CLINICAL STUDY PROTOCOL X14024

ALISERTIB (MLN8237)

Phase 2 trial of alisertib (MLN8237) in salvage malignant mesothelioma

Protocol Number:	X14024
Indication:	Malignant Mesothelioma
Phase:	2
Sponsor:	MD Anderson Cancer Center
Study Supporter:	Millennium Pharmaceuticals, Inc.
Therapeutic Area:	Oncology

Protocol History

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PROTOCOL SUMMARY

Study Title: Phase 2 trial of alisertib in salvage malignant mesothelioma
Phase: 2

Objectives
The primary objective of this study is:

- To assess 4-month disease control rate (DCR) in pre-treated patients with unresectable malignant pleural mesothelioma (MPM) treated with alisertib

The secondary objectives of this study are:

- To assess the response rate (confirmed and unconfirmed complete + partial responses)
- To assess the progression-free survival.
- To assess overall survival.
- To evaluate the side effects and toxicities associated with this treatment regimen.
- To collect archival tissue, blood, pleural effusion fluid and plasma for correlative studies.

The Exploratory Objectives of this study are:

- To collect archival or new tissue, blood and pleural effusion fluid for correlative studies. Tissue biomarkers to be evaluated include aurora kinase pathway and c-myc gene amplification
- Next generation sequencing (NGC) will be conducted on adequate tumor tissue specimens.

Patient Population
Pre-treated Malignant Mesothelioma patients with any histology and any primary tumor site. Patients can have up to 4 prior lines of therapy. Patients must have received at least 1 platinum-pemetrexed or platinum-doublet or pemetrexed-based chemotherapy.

Number of Patients
53 eligible patients, 58 maximum patients (assumes 5 inevaluable patients)

Study Design and Methodology
This is single-arm phase II trial in pre-treated malignant mesothelioma patients who will receive oral alisertib monotherapy. 1 cycle is 21 days. Alisertib will be given 50 mg po BID on days 1-7. A treatment break will occur on days 8-21.

Patients will be radiographically evaluated every 2 cycles of therapy for efficacy.
Patients will remain on trial until unacceptable toxicity, withdrawal of consent, or disease progression.

Duration of Study
3.2 years

Treatments Administered
Alisertib will be administered PO at 50 mg BID for 7 days in each treatment cycle, followed by a 14-day, treatment-free period, for a 21-day cycle.

Efficacy Data Collected
The following evaluations will be conducted to assess the efficacy of alisertib:

- Radiographic imaging (PET-CT, chest CT, or abdominal-pelvic CT scans)
- Tumor measurements by modified RECIST (preferred) or RECIST (if modified RECIST is not possible)

The following specimens will be collected at baseline for storage for subsequent studies.

- Baseline tissue specimen block (archived tissue block is acceptable)
- Baseline tumor tissue biopsy if a baseline tissue specimen block is not available. (at least 10 unstained slides or a tumor block are requested)
- Baseline blood specimen

Safety Data Collected
The following evaluations will be conducted to assess the safety of alisertib:

- Laboratory values (standard of care hematologic, liver and renal function, electrolytes)
- History and physical exam during each clinic visit
- Toxicity will be evaluated according to the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE), Version 4.03, effective14 June 2010 [(16)]. These criteria are provided in the Study Manual and are available online at http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm

Statistical Procedures
The primary objective of this study is to assess the efficacy of alisertib in pretreated patients with unresectable malignant mesothelioma (pleural or peritoneal). The primary endpoint is the disease control rate at 4 months in patients with unresectable malignant pleural mesothelioma (MPM) treated with alisertib. The trial will be conducted by the Simon's minimax two-stage design and the disease control rate at 4 months will be estimated accordingly.[2]

It is assumed that the new regimen will have a target disease control rate of 50% at 4 months. A disease control rate of 30% or lower is considered a failure and the new regimen will be rejected under this circumstance. When the probability of accepting a "bad" regimen (i.e. disease control rate<=30%) is 0.05 and the probability of rejecting a "good" regimen

(i.e. disease control rate >=50%) is 0.10, Simon's minimax design requires to enter 24 patients in the first stage. If 7 or less patients are alive and free of disease progression to the treatment at 4 months, the trial will be stopped and the regimen will be declared as ineffective. If there are 8 or more patients are alive and progression-free at 4 months, 29 more patients will be entered in the study to reach a total of 53 patients. By the end of the study, the new regimen will be rejected if disease control rate is less than or equal to 21/53 and will be accepted otherwise. The operating characteristics of the trial are given as follows. When the true disease control rate is 0.30 the probability of stopping the trial early is 57%. On the other hand, if the true disease control rate is 0.50, the probability to stop the trial early is 3%. The expected sample sizes are 36.6 and 52.1 when the true response rates are 0.3 and 0.5, respectively.

This design has the minimax property of minimizing the maximum sample size under null hypothesis. If the number of disease control required for moving the trial to the second stage has not been achieved, the patient enrollment will be halted until enough responses observed.

Analysis of efficacy results will be conducted using standard statistical methods. Descriptive statistics including mean, standard deviation, median, and range will be provided for continuous variables such as age. Frequency tables will be used to summarize categorical variables such as gender, and response. The standard error and confidence interval on estimating the disease control rate will be provided. The distribution of time-to-event endpoints, such as time to progression and overall survival, will be estimated using the method of Kaplan and Meier. Comparison of time-to-event endpoints by important patient characteristics will be made using the log-rank test. Cox proportional hazard regression will be employed for multi-covariate analysis on time-to-event outcomes when appropriate.

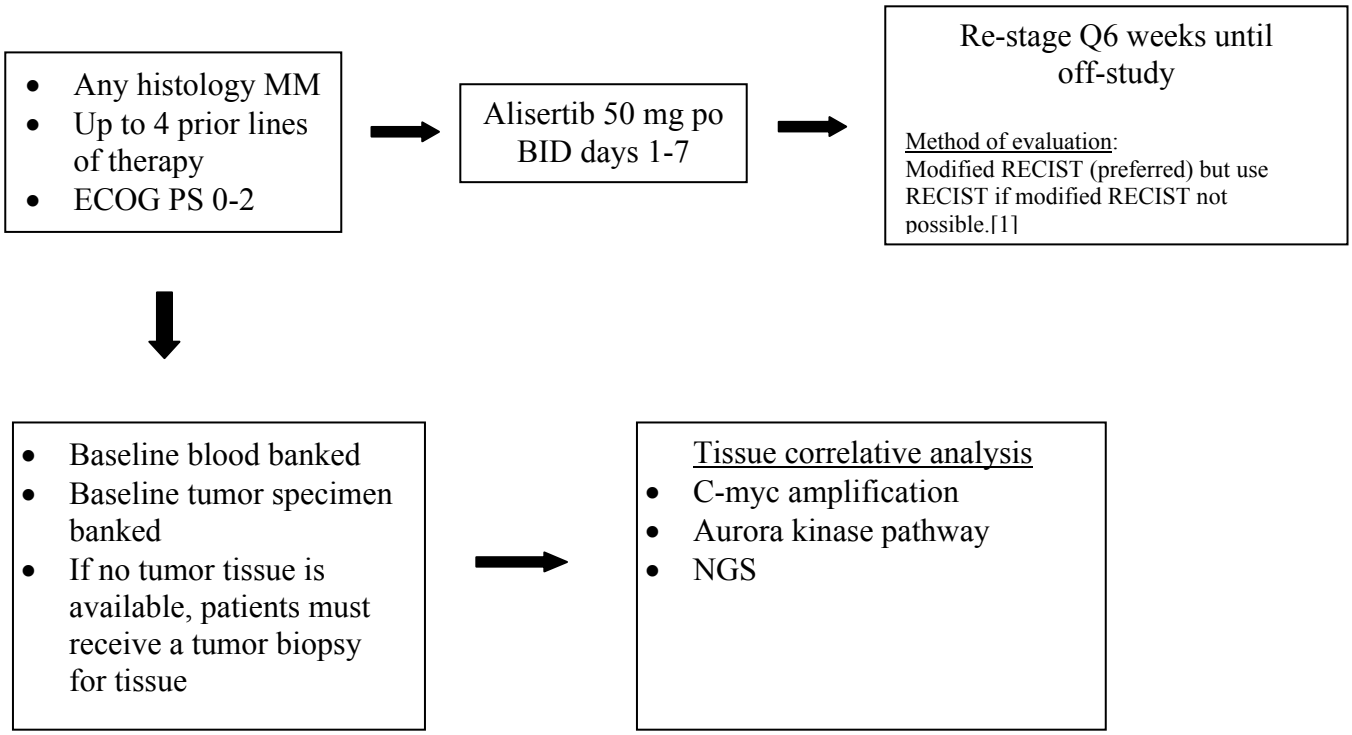
TABLE OF CONTENTS

(To update the TOC, right click it and choose **Update Field**. When prompted, choose **Update entire table,** and click **OK**.)

LIST OF IN-TEXT TABLES

(To update the List of Tables, right click it and choose **Update Field**. When prompted, choose **Update entire table**, and click **OK**.)

Study Flow Diagram



Schedule of Events

Required studies	Prestudy[a]	Cycle[a] 1		Cycle 2			Cycle 3		Cycle 4			Cycle 5		Cycle 6+ Restage every 2 cycles			Follow Up[d]
		D1	D 1-7	D1	D 1-7	W K 3	D1	D 1-7	D1	D 1-7	W K 3	D1	D 1-7	D1	D 1-7	WK 3	
History and physical exam	X	X		X			X					X					
Weight and PS	X	X		X			X					X					
Concurrent medications	X	X		X			X					X					
Toxicity evaluation	X	X		X			X					X				X	X[d]
Electrocardiogram	X																
LABORATORY																	
CBC/diff/platelets	X	X		X			X					X					
Urine Analysis	X																
PT, PTT	X																
Bilirubin, AST, ALT	X	X		X			X					X					
Electrolytes[b], Creatinine Clearance	X	X		X			X					X					
Serum βHCG Pregnancy Test	X																
SCANS																	
Imaging/Restaging scans	X					X					X					X	

SPECIMENS																	
Tumor Tissue Collection[e]	X																
Blood for banking[c] (optional)	X						X										
Pleural Effusion Fluid[f] (optional)																	
TREATMENT																	
Alisertib			X		X			X		X			X		X		
FOLLOW UP																	
Chart review or phone call																	X[d]

a. There is a 14-day window for baseline and ±3 days for clinic visits at every study cycle.
b. Electrolytes panel includes Carbon Dioxide, Chloride, Potassium, and Sodium
c. 24mL of blood will be collected each time at baseline, cycle 3 day 1 and at progression.
d: Toxicities and survival follow-up will be done through chart review or phone call 30 days post treatment, at 3 months and every 6 months thereafter.
e: Leftover tissue collected on study will be stored in a research bank, if allowed.
f: Pleural effusion fluid will be collected whenever available.

LIST OF ABBREVIATIONS

(Update with additional abbreviations/remove un-used abbreviations as necessary.)

Abbreviation	Definition
°C	degrees Celsius
μM	micromolar
20S	20S proteasome subunit
AE	adverse event
ANC	absolute neutrophil count
Bc1-2	B-cell lymphoma-2; a gene that inhibits apoptosis
BSA	body surface area
CAM	cell adhesion molecules
cm	centimeter
CR	complete response
CTCAE	(NCI) Common Terminology Criteria for Adverse Events
CTEP	Cancer Therapy Evaluation Program
dL	deciliter
DLT	dose-limiting toxicity
DNA	deoxyribonucleic acid
ECT	enteric-coated tablet
FDA	Food and Drug Administration
GCP	Good Clinical Practice
GLP	Good Laboratory Practice
ht	height
IκB	I kappa B kinase; cytokine response kinase that activates transcription factor NF-kappa b at serine 32 and 36
ICAM-1	intercellular adhesion molecule 1
ICH	International Conference on Harmonisation
IEC	independent ethics committee
IND	Investigational New Drug
IRB	institutional review board
IV	intravenous
IκBα	I kappa B alpha-associated protein kinase
kg	kilogram
Ki	inhibitory constant
lbs	pounds

Abbreviation	Definition
m^2	square meters
mg	milligram
min	minute
mL	milliliter
mm^3	cubic millimeters
mmol	millimole
MTD	maximum tolerated dose
NCI	National Cancer Institute
NF-κB	nuclear factor-κB
ng	nanogram
nM	Nanomole
OS	oral solution
p21	p21(ras) farnesyl-protein transferase
p27	cyclin-dependent kinase inhibitor
p53	tumor suppressor protein with molecular weight of 53 kDa
PIC	powder-in-capsule
SAE	serious adverse event
US	United States
USP	United States Pharmacopeia
VCAM-1	vascular cell adhesion molecule 1
w/w	weight-to-weight ratio
wt	Weight

1. INTRODUCTION AND STUDY RATIONALE

1.1 Overview of the Disease

Unresectable malignant mesothelioma is a challenging disease that has typically had poor responses to chemotherapy. Response rates to systemic chemotherapy have ranged from 3% to 30%.[3] In the front-line setting, the Evaluation of Mesothelioma in a Phase III study of Pemetrexed with Cisplatin randomized 456 patients to single agent cisplatin or the combination of cisplatin and pemetrexed. This study reported improvement in median overall survival (12.1 months vs. 9.3 months; p=0.02).[4] There are currently no Food and Drug Administration (FDA) approved agents in the salvage setting for mesothelioma. Novel agents for refractory patients are desperately needed to improve the therapeutic landscape.

1.1.1 Aurora A Kinases and the Aurora A Kinase Inhibitor Alisertib (MLN8237)

Alisertib (International Proprietary Name, also known as MLN8237) is a selective small molecule inhibitor of Aurora A kinase that is being developed for the treatment of advanced malignancies. Aurora A kinase belongs to a highly conserved family of serine/threonine protein kinases that also includes Aurora B and Aurora C. Aurora A and Aurora B are expressed in all actively dividing cells, while Aurora C expression is largely restricted to dividing germ cells.[5] Aurora A localizes to centrosomes and the proximal mitotic spindle during mitosis where it functions in a diverse set of mitotic processes.

The evidence supporting Aurora A kinase as a therapeutic target for the treatment of malignancies comes from several sources. First, the Aurora A kinase gene is amplified, overexpressed, or both in many tumors, including colon, breast, pancreatic, and bladder cancers, as well as certain lymphomas, leukemias, and myeloma.[3, 6-9] Aurora A overexpression in human cancers has been correlated with increased aneuploidy and centrosome amplification.[10] Second, forced overexpression of Aurora A kinase in experimental models results in the transformation of normal cells, suggesting that Aurora A overexpression may be oncogenic.[6] Lastly, in a number of different experimental systems, Aurora A inhibition leads to mitotic delays and severe chromosome alignment and segregation defects, followed by cell death.[11-14] Overall, the essential role of Aurora A in mitotic progression and its dysregulation in certain cancers makes it an attractive therapeutic target.

Given the obligatory role of mitosis in tumor proliferation, an Aurora A inhibitor would be expected to have potential applications across a broad range of human tumors. Indeed, alisertib has demonstrated activity against a variety of nonclinical solid tumor and

hematological malignancy models grown in vitro and in vivo, as described below. Alisertib is also expected to be toxic to proliferating normal tissues, such as the bone marrow, gastrointestinal (GI) epithelium, and hair follicles because any cell that is in mitosis, where Aurora A is expressed and active, should be susceptible to the effects of an Aurora A kinase inhibitor.

1.2 Preclinical Experience with Alisertib

1.2.1 In Vitro Studies

Alisertib is an ATP-competitive and reversible inhibitor of Aurora A kinase in vitro with an inhibition constant (Ki) of 0.43 nM. The data from both enzymatic and cell-based assays demonstrated that alisertib is a selective and potent inhibitor of Aurora A kinase. Alisertib inhibited proliferation of a wide variety of tumor cell lines grown in culture. Moreover, treatment of tumor cell lines with alisertib induced phenotypes consistent with Aurora A kinase inhibition, including mitotic spindle defects, mitotic delay, and apoptosis.[3, 6-9] For further details, refer to the Investigators' Brochure (IB).

1.2.2 In Vivo Studies

Alisertib demonstrated antitumor activity when administered orally on a daily basis for approximately 21 days (maximal tumor growth inhibition [TGI] > 90%) in several experimental human solid and hematologic tumor models grown as xenografts in immunocompromised mice. The maximally efficacious dose (ED) for each model varied: between 10 and 30 mg/kg if given once daily (QD) and 20 mg/kg if given twice daily (BID). Studies in the HCT-116 colon tumor model showed that less frequent dosing (eg, 5 days on followed by 5 days off) was also efficacious, demonstrating that continuous dosing is not necessary for antitumor activity. A single oral dose of alisertib given to nude mice bearing subcutaneous HCT-116 human colon tumors resulted in inhibition of activated Aurora A kinase and an increase in mitotic cells. Therefore, mitotic index (MI) can be used as a pharmacodynamic marker of alisertib in some in vivo settings. The relationship between pharmacokinetics (PK), pharmacodynamics, and efficacy was further studied in HCT-116 xenografts using oral dosing and subcutaneous osmotic mini-pumps. Both a pharmacodynamic response and efficacy (antitumor activity) were achieved using either route of administration. The data from these studies suggest that the maximum pharmacodynamic effect (mitotic accumulation) and efficacy are achieved at steady state plasma concentrations of 1-μM. Moreover, the maximally efficacious oral doses of alisertib in the HCT-116 model (30 mg/kg QD) resulted in plasma concentrations of 1 μM for 8 to 12

hours postdose. Plasma concentrations of alisertib associated with saturating levels of pharmacodynamic and antitumor activity (1 μM) were exceeded at the recommended phase 2 dose (RP2D) of alisertib in patients (50 mg BID).To determine whether alisertib would enhance the antitumor effects of standard of care agents in solid and hematologic malignancies, nonclinical combination studies were performed. Combination therapy with alisertib and docetaxel resulted in additive or synergistic effects during the dosing period, with prolonged tumor growth delay in multiple solid tumor xenograft models after terminating treatment. These effects were also observed in alternative intermittent dosing schedules. In DLBCL xenograft models, combination therapy with alisertib and rituximab resulted in synergistic, additive, or subadditive effects depending on the dose and model; however, prolonged tumor growth delays were observed in every case after terminating treatment, and in some cases complete cures were maintained.

1.2.3 Safety Pharmacology, Toxicology, and Drug Metabolism

Safety pharmacology studies with alisertib did not identify significant adverse effects in nonclinical studies, including in the central nervous system (CNS) and cardiovascular systems. No alisertib-related effects on clinical signs or physical examination findings indicative of impaired respiratory function (ie, labored or shallow breathing), or microscopic changes in the lungs of animals that survived until scheduled termination, were noted at tolerated doses in Good Laboratory Practice (GLP)-compliant, repeat-dose, toxicology studies. Alisertib exhibited minimal activity against the rapidly activating component of I_{Kr}, which is encoded by hERG (IC_{50} and Ki > 100 μM). Alisertib had in vitro activity against the GABAAα1 benzodiazepine binding site (Ki = 290 nM).

The dose-limiting toxicities (DLTs) for alisertib in both rats and dogs after repeat daily oral dosing for 2 cycles (each cycle consisted of 7 consecutive days separated by a 14-day dose holiday) or for 6 cycles (each cycle consisted of 21 consecutive days of dosing separated by a 7-day dose holiday) were consistent with inhibition of Aurora A kinase by alisertib. Principal findings in toxicology studies in rats and dogs included gastrointestinal (GI) signs, panleukopenia, decreased reticulocyte counts, and increased mitotic figures and apoptosis (single-cell necrosis) in tissues with a high basal cellular replication rate. These findings are indicative of toxicity to rapidly replicating cell populations and are consistent with the outcomes associated with Aurora A kinase inhibition. No off-target effects were seen in the GLP-compliant toxicology studies.Alisertib was negative in the bacterial reverse mutation assay (Ames assay) both in the absence and presence of Aroclor™ 1254-induced rat liver S9

fractions. In a rat bone marrow micronucleus assay, alisertib was considered to be equivocal for clastogenicity.

Alisertib is metabolized by multiple phase I (cytochrome P450 [CYP]3A4, CYP2C9, CYP2C19, and CYP1A2) and phase II (uridine diphosphate glucuronosyltransferase [UGT] 1A1, 1A3, and 1A8) enzymes. Using human liver microsomes with the appropriate cofactors, the percent contribution of CYP and UGT was calculated to be 13.1% and 86.9%, respectively, showing that CYP isozymes play a minor role in the metabolism of MLN8237. MLN8237 is unlikely to inhibit the 5 major CYP enzymes, 1A2, 2C9, 2C19, 2D6, and 3A4/5 (IC_{50} > 100 μM) when administered at the projected human efficacious dose. MLN8237 is not a mechanism-based inhibitor of CYP3A4/5. Alisertib inhibited the P-glycoprotein (Pgp)-mediated efflux of paclitaxel (Taxol®) in Caco 2 cells with an IC_{50} of 4.0 μM.

Detailed information regarding the nonclinical pharmacology and toxicology of alisertib may be found in the IB.

1.3 Clinical Experience

As of 29 March 2012, the following company-sponsored alisertib studies were in progress or completed: 6 single-agent phase 1 studies, 3 single-agent phase 2 studies, 1 single-agent phase 1/2 study, and 3 combination studies.

Alisertib for clinical studies is being developed in 2 dosage formulations: Enteric-coated-tablet (ECT) and oral solution (OS).

The dose-escalation, phase 1 study, C14007, evaluated multiple dose levels from 10 to 60 mg BID for 7 days in repeat, 21-day cycles and 50 mg BID has been determined to be the MTD.

Alisertib is structurally related to the benzodiazepines (BZD) (eg, diazepam, lorazepam) and also has activity against the GABAAα1 BZD receptor. BZD-like effects (eg, somnolence, confusion, memory loss) have been observed to be associated with the onset of maximal plasma concentration (eg, T_{max} [time to maximum plasma concentration]). CNS effects associated with peak plasma levels have been generally managed by administration of divided doses (eg, BID administration), although dose reductions have sometimes been required. While CNS effects attributed to alisertib were also generally reversible and manageable by dose delay or reduction, the causal relationship, and thus optimal approach to

management, were sometimes confounded by diverse causes including, but not limited to, concomitant medications (eg, narcotic analgesics, antianxiety medications), comorbidities (eg, infection, anemia, electrolyte abnormalities), or progressive malignancy (eg, brain metastases).

The clinical experience with alisertib includes treatment with multiple doses and schedules and is summarized in the IB.

1.4 Pharmacokinetics

Upon oral administration to patients with advanced nonhematologic malignancies, absorption of alisertib was fast, with peak plasma concentrations generally achieved by 3 hours postdose. Negligible urinary excretion of alisertib was observed in humans. The renal clearance of alisertib in humans was less than 0.1% of apparent oral clearance. Steady-state plasma exposures of alisertib increased in an approximately dose-proportional manner over the range of 5 to 200 mg/day in patients with advanced solid tumors. Overall mean steady-state terminal half-life following multiple-dose administration in patients with nonhematologic malignancies was approximately 22 hours. The overall mean peak/trough ratios were 2.6 and 5.0 for BID and QD dosing, respectively. The overall mean accumulation ratios were 2.8 and 1.9 for BID and QD dosing, respectively. Pharmacokinetic steady-state conditions were approximately achieved by Day 7 following daily oral administration. The PK properties of alisertib in patients with hematologic malignancies were generally consistent with those observed in patients with nonhematologic malignancies. Based on PK and genotype data in patients with nonhematologic malignancies, there was a substantial overlap in exposures (dose-normalized steady-state area under the plasma concentration versus time curve [AUC]) of alisertib in patients with 0, 1, or 2 copies of the UGT1A1 *28 allele, indicating the lack of readily apparent effects of UGT1A1 genotype on alisertib systemic exposure.

Clinical pharmacokinetic data available as of 20 April 2012 are summarized in the IB. Upon oral administration to patients with advanced nonhematologic malignancies, absorption of alisertib was fast, with peak plasma concentrations generally achieved by 2 hours post dose. Negligible urinary excretion of alisertib was observed in humans. The renal clearance of alisertib in humans was less than 0.1% of apparent oral clearance. Steady-state plasma exposures of alisertib increased in an approximately dose proportional manner over the range of 5 to 200 mg/day in patients with advanced solid tumors. Overall mean steady-state terminal half-life following multiple-dose administration in patients with nonhematologic

malignancies was approximately 22 hours. The overall mean peak/trough ratios were 2.6 and 5.0 for BID and QD dosing, respectively. The overall mean accumulation ratios were 2.8 and 1.9 for BID and QD dosing, respectively. Pharmacokinetic steady-state conditions were approximately achieved by Day 7 following daily oral administration.

Based on the results of a population PK analysis in 294 adult cancer patients, the apparent oral clearance of alisertib CL/F was unaffected by age, body weight, BSA, or the UGT1A1 genotype (number of *28 alleles). These results support the use of a common fixed starting dose of alisertib independent of UGT1A1 genotype status, age or body size in the adult patient population, in the ongoing and planned clinical trials.

The absolute bioavailability of alisertib in humans has not been determined; however, the single-dose pharmacokinetics of a prototype oral solution formulation of alisertib (25-mg dose) were characterized in a cross-over relative bioavailability evaluation in Study C14010 in 15 patients with nonhematologic malignancies.

The effect of a standardized high-fat meal on the PK of single-dose alisertib administered as a 50-mg strength was evaluated in 14 patients with advanced solid tumors. The lack of an effect of food on alisertib AUC_{inf} observed in this study supports the conclusion of the lack of a clinically meaningful effect of food on the PK of alisertib. The results of this study, therefore, support a recommendation that alisertib may be dosed without regard to the timing of meals in future clinical studies, unless otherwise specified in the clinical study protocol.

1.5 Potential Risks and Benefits

Seven-hundred fourteen patients (excluding 13 patients from a company-sponsored, non-US IND study in Japan) have been treated with alisertib as of 29 March 2012. Clinical safety data includes experience from patients who received multiple cycles followed by treatment-free periods between each cycle, and from patients who reduced or discontinued treatment. Based on the available clinical data, drug abuse, dependency, and drug withdrawal effects were not observed.

To date, the observed risks associated with alisertib treatment, as detailed in the Safety Management Attachment of the IB, include: (1) reversible myelosuppression including leukopenia, neutropenia, febrile neutropenia, lymphopenia, thrombocytopenia, and anemia; (2) GI toxicity including stomatitis/mucositis/oral pain, nausea, vomiting, anorexia, abdominal pain, dyspepsia, diarrhea, and dehydration; (3) sedation, somnolence, confusional

state, disorientation (and associated memory loss), and gait disturbances; (4) alopecia; (5) asthenia/fatigue; (6) fever, (7) infection, (8) abnormal liver function tests (including aspartate transaminase [AST], alanine transaminase [ALT], bilirubin, alkaline phosphatase [ALP], and gamma glutamyl transferase [GGT]), and (9) rash, which may include bullous dermatitis, and palmar-plantar erythrodysaesthesia syndrome.While these toxicities are potentially associated with risk or discomfort to the patient, they are anticipated to be reversible.

To mitigate the inherent risks in clinical studies of alisertib, patients are evaluated frequently while they are receiving treatment.

Because alisertib inhibits Aurora A kinase, it is possible that alisertib may interfere with cancer growth and cause cancer cell death. Preclinical results indicate that alisertib is not a major substrate for efflux mechanisms that have been associated with cross-resistance between some types of anticancer agents. Thus, alisertib has potential through a potentially non-cross resistant pathway as compared to other agents the patients may have received. The clinical utility of these effects will be investigated in current and future studies.

This study will be conducted in compliance with the protocol, applicable regulatory requirements, and International Conference on Harmonisation (ICH) Good Clinical Practice (GCP), guidelines.

1.6 Study rationale and selection of drug doses

Study Rationale

There are 3 subtypes of aurora kinases (A, B, C), but only aurora kinase A and B are potential therapeutic targets. Aurora kinase A inhibition leads to mitotic delay, monopolar spindles, and chromosomal segregation errors whereas aurora kinase B inhibition causes dysfunctional cytokinesis, polyploidy, and apoptosis. Preclinical studies have strongly suggested aurora kinases to be a relevant therapeutic target in mesothelioma patients. Several independent groups have reported that aurora kinase gene expression is upregulated in mesothelioma tumor tissue and is a negative prognostic factor.[15, 16](also our group – publication pending). Crispi et al.[17] have also shown that inhibition of mesothelioma cell lines with an aurora kinase inhibitor ZM447439 led to cell growth arrest and was target specific. Kim et al. also reported on irradiated mesothelioma cells and how inhibition of surviving and aurora kinase B resulted in mitotic cell arrest.

There are several aurora kinase inhibitors under investigation. For aurora kinase A inhibitors, alisertib, (MLN8237, Millenium) are under investigation in phase I and II trials. Preliminary results suggest dose limiting toxicity to be myelosuppression and mucositis. Aurora kinase B inhibitors, GSK1070916A (GlaxoSmithKline) and AZD1152 (AstraZeneca) are also under investigation in phase I and II trials in solid tumors and AML.

Several additional phase I and II trials of pan-aurora kinase inhibitor trials are underway in solid tumors. Alisertib (International Proprietary Name, also known as MLN8237) is a selective small molecule inhibitor of Aurora A kinase that is being developed for the treatment of advanced malignancies.

To date, there are no aurora kinase inhibitor-specific mesothelioma trials and the proposed study would be the first to assess this class of drug in the disease. Based on the preclinical data reported in the literature and our own profiling studies, we believe that aurora kinase is a feasible and relevant target in mesothelioma therapeutics. Alisertib is a novel compound that has a reasonable safety profile and may show efficacy in this population of patients.

1.6.1. Selection of Drug Dosages

The alisertib dose employed in this study will be 50 mg po BID (enteric coated tablet ECT) for 7 days then a 2 week treatment free period for a 21-day cycle. BID doses should be taken at least over 6 hours apart. This phase II dosing was established from phase I studies detailed in Section 1.3.

2. STUDY OBJECTIVES

2.1 Primary Objective

The primary objective of this study is to:

- To assess the 4-month disease control rate (DCR) in pre-treated patients with unresectable malignant pleural mesothelioma (MPM) treated with alisertib.

2.2 Secondary Objectives

The secondary objectives of this study are:

- To assess the response rate (confirmed and unconfirmed complete + partial responses)
- To assess the progression-free survival
- To assess overall survival.
- To evaluate the side effects and toxicities associated with this treatment regimen.
- To collect archival tissue, blood, pleural effusion fluid and plasma for correlative studies.

2.3 Exploratory Objectives

- To collect archival or new tissue, blood and pleural effusion fluid for correlative studies. Tissue biomarkers to be evaluated include aurora kinase pathway and c-myc gene amplification.

- Next generation sequencing (NGS) will be conducted on adequate tumor tissue specimens.

3. INVESTIGATIONAL PLAN

This is an investigator-initiated study. The principal investigator, Dr. Anne Tsao, is conducting the study.

3.1 Overall Design and Plan of the Study

This is a standard single-arm, single institution phase II trial that will enroll patients with malignant mesothelioma and treat them with alisertib monotherapy. The patients will be monitored for efficacy by either modified RECIST (preferred) or RECIST criteria (if modified RECIST cannot be performed) every 6 weeks. Patients with disease progression by either modified RECIST or RECIST criteria will be taken off active treatment from the trial and begin long-term follow-up.

The primary endpoint is 4-month disease-control rate. Secondary and exploratory endpoints include response rates, progression-free survival, overall survival, safety/toxicity profile, and collection of archival or fresh tissue and blood for correlative studies (See Section 5.6).

The alisertib dose employed in this study will be 50 mg po BID (enteric coated tablet ECT) for 7 days then a 2 week treatment free period for a 21-day cycle. BID doses should be taken at least over 6 hours apart.

Enrollment is defined as the first day of alisertib treatment (ie, Day 1 of Cycle 1).

Evaluable patients are defined as completing at least 1 cycle of alisertib treatment and having a documented radiographic response.

Radiographic assessments will occur every 6 weeks using modified RECIST (preferred) or RECIST (if modified RECIST is not feasible).

3.2 Selection of Patients

The total number of patients to be enrolled on this study is 58 subjects for 53 evaluable patients.

3.2.1 Inclusion Criteria

Each patient must meet all of the following inclusion criteria to be enrolled in the study:

- Voluntary written informed consent before performance of any study-related procedure not part of normal medical care, with the understanding that consent may be withdrawn by the subject at any time without prejudice to future medical care.

- Female subject is either:

    - post-menopausal for at least one year before the screening visit, or

    - surgically sterilized, or

    - willing to use an acceptable method of birth control (ie, a hormonal contraceptive, intra-uterine device, diaphragm with spermicide, condom with spermicide, or abstinence) for the duration of the study and at least 1 month after the last dose of alisertib.

- Male subject, even if surgically sterilized (ie, status postvasectomy), agrees to use an acceptable barrier method for contraception (condom with a spermicidal agent), or completely abstain from heterosexual intercourse during the entire study treatment period through 4 months after the last dose of alisertib.

- Absolute neutrophil count (ANC) > 1500/mm^3, platelets > 100,000/mm^3, Hgb > 9 g/dL. Total bilirubin ≤ 1.5 x upper limit of nomal (ULN), SGOT (AST) and SGPT (ALT)< 2.5 x ULN. AST and/or ALT may be up to 5X ULN if with known liver mets

- Adequate renal function as defined by: Calculated creatinine clearance must be ≥ 30 mL/minute(see Cockcroft-Gault formula in Section 9.5)

- Eastern Cooperative Oncology Group (ECOG) performance status 0-2 (refer to Section 9.4)
- Pathologic diagnosis of malignant mesothelioma (any primary site is acceptable)

- Have unresectable malignant mesothelioma (any histology)

- Received at least one prior pemetrexed-based chemotherapy for unresectable disease, unless within 3 months of receiving platinum-pemetrexed therapy for neoadjuvant or adjuvant treatment that has been unsuccessful.

- Up to 4 prior lines of systemic therapy (biologic or chemotherapy) are allowed. Maintenance therapy after 4-6 cycles of front-line chemotherapy is still considered 1 line of therapy and is not considered 2 separate therapies.

- Patients must have measurable disease by modified RECIST or RECIST. Examinations for assessment of measurable disease must have been completed within 28 days prior to registration.

- Patient must be >/= 18 years of age

3.2.2 Exclusion Criteria

Patients meeting any of the following exclusion criteria are not to be enrolled in the study.

- Radiation therapy to more than 25% of the bone marrow. Whole pelvic radiation is considered to be over 25%.

- Prior allogeneic bone marrow or organ transplantation

- Known GI disease or GI procedures that could interfere with the oral absorption or tolerance of alisertib. Examples include, but are not limited to partial gastrectomy, history of small intestine surgery with significant removel of the small intestine, and celiac disease

- Known history of *uncontrolled* sleep apnea syndrome and other conditions that could result in excessive daytime sleepiness, such as severe chronic obstructive pulmonary disease. Patients who use CPAP or BIPAP at night and have controlled sleep apnea syndrome are allowed.

- Requirement for constant administration of proton pump inhibitor, H2 antagonist, or pancreatic enzymes. Intermittent uses of antacids or H2 antagonists are allowed as described in Section 3.4.

- Systemic infection requiring IV antibiotic therapy within 14 days preceding the first dose of study drug, or other severe infection.

- Myocardial infarction within 6 months prior to enrollment or has New York Heart Association (NYHA) Class III or IV heart failure (see Section 9.3), uncontrolled angina, severe uncontrolled ventricular arrhythmias, or electrocardiographic

evidence of acute ischemia or active conduction system abnormalities. Prior to study entry, any ECG abnormality at Screening has to be documented by the investigator as not medically relevant.

- Female subject who is pregnant or breast-feeding. Confirmation that the subject is not pregnant must be established by a negative serum Beta-human chorionic gonadotropin (Beta-hCG) pregnancy test result obtained during screening. Pregnancy testing is not required for post-menopausal or surgically sterilized women.

- Patient has received other investigational drugs with 14 days before enrollment

- Serious medical or psychiatric illness likely to interfere with participation in this clinical study.

- Other severe acute or chronic medical or psychiatric condition, including uncontrolled diabetes, malabsorption, resection of the pancreas or upper small bowel, requirement for pancreatic enzymes, any condition that would modify small bowel absorption of oral medications, or laboratory abnormality that may increase the risk associated with study participation or investigational product administration or may interfere with the interpretation of study results and, in the judgment of the investigator, would make the patient inappropriate for enrollment in this study.

- Diagnosed or treated for another malignancy within 3 years of enrollment, with the exception of complete resection of basal cell carcinoma or squamous cell carcinoma of the skin, an in situ malignancy, or low-risk prostate cancer after curative therapy.

- Treatment with clinically significant enzyme inducers, such as the enzyme-inducing antiepileptic drugs phenytoin, carbamazepine or phenobarbital, or rifampin, rifabutin, rifapentine or St. John's wort within 14 days prior to the first dose of alisertib and during the study.

- Known history of human immunodeficiency virus (HIV) infection, hepatitis B, or hepatitis C. Testing is not required in the absence of clinical findings or suspicion. For guidance in defining active infection for hepatitis B, please refer to the WHO guidelines. (World Health Organization, Global Alert and Response

(GAR), Hepatitis B. who.int/csr/disease/hepatitis/whocdscsrlyo20022/en/index4.html)

- Prior administration of an Aurora A kinase-targeted agent, including alisertib
- Receipt of corticosteroids within 7 days prior to the first dose of study treatment, unless patient has been taking a continuous dose of no more than 15 mg/day of prednisone for at least 1 month prior to first dose of study treatment. Low dose steroid use for the control of nausea and vomiting will be allowed. Topical steroid use is permitted. Inhaled steroids are permitted.
- Inability to swallow oral medication or inability or unwillingness to comply with the administration requirements related to alisertib.
- Administration of myeloid growth factors or platelet transfusion within 14 days prior to the first dose of study treatment.
- Persons who are incarcerated at time of enrollment (e.g., prisoners) or likely to become incarcerated during the study.

3.3 Study Treatments

Alisertib drug product is supplied as the ECT dosage form in 10 mg strength, with dose strength expressed as the milligrams of active drug (free acid). The key formulation excipients of the alisertib tablet formulation that aid in the in vivo absorption of the drug are the buffer (sodium bicarbonate), the surfactant (sodium lauryl sulfate), and the enteric coating.

3.3.1 Preparation, Handling, and Storage of Drugs

Alisertib ECT are packaged in a 60-cc high-density polyethylene (HDPE) bottle with a rayon coil, induction seal, desiccant packs, and a polypropylene child-resistant cap.

Alisertib is an anticancer drug, and as with other potentially toxic compounds, caution should be exercised when handling MLN8237.

Drug Administration and Dosage Schedule

The alisertib dose employed in this study will be 50 mg po BID (enteric coated tablet ECT) for 7 days then a 2 week treatment free period for a 21-day cycle. This phase II dosing was established from phase I studies detailed in Section 1.3.

Patients will be instructed to take each oral dose of alisertib with 8 ounces (1 cup, 240 mL) of water. For BID dosing, the doses must be taken at least 6 hours apart.

Patients will be treated until disease progression by modified RECIST or RECIST criteria, withdrawal of consent, or unacceptable side effects.

Alisertib Administration

Alisertib will be given PO in a dosage of 50 mg BID for 7 Days (Days 1-7) of each 21-day treatment cycle.

Alisertib will be supplied as 10 mg ECT, with the dose strength expressed as milligrams of active drug (free acid). All tablets are to be ingested whole; patients who have difficulty swallowing tablets will be excluded from the study. Antiemetogenic agents may be administered at the discretion of the investigator.

Neutralizing antacids and calcium-containing supplements cannot be taken from 2 hours prior to alisertib dosing until up to 2 hours after dosing

Although not prohibited, the use of benzodiazepines for the prophylaxis or treatment of nausea or vomiting is discouraged because of the potential benzodiazepine-like effects of alisertib.

Study drug will be administered only to eligible patients under the supervision of the investigator or identified subinvestigator(s).

Accountability and Mechanism of Drug Destruction

The principal investigator and/or the designated study personnel is responsible for maintaining accurate dispensing records of the study drug. All study drugs must be accounted for, including study drug accidentally or deliberately destroyed. All discrepancies between amounts of study drug dispensed and amounts returned must be documented. Accountability records will include dates, quantities, lot numbers, expiration dates and patient numbers. Under no circumstances will the site principal investigator allow the investigational drug to be used other than as directed by the protocol without prior Millennium approval. If appropriate, drug storage, drug dispensing, and drug accountability may be delegated to the pharmacy section of the investigative site.

Documentation indicating study drug was destroyed will be sent to Millennium.

3.3.2 Dose Modification and Delay

Toxicity will be evaluated according to the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE), Version 4.03, effective 14 June 2010 [16]. These criteria are provided in the Study Manual and are available online at http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm

To manage excessive toxicity, reduction of the total alisertib dose can be done by reducing the daily dose administered and/or by interruption of the schedule treatment within a cycle.

3.3.3 Criteria for Retreatment and Dose Delays

Treatment with alisertib will be repeated every 21 days. In order for a new cycle of therapy to begin, the patient must meet the following criteria:

- ANC must be $\geq 1{,}500/mm^3$
- Platelet count must be $\geq 75{,}000/mm^3$.

If the patient fails to meet the above-cited criteria for retreatment, then initiation of the next cycle of therapy should be delayed for up to 1 week. At the end of that week, the patient should be re-evaluated to determine whether the criteria for retreatment have been met. Should treatment need to be delayed for more than 1 week (ie, a rest period of more than 21 days) because of incomplete recovery from treatment-related toxicity, the dose of alisertib will be reduced (Table 3-1~~Table 3-1~~) to 40 mg BID (Dose Level -1) when therapy resumes. A second dose reduction to 30 mg BID (Dose Level -2) may occur should treatment need to be delayed for more than 1 week because of incomplete recovery from treatment-related toxicity on the reduced dosage of 40 mg BID. Patients who require further dose reductions (Dose Level -3) will be taken off active treatment from the study and begin long-term follow-up. Should treatment need to be delayed for more than 2 weeks at any dose, therapy with alisertib will be discontinued.

Table 3-1 Table of Dose Adjustments

Dose Level	Dose	Schedule	Cycle Length
1	50 mg	PO BID day 1-7	21 days
-1	40 mg	PO BID day 1-7	21 days

-2	30 mg	PO BID day 1-7	21 days
-3		Discontinue	

Level 1 is the starting dose.

3.3.4 Dose Modifications for Hematological Toxicity

If a patient experiences any of the following hematological toxicities during the dosing period, dosing will be discontinued for the remainder of that cycle and the dose will be decreased 1 level for all subsequent cycles of treatment.

- Grade 4 neutropenia (ANC < 500 cells/mm^3) lasting more than 7 consecutive days
- Grade 4 thrombocytopenia (platelet count < 25,000/µL) lasting more than 7 consecutive days
- Platelet count less than 10,000/µL at any time
- Grade 3 neutropenia with fever or infection, or both, where fever is defined as an oral temperature greater than 38.3°C
- Grade 3 thrombocytopenia with clinically significant bleeding

3.3.5 Dose Modifications for Non-Hematological Toxicities

If a patient experiences any of the following toxicities during the dosing period, dosing will be discontinued for the remainder of that cycle and the dose will be decreased 1 level for all subsequent cycles of treatment, and treatment may resume after drug-related toxicities have resolved to ≤ Grade 1 or to baseline.

- Any Grade 3 nonhematological toxicity that is considered by the investigator to be related to study drug other than:
    - Grade 3 or greater nausea or emesis, or both, that occurs in the absence of optimal antiemetic therapy (5-hydroxytryptamine 3 [5-HT3] serotonin receptor antagonist);
    - Grade 3 or greater diarrhea that occurs in the absence of optimal supportive therapy with loperamide or a comparable anti-diarrheal;
    - Grade 3 fatigue that lasts less than 1 week

- Grade 2 non-hematological toxicities that are considered by the investigator to be related to study drug and in the opinion of the investigator require dose reduction.

In general, study drug treatment should be discontinued if a patient experiences a Grade 4 toxicity. If, in the opinion of the investigator and Millennium it is in the patient's interest to continue therapy with alisertib, then after recovery from the toxicity or toxicities in question to ≤ Grade 1 or to baseline values, the dose of alisertib should be reduced by at least 1 dose level with subsequent cycles of therapy.

When a dose reduction of alisertib is required, no re-escalation of dose will be permitted. If a patient requires more than 2 dose reductions, therapy with alisertib will be discontinued.

3.3.6 Packaging and Labeling

The study drug, provided by Millennium, will be labeled and handled at the investigative site as open-label material; packaging labels will fulfill all requirements specified by governing regulations. Alisertib will be supplied as ECT in 10 mg strength. The 60-cc HDPE bottles will have a child-resistant cap and be labeled for take-home use. Patients will receive instructions for home use of alisertib, including the requirement that Alisertib be administered as intact tablets.

3.4 Excluded Concomitant Medications and Procedures

The following medications and procedures are prohibited during the study:

- Any antineoplastic therapy other than alisertib
- Alternative therapy, including palliative radiotherapy, for definitive treatment of the patient's malignancy
- Any investigational anti-cancer therapy other than alisertib.
- Requirement for administration of any proton pump inhibitor. Use of any PPI in either continued or intermittent use will be prohibited during the conduct of the study and patients must discontinue any use of PPI within five days prior to the first dose of alisertib. Patients may be administered alternative agents to manage gastric acidity or reflux (eg, H2 receptor antagonists, antacids) with exceptions described below.

- Histamine-2 (H2) receptor antagonists are not permitted from the day prior (Day -1) through to the end of alisertib dosing (e.g., Day 7), except as required for premedication for a protocol-specific agent (eg, taxane).

- Enzyme inducers, such as the enzyme-inducing antiepileptic drugs phenytoin, carbamazepine or phenobarbital, or rifampin, rifabutin, rifapentine or St. John's wort within 14 days prior to the first dose of alisertib.

3.5 Permitted Concomitant Medications and Procedures

Myeloid growth factors to treat patients with neutropenia according to the American Society of Clinical Oncology (ASCO) Guidelines.[1] When applicable (eg, Phase 1 studies) use of myeloid growth factors should be avoided (if medically appropriate) in Cycle 1 until patients have developed a DLT or dose-limiting Grade 4 neutropenia. Antiemetic agents may be administered at the discretion of the investigator but are not commonly required as a prophylactic agent. All other manifestations of the patient's malignancy should be treated at the discretion of the investigator.

Antacids are permitted; however, they should be administered more than 2 hours before or 2 hours after administration of alisertib.

Medications with potential CNS effects are not prohibited in this study, but it is recommended that their use be minimized to avoid confusion in the interpretation of CNS effects should they occur during the course of treatment with alisertib. Because of alisertib's structural and pharmacological similarity to the benzodiazepines, concomitant therapy with benzodiazepines is discouraged but not prohibited.

In appropriate settings, such as combinations with agents known to produce frequent thrombocytopenia, restricted uses of anticoagulants should be considered.

All other medical conditions should be treated at the discretion of the investigator in accordance with local community standards of medical care.

3.6 Precautions and Restrictions

Patients are to be instructed to limit the use of alcohol while enrolled in this study. Patients should consume no more than 1 standard unit of alcohol per day during the study and for 30 days from the last dose of alisertib. A standard unit of alcohol is defined as a 12 oz beer (350 mL), 1.5 oz (45 mL) of 80-proof alcohol, or one 6-oz (175 mL) glass of wine.

It is not known what effects alisertib has on human pregnancy or development of the embryo or fetus. Therefore, male patients should avoid impregnating a female partner.

Male patients, even if surgically sterilized (ie, status post-vasectomy) must agree to one of the following:

Practice effective barrier contraception during the entire study treatment period and through four months after the last dose of study drug, or completely abstain from heterosexual intercourse.

3.7 Management of Clinical Events

3.7.1 Nausea and Vomiting

Prophylactic antiemetic therapy will not be used in this study unless it becomes clear that alisertib causes acute nausea and vomiting. If prophylactic antiemetic therapy is needed, 5-HT_3 receptor antagonists (without corticosteroids) should be tried first. Because of the potential of benzodiazepines to cause sedation, the use of benzodiazepines for antiemetic prophylaxis should be reserved for patients who cannot be satisfactorily managed otherwise.

Although this study will not initially employ prophylactic antiemetics, there is no prohibition against antiemetic use in the management of a patient who develops nausea or vomiting, or both.

3.7.2 Diarrhea

Antidiarrheal medications will not be used prophylactically; however, patients will be instructed to take loperamide, 4 mg, at the occurrence of the first loose stool and then 2 mg every 2 hours until they are diarrhea-free for at least 12 hours. During the night, patients may take 4 mg of loperamide every 4 hours. Fluid intake should be maintained to avoid dehydration.

3.7.3 Central Nervous System Effects

If a patient experiences excessive sedation believed to be related to alisertib, treatment with alisertib should be interrupted. Patients whose sedation is not considered immediately life-threatening should be carefully monitored and given appropriate supportive care.

3.7.4 Treatment Compliance

All drug(s) will be administered to eligible patients under the supervision of the investigator or identified sub-investigator(s). The research staff will maintain records of drug receipt (if applicable), drug preparation, and dispensing, including the applicable lot numbers, patients' height, body weight, and body surface area (see Section 9.5), and total drug administered in milliliters and milligrams. Any discrepancy between the calculated dose and dose administered and the reason for the discrepancy must be recorded in the source documents.

3.8 Duration of Treatment and Patient Participation

Once patients register and enroll on this trial, they will be monitored per study guidelines while on treatment.

Patients enrolled and treated on this trial will continue with therapy until:

1) Unacceptable toxicities requiring removal from study
2) Disease progression defined by modified RECIST or RECIST
3) Patient withdraws consent
4) Treating physician removes patient from the trial for the reasons outlined in Section 3.9.

Once patients are taken off active treatment and begin long-term follow-up, they will be monitored for both first disease progression and overall survival.

3.9 Termination of Treatment and/or Study Participation

Patients will be informed that they have the right to withdraw from the study at any time for any reason, without prejudice to their medical care. The investigator also has the right to withdraw patients from the study for any of the following reasons:

- Intercurrent illness
- Occurrence of an unacceptable adverse event
- A treatment cycle delay or alisertib interruption of> 2 weeks because of toxicity
- Patient request

- Protocol violations
- Non-compliance
- Administrative reasons
- Failure to return for follow-up
- General or specific changes in the patient's condition unacceptable for further treatment in the judgment of the investigator
- Progressive disease at any time

At the time of withdrawal, all study procedures outlined for the End of Study visit should be completed. The primary reason for a patient's withdrawal from the study is to be recorded in the source documents.

3.10 Efficacy and Safety Measurements

3.10.1 Efficacy Measurements

Patients will be re-staged every 2 cycles of therapy (6 weeks). 1 cycle = 21 days or 3 weeks. Imaging studies will consist of any of the following studies: PET-CT scans, chest CT scans, abdominal-pelvic CT scans. Modified RECIST (preferred) or RECIST (if modified RECIST cannot be used) measurements will be performed after every 2 cycles of therapy. The radiologist or the PI will review and determine response based on the RECIST. Examples of these measurements are described in Tsao et al.[1]

3.10.2 Safety Measurements

Patients will be evaluated with a history and physical exam during each clinic visit. Baseline laboratory values will be obtained during each clinic visit. All toxicities reported will be recorded by the research RN and graded using the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE), Version 4.03, effective 14 June 2010 [(16)]. These criteria are provided in the Study Manual and are available online at http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

3.11 Study Drug Administration

Alisertib will be administered PO at 50 mg BID for 7 days in each treatment cycle, followed by a 14-day, treatment-free period.

Patients will be instructed to take each oral dose of alisertib with 8 ounces (1 cup, 240 mL) of water. For BID dosing, the doses must be taken at least 6 hours apart.

Alisertib will be supplied as 10 mg ECT, with the dose strength expressed as milligrams of active drug (free acid); higher strengths may be supplied depending on the observed MTD. All tablets are to be ingested whole; patients who have difficulty swallowing tablets will be excluded from the study. Antiemetogenic agents may be administered at the discretion of the investigator. Although not prohibited, the use of benzodiazepines for the prophylaxis or treatment of nausea or vomiting is discouraged because of the potential benzodiazepine-like effects of alisertib.

Study drug will be administered only to eligible patients under the supervision of the investigator or identified subinvestigator(s).

3.12 Description of Investigational Agents

Alisertib drug product is supplied as the ECT dosage form in 10 mg strengths with dose strength expressed as the milligrams of active drug (free acid); other strengths may be supplied based on the observed MTD. The key formulation excipients of the alisertib tablet formulation that aid in the in vivo absorption of the drug are the buffer (sodium bicarbonate), the surfactant (sodium lauryl sulfate), and the enteric coating.

3.13 Preparation, Reconstitution, and Dispensation

Alisertib ECT are packaged (10 tablets to a bottle) in a 60-cc high-density polyethylene (HDPE) bottle with a child-resistant cap.

Alisertib is an anticancer drug, and as with other potentially toxic compounds, caution should be exercised when handling alisertib. It is recommended that gloves and protective garments be worn during preparation.

3.14 Packaging and Labeling

The packaged and labeled study drug, alisertib ECT, will be provided by Millennium and will be handled at the investigative site as open-label material. The labels on the study drug will fulfill all requirements specified by governing regulations. Ten alisertib ECT are packaged into each 60-cc HDPE bottle. Alisertib will be supplied as ECT in 10 mg or 50 mg strengths. The bottles will have a child-resistant cap and be labeled for take-home use. Patients will receive instructions for home use of alisertib, including the requirement that alisertib be administered as intact tablets.

As required by local regulations, any modifications to the plan for drug supply or storage will be communicated to the investigator and detailed in the Study Manual.

3.15 Storage, Handling, and Accountability

Tablets should remain in the bottle provided until use. The container should be stored at the investigative site at controlled room temperature (20-25°C; 68-77°F; excursions are permitted from 15-30°C; 59-86°F) and used before the retest expiry date provided by Millennium. Containers should be kept closed during storage.

Because alisertib is an investigational agent, it should be handled with due care. In case of contact with broken tablets, raising dust should be avoided during the cleanup operation.

The product may be harmful by inhalation, ingestion, or skin absorption. Gloves and protective clothing should be worn during preparation and the cleanup operation. The area should be ventilated and the spill site washed after material pick-up is complete. The spilled material should be disposed of as hazardous medical waste in compliance with federal, state, and local regulations.

In case of contact with the powder (eg, from a broken tablet), skin should be washed immediately with soap and copious amounts of water for at least 15 minutes.

In case of contact with the eyes, copious amounts of water should be used to flush the eyes for at least 15 minutes. Medical personnel should be notified.

Patients are to be instructed on proper storage, accountability, and administration of alisertib, including that alisertib is to be taken as intact tablets.

4. ADVERSE EVENTS

4.1.1 Adverse Event Definition

Adverse event (AE) means any untoward medical occurrence in a patient or subject administered a pharmaceutical product; the untoward medical occurrence does not necessarily have a causal relationship with this treatment. An AE 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 it is related to the medicinal product. This includes any newly occurring event, or a previous condition that has increased in severity or frequency since the administration of study drug.

For this protocol an abnormal laboratory value will not be assessed as an AE unless that value leads to discontinuation or delay in treatment, dose modification, therapeutic intervention, or is considered by the investigator to be a clinically significant change from baseline.

Adverse events are documented in the Case Report Forms (CRF) and CORe is the database for AE collection. The PI and the designees will be responsible for assigning attribution to study drug, and how this will be documented.

4.1.2 Serious Adverse Event Definition

Serious adverse event (SAE) means any untoward medical occurrence that at any dose:

- Results in **death**.
- Is **life-threatening** (refers to an AE in which the patient was at risk of death at the time of the event. It does not refer to an event which hypothetically might have caused death if it were more severe).
- Requires **inpatient hospitalization or prolongation of an existing hospitalization** (see clarification below on planned hospitalizations in Section **Error! Reference source not found.**).
- Results in **persistent or significant disability or incapacity**. (Disability is defined as a substantial disruption of a person's ability to conduct normal life functions).

- Is a **congenital anomaly/birth defect**.

- Is a **medically important event**. This refers to an AE that may not result in death, be immediately life threatening, or require hospitalization, but may be considered serious when, based on appropriate medical judgment, may jeopardize the patient, require medical or surgical intervention to prevent 1 of the outcomes listed above, or involves suspected transmission via a medicinal product of an infectious agent. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse; any organism, virus, or infectious particle (eg, prion protein transmitting Transmissible Spongiform Encephalopathy), pathogenic or nonpathogenic, is considered an infectious agent. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse; any organism, virus, or infectious particle (eg, prion protein transmitting Transmissible Spongiform Encephalopathy), whether pathogenic or non-pathogenic, is considered an infectious agent.

- Clarification should be made between a serious AE (SAE) and an AE that is considered severe in intensity (Grade 3 or 4), because the terms serious and severe are NOT synonymous. The general term *severe* is often used to describe the intensity (severity) of a specific event; the event itself, however, may be of relatively minor medical significance (such as a Grade 3 headache). This is NOT the same as *serious*, which is based on patient/event outcome or action criteria described above, and is usually associated with events that pose a threat to a patient's life or ability to function. A severe AE (Grade 3 or 4) does not necessarily need to be considered serious. For example, a white blood cell count of 1000/mm^3 to less than 2000 is considered Grade 3 (severe) but may not be considered serious. Seriousness (not intensity) serves as a guide for defining regulatory reporting obligations.

4.1.3 Serious Adverse Event Reporting (SAE) Reporting

An adverse event or suspected adverse reaction is considered "serious" if, in the view of either the investigator or the sponsor, it results in any of the following outcomes:

- Death

- A life-threatening adverse drug experience – any adverse experience that places the patient, in the view of the initial reporter, at immediate risk of death from the adverse experience as it occurred. It does not include an adverse experience that, had it occurred in a more severe form, might have caused death.
- Inpatient hospitalization or prolongation of existing hospitalization
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions.
- A congenital anomaly/birth defect.

Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse drug experience when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse (21 CFR 312.32).

- Important medical events as defined above, may also be considered serious adverse events. Any important medical event can and should be reported as an SAE if deemed appropriate by the Principal Investigator or the IND Sponsor, IND Office.
- All events occurring during the conduct of a protocol and meeting the definition of a SAE must be reported to the IRB in accordance with the timeframes and procedures outlined in "The University of Texas M. D. Anderson Cancer Center Institutional Review Board Policy for Investigators on Reporting Serious Unanticipated Adverse Events for Drugs and Devices". Unless stated otherwise in the protocol, all SAEs, expected or unexpected, must be reported to the IND Office, regardless of attribution (within 5 working days of knowledge of the event).
- **All life-threatening or fatal events,** that are unexpected, and related to the study drug, must have a written report submitted within **24 hours** (next working day) of knowledge of the event to the Safety Project Manager in the IND Office.
- Unless otherwise noted, the electronic SAE application (eSAE) will be utilized for safety reporting to the IND Office and MDACC IRB.
- Serious adverse events will be captured from the time of the first protocol-specific intervention, until 30 days after the last dose of drug, unless the participant withdraws consent. Serious adverse events must be followed until clinical recovery is complete and laboratory tests have returned to baseline, progression of the event has stabilized, or there has been acceptable resolution of the event.
- Additionally, any serious adverse events that occur after the 30 day time period that are related to the study treatment must be reported to the IND Office. This may include the development of a secondary malignancy.

Reporting to FDA:

- Serious adverse events will be forwarded to FDA by the IND Sponsor (Safety Project Manager IND Office) according to 21 CFR 312.32.

It is the responsibility of the PI and the research team to ensure serious adverse events are reported according to the Code of Federal Regulations, Good Clinical Practices, the protocol guidelines, the sponsor's guidelines, and Institutional Review Board policy.

4.2 Communication between Investigator and Millennium

AEs may be spontaneously reported by the patient and/or in response to an open question from study personnel or revealed by observation, physical examination, or other diagnostic procedures. Any clinically relevant deterioration in laboratory assessments or other clinical finding is considered an AE. When possible, signs and symptoms indicating a common underlying pathology should be noted as one comprehensive event. AEs which are serious must be reported to Millennium Pharmacovigilance (or designee) from the first dose of alisertib up to and including 30 days after administration of the last dost of alisertib. Any SAE that occurs at any time after completion of alisertib treatment or after the designated follow-up period that the investigator and/or sub-investigator considers to be related to any study drug must be reported to Millennium Pharmacovigilance (or designee). Planned hospital admissions or surgical procedures for an illness or disease that existed before the patient was enrolled in the trial are not to be considered AEs unless the condition deteriorated in an unexpected manner during the trial (e.g., surgery was performed earlier or later than planned). All SAEs should be monitored until they are resolved or are clearly determined to be due to a patient's stable or chronic condition or intercurrent illness(es).

Since this is an investigator-initiated study, the principal investigator Dr. Anne Tsao, , is responsible for reporting serious adverse events (SAEs) to any regulatory agency and to the investigator's EC or IRB. Regardless of expectedness or causality, all SAEs must also be reported to Millennium Pharmacovigilance or designee as soon as possible, but no later than 4 calendar days of the investigator's observation or awareness of the event. See below for contact information for the reporting of SAEs to Millennium Pharmacovigilance.

The -investigator should fax the SAE Form within four calendar days after becoming aware of the event. A sample of an SAE Form will be provided. Follow-up information on the SAE may be requested by Millennium. The SAE report must include event term(s), serious criteria, and the investigator's or sub-investigator's determination of both the intensity of the event(s) and the relationship of the event(s) to study drug administration. Intensity for each SAE, including any lab abnormalities, will be determined by using the NCI CTCAE version used at your institution, as a guideline, whenever possible. The criteria are available online at http://ctep.cancer.gov/reporting/ctc.html.

In the event that this is a multisite study, the -investigator is responsible to ensure that the SAE reports are sent to Millennium Pharmacovigilance (or designee) from all sites participating in the study. Sub-investigators must report all SAEs to so that the principal investigator can meet his/her foregoing reporting obligations to the required regulatory agencies and to Millennium Pharmacovigilance, unless otherwise agreed between the principal investigator and sub-investigator(s).

Relationship to all study drugs for each SAE will be determined by the investigator or sub-investigator by responding yes or no to the question: Is there a reasonable possibility that the AE is associated with the study drug(s)?

The principal investigator must also provide Millennium Pharmacovigilance with a copy of all communications with applicable regulatory authorities related to the study or study drug(s), including, but not limited to, telephone conversation logs, as soon as possible but no later than 4 calendar days of such communication.

SAE and Pregnancy Reporting Contact Information

Millennium Pharmacovigilance
SAE and Pregnancy Reporting Contact Information:

Millennium Pharmacovigilance or Designee
SAE and Pregnancy Reporting Contact Information
FAX Number 1-800-963-6290
Email: TakedaOncoCases@cognizant.com

4.3 Procedures for Reporting Drug Exposure during Pregnancy and Birth Events

If a woman becomes pregnant or suspects that she is pregnant while participating in this study, she must inform the investigator immediately and must permanently discontinue study drug(s). The investigator must fax a completed Pregnancy Form to the Millennium Department of Pharmacovigilance or designee (or designee; see Section 4.2 for contact information) immediately. The pregnancy must be followed for the final pregnancy outcome (ie, delivery, still birth, miscarriage) and Millennium Pharmacovigilance will request this information from the investigator.

If a female partner of a male patient becomes pregnant during the male patient's participation in this study, this must be reported to Millennium Pharmacovigilance (or

designee) immediately (see Section 4.2 for contact information). Every effort should be made to follow the pregnancy for the final pregnancy outcome.

4.4 Product Complaints

A product complaint is a verbal, written, or electronic expression that implies dissatisfaction regarding the identity, strength, purity, quality, or stability of a drug product. Individuals who identify a potential product complaint situation should immediately contact MedComm Solutions (see below) and report the event. Whenever possible, the associated product should be maintained in accordance with the label instructions pending further guidance from a Millennium Quality representative.

For Product Complaints, call MedComm Solutions at 877-674-3784 (877 MPI DRUG) (US and International)

Product complaints in and of themselves are not AEs. If a product complaint results in an SAE, an SAE form should be completed and sent to Millennium Pharmacovigilance (refer to Section 8.2).

A product complaint is a verbal, written, or electronic expression that implies dissatisfaction regarding the identity, strength, purity, quality, or stability of a drug product. Individuals who identify a potential product complaint situation should immediately contact MedComm Solutions (see below) and report the event. Whenever possible, the associated product should be maintained in accordance with the label instructions pending further guidance from a Millennium Quality representative.

A medication error is a preventable event that involves an identifiable patient and that leads to inappropriate medication use, which may result in patient harm. While overdoses and underdoses constitute medication errors, doses missed inadvertently by a patient do not. Individuals who identify a potential medication error situation should immediately contact MedComm Solutions (see below) and report the event.

5. STATISTICAL PROCEDURES

5.1 Study Design and Overview of Primary and Secondary Endpoints

The primary objective of this study is to:

- Assess 4-month disease control rate (DCR) in pre-treated patients with unresectable MPM treated with alisertib

The secondary objectives of this study are to:

- To assess the response rate (confirmed and unconfirmed complete + partial responses)
- To assess the progression-free survival.
- To assess overall survival.
- To evaluate the side effects and toxicities associated with this treatment regimen.
- To collect archival tissue, blood, pleural effusion and plasma for correlative studies.

The primary endpoint is the disease control rate at 4 months in patients with unresectable malignant pleural mesothelioma (MPM) treated with alisertib. The trial will be conducted by the Simon's minimax two-stage design and the disease control rate at 4 months will be estimated accordingly (Simon, 1989)

It is assumed that the new regimen will have a target disease control rate of 50% at 4 months. A disease control rate of 30% or lower is considered a failure and the new regimen will be rejected under this circumstance. When the probability of accepting a "bad" regimen (i.e. disease control rate<=30%) is 0.05 and the probability of rejecting a "good" regimen (i.e. disease control rate >=50%) is 0.10, Simon's minimax design requires to enter 24 patients in the first stage. If 7 or less patients are free of disease progression to the treatment at 4 months, the trial will be stopped and the regimen will be declared as ineffective. If there are 8 or more patients are alive and progression-free at 4 months, 29 more patients will be entered in the study to reach a total of 53 patients. By the end of the study, the new regimen will be rejected if disease control rate is less than or equal to 21/53 and will be accepted otherwise. The operating characteristics of the trial are given as follows. When the true disease control rate is 0.30 the probability of stopping the trial early is 57%. On the other

hand, if the true disease control rate is 0.50, the probability to stop the trial early is 3%. The expected sample sizes are 36.6 and 52.1 when the true response rates are 0.3 and 0.5, respectively. This design has the minimax property of minimizing the maximum sample size under null hypothesis. If the number of disease control required for moving the trial to the second stage has not been achieved, the patient enrollment will be halted until enough responses observed.

Descriptive statistics including mean, standard deviation, median, and range will be provided for continuous variables such as age. Frequency tables will be used to summarize categorical variables such as gender, and response. The standard error and confidence interval on estimating the disease control rate will be provided. The distribution of time-to-event endpoints, such as time to progression and overall survival, will be estimated using the method of Kaplan and Meier. Comparison of time-to-event endpoints by important patient characteristics will be made using the log-rank test. Cox proportional hazard regression will be employed for multi-covariate analysis on time-to-event outcomes when appropriate.

5.2 Sample Size Estimation/Accrual Rate

It is anticipated to accrue 1-2 patients per month.

Sample size = 53 evaluable patients, 58 maximum patients (estimating 5 inevaluable patients)

5.3 Randomization and Stratification Factors

None.

5.4 Evaluation of Efficacy

Patients will be re-staged every 2 cycles of therapy. 1 cycle = 21 days or 3 weeks. Imaging studies will consist of *any* of the following studies: PET-CT scans, chest CT scans, abdominal-pelvic CT scans. Modified RECIST (preferred) or RECIST (if modified RECIST cannot be used) measurements will be performed after every 2 cycles of therapy. The radiologist or the PI will review and determine response based on the RECIST and the data will be documented in the Case Report Form (CRF). Examples of these measurements are described in Tsao et al.[1]

The primary objective of this study is to assess the efficacy of alisertib in pretreated patients with unresectable malignant mesothelioma (pleural or peritoneal). The primary endpoint is the disease control rate at 4 months in patients with unresectable malignant pleural mesothelioma (MPM) treated with alisertib. The trial will be conducted by the Simon's minimax two-stage design and the disease control rate at 4 months will be estimated accordingly (Simon, 1989).

Standard RECIST measurement definitions will be used.

Complete Response (CR): Complete disappearance of all measurable and non-measurable disease. No new lesions.

Partial Response (PR): Greater than or equal to 30% decrease under baseline of the sum of longest diameters of all target measurable lesions. No unequivocal progression or non-measurable disease. No new lesions.

Stable disease (SD): Does not qualify for CR or PR or Progression.

Progression (PD): One or more of the following must occur: 20% increase in the sum of longest diameters of target measurable lesions over smallest sum observed (over baseline if no decrease during therapy). Unequivocal progression of non-measurable disease in the opinion of the treating physician (an explanation must be provided). Appearance of any new lesion/site. Death due to disease without prior documentation of progression and without symptomatic deterioration.

5.5 Evaluation of Safety

Patients will be evaluated with a history and physical exam during each clinic visit. Baseline laboratory values will be obtained during each clinic visit. All toxicities reported will be recorded by the research RN and graded using the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE), Version 4.03, effective 14 June 2010 [16]. These criteria are provided in the Study Manual and are available online at http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

5.6 Correlative Studies

Blood Specimens will be collected at baseline (pretreatment), cycle 3 – day 1 and at progression and banked at MD Anderson Cancer for subsequent analysis. At each time

point, blood will be collected into three (3) x 8 mL EDTA Vaccutainer top tubes separated; plasma and buffy coat will be collected and stored at -70 until analysis.

Pleural Effusion Fluid: in cases when pleural effusion fluid is available, a 10 mL aliquot of the pleural effusion fluid will be collected in a sterile container, aliqouted and stored at -70C until analysis.

Patients must have either archived baseline tumor specimens available and adequate for biomarker analysis or must undergo a tumor tissue biopsy. Tumor specimens will be batched until the end of the clinical trial and subsequently evaluated for aurora kinase pathway molecules by immunohistochemistry, fluorescent in situ hybridization (FISH) for c-myc gene amplification, and next-generation sequencing. Correlation of biomarkers to patient demographics and clinical outcome will be analyzed by the below statistical analysis.

Descriptive statistics including mean, standard deviation, median, and range will be provided for continuous variables such as age. Frequency tables will be used to summarize categorical variables such as gender, and response. The standard error and confidence interval on estimating the disease control rate will be provided. The distribution of time-to-event endpoints, such as time to progression and overall survival, will be estimated using the method of Kaplan and Meier. Comparison of time-to-event endpoints by important patient characteristics will be made using the log-rank test. Cox proportional hazard regression will be employed for multi-covariate analysis on time-to-event outcomes when appropriate.

5.6.1. Tissue Biomarker Analysis

A total of 10 unstained slides or a tumor block will be requested from each patient. Archived baseline tissue blocks or unstained slides will be accepted. If no baseline tissue is available, then patients will be asked to undergo a tumor tissue biopsy. The tumor tissue will be subjected to immunohistochemistry analysis of aurora kinase pathway biomarkers and fluorescent in situ hybridization analysis of the copy numbers of the c-myc gene.

5.6.1.1. Immunohistochemistry

Standard immunohistochemistry (IHC) will be conducted on tumor tissue slides for aurora kinase pathway biomarkers. The precise antibodies are to be determined. A general methodology for standard IHC is described below.

We will perform standard IHC studies of aurora kinase pathway biomarkers and evaluate them in subcellular locations (cytoplasmic, stromal, membrane, and nuclear. The histology sections will be incubated with primary antibodies TBD for a predescribed amount of time at room temperature. Tissue sections will then be incubated with the secondary antibody (TBD) for 30 min, after which diaminobenzidine chromogen was applied for 5 min. Tumor malignant cells and stroma will be evaluated using the same methodology for protein expressions. Briefly, for each marker, two experienced thoracic pathologists (I.I.W. and J.F.) will examine both the intensity and extent of immunostaining by light microscopy using a ×20 magnification objective. Immunoreactivity of all antibodies will be detected in the cytoplasm of malignant cell and stroma fibroblast, and in the cytoplasm and nucleus. Cytoplasmic or nuclear expressions will be quantified using a four-value intensity score (0, none; 1+, weak; 2+, moderate; and 3+, strong) and the percentage (0-100%) of the extent of reactivity. A final expression score was obtained by multiplying the intensity and reactivity extension values (range, 0-300).

5.6.1.2. c-myc copy number analysis rationale for study

We[18] have previously evaluated potential therapeutic targets for malignant pleural mesothelioma (MPM) and one of the most common chromosomal amplification sites in cancer tissues is the 8q24 region which contains the genes *C-MYC* and *PVT1* [19-21]. *C-MYC* encodes a transcription factor that regulates the expression of multiple genes involved in cellular responses such as growth, proliferation, apoptosis, and differentiation [22, 23]. Deregulated amplification and expression of the *MYC* locus occurs in ~30% of human cancers, including colon, prostate and breast carcinomas, and has been associated with poor prognosis [19, 24, 25]. *PVT1* is a candidate oncogene located adjacent to the *MYC* locus on chromosomal region 8q24 [25-27]. *PVT1* has been shown to act as a non-coding RNA with many alternatively spliced isoforms [20]. The *PVT1* locus has recently been found to contain a cluster of at least six microRNAs (*miRNAs*) (*miR-1204*, *-1205*, *-1206*, *-1207-3p*, *-1207-5p*, and *-1208*) that span the *PVT1* region, adding further complexity to the locus [20]. *PVT1* copy number gains (CNGs) and *PVT1* overexpression both have been implicated in the pathophysiology of many tumors, including breast and ovarian cancers and acute myeloid leukemia [26, 28]. Additionally, *PVT1* alteration has been shown to contribute to tumor survival and chemoresistance [28, 29].

We sought to elucidate these roles and the specific mechanisms of action of *C-MYC* and *PVT1* involved in the pathogenesis of malignant pleural mesothelioma (MPM) by characterizing the molecular abnormalities found in the 8q24 locus in malignant pleural mesothelioma (MPM) cell lines and in specimens from surgically resected malignant pleural mesotheliomas. The *miRNA* (*miR-1204, -1205, 1206, -1207 3p, 1207-5p*, and *-1208*) expression in malignant pleural mesothelioma (MPM) cell lines was evaluated. We also determined the biological impact of siRNA-mediated *C-MYC* and *PVT1* abrogation on malignant pleural mesothelioma (MPM) cellular processes such as apoptosis, cell proliferation, and response to cisplatin and then determined the effect of *C-MYC*, *PVT1*, and *miR-1204 knockdown* on the expression levels of apoptosis related genes. Finally, we studied *C-MYC* and *PVT1* copy number and gene expression in malignant pleural mesothelioma (MPM) tumor specimens.[18]

Our copy number analysis revealed copy number gains (CNGs) in chromosomal region 8q24 in three of five malignant pleural mesothelioma (MPM) cell lines. MicroRNA analysis showed high *miR-1204* expression in MSTO-211H cell lines with ≥4 copies of *PVT1*. Knockdown by siRNA showed increased PARP-C levels in MSTO-211H transfected with *siPVT1* but not in cells transfected with si*C-MYC*. *C-MYC* and *PVT1* knockdown reduced cell proliferation and increased sensitivity to cisplatin. Analysis of the expression of apoptosis-related genes in the MSTO-211H cell line suggested that *C-MYC* maintains a balance between pro-apoptotic and anti-apoptotic gene expression, whereas *PVT1* and to a lesser extent *miR-1204*, upregulate pro-apoptotic genes and downregulate anti-apoptotic genes. FISH analysis of malignant pleural mesothelioma (MPM) tumor specimens showed a high frequency of both CNGs (11/75) and trisomy (three copies; 11/75) for the *C-MYC* locus.[18]

We additionally determined *C-MYC* and *PVT1* mRNA expression levels in a subset of 55 malignant pleural mesothelioma (MPM) tumor specimens paired with 41 normal tissues from our database using Affymetrix U133 plus 2.0 chips. We found significant differences in *C-MYC* and *PVT1* gene expression between the normal tissues and the tumor samples (Fig. 3F and 3G; $p < 0.05$).

Our preclinical results suggest that *C-MYC and PVT1* amplification promotes a malignant phenotype of malignant pleural mesothelioma (MPM), with *C-MYC* amplification stimulating cell proliferation and *PVT1* both stimulating proliferation and inhibiting apoptosis.

This is significantly relevant to this protocol as c-myc expression and pathway upregulation has been identified as a potential biomarker in aurora kinase inhibition in other tumor types (personal communication Millennium).

5.6.1.2.1. Methodology c-myc copy number analysis measured by quatitative PCR and fluorescence in situ hybridization (FISH)

Isolation of DNA and Copy Number Profiling
DNA was extracted from cell lines using DNAzol Reagent (Life Technologies, Grand Island, NY) and whole-genome single nucleotide polymorphism (SNP) array profiling can be performed using Affymetrix SNP 6.0 chips (Agilent Technologies, Santa Clara, CA). Copy number gains (CNGs) were identified using the SNP-Fast Adaptive States Segmentation Technique 2 algorithm in Nexus 5.1 software (BioDiscovery, Hawthorne, CA) with the significance threshold for segmentation setting at $p < 5 \times 10^{-7}$. CNGs were defined with log2 ratio values of 0.2, and two or more than two CNGs were defined by log2 ratio values of 0.7.

Copy Number Analysis
We used fluorescence in situ hybridization (FISH) and real-time quantitative PCR (q-PCR) to quantify 8q24 CNGs in malignant pleural mesothelioma (MPM) tumor specimens. We used directly labeled fluorescent chromosomal centromeric probes (CEP 8, SpectrumGreen) for chromosome 8 and locus-specific probes (LSI) for regions 8q24.12-q13 (*C-MYC* Spectrum Orange) (Vysis, Abbott Laboratories, Chicago, IL). Fluorescence in situ

hybridization (FISH) was performed according to the manufacturer's instructions. Copy number analysis was performed in 50 nuclei per tumor in at least four areas. Copy number alteration was defined as the presence of more than two gene copies per cell on average of the 50 cells. Trisomy was defined as the presence of three copy number alterations and CNG was defined the presence of at least four copies. To enrich for malignant cell content for q-PCR analysis, tumor tissues were manually microdissected for subsequent DNA extraction from FFPE tissue sections. Tumor DNA was extracted using the Pico Pure DNA Extraction Kit (Arcturus, Life Technologies, Grand Island, NY) according to the manufacturer's instructions. DNA samples with proportions of microdissected tumor cell greater than 70% were qualified for qPCR analysis. *MYC* and *PVT1* gene copy numbers were examined by q-PCR using the ABI 7300 real time PCR system (Applied Biosystems, Grand Island, NY). A gene copy number ≥4 was considered as CNG [30].

5.7 Interim Analysis

Interim analysis will be conducted as per the Simon's 2-stage design described in Section 5.1.

6. ADMINISTRATIVE REQUIREMENTS

6.1 Good Clinical Practice

The study will be conducted in accordance with the International Conference on Harmonisation (ICH) for Good Clinical Practice (GCP) and the appropriate regulatory requirement(s). The investigator will be thoroughly familiar with the appropriate use of the drug as described in the protocol and Investigator's Brochure. Essential clinical documents will be maintained to demonstrate the validity of the study and the integrity of the data collected. Master files should be established at the beginning of the study, maintained for the duration of the study and retained according to the appropriate regulations.

6.2 Ethical Considerations

The study will be conducted in accordance with ethical principles founded in the Declaration of Helsinki (see Section 9.1). The IRB/IEC will review all appropriate study documentation in order to safeguard the rights, safety and well-being of the patients. The study will only be conducted at sites where IRB/IEC approval has been obtained. The protocol, Investigator's Brochure, informed consent, advertisements (if applicable), written information given to the patients (including diary cards), safety updates, annual progress reports, and any revisions to these documents will be provided to the IRB/IEC by the investigator. Millennium requests that informed consent documents be reviewed by Millennium or designee prior to IRB/IEC submission.

6.3 Patient Information and Informed Consent

After the study has been fully explained, written informed consent will be obtained from either the patient or his/her guardian or legal representative prior to study participation. The method of obtaining and documenting the informed consent and the contents of the consent will comply with ICH-GCP and all applicable regulatory requirement(s).

6.4 Patient Confidentiality

For the purpose of this study at MD Anderson Cancer Center, all patients will be registered in the Clinical Oncology Research System (CORE). All study related data will be captured in the Protocol Data Management System (PDMS).In order to maintain patient privacy, all data capture records, drug accountability records, study reports and communications will identify the patient by initials and the assigned patient number. The investigator will grant monitor(s) and auditor(s) from Millennium or its designees and regulatory authority(ies) access to the patient's original medical records for verification of data gathered on the data capture records and to audit the data collection process. The patient's confidentiality will be maintained and will not be made publicly available to the extent permitted by the applicable laws and regulations.

6.5 Protocol Compliance

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

Any departures from the protocol must be fully documented in the source documents.

6.6 On-site Audits

Regulatory authorities, the IEC/IRB and/or Millennium's clinical quality assurance group may request access to all source documents, data capture records, and other study

documentation for on-site audit or inspection. Direct access to these documents must be guaranteed by the investigator, who must provide support at all times for these activities.

6.7 Drug Accountability

Accountability for alisertib at all study sites is the responsibility of the Principal Investigator. The investigator will ensure that the drug is used only in accordance with this protocol. Drug accountability records indicating the drug's delivery date to the site (if applicable), inventory at the site (if applicable), use by each patient, and return to Millennium or disposal of the drug (if applicable and if approved by Millennium) will be maintained by the clinical site. Accountability records will include dates, quantities, lot numbers, expiration dates (if applicable), and patient numbers.

6.8 Premature Closure of the Study

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

Circumstances that may warrant termination include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to patients
- Failure to enter patients at an acceptable rate
- Insufficient adherence to protocol requirements
- Insufficient complete and/or evaluable data
- Plans to modify, suspend or discontinue the development of the drug

Should the study be closed prematurely, all study materials must be returned to Millennium.

6.9 Record Retention

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

7. USE OF INFORMATION

All information regarding alisertib supplied by Millennium to the investigator is privileged and confidential information. The investigator may use this information to accomplish the study, but may not use it for other purposes without consent from Millennium. In accordance with the agreement between Millennium and the principal investigator complete study data must be provided to Millennium. Upon completion of the clinical study and evaluation of results by Millennium, the hospital or institution and/or investigator may publish or disclose the clinical trial results pursuant to the terms contained in the applicable Clinical Trial Agreement.

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9. APPENDICES

9.1 Declaration of Helsinki

World Medical Association Declaration of Helsinki:

Ethical Principles for Medical Research Involving Human Subjects

Adopted by the 18th WMA General Assembly Helsinki, Finland, June 1964 and amended by the 29th WMA General Assembly, Tokyo, Japan, October 1975 35th WMA General Assembly, Venice, Italy, October 1983 41st WMA General Assembly, Hong Kong, September 1989 48th WMA General Assembly, Somerset West, Republic of South Africa, October 1996 and the 52nd WMA General Assembly, Edinburgh, Scotland, October 2000

A. INTRODUCTION

1. The World Medical Association has developed the Declaration of Helsinki as a statement of ethical principles to provide guidance to physicians and other participants in medical research involving human subjects. Medical research involving human subjects includes research on identifiable human material or identifiable data.
2. It is the duty of the physician to promote and safeguard the health of the people. The physician's knowledge and conscience are dedicated to the fulfillment of this duty.
3. The Declaration of Geneva of the World Medical Association binds the physician with the words, "The health of my patient will be my first consideration," and the International Code of Medical Ethics declares that, "A physician shall act only in the patient's interest when providing medical care which might have the effect of weakening the physical and mental condition of the patient."
4. Medical progress is based on research which ultimately must rest in part on experimentation involving human subjects.
5. In medical research on human subjects, considerations related to the well-being of the human subject should take precedence over the interests of science and society.
6. The primary purpose of medical research involving human subjects is to improve prophylactic, diagnostic and therapeutic procedures and the understanding of the aetiology and pathogenesis of disease. Even the best proven prophylactic, diagnostic, and therapeutic methods must continuously be challenged through research for their effectiveness, efficiency, accessibility and quality.
7. In current medical practice and in medical research, most prophylactic, diagnostic and therapeutic procedures involve risks and burdens.
8. Medical research is subject to ethical standards that promote respect for all human beings and protect their health and rights. Some research populations are vulnerable and need special protection. The particular needs of the economically and medically disadvantaged must be recognized. Special attention is also required for those who cannot give or refuse consent for themselves, for those who may be subject to giving consent under duress, for those who will not benefit personally from the research and for those for whom the research is combined with care.

9. Research Investigators should be aware of the ethical, legal and regulatory requirements for research on human subjects in their own countries as well as applicable international requirements. No national ethical, legal or regulatory requirement should be allowed t reduce or eliminate any of the protections for human subjects set forth in this Declaration.

B. BASIC PRINCIPLES FOR ALL MEDICAL RESEARCH

1. It is the duty of the physician in medical research to protect the life, health, privacy, and dignity of the human subject.
2. Medical research involving human subjects must conform to generally accepted scientific principles, be based on a thorough knowledge of the scientific literature, other relevant sources of information, and on adequate laboratory and, where appropriate, animal experimentation.
3. Appropriate caution must be exercised in the conduct of research which may affect the environment, and the welfare of animals used for research must be respected.
4. The design and performance of each experimental procedure involving human subjects should be clearly formulated in an experimental protocol. This protocol should be submitted for consideration, comment, guidance, and where appropriate, approval to a specially appointed ethical review committee, which must be independent of the investigator, the sponsor or any other kind of undue influence. This independent committee should be in conformity with the laws and regulations of the country in which the research experiment is performed. The committee has the right to monitor ongoing trials. The researcher has the obligation to provide monitoring information to the committee, especially any serious adverse events. The researcher should also submit to the committee, for review, information regarding funding, sponsors, institutional affiliations, other potential conflicts of interest and incentives for subjects.
5. The research protocol should always contain a statement of the ethical considerations involved and should indicate that there is compliance with the principles enunciated in this Declaration.
6. Medical research involving human subjects should be conducted only by scientifically qualified persons and under the supervision of a clinically competent medical person. The responsibility for the human subject must always rest with a medically qualified person and never rest on the subject of the research, even though the subject has given consent.
7. Every medical research project involving human subjects should be preceded by careful assessment of predictable risks and burdens in comparison with foreseeable benefits to the subject or to others. This does not preclude the participation of healthy volunteers in medical research. The design of all studies should be publicly available.
8. Physicians should abstain from engaging in research projects involving human subjects unless they are confident that the risks involved have been adequately assessed and can be satisfactorily managed. Physicians should cease any investigation if the risks are found to outweigh the potential benefits or if there is conclusive proof of positive and beneficial results.
9. Medical research involving human subjects should only be conducted if the importance of the objective outweighs the inherent risks and burdens to the subject. This is especially important when the human subjects are healthy volunteers.
10. Medical research is only justified if there is a reasonable likelihood that the populations

in which the research is carried out stand to benefit from the results of the research.

11. The subjects must be volunteers and informed participants in the research project.
12. The right of research subjects to safeguard their integrity must always be respected. Every precaution should be taken to respect the privacy of the subject, the confidentiality of the patient's information and to minimize the impact of the study on the subject's physical and mental integrity and on the personality of the subject.
13. In any research on human beings, each potential subject must be adequately informed of the aims, methods, sources of funding, any possible conflicts of interest, institutional affiliations of the researcher, the anticipated benefits and potential risks of the study and the discomfort it may entail. The subject should be informed of the right to abstain from participation in the study or to withdraw consent to participate at any time without reprisal. After ensuring that the subject has understood the information, the physician should then obtain the subject's freely-given informed consent, preferably in writing. If the consent cannot be obtained in writing, the non-written consent must be formally documented and witnessed.
14. When obtaining informed consent for the research project the physician should be particularly cautious if the subject is in a dependent relationship with the physician or may consent under duress. In that case the informed consent should be obtained by a well-informed physician who is not engaged in the investigation and who is completely independent of this relationship.
15. For a research subject who is legally incompetent, physically or mentally incapable of giving consent or is a legally incompetent minor, the investigator must obtain informed consent from the legally authorized representative in accordance with applicable law. These groups should not be included in research unless the research is necessary to promote the health of the population represented and this research cannot instead be performed on legally competent persons.
16. When a subject deemed legally incompetent, such as a minor child, is able to give assent to decisions about participation in research, the investigator must obtain that assent in addition to the consent of the legally authorized representative.
17. Research on individuals from whom it is not possible to obtain consent, including proxy or advance consent, should be done only if the physical/mental condition that prevents obtaining informed consent is a necessary characteristic of the research population. The specific reasons for involving research subjects with a condition that renders them unable to give informed consent should be stated in the experimental protocol for consideration and approval of the review committee. The protocol should state that consent to remain in the research should be obtained as soon as possible from the individual or a legally authorized surrogate.
18. Both authors and publishers have ethical obligations. In publication of the results of research, the investigators are obliged to preserve the accuracy of the results. Negative as well as positive results should be published or otherwise publicly available. Sources of funding, institutional affiliations and any possible conflicts of interest should be declared in the publication. Reports of experimentation not in accordance with the principles laid down in this Declaration should not be accepted for publication.

C. ADDITIONAL PRINCIPLES FOR MEDICAL RESEARCH COMBINED WITH MEDICAL CARE

1. The physician may combine medical research with medical care, only to the extent that the research is justified by its potential prophylactic, diagnostic or therapeutic value. When medical research is combined with medical care, additional standards apply to protect the patients who are research subjects.
2. The benefits, risks, burdens and effectiveness of a new method should be tested against those of the best current prophylactic, diagnostic, and therapeutic methods. This does not exclude the use of placebo, or no treatment, in studies where no proven prophylactic, diagnostic or therapeutic method exists.
3. At the conclusion of the study, every patient entered into the study should be assured of access to the best proven prophylactic, diagnostic and therapeutic methods identified by the study.
4. The physician should fully inform the patient which aspects of the care are related to the research. The refusal of a patient to participate in a study must never interfere with the patient-physician relationship.
5. In the treatment of a patient, where proven prophylactic, diagnostic and therapeutic methods do not exist or have been ineffective, the physician, with informed consent from the patient, must be free to use unproven or new prophylactic, diagnostic and therapeutic measures, if in the physician's judgment it offers hope of saving life, re-establishing health or alleviating suffering. Where possible, these measures should be made the object of research, designed to evaluate their safety and efficacy. In all cases, new information should be recorded and, where appropriate, published. The other relevant guidelines of this Declaration should be followed.

9.2 Common Terminology Criteria for Adverse Events Version 4.03

http://ctep.cancer.gov/reporting/ctc.html

9.3 New York Heart Association Classification of Cardiac Disease

The following table presents the NYHA classification of cardiac disease.

Class	Functional Capacity	Objective Assessment
I	Patients with cardiac disease but without resulting limitations of physical activity. Ordinary physical activity does not cause undue fatigue, palpitation, dyspnea, or anginal pain.	No objective evidence of cardiovascular disease.
II	Patients with cardiac disease resulting in slight limitation of physical activity. They are comfortable at rest. Ordinary physical activity results in fatigue, palpitation, dyspnea, or anginal pain.	Objective evidence of minimal cardiovascular disease.
III	Patients with cardiac disease resulting in marked limitation of physical activity. They are comfortable at rest. Less than ordinary activity causes fatigue, palpitation, dyspnea, or anginal pain.	Objective evidence of moderately severe cardiovascular disease.
IV	Patients with cardiac disease resulting in inability to carry on any physical activity without discomfort. Symptoms of heart failure or the anginal syndrome may be present even at rest. If any physical activity is undertaken, discomfort is increased.	Objective evidence of severe cardiovascular disease.

Source: The Criteria Committee of New York Heart Association. Nomenclature and Criteria for Diagnosis of Diseases of the Heart and Great Vessels. 9th Ed. Boston, MA: Little, Brown & Co; 1994:253-256.

9.4 Eastern Cooperative Oncology Group Scale for Performance Status

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

Source: Oken MM, Creech RH, Tormey DC, Horton J, Davis TE, McFadden ET et al. Toxicity and response criteria of the Eastern Cooperative Oncology Group. Am J Clin Oncol 1982; 5 (6):649-55.

9.5 Body Surface Area and Creatinine Clearance Calculations

Body surface area (BSA) should be calculated using a standard nomogram that yields the following results in meters squared (m^2):

$$\text{BSA} = \sqrt{\frac{Ht(inches) \times Wt(lbs)}{3131}}$$

or

$$\text{BSA} = \sqrt{\frac{Ht(cm) \times Wt(kg)}{3600}}$$

Creatinine clearance (CrCl) can be calculated using the Cockroft-Gault equation as follows:

$$\text{CrCl (ml/min)} = \frac{(140\text{-age}) \text{ (body weight in kg)}}{(72 \times \text{serum creatinine in mg/dL})}$$

OR

$$\frac{(140\text{-age}) \text{(body weight in kg)}}{(0.81 \text{ x serum creatinine in } \mu\text{mol/L})}$$

For females, use 85% of calculated CrCl value.

Source: Cockcroft DW, Gault MH. Prediction of creatinine clearance from serum creatinine. Nephron 1976;16(1):31-41.

Note: In markedly obese patients, the Cockroft-Gault formula will tend to overestimate the creatinine clearance. (Adipose tissue tends to contribute little creatinine requiring renal clearance.)