guidelines in Table 2.

Table 2: Dose Reduction for ALIMTA (single-agent or in combination) and Cisplatin - Nonhematologic Toxicities[a,b]

	Dose of ALIMTA (mg/m^2)	Dose of Cisplatin (mg/m^2)
Any Grade 3 or 4 toxicities except mucositis	75% of previous dose	75% of previous dose
Any diarrhea requiring hospitalization (irrespective of Grade) or Grade 3 or 4 diarrhea	75% of previous dose	75% of previous dose
Grade 3 or 4 mucositis	50% of previous dose	100% of previous dose

[a] NCI Common Toxicity Criteria (CTC).
[b] Excluding neurotoxicity (see Table 3).

In the event of neurotoxicity, the recommended dose adjustments for ALIMTA and cisplatin are described in Table 3. Patients should discontinue therapy if Grade 3 or 4 neurotoxicity is experienced.

Table 3: Dose Reduction for ALIMTA (single-agent or in combination) and Cisplatin - Neurotoxicity

CTC Grade	Dose of ALIMTA (mg/m^2)	Dose of Cisplatin (mg/m^2)
0-1	100% of previous dose	100% of previous dose
2	100% of previous dose	50% of previous dose

Discontinuation Recommendation

ALIMTA therapy should be discontinued if a patient experiences any hematologic or nonhematologic Grade 3 or 4 toxicity after 2 dose reductions or immediately if Grade 3 or 4 neurotoxicity is observed.

Renally Impaired Patients

In clinical studies, patients with creatinine clearance ≥45 mL/min required no dose adjustments other than those recommended for all patients. Insufficient numbers of patients with creatinine clearance below 45 mL/min have been treated to make dosage recommendations for this group of patients *[see Clinical Pharmacology (12.3)]*. Therefore, ALIMTA should not be administered to patients whose creatinine clearance is <45 mL/min using the standard Cockcroft and Gault formula (below) or GFR measured by Tc99m-DPTA serum clearance method:

$$\text{Males: } \frac{[140 - \text{Age in years}] \times \text{Actual Body Weight (kg)}}{72 \times \text{Serum Creatinine (mg/dL)}} = \text{mL/min}$$

Females: Estimated creatinine clearance for males × 0.85

Caution should be exercised when administering ALIMTA concurrently with NSAIDs to patients whose creatinine clearance is <80 mL/min *[see Drug Interactions (7.1)]*.

2.5 Preparation and Administration Precautions

As with other potentially toxic anticancer agents, care should be exercised in the handling and preparation of infusion solutions of ALIMTA. The use of gloves is recommended. If a solution of ALIMTA contacts the skin, wash the skin immediately and thoroughly with soap and water. If ALIMTA contacts the mucous membranes, flush thoroughly with water. Several published guidelines for handling and disposal of anticancer agents are available *[see References (15)]*.

ALIMTA is not a vesicant. There is no specific antidote for extravasation of ALIMTA. To date, there have been few reported cases of ALIMTA extravasation, which were not assessed as serious by the investigator. ALIMTA extravasation should be managed with local standard practice for extravasation as with other non-vesicants.

2.6 Preparation for Intravenous Infusion Administration

1. Use aseptic technique during the reconstitution and further dilution of ALIMTA for intravenous infusion administration.
2. Calculate the dose of ALIMTA and determine the number of vials needed. Vials contain either 100 mg or 500 mg of ALIMTA. The vials contain an excess of ALIMTA to facilitate delivery of label amount.
3. Reconstitute each 100-mg vial with 4.2 ml of 0.9% Sodium Chloride Injection (preservative free). Reconstitute each 500-mg vial with 20 mL of 0.9% Sodium Chloride Injection (preservative free). Reconstitution of either size vial gives a

solution containing 25 mg/mL ALIMTA. Gently swirl each vial until the powder is completely dissolved. The resulting solution is clear and ranges in color from colorless to yellow or green-yellow without adversely affecting product quality. The pH of the reconstituted ALIMTA solution is between 6.6 and 7.8. FURTHER DILUTION IS REQUIRED.

4. Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration, whenever solution and container permit. If particulate matter is observed, do not administer.
5. An appropriate quantity of the reconstituted ALIMTA solution must be further diluted into a solution of 0.9% Sodium Chloride Injection (preservative free), so that the total volume of solution is 100 ml. ALIMTA is administered as an intravenous infusion over 10 minutes.
6. Chemical and physical stability of reconstituted and infusion solutions of ALIMTA were demonstrated for up to 24 hours following initial reconstitution, when stored at refrigerated or ambient room temperature [see USP Controlled Room Temperature] and lighting. When prepared as directed, reconstitution and infusion solutions of ALIMTA contain no antimicrobial preservatives. Discard any unused portion.

Reconstitution and further dilution prior to intravenous infusion is only recommended with 0.9% Sodium Chloride Injection (preservative free). ALIMTA is physically incompatible with diluents containing calcium, including Lactated Ringer's Injection, USP and Ringer's Injection, USP and therefore these should not be used. Coadministration of ALIMTA with other drugs and diluents has not been studied, and therefore is not recommended. ALIMTA is compatible with standard polyvinyl chloride (PVC) administration sets and intravenous solution bags.

3 DOSAGE FORMS AND STRENGTHS

ALIMTA, pemetrexed for injection, is a white to either light-yellow or green-yellow lyophilized powder available in sterile single-use vials containing 100 mg or 500 mg pemetrexed.

4 CONTRAINDICATIONS

ALIMTA is contraindicated in patients who have a history of severe hypersensitivity reaction to pemetrexed or to any other ingredient used in the formulation.

5 WARNINGS AND PRECAUTIONS

5.1 Premedication Regimen

Need for Folate and Vitamin B_{12} Supplementation

Patients treated with ALIMTA must be instructed to take folic acid and vitamin B_{12} as a prophylactic measure to reduce treatment-related hematologic and GI toxicity *[see Dosage and Administration (2.3)]*. In clinical studies, less overall toxicity and reductions in Grade 3/4 hematologic and nonhematologic toxicities such as neutropenia, febrile neutropenia, and infection with Grade 3/4 neutropenia were reported when pretreatment with folic acid and vitamin B_{12} was administered.

Corticosteroid Supplementation

Skin rash has been reported more frequently in patients not pretreated with a corticosteroid in clinical trials. Pretreatment with dexamethasone (or equivalent) reduces the incidence and severity of cutaneous reaction *[see Dosage and Administration (2.3)]*.

5.2 Bone Marrow Suppression

ALIMTA can suppress bone marrow function, as manifested by neutropenia, thrombocytopenia, and anemia (or pancytopenia) *[see Adverse Reactions (6.1)]*; myelosuppression is usually the dose-limiting toxicity. Dose reductions for subsequent cycles are based on nadir ANC, platelet count, and maximum nonhematologic toxicity seen in the previous cycle *[see Dosage and Administration (2.4)]*.

5.3 Decreased Renal Function

ALIMTA is primarily eliminated unchanged by renal excretion. No dosage adjustment is needed in patients with creatinine clearance ≥45 mL/min. Insufficient numbers of patients have been studied with creatinine clearance <45 mL/min to give a dose recommendation. Therefore, ALIMTA should not be administered to patients whose creatinine clearance is <45 mL/min *[see Dosage and Administration (2.4)]*.

One patient with severe renal impairment (creatinine clearance 19 mL/min) who did not receive folic acid and vitamin B_{12} died of drug-related toxicity following administration of ALIMTA alone.

5.4 Use with Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) with Mild to Moderate Renal Insufficiency

Caution should be used when administering NSAIDs concurrently with ALIMTA to patients with mild to moderate renal insufficiency (creatinine clearance from 45 to 79 mL/min) *[see Drug Interactions (7.1)]*.

5.5 Required Laboratory Monitoring

Patients should not begin a new cycle of treatment unless the ANC is ≥1500 cells/mm^3, the platelet count is ≥100,000 cells/mm^3, and creatinine clearance is ≥45 mL/min *[see Dosage and Administration (2.4)]*.

5.6 Pregnancy Category D

Based on its mechanism of action, ALIMTA can cause fetal harm when administered to a pregnant woman. Pemetrexed administered intraperitoneally to mice during organogenesis was embryotoxic, fetotoxic and teratogenic in mice at greater than 1/833rd the recommended human dose. If ALIMTA 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 should be advised to use effective contraceptive measures to prevent pregnancy during treatment with ALIMTA *[see Use in Specific Populations (8.1)]*.

6 ADVERSE REACTIONS

6.1 Clinical Trials Experience

Because clinical trials are conducted under widely varying conditions, adverse reactions rates cannot be directly compared to rates in other clinical trials and may not reflect the rates observed in clinical practice.

In clinical trials, the most common adverse reactions (incidence ≥20%) during therapy with ALIMTA as a single-agent were fatigue, nausea, and anorexia. Additional common adverse reactions (incidence ≥20%) during therapy with ALIMTA when used in combination with cisplatin included vomiting, neutropenia, leukopenia, anemia, stomatitis/pharyngitis, thrombocytopenia, and constipation.

Non-Small Cell Lung Cancer (NSCLC) - Combination with Cisplatin

Table 4 provides the frequency and severity of adverse reactions that have been reported in >5% of 839 patients with NSCLC who were randomized to study and received ALIMTA plus cisplatin and 830 patients with NSCLC who were randomized to study and received gemcitabine plus cisplatin. All patients received study therapy as initial treatment for locally advanced or metastatic NSCLC and patients in both treatment groups were fully supplemented with folic acid and vitamin B_{12}.

Table 4: Adverse Reactions in Fully Supplemented Patients Receiving ALIMTA plus Cisplatin in NSCLC[a]

Reaction[b]	ALIMTA/cisplatin (N=839)		Gemcitabine/cisplatin (N=830)	
	All Grades Toxicity (%)	Grade 3-4 Toxicity (%)	All Grades Toxicity (%)	Grade 3-4 Toxicity (%)
All Adverse Reactions	90	37	91	53
Laboratory				
Hematologic				
Anemia	33	6	46	10
Neutropenia	29	15	38	27
Leukopenia	18	5	21	8
Thrombocytopenia	10	4	27	13
Renal				
Creatinine elevation	10	1	7	1
Clinical				
Constitutional Symptoms				
Fatigue	43	7	45	5
Gastrointestinal				
Nausea	56	7	53	4
Vomiting	40	6	36	6
Anorexia	27	2	24	1
Constipation	21	1	20	0
Stomatitis/Pharyngitis	14	1	12	0
Diarrhea	12	1	13	2
Dyspepsia/Heartburn	5	0	6	0
Neurology				
Neuropathy-sensory	9	0	12	1
Taste disturbance	8	0[c]	9	0[c]
Dermatology/Skin				
Alopecia	12	0[c]	21	1[c]
Rash/Desquamation	7	0	8	1

[a] For the purpose of this table a cut off of 5% was used for inclusion of all events where the reporter considered a possible relationship to ALIMTA.

[b] Refer to NCI CTC Criteria version 2.0 for each Grade of toxicity.

[c] According to NCI CTC Criteria version 2.0, this adverse event term should only be reported as Grade 1 or 2.

No clinically relevant differences in adverse reactions were seen in patients based on histology.

In addition to the lower incidence of hematologic toxicity on the ALIMTA and cisplatin arm, use of transfusions (RBC and platelet) and hematopoietic growth factors was lower in the ALIMTA and cisplatin arm compared to the gemcitabine and cisplatin arm.

The following additional adverse reactions were observed in patients with non-small cell lung cancer randomly assigned to receive ALIMTA plus cisplatin.

Incidence 1% to 5%

Body as a Whole — febrile neutropenia, infection, pyrexia
General Disorders — dehydration
Metabolism and Nutrition — increased AST, increased ALT
Renal — creatinine clearance decrease, renal failure
Special Senses — conjunctivitis

Incidence Less than 1%

Cardiovascular — arrhythmia
General Disorders — chest pain
Metabolism and Nutrition — increased GGT
Neurology — motor neuropathy

Non-Small Cell Lung Cancer (NSCLC) - Maintenance

Table 5 provides the frequency and severity of adverse reactions that have been reported in >5% of 438 patients with NSCLC who received ALIMTA and 218 patients with NSCLC who received placebo. All patients received study therapy immediately following 4 cycles of platinum-based treatment for locally advanced or metastatic NSCLC. Patients in both study arms were fully supplemented with folic acid and vitamin B_{12}.

Table 5: Adverse Reactions in Patients Receiving ALIMTA versus Placebo in NSCLC[a]

Reaction[b]	ALIMTA (N=438)		Placebo (N=218)	
	All Grades Toxicity (%)	Grade 3-4 Toxicity (%)	All Grades Toxicity (%)	Grade 3-4 Toxicity (%)
All Adverse Reactions	66	16	37	4
Laboratory				
Hematologic				
Anemia	15	3	6	1
Neutropenia	6	3	0	0
Leukopenia	6	2	1	1
Hepatic				
Increased ALT	10	0	4	0
Increased AST	8	0	4	0
Clinical				
Constitutional Symptoms				
Fatigue	25	5	11	1
Gastrointestinal				
Nausea	19	1	6	1
Anorexia	19	2	5	0
Vomiting	9	0	1	0
Mucositis/stomatitis	7	1	2	0
Diarrhea	5	1	3	0
Infection	5	2	2	0
Neurology				
Neuropathy-sensory	9	1	4	0
Dermatology/Skin				
Rash/Desquamation	10	0	3	0

[a] For the purpose of this table a cut off of 5% was used for inclusion of all events where the reporter considered a possible relationship to ALIMTA.

[b] Refer to NCI CTCAE Criteria version 3.0 for each Grade of toxicity.

No clinically relevant differences in Grade 3/4 adverse reactions were seen in patients based on age, gender, ethnic origin, or histology except a higher incidence of Grade 3/4 fatigue for Caucasian patients compared to non-Caucasian patients (6.5% versus 0.6%).

Safety was assessed by exposure for patients who received at least one dose of ALIMTA (N=438). The incidence of adverse reactions was evaluated for patients who received ≤6 cycles of ALIMTA, and compared to patients who received >6 cycles of ALIMTA. Increases in adverse reactions (all grades) were observed with longer exposure; however no clinically relevant differences in Grade 3/4 adverse reactions were seen.

Consistent with the higher incidence of anemia (all grades) on the ALIMTA arm, use of transfusions (mainly RBC) and erythropoiesis stimulating agents (ESAs; erythropoietin and darbepoetin) were higher in the ALIMTA arm compared to the placebo arm (transfusions 9.5% versus 3.2%, ESAs 5.9% versus 1.8%).

The following additional adverse reactions were observed in patients with non-small cell lung cancer who received ALIMTA.

Incidence 1% to 5%

Dermatology/Skin — alopecia, pruritis/itching
Gastrointestinal — constipation
General Disorders — edema, fever (in the absence of neutropenia)
Hematologic — thrombocytopenia
Renal — decreased creatinine clearance, increased creatinine, decreased glomerular filtration rate
Special Senses — ocular surface disease (including conjunctivitis), increased lacrimation

Incidence Less than 1%

Cardiovascular — supraventricular arrhythmia
Dermatology/Skin — erythema multiforme
General Disorders — febrile neutropenia, allergic reaction/hypersensitivity
Neurology — motor neuropathy
Renal — renal failure

Non-Small Cell Lung Cancer (NSCLC) – After Prior Chemotherapy

Table 6 provides the frequency and severity of adverse reactions that have been reported in >5% of 265 patients randomly assigned to receive single-agent ALIMTA with folic acid and vitamin B_{12} supplementation and 276 patients randomly assigned to receive single-agent docetaxel. All patients were diagnosed with locally advanced or metastatic NSCLC and received prior chemotherapy.

Table 6: Adverse Reactions in Fully Supplemented Patients Receiving ALIMTA versus Docetaxel in NSCLC[a]

Reaction[b]	ALIMTA (N=265)		Docetaxel (N=276)	
	All Grades Toxicity (%)	Grades 3-4 Toxicity (%)	All Grades Toxicity (%)	Grades 3-4 Toxicity (%)
Laboratory				
Hematologic				
Anemia	19	4	22	4
Leukopenia	12	4	34	27
Neutropenia	11	5	45	40
Thrombocytopenia	8	2	1	0
Hepatic				
Increased ALT	8	2	1	0
Increased AST	7	1	1	0
Clinical				
Gastrointestinal				
Nausea	31	3	17	2
Anorexia	22	2	24	3
Vomiting	16	2	12	1
Stomatitis/Pharyngitis	15	1	17	1
Diarrhea	13	0	24	3
Constipation	6	0	4	0
Constitutional Symptoms				
Fatigue	34	5	36	5
Fever	8	0	8	0
Dermatology/Skin				
Rash/Desquamation	14	0	6	0
Pruritis	7	0	2	0
Alopecia	6	1[c]	38	2[c]

[a] For the purpose of this table a cut off of 5% was used for inclusion of all events where the reporter considered a possible relationship to ALIMTA.

[b] Refer to NCI CTC Criteria for lab values for each Grade of toxicity (version 2.0).

[c] According to NCI CTC Criteria version 2.0, this adverse event term should only be reported as Grade 1 or 2.

No clinically relevant differences in adverse reactions were seen in patients based on histology.

Clinically relevant adverse reactions occurring in <5% of patients that received ALIMTA treatment but >5% of patients that received docetaxel include CTC Grade 3/4 febrile neutropenia (1.9% ALIMTA, 12.7% docetaxel).

The following additional adverse reactions were observed in patients with non-small cell lung cancer randomly assigned to receive ALIMTA.

Incidence 1% to 5%

Body as a Whole — abdominal pain, allergic reaction/hypersensitivity, febrile neutropenia, infection
Dermatology/Skin — erythema multiforme
Neurology — motor neuropathy, sensory neuropathy
Renal — increased creatinine

Incidence Less than 1%

Cardiovascular — supraventricular arrhythmias

Malignant Pleural Mesothelioma (MPM)

Table 7 provides the frequency and severity of adverse reactions that have been reported in >5% of 168 patients with mesothelioma who were randomly assigned to receive cisplatin and ALIMTA and 163 patients with mesothelioma randomly assigned to receive single-agent cisplatin. In both treatment arms, these chemonaive patients were fully supplemented with folic acid and vitamin B_{12}.

Table 7: Adverse Reactions in Fully Supplemented Patients Receiving ALIMTA plus Cisplatin in MPM[a]

Reaction[b]	ALIMTA/cisplatin (N=168)		Cisplatin (N=163)	
	All Grades Toxicity (%)	Grade 3-4 Toxicity (%)	All Grades Toxicity (%)	Grade 3-4 Toxicity (%)
Laboratory				
Hematologic				
Neutropenia	56	23	13	3
Leukopenia	53	15	17	1
Anemia	26	4	10	0
Thrombocytopenia	23	5	9	0
Renal				
Creatinine elevation	11	1	10	1
Creatinine clearance decreased	16	1	18	2
Clinical				
Eye Disorder				
Conjunctivitis	5	0	1	0
Gastrointestinal				
Nausea	82	12	77	6
Vomiting	57	11	50	4
Stomatitis/Pharyngitis	23	3	6	0
Anorexia	20	1	14	1
Diarrhea	17	4	8	0
Constipation	12	1	7	1
Dyspepsia	5	1	1	0
Constitutional Symptoms				
Fatigue	48	10	42	9
Metabolism and Nutrition				
Dehydration	7	4	1	1
Neurology				
Neuropathy-sensory	10	0	10	1
Taste Disturbance	8	0[c]	6	0[c]
Dermatology/Skin				
Rash	16	1	5	0
Alopecia	11	0[c]	6	0[c]

[a] For the purpose of this table a cut off of 5% was used for inclusion of all events where the reporter considered a possible relationship to ALIMTA.

[b] Refer to NCI CTC Criteria version 2.0 for each Grade of toxicity except the term "creatinine clearance decreased" which is derived from the CTC term "renal/genitourinary-other".

[c] According to NCI CTC Criteria version 2.0, this adverse event term should only be reported as Grade 1 or 2.

The following additional adverse reactions were observed in patients with malignant pleural mesothelioma randomly assigned to receive ALIMTA plus cisplatin.

Incidence 1% to 5%

Body as a Whole — febrile neutropenia, infection, pyrexia
Dermatology/Skin — urticaria
General Disorders — chest pain
Metabolism and Nutrition — increased AST, increased ALT, increased GGT
Renal — renal failure

Incidence Less than 1%

Cardiovascular — arrhythmia
Neurology — motor neuropathy

Effects of Vitamin Supplementations

Table 8 compares the incidence (percentage of patients) of CTC Grade 3/4 toxicities in patients who received vitamin supplementation with daily folic acid and vitamin B_{12} from the time of enrollment in the study (fully supplemented) with the incidence in patients who never received vitamin supplementation (never supplemented) during the study in the ALIMTA plus cisplatin arm.

Table 8: Selected Grade 3/4 Adverse Events Comparing Fully Supplemented versus Never Supplemented Patients in the ALIMTA plus Cisplatin arm (% incidence)

Adverse Event[a] (%)	**Fully Supplemented Patients** (N=168)	**Never Supplemented Patients** (N=32)
Neutropenia/granulocytopenia	23	38
Thrombocytopenia	5	9
Vomiting	11	31
Febrile neutropenia	1	9
Infection with Grade 3/4 neutropenia	0	6
Diarrhea	4	9

[a] Refer to NCI CTC criteria for lab and non-laboratory values for each grade of toxicity (Version 2.0).

The following adverse events were greater in the fully supplemented group compared to the never supplemented group: hypertension (11%, 3%), chest pain (8%, 6%), and thrombosis/embolism (6%, 3%).

Subpopulations

No relevant effect for ALIMTA safety due to gender or race was identified, except an increased incidence of rash in men (24%) compared to women (16%).

Additional Clinical Trials Experience

Across clinical trials, sepsis, which in some cases was fatal, occurred in approximately 1% of patients.
Cases of esophagitis have been reported in clinical trials.

6.2 Postmarketing Experience

The following adverse reactions have been identified during post-approval use of ALIMTA. Because these reactions are reported voluntarily from a population of uncertain size, it is not always possible to reliably estimate their frequency or establish a causal relationship to drug exposure.

These reactions have occurred with ALIMTA when used as a single-agent and in combination therapies.

Gastrointestinal — colitis
General Disorders and Administration Site Conditions — edema
Injury, poisoning, and procedural complications — Radiation recall has been reported in patients who have previously received radiotherapy.
Respiratory — interstitial pneumonitis
Skin — Bullous conditions, including Stevens-Johnson syndrome and toxic epidermal necrolysis. Some cases were fatal.

7 DRUG INTERACTIONS

7.1 Non-Steroidal Anti-Inflammatory Drugs (NSAIDs)

Although ibuprofen (400 mg four times a day) can decrease the clearance of pemetrexed, it can be administered with ALIMTA in patients with normal renal function (creatinine clearance ≥80 mL/min). No dose adjustment of ALIMTA is needed with concomitant NSAIDs in patients with normal renal function *[see Clinical Pharmacology (12.3)]*.

Caution should be used when administering NSAIDs concurrently with ALIMTA to patients with mild to moderate renal insufficiency (creatinine clearance from 45 to 79 mL/min). NSAIDs with short elimination half-lives (e.g., diclofenac, indomethacin) should be avoided for a period of 2 days before, the day of, and 2 days following administration of ALIMTA.

In the absence of data regarding potential interaction between ALIMTA and NSAIDs with longer half-lives (e.g., meloxicam, nabumetone), patients taking these NSAIDs should interrupt dosing for at least 5 days before, the day of, and 2 days following ALIMTA administration. If concomitant administration of NSAIDs is necessary, patients should be monitored closely for toxicity, especially myelosuppression, renal, and gastrointestinal toxicity.

7.2 Nephrotoxic Drugs

ALIMTA is primarily eliminated unchanged renally as a result of glomerular filtration and tubular secretion. Concomitant administration of nephrotoxic drugs could result in delayed clearance of ALIMTA. Concomitant administration of substances that are also tubularly secreted (e.g., probenecid) could potentially result in delayed clearance of ALIMTA.

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

Teratogenic Effects - Pregnancy Category D *[see Warnings and Precautions (5.6)]*

Based on its mechanism of action, ALIMTA can cause fetal harm when administered to a pregnant woman. There are no adequate and well controlled studies of ALIMTA in pregnant women. Pemetrexed was embryotoxic, fetotoxic, and teratogenic in mice. In mice, repeated intraperitoneal doses of pemetrexed when given during organogenesis caused fetal malformations (incomplete ossification of talus and skull bone; about 1/833rd the recommended intravenous human dose on a mg/m^2 basis), and cleft palate (1/33rd the recommended intravenous human dose on a mg/m^2 basis). Embryotoxicity was characterized by increased embryo-fetal deaths and reduced litter sizes. If ALIMTA 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 use effective contraceptive measures to prevent pregnancy during the treatment with ALIMTA.

8.3 Nursing Mothers

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

8.4 Pediatric Use

Efficacy of ALIMTA in pediatric patients has not been demonstrated. ALIMTA was administered as an intravenous infusion over 10 minutes on Day 1 of a 21 day cycle to pediatric patients with recurrent solid tumors in a Phase 1 study (32 patients) and a Phase 2 study (72 patients). All patients received pretreatment with vitamin B_{12} and folic acid supplementation and dexamethasone. The dose escalation in the Phase 1 study determined the maximum tolerated dose was 1910 mg/m^2 and this dose (or 60 mg/kg for patients <12 months old) was evaluated in the Phase 2 study of patients with relapsed or refractory osteosarcoma, Ewing sarcoma/peripheral PNET, rhabdomyosarcoma, neuroblastoma, ependymoma, medulloblastoma/supratentorial PNET, or non-brainstem high grade glioma. No responses were observed among the 72 patients in this Phase 2 trial. The most common toxicities reported were hematological (leukopenia, neutropenia/granulocytopenia, anemia, thrombocytopenia, and lymphopenia), liver function abnormalities (increased ALT/AST), fatigue, and nausea.

The single dose pharmacokinetics of ALIMTA administered in doses ranging from 400 to 2480 mg/m^2 were evaluated in the Phase 1 trial in 22 patients (13 males and 9 females) aged 4 to 18 years (average age 12 years). Pemetrexed exposure (AUC and C_{max}) appeared to increase proportionally with dose. The average pemetrexed clearance (2.30 L/h/m^2) and half-life (2.3 hours) in pediatric patients were comparable to values reported in adults.

8.5 Geriatric Use

ALIMTA is known to be substantially excreted by the kidney, and the risk of adverse reactions to this drug may be greater in patients with impaired renal function. Because elderly patients are more likely to have decreased renal function, care should be taken in dose selection. Renal function monitoring is recommended with administration of ALIMTA. No dose reductions other than those recommended for all patients are necessary for patients 65 years of age or older *[see Dosage and Administration (2.4)]*.

In the initial treatment non-small cell lung cancer clinical trial, 37.7% of patients treated with ALIMTA plus cisplatin were ≥65 years and Grade 3/4 neutropenia was greater as compared to patients <65 years (19.9% versus 12.2%). For patients <65 years, the HR for overall survival was 0.96 (95% CI: 0.83, 1.10) and for patients ≥65 years the HR was 0.88 (95% CI: 0.74, 1.06) in the intent to treat population.

In the maintenance non-small cell lung cancer trial 33.3% of patients treated with ALIMTA were ≥65 years and no differences were seen in Grade 3/4 adverse reactions as compared to patients <65 years. For patients <65 years, the HR for overall survival was 0.74 (95% CI: 0.58, 0.93) and for patients ≥65 years the HR was 0.88 (95% CI: 0.65, 1.21) in the intent to treat population.

In the non-small cell lung cancer trial after prior chemotherapy, 29.7% patients treated with ALIMTA were ≥65 years and Grade 3/4 hypertension was greater as compared to patients <65 years. For patients <65 years, the HR for overall survival was 0.95 (95% CI: 0.76, 1.19), and for patients ≥65 years the HR was 1.15 (95% CI: 0.79, 1.68) in the intent to treat population.

The mesothelioma trial included 36.7% patients treated with ALIMTA plus cisplatin that were ≥65 years, and Grade 3/4 fatigue, leukopenia, neutropenia, and thrombocytopenia were greater as compared to patients <65 years. For patients <65 years, the HR for overall survival was 0.71 (95% CI: 0.53, 0.96) and for patients ≥65 years, the HR was 0.85 (95% CI: 0.59, 1.22) in the intent to treat population.

8.6 Patients with Hepatic Impairment

There was no effect of elevated AST, ALT, or total bilirubin on the pharmacokinetics of pemetrexed *[see Clinical Pharmacology (12.3)]*.

Dose adjustments based on hepatic impairment experienced during treatment with ALIMTA are provided in Table 2 *[see Dosage and Administration (2.4)]*.

8.7 Patients with Renal Impairment

ALIMTA is known to be primarily excreted by the kidneys. Decreased renal function will result in reduced clearance and greater exposure (AUC) to ALIMTA compared with patients with normal renal function *[see Dosage and Administration (2.4) and Clinical Pharmacology (12.3)]*. Cisplatin coadministration with ALIMTA has not been studied in patients with moderate renal impairment.

8.8 Gender

In the initial treatment non-small cell lung cancer trial, 70% of patients were males and 30% females. For males the HR for overall survival was 0.97 (95% CI: 0.85, 1.10) and for females the HR was 0.86 (95% CI: 0.70, 1.06) in the intent to treat population.

In the maintenance non-small cell lung cancer trial, 73% of patients were males and 27% females. For males the HR for overall survival was 0.78 (95% CI: 0.63, 0.96) and for females the HR was 0.83 (95% CI: 0.56, 1.21) in the intent to treat population.

In the non-small cell lung cancer trial after prior chemotherapy, 72% of patients were males and 28% females. For males the HR for overall survival was 0.95 (95% CI: 0.76, 1.19) and for females the HR was 1.28 (95% CI: 0.86, 1.91) in the intent to treat population.

In the mesothelioma trial, 82% of patients were males and 18% females. For males the HR for overall survival was 0.85 (95% CI: 0.66, 1.09) and for females the HR was 0.48 (95% CI: 0.27, 0.85) in the intent to treat population.

8.9 Race

In the initial treatment non-small cell lung cancer trial, 78% of patients were Caucasians, 13% East/Southeast Asians, and 9% others. For Caucasians, the HR for overall survival was 0.92 (95% CI: 0.82, 1.04), for East/Southeast Asians the HR was 0.86 (95% CI: 0.61, 1.21), and for others the HR was 1.24 (95% CI: 0.84, 1.84) in the intent to treat population.

In the maintenance non-small cell lung cancer trial, 65% of patients were Caucasians, 23% East Asian, and 12% others. For Caucasians the HR for overall survival was 0.77 (95% CI: 0.62, 0.97), for East Asians was 1.05 (95% CI: 0.70, 1.59) and for others the HR was 0.46 (95% CI: 0.26, 0.79) in the intent to treat population.

In the non-small cell lung cancer trial after prior chemotherapy, 71% of patients were Caucasians and 29% others. For Caucasians the HR for overall survival was 0.91 (95% CI: 0.73, 1.15) and for others the HR was 1.27 (95% CI: 0.87, 1.87) in the intent to treat population.

In the mesothelioma trial, 92% of patients were Caucasians and 8% others. For Caucasians, the HR for overall survival was 0.77 (95% CI: 0.61, 0.97) and for others the HR was 0.86 (95% CI: 0.39, 1.90) in the intent to treat population.

10 OVERDOSAGE

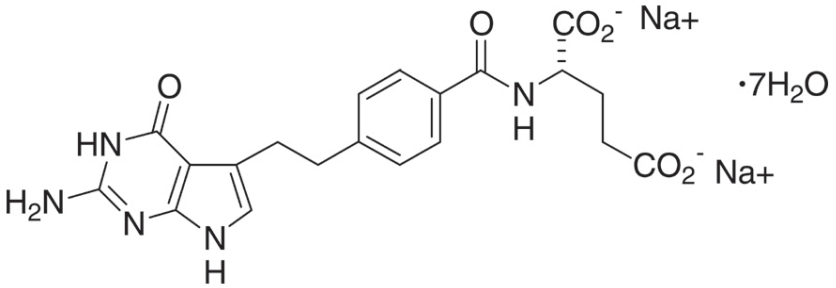
There have been few cases of ALIMTA overdose. Reported toxicities included neutropenia, anemia, thrombocytopenia, mucositis, and rash. Anticipated complications of overdose include bone marrow suppression as manifested by neutropenia, thrombocytopenia, and anemia. In addition, infection with or without fever, diarrhea, and mucositis may be seen. If an overdose occurs, general supportive measures should be instituted as deemed necessary by the treating physician.

In clinical trials, leucovorin was permitted for CTC Grade 4 leukopenia lasting ≥3 days, CTC Grade 4 neutropenia lasting ≥3 days, and immediately for CTC Grade 4 thrombocytopenia, bleeding associated with Grade 3 thrombocytopenia, or Grade 3 or 4 mucositis. The following intravenous doses and schedules of leucovorin were recommended for intravenous use: 100 mg/m^2, intravenously once, followed by leucovorin, 50 mg/m^2, intravenously every 6 hours for 8 days.

The ability of ALIMTA to be dialyzed is unknown.

11 DESCRIPTION

Pemetrexed disodium heptahydrate has the chemical name L-Glutamic acid, *N*-[4-[2-(2-amino-4,7-dihydro-4-oxo-1*H*-pyrrolo[2,3-*d*]pyrimidin-5-yl)ethyl]benzoyl]-, disodium salt, heptahydrate. It is a white to almost-white solid with a molecular formula of $C_{20}H_{19}N_5Na_2O_6 \cdot 7H_2O$ and a molecular weight of 597.49. The structural formula is as follows:



ALIMTA is supplied as a sterile lyophilized powder for intravenous infusion available in single-dose vials. The product is a white to either light yellow or green-yellow lyophilized solid. Each 100-mg or 500-mg vial of ALIMTA contains pemetrexed disodium equivalent to 100 mg pemetrexed and 106 mg mannitol or 500 mg pemetrexed and 500 mg mannitol, respectively. Hydrochloric acid and/or sodium hydroxide may have been added to adjust pH.

12 CLINICAL PHARMACOLOGY

12.1 Mechanism of Action

ALIMTA, pemetrexed for injection, is a folate analog metabolic inhibitor that exerts its action by disrupting folate-dependent metabolic processes essential for cell replication. In vitro studies have shown that pemetrexed inhibits thymidylate synthase (TS),

dihydrofolate reductase (DHFR), and glycinamide ribonucleotide formyltransferase (GARFT), which are folate-dependent enzymes involved in the de novo biosynthesis of thymidine and purine nucleotides. Pemetrexed is taken into cells by membrane carriers such as the reduced folate carrier and membrane folate binding protein transport systems. Once in the cell, pemetrexed is converted to polyglutamate forms by the enzyme folylpolyglutamate synthetase. The polyglutamate forms are retained in cells and are inhibitors of TS and GARFT. Polyglutamation is a time- and concentration-dependent process that occurs in tumor cells and, is thought to occur to a lesser extent, in normal tissues. Polyglutamated metabolites are thought to have an increased intracellular half-life resulting in prolonged drug action in malignant cells.

12.2 Pharmacodynamics

Preclinical studies have shown that pemetrexed inhibits the in vitro growth of mesothelioma cell lines (MSTO-211H, NCI-H2052). Studies with the MSTO-211H mesothelioma cell line showed synergistic effects when pemetrexed was combined concurrently with cisplatin.

Absolute neutrophil counts (ANC) following single-agent administration of ALIMTA to patients not receiving folic acid and vitamin B_{12} supplementation were characterized using population pharmacodynamic analyses. Severity of hematologic toxicity, as measured by the depth of the ANC nadir, correlates with the systemic exposure, or area under the curve (AUC) of pemetrexed. It was also observed that lower ANC nadirs occurred in patients with elevated baseline cystathionine or homocysteine concentrations. The levels of these substances can be reduced by folic acid and vitamin B_{12} supplementation. There is no cumulative effect of pemetrexed exposure on ANC nadir over multiple treatment cycles.

Time to ANC nadir with pemetrexed systemic exposure (AUC), varied between 8 to 9.6 days over a range of exposures from 38.3 to 316.8 mcg•hr/mL. Return to baseline ANC occurred 4.2 to 7.5 days after the nadir over the same range of exposures.

12.3 Pharmacokinetics

Absorption

The pharmacokinetics of ALIMTA administered as a single-agent in doses ranging from 0.2 to 838 mg/m^2 infused over a 10-minute period have been evaluated in 426 cancer patients with a variety of solid tumors. Pemetrexed total systemic exposure (AUC) and maximum plasma concentration (C_{max}) increase proportionally with dose. The pharmacokinetics of pemetrexed do not change over multiple treatment cycles.

Distribution

Pemetrexed has a steady-state volume of distribution of 16.1 liters. In vitro studies indicate that pemetrexed is approximately 81% bound to plasma proteins. Binding is not affected by degree of renal impairment.

Metabolism and Excretion

Pemetrexed is not metabolized to an appreciable extent and is primarily eliminated in the urine, with 70% to 90% of the dose recovered unchanged within the first 24 hours following administration. The clearance decreases, and exposure (AUC) increases, as renal function decreases. The total systemic clearance of pemetrexed is 91.8 mL/min and the elimination half-life of pemetrexed is 3.5 hours in patients with normal renal function (creatinine clearance of 90 mL/min).

The pharmacokinetics of pemetrexed in special populations were examined in about 400 patients in controlled and single arm studies.

In vitro studies indicate that pemetrexed is a substrate of OAT3 (organic anion transporter 3), a transporter that may play a role in active secretion of pemetrexed.

Effect of Age

No effect of age on the pharmacokinetics of pemetrexed was observed over a range of 26 to 80 years.

Effect of Gender

The pharmacokinetics of pemetrexed were not different in male and female patients.

Effect of Race

The pharmacokinetics of pemetrexed were similar in Caucasians and patients of African descent. Insufficient data are available to compare pharmacokinetics for other ethnic groups.

Effect of Hepatic Insufficiency

There was no effect of elevated AST, ALT, or total bilirubin on the pharmacokinetics of pemetrexed. However, studies of hepatically impaired patients have not been conducted *[see Dosage and Administration (2.4) and Use in Specific Populations (8.6)]*.

Effect of Renal Insufficiency

Pharmacokinetic analyses of pemetrexed included 127 patients with reduced renal function. Plasma clearance of pemetrexed decreases as renal function decreases, with a resultant increase in systemic exposure. Patients with creatinine clearances of 45, 50, and 80 mL/min had 65%, 54%, and 13% increases, respectively in pemetrexed total systemic exposure (AUC) compared to patients with creatinine clearance of 100 mL/min *[see Warnings and Precautions (5.4) and Dosage and Administration (2.4)]*.

Effect of Third Space Fluid

The effect of third space fluid, such as pleural effusion and ascites, on ALIMTA is not fully defined. A study of ALIMTA 500 mg/m^2 was performed in 31 solid tumor patients with stable third space fluid (All but 2 of the 31 patients included in study had mild or moderate amounts of third space fluid). Moderate pleural effusion was defined in the study as less than 1/3 the way up on one side with obscuring of the entire hemidiaphragm. Moderate ascites was defined as that detectable on physical exam. The pemetrexed plasma concentrations in these patients were comparable to those observed in previous clinical trials in patients without third space fluid collections. Thus, drainage of mild or moderate third space fluid collection prior to ALIMTA treatment should be considered, but is probably not necessary. The effect of severe third space fluid on pharmacokinetics is not known.

Effect of Ibuprofen

Ibuprofen doses of 400 mg four times a day reduce pemetrexed's clearance by about 20% (and increase AUC by 20%) in patients with normal renal function. The effect of greater doses of ibuprofen on pemetrexed pharmacokinetics is unknown *[see Drug Interactions (7.1)]*.

Effect of Aspirin

Aspirin, administered in low to moderate doses (325 mg every 6 hours), does not affect the pharmacokinetics of pemetrexed. The effect of greater doses of aspirin on pemetrexed pharmacokinetics is unknown.

Effect of Cisplatin

Cisplatin does not affect the pharmacokinetics of pemetrexed and the pharmacokinetics of total platinum are unaltered by pemetrexed.

Effect of Vitamins

Coadministration of oral folic acid or intramuscular vitamin B_{12} does not affect the pharmacokinetics of pemetrexed.

Drugs Metabolized by Cytochrome P450 Enzymes

Results from in vitro studies with human liver microsomes predict that pemetrexed would not cause clinically significant inhibition of metabolic clearance of drugs metabolized by CYP3A, CYP2D6, CYP2C9, and CYP1A2.

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

No carcinogenicity studies have been conducted with pemetrexed. Pemetrexed was clastogenic in the in vivo micronucleus assay in mouse bone marrow but was not mutagenic in multiple in vitro tests (Ames assay, CHO cell assay). Pemetrexed administered at i.v. doses of 0.1 mg/kg/day or greater to male mice (about 1/1666 the recommended human dose on a mg/m^2 basis) resulted in reduced fertility, hypospermia, and testicular atrophy.

14 CLINICAL STUDIES

14.1 Non-Small Cell Lung Cancer (NSCLC) - Combination with Cisplatin

A multi-center, randomized, open-label study in 1725 chemonaive patients with Stage IIIb/IV NSCLC was conducted to compare the overall survival following treatment with ALIMTA in combination with cisplatin (AC) versus gemcitabine in combination with cisplatin (GC). ALIMTA was administered intravenously over 10 minutes at a dose of 500 mg/m^2 with cisplatin administered intravenously at a dose of 75 mg/m^2 after ALIMTA administration, on Day 1 of each 21-day cycle. Gemcitabine was administered at a dose of 1250 mg/m^2 on Day 1 and Day 8, and cisplatin was administered intravenously at a dose of 75 mg/m^2 after administration of gemcitabine, on Day 1 of each 21-day cycle. Treatment was administered up to a total of 6 cycles, and patients in both treatment arms received folic acid, vitamin B_{12}, and dexamethasone *[see Dosage and Administration (2.3)]*.

Patient demographics of the intent to treat (ITT) population are shown in Table 9. The demographics and disease characteristics were well balanced.

Table 9: First-Line Therapy: Summary of Patient Characteristics in Study of NSCLC

Patient characteristic	**ALIMTA plus Cisplatin (AC) (N=862)**	**Gemcitabine plus Cisplatin (GC) (N=863)**
Age (yrs)		
Median (range)	61.1 (28.8-83.2)	61.0 (26.4-79.4)
Gender		
Male/Female	70.2%/29.8%	70.1%/29.9%
Origin		
Caucasian	669 (77.6%)	680 (78.8%)
Hispanic	27 (3.1%)	23 (2.7%)
Asian	146 (16.9%)	141 (16.3%)
African descent	18 (2.1%)	18 (2.1%)
Stage at Entry		
IIIb/IV	23.8%/76.2%	24.3%/75.7%
Histology		
Nonsquamous NSCLC[a]	618 (71.7%)	634 (73.5%)
Adenocarcinoma	436 (50.6%)	411 (47.6%)
Large cell	76 (8.8%)	77 (8.9%)
Other[b]	106 (12.3%)	146 (16.9%)
Squamous	244 (28.3%)	229 (26.5%)
ECOG PS[c,d]		
0/1	35.4%/64.6%	35.6%/64.3%
Smoking History[e]		
Ever/never smoker	83.1%/16.9%	83.9%/16.1%

[a] Includes adenocarcinoma, large cell, and other histologies except those with squamous cell type.

[b] The subgroup of "other" represents patients with a primary diagnosis of NSCLC whose disease did not clearly qualify as adenocarcinoma, squamous cell carcinoma, or large cell carcinoma.

[c] Eastern Cooperative Oncology Group Performance Status.

[d] ECOG PS was not reported for all randomized patients. Percentages are representative of N=861 for the ALIMTA plus cisplatin arm, and N=861 for the gemcitabine plus cisplatin arm.

[e] Smoking history was collected for 88% of randomized patients (N=757 for the ALIMTA plus cisplatin arm and N=759 for the gemcitabine plus cisplatin arm).

Patients received a median of 5 cycles of treatment in both study arms. Patients treated with ALIMTA plus cisplatin received a relative dose intensity of 94.8% of the protocol-specified ALIMTA dose intensity and 95.0% of the protocol-specified cisplatin dose intensity. Patients treated with gemcitabine plus cisplatin received a relative dose intensity of 85.8% of the protocol-specified gemcitabine dose intensity and 93.5% of the protocol-specified cisplatin dose intensity.

The primary endpoint in this study was overall survival. The median survival time was 10.3 months in the ALIMTA plus cisplatin treatment arm and 10.3 months in the gemcitabine plus cisplatin arm, with an adjusted hazard ratio of 0.94.

Table 10: First-Line Therapy: Efficacy in NSCLC - ITT Population

	ALIMTA plus Cisplatin (N=862)	**Gemcitabine plus Cisplatin (N=863)**
Median overall survival (95% CI)	10.3 mos (9.8-11.2)	10.3 mos (9.6-10.9)
Adjusted hazard ratio (HR)[a,b] (95% CI)	0.94 (0.84-1.05)	
Median progression-free survival (95% CI)	4.8 mos (4.6-5.3)	5.1 mos (4.6-5.5)
Adjusted hazard ratio (HR)[a,b] (95% CI)	1.04 (0.94-1.15)	
Overall response rate (95% CI)	27.1% (24.2-30.1)	24.7% (21.8-27.6)

[a] Adjusted for gender, stage, basis of diagnosis, and performance status.

[b] A HR that is less than 1.0 indicates that survival is better in the AC arm than in the GC arm. Alternatively, a HR that is greater than 1.0 indicates survival is better in the GC arm than in the AC arm.

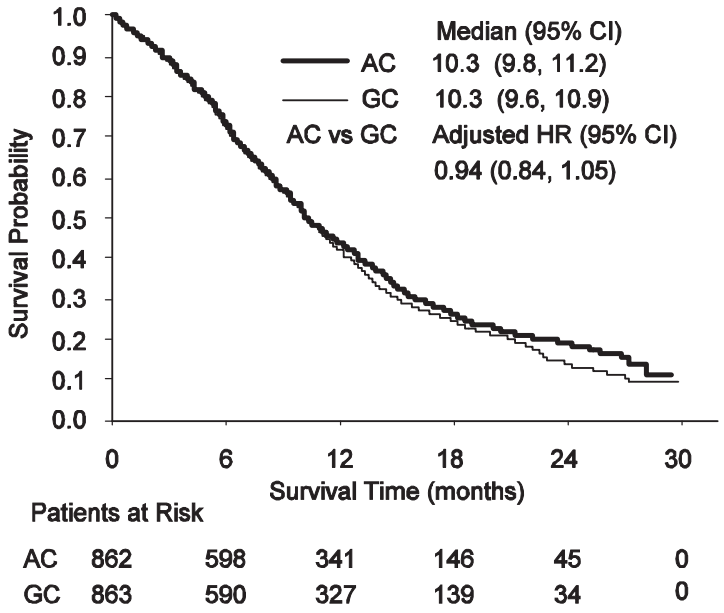


Figure 1: Kaplan-Meier Curves for Overall Survival ALIMTA plus Cisplatin (AC) versus Gemcitabine plus Cisplatin (GC) in NSCLC - ITT Population.

A pre-specified analysis of the impact of NSCLC histology on overall survival was examined. Clinically relevant differences in survival according to histology were observed and are shown in Table 11. This difference in treatment effect for ALIMTA based on histology demonstrating a lack of efficacy in squamous cell histology was also observed in the single-agent, second-line study and the maintenance study *[see Clinical Studies (14.2, 14.3)]*.

Table 11: First-Line Therapy: Overall Survival in NSCLC Histologic Subgroups

Histology Subgroup	**Median Overall Survival in Months (95% CI)**	**Unadjusted Hazard**	**Adjusted Hazard Ratio (HR)[a,b,c]**

	ALIMTA plus Cisplatin		Gemcitabine plus Cisplatin		**Ratio (HR)[a,b] (95% CI)**	**(95% CI)**
Nonsquamous NSCLC[d] (N=1252)	11.0 (10.1-12.5)	N=618	10.1 (9.3-10.9)	N=634	0.84 (0.74-0.96)	0.84 (0.74-0.96)
Adenocarcinoma (N=847)	12.6 (10.7-13.6)	N=436	10.9 (10.2-11.9)	N=411	0.84 (0.71-0.98)	0.84 (0.71-0.99)
Large Cell (N=153)	10.4 (8.6-14.1)	N=76	6.7 (5.5-9.0)	N=77	0.68 (0.48-0.97)	0.67 (0.48-0.96)
Other[e] (N=252)	8.6 (6.8-10.2)	N=106	9.2 (8.1-10.6)	N=146	1.12 (0.84-1.49)	1.08 (0.81-1.45)
Squamous Cell (N=473)	9.4 (8.4-10.2)	N=244	10.8 (9.5-12.1)	N=229	1.22 (0.99-1.50)	1.23 (1.00-1.51)

[a] A HR that is less than 1.0 indicates that survival is better in the AC arm than in the GC arm. Alternatively, a HR that is greater than 1.0 indicates survival is better in the GC arm than in the AC arm.

[b] Unadjusted for multiple comparisons.

[c] HRs adjusted for ECOG PS, gender, disease stage, and basis for pathological diagnosis (histopathological/cytopathological).

[d] Includes adenocarcinoma, large cell, and other histologies except those with squamous cell type.

[e] The subgroup of "other" represents patients with a primary diagnosis of NSCLC whose disease did not clearly qualify as adenocarcinoma, squamous cell carcinoma, or large cell carcinoma.

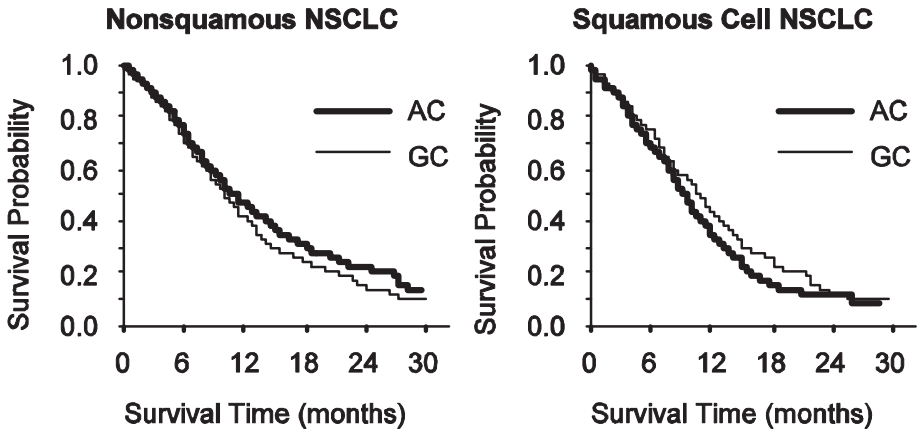


Figure 2: Kaplan-Meier Curves for Overall Survival ALIMTA plus Cisplatin (AC) versus Gemcitabine plus Cisplatin (GC) in NSCLC - Nonsquamous NSCLC and Squamous Cell NSCLC.

14.2 Non-Small Cell Lung Cancer - Maintenance

A multi-center, randomized, double-blind, placebo-controlled study was conducted in 663 patients with Stage IIIb/IV NSCLC who did not progress after four cycles of platinum-based chemotherapy. Patients who did not progress were randomized 2:1 to receive ALIMTA or placebo immediately following platinum-based chemotherapy. ALIMTA was administered intravenously over 10 minutes at a dose of 500 mg/m^2 on Day 1 of each 21-day cycle, until disease progression. Patients in both study arms received folic acid, vitamin B_{12}, and dexamethasone *[see Dosage and Administration (2.3)]*.

The study was designed to demonstrate superior progression-free survival and overall survival of ALIMTA over placebo. Progression-free survival (PFS) was assessed by independent review. Patient characteristics of the intent to treat (ITT) population are shown in Table 12. The demographics and baseline disease characteristics were well balanced between study arms.

Table 12: Maintenance Therapy: Summary of Patient Characteristics in Study of NSCLC

Patient characteristic	**ALIMTA (N=441)**	**Placebo (N=222)**
Age (yrs)		
Median (range)	60.6 (25.6-82.6)	60.4 (35.4-78.5)
Gender		
Male/Female	73.0%/27.0%	72.5%/27.5%
Ethnic Origin		
Caucasian	279 (63.3%)	149 (67.1%)
East Asian	104 (23.6%)	50 (22.5%)

Other	58 (13.2%)	23 (10.4%)
Stage at Entry[a]		
IIIb/IV	18.0%/82.0%	21.2%/78.8%
Histology (%)		
Nonsquamous NSCLC[b]	325 (73.7%)	156 (70.3%)
Adenocarcinoma	222 (50.3%)	106 (47.7%)
Large cell	10 (2.3%)	10 (4.5%)
Other[c]	93 (21.1%)	40 (18.0%)
Squamous	116 (26.3%)	66 (29.7%)
ECOG PS[d]		
0/1	40.1%/59.9%	38.3%/61.7%
Smoking History[e]		
Ever/never smoker	74.1%/25.9%	71.5%/28.5%
Time from start of induction therapy to study randomization (months)		
Median (range)	3.25 (1.6-4.8)	3.29 (2.7-5.1)

[a] Stage at Entry was not reported for all randomized patients. Percentages are representative of N=440 for the ALIMTA arm and N=222 for the placebo arm.

[b] Includes patients with adenocarcinoma, large cell, and other histologic diagnoses.

[c] The subgroup of "Other" represents patients with a primary diagnosis of NSCLC whose disease did not clearly qualify as adenocarcinoma, large cell carcinoma, or squamous cell carcinoma.

[d] Eastern Cooperative Oncology Group Performance Status (ECOG PS) was not reported for all randomized patients. Percentages are representative of N=439 for the ALIMTA arm, and N=222 for the placebo arm.

[e] Smoking history was not reported for all randomized patients. Percentages are representative of N=437 for the ALIMTA arm and N=221 for the placebo arm.

Patients received a median of 5 cycles of ALIMTA and 3.5 cycles of placebo. Patients randomized to ALIMTA received a relative dose intensity of 95.7%. A total of 213 patients (48.3%) completed ≥6 cycles and a total of 98 patients (22.6%) completed ≥10 cycles of treatment with ALIMTA.

In the overall study population, ALIMTA was statistically superior to placebo in terms of overall survival (OS) (median 13.4 months versus 10.6 months, HR=0.79 (95% CI: 0.65-0.95), p-value=0.012) and PFS (median 4.0 months versus 2.0 months, HR=0.60 (95% CI: 0.49-0.73), p-value<0.00001). A difference in treatment outcomes was observed according to histologic classification. For the population of patients with nonsquamous NSCLC, ALIMTA was superior to placebo for OS (median 15.5 months versus 10.3 months, HR=0.70 (95% CI: 0.56-0.88)) and PFS (median 4.4 months versus 1.8 months, HR=0.47 (95% CI: 0.37-0.60)). For the population of patients with squamous NSCLC, ALIMTA did not improve OS compared to placebo (median 9.9 months versus 10.8 months, HR=1.07 (95% CI: 0.77-1.50)) or PFS (median 2.4 months versus 2.5 months, HR=1.03 (95% CI: 0.71-1.49)). This difference in treatment effect for ALIMTA based on histology demonstrating lack of benefit in squamous cell histology was also observed in the first-line and second-line studies. *[see Clinical Studies (14.1, 14.3)]*

Efficacy results for the overall patient population are presented in Table 13 and Figure 3, and efficacy results by pre-specified histologic subgroups are presented in Table 14 and Figure 4, below.

Table 13: Maintenance Therapy: Efficacy of ALIMTA versus Placebo in NSCLC - ITT Population

Efficacy Parameter[a,b]	**ALIMTA (N=441)**	**Placebo (N=222)**
Median overall survival[c] (95% CI)	13.4 mos (11.9-15.9)	10.6 mos (8.7-12.0)
Hazard ratio (HR)[c] (95% CI)	0.79 (0.65-0.95)	
p-value	p=0.012	
Median progression-free survival (95% CI)	4.0 mos (3.1-4.4)	2.0 mos (1.5-2.8)
Hazard ratio (HR)[c] (95% CI)	0.60 (0.49-0.73)	
p-value	p<0.00001	

[a] PFS and OS were calculated from time of randomization, after completion of 4 cycles of induction platinum-based chemotherapy.

[b] Values for PFS given based on independent review (ALIMTA N=387, Placebo N=194).

[c] Unadjusted hazard ratios are provided. A HR <1.0 indicates that the result is better in the ALIMTA arm than in the placebo arm.

Table 14: Maintenance Therapy: Efficacy in NSCLC by Histologic Subgroups[a]

	Overall Survival		**Progression-Free Survival[b]**	
	ALIMTA	**Placebo**	**ALIMTA**	**Placebo**
	Median (months)	**Median (months)**	**Median (months)**	**Median (months)**
	HR[c] (95% CI)		**HR[c] (95% CI)**	

Nonsquamous NSCLC[d] N=481	**15.5**	**10.3**	**4.4**	**1.8**
	0.70 (0.56-0.88)		**0.47 (0.37-0.60)**	
Adenocarcinoma N=328	16.8	11.5	4.6	2.7
	0.73 (0.56-0.96)		0.51 (0.38-0.68)	
Large cell carcinoma N=20	8.4	7.9	4.5	1.5
	0.98 (0.36-2.65)		0.40 (0.12-1.29)	
Other[e] N=133	11.3	7.7	4.1	1.6
	0.61 (0.40-0.94)		0.44 (0.28-0.68)	
Squamous cell N=182	**9.9**	**10.8**	**2.4**	**2.5**
	1.07 (0.77-1.50)		**1.03 (0.71-1.49)**	

[a] PFS and OS were calculated from time of randomization, after completion of 4 cycles of induction platinum-based chemotherapy. All results unadjusted for multiple comparisons.

[b] Values for PFS are given based on independent review (ALIMTA N=387, Placebo N=194).

[c] Unadjusted hazard ratios are provided. A HR <1.0 indicates that the result is better in the ALIMTA arm than in the placebo arm. A HR >1.0 indicates that the result is better in the placebo arm than in the ALIMTA arm.

[d] Includes patients with adenocarcinoma, large cell carcinoma, and other histology.

[e] The subgroup of "Other" represents patients with a primary diagnosis of NSCLC whose disease did not clearly qualify as adenocarcinoma, large cell carcinoma, or squamous cell carcinoma.

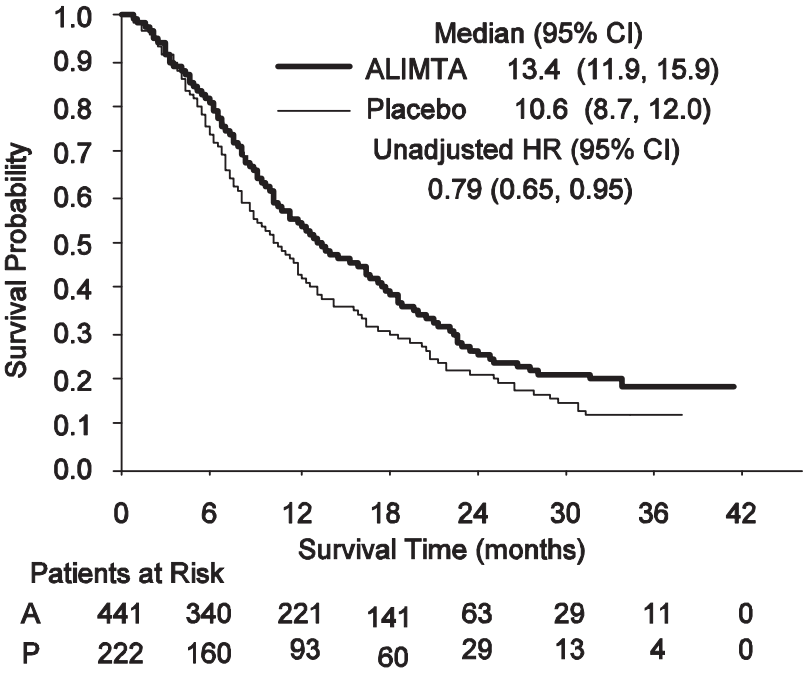


Figure 3: Kaplan-Meier Curve for Overall Survival ALIMTA (A) versus Placebo (P) in NSCLC - ITT Population.

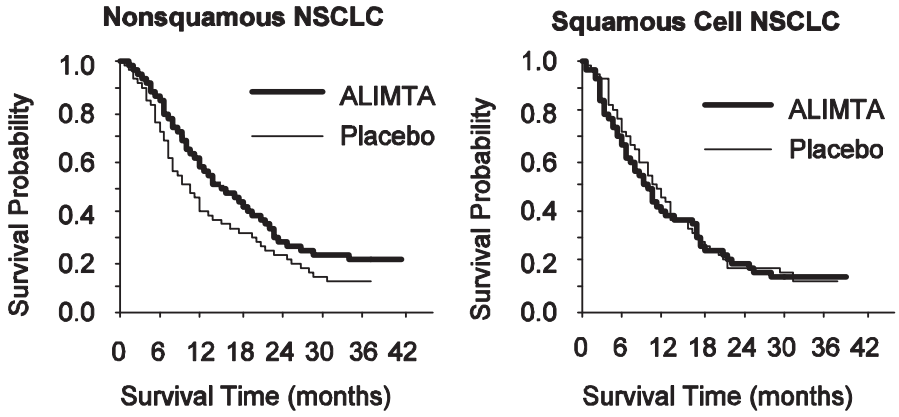


Figure 4: Kaplan-Meier Curves for Overall Survival ALIMTA versus Placebo in NSCLC - Nonsquamous NSCLC and Squamous Cell NSCLC.

14.3 Non-Small Cell Lung Cancer - After Prior Chemotherapy

A multi-center, randomized, open label study was conducted in patients with Stage III or IV NSCLC after prior chemotherapy to compare the overall survival following treatment with ALIMTA versus docetaxel. ALIMTA was administered intravenously over 10 minutes at a dose of 500 mg/m^2 and docetaxel was administered at 75 mg/m^2 as a 1-hour intravenous infusion. Both drugs were given on Day 1 of each 21-day cycle. All patients treated with ALIMTA received vitamin supplementation with folic acid and vitamin B_{12}. The study was intended to show either an overall survival superiority or non-inferiority of ALIMTA to docetaxel. Patient demographics of the intent to treat (ITT) population are shown in Table 15.

Table 15: Second-Line Therapy: Summary of Patient Characteristics in NSCLC Study

Patient characteristic	**ALIMTA** (N=283)	**Docetaxel** (N=288)
Age (yrs)		
Median (range)	59 (22-81)	57 (28-87)
Gender (%)		
Male/Female	68.6/31.4	75.3/24.7
Stage at Entry (%)		
III/IV	25.1/74.9	25.3/74.7
Diagnosis/Histology (%)		
Adenocarcinoma	154 (54.4)	142 (49.3)
Squamous	78 (27.6)	94 (32.6)
Bronchoalveolar	4 (1.4)	1 (0.3)
Other	47 (16.6)	51 (17.7)
Performance Status (%)[a]		
0-1	234 (88.6)	240 (87.6)
2	30 (11.4)	34 (12.4)

[a] Performance status was not reported for all randomized patients. Percentages are representative of N=264 for the ALIMTA arm and N=274 for the docetaxel arm.

The primary endpoint in this study was overall survival. The median survival time was 8.3 months in the ALIMTA treatment arm and 7.9 months in the docetaxel arm, with a hazard ratio of 0.99 (see Table 16). The study did not show an overall survival superiority of ALIMTA.

Table 16: Efficacy of ALIMTA versus Docetaxel in Non-Small Cell Lung Cancer - ITT Population

	ALIMTA (N=283)	Docetaxel (N=288)
Median overall survival (95% CI)	8.3 mos (7.0-9.4)	7.9 mos (6.3-9.2)
Hazard ratio (HR) (95% CI)	0.99 (0.82-1.20)	
Median progression-free survival (95% CI)	2.9 mos (2.4-3.1)	2.9 mos (2.7-3.4)
Hazard ratio (HR) (95% CI)	0.97 (0.82-1.16)	
Overall response rate (95% CI)	8.5% (5.2-11.7)	8.3% (5.1-11.5)

A retrospective analysis of the impact of NSCLC histology on overall survival was examined. Clinically relevant differences in survival according to histology were observed and are shown in Table 17. This difference in treatment effect for ALIMTA based on histology demonstrating a lack of efficacy in squamous cell histology was also observed in the first-line combination study and in the maintenance study *[see Clinical Studies (14.1, 14.2)]*.

Table 17: Second-Line Therapy: Overall Survival of ALIMTA versus Docetaxel in NSCLC by Histologic Subgroups

Histology Subgroup	**Median Overall Survival in Months (95% CI)**				**Unadjusted Hazard Ratio (HR)[a,b] (95% CI)**	**Adjusted Hazard Ratio (HR)[a,b,c] (95% CI)**
	ALIMTA		Docetaxel			
Nonsquamous NSCLC[d] (N=399)	9.3 (7.8-9.7)	N=205	8.0 (6.3-9.3)	N=194	0.89 (0.71-1.13)	0.78 (0.61-1.00)
Adenocarcinoma (N=301)	9.0 (7.6-9.6)	N=158	9.2 (7.5-11.3)	N=143	1.09 (0.83-1.44)	0.92 (0.69-1.22)
Large Cell	12.8	N=18	4.5	N=29	0.38	0.27

(N=47)	(5.8-14.0)		(2.3-9.1)		(0.18-0.78)	(0.11-0.63)
Other[e] (N=51)	9.4 (6.0-10.1)	N=29	7.9 (4.0-8.9)	N=22	0.62 (0.32-1.23)	0.57 (0.27-1.20)
Squamous Cell (N=172)	6.2 (4.9-8.0)	N=78	7.4 (5.6-9.5)	N=94	1.32 (0.93-1.86)	1.56 (1.08-2.26)

[a] A HR that is less than 1.0 indicates that survival is better in the ALIMTA arm than in the docetaxel arm. Alternatively, a HR that is greater than 1.0 indicates survival is better in the docetaxel arm than in the ALIMTA arm.

[b] Unadjusted for multiple comparisons.

[c] HRs adjusted for ECOG PS, time since prior chemotherapy, disease stage, and gender.

[d] Includes adenocarcinoma, large cell, and other histologies except those with squamous cell type.

[e] The subgroup of "other" represents patients with a primary diagnosis of NSCLC whose disease did not clearly qualify as adenocarcinoma, squamous cell carcinoma, or large cell carcinoma.

14.4 Malignant Pleural Mesothelioma

A multi-center, randomized, single-blind study in 448 chemonaive patients with malignant pleural mesothelioma (MPM) compared survival in patients treated with ALIMTA in combination with cisplatin to survival in patients receiving cisplatin alone. ALIMTA was administered intravenously over 10 minutes at a dose of 500 mg/m^2 and cisplatin was administered intravenously over 2 hours at a dose of 75 mg/m^2 beginning approximately 30 minutes after the end of administration of ALIMTA. Both drugs were given on Day 1 of each 21-day cycle. After 117 patients were treated, white cell and GI toxicity led to a change in protocol whereby all patients were given folic acid and vitamin B_{12} supplementation.

The primary analysis of this study was performed on the population of all patients randomly assigned to treatment who received study drug (randomized and treated). An analysis was also performed on patients who received folic acid and vitamin B_{12} supplementation during the entire course of study therapy (fully supplemented), as supplementation is recommended *[see Dosage and Administration (2.3)]*. Results in all patients and those fully supplemented were similar. Patient demographics are shown in Table 18.

Table 18: Summary of Patient Characteristics in MPM Study

Patient characteristic	Randomized and Treated Patients		Fully Supplemented Patients	
	ALIMTA/cis (N=226)	Cisplatin (N=222)	ALIMTA/cis (N=168)	Cisplatin (N=163)
Age (yrs)				
Median (range)	61 (29-85)	60 (19-84)	60 (29-85)	60 (19-82)
Gender (%)				
Male	184 (81.4)	181 (81.5)	136 (81.0)	134 (82.2)
Female	42 (18.6)	41 (18.5)	32 (19.0)	29 (17.8)
Origin (%)				
Caucasian	204 (90.3)	206 (92.8)	150 (89.3)	153 (93.9)
Hispanic	11 (4.9)	12 (5.4)	10 (6.0)	7 (4.3)
Asian	10 (4.4)	4 (1.9)	7 (4.2)	3 (1.8)
African descent	1 (0.4)	0	1 (0.6)	0
Stage at Entry (%)				
I	16 (7.1)	14 (6.3)	15 (8.9)	12 (7.4)
II	35 (15.6)	33 (15.0)	27 (16.2)	27 (16.8)
III	73 (32.4)	68 (30.6)	51 (30.5)	49 (30.4)
IV	101 (44.9)	105 (47.2)	74 (44.3)	73 (45.3)
Unspecified	1 (0.4)	2 (0.9)	1 (0.6)	2 (1.2)
Diagnosis/Histology[a] (%)				
Epithelial	154 (68.1)	152 (68.5)	117 (69.6)	113 (69.3)
Mixed	37 (16.4)	36 (16.2)	25 (14.9)	25 (15.3)
Sarcomatoid	18 (8.0)	25 (11.3)	14 (8.3)	17 (10.4)
Other	17 (7.5)	9 (4.1)	12 (7.1)	8 (4.9)
Baseline KPS[b] (%)				
70-80	109 (48.2)	97 (43.7)	83 (49.4)	69 (42.3)
90-100	117 (51.8)	125 (56.3)	85 (50.6)	94 (57.7)

[a] Only 67% of the patients had the histologic diagnosis of malignant mesothelioma confirmed by independent review.

[b] Karnofsky Performance Scale.

Table 19 and Figure 5 summarize the survival results for all randomized and treated patients regardless of vitamin supplementation status and those patients receiving vitamin supplementation from the time of enrollment in the trial.

Table 19: Efficacy of ALIMTA plus Cisplatin versus Cisplatin in Malignant Pleural Mesothelioma

Efficacy Parameter	Randomized and Treated Patients		Fully Supplemented Patients	
	ALIMTA/cis (N=226)	Cisplatin (N=222)	ALIMTA/cis (N=168)	Cisplatin (N=163)
Median overall survival (95% CI)	12.1 mos (10.0-14.4)	9.3 mos (7.8-10.7)	13.3 mos (11.4-14.9)	10.0 mos (8.4-11.9)
Hazard ratio	0.77		0.75	
Log rank p-value[a]	0.020		0.051	

[a] p-value refers to comparison between arms.

Similar results were seen in the analysis of patients (N=303) with confirmed histologic diagnosis of malignant pleural mesothelioma. There were too few non-white patients to assess possible ethnic differences. The effect in women (median survival 15.7 months with the combination versus 7.5 months on cisplatin alone), however, was larger than the effect in males (median survival 11 versus 9.4 respectively). As with any exploratory analysis, it is not clear whether this difference is real or is a chance finding.

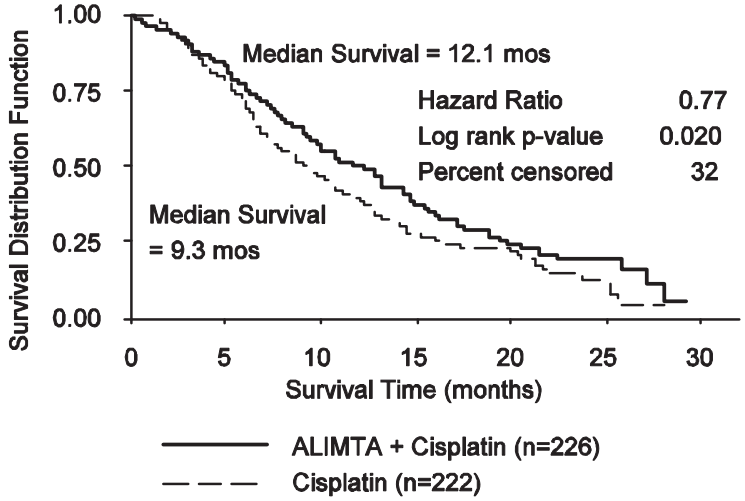


Figure 5: Kaplan-Meier Estimates of Survival Time for ALIMTA plus Cisplatin and Cisplatin Alone in all Randomized and Treated Patients.

Objective tumor response for malignant pleural mesothelioma is difficult to measure and response criteria are not universally agreed upon. However, based upon prospectively defined criteria, the objective tumor response rate for ALIMTA plus cisplatin was greater than the objective tumor response rate for cisplatin alone. There was also improvement in lung function (forced vital capacity) in the ALIMTA plus cisplatin arm compared to the control arm.

Patients who received full supplementation with folic acid and vitamin B_{12} during study therapy received a median of 6 and 4 cycles in the ALIMTA/cisplatin (N=168) and cisplatin (N=163) arms, respectively. Patients who never received folic acid and vitamin B_{12} during study therapy received a median of 2 cycles in both treatment arms (N=32 and N=38 for the ALIMTA/cisplatin and cisplatin arm, respectively). Patients receiving ALIMTA in the fully supplemented group received a relative dose intensity of 93% of the protocol specified ALIMTA dose intensity; patients treated with cisplatin in the same group received 94% of the projected dose intensity. Patients treated with cisplatin alone had a dose intensity of 96%.

15 REFERENCES

1. Preventing Occupational Exposures to Antineoplastic and Other Hazardous Drugs in Health Care Settings. NIOSH Alert 2004-165.
2. OSHA Technical Manual, TED 1-0.15A, Section VI: Chapter 2. Controlling Occupational Exposure to Hazardous Drugs. OSHA, 1999. http://www.osha.gov/dts/osta/otm/otm_vi/otm_vi_2.html
3. American Society of Health-System Pharmacists. ASHP guidelines on handling hazardous drugs. *Am J Health-Syst Pharm*. 2006; 63:1172-1193.
4. Polovich, M., White, J. M., & Kelleher, L. O. (eds.) 2005. Chemotherapy and biotherapy guidelines and recommendations for practice (2nd. ed.) Pittsburgh, PA: Oncology Nursing Society.

16 HOW SUPPLIED/STORAGE AND HANDLING

16.1 How Supplied

ALIMTA, pemetrexed for injection, is available in sterile single-use vials containing 100 mg pemetrexed.
NDC 0002-7640-01 (VL7640): single-use vial with ivory flip-off cap individually packaged in a carton.
ALIMTA, pemetrexed for injection, is available in sterile single-use vials containing 500 mg pemetrexed.
NDC 0002-7623-01 (VL7623): single-use vial with ivory flip-off cap individually packaged in a carton.

16.2 Storage and Handling

ALIMTA, pemetrexed for injection, should be stored at 25°C (77°F); excursions permitted to 15-30°C (59-86°F) [see USP Controlled Room Temperature].

Chemical and physical stability of reconstituted and infusion solutions of ALIMTA were demonstrated for up to 24 hours following initial reconstitution, when stored refrigerated, 2-8°C (36-46°F), or at 25°C (77°F), excursions permitted to 15-30°C (59-86°F) [see USP Controlled Room Temperature]. When prepared as directed, reconstituted and infusion solutions of ALIMTA contain no antimicrobial preservatives. Discard unused portion *[see Dosage and Administration (2.5)]*.

ALIMTA is not light sensitive.

17 PATIENT COUNSELING INFORMATION

See FDA-Approved Patient Labeling (PPI)

Patients should be instructed to read the patient package insert carefully.

17.1 Need for Folic Acid and Vitamin B_{12}

Patients treated with ALIMTA must be instructed to take folic acid and vitamin B_{12} as a prophylactic measure to reduce treatment-related hematologic and gastrointestinal toxicity *[see Dosage and Administration (2.3)]*.

17.2 Low Blood Cell Counts

Patients should be adequately informed of the risk of low blood cell counts and instructed to immediately contact their physician should any sign of infection develop including fever. Patients should also contact their physician if bleeding or symptoms of anemia occur.

17.3 Gastrointestinal Effects

Patients should be instructed to contact their physician if persistent vomiting, diarrhea, or signs of dehydration appear.

17.4 Concomitant Medications

Patients should be instructed to inform the physician if they are taking any concomitant prescription or over-the-counter medications including those for pain or inflammation such as non-steroidal anti-inflammatory drugs *[see Drug Interactions (7.1)]*.

Literature revised December 19, 2011

Lilly USA, LLC

Indianapolis, IN 46285, USA

PV 8921 AMP

APPENDIX D

PLATINOL Prescribing Information

Rx only

PLATINOL®
(cisplatin for injection, USP)

WARNING

PLATINOL (cisplatin for injection, USP) should be administered under the supervision of a qualified physician experienced in the use of cancer chemotherapeutic agents. Appropriate management of therapy and complications is possible only when adequate diagnostic and treatment facilities are readily available.

Cumulative renal toxicity associated with PLATINOL is severe. Other major dose-related toxicities are myelosuppression, nausea, and vomiting.

Ototoxicity, which may be more pronounced in children, and is manifested by tinnitus, and/or loss of high frequency hearing and occasionally deafness, is significant.

Anaphylactic-like reactions to PLATINOL have been reported. Facial edema, bronchoconstriction, tachycardia, and hypotension may occur within minutes of PLATINOL administration. Epinephrine, corticosteroids, and antihistamines have been effectively employed to alleviate symptoms (see **WARNINGS** and **ADVERSE REACTIONS**).

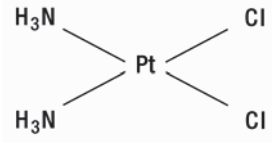
Exercise caution to prevent inadvertent PLATINOL overdose. Doses greater than 100 mg/m^2/cycle once every 3 to 4 weeks are rarely used. Care must be taken to avoid inadvertent PLATINOL overdose due to confusion with PARAPLATIN® (carboplatin) or prescribing practices that fail to differentiate daily doses from total dose per cycle.

DESCRIPTION

PLATINOL® (cisplatin for injection, USP) is a white to light yellow lyophilized powder. Each vial of PLATINOL contains 50 mg cisplatin, 450 mg Sodium Chloride, USP, and 500 mg Mannitol, USP.

The active ingredient, cisplatin, is a yellow to orange crystalline powder with the molecular formula $PtCl_2H_6N_2$, and a molecular weight of 300.1. Cisplatin is a heavy

metal complex containing a central atom of platinum surrounded by two chloride atoms and two ammonia molecules in the cis position. It is soluble in water or saline at 1 mg/mL and in dimethylformamide at 24 mg/mL. It has a melting point of 207° C.



CLINICAL PHARMACOLOGY

Plasma concentrations of the parent compound, cisplatin, decay monoexponentially with a half-life of about 20 to 30 minutes following bolus administrations of 50 or 100 mg/m^2 doses. Monoexponential decay and plasma half-lives of about 0.5 hour are also seen following 2-hour or 7-hour infusions of 100 mg/m^2. After the latter, the total body clearances and volumes of distribution at steady-state for cisplatin are about 15 to 16 L/h/m^2 and 11 to 12 L/m^2.

Due to its unique chemical structure, the chlorine atoms of cisplatin are more subject to chemical displacement reactions by nucleophiles, such as water or sulfhydryl groups, than to enzyme-catalyzed metabolism. At physiological pH in the presence of 0.1M NaCl, the predominant molecular species are cisplatin and monohydroxymonochloro *cis*-diammine platinum (II) in nearly equal concentrations. The latter, combined with the possible direct displacement of the chlorine atoms by sulfhydryl groups of amino acids or proteins, accounts for the instability of cisplatin in biological matrices. The ratios of cisplatin to total free (ultrafilterable) platinum in the plasma vary considerably between patients and range from 0.5 to 1.1 after a dose of 100 mg/m^2.

Cisplatin does not undergo the instantaneous and reversible binding to plasma proteins that is characteristic of normal drug-protein binding. However, the platinum from cisplatin, but not cisplatin itself, becomes bound to several plasma proteins, including albumin, transferrin, and gamma globulin. Three hours after a bolus injection and two hours after the end of a three-hour infusion, 90% of the plasma platinum is protein bound. The complexes between albumin and the platinum from cisplatin do not dissociate to a significant extent and are slowly eliminated with a minimum half-life of five days or more.

Following cisplatin doses of 20 to 120 mg/m^2, the concentrations of platinum are highest in liver, prostate, and kidney; somewhat lower in bladder, muscle, testicle, pancreas, and spleen; and lowest in bowel, adrenal, heart, lung, cerebrum, and cerebellum. Platinum is present in tissues for as long as 180 days after the last administration. With the exception of intracerebral tumors, platinum concentrations in tumors are generally somewhat lower than the concentrations in the organ where the tumor is located. Different metastatic sites in the same patient may have different platinum concentrations. Hepatic metastases have the highest platinum concentrations, but these are similar to the platinum concentrations in normal liver. Maximum red blood cell concentrations of platinum are reached within 90 to 150 minutes after a 100 mg/m^2 dose of cisplatin and decline in a biphasic manner with a terminal half-life of 36 to 47 days.

Over a dose range of 40 to 140 mg cisplatin/m^2 given as a bolus injection or as infusions varying in length from 1 hour to 24 hours, from 10% to about 40% of the administered platinum is excreted in the urine in 24 hours. Over five days following administration of 40 to 100 mg/m^2 doses given as rapid, 2- to 3-hour, or 6- to 8-hour infusions, a mean of 35% to 51% of the dosed platinum is excreted in the urine. Similar mean urinary recoveries of platinum of about 14% to 30% of the dose are found following five daily administrations of 20, 30, or 40 mg/m^2/day. Only a small percentage of the administered platinum is excreted beyond 24 hours post-infusion and most of the platinum excreted in the urine in 24 hours is excreted within the first few hours. Platinum-containing species excreted in the urine are the same as those found following the incubation of cisplatin with urine from healthy subjects, except that the proportions are different.

The parent compound, cisplatin, is excreted in the urine and accounts for 13% to 17% of the dose excreted within one hour after administration of 50 mg/m^2. The mean renal clearance of cisplatin exceeds creatinine clearance and is 62 and 50 mL/min/m^2 following administration of 100 mg/m^2 as 2-hour or 6- to 7-hour infusions, respectively.

The renal clearance of free (ultrafilterable) platinum also exceeds the glomerular filtration rate indicating that cisplatin or other platinum-containing molecules are actively secreted by the kidneys. The renal clearance of free platinum is nonlinear and variable and is dependent on dose, urine flow rate, and individual variability in the extent of active secretion and possible tubular reabsorption.

There is a potential for accumulation of ultrafilterable platinum plasma concentrations whenever cisplatin is administered on a daily basis but not when dosed on an intermittent basis.

No significant relationships exist between the renal clearance of either free platinum or cisplatin and creatinine clearance.

Although small amounts of platinum are present in the bile and large intestine after administration of cisplatin, the fecal excretion of platinum appears to be insignificant.

INDICATIONS AND USAGE

PLATINOL (cisplatin for injection, USP) is indicated as therapy to be employed as follows:

Metastatic Testicular Tumors

In established combination therapy with other approved chemotherapeutic agents in patients with metastatic testicular tumors who have already received appropriate surgical and/or radiotherapeutic procedures.

Metastatic Ovarian Tumors

In established combination therapy with other approved chemotherapeutic agents in patients with metastatic ovarian tumors who have already received appropriate surgical and/or radiotherapeutic procedures. An established combination consists of PLATINOL and cyclophosphamide. PLATINOL, as a single agent, is indicated as secondary therapy in patients with metastatic ovarian tumors refractory to standard chemotherapy who have not previously received PLATINOL therapy.

Advanced Bladder Cancer

PLATINOL is indicated as a single agent for patients with transitional cell bladder cancer which is no longer amenable to local treatments, such as surgery and/or radiotherapy.

CONTRAINDICATIONS

PLATINOL is contraindicated in patients with preexisting renal impairment. PLATINOL should not be employed in myelosuppressed patients, or in patients with hearing impairment.

PLATINOL is contraindicated in patients with a history of allergic reactions to PLATINOL or other platinum-containing compounds.

WARNINGS

PLATINOL (cisplatin for injection, USP) produces cumulative nephrotoxicity which is potentiated by aminoglycoside antibiotics. The serum creatinine, blood urea nitrogen (BUN), creatinine clearance, and magnesium, sodium, potassium, and calcium levels should be measured prior to initiating therapy, and prior to each subsequent course. At the recommended dosage, PLATINOL should not be given more frequently than once every 3 to 4 weeks (see **ADVERSE REACTIONS**). Elderly patients may be more susceptible to nephrotoxicity (see **PRECAUTIONS: Geriatric Use**).

There are reports of severe neuropathies in patients in whom regimens are employed using higher doses of PLATINOL or greater dose frequencies than those recommended. These neuropathies may be irreversible and are seen as paresthesias in a stocking-glove distribution, areflexia, and loss of proprioception and vibratory sensation. Elderly patients may be more susceptible to peripheral neuropathy (see **PRECAUTIONS: Geriatric Use**).

Loss of motor function has also been reported.

Anaphylactic-like reactions to PLATINOL have been reported. These reactions have occurred within minutes of administration to patients with prior exposure to PLATINOL, and have been alleviated by administration of epinephrine, corticosteroids, and antihistamines.

Since ototoxicity of PLATINOL is cumulative, audiometric testing should be performed prior to initiating therapy and prior to each subsequent dose of drug (see **ADVERSE REACTIONS**).

PLATINOL can cause fetal harm when administered to a pregnant woman. PLATINOL is mutagenic in bacteria and produces chromosome aberrations in animal cells in tissue culture. In mice PLATINOL is teratogenic and embryotoxic. If this drug 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. Patients should be advised to avoid becoming pregnant.

The carcinogenic effect of PLATINOL was studied in BD IX rats. PLATINOL was administered intraperitoneally (i.p.) to 50 BD IX rats for 3 weeks, 3 X 1 mg/kg body weight per week. Four hundred and fifty-five days after the first application, 33 animals died, 13 of them related to malignancies: 12 leukemias and 1 renal fibrosarcoma.

The development of acute leukemia coincident with the use of PLATINOL has been reported. In these reports, PLATINOL was generally given in combination with other leukemogenic agents.

Injection site reactions may occur during the administration of PLATINOL (see **ADVERSE REACTIONS**). Given the possibility of extravasation, it is recommended to closely monitor the infusion site for possible infiltration during drug administration. A specific treatment for extravasation reactions is unknown at this time.

PRECAUTIONS

Peripheral blood counts should be monitored weekly. Liver function should be monitored periodically. Neurologic examination should also be performed regularly (see **ADVERSE REACTIONS**).

Drug Interactions

Plasma levels of anticonvulsant agents may become subtherapeutic during cisplatin therapy.

In a randomized trial in advanced ovarian cancer, response duration was adversely affected when pyridoxine was used in combination with altretamine (hexamethylmelamine) and PLATINOL.

Carcinogenesis, Mutagenesis, Impairment of Fertility

See **WARNINGS**.

Pregnancy

Category D. See **WARNINGS**.

Nursing Mothers

Cisplatin has been reported to be found in human milk; patients receiving PLATINOL should not breast-feed.

Pediatric Use

Safety and effectiveness in pediatric patients have not been established.

Geriatric Use

Insufficient data are available from clinical trials of cisplatin in the treatment of metastatic testicular tumors or advanced bladder cancer to determine whether elderly patients respond differently than younger patients. In four clinical trials of combination chemotherapy for advanced ovarian carcinoma, 1484 patients received cisplatin either in combination with cyclophosphamide or paclitaxel. Of these, 426 (29%) were older than 65 years. In these trials, age was not found to be a prognostic factor for survival. However, in a later secondary analysis for one of these trials, elderly patients were found to have shorter survival compared with younger patients. In all four trials, elderly patients experienced more severe neutropenia than younger patients. Higher incidences of severe thrombocytopenia and leukopenia were also seen in elderly compared with younger patients, although not in all cisplatin-containing treatment arms. In the two trials where nonhematologic toxicity was evaluated according to age, elderly patients had a numerically higher incidence of peripheral neuropathy than younger patients. Other reported clinical experience suggests that elderly patients may be more susceptible to myelosuppression, infectious complications, and nephrotoxicity than younger patients.

Cisplatin is known to be substantially excreted by the kidney and is contraindicated in patients with preexisting renal impairment. Because elderly patients are more likely to have decreased renal function, care should be taken in dose selection, and renal function should be monitored.

ADVERSE REACTIONS

Nephrotoxicity

Dose-related and cumulative renal insufficiency, including acute renal failure, is the major dose-limiting toxicity of PLATINOL. Renal toxicity has been noted in 28% to 36% of patients treated with a single dose of 50 mg/m^2. It is first noted during the second week after a dose and is manifested by elevations in BUN and creatinine, serum uric acid and/or a decrease in creatinine clearance. **Renal toxicity becomes more prolonged and severe with repeated courses of the drug. Renal function must return to normal**

before another dose of PLATINOL can be given. Elderly patients may be more susceptible to nephrotoxicity (see **PRECAUTIONS: Geriatric Use**).

Impairment of renal function has been associated with renal tubular damage. The administration of PLATINOL using a 6- to 8-hour infusion with intravenous hydration, and mannitol has been used to reduce nephrotoxicity. However, renal toxicity still can occur after utilization of these procedures.

Ototoxicity

Ototoxicity has been observed in up to 31% of patients treated with a single dose of PLATINOL 50 mg/m^2, and is manifested by tinnitus and/or hearing loss in the high frequency range (4000 to 8000 Hz). Decreased ability to hear normal conversational tones may occur. Deafness after the initial dose of PLATINOL has been reported. Ototoxic effects may be more severe in children receiving PLATINOL. Hearing loss can be unilateral or bilateral and tends to become more frequent and severe with repeated doses. Ototoxicity may be enhanced with prior or simultaneous cranial irradiation. It is unclear whether PLATINOL-induced ototoxicity is reversible. Ototoxic effects may be related to the peak plasma concentration of PLATINOL. Careful monitoring of audiometry should be performed prior to initiation of therapy and prior to subsequent doses of PLATINOL.

Vestibular toxicity has also been reported.

Ototoxicity may become more severe in patients being treated with other drugs with nephrotoxic potential.

Hematologic

Myelosuppression occurs in 25% to 30% of patients treated with PLATINOL. The nadirs in circulating platelets and leukocytes occur between days 18 to 23 (range 7.5 to 45) with most patients recovering by day 39 (range 13 to 62). Leukopenia and thrombocytopenia are more pronounced at higher doses (>50 mg/m^2). Anemia (decrease of 2 g hemoglobin/100 mL) occurs at approximately the same frequency and with the same timing as leukopenia and thrombocytopenia. Fever and infection have also been reported in patients with neutropenia. Potential fatalities due to infection (secondary to myelosuppression) have been reported. Elderly patients may be more susceptible to myelosuppression (see **PRECAUTIONS: Geriatric Use**).

In addition to anemia secondary to myelosuppression, a Coombs' positive hemolytic anemia has been reported. In the presence of cisplatin hemolytic anemia, a further course of treatment may be accompanied by increased hemolysis and this risk should be weighed by the treating physician.

The development of acute leukemia coincident with the use of PLATINOL has been reported. In these reports, PLATINOL was generally given in combination with other leukemogenic agents.

Gastrointestinal

Marked nausea and vomiting occur in almost all patients treated with PLATINOL, and may be so severe that the drug must be discontinued. Nausea and vomiting may begin within 1 to 4 hours after treatment and last up to 24 hours. Various degrees of vomiting, nausea and/or anorexia may persist for up to 1 week after treatment.

Delayed nausea and vomiting (begins or persists 24 hours or more after chemotherapy) has occurred in patients attaining complete emetic control on the day of PLATINOL therapy.

Diarrhea has also been reported.

OTHER TOXICITIES

Vascular toxicities coincident with the use of PLATINOL in combination with other antineoplastic agents have been reported. The events are clinically heterogeneous and may include myocardial infarction, cerebrovascular accident, thrombotic microangiopathy (hemolytic-uremic syndrome [HUS]), or cerebral arteritis. Various mechanisms have been proposed for these vascular complications. There are also reports of Raynaud's phenomenon occurring in patients treated with the combination of bleomycin, vinblastine with or without PLATINOL. It has been suggested that hypomagnesemia developing coincident with the use of PLATINOL may be an added, although not essential, factor associated with this event. However, it is currently unknown if the cause of Raynaud's phenomenon in these cases is the disease, underlying vascular compromise, bleomycin, vinblastine, hypomagnesemia, or a combination of any of these factors.

Serum Electrolyte Disturbances

Hypomagnesemia, hypocalcemia, hyponatremia, hypokalemia, and hypophosphatemia have been reported to occur in patients treated with PLATINOL and are probably related to renal tubular damage. Tetany has been reported in those patients with hypocalcemia and hypomagnesemia. Generally, normal serum electrolyte levels are restored by administering supplemental electrolytes and discontinuing PLATINOL.

Inappropriate antidiuretic hormone syndrome has also been reported.

Hyperuricemia

Hyperuricemia has been reported to occur at approximately the same frequency as the increases in BUN and serum creatinine.

It is more pronounced after doses greater than 50 mg/m^2, and peak levels of uric acid generally occur between 3 to 5 days after the dose. Allopurinol therapy for hyperuricemia effectively reduces uric acid levels.

Neurotoxicity

See **WARNINGS**.

Neurotoxicity, usually characterized by peripheral neuropathies, has been reported. The neuropathies usually occur after prolonged therapy (4 to 7 months); however, neurologic symptoms have been reported to occur after a single dose. Although symptoms and signs of PLATINOL neuropathy usually develop during treatment, symptoms of neuropathy may begin 3 to 8 weeks after the last dose of PLATINOL. PLATINOL therapy should be discontinued when the symptoms are first observed. The neuropathy, however, may progress further even after stopping treatment. Preliminary evidence suggests peripheral neuropathy may be irreversible in some patients. Elderly patients may be more susceptible to peripheral neuropathy (see **PRECAUTIONS: Geriatric Use**).

Lhermitte's sign, dorsal column myelopathy, and autonomic neuropathy have also been reported.

Loss of taste, seizures, leukoencephalopathy, and reversible posterior leukoencephalopathy syndrome (RPLS) have also been reported.

Muscle cramps, defined as localized, painful, involuntary skeletal muscle contractions of sudden onset and short duration, have been reported and were usually associated in patients receiving a relatively high cumulative dose of PLATINOL and with a relatively advanced symptomatic stage of peripheral neuropathy.

Ocular Toxicity

Optic neuritis, papilledema, and cerebral blindness have been reported in patients receiving standard recommended doses of PLATINOL. Improvement and/or total recovery usually occurs after discontinuing PLATINOL. Steroids with or without mannitol have been used; however, efficacy has not been established.

Blurred vision and altered color perception have been reported after the use of regimens with higher doses of PLATINOL or greater dose frequencies than recommended in the package insert. The altered color perception manifests as a loss of color discrimination, particularly in the blue-yellow axis. The only finding on funduscopic exam is irregular retinal pigmentation of the macular area.

Anaphylactic-Like Reactions

Anaphylactic-like reactions have been reported in patients previously exposed to PLATINOL. The reactions consist of facial edema, wheezing, tachycardia, and hypotension within a few minutes of drug administration. Reactions may be controlled by intravenous epinephrine with corticosteroids and/or antihistamines as indicated. Patients receiving PLATINOL should be observed carefully for possible anaphylactic-like reactions and supportive equipment and medication should be available to treat such a complication.

Hepatotoxicity

Transient elevations of liver enzymes, especially SGOT, as well as bilirubin, have been reported to be associated with PLATINOL administration at the recommended doses.

Other Events

Cardiac abnormalities, hiccups, elevated serum amylase, rash, alopecia, malaise, asthenia, and dehydration have been reported.

Local soft tissue toxicity has been reported following extravasation of PLATINOL. Severity of the local tissue toxicity appears to be related to the concentration of the

PLATINOL solution. Infusion of solutions with a PLATINOL concentration greater than 0.5 mg/mL may result in tissue cellulitis, fibrosis, necrosis, pain, edema, and erythema.

OVERDOSAGE

Caution should be exercised to prevent inadvertent overdosage with PLATINOL. Acute overdosage with this drug may result in kidney failure, liver failure, deafness, ocular toxicity (including detachment of the retina), significant myelosuppression, intractable nausea and vomiting and/or neuritis. In addition, death can occur following overdosage.

No proven antidotes have been established for PLATINOL overdosage. Hemodialysis, even when initiated four hours after the overdosage, appears to have little effect on removing platinum from the body because of PLATINOL's rapid and high degree of protein binding. Management of overdosage should include general supportive measures to sustain the patient through any period of toxicity that may occur.

DOSAGE AND ADMINISTRATION

PLATINOL is administered by slow intravenous infusion. PLATINOL SHOULD NOT BE GIVEN BY RAPID INTRAVENOUS INJECTION.

Note: Needles or intravenous sets containing aluminum parts that may come in contact with PLATINOL should not be used for preparation or administration. Aluminum reacts with PLATINOL, causing precipitate formation and a loss of potency.

Metastatic Testicular Tumors

The usual PLATINOL dose for the treatment of testicular cancer in combination with other approved chemotherapeutic agents is 20 mg/m^2 IV daily for 5 days per cycle.

Metastatic Ovarian Tumors

The usual PLATINOL dose for the treatment of metastatic ovarian tumors in combination with cyclophosphamide is 75 to 100 mg/m^2 IV per cycle once every 4 weeks (DAY 1).

The dose of cyclophosphamide when used in combination with PLATINOL is 600 mg/m^2 IV once every 4 weeks (DAY 1).

For directions for the administration of cyclophosphamide, refer to the cyclophosphamide package insert.

In combination therapy, PLATINOL and cyclophosphamide are administered sequentially.

As a single agent, PLATINOL should be administered at a dose of 100 mg/m^2 IV per cycle once every 4 weeks.

Advanced Bladder Cancer

PLATINOL should be administered as a single agent at a dose of 50 to 70 mg/m^2 IV per cycle once every 3 to 4 weeks depending on the extent of prior exposure to radiation therapy and/or prior chemotherapy. For heavily pretreated patients an initial dose of 50 mg/m^2 per cycle repeated every 4 weeks is recommended.

All Patients

Pretreatment hydration with 1 to 2 liters of fluid infused for 8 to 12 hours prior to a PLATINOL dose is recommended. The drug is then diluted in 2 liters of 5% Dextrose in 1/2 or 1/3 normal saline containing 37.5 g of mannitol, and infused over a 6- to 8-hour period. If diluted solution is not to be used within 6 hours, protect solution from light. Adequate hydration and urinary output must be maintained during the following 24 hours.

A repeat course of PLATINOL should not be given until the serum creatinine is below 1.5 mg/100 mL, and/or the BUN is below 25 mg/100 mL. A repeat course should not be given until circulating blood elements are at an acceptable level (platelets ≥100,000/mm^3, WBC ≥4000/mm^3). Subsequent doses of PLATINOL should not be given until an audiometric analysis indicates that auditory acuity is within normal limits.

PREPARATION OF INTRAVENOUS SOLUTIONS

Preparation Precautions

Caution should be exercised in handling the powder and preparing the solution of cisplatin. Procedures for proper handling and disposal of anticancer drugs should be utilized. Several guidelines on this subject have been published.[1-4] To minimize the risk

of dermal exposure, always wear impervious gloves when handling vials and IV sets containing PLATINOL for injection.

Skin reactions associated with accidental exposure to cisplatin may occur. The use of gloves is recommended. If PLATINOL powder or PLATINOL solution contacts the skin or mucosa, immediately and thoroughly wash the skin with soap and water and flush the mucosa with water. More information is available in the references listed below.

Instructions for Preparation

The 50 mg vials should be reconstituted with 50 mL of Sterile Water for Injection, USP. Each mL of the resulting solution will contain 1 mg of PLATINOL.

Reconstitution as recommended results in a clear, colorless to slight yellow solution.

The reconstituted solution should be used intravenously only and should be administered by IV infusion over a 6- to 8-hour period (see **DOSAGE AND ADMINISTRATION**).

Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration, whenever solution and container permit.

NOTE TO PHARMACIST: Exercise caution to prevent inadvertent PLATINOL overdosage. Please call prescriber if dose is greater than 100 mg/m^2 per cycle. Aluminum and flip-off seal of vial have been imprinted with the following statement:

CALL DR. IF DOSE>100 MG/M^2/CYCLE.

STABILITY

Unopened vials of dry powder are stable for the lot life indicated on the package when stored at room temperature (25° C, 77° F).

The reconstituted solution is stable for 20 hours at room temperature (25° C, 77° F). Solution removed from the amber vial should be protected from light if it is not to be used within six hours.

Important Note: Once reconstituted, the solution should be kept at room temperature (25° C, 77° F). If the reconstituted solution is refrigerated a precipitate will form.

HOW SUPPLIED

PLATINOL® (cisplatin for injection, USP)

NDC 0015-3072-20—Each amber vial contains 50 mg of cisplatin

REFERENCES

1. NIOSH Alert: Preventing occupational exposures to antineoplastic and other hazardous drugs in healthcare settings. 2004. U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health, DHHS (NIOSH) Publication No. 2004-165.

2. OSHA Technical Manual, TED 1-0.15A, Section VI: Chapter 2. Controlling occupational exposure to hazardous drugs. OSHA, 1999. http://www.osha.gov/dts/osta/otm/otm_vi/otm_vi_2.html.

3. American Society of Health-System Pharmacists. ASHP guidelines on handling hazardous drugs. *Am J Health-Syst Pharm*. 2006;63:1172-1193.

4. Polovich M, White JM, Kelleher LO, eds. 2005. Chemotherapy and biotherapy guidelines and recommendations for practice. 2nd ed. Pittsburgh, PA: Oncology Nursing Society.

Manufactured for:
Bristol-Myers Squibb Company
Princeton, New Jersey 08543 USA
Made in Italy

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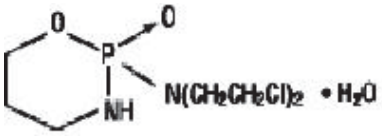
APPENDIX E

CYCLOPHOSPHAMIDE Prescribing Information

CYCLOPHOSPHAMIDE - cyclophosphamide injection, powder, for solution
Baxter Healthcare Corporation

DESCRIPTION
Cyclophosphamide for Injection, USP is a sterile white powder containing cyclophosphamide monohydrate. Cyclophosphamide is a synthetic antineoplastic drug chemically related to the nitrogen mustards. Cyclophosphamide is a white crystalline powder with the molecular formula $C_7H_{15}Cl_2N_2O_2P \cdot H_2O$ and a molecular weight of 279.1. The chemical name for cyclophosphamide is 2-[bis(2-chloroethyl)amino]tetrahydro-2H-1,3,2-oxazaphosphorine 2-oxide monohydrate. Cyclophosphamide is soluble in water, saline, or ethanol and has the following structural formula:



CLINICAL PHARMACOLOGY
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. The mechanism of action is thought to involve cross-linking of tumor cell DNA.
Cyclophosphamide is well absorbed after oral administration with a bioavailability greater than 75%. The unchanged drug has an elimination half-life of 3 to 12 hours. It is eliminated primarily in the form of metabolites, but from 5 to 25% of the dose is excreted in urine as unchanged drug. Several cytotoxic and noncytotoxic metabolites have been identified in urine and in plasma. Concentrations of metabolites reach a maximum in plasma 2 to 3 hours after an intravenous dose. Plasma protein binding of unchanged drug is low but some metabolites are bound to an extent greater than 60%. It has not been demonstrated that any single metabolite is responsible for either the therapeutic or toxic effects of cyclophosphamide. Although elevated levels of metabolites of cyclophosphamide have been observed in patients with renal failure, increased clinical toxicity in such patients has not been demonstrated.

INDICATIONS AND USAGE

Malignant Diseases
Cyclophosphamide, although effective alone in susceptible malignancies, is more frequently used concurrently or sequentially with other antineoplastic drugs. The following malignancies are often susceptible to cyclophosphamide treatment:
1. 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.

2. Multiple myeloma.

3. 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 in children (cyclophosphamide given during remission is effective in prolonging its duration).

4. Mycosis fungoides (advanced disease).

5. Neuroblastoma (disseminated disease).

6. Adenocarcinoma of the ovary.

7. Retinoblastoma.

8. Carcinoma of the breast.

Nonmalignant Disease Biopsy Proven "Minimal Change" Nephrotic Syndrome in Children:
Cyclophosphamide is useful in carefully selected cases of biopsy proven "minimal change" nephrotic syndrome in children but should not be used as primary therapy. In children whose disease fails to respond adequately to appropriate adrenocorticosteroid therapy or in whom the adrenocorticosteroid therapy produces or threatens to produce intolerable side effects, cyclophosphamide may induce a remission. Cyclophosphamide is not indicated for the nephrotic syndrome in adults or for any other renal disease.

CONTRAINDICATIONS
Continued use of cyclophosphamide is contraindicated in patients with severely depressed bone marrow function. Cyclophosphamide is contraindicated in patients who have demonstrated a previous hypersensitivity to it. See WARNINGS and PRECAUTIONS sections.

WARNINGS

Carcinogenesis, Mutagenesis, and Impairment of Fertility
Second malignancies have developed in some patients treated with cyclophosphamide used alone or in association with other antineoplastic drugs and/or modalities. Most frequently, they have been urinary bladder, myeloproliferative, or lymphoproliferative malignancies. Second malignancies most frequently were detected in patients treated for primary myeloproliferative or lymphoproliferative malignancies or nonmalignant disease in which immune processes are believed to be involved pathologically. In some cases, the second malignancy developed several years after cyclophosphamide treatment had been discontinued. In a single breast cancer trial utilizing two to four times the standard dose of cyclophosphamide in conjunction with doxorubicin a small number of cases of secondary acute myeloid leukemia occurred within two years of treatment initiation. Urinary bladder malignancies generally have occurred in patients who previously had hemorrhagic cystitis. In patients treated with cyclophosphamide-containing regimens for a variety of solid tumors, isolated case reports of secondary malignancies have been published. One case of carcinoma of the renal pelvis was reported in a patient receiving long-term cyclophosphamide therapy for cerebral vasculitis. The possibility of cyclophosphamide-induced malignancy should be considered in any benefit-to-risk assessment for use of the drug.
Cyclophosphamide can cause fetal harm when administered to a pregnant woman and such abnormalities have been reported following cyclophosphamide therapy in pregnant women. Abnormalities were found in two infants and a six-month old fetus born to women treated with cyclophosphamide. Ectrodactylia was found in two of the three cases. Normal infants have also been born to women treated with cyclophosphamide during pregnancy, including the first trimester. If this drug is used during pregnancy, or if the patient becomes pregnant while taking (receiving) 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.
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.
Amenorrhea associated with decreased estrogen and increased gonadotropin secretion develops in a significant proportion of women treated with cyclophosphamide. Affected patients generally resume regular menses within a few months after cessation of therapy. Girls treated with cyclophosphamide during prepubescence 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 prepubescence has been reported. Girls treated with cyclophosphamide during prepubescence subsequently have conceived.
Men treated with cyclophosphamide may develop oligospermia or azoospermia associated with increased gonadotropin but normal testosterone secretion. Sexual potency and libido are unimpaired in these patients. Boys treated with cyclophosphamide during prepubescence 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. Men temporarily rendered sterile by cyclophosphamide have subsequently fathered normal children.

Urinary System
Hemorrhagic cystitis may develop in patients treated with cyclophosphamide. Rarely, this condition can be severe and even fatal. Fibrosis of the urinary bladder, sometimes extensive, also may develop with or without accompanying cystitis. Atypical urinary bladder epithelial cells may appear in the urine. These adverse effects appear to depend on the dose of cyclophosphamide and the duration of therapy. Such bladder injury is thought to be due to cyclophosphamide metabolites excreted in the urine. Forced fluid intake helps to assure an ample output of urine, necessitates frequent voiding, and reduces the time the drug remains in the bladder. This helps to prevent cystitis. Hematuria usually resolves in a few days after cyclophosphamide treatment is stopped, but it may persist. Medical and/or surgical supportive treatment may be required, rarely, to treat protracted cases of severe hemorrhagic cystitis. It is usually necessary to discontinue cyclophosphamide therapy in instances of severe hemorrhagic cystitis.

Cardiac Toxicity
Although a few instances of cardiac dysfunction have been reported following use of recommended doses of cyclophosphamide, no causal relationship has been established. Acute cardiac toxicity has been reported with doses as low as 2.4 g/m^2 to as high as 26 g/m^2, usually as a portion of an intensive antineoplastic multi-drug regimen or in conjunction with transplantation procedures. In a few instances with high doses of cyclophosphamide, severe, and sometimes fatal, congestive heart failure has occurred after the first cyclophosphamide dose. Histopathologic examination has primarily shown hemorrhagic myocarditis. Hemopericardium has occurred secondary to hemorrhagic myocarditis and myocardial necrosis. Pericarditis has been reported independent of any hemopericardium. No residual cardiac abnormalities, as evidenced by electrocardiogram or echocardiogram appear to be present in patients surviving episodes of apparent cardiac toxicity associated with high doses of cyclophosphamide.
Cyclophosphamide has been reported to potentiate doxorubicin-induced cardiotoxicity.

Infections
Treatment with cyclophosphamide may cause significant suppression of immune responses. Serious, sometimes fatal, infections may develop in severely immunosuppressed patients. Cyclophosphamide treatment may not be indicated, or should be interrupted, or the dose reduced, in patients who have or who develop viral, bacterial, fungal, protozoan, or helminthic infections.

Other
Anaphylactic reactions have been reported; death has also been reported in association with this event. Possible cross-sensitivity with other alkylating agents has been reported.

PRECAUTIONS

General
Special attention to the possible development of toxicity should be exercised in patients being treated with cyclophosphamide if any of the following conditions are present.
1. Leukopenia
2. Thrombocytopenia
3. Tumor cell infiltration of bone marrow
4. Previous X-ray therapy
5. Previous therapy with other cytotoxic agents
6. Impaired hepatic function
7. Impaired renal function

Laboratory Tests
During treatment, the patient's hematologic profile (particularly neutrophils and platelets) should be monitored regularly to determine the degree of hematopoietic suppression. Urine should also be examined regularly for red cells which may precede hemorrhagic cystitis.

Drug Interactions
The rate of metabolism and the leukopenic activity of cyclophosphamide reportedly are increased by chronic administration of high doses of phenobarbital.
The physician should be alert for possible combined drug actions, desirable or undesirable, involving cyclophosphamide even though cyclophosphamide has been used successfully concurrently with other drugs, including other cytotoxic drugs.
Cyclophosphamide treatment, which causes a marked and persistent inhibition of cholinesterase activity, potentiates the effect of succinylcholine chloride.
If a patient has been treated with cyclophosphamide within 10 days of general anesthesia, the anesthesiologist should be alerted.

Adrenalectomy
Since cyclophosphamide has been reported to be more toxic in adrenalectomized dogs, adjustment of the doses of both replacement steroids and cyclophosphamide may be necessary for the adrenalectomized patient.

Wound Healing
Cyclophosphamide may interfere with normal wound healing.

Carcinogenesis, Mutagenesis, and Impairment of Fertility
See WARNINGS section for information on carcinogenesis, mutagenesis, and impairment of fertility.

Pregnancy
Pregnancy Category D—See WARNINGS section.

Nursing Mothers
Cyclophosphamide is excreted in breast milk. Because of the potential for serious adverse reactions and the potential for tumorigenicity shown for cyclophosphamide in humans, 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.

Pediatric Use
The safety profile of cyclophosphamide in pediatric patients is similar to that of the adult population (see ADVERSE REACTIONS section).

Geriatric Use
Insufficient data from clinical studies of cyclophosphamide for malignant lymphoma, multiple myeloma, leukemia, mycosis fungoides, neuroblastoma, retinoblastoma, and breast carcinoma are available for patients 65 years of age and older to determine whether they respond differently than younger patients. In two clinical trials in which cyclophosphamide was compared with paclitaxel, each in combination with cisplatin, for the treatment of advanced ovarian carcinoma, 154 (28%) of 552 patients who received cyclophosphamide plus cisplatin were 65 years or older. Subset analyses (<65 versus >65 years) from these trials, published reports of clinical trials of cyclophosphamide-containing regimens in breast cancer and non-Hodgkin's lymphoma, and postmarketing experience suggest that elderly patients may be more susceptible to cyclophosphamide toxicities. In general, dose selection for an elderly patient should be cautious, usually starting at the low end of the dosing range and adjusting as necessary based on patient response (see DOSAGE AND ADMINISTRATION: Treatment of Malignant Diseases).

ADVERSE REACTIONS
Information on adverse reactions associated with the use of cyclophosphamide is arranged according to body system affected or type of reaction. The adverse reactions are listed in order of decreasing incidence. The most serious adverse reactions are described in the WARNINGS section.

Reproductive System
See WARNINGS section for information on impairment of fertility.

Digestive System
Nausea and vomiting commonly 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. These adverse drug effects generally remit when cyclophosphamide treatment is stopped.

Skin and Its Structures
Alopecia occurs commonly in patients treated with cyclophosphamide. The hair can be expected to grow back after treatment with the drug or even during continued drug treatment, though it may be different in texture or color. Skin rash occurs occasionally in patients receiving the drug. Pigmentation of the skin and changes in nails can occur. Very rare reports of Stevens-Johnson syndrome and toxic epidermal necrolysis have been received during postmarketing surveillance; due to the nature of spontaneous adverse event reporting, a definitive causal relationship to cyclophosphamide has not been established.

Hematopoietic System
Leukopenia occurs in patients treated with cyclophosphamide, is related to the dose of drug, and can be used as a dosage guide. Leukopenia of less than 2000 cells/mm3 develops commonly in patients treated with an initial loading dose of the drug, and less frequently in patients maintained on smaller doses. 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.
Thrombocytopenia or anemia develop occasionally in patients treated with cyclophosphamide. These hematologic effects usually can be reversed by reducing the drug dose or by interrupting treatment. Recovery from leukopenia usually begins in 7 to 10 days after cessation of therapy.

Urinary System
See WARNINGS section for information on cystitis and urinary bladder fibrosis.
Hemorrhagic ureteritis and renal tubular necrosis have been reported to occur in patients treated with cyclophosphamide. Such lesions usually resolve following cessation of therapy.

Infections
See WARNINGS section for information on reduced host resistance to infections.

Carcinogenesis
See WARNINGS section for information on carcinogenesis.

Respiratory System
Interstitial pneumonitis has been reported as part of the postmarketing experience. Interstitial pulmonary fibrosis has been reported in patients receiving high doses of cyclophosphamide over a prolonged period.

Other
Anaphylactic reactions have been reported; death has also been reported in association with this event. Possible cross-sensitivity with other alkylating agents has been reported. SIADH (syndrome of inappropriate ADH secretion) has been reported with the use of cyclophosphamide. Malaise and asthenia have been reported as part of the postmarketing experience.

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.

DOSAGE AND ADMINISTRATION

Treatment of Malignant Diseases Adults and Children
When used as the only oncolytic drug therapy, the initial course of cyclophosphamide for patients with no hematologic deficiency usually consists of 40 to 50 mg/kg given intravenously in divided doses over a period of 2 to 5 days. Other intravenous regimens include 10 to 15 mg/kg given every 7 to 10 days or 3 to 5 mg/kg twice weekly.
Oral cyclophosphamide dosing is usually in the range of 1 to 5 mg/kg/day for both initial and maintenance dosing.
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. Transient decreases in the total white blood cell count to 2000 cells/mm^3 (following short courses) or more persistent reduction to 3000 cells/mm^3 (with continuing therapy) are tolerated without serious risk of infection if there is no marked granulocytopenia.
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.
Cyclophosphamide and its metabolites are dialyzable although there are probably quantitative differences depending upon the dialysis system being used. Patients with compromised renal function may show some measurable changes in pharmacokinetic parameters of cyclophosphamide metabolism, but there is no consistent evidence indicating a need for cyclophosphamide dosage modification in patients with renal function impairment.

Treatment of Nonmalignant Diseases Biopsy Proven "Minimal Change'' Nephrotic Syndrome in Children
An oral dose of 2.5 to 3 mg/kg daily for a period of 60 to 90 days is recommended. In males, the incidence of oligospermia and azoospermia increases if the duration of cyclophosphamide treatment exceeds 60 days. Treatment beyond 90 days increases the probability of sterility. Adrenocorticosteroid therapy may be tapered and discontinued during the course of cyclophosphamide therapy. See PRECAUTIONS section concerning hematologic monitoring.

Preparation and Handling of Solutions
Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration, whenever solution and container permit.
Cyclophosphamide should be prepared for parenteral use by adding 0.9% sterile sodium chloride solution if injected directly.
Cyclophosphamide should be prepared for parenteral use by infusion by adding Sterile Water for Injection, USP. Cyclophosphamide, constituted in water, is hypotonic and should not be injected directly. Add the diluent to the vial and shake it vigorously to dissolve. If the powder fails to dissolve immediately and completely, it is advisable to allow the vial to stand for a few minutes. Use the quantity of diluent shown below to constitute the product:

Dosage Strength	Cyclophosphamide for Injection Contains Cyclophosphamide Monohydrate	Quantity of Diluent
500 mg	534.5 mg	25 mL
1 g	1069.0 mg	50 mL
2 g	2138.0 mg	100 mL

Solutions of cyclophosphamide may be injected intravenously, intramuscularly, intraperitoneally, or intrapleurally if constituted by adding 0.9% sodium chloride solution, or they may be infused intravenously in the following:
Dextrose Injection, USP (5% dextrose)
Dextrose and Sodium Chloride Injection, USP (5% dextrose and 0.9% sodium chloride)
5% Dextrose and Ringer's Injection
Lactated Ringer's Injection, USP
Sodium Chloride Injection, USP (0.45% sodium chloride)
Sodium Lactate Injection, USP (1/6 molar sodium lactate)
Constituted cyclophosphamide is chemically and physically stable for 24 hours at room temperature or for six days in the refrigerator; it does not contain any antimicrobial preservative and thus care must be taken to assure the sterility of prepared solutions.
The osmolarities of solutions of cyclophosphamide constituted with water and 0.9% sodium chloride solution are found in the following table:

Cyclophosphamide and Diluent	mOsm/L
5 mL water per 100 mg cyclophosphamide (anhydrous)	74
5 mL 0.9% sodium chloride solution per 100 mg cyclophosphamide (anhydrous)	374

Isotonic 0.9% sodium chloride solution has an osmolarity of 289 mOsm/L.
Cyclophosphamide solution in water is hypotonic.
Extemporaneous liquid preparations of cyclophosphamide for oral administration may be prepared by dissolving cyclophosphamide in Aromatic Elixir, N.F.& Such preparations should be stored under refrigeration in glass containers and used within 14 days.

HOW SUPPLIED
Cyclophosphamide for Injection, USP contains cyclophosphamide monohydrate and is supplied in vials for single dose use.
NDC 10019-955-01 500 mg vial, carton of 1
NDC 10019-956-01 1.0 g vial, carton of 1
NDC 10019-957-01 2.0 g vial, carton of 1
Store vials at or below 25°C (77°F) [see USP Controlled Room Temperature]. During transport or storage of cyclophosphamide vials, temperature influences can lead to melting of the active ingredient, cyclophosphamide. Vials containing melted substance can be visually differentiated. Melted cyclophosphamide is a clear or yellowish viscous liquid usually found as a connected phase or in droplets in the affected vials. Do not use cyclophosphamide vials if there are signs of melting.
Procedures for proper handling and disposal of anticancer drugs should be considered. Several guidelines on this subject have been published.[1-8] There is no general agreement that all of the procedures recommended in the guidelines are necessary or appropriate.

REFERENCES
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4. National Study Commission on Cytotoxic Exposure—Recommendations for Handling Cytotoxic Agents. Available from Louis P. Jeffrey, Sc.D., Chairman, National Study Commission on Cytotoxic Exposure, Massachusetts College of Pharmacy and Allied Health Sciences, 179 Longwood Avenue, Boston, Massachusetts 02115.

5. Clinical Oncological Society of Australia. Guidelines and Recommendations for Safe Handling of Antineoplastic Agents. Med J Australia 1983; 1:426-428.

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7. American Society of Hospital Pharmacists Technical Assistance Bulletin on Handling Cytotoxic and Hazardous Drugs. Am J Hosp Pharm 1990; 47:1033-1049.

8. Controlling Occupational Exposure to Hazardous Drugs. (OSHA WORK PRACTICE GUIDELINES). Am J Health Syst Pharm 1996; 53:1669-1685.

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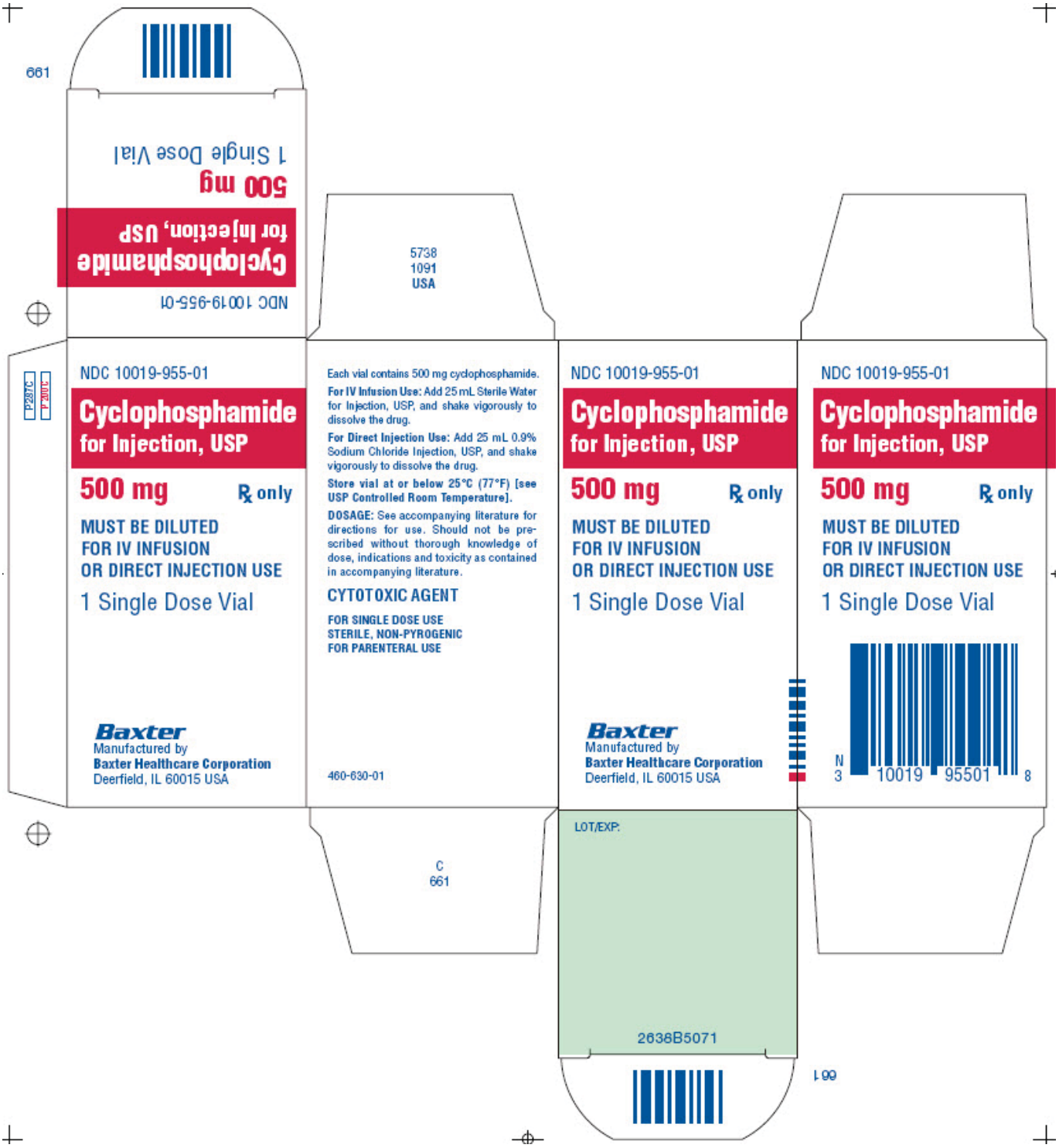
PACKAGE LABEL - PRINCIPAL DISPLAY PANEL

Container Label

NDC 10019-955-50
Cyclophosphamide
for Injection, USP
500 mg
CYTOTOXIC AGENT
FOR SINGLE DOSE USE
STERILE, NON-PYROGENIC
FOR PARENTERAL USE
Rx only
Baxter
Manufactured by **Baxter Healthcare Corporation**
Deerfield, IL 60015 USA
460-629-01

Carton Label



NDC 10019-955-01
Cyclophosphamide
for Injection, USP
500 mg
Rx only
MUST BE DILUTED
FOR IV INFUSION
OR DIRECT INJECTION USE
1 Single Dose Vial

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