Weill Cornell Medical College

A Phase II Trial of the Aurora Kinase A Inhibitor MLN8237 in Patients with Metastatic Castrate Resistant and Neuroendocrine Prostate Cancer

Study Chair: **Himisha Beltran, MD**
Div of Hematology & Medical Oncology
New York Presbyterian – Weill Cornell Medical College
525 East 68th Street, Box 403
New York, NY 10065
Tel: (646) 962-2072
Fax: (646) 962-1603
Hip9004@med.cornell.edu

This is an investigator-initiated study. The principal investigator Himisha Beltran, who may also sometimes be referred to as the sponsor-investigator, is conducting the study and acting as the sponsor. Therefore, the legal/ethical obligations of the principal investigator include both those of a sponsor and those of an investigator.

WCMC Co-Investigators:

David M. Nanus, MD
Scott T. Tagawa, MD
Caryn Ecker, NP
Florita Martin, PA

Statistician:

Paul Christos, Dr.PH
Weill Cornell Medical College
Division of Biostatistics and Epidemiology
pac2001@med.cornell.edu

WCMC Collaborating-Investigators:

Mark Rubin, MD
Weill Cornell Medical College
Department of Pathology
1300 York Avenue, Rm C-440
New York, NY 10065
Tel: (212) 746- 6313
Fax: 212-746-8816
rubinma@med.cornell.edu

Coordinating Data Manager:

Terra J. McNary
Weill Cornell Medical College
New York, NY 10021
Tel: (646) 962-9348
Fax: (646) 962-1611
tem2006@med.cornell.edu

Coordinating Regulatory Manager:

Irene Karpenko
Weill Cornell Medical College
E. 61st Street, New York, NY 10065
Tel: (646) 962-9346
Fax: (646) 962-1610
irk2006@med.cornell.edu

INVESTIGATOR AGREEMENT

I have read the protocol entitled "A Phase II Trial of the Aurora Kinase A Inhibitor MLN8237 in Patients with Metastatic Castrate Resistant and Neuroendocrine Prostate Cancer**", version 2, dated May 14, 2014.**

I agree to conduct the study as detailed herein and in compliance with ICH Guidelines for Good Clinical Practice and applicable regulatory requirements and to inform all who assist me in the conduct of this study of their responsibilities and obligations.

Principal investigator printed name	
Principal investigator signature	**Date**
Investigational site or name of institution and location (printed)	

Protocol summary

WCMC IRB Protocol Number: 1210013164
Title of Study: A Phase II Trial of the Aurora Kinase A Inhibitor MLN8237 in Patients with Metastatic Castrate Resistant and Neuroendocrine Prostate Cancer
Study Chair: Himisha Beltran
Study Center(s): WCMC and up to 10 additional sites
Number of Patients: 60 (multi-institutional, estimated accrual 3 per month, duration of enrollment - 24 months)
Concept and Rationale: The amount of neuroendocrine differentiation increases in late stages of prostate cancer and after exposure to androgen deprivation therapy, and contributes to the development of castrate resistance. Transformation to a predominantly androgen receptor (AR)- negative, neuroendocrine phenotype occurs in approximately 25% of late stage prostate cancer; these have been termed neuroendocrine or anaplastic prostate cancer. As more potent anti-androgen therapies are being introduced into the clinical arena, it is predicted that the incidence of neuroendocrine prostate cancer (NEPC) will escalate. There is currently no proven effective therapy for NEPC, and most patients survive less than one year. Aurora kinase A (AURKA) and N-myc (MYCN) are significantly overexpressed and amplified in NEPC compared to prostate adenocarcinoma, and these proteins cooperate to directly induce neuroendocrine differentiation (Beltran et al, *Cancer Discovery* 2011). In preclinical models, aurora kinase inhibition resulted in dramatic and preferential anti-tumor activity in NEPC with suppression of neuroendocrine marker expression. MLN8237 is an orally administered Aurora kinase A inhibitor that has demonstrated broad antitumor activity in vitro and in vivo, and the RP2D as monotherapy in adults with refractory solid tumors was 50 mg BID for 7 days in 21-day cycles.
Primary Objective(s): The primary endpoint is the proportion of patients who are free from radiographic progression 6 months following initiation of treatment with MLN8237.
Secondary Objective(s): To determine the radiologic response rate, overall survival (OS), radiographic progression free survival (rPFS), PSA response rate, circulating tumor cell (CTC) response, and serum neuroendocrine markers in response to therapy. **Exploratory objectives:** To evaluate molecular biomarkers archival and metastatic tissue, including IHC and FISH for Aurora kinase A and N-myc overexpression and amplification, neuroendocrine markers, AR status, ERG fusion, and other genomic mutations determined by sequencing. To evaluate plasma DNA for AURKA and MYCN amplification.
Primary Endpoint(s): Radiographic progression is measured using modified RECIST 1.1 criteria.
Secondary Endpoint(s): Overall Survival is defined as time from first treatment until death. rPFS will be defined as the time from first treatment day until objective or symptomatic radiologic progression. Progression will be determined by RECIST 1.1 and bone progression is per PCWG2 criteria. PSA progression will be evaluated but will not be considered PD; PSA progression is defined as increase in PSA value of $\geq$ 25% and an absolute increase of $\geq$ 2 ng/mL over baseline or nadir. Serum chromogranin A and neuron specific enolase response is defined as obtaining >50% reduction from baseline. CTC enumeration counts will be classified into favorable ($\leq$5) or unfavorable (>5); baseline and post-cycle 2 classification will be compared. In addition, % changes in counts will be assessed. Molecular analysis will be performed on pre-treatment tissue specimens and

plasma DNA.

Study Design:
This is a multi-institutional single-arm, open-label Phase 2 trial evaluating MLN8237 in patients with histologically confirmed or clinically suspected metastatic neuroendocrine prostate cancer (see inclusion criteria for full list of eligibility). All subjects will have a mandatory metastatic tumor biopsy at time of enrollment for research purposes. Subjects will be treated with MLN8237 at 50 mg twice daily for 7 days repeated every 21 days. Individual dose reductions will be made on the basis of the AEs observed. Therapy will continue until disease progression, unacceptable toxicity as a result of MLN8237, or withdrawal of patient consent. Patients will be followed with history, physical, and blood tests at each visit to monitor for toxicity. Response and progression will be evaluated by CT/MRI scan and bone scan after every 3 cycles and determined using RECIST v1.1. PSA and investigational tumor markers (assessed centrally) will be followed every cycle. CTC counts by CellSearch will be performed at baseline, at 4-6 weeks, and upon progression. Patients will be followed for survival endpoints following completion of this study until death.

Main Criteria for Inclusion/Exclusion (summary):

1) Metastatic prostate carcinoma and at least one of the following:
   a) Histologic diagnosis of small cell or neuroendocrine prostate cancer
   b) Histologic diagnosis of prostate adenocarcinoma plus $\geq$ 50% immunohistochemical staining for neuroendocrine markers (such as chromogranin, synaptophysin or neuron specific enolase)
   c) Development of liver metastases in the absence of PSA progression as defined by Prostate Cancer Working Group 2 (PCWG2) criteria.
   d) Serum chromogranin A level >5 x upper limit of normal and/or serum neuron specific enolase (NSE) >2x upper limit of normal

2) Patients with pure small cell neuroendocrine carcinoma on histology are not required to have received prior androgen deprivation therapy (ADT) or castrate levels of testosterone. Other patients are required to have surgical or ongoing chemical castration, with baseline testosterone level <50ng/dL.

3) ECOG performance status 0-2
4) Willing and able to give informed consent
5) Estimated life expectancy >3months

Key Exclusion criteria:
1) Active serious comorbid illness which in investigator's opinion will preclude completion of this study or interfere with determination of causality o f any adverse effects experienced in this study
2) Absolute neutrophil count <1,500/mm3
3) Platelet count < 100/mm3
4) Hemoglobin < 9.0 g/dL
5) Total bilirubin > ULN, SGOT (AST) and SGPT (ALT)> 1.5 x ULN. AST and/or ALT may be up to 5X ULN if with known liver metastases.
6) inadequate renal function as defined by creatinine >1.5 x ULN. If creatinine >1.5 ULN, calculated creatinine clearance must be $\geq$ 40 mL/minute (Cockcroft-Gault)
7) Patient has received other investigational drugs with 14 days before treatment

Intervention and Mode of Delivery:
Subjects will be treated with MLN8237 at 50 mg twice daily for 7 days repeated every 21 days

Duration of Intervention and Evaluation:

Patients will continue therapy until disease progression or unacceptable toxicity as a result of MLN8237. Patients will be followed with history, physical, and blood tests at each visit to monitor for toxicity. Response and progression will be evaluated by CT/MRI scan and bone scan every 3 cycles and determined using RECIST v1.1. PSA and serum chromogranin A and NSE will be followed every cycle. CTC counts will be performed at baseline, at 4-6 weeks, and upon progression. Patients will be followed for survival endpoints following completion of this study until death.

Statistical Methods:

The primary endpoint is the proportion of patients who are free from radiographic progression at 6 months, following treatment with MLN8237. Based on the phase II trial by Meulenbeld et al (BJUI 2012), the proposed null hypothesis (H0) is that ≤15% of patients will be radiographic progression-free at 6 months and the alternative hypothesis (Ha) is that ≥30% of patients will be radiographic progression-free at 6 months.

Sample size recommendations for the phase II design are determined according to Ahern's exact single-stage phase II design (A'Hern RP, 2001). We project a 6-month radiographic progression-free proportion of 15%, below which the response will be unacceptable, and a 6-month radiographic progression-free proportion of 30%, above which the regimen will be considered worthy of further exploration. The null hypothesis that the 6-month radiographic progression-free proportion is less than or equal to 15% will be tested against the alternative hypothesis that the 6-month radiographic progression-free proportion is greater than or equal to 30%.

The sample size computations were performed assuming a 5% level of significance and 80% power. A total of 48 patients will be required to enroll in the study. The treatment will be declared effective and worthy of further testing if 12 or more patients are free from radiographic progression at 6 months among the 48 patients entered into the study. This exact single-stage design yields a ≥ 0.80 probability of a positive result if the true percentage of patients who are free from radiographic progression at 6 months is ≥30%. It yields a ≥ 0.95 probability of a negative result if the true percentage of patients who are free from radiographic progression at 6 months is ≤15%. Assuming 10-20% of patients will be unevaluable/ineligible, we anticipate that a total 60 patients will be enrolled in the study.

Approximately 20% of patients are expected to have histologic entry criteria. With a sample size of approximately 12 patients meeting such criteria (i.e., 20% of 60 enrolled patients), a 95% confidence interval for the 6-month radiographic progression-free proportion in this subgroup of 12 patients can be expected to be within ± 25.9% of the true 6-month radiographic progression-free proportion in this subgroup. This calculation assumes a 6-month radiographic progression-free proportion of 30% in patients with biopsy positive NEPC. This calculation is for descriptive/exploratory purposes only, with the intent of estimating the 6-month radiographic progression-free proportion among the small subgroup of patients with biopsy positive NEPC.

Analysis Plan for Endpoints:

Primary Endpoint:

The primary endpoint of 6-month radiographic progression-free proportion will be estimated and a 95% confidence interval will be estimated via binomial proportions.

Secondary Clinical Endpoints:
With adequate follow-up time, secondary endpoints of response rate and overall survival (OS) will be assessed. Radiologic response rate will be estimated and a 95% confidence interval will be estimated via binomial proportions. For OS and rPFS, Kaplan-Meier survival analysis and 95% confidence intervals will be calculated using Greenwood's formulae.

The frequency of subjects experiencing toxicities will be tabulated. Toxicities will be assessed and graded according to CTCAE v. 4.0 terminology. Exact 95% confidence intervals around the toxicity proportions will be calculated to assess the precision of the obtained estimates.

Exploratory objectives/correlative studies will be evaluated using descriptive statistics, graphical methods, and statistical modeling, as appropriate, to explore the relationship between response and Aurora –A and N-myc overexpression and/or amplification in circulating tumor cells or archival tissue.

Funding, Regulatory, and Feasibility Issues:
Study drug will be provided by Millennium Pharmaceuticals. A research grant from Millennium Pharmaceuticals to WCMC will be used to fund start up costs and per-subject reimbursement for additional sites (i.e. additional sites will be funded through WCMC). Correlative studies will be supplemented with funding from other grants of the investigators, including the Prostate Cancer Foundation, Damon Runyon Cancer Research Foundation, and the United States Department of Defense.

Patient Acceptability/Ethics and Consent Issues:
The patients eligible for this study have an aggressive subtype of prostate cancer that is currently incurable with a poor prognosis of less than 1 year. There is currently no standard treatment. Based on extensive preclinical data, there is strong biologic rationale for this study. MLN8237 is orally given and conveniently administered for patients. The study will be conducted in accordance with ethical principles founded in the Declaration of Helsinki. The IRB/IEC will review all appropriate study documentation to safeguard the rights, safety, and well-being of the patients. After the study has been fully explained, written informed consent will be obtained from either the patient or his/her guardian or legal representative before study participation.

	Screening	C1D1	C1D2-7	C1D8*	C1D15*	C_D1*	C_D2-7	C_D8*	After every 3 cycles	Progression	End of Study[12]
Informed Consent	X										
ECG	X										
Medical History	X[1]	X		X	X	X		X		X	X
Physical Exam	X	X		X	X	X		X		X	X
Performance Status	X	X		X	X	X		X		X	X
Vital Signs[2]	X	X		X	X	X		X		X	X
CBC[3]	X	X		X	X	X		X		X	X
Chemistries[4]	X	X		X	X	X		X		X	X
PSA	X	X				X				X	X
Testosterone	X									X	X
CTC count[5]		X				X				X	X
Serum CgA, NSE[6]	X	X				X				X	X
Serum CEA[7]		X							X	X	X
blood for research[8]		X		X						X	X
CT/MRI[9]	X								X[9]	X	X
Bone scan	X								X	x	X
Archival Tissue	X										
Metastatic Biopsy[10]	X									(Optional)	
MLN8237		X	X[11]				X				
Adverse Event Monitoring		X	X	X	X	X	X	X		X	X
Concurrent Medications	X	X		x	X	X		X		X	X

* Window for study visits is +/- 3 days

1 Comprehensive history required for enrollment; interval history satisfactory for subsequent visits

2 Vital signs must include temperature, blood pressure, heart rate, respiratory rate, weight and height at least once during screening (prior to C1D1); subsequent vitals must include at least blood pressure, heart rate, respiratory rate, and weight

3 CBC must include differential for WBC; CBC may be performed in any CLIA certified laboratory up to 3 days prior to chemotherapy administration

4	Chemistry may be performed at any CLIA certified laboratory up to 3 days prior to chemotherapy administration and must include electrolytes, AST, ALT, total bilirubin, alkaline phosphatase; D1 chemistry for all cycles must also include uric acid and LDH; C1D8 labs must also include uric acid and LDH
5	CTC counts are encouraged, but are not mandatory and must be performed via CellSearch methodology at the following time points: prior to chemotherapy C1D1 (or within one month prior to treatment), C3D1, and at progression or end of study.
6	Serum chromogranin and neuron specific enolase should be performed locally during screening, then centrally Day 1 of each cycle and at progression
7	Serum carcinoembryonic acid (CEA) level is performed centrally C1D1, after every 3 cycles (at time of scans), and at progression
8	Blood for research will be collected at the following time points: C1D1, C1D8, and at progression or end of study. On C1D1 sample, PBMC and plasma will be isolated separately. At other time points, plasma will be collected.
9	CT/MRI of abdomen/pelvis plus CT of chest; imaging modality should remain constant for the duration of the study (i.e. if baseline scan was MRI, follow up scans should be MRI). See section 7.0 for details
10	Biopsy of metastatic tumor can be performed at any time after informed consent prior to C1D1
11	MLN8237 will be given D1-7 of every cycle
12	Must occur >30 days from last dose of MLN8237; research lab procedures performed as part of progression visit do not need to be repeated

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Background

1.1 Neuroendocrine prostate cancer

Prostate cancer is a leading cause of cancer mortality in men worldwide (Siegel et al, *Cancer Statistics*, 2012). Those that die from prostate cancer die from metastatic castration resistant prostate cancer (CRPC), tumors that progress after surgical or medical castration. We know now that many CRPC tumors remain dependent on androgen receptor (AR) signaling due to AR gene amplification, intra-tumoral androgen production, constitutive activation of AR, or other proposed mechanisms (Knudsen et al, *Clin Cancer Res* 2009). This knowledge has led to the clinical development of novel and highly potent drugs that block AR signaling, through inhibition of hormone production (e.g. abiraterone acetate) or AR receptor binding (e.g. enzalutamide).

An often under-recognized late manifestation of prostate cancer is the development of neuroendocrine prostate cancer (NEPC), which is considered a hormone refractory (AR negative) subtype of prostate cancer (Tagawa et al, *Textbook of Uncommon Cancer*, 4th Ed, 2012). These tumors do not secrete prostate specific antigen (PSA), and should be suspected in a patient with progressive disease (especially visceral or lytic bone metastases) in the setting of a low or modestly elevated PSA. Based on autopsy series and other studies, NEPC may represent approximately 25% of late stage prostate cancer (Aparicio et al, *Cancer Discovery* 2011). Transformation from prostate adenocarcinoma to NEPC is believed to be promoted by androgen deprivation therapy (Hirano et al, *Eur Urol* 2004), and many are concerned that with the introduction of novel potent AR-targeted drugs into the clinic for CRPC the incidence of NEPC will escalate (Beltran et al, *J Clin Oncol* 2012).

Histologically NEPC can appear like small cell carcinoma of other primary sites, does not express AR or secrete PSA (Palmgren et al, *Semin Oncol* 2007), and can sometimes be misdiagnosed. Immunohistochemical staining of tumors are often positive for neuroendocrine markers chromogranin A, synaptophysin, or neuron specific enolase (NSE), and elevated serum levels of chromogranin A, NSE, and carcinoembryonic antigen can support the diagnosis. Gene fusions involving the *ERG* gene (most commonly rearranged with the *TMPRSS2* gene), are prostate-specific molecular alterations and present in approximately 50% of all prostate cancers (Tomlins et al, *Science* 2005), with a similar frequency in prostate adenocarcinoma and NEPC (Lotan et al, *Mod Pathol* 2011, Scheble et al,

Histopathology 2010). This suggests that the molecular events associated with NEPC pathogenesis differs from small cell carcinomas of other primary sites, and likely has same cell of origin as prostate adenocarcinoma.

There is little prospective data regarding treatment of patients with NEPC. Platinum based chemotherapy regimens similar to those used to treat small cell lung carcinomas are often used (Papendreou et al, *JCO* 2002). However, relapses are common and most patients with NEPC survive less than one year.

1.2 Aurora kinase A and Neuroendocrine prostate cancer

In attempts to identify biomarkers and develop new treatment strategies for patients with NEPC, we performed massively parallel, paired end RNA- sequencing (RNA-Seq) and genomic analysis using oligonucleotide arrays of prostate tumors and compared the expression profile of NEPC with prostate adenocarcinoma (PCA) and benign prostate (Beltran et al, *Cancer Discovery* 2011).

There were significant molecular differences between NEPC and PCA, despite likely clonal origin (based on concordance of molecular alterations between primaries and metastases and between NEPC and PCA foci of mixed tumors). Aurora kinase A (AURKA) was significantly overexpressed and amplified in NEPC vs PCA ($P<0.001$), and this was independent of tumor proliferation (Ki67 expression). AURKA encodes Aurora kinase A, a serine/threonine kinase involved in mitotic spindle formation, centrosome separation, and G2-M transition during the cell cycle (Marumoto et al, *Genes to Cells* 2002). Beyond its role mitosis, Aurora- kinase A also displays oncogenic properties (can transform rat fibroblasts in vitro and produce tumors in athymic mice), though its full range of function is not well elucidated (Katayama et al, *Cancer and Metastasis Reviews* 2003). In neuroblastoma, AURKA is found to stabilize N-myc, an oncogene frequently amplified in neuroblastoma and associated with poor prognosis (Otto et al, *Cancer Cell* 2009). N-myc is not normally present in prostate, but we also found its gene (MYCN) to be overexpressed and amplified in NEPC compared to PCA ($P<0.001$). We validated these findings by screening prostate samples from a large cohort of patients using immunohistochemistry (IHC) and fluorescent in situ hybridization (FISH), and discovered gene amplification of AURKA and MYCN in 40% of NEPC, 5% of PCA, and none of the benign prostate tissue. Furthermore, concurrent AURKA and MYCN amplification is seen in 75% of primary prostate

tumors from patients that late develop NEPC and with a high concordance between primary tumor and metastases (suggesting that these lesions are early events and may be prognostic).

When we introduced AURKA or MYCN into benign prostate epithelial cells (RWPE-1) and prostate adenocarcinoma cells (LNCaP), MYCN induced AURKA expression and kinase activity by stabilizing Aurora A protein, and AURKA induced MYCN. Furthermore, either AURKA or MYCN could induce expression of the neuroendocrine markers, synaptophysin or neuron-specific enolase, not normally expressed in RWPE-1 or LNCaP cells. This data suggests that AURKA and MYCN are involved in prostate neuroendocrine differentiation.

LNCaP transfected with N-myc were phenotypically similar to NEPC, with downregulation of AR and androgen regulated genes (NKX3-1, TMPRSS2), and chromatin immunoprecipitation revealed that N-myc directly binds promoters of NSE, SYP, and AR to regulate their expression and modulate the neuroendocrine phenotype. N-myc amplified neuroblastoma cells (IMR-32) also demonstrated N-myc binding to NSE and SYP but not AR, suggesting that N-myc binding of AR promoter is prostate specific.

Based on these data, we hypothesized that aurora kinase inhibition would demonstrate preferential anti-tumor effect in NEPC compared to PCA. In support of this, we observed enhanced *in vitro* and *in vivo* sensitivity of NEPC cells and xenografts treated with the aurora kinase inhibitor PHA-739358 (Danusertib, Nerviano), compared to PCA models and benign prostate cells, with > 50% tumor shrinkage in NEPC xenografts and minimal to no effect in PCA. Notably, neuroendocrine marker expression (synaptophysin) was completely suppressed in the treated NEPC xenografts, again supporting a role of Aurora kinase in modulating the neuroendocrine phenotype. Knockdown of AURKA with siRNA in NEPC cells (NCI-H660) also resulted in significant cell death (50-60% decreased viability) and suppression of neuroendocrine marker expression.

MLN8237 is an orally administered Aurora kinase A inhibitor that has demonstrated broad antitumor activity in vitro and in vivo, and the RP2D as monotherapy in adults with refractory solid tumors was 50 mg BID for 7 days in 21-day cycles (Dees *et al.*, ASCO 2010).

We hypothesize that NEPC patients (and a subset of PCA) will preferentially benefit from MLN8237, based on:

1) Aurora-A and its inducer, N-myc, can drive the NEPC phenotype and are overexpressed and amplified in NEPC and a small subset of PCA;

2) Knockdown of Aurora kinase A (AURKA) resulted in decreased viability and suppression of neuroendocrine marker expression in NCI-H660 (NEPC) cells;

3) Aurora kinase inhibitor therapy demonstrate significant and preferential anti-tumor effect in NEPC xenografts and results in complete reversal of neuroendocrine marker expression.

1.3. Study Drug (MLN8237, alisertib)

1.3.1. Aurora A Kinases and the Aurora A Kinase Inhibitor MLN8237

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 (Nigg et al, *Nature Reviews* Molecular Cell Biology 2001). Aurora A localizes to centrosomes and the proximal mitotic spindle during mitosis where it functions in a diverse set of mitotic processes. Aurora A overexpression in human cancers has been correlated with increased aneuploidy and centrosome amplification (Sen et al, *Journal of National Cancer Institute* 2002). Forced overexpression of Aurora A kinase in experimental models results in the transformation of normal cells, suggesting that Aurora A overexpression may be oncogenic. 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 (Hoar et al, *Molecular & Cell Biology* 2007). Overall, the essential role of Aurora A in mitotic progression and its dysregulation in certain cancers made it an attractive therapeutic target, and therefore has been initially developed for treatment of a wide variety of tumor types. MLN8237 has demonstrated activity against a variety of nonclinical solid tumor and hematological malignancy models grown in vitro and in vivo (Goldberg et al, *Blood* 2010), as described below. MLN8237 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.

1.3.2. Preclinical Experience with MLN8237

1.3.2.1. In Vitro Studies. MLN8237 is an adenosine triphosphate (ATP)-competitive and reversible inhibitor of Aurora A kinase in vitro with an inhibition constant (K_i) of 0.43 nM. In cultured HCT-116 human colorectal tumor cells, MLN8237 produces 50% inhibition of Aurora A kinase activity at a concentration of 6.7 nM. It is approximately 200-fold more selective for Aurora A kinase than the structurally related family member, Aurora B kinase (half maximal inhibitory concentration [IC_{50}] = 1534 nM). Moreover, in enzyme assays, MLN8237 is selective for Aurora A kinase when compared to other kinases and receptors. MLN8237 has affinity for the gamma acid alpha 1 ($GABA_A$ $\alpha 1$) receptor benzodiazepine (BZD) binding site (K_i = 290 nM). The consequences of $GABA_A$ binding in rat and dog safety pharmacology studies are discussed below.

Consistent with the mechanism of action for an Aurora A kinase inhibitor, MLN8237 treatment results in formation of abnormal mitotic spindles, an accumulation of mitotic cells, and a decrease in the proliferation of a broad range of tumor cell lines grown in culture.

The in vitro antiproliferative effect of MLN8237 was quantified in tumor cell lines derived from a variety of malignancies, including colon (3 cell lines), breast (1 cell line), lung (1), ovary (1), prostate (1), pancreas (1), and lymphoid (1). MLN8237 inhibited proliferation with lethal concentrations for 50% cell-growth inhibitor concentrations (GI_{50} values) ranging from 16 to 469 nM, demonstrating that MLN8237 is a potent inhibitor of proliferation in diverse human tumor cell lines.

1.3.2.2. In Vivo Studies

MLN8237 has demonstrated broad antitumor activity in a diverse array of experimental human tumor xenografts when dosed QD or BID. These include 2 colon models (HCT-116 and DLD-1), 2 lung models (H460 and Calu-6), 1 breast model (MDA-MB-231 FP4), 1 prostate model (CWR22 RV-1 Luc1.17) and 4 DLBCL models (Ly19, WSU, Ly7, PHTX-22-06). Statistically significant tumor growth inhibition (TGI) was observed with MLN8237 given at 30 mg/kg QD or less in all models but the Calu-6 model. At 20 mg/kg BID or less, statistically significant TGI was observed in all models tested, including Calu-6. Taken together, these results demonstrated that MLN8237 has broad antitumor activity in many experimental human tumor models.

To define the plasma concentration of MLN8237 that inhibits Aurora A, the relationship between pharmacokinetics (PK) and pharmacodynamics was assessed at the steady-state concentration (C_{ss}). Steady-state MLN8237 concentrations were achieved using osmotic mini-pumps implanted subcutaneously (SC) in mice bearing HCT-116 xenografts. The relationship between the C_{ss} of MLN8237 and the tumor mitotic index fit the sigmoid maximum effect (E_{max}) model with an estimated efficacious concentration producing 90% of the maximal possible response (EC_{90}) of approximately 1 μM.

The HCT-116 tumor model was also used to determine the antitumor activity of MLN8237 given either QD or BID. MLN8237 demonstrated dose-dependent TGI whether administered QD or BID. TGI with BID administration of 3 mg/kg (TGI = 70%) and 10 mg/kg (TGI = 102%) was greater than with QD administration when measured on the last day of treatment (Day 21).

The relationship between efficacy and systemic exposure was investigated using osmotic mini-pumps implanted SC to deliver sustained release of MLN8237 over 12 days to HCT-116 xenografts. The relationship between the efficacy (TGI) on Day 12 and the average plasma concentration (C_{avg}) values on Days 1, 3, 6, 8 and 12 fit the sigmoid E_{max} model. At a C_{avg} of 1 μM sustained exposure in the 10 mg/mL group the TGI was 88.3%, which was between that observed in the 10 mg/kg (TGI 82.1%) and 30 mg/kg (TGI 93.1%) orally dosed groups.

1.3.3. Safety Pharmacology, Toxicology, and Drug Metabolism

Safety pharmacology studies evaluating the central nervous system (CNS) and cardiovascular effects of MLN8237 did not reveal significant adverse effects at the exposures anticipated to be required for human efficacy. MLN8237 has a low potential to prolong the QT interval on the electrocardiogram (ECG), based on its low in vitro activity against the potassium ion channel encoded by human ether-à-go-go related gene (hERG) and its lack of an effect on the QTcV interval in dogs. MLN8237 had minimal activity (< 40% inhibition) against all receptor ligand interactions examined except GABAAα1 benzodiazepine. Although MLN8237 did elicit behavioral CNS effects attributed to its binding to the GABAAα1 benzodiazepine, these effects occurred at dose levels which exceeded the repeat-administration daily oral maximum tolerated dose (MTD) of MLN8237.

The predominant effects of MLN8237 in Sprague-Dawley rats included peripheral blood cytopenias secondary to myelosuppression and increased mitotic figures/single cell necrosis (apoptosis) in tissues with a high basal cellular replication rate, consistent with the known mechanism of action of MLN8237. These effects were also the effects that predominated in repeat-dose studies in beagle dogs. CNS effects, consistent with the activity of MLN8237 at the GABAAα1 benzodiazepine receptor, were observed only in beagle dogs following a single oral dose of 5 mg/kg, which exceeds the daily oral repeat-administration MTD in this species.

MLN8237 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. MLN8237 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 MLN8237 may be found in the IB.

1.3.4. Clinical Experience

Clinical experience with MLN8237 (alisertib) includes phase 1 and 2 studies in both solid tumors and heme lymphatic malignancies, described below. A phase 3 study in Peripheral T-cell Lymphoma (PTCL) began in early 2012.

MLN8237 for clinical studies is being developed in 2 dosage formulations: enteric-coated-tablet (ECT), and oral solution (OS: for pediatric use). Initial studies employed a powder-in-capsule (PIC) formulation, and more recent studies using current formulations all have evaluated safety, PK, relative bioavailability (in reference to the PIC), and antitumor activity after administration of the ECT formulation.

Using the ECT formulation, the dose-escalation, phase 1 portion of an ongoing study, C14007, has 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.

In Study C14007, which includes a phase 1 dose escalation using the same ECT formulation employed in this study, multiple dose levels up to 840 mg total cycle dose (60 mg BID for 7 days) were evaluated. At the maximum administered dose of 60 mg BID for 7 days administered to patients with advanced solid tumors, 3 patients (out of 3 enrolled) developed myelosuppression during the treatment-free period which was considered intolerable and above the MTD of 50 mg BID. In summary, 700 mg total cycle dose (eg, 50 mg BID administered daily for 7 days) represents the ceiling for dose escalation in these combinations with MLN8237 planned for this current study.

The predominant toxicities reflect the mechanism of action in proliferating tissues (bone marrow, GI epithelium, and hair follicles). The suggested management of these toxicities is based on standard clinical paradigms for an anti-proliferative chemotherapeutic agent. Using a treatment-free period for recovery between each cycle of drug administration, the clinical experience from multiple phase 1 through 2 studies indicate that major toxicities can be managed to allow repeat treatment cycles over periods extending beyond 12 months.

MLN8237 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 MLN8237 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 MLN8237 includes treatment with multiple doses and schedules as described in the IB.

1.3.5. Pharmacokinetics

Based on an integrated assessment of the currently available PK data from PIC formulationacross two solid tumor studies C14001 and C14002, it can be concluded that MLN8237 absorption is fast, with overall median T_{max} of 2 hours postdose. Overall mean steady-state terminal half-life following multiple dose administration in patients with non-hematologic malignancies was 23 hours. The overall mean peak/trough ratios were 2.5 and 5.2 for BID and QD dosing, respectively. The overall mean accumulation ratios were 2.9 and 1.8 for BID and QD dosing, respectively. Pharmacokinetic steady-state conditions were approximately achieved by Day 7 following daily oral administration. The steady-state exposure of MLN8237 increases approximately in a dose proportional manner over the range from 5 to 200 mg daily dose. Pharmacokinetic variability was high following administration as PIC (%CV in steady-state dose-normalized $AUC_{0\text{-}24hr}$ of 78%). At the recommended phase 2 dose of 50 mg BID, the mean steady-state average concentration (Css,avg) exceeded the preclinical projected efficacious plasma concentration associated with saturating levels of pharmacodynamic and antitumor activity in HCT-116 mouse xenograft studies (1 μM). The pharmacokinetic properties of MLN8237 in Protocol C14003 in patients with hematologic malignancies are generally consistent with those observed in patients with non-hematologic malignancies.

Glucuronidation is expected to be a major route of MLN8237 clearance and in vitro metabolism studies indicate that UGT1A1 is a contributor to MLN8237 glucuronidation. Preliminary results of dose-normalized steady-state exposure of MLN8237 by UGT1A1 genotype are available in 67 patients in Protocols C14001 and C14002. The geometric mean of dose-normalized steady-state $AUC_{0\text{-}24hr}$ in *28/*28 genotype group (N = 9) was approximately 40% higher than that observed in the wt/wt genotype group (N = 36).

The MLN8237 molecules exhibits pH-dependent solubility, and the variability in gastric pH likely represents an important source of variability in MLN8237 oral absorption following administration of an immediate release formulation like the PIC. Emerging clinical data from enteric-coated tablet (ECT) formulation indicated that the steady-state systemic exposures of MLN8237 administered as the ECT

formulation are generally comparable to those observed with the PIC formulation. Based on preliminary results from 14 patients who received either ECT or PIC in a crossover design, the relative bioavailability of the ECT formulation in reference to the PIC formulation is estimated to be approximately 90%. Therefore the MTD of 50 mg BID determined for the ECT formulation in protocol C14007 is consistent with the estimated relative bioavailability of ECT versus PIC and the PIC MTD of 50 mg BID. These findings support continued use of the ECT formulation in the ongoing and future clinical development of MLN8237.

Further details regarding the clinical PK of MLN8237 are provided in the MLN8237 Investigator Brochure.

1.3.6. Potential Risks and Benefits

The safety risks of MLN8237 (alisertib) treatment are: (1) leukopenia, neutropenia, febrile neutropenia, and lymphopenia with a potential increased susceptibility to infection, (2) thrombocytopenia with a potential increased risk of bleeding; (3) anemia; (4) gastrointestinal (GI) toxicity resulting in stomatitis/mucositis, nausea, vomiting, anorexia, abdominal pain, dyspepsia, diarrhea, dehydration, and potentially mucosal/GI bleeding, and sepsis; (5) alopecia; (6) asthenia/fatigue; and (7) fever, and (8) benzodiazepine-like effects including sedation, somnolence, sleep disorders, confusion, disorientation and associated memory loss and gait disturbances (which were reversible in clinical studies with cessation of treatment or dose reduction). Skin changes including hand-foot syndrome have been reported in some patients. 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 withdrawal were not observed.

While these toxicities are potentially associated with risk or discomfort to the patient, they are anticipated to be reversible. MLN8237 has not led to major neurotoxicities or fluid retention as a single agent. However, because there is only limited human experience with MLN8237, it is possible that MLN8237 will have other toxicities that have not been observed in or predicted from its evaluation in rats and dogs and from ongoing studies in humans. MLN8237 has a low potential to prolong the QT interval in vivo based upon its extremely weak in vitro binding to hERG (IC_{50} and K_i both > 100 μM).

To mitigate the inherent risks in clinical studies of MLN8237, patients are evaluated frequently while they are receiving treatment.

Because MLN8237 inhibits Aurora A kinase, it is possible that MLN8237 may interfere with cancer growth and cause cancer cell death 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.

1.4. Correlative Studies Background.

Blood: Shedding of circulating tumor cells (CTCs) into the circulation is common in advanced prostate cancer. The CellSearch (Veridex LLC, Warren, NJ) automated enrichment system capture device is an FDA-cleared technology for detecting and quantifying EpCAM expressing tumor cells in the blood and has prognostic value. A CTC count $\geq$ 5 per 7.5 mL of blood is considered unfavorable and <5 is favorable; furthermore, changes in CTC count on therapy is reflective of prognosis and therefore serial CTC counts are being validated prospectively as alternative biomarker of tumor response for patients with advanced prostate cancer. CTC counts by CellSearch are encouraged as part of this study, but are not mandatory. Blood will also be obtained on day 1 of each cycle and evaluated for neuroendocrine markers (serum chromogranin and NSE), lactate dehydrogenase (LDH), and prostate specific antigen (PSA).

In addition, blood for research (plasma and PBMC's) will be collected for research on C1D1, C1D8, and at progression or end of study. Plasma DNA will be evaluated for Aurora A and N-myc amplification and correlated with response to therapy and tumor tissue status. In addition, we will evaluate for concordance between plasma DNA molecular alterations and matched primary prostate cancer tissue and metastatic samples. We will correlate these results with clinical response to MLN8237.

Archival Tissue: Formalin fixed paraffin embedded (FFPE) archival prostate tumor tissue will be obtained from all patients (prostatectomy specimens or prostate biopsies) and evaluated for AR, ERG, aurora kinase A, N-myc protein expression, copy number changes, and/or translocation (in case of ERG). We hypothesize that high-risk primary prostate tumors harbor genetic alterations that predispose to clinical progression and the development of NEPC. Using the MiSeq technology (Illumina), we will perform targeted exome sequencing of matched primary tumors from 30 patients that later developed

metastatic NEPC. We will take a focused approached to examine non-synonymous recurrent mutations in FFPE tissue. Through molecular classification and correlation with clinical features and between matched primary tumors and metastases, we will gain insight into genetic alterations that arise early and predispose to the development of NEPC and will correlate with outcomes and clinical response to MLN8237.

Metastatic Tissue: A tumor biopsy of metastatic site is required for all patients and will also be evaluated for AR, ERG, aurora kinase A, N-myc protein expression, copy number changes, and/or translocation (in case of ERG). Biopsy is performed for research and must be performed prior to Cycle 1 Day 1, but results not needed before proceeding. We will perform exome and transcriptome sequencing of approximately 30 metastatic tumors from patients enrolled on study. We will determine the spectrum of mutations associated with NEPC, and compare to >150 localized prostate cancer whole exomes (n=120) and whole genomes (n=30) that we recently sequenced in collaboration with the Broad Institute. Highly ranked and potential driving mutations will be integrated with gene expression data (RNA-Seq) and protein expression (IHC) and correlated with clinical features, treatment response to MLN8237, and outcomes. We will generate a comprehensive genetic profile and characterize the spectrum of actionable and informative mutations in NEPC. An optional metastatic biopsy at time of progression will be obtained in consenting patients; these tumors will be evaluated for molecular changes associated with progression on MLN8237.
Extensive genomic profiling of metastatic and primary tumor specimens will help establish novel prognostic biomarkers and those that may predict response to MLN8237. This study will also identify molecular alterations associated with disease progression and treatment resistance, bring new insight into the mutational spectrum of advanced disease, and identify additional therapeutic targets. We hypothesize that advanced prostate tumors acquire driving mutations in response to therapy that allows them to evade therapy and transform to a NEPC phenotype.

2.0 Study objectives

2.1 Primary objectives

- The primary endpoint is the proportion of patients who are free from radiographic progression at 6 months following treatment with MLN8237.

2.2 Secondary objectives

- To determine the overall survival with MLN8237
- To evaluate the objective response rate
- To determine the progression free survival with MLN8237
- To determine the PSA response rate with MLN8237
- To determine baseline and changes in circulating tumor cell count in response to therapy and at disease progression
- To determine serum neuroendocrine marker response with MLN8237

2.3 Exploratory Objectives

- To determine Aurora kinase A and N-myc gene amplification status plasma DNA at baseline and correlate with archival and metastatic tumor Aurora kinase A and N-myc status, other pathologic findings (neuroendocrine markers, AR status), and correlate with response to therapy with MLN8237
- To evaluate for Aurora kinase A and N-myc overexpression and amplification in archival and metastatic tumor tissue and correlate with other pathologic findings (including neuroendocrine markers, AR status, ERG status), and correlate with response to therapy with MLN8237

3.0 Study design. This is a multi-institutional single-arm, open-label Phase 2 trial evaluating MLN8237 in patients with histologically confirmed or clinically suspected neuroendocrine prostate cancer (see inclusion criteria for full list of eligibility). Subjects will be treated with MLN8237 at 50 mg twice daily for 7 days repeated every 21 days. Individual dose reductions will be made on the basis of the AEs observed. Therapy will continue until disease progression, unacceptable toxicity as a result of MLN8237, or withdrawal of patient consent. Patients will be followed with history, physical, and blood tests at each visit to monitor for toxicity. Response and progression will be evaluated by CT/MRI scan and bone scan every 3 cycles and determined using RECIST v1.1 with PCWG2 modifications. PSA and serum chromogranin A and NSE will be followed every cycle. CTC counts will be performed at baseline, at 4-6 weeks, and upon progression. Patients will be followed for survival endpoints following completion of this study until death.

4.0 Subjects

4.1 Recruitment. These patients will be recruited from the existing and referred patient population at Weill Cornell Medical College (WCMC)-New York Presbyterian Hospital (NYPH) and additional institutions.

Patients are initially chosen based on a) MD evaluation; b) outpatient interview; c) criteria for inclusion and exclusion (as described below). This study will be explained to the patient in the physician's office or other appropriate professional setting. The patient will be given a copy of IRB approved written consent form to take home for a thorough reading and consideration. Should the patient wish to enter the study, he will return to physician's office for further discussion and to complete the informed consent process. Only the investigators will obtain a subject's informed consent.

4.2 Number of patients. Up to 60 patients will be treated, with at least 30 patients requiring histologically proven NEPC (Inclusion criteria 4.3.1.1 or 4.3.1.2). While all registered subjects will be included in the final intent to treat analysis, only subjects who receive at least one dose of study drug will be considered eligible to toxicity analysis. Subjects who are enrolled, but do not complete at least one planned cycle with MLN8237 may be replaced.

4.3 Inclusion criteria

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

4.3.1. Metastatic prostate carcinoma and at least one of the following:

4.3.1.1. Histologic diagnosis of small cell or neuroendocrine prostate cancer

4.3.1.2. Histologic diagnosis of prostate adenocarcinoma plus $\geq$ 50% immunohistochemical staining for neuroendocrine markers (such as chromogranin, synaptophysin or neuron specific enolase)

4.3.1.3. Development of liver metastases in the absence of PSA progression as defined by PCWG2 criteria

4.3.1.4. Serum chromogranin A level >5 x upper limit of normal and/or serum neuron specific enolase (NSE) >2x upper limit of normal

4.3.3. Patients with pure small cell neuroendocrine carcinoma on histology are not required to have received prior androgen deprivation therapy (ADT) or castrate levels of testosterone. Other patients are required to have surgical or ongoing chemical castration, with baseline testosterone level <50 ng/dL.

4.3.4. Patients capable of fathering children must agree to use an effective method of contraception for the duration of the trial and should continue use for 4 months after last dose of study drug

4.3.5. Subjects must be able to take oral medication and to maintain a fast as required for 2 hours before and 1 hour after MLN8237 administration.

4.3.6. ANC > 1500/mm³, platelets > 100,000/mm³, Hgb > 9 g/dL. Values must be obtained without need for myeloid growth factor or platelet transfusion support within 14 days, however, erythrocyte growth factor is allowed as per published ASCO guidelines.

4.3.7. Total bilirubin ≤ ULN, SGOT (AST) and SGPT (ALT)< 1.5 x ULN. AST and/or ALT may be up to 5X ULN if with known liver metastases provided bilirubin is normal.

4.3.8. Adequate renal function as defined by serum creatinine ≤ 1.5 x ULN. If creatinine >1.5 x ULN, calculated or measured creatinine clearance must be ≥ 40 mL/minute (Cockcroft-Gault).

4.3.13. ECOG performance status 0-2

4.3.14. Estimated life expectancy > 3 months

4.3.15. 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.

4.3.16. Age >18 years

4.4. Exclusion criteria

Patients meeting any of the following exclusion criteria are ineligible for treatment in the study:

4.4.1 Radiation therapy to 25% of bone marrow within 2 weeks of first dose

4.4.2 Residual > Grade 2 toxicity from prior treatment must have resolved with the exception of those explicitly described elsewhere in entry criteria

4.4.3 Known history of uncontrolled sleep apnea syndrome and other conditions that could result in excessive daytime sleepiness, such as severe chronic obstructive pulmonary disease; requirement for supplemental oxygen.

4.4.4 Requirement for constant administration of proton pump inhibitor, H2 antagonist, or pancreatic enzymes. Intermittent uses of antacids or H2 antagonists are allowed (see section 5.5)

4.4.5 Severe or uncontrolled systemic infection

4.4.6 Myocardial infarction within 6 months prior to enrollment or has New York Heart Association (NYHA) Class III or IV heart failure, 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.

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

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

4.4.9 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.

4.4.10 Currently active other malignancy excluding controlled non-melanoma skin cancer. Patients are considered NOT to have "currently active" malignancy if they have completed any necessary therapy and are considered by their physician to be at less than 30% risk of relapse.

4.4.11 Treatment with 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 MLN8237 and during the study

4.4.12 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.

4.5 Registration procedure

Patients will be centrally registered with the Weill Cornell Medical College (WCMC), Department of Medicine Clinical Trials Office. To register a patient, fax the following documents to the Clinical Trials

Office at 646-962-1610 AND email notification to guonc@med.cornell.edu or scan/email all documents to the email addresses listed above. Note that the study email address will not accept attachments > 5 MB, so large attachments should be split.

- WCMC Patient registration form
- First and last page of the fully executed informed consent form, plus additional pages if checkboxes for correlative studies are required.
- Fully executed HIPAA research authorization form (if separate from the consent document)
- Eligibility checklist signed and dated by investigator and research nurse
- Documentation of any eligibility waivers granted

In addition, entry of screening information into WCMC web-based system (REDCap) should be completed.

Central registration information is reviewed and entered into the HemOnc centralized research database. These documents should be emailed or faxed Monday to Friday from 9:00 AM to 4:45 PM EST. Patients will be assigned a sequence number for the protocol. The registering institution will then be faxed or emailed a copy of the sequence number as confirmation of a completed registration. Subjects should NOT receive any study medication prior to receipt of registration confirmation.

Registration of patients cannot occur until the Coordinating Center has received proper documentation from the registering institution of IRB approval, including a copy of the current approval letter, stamped consent and signed FDA Form 1572. These documents may be faxed to the Coordinating Center at 646-962-1610 or emailed to guonc@med.cornell.edu

4.6 Duration of follow-up. Patients will be followed every 3 months for up to 3 years. Following 3 years, patients will be followed yearly until death.

5. Patient Evaluation

5.1. Screening Period

The following procedures must be completed no more than 4 weeks from enrollment.

- Informed Consent
- Demographics
- Medical History
- Previous therapy
  - Surgical report
  - Radiotherapy report

    - Previous systemic (hormonal, chemotherapy, other) therapy – drugs, doses, dates of therapy
- Complete Physical Exam including height and weight
- Vital Signs
- ECOG Performance Status
- Electrocardiogram
- CBC with differential and platelet count.
- Electrolytes, BUN, Creatinine, LDH,
- chromogranin A, neuron specific enolase
- Total protein, albumin, total bilirubin, AST, ALT, Alkaline phosphatase
    - Direct bilirubin is required for those patients with Gilbert's syndrome
- PSA
- Testosterone
- Consider coagulation profile if needed for biopsy
- Labs for research (up to 4 weeks prior to treatment)
- CT or MRI (abdomen-pelvis) plus CT of chest, within 4 weeks of treatment
- Bone scan, within 4 weeks of treatment
    - Any confirmatory tests to assess equivocal results of bone scan should also be completed within a month of enrollment
- Tumor tissue: archival tumor tissue must be requested at time of screening. Metastatic tumor biopsy is mandatory and may be completed at any time after informed consent prior to treatment (for research purposes only and results not needed prior to proceeding to therapy)

The following tests need to be performed with results within eligibility criteria range within 3 days of C1D1:

- CBC (absolute neutrophil count, hemoglobin, platelet count)
- Liver function tests (total bilirubin, AST, ALT, Alkaline phosphatase)

5.2 Treatment period

5.2.1 Cycle 1, Day 1

- Targeted Physical Examination with vital signs and weight
- ECOG Performance Status (PS)
- Adverse event evaluation
- Concomitant medications
- Concomitant procedures
- Up to 3 days prior to treatment the following labs will be performed:
  - CBC with differential and platelet count
  - Electrolytes, BUN, Creatinine
  - Total protein, albumin, total bilirubin, AST, ALT, Alkaline phosphatase, Lactate dehydrogenase (LDH), uric acid
  - Serum level of chromogranin A, neuron specific enolase, carcinoembryonic acid (to be performed centrally)
  - CTC count (CellSearch methodology, accepted up to 4 weeks prior to treatment, to be performed locally)—optional
  - PSA, Plasma and PBMC for research (or within one month prior to treatment, to be performed centrally)—Do not need to perform if done at screening
  - Administration of MLN8237

5.2.3 Cycle 1, Days 2-7

- Administration of MLN8237

5.2.4 Cycle 1, Day 8 (Visit window +/- 3 days)

- Targeted Physical Examination with vital signs and weight
- ECOG PS
- Adverse event evaluation
- Concomitant medications
- Concomitant procedures
- Up to 3 days prior to C1D8 the following labs will be performed:
  - CBC with differential and platelet count
  - Electrolytes, BUN, Creatinine

- Total protein, albumin, total bilirubin, AST, ALT, Alkaline phosphatase, Lactate dehydrogenase (LDH), uric acid
- Blood for research (central)

5.2.5 Cycle 1, Day 15 (Visit window +/- 3 days)

- Targeted Physical Examination with vital signs and weight
- ECOG PS
- Adverse event evaluation
- Concomitant medications
- Concomitant procedures
- Up to 3 days prior to C1D15 the following labs will be performed:
  - CBC with differential and platelet count
  - Electrolytes, BUN, Creatinine
  - Total protein, albumin, total bilirubin, AST, ALT, Alkaline phosphatase

5.2.6 Day 1 treatment days of subsequent cycles (cycle 2 and beyond – see specifics for C3D1 below) Visit window +/- 3 days. Laboratory evaluation for determination of study drug dosing (e.g. CBC and chemistry) may be performed up to 3 days prior to treatment.

- Targeted Physical Examination with vital signs and weight
- ECOG Performance Status assessment
- CBC with differential and platelet count
- Electrolytes, BUN, Creatinine
- Total protein, albumin, total bilirubin, AST, ALT, Alkaline phosphatase, Lactate dehydrogenase (LDH), uric acid
- PSA
- Chromogranin A, neuron specific enolase (central labs)
- Adverse event evaluation
- Concomitant medications
- Concomitant procedures
- Administration of MLN8237

5.2.6.1 Cycle 3, Day 1 (Visit window +/- 3 days)

Follow protocol procedures for D1 of subsequent cycles AND:

- CTC count (CellSearch, performed locally)- optional

5.2.7. Days 2-7 treatment days of subsequent cycles (cycle 2 and beyond)

- Administration of MLN8237

5.2.8 Day 8 of subsequent cycles (cycle 2 and beyond)- (Visit window +/- 3 days)

- Adverse event evaluation
- Targeted Physical Examination with vital signs and weight
- ECOG PS
- Up to 3 days prior to D8 the following labs will be performed:
  - CBC with differential and platelet count
  - Electrolytes, BUN, Creatinine
  - Total protein, albumin, total bilirubin, AST, ALT, LDH, Alkaline phosphatase,

5.2.9 Day 15 of subsequent cycles (cycle 2 and beyond)

- Evaluation of subjects on D15 of each cycle (except cycle 1) is at the discretion of the investigator

5.2.10 Radiographic evaluation – See Section 7.0

To be performed after cycle 3, and following every 3rd subsequent cycle, or as clinically indicated. Consideration should be made for confirmatory scans after 4 weeks should criteria for PR or CR by RECIST be met at any imaging time point.

5.2.11 At progression

- Targeted Physical Examination with vital signs and weight
- Adverse event evaluation
- ECOG Performance Status

- PSA
- CBC with differential and platelet count
- Electrolytes, BUN, Creatinine
- Total protein, albumin, total bilirubin, AST, ALT, Alkaline phosphatase, Lactate dehydrogenase (LDH), uric acid
- Serum level of chromogranin A, neuron specific enolase, carcinoembryonic acid- central labs (can be performed at progression or end of study)
- CTC count (CellSearch)- optional
- Blood for research
- Optional metastatic tumor biopsy for research
- CT or MRI (abdomen-pelvis) plus CT of chest within 2 weeks of progression visit
- Bone scan

5.2.12 End of study (must occur > 30 days after last dose of study drug; research lab procedures performed as part of progression visit do not need to be repeated).

- Targeted Physical Examination with vital signs and weight
- Adverse event evaluation
- ECOG Performance Status
- PSA
- CBC with differential and platelet count
- Electrolytes, BUN, Creatinine
- Total protein, albumin, total bilirubin, AST, ALT, Alkaline phosphatase, Lactate dehydrogenase (LDH), uric acid
- Serum level of chromogranin A, neuron specific enolase, carcinoembryonic acid- central labs(can be performed at progression or end of study)
- CTC count (CellSearch) - optional
- Blood for research (can be performed at progression or end of study)

Standard lab evaluations (including CBC, chemistry exams) may be performed at approved CLIA certified local laboratories with the investigator's pre-approval.

5.2.13 Long term follow up

Subjects should be followed for survival every 3 months for up to 3 years following end of study visit (in-office visits at study institutions are not necessary)

6.0 Correlative Studies

6.1 CTC enumeration by CellSearch (encouraged, but not required)

CTC counts by CellSearch (Veridex) methodology will be performed prior to study drug initiation (within 1 month), after study drug completion C2, and at progression. Samples should be analyzed by a CLIA certified lab and billed to subject's insurance. CTC counts are optional for institutions not able to perform this procedure due to insurance reimbursement or may arrange for central CTC enumeration (in this case, subjects will not be billed, but funding will be taken out of per-subject reimbursement for that site).

6.2 Blood for research analysis:

PBMCs and plasma for research will be collected on C1D1 (or within 4 weeks prior to treatment). Plasma will also be collected at the following time points: C1D8, and at progression or end of study. This will be collected for research purposes only and will not be billed to the subject. Blood samples should be collected, processed and shipped to the central laboratory per specifications in the Lab Manual.

6.3 Serum tumor markers

Serum for central analysis of chromogranin A and neuron specific enolase (and CEA at some time points) will be collected on D1 of every cycle and at progression/end of study. Blood samples should be collected, processed and shipped to the central laboratory per specifications in the Lab Manual.

6.4 Archival Tissue. Tissue blocks or 10 unstained slides (10 microns each) and 5 unstained slides (40 microns each) with corresponding H&E slide will be sent at time of enrollment.

6.5 Metastatic Biopsy tissue. Will be performed per institutional protocol, but should include at least 1 core biopsy; fresh tissue should be processed and shipped to the central laboratory per specifications in the Lab Manual.

7.0 Radiographic evaluation

The following radiographic evaluations must be performed according to the schedule outlined below:

- Bone scan, CT/MRI abdomen/pelvis, and CT of chest at baseline
- Bone scan, CT/MRI of abdomen/pelvis, and CT of chest will be repeated after every 3rd cycle until the subject is off-study
- In addition, scans will be performed for subjects with clinical signs or symptoms of progression at the investigators discretion
- Every effort should be made to use the same imaging technique for follow up evaluation as was utilized at baseline
- Should RECIST criteria for PR or CR be met at any imaging time point, investigators are urged to consider a repeat scan at least 4 weeks following the scan demonstrating response. In this instance, or if imaging is performed for clinical purposes earlier than scheduled per protocol, it is permissible to "reset" future imaging time points to every 3rd cycle following the last scan.

8.0 Treatment Plan

8.1. Administration of MLN8237. Treatment will be administered only to eligible patients under the supervision of the investigator or identified co-investigator(s). Treatment will be administered on an outpatient basis. MLN8237 will be given PO in a dosage of 50 mg BID for 7 Days (Days 1-7) of each 21-day treatment cycle. The study drug will be administered on an empty stomach with the patient remaining NPO (nothing by mouth), except for water and prescribed medications, for 2 hours before and 1 hour after each dose. Patients will be instructed to take each oral dose of MLN8237 with 8 ounces (1 cup, 240 mL) of water. Appropriate dose modifications/delays for MLN8237 in section 5.2.

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

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 MLN8237.

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

8.2. 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. These criteria are available online at http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm

8.2.1 Criteria for Retreatment and Dose Delay

Treatment with MLN8237 will be repeated every 21 days. In order for a new cycle of therapy to begin, the patient's ANC must be $\geq 1{,}500/mm^3$ and the platelet count must be $\geq 75{,}000/mm^3$. In addition, all other non-hematologic toxicity considered by the investigator to be related to therapy with MLN8237 must have resolved to ≤ Grade 2 or to the patient's baseline values before a new cycle of therapy may begin.

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 MLN8237 will be reduced 1 level (Table 0-1) to 40 mg BID when therapy resumes. A second dose reduction to 30 mg BID 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 will be removed from the study. Should treatment need to be delayed for more than 3 weeks at any dose, therapy with MLN8237 will be discontinued.

Table 0-1 Table of Dose Adjustments

Dose Level	Dose	Schedule	Cycle Length
1	50 mg	PO BID	
-1	40 mg	PO BID	
-2	30 mg	PO BID	
-3		Discontinue	

Level 1 is the starting dose.

8.2.3. Dose Modifications for Hematological Toxicity

If a patient experiences any of the following hematological toxicities, 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 3 neutropenia with fever or infection, or both, where fever is defined as an oral temperature greater than 38.5°C

- Grade 4 neutropenia (ANC < 500 cells/mm^3) lasting more than 7 consecutive days. Patients experiencing Gr 4 neutropenia at any time should undergo repeat CBC within 5 days
- Grade 3 thrombocytopenia with clinically significant bleeding
- Grade 4 thrombocytopenia (platelet count < 25,000/μL) lasting more than 7 consecutive days.
- Patients experiencing Gr 4 thrombocytopenia at any time should undergo repeat CBC within 5 days.
- Platelet count less than 10,000/μL at any time

8.2.4. Dose Modifications for Nonhematological Toxicities

If a patient experiences any of the following toxicities during the dosing period (i.e. D1-7 of the cycle), 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 arthralgias/myalgias
    - 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
    - Grade 3 fatigue that lasts less than 1 week
- Grade 2 nonhematological 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 any Grade 4 non-hematologic toxicity. If, in the opinion of the investigator and study sponsor it is in the patient's interest to continue therapy with MLN8237, then after recovery from the toxicity or toxicities in question to ≤ Grade 1 or to baseline values, the dose of MLN8237 should be reduced by at least 1 dose level with subsequent cycles of therapy. When a dose reduction of MLN8237 is required, no re-escalation of dose will be permitted. If a patient requires more than 2 dose reductions, therapy with MLN8237 will be discontinued. Any additional dose delays or dose modifications should be discussed with the Study Chair.

8.3. 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. MLN8237 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 MLN8237, including the requirement that MLN8237 be administered as intact tablets.

8.4. Excluded Concomitant Medications and Procedures

The following medications and procedures are prohibited during the study:

- Any antineoplastic therapy, other than LHRH agonists/antagonists. Bisphosphonates and denosomab are allowed.
- Any investigational therapy other than MLN8237
- Requirement for constant administration of any proton pump inhibitor. 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 MLN8237 dosing (Day 7)

8.5. Permitted Concomitant Medications and Procedures

Myeloid or erythroid growth factors to treat patients with neutropenia or anemia according to the American Society of Clinical Oncology (ASCO) Guidelines. 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 MLN8237.

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 MLN8237. Because of MLN8237'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.

8.6. Precautions and Restrictions

Food and drinks other than water and prescribed medications are not permitted for 2 hours preceding and 1 hour following each dose of MLN8237.

Patients should not drive, operate dangerous tools or machinery, or engage in any other potentially hazardous activity that requires full alertness and coordination if they experience sedation while enrolled in this study.

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 MLN8237. 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 MLN8237 has on human pregnancy or development of the embryo or fetus. Therefore, patients should avoid impregnating a female partner. Even if surgically sterilized (ie, status postvasectomy) 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.

8.7 Management of Clinical Events

8.7.1. Nausea and Vomiting

Prophylactic antiemetic therapy will not be used in this study unless it becomes clear that MLN8237 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.

8.7.2. 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.

8.7.3. Central Nervous System Effects

If a patient experiences excessive sedation believed to be related to MLN8237, treatment with MLN8237 should be interrupted. Patients whose sedation is not considered immediately life-threatening should be carefully monitored and given appropriate supportive care. If the patient's level of consciousness is considered to be life-threatening, necessary measures should be instituted to secure the airway, ventilation, and intravenous access. Flumazenil (Romazicon®) is a selective benzodiazepine receptor antagonist that is intended as an adjunct to, not as a substitute for, the proper management of benzodiazepine overdose. Although there is neither preclinical nor clinical experience with flumazenil and MLN8237, the use of flumazenil should be considered if the level of MLN8237-associated sedation is considered to be life-threatening. Patients treated with flumazenil should be monitored for resedation, respiratory depression, and other residual benzodiazepine effects for an appropriate period after treatment. Continued monitoring is particularly important in the case of MLN8237 given its half-life and the comparatively brief half-life of flumazenil in the CNS (20-30 minutes). Flumazenil should be administered according to its label. CNS stimulants such as modafinil (Provigil®), 100 mg/day to 300 mg/day or methylphenidate (Ritalin®), 10 mg/day to 60 mg/day, may be used to counteract daytime somnolence.

8.8. Treatment Compliance

All drug will be administered to eligible patients under the supervision of the investigator or identified sub-investigator(s). The pharmacist will maintain records of drug receipt (if applicable), drug preparation, and dispensing, including the applicable lot numbers, patients' study ID number, and total drug administered in milligrams. Any discrepancy between the prescribed dose and dose administered and the reason for the discrepancy must be recorded in the source documents.

8.9 Duration of Treatment and Patient Participation

Patients will continue therapy until disease progression, unacceptable toxicity as a result of MLN8237, investigator's discretion, or withdrawal of patient consent. Patients will be followed with history, physical, and blood tests at each visit to monitor for toxicity. Patients will be followed for survival

endpoints following completion of this study until death for up to 3 years after completion of other study drug procedures.

8.10 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 will withdraw patients from the study for any of the following reasons:

- Occurrence of an unacceptable adverse event
- A treatment cycle delay of >3 weeks because of toxicity
- Patient request
- 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.

9.0 Study Drug Administration

MLN8237 will be administered PO at a dosage of 50 mg BID for 7 days in each treatment cycle, followed by a 14-day, treatment-free period. The study drug will be administered on an empty stomach with the patient remaining nothing by mouth (NPO), except for water and prescribed medications, for 2 hours before and 1 hour after each dose. Patients will be instructed to take each oral dose of MLN8237 with 8 ounces (1 cup, 240 mL) of water. For BID dosing, the doses must be taken at least 6 hours apart.

MLN8237 will be supplied as 10 mg ECT, with the dose strength expressed as milligrams of active drug (free acid); this strength will support the need for dose reduction if applicable. 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 MLN8237.

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

9.1 Description of Investigational Agents

MLN8237 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 MLN8237 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.

9.2 Preparation, Reconstitution, and Dispensation

MLN8237 ECT are packaged (10 tablets to a bottle) in a 60-cc high-density polyethylene (HDPE) bottle with a child-resistant cap. MLN8237 is an anticancer drugs, and as with other potentially toxic compounds, caution should be exercised when handling MLN8237. It is recommended that gloves and protective garments be worn during preparation.

9.3 Packaging and Labeling

The packaged and labeled study drug, MLN8237 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 MLN8237 ECT are packaged into each 60-cc HDPE bottle. MLN8237 will be supplied as ECT in 10 mg strength. The bottles will have a child-resistant cap and be labeled for take-home use. Patients will receive instructions for home use of MLN8237, including the requirement that MLN8237 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.

9.4. 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 MLN8237 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.

Patients are to be instructed on proper storage, accountability, and administration of MLN8237, including that MLN8237 is to be taken as intact tablets.

10.0. ADVERSE EVENTS

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.

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
- 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 one 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.
- With respect to the suspected transmission via a medicinal product of an infectious agent; 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 the terms *serious* and *severe* because they ARE NOT the same. The term *severe* is often used to describe the intensity (severity) of a specific event (as in mild, moderate, or severe myocardial infarction); the event itself, however, may be of relatively minor medical significance (such as a severe 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 functioning. A severe AE does not necessarily need to be considered serious. For example, persistent nausea of several hours duration may be considered severe nausea but may not be considered an SAE. On the other hand, a stroke resulting in only a minor degree of disability may be considered mild but would be defined as an SAE based on the above noted criteria. Seriousness (not severity) serves as a guide for defining regulatory reporting obligations.

Procedures for Reporting Serious Adverse Events (SAEs)

All SAEs occurring on this study will be reported to WCMC within 24 hours of Investigator notification of the event. SAEs should be reported to individual site's IRB per local guidelines. SAEs reported to WCMC will be reported on the institutional SAE reporting form and comprehensive AE & IND Reporting table and will be distributed to all study sites. WCMC forms may be downloaded http://www.med.cornell.edu/research/for_pol/ins_rev_boa.html

Adverse events (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 must be reported to WCMC (which will notify Millennium Pharmacovigilance). AEs which are serious must be reported to WCMC from first dose of MLN8237 up to and including 30 days after administration of the last dose of MLN8237. When possible, signs and symptoms indicating a common underlying pathology should be noted as one comprehensive event. Any SAE that occurs at any time after completion of MLN8237 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 the WCMC for further reporting to Millennium Pharmacovigilance. 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 (eg, 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).
This is an investigator-initiated study. The principal investigator, Himisha Beltran (who may also sometimes be referred to as the sponsor-investigator), is conducting the study and acting as the sponsor. Therefore, the legal/ethical obligations of the principal investigator include both those of a sponsor and those of an investigator.

Sponsor-investigator must report all SAEs, regardless of expectedness or relationship with any study drug, to Millennium Pharmacovigilance (or designee) as soon as possible, but no later than 5 calendar days of the sponsor-investigator's observation or awareness of the event. In the event that this is a multisite study, the sponsor-investigator is responsible to ensure that the SAE reports are sent to Millennium Pharmacovigilance (or designee) from all sites participating in the study. Subinvestigators must report all SAEs to the sponsor-investigator so that the sponsor-investigator can meet his/her foregoing reporting obligations to Millennium Pharmacovigilance, unless otherwise agreed between the sponsor-investigator and subinvestigator(s). Millennium Pharmacovigilance (or designee) may request follow-up information to a reported SAE, which the sponsor-investigator will be responsible for providing to Millennium Pharmacovigilance (or designee).

Millennium will provide a sample SAE Report Form representative of the information Millennium Pharmacovigilance may request in follow-up.

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 abnormality, will be determined by using the NCI CTCAE, version 4.03. The criteria are available online at http://ctep.cancer.gov/reporting/ctc.html.

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)?

Sponsor-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 5 calendar days of such communication.

Sponsor-investigator will be responsible for forwarding such reports to any subinvestigator(s).

Millennium Pharmacovigilance SAE and Pregnancy Reporting Contact Information: North America PPD, Inc. Safety and Medical Management, US Fax: +1 888-488-9697 Hotline number (available 24/7): 1-800-201-8725

Suggested Reporting Form:

- SAE Report Form
- US FDA MedWatch 3500A: http://www.fda.gov/Safety/MedWatch/HowToReport/DownloadForms/default.htm
- Any other form deemed appropriate by the sponsor-investigator

Data Safety Monitoring Board.

This study will utilize the Weill Cornell Medical College (WCMC) Institutional Data Safety Monitoring Board (DSMB) and follow its policies and procedures for monitoring this multicenter study for safety concerns, with ongoing updates from the Study Chair on a continuous basis.

The Weill Cornell DSMB is comprised of medical specialists and advisors on human rights issues in human subjects research. The DSMB currently has 9 members, meets at quarterly intervals during the year, and carries out ongoing review of protocols submitted throughout the year. Once a protocol has been submitted and approved by the Institutional Review Board (IRB) and is recommended for oversight by the DSMB, an independent research monitor will be assigned.

The DSMB evaluates the accumulated data from the study in order to monitor the safety of subjects throughout the trial and reviews the risks and benefits, as well as the efficacy, of the study. The DSMB will also evaluate the overall trial conduct and progress. Ultimately, the DSMB validates the continuation of the trial or determines if a study needs modification or termination.

Reports to the DSMB will include the following items for review:

1. Completed DSMB Periodic Review Form.
2. Synopsis of the study to date.
3. IRB approved consent form.
4. IRB current protocol.
5. Summary table of study results.
6. Adverse event table.
7. Data safety monitoring plan.

Safety monitoring is carried out to ensure and maintain the scientific integrity of human subject research projects and to protect the safety of human subjects. Safety monitoring can be viewed as any process during a clinical trial that involves the review of accumulated outcome data for groups of patient-subjects to determine if any of the treatment procedures practiced should be altered or stopped. NIH

Guidelines (1998, 2000) specify that all clinical trials should have a system in place for appropriate oversight and monitoring to ensure the safety of participants and the validity of the data.

Monitoring activities will be commensurate with the nature, size, and complexity of the trial in accordance with institutional policies and will be determined after IRB and DSMB review of the protocol immediately prior to study activation. For a small, single-center study, the monitoring is usually performed by a statistician in conjunction with a Safety Officer. For those single-site, high risk trials, a DSMB may be appropriate. For larger, single or multi-site studies, the monitoring is usually performed by a committee, often called a Data Safety Monitoring Board (DSMB). Ongoing review of the data by an independent individual or committee assures the investigators, the IRB, the study's sponsor, and the funding agency that the trial can continue without jeopardizing subjects' safety.

Weill Cornell Medical College requires that all research approved by the WCMC IRB include an appropriate plan for the monitoring of data to ensure the safety of human subjects. The research monitor, Peter Martin, MD, will be providing an unbiased written report to the USAMRMC Office of Research Protections (ORP) Human Research Protection Office (HRPO). Research supported by Federal agencies will be monitored according to all regulations and guidelines of the relevant Federal agency.

For this study, the initial planned report to be submitted to the DSMB will occur no later than after 19 evaluable subjects have received therapy on study or every 12 months (whichever event occurs sooner). Should at least 4 subjects experience a response as defined by the protocol prior to enrollment of the 19^{th} subject, we will notify the DSMB and plan to continue enrollment without a halt after subject #19. In addition, attributable, unexpected grade $\geq$ 3 SAE's will be reported to the DSMB within 24 hours of study team awareness of the event.

Procedures for Reporting Pregnancy of a Female Partner of Male Patient and Birth Events

If a female partner of a male patient becomes pregnant during the male patient's participation in this study, this must be reported to the Study Chair immediately. Every effort should be made to follow the pregnancy for the final pregnancy outcome (ie, delivery, still birth, miscarriage) and Study Chair or Millennium Pharmacovigilance will request this information from the investigator.

Product Complaints

A product complaint is a verbal, written, or electronic expression which 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
1-877-674-3784 (877-MPI-DRUG)

Product complaints in and of themselves are not AEs. If a product complaint or medication error results in an SAE, an SAE form should be completed and sent to PPD.

11. Removal from study

Patients may be removed from study for any of the following reasons:

- Disease progression
- Physician determination that discontinuation of MLN8237 is in the patient's best interest
- Withdrawal of patient consent
- Noncompliance with protocol-specified procedures which in the opinion of the investigator will expose subjects to undue risk or will significantly effect interpretation of study results

12. Statistical Considerations.

12.1. Sample Size Justification:

The primary endpoint is the proportion of patients who are free from radiographic progression at 6 months following treatment with MLN8237. Based on the phase II trial by Meulenbeld et al (BJUI 2012), the proposed null hypothesis (H0) is that ≤15% of patients will be radiographic progression-free at 6 months and the alternative hypothesis (Ha) is that ≥30% of patients will be radiographic progression-free at 6 months.

Sample size recommendations for the phase II design are determined according to Ahern's exact single-stage phase II design (A'Hern RP, 2001). We project a 6-month radiographic progression-free proportion of 15%, below which the response will be unacceptable, and a 6-month radiographic progression-free proportion of 30%, above which the regimen will be considered worthy of further exploration. The null hypothesis that the 6-month radiographic progression-free proportion is less than or equal to 15% will be tested against the alternative hypothesis that the 6-month radiographic progression-free proportion is greater than or equal to 30%.

The sample size computations were performed assuming a 5% level of significance and 80% power. A total of 48 patients will be required to enroll in the study. The treatment will be declared effective and worthy of further testing if 12 or more patients are free from radiographic progression at 6 months among the 48 patients entered into the study. This exact single-stage design yields a ≥ 0.80 probability of a positive result if the true percentage of patients who are free from radiographic progression at 6 months is ≥30%. It yields a ≥ 0.95 probability of a negative result if the true percentage of patients who are free from radiographic progression at 6 months is ≤15%. Assuming 10-20% of patients will be unevaluable/ineligible, we anticipate that a total 60 patients will be enrolled in the study.

Approximately 20% of patients are expected to have histologic entry criteria. With a sample size of approximately 12 patients meeting such criteria (i.e., 20% of 60 enrolled patients), a 95% confidence interval for the 6-month radiographic progression-free proportion in this subgroup of 12 patients can be expected to be within ± 25.9% of the true 6-month radiographic progression-free proportion in this subgroup. This calculation assumes a 6-month radiographic progression-free proportion of 30% in patients with biopsy positive NEPC. This calculation is for descriptive/exploratory purposes only, with the intent of estimating the 6-month radiographic progression-free proportion among the small subgroup of patients with biopsy positive NEPC.

Analysis Plan for Endpoints:

Primary Endpoint:

The primary endpoint of 6-month radiographic progression-free proportion will be estimated and a 95% confidence interval will be estimated via binomial proportions.

Secondary Clinical Endpoints:
With adequate follow-up time, secondary endpoints of response rate and overall survival (OS) will be assessed. Radiologic response rate will be estimated and a 95% confidence

interval will be estimated via binomial proportions. For OS, Kaplan-Meier survival analysis and 95% confidence intervals will be calculated using Greenwood's formulae.

The frequency of subjects experiencing toxicities will be tabulated. Toxicities will be assessed and graded according to CTCAE v. 4.0 terminology. Exact 95% confidence intervals around the toxicity proportions will be calculated to assess the precision of the obtained estimates.

Exploratory objectives/correlative studies will be evaluated using descriptive statistics, graphical methods, and statistical modeling, as appropriate, to explore the relationship between response and Aurora –A and N-myc overexpression and/or amplification in circulating tumor cells or archival tissue.

REFERENCES:

A'Hern RP. Sample size tables for exact single-stage phase II designs. Statistics in Medicine. 2001;20:859-866.

12.3. Measurement of Effect

Progressive standard prostate cancer is often manifest by rising PSA levels, new lesions on bone scan, new disease-related symptoms and increasing size of a measurable soft tissue mass. Response is commonly assessed either biochemically (PSA change) or by change in size of a measurable lesion/s. Given that patients with NEPC do not often secrete PSA and all patients on study will have measurable disease, the primary outcome will be determined by radiologic response and progression. PSA response rate is a secondary endpoint.

12.3.1 Change in lesion size

Complete response (CR) is defined as complete disappearance of all measurable and evaluable lesions by physical examination or imaging studies and normalization of PSA with no appearance of new lesions for > 1 month. Partial response (PR) is defined as a 30%

or greater reduction in the sum longest unidimensional diameter of all measurable lesions. There may be no new lesions. Stable Disease (SD) is characterized by patients who do not meet the criteria of PR and who are without signs of progressive disease for at least 1 month. Disease Progression (DP) is defined as a greater than 20% increase in the sum longest unidimensional diameters of the indicator lesions or the appearance of new lesions. Bone scan progression (evaluable disease only) is defined by PCWG2 criteria. Per consensus guidelines in CRPC, to be considered measurable, lymph nodes need to be at least 2 cm in greatest dimension and 1.5 cm in short axis. [32 and RECIST 1.1]

12.3.2 Serum marker response (PSA, chromogranin, neuron specific enolase)

Serum PSA, chromogranin, and neuron specific enolase (NSE) responses are determined by comparing the nadir level after therapy to the baseline, pre-treatment level determined just prior to initiating therapy. Declines of ≥ 30% and 50%, confirmed by a value ≥ 2 weeks later, will be reported. Serum marker progression will be defined as a rise of > 25% above either the pretreatment level or the nadir level (whichever is lowest). PSA must increase by > 2 ng/ml to be considered progression. Confirmation requires a second consecutive rising PSA, chromogranin, or NSE at least 2 weeks apart. Marker stabilization is referred to as any set of values that do not meet the criteria for response or PSA progression.

12.3.3 CTC count response (CellSearch). Baseline CTC counts of < 5 when measured by CellSearch methodology have been associated with better prognosis (overall survival).[34, 35] In addition, changes in CTC counts after initial therapy have been demonstrated to be predictive.[36] We will explore the prognostic and predictive significance of 4 groups as has been described:

Baseline < 5, follow up < 5

Baseline $\geq$ 5, follow up < 5

Baseline < 5, follow up $\geq$ 5

Baseline $\geq$ 5, follow up $\geq$ 5

In addition, we will report individual subject's CTC count change from baseline.

13.0 Data Collection The data collection plan for this study is to utilize a study-specific (WCMC CTSC-supported) REDCap database to capture all treatment, toxicity, and efficacy data for all enrolled patients.

13.1 Confidentiality In order to protect confidentiality of the data, patient full names will be removed and data coded with patient initials and study subject numbers. All primary data will be kept in locked file cabinets or password-protected computers and available only to the investigators and appropriate regulatory personnel (e.g., IRB, FDA, etc.).

14.0 ADMINISTRATIVE REQUIREMENTS

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.

Ethical Considerations

The study will be conducted in accordance with ethical principles founded in the Declaration of Helsinki (see Section 0). 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.

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).

Patient Confidentiality

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 WCMC 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.

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). Significant 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.

On-site Audits

Regulatory authorities, the IEC/IRB, WCMC personnel or their delegates 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.

Drug Accountability

Accountability for the drug 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.
All material containing MLN8237 will be returned to Millennium Pharmaceuticals, Inc.

Premature Closure of the Study

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

Record Retention

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

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APPENDIX 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 to 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 judgement 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.

APPENDIX 2

Common Terminology Criteria for Adverse Events Version 4.0x

Available at:

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

APPENDIX 3

Study Steering Committee

The initial protocol has been designed by WCMC investigators in collaboration from external investigators and input from Millennium Pharmaceuticals.

In the event that protocol discrepancies arise or future amendments are deemed necessary, a discussion with investigators may occur. Ultimate decisions affecting the protocol, including amendments, future analyses, and presentation/publication will be determined by the Steering Committee, comprised of:

- WCMC Core Investigators (Beltran, Christos, Tagawa)
- PCCTC Sponsor
- Additional external Investigator