CLINICAL PHASE Ib/II TRIAL OF L-NMMA PLUS TAXANE CHEMOTHERAPY IN THE TREATMENT OF REFRACTORY LOCALLY ADVANCED OR METASTATIC TRIPLE NEGATIVE BREAST CANCER PATIENTS.

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Abbreviation	Definition
ALP	alkaline phosphatase
ALT	alanine aminotransferase
ANC	absolute neutrophil count
aPTT	activated partial thromboplastin time
ASCO	American Society of Clinical Oncology
AST	aspartate aminotransferase
ATA	antitherapeutic antibody
AUC	area under the plasma concentration versus time curve
°C	degrees Celsius
μM	Micromolar
AE	adverse event
BSA	body surface area
BUN	blood urea nitrogen
CBC	complete blood count
Cm	Centimeter
CO	cardiac output
CR	complete response
CTCAE	(NCI) Common Terminology Criteria for Adverse Events
CTEP	Cancer Therapy Evaluation Program
dL	Deciliter
CL	clearance, IV dosing
C_{max}	single-dose maximum (peak) concentration
CO_2	carbon dioxide
COPD	chronic Obstructive Pulmonary Disease
CSF	cerebral spinal fluid
CT	computed tomography
CSF	cerebral spinal fluid
CT	computed tomography
DLT	dose-limiting toxicity
DNA	deoxyribonucleic acid
ECT	enteric-coated tablet
FDA	Food and Drug Administration
FPA	fundus pulsation amplitude
GCP	Good Clinical Practice
G-CSF	granulocyte colony stimulating factor
GEMM	genetically engineered mouse models
GGT	gamma glutamyl transferase
GI	Gastrointestinal
GLP	Good Laboratory Practice
Hb	Hemoglobin
Hct	Hematocrit
ht	Height
HDPE	high-density polyethylene

Abbreviation	Definition
HIV	human immunodeficiency virus
HMCC	Houston Methodist Cancer Center
HMRI	Houston Methodist Research Institute
HNSTD	highest nonseverely toxic dose
HDPE	high-density polyethylene
HR	heart rate
IEC	independent ethics committee
IND	Investigational New Drug
IRB	institutional review board
IV	Intravenous
kg	Kilogram
Ki	inhibitory constant
Lbs	Pounds
LDA	limiting dilution assays
L-NMMA	NG-monomethyl-l-arginine
m^2	square meters
mg	Milligram
min	Minute
mL	milliliter
mm^3	cubic millimeters
Mmol	Millimole
MSFE	mammosphere formation efficiency
MTD	maximum tolerated dose
NCI	National Cancer Institute
NF-κB	nuclear factor-κB
ng	nanogram
nM	Nanomole
NO	nitric oxide
NYHA	New York Heart Association
ORR	Objective response rate
OS	oral solution
p21	p21(ras) farnesyl-protein transferase
p27	cyclin-dependent kinase inhibitor
p53	tumor suppressor protein with molecular weight of 53 kDa
PIC	powder-in-capsule
RP2D	recommended phase 2 dose
RP56976	Docetaxel
SAE	serious adverse event
TCGA	The Cancer Genome Atlas
TGD	tumor growth delay
TGI	tumor growth inhibition
TK	Toxicokinetics
TNBC	triple Négative Brest cancer
TNF-R	tumor necrosis factor-receptor
US	United States
USP	United States Pharmacopeia
ULN	upper limit of the normal range
w/w	weight-to-weight ratio

Abbreviation	Definition
wt	Weight

Glossary of terms

Assessment	A procedure used to generate data required by the study
Baseline	For efficacy evaluations, the baseline assessment will be the last available assessment before or on the date of randomization. For safety evaluations (i.e. laboratory assessments and vital signs), the baseline assessment will be the last available assessment before or on the start date of study treatment. The value obtained at baseline assessments, referred to as "baseline value" will be used as reference for the patient.
Control drug	A study treatment used as a comparator to reduce assessment bias, preserve blinding of investigational drug, assess internal study validity, and/or evaluate comparative effects of the investigational drug
Dose level	The dose of drug given to the patient (total daily or weekly etc.)
Enrollment	Point/time of patient entry into the study; the point at which informed consent must be obtained (i.e. prior to starting any of the procedures described in the protocol)
Investigational drug	The study treatment whose properties are being tested in the study; this definition is consistent with US CFR 21 Section 312.3 and is synonymous with "investigational new drug."
Investigational treatment	Drug whose properties are being tested in the study as well as their associated placebo and active treatment controls (when applicable). This also includes approved drugs used outside of their indication/approved dosage, or that are tested in a fixed combination. Investigational treatment generally does not include other study treatments administered as concomitant background therapy required or allowed by the protocol when used in within approved indication/dosage
Medication number	A unique identifier on the label of each study treatment package which is linked to one of the treatment groups of a study
Other study treatment	Any drug administered to the patient as part of the required study procedures that was not included in the investigational treatment
Patient	A unique identifying number assigned to each patient/subject/healthy volunteer who enrolls in the study
Period	A subdivision of the study timeline; divides stages into smaller functional segments such as screening, baseline, titration, washout, etc.
Premature patient withdrawal	Point/time when the patient exits from the study prior to the planned completion of all study treatment administration and/or assessments; at this time all study treatment administration is discontinued and no further assessments are planned, unless the patient will be followed for progression and/or survival
Stop study participation	Point/time at which the patient came in for a final evaluation visit or when study treatment was discontinued whichever is later
Study treatment	Includes any drug or combination of drugs in any study arm administered to the patient (subject) as part of the required study procedures, including placebo and active drug run-ins. In specific examples, it is important to judge investigational treatment component relationship relative to a study treatment combination; study treatment in this case refers to the investigational and non-investigational treatments in combination.
Study treatment discontinuation	Point/time when patient permanently stops taking study treatment for any reason; may or may not also be the point/time of premature patient withdrawal
Supportive treatment	Refers to any treatment required by the exposure to a study treatment, e.g. premedication of vitamin supplementation and corticosteroid for pemetrexed disodium.
Variable	Identifier used in the data analysis; derived directly or indirectly from data collected using specified assessments at specified time points

1. Background

Despite the significant advances in breast cancer biology, there has been limited progress in the treatment of advanced breast cancer, with little change in the overall survival for women with treatment resistant metastatic breast cancer over the last several decades[1]. It is notable that approximately 40,000 women with metastatic breast cancer die each year because of treatment resistance and failure of current therapies. Our research focuses on delineating mechanisms of treatment resistance to identify novel treatment approaches to improve patient outcome. Classical models of carcinogenesis can be described as random or "stochastic" in which any cell can be transformed by accumulating the right combination of mutations. An alternative model is that subpopulations or clones of cells retain key stem-like properties including the capacity for self-renewal that drives carcinogenesis, as well as differentiation which contributes to cellular heterogeneity. Experimental evidence supporting this intratumoral clonal heterogeneity was first reported in human leukemia by John Dick et al[2]. These concepts were then extended to solid tumors by some groups demonstrating that human breast cancers were driven by stem-like cells characterized by cell surface expression of $CD44^{+}/CD24^{-/low}$[3]. Large-scale sequencing analyses of solid cancers have provided further evidence of the extensive heterogeneity within individual tumors[4]. This intratumoral heterogeneity[5-7] may be a major contributor to treatment resistance and treatment failure. Different subpopulations may be associated with heterogeneous protein function that may foster tumor adaptation and lead to therapeutic failure through Darwinian selection. Accordingly, the subpopulations of cells with stem-like properties within a heterogeneous bulk tumor have been shown to be responsible for tumor initiation and recurrence[3,8-12]. Three groups have recently and independently provided direct and functional evidence for the presence of cells with stem-like properties by lineage tracing experiments in glioblastomas (GBM), squamous skin tumors, and intestinal adenomas, further corroborating the hierarchical nature of cancer[13-15]. These independent groups confirm that only a fraction of cells within the bulk tumor have clonogenic potential[13-15] and that this fraction is intrinsically resistant to chemotherapy[13].

2. Significance

We were one of the first groups to demonstrate that breast cancer cells with stem-like properties were intrinsically resistant to conventional therapy[9,16]. Since these initial observations, other groups have confirmed this view by establishing the resistance of these cells to conventional chemotherapy and radiation therapy[17-20]. These and other studies have also supported our finding that an increase in the stem-like cell population is associated with a worse prognosis[3,17,19-21]. These findings have fundamental clinical implications. Current development of cancer therapeutics is largely based on identifying agents with the ability to cause bulk tumor regression in animal models or in clinical trials; however, an exclusive focus on drugs that elicit tumor regression by killing actively cycling or fully differentiated cells may spare the critical population of therapy-resistant cells. We have recently extended these observations to breast cancer and have shown that subpopulations of chemoresistant cells within the bulk primary tumor had the propensity to metastasize through an array of different adaptive mechanisms.

We have also identified a tumorigenic signature from patient breast cancer biopsies[16] and, subsequently, used a functional approach to identify novel targets for treatment resistance from this gene set. Using shRNA knockdown of the 477 genes in our tumorigenic signature, we performed a high throughput mammosphere formation efficiency (MSFE) screen. This approach identified two target proteins, RPL39 and MLF2. RPL39 was previously recognized as a component of the 60S ribosomal complex located on chromosome X (XQ24) with a proposed role in spermatogenesis and protein translation[22,23]. MLF2 is located on chromosome 12 and may participate in chromosomal aberrations and cellular defense responses[24]. While little is known about the role of RPL39 in cancer, there is even more limited knowledge available on MLF2. A series of amino acid modifications of MLF2 on Ser 144, 152 and 238[25-27] and a somatic mutation (Phe80Cys) has been linked to colorectal cancer[28]. Notably, RPL39 and MLF2 overexpression increased cell migration, proliferation and mammosphere formation, suggesting a potentially important function for these two genes in cancer. Comprehensive understanding of the mechanisms of RPL39 and MLF2 is a salient prerequisite for the confirmation of these two genes as novel cancer targets. By mutual exclusivity analysis of RPL39 and MLF2

using The Cancer Genome Atlas (TCGA) database, RPL39 and MLF2 were found to exclusively co-occur ($p<0.00001$), suggesting a shared mechanistic pathway for both genes. Using microarray analysis, we identified "cellular effects of sildenafil (Viagra)" i.e. nitric oxide (NO) signaling as the primary pathway that linked both RPL39 and MLF2. We then confirmed the role of NO signaling by inducing iNOS (inducible nitric oxide synthase) protein with overexpression of RPL39 and MLF2 and reducing iNOS protein levels with siRNA (small interfering ribonucleic acid) silencing of RPL39 and MLF2. In literature, the role of NOS signaling in breast cancer biology has not been extensively studied. Reports to date suggest that high NO concentrations are cytotoxic to cancer cells whereas lower NO concentrations can enhance tumor growth[29-31]. iNOS expression has been correlated with increased tumor grade and aggressiveness of breast tumor cells[32,33], specifically in basal-like triple negative breast cancer (TNBC)[32]. Increased iNOS expression has been correlated with treatment resistance in GBM[34,35]. *In vitro* anti-tumor activity of iNOS inhibitors has been previously reported in epidermoid carcinoma[36], oral[37], hepatic[38], glioblastoma[39], and breast cancer[35,40-42]. Collectively, this lead us to ask whether inhibition of NO signaling could target TNBC therapeutically.

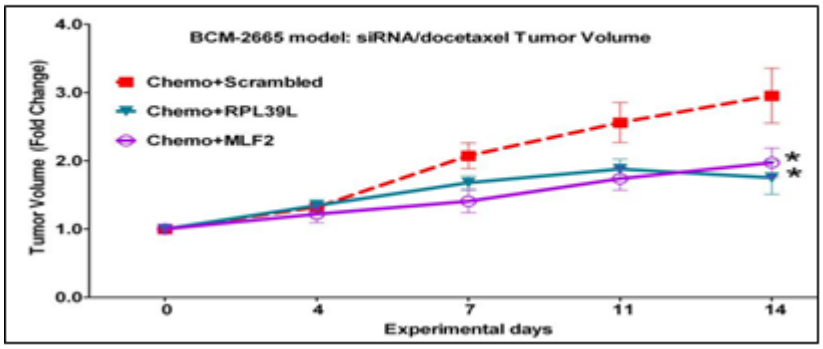
Here, we hypothesize that targeting all cells within heterogeneous bulk tumor, particularly those cells that have metastatic propensity, will be essential for elimination of cancer.

2.1 Approach And Preliminary Studies

We have previously identified two novel cancer genes (RPL39 and MLF2) that play a role in treatment resistance and lung metastases. We determined that upregulation of NO signaling was a common mechanistic pathway for both genes. We next determined that inhibition of NO signaling with L-NMMA diminishes the number of treatment resistant cells, as well as lung metastases in human TNBC cell lines. Thus, we have identified a novel target for TNBC as well as a potential treatment, a re-purposed drug L-NMMA, to be further evaluated in the phase 1b clinical trial, as proposed here. Secondly, we have developed one of the largest series of twenty-seven well-characterized patient-derived xenograft (PDX) models. Transcriptome and proteome analysis indicate that the serially transplanted xenografts are similar, thus confirming that we have generated a renewable source of clinically relevant patient-derived human breast cancers for *in vivo* experiments. Thirdly, we have genetically engineered mouse models (GEMM) with metastatic potential, with intact immune system. Fourthly, access to GMP grade L-NMMA from ArgiNox Pharmaceuticals Inc. will enable us to conduct a phase 1b clinical study. This investigation seeks to establish a novel approach in overcoming treatment-resistant triple negative breast cancer for which no targeted therapies exist and where most women will eventually die from their disease.

APPROACH: <u>Preliminary data:</u> Derivation of a tumorigenic gene signature

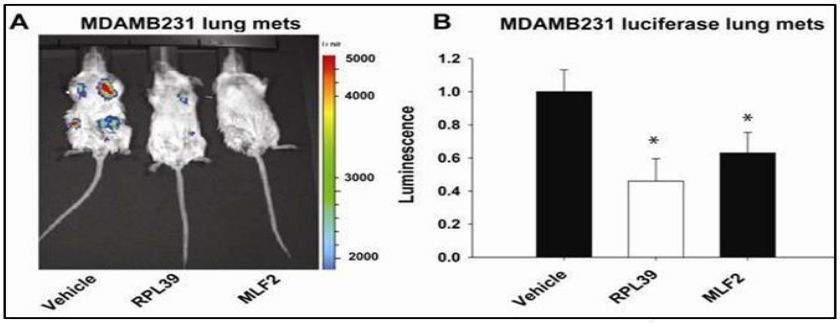
Fig 1. Significant decrease in tumor volume in patient-derived xenograft model treated with docetaxel+siRNA (RPL39 and MLF2) compared to chemotherapy alone.



We previously reported that the proportion of treatment resistant cells with stem-like properties increases after chemotherapy[9]. In matched biopsies, the relative proportion of cells bearing stem cell marker $CD44^{+}/CD24^{-/low}$ and MSFE increased significantly after chemotherapy ($p<0.001$)[9]. We isolated tumorigenic populations from patient biopsies by two selection criteria: expression of $CD44^{+}/CD24^{-/low}$ or survival in MS assay. These populations were evaluated by comparative gene expression analysis: $CD44^{+}/CD24^{-/low}$ and MS vs. non-tumorigenic cells. The number of genes in the two separate "enrichment" comparisons greatly exceeded chance according to multiple statistical tests. Hence, we defined a "$CD44^{+}/CD24^{-/low}$/MS gene signature" of the 477 genes present in the significant overlap between both enrichment methods[16].

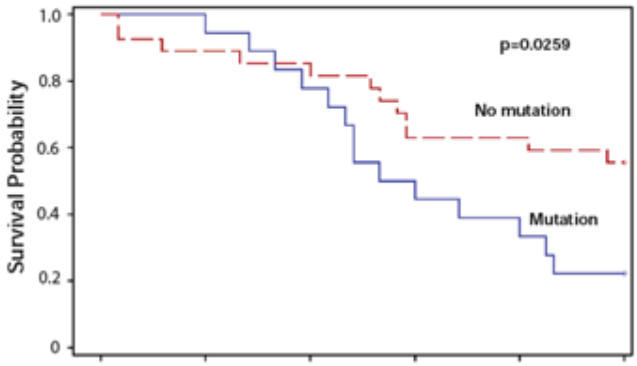
Identification of RPL39 and MLF2 By shRNAs knockdown of the ~477 genes[45] using 1124 shRNAs (~2-3 shRNA per gene) in high-throughput screening, two top candidate genes, RPL39 and MLF2 were identified. In PDX and other models, animals treated with RPL39 and MLF2 siRNAs showed a significant decrease in tumor volume compared to chemotherapy (Fig 1), together with significant reduction in CD44+/CD24-/low and MSFE, as well as decreased tumor initiation in limiting dilution assays (LDA). Additionally, RPL39 and MLF2 siRNA significantly reduced lung metastasis (Fig 2). Importantly, we described potential activating mutations in RPL39 and MLF2 that had not been previously identified in TCGA in lung metastases from breast cancer patients. Primary tumors were sequenced in TCGA and were unlikely to be of sufficient depth of coverage to detect small subclones of these treatment resistant cells. Next, in paired patient primary and lung metastatic samples, we determined that these activating mutations were present only in metastatic lesions, thus confirming **our hypothesis that small subpopulations of chemoresistant cells within the bulk primary had the propensity to metastasize**, **and that targeting this intra-tumoral heterogeneity is essential for the eradication of cancer.**

Fig 2. Effect of RPL39 and MLF2 siRNA on lung metastasis. (A) RPL39 and MLF2 siRNA treated mice 6weeks after primary tumor injection. (B) Reduced luminescence observed with RPL39 and MLF2 siRNA treatment



Importantly, patients with these activating mutations had a significantly worse survival (Fig 3). In summary, we have shown that silencing two novel genes (RPL39 and MLF2) in PDX and other xenograft models dramatically reduced primary tumor volume and lung metastases, thus improving overall survival.

Fig 3. Significantly worse survival in patients with activating mutations in RPL39/MLF2 by Kaplan Meier analysis



RPL39, MFL2, and nitric oxide signaling Mutual exclusivity analysis of MLF2 and RPL39 using TCGA database determined that RPL39 and MLF2 co-occur exclusively with p<0.00001, thus indicating a common mechanistic pathway for both genes. To gain insight to this mechanistic pathway, siRNA-treated PDX tumors described above were analyzed by gene expression microarray profiling. Ingenuity Pathway Analysis revealed that the pathway associated with "cellular effects of sildenafil (Viagra)" or nitric oxide (NO) signaling was common to both RPL39 and MLF2. Consequently, we specifically addressed the role of NO signaling.

A significant increase in inducible nitric oxide synthase (iNOS) was observed with overexpression of RPL39 or MLF2, whereas a decrease in iNOS and eNOS was seen with knockdown of RPL39 and MFL2 (Fig 4).

Efficacy of the small molecule pan-NOS inhibitor LNMMA as a re-purposed cancer therapeutic

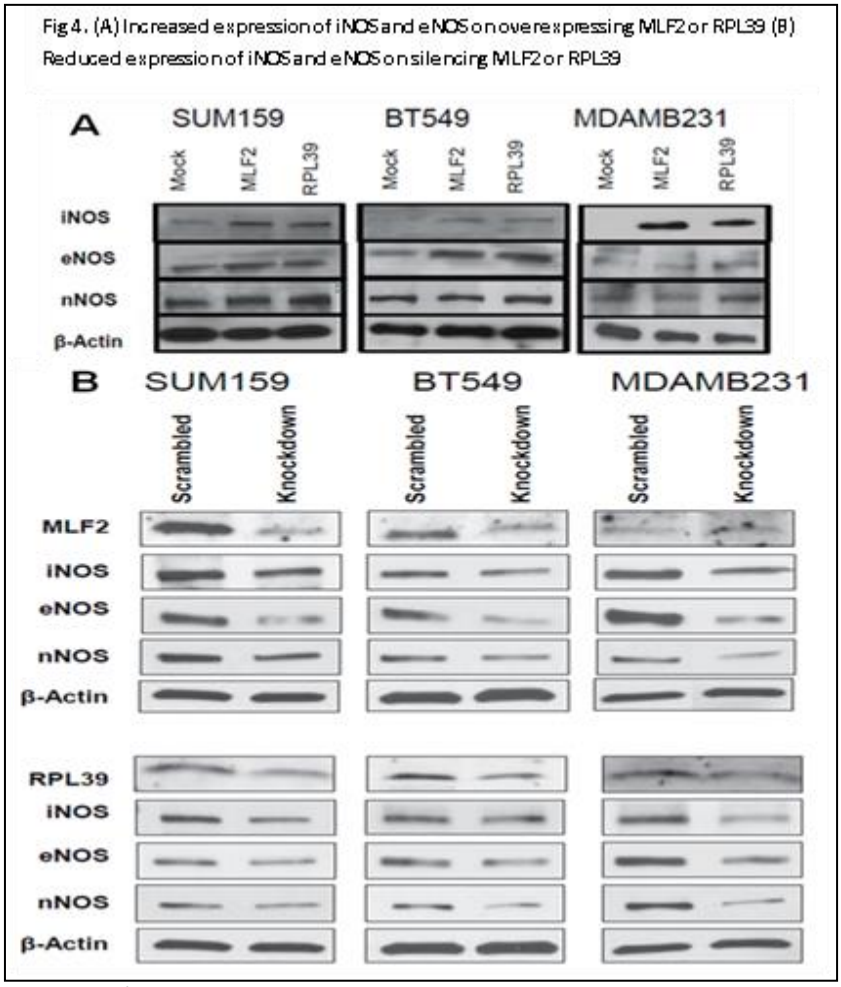


Fig 4. (A) Increased expression of iNOS and eNOS on overexpressing MLF2 or RPL39 (B) Reduced expression of iNOS and eNOS on silencing MLF2 or RPL39

As siRNA-based therapies are still in early clinical development, we sought to determine if the small molecule pan-NOS inhibitor LNMMA could be an effective targeted therapy for treatment resistant and metastatic TNBC. Specifically, this drug was selected as it has been tested in several thousand patients by **Dr. Steven Gross** and colleagues in a randomized phase 3 study for cardiogenic shock (reviewed from Kilbourn[46]), The study was conducted to established the safety of LNMMA, but failed to reach its efficacy goal for cardiogenic shock. Thus, LNMMA could be re-purposed as therapy for treatment resistant metastatic triple negative breast cancer, for which no effective targeted therapy currently exists. *In vitro,* LNMMA potently and significantly decreased proliferation, MSFE, and migration.

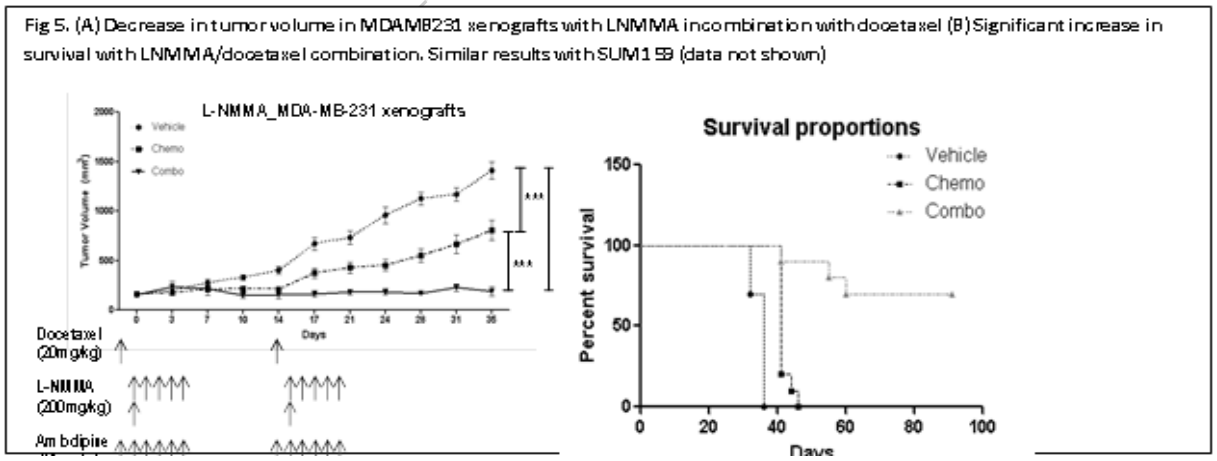
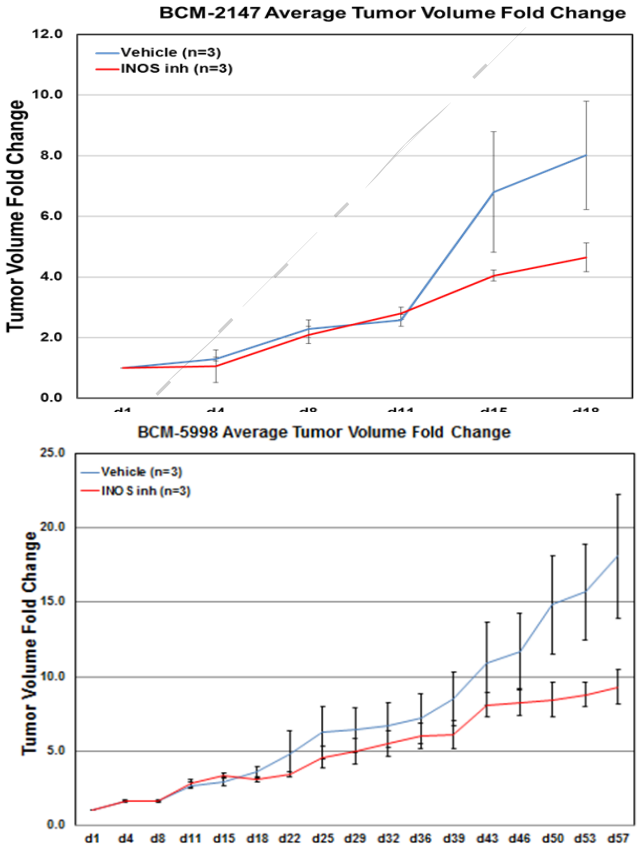


Fig 5. (A) Decrease in tumor volume in MDAMB231 xenografts with LNMMA in combination with docetaxel (B) Significant increase in survival with LNMMA/docetaxel combination. Similar results with SUM159 (data not shown)

LNMMA is associated with an acute blood pressure (BP) elevation through inhibition of constitutive endothelial nitric oxide synthase (eNOS)[46]. To overcome LNMMA-induced hypertension, we tested an attenuated treatment regimen of LNMMA with the anti-hypertensive amlodipine, for two cycles in two different mouse cancer models (MDA-MB-231 and SUM159 xenografts), together with docetaxel chemotherapy. LNMMA was given after chemotherapy for 5 days at 200 mg/kg, which is comparable to previous clinical doses[47] . Our

results indicate that the increase in blood pressure (mean systolic pressure of 147 mmHg compared to basal levels of 120 mmHg) was reversed by the calcium channel blocker amlodipine (10 mg daily [QD] for 6 days, i.p). This elevation in BP was transient and disappeared within 24 hours after the last injection of LNMMA. Of note, this combination of LNMMA and docetaxel decreased tumor growth in a MDA-MB-231 orthotopic model (Fig 5). This dose regimen also showed improvement in survival. Similar results were found in SUM159 xenografts (data not shown). Overall, these data indicate that the dose regimen proposed here is effective and safe in reducing tumor growth and metastases (data not shown) without elevations in BP. Limiting dilution data in both SUM 159 and MDA-MB-231 showed a significant decrease in tumor initiating capacity with NOS inhibition.

Fig 6. Significant decrease in volume in two PDX models (BCM 2147 and BCM 5898) with LNMMA vs. vehicle.



Preclinical in vivo results in patient derived xenografts

We evaluated the effect of LNMMA in two basal-like TNBC PDX models (BCM 5898 and BCM 2147). BCM 5898 had differential higher expression of iNOS pathways compared to BCM 2147 (Fig 8). LNMMA (200 mg/kg, i.p for 5 days) every three-week cycle, compared to vehicle. This preliminary data in only two TNBC PDX models demonstrated a statistically significant reduction in tumor volume with LNMMA in both models (Fig 6). This dose of LNMMA in mouse models is equivalent to 10-15 mg/kg in humans, as is used in this proposal (FASEB J. 22, 659-661:2007).

In summary, we describe LNMMA as an effective small molecule inhibitor targeted against treatment resistance and metastases. Our findings lay the foundation for developing new therapies for women with treatment-refractory triple negative breast cancer for whom prognosis is extremely poor.

2.2 LNMMA Clinical Experience

Randomized phase 3 study in cardiogenic shock

A Randomized Controlled Phase III Trial, testing the effect of Tilarginine Acetate in patients with Acute Myocardial Infarction and Cardiogenic Shock was completed in 2007.[67] **TRIUMPH** was a prospective, international, multicenter, randomized, double-blind, placebo-controlled trial that tested the hypothesis that Tilarginine compared with placebo would reduce by 25% 30-day all-cause mortality in patients with MI complicated by cardiogenic shock despite successful revascularization of the infarct artery.

The study was conducted at 130 centers in 8 countries in North America and Europe. Participants, or their legally authorized representatives, provided written informed consent. Institutional review board or ethics committee approval was obtained at all sites. Inclusion required all of the following: (1) MI, confirmed by ischemic symptoms for at least 30 minutes with elevated cardiac markers and/or ST-segment elevation or left bundle-branch block; (2) patency (<70% stenosis) of the infarct artery, either occurring spontaneously and confirmed at angiography or after percutaneous revascularization; (3) refractory cardiogenic shock of less than 24 hours' duration, confirmed by peripheral signs of tissue hypoperfusion and systolic blood pressure less than

100mmHg despite vasopressor therapy (dopamine≥7 μg/kg per minute or norepinephrine or epinephrine ≥0.15 μg/kg per minute) continuing longer than 1 hour after infarct artery patency; (4) clinical or hemodynamic evidence of elevated left ventricular filling pressures; and (5) left ventricular ejection fraction of less than 40%. Hemodynamics and requirement for vasopressor treatment were reconfirmed after randomization just prior to study drug administration; patients with resolving shock were excluded. Between January 2005 and August 2006, 398 met the study inclusion criteria and were enrolled. Baseline characteristics of the population are shown in TABLE 1. All baseline characteristics were well balanced between the treatment groups. More than one quarter of the population was older than 75 years; the majority were male and of white race. More than half of the patients had hypertension and one third had diabetes; 84 (21%) had a history of heart failure and almost a third of those had advanced heart failure symptoms in the 6 weeks prior to enrollment. A quarter of the patients had baseline creatinine levels of 1.7 mg/dL (150 mol/L) or higher.

	Tilarginine (n = 206)	Placebo (n = 190)	Overall (N = 396)	P Value
Demographics				
Age, median (IQR), y	67 (57-76)	68 (56-76)	67 (56-76)	.98
Age ≥75 y, No. (%)	55 (27)	50 (27)	105 (27)	.96
Male sex, No. (%)	152 (74)	133 (70)	285 (72)	.40
White race, No. (%)	189 (92)	173 (91)	362 (92)	.68
Height, median (IQR), cm	173 (165-178)	170 (165-176)	172 (165-178)	.27
Weight, median (IQR), kg	80 (68-90)	75 (67-85)	76 (67-90)	.19
Medical history, No. (%)				
Hypertension	114 (56)	112 (60)	226 (58)	.43
Diabetes mellitus	75 (36)	58 (31)	126 (34)	.23
Current tobacco use	68 (34)	58 (31)	134 (33)	.49
Prior MI	60 (29)	49 (26)	109 (28)	.48
Prior CABG	14 (6.8)	15 (7.9)	29 (7.3)	.68
Prior heart failure	44 (22)	40 (21)	84 (21)	.94
Among those with heart failure, highest NYHA class in 6 wk				
I	6 (14)	3 (8)	9 (11)	.59
II	17 (39)	12 (32)	29 (35)	
III	12 (27)	11 (29)	23 (28)	
IV	9 (21)	12 (32)	21 (26)	
Prior cerebrovascular disease	12 (5.9)	18 (9.5)	30 (7.6)	.18
COPD	21 (10.2)	11 (5.8)	32 (8.1)	.11
Blood pressure, median (IQR), mm Hg†				
Systolic	88 (80-95)	89 (81-93)	88 (80-95)	.69
Diastolic	50 (43-60)	53 (45-60)	52 (43-60)	.54
Electrocardiogram findings, No. (%)				
ST-segment elevation	163 (79)	144 (76)	307 (78)	.43
Q wave	58 (28)	53 (28)	111 (28)	.95
Left bundle-branch block	27 (13)	21 (11)	48 (12)	.53
ST-segment depression	41 (20)	47 (25)	88 (22)	.25
Infarct artery, No. (%)				
Left main	23 (11)	24 (13)	47 (12)	.73
Left anterior descending	125 (61)	106 (56)	231 (58)	
Left circumflex	29 (14)	25 (13)	54 (14)	
Right coronary artery	23 (11)	26 (14)	49 (12)	
Bypass graft	6 (2.9)	9 (4.7)	15 (3.8)	
Extent of disease (≥50%), No. (%)				
1 vessel	89 (45)	84 (45)	174 (45)	>.99
2 vessels	43 (22)	33 (18)	76 (20)	
3 vessels or left main	68 (34)	70 (37)	138 (36)	
Left ventricular ejection fraction, median (IQR), %†	27 (20-32)	27 (20-33)	27 (20-32)	.72
Creatinine, median (IQR), mg/dL‡	1.3 (0.9-1.7)	1.4 (1.0-1.9)	1.3 (0.9-1.7)	.32
Time interval, median (IQR), h				
MI to shock	5.0 (1.9-10.3)	4.2 (1.0-11.7)	4.6 (1.5-10.9)	.33
Shock to open infarct artery	1.2 (0.3-2.9)	1.6 (0.2-3.2)	1.4 (0.3-3.0)	.21
Open infarct artery to randomization	5.1 (2.8-13.4)	5.1 (2.4-12.8)	5.1 (2.5-13.3)	.81

Table 1. Baseline Patient Characteristics*
Abbreviations: CABG, coronary artery bypass graft surgery; COPD, chronic obstructive pulmonary disease; ECG, electrocardiogram; IQR, interquartile range; MI, myocardial infarction;
NYHA, New York Heart Association.
SI conversion factor: To convert creatinine to µmol/L, multiply values by 88.4.
*The 2 patients for whom treatment assignment is unknown were not included (Figure 1).
†Blood pressure and ejection fraction were measured on support measures.
‡Baseline creatinine available only for 158 of 396 (40%) patients.

	Tilarginine (n = 206)	Placebo (n = 190)	Overall (N = 396)	P Value
No. of vasopressors, No. (%)				
1	126 (61)	132 (70)	258 (65)	.49
2	62 (30)	45 (24)	107 (27)	
3	9 (4.4)	8 (4.2)	17 (4.3)	
4	7 (3.4)	4 (2.1)	11 (2.8)	
Dopamine, No. (%)	139 (68)	135 (71)	274 (69)	.44
Dose, median (IQR), μg/kg per minute	10.0 (7.2-14.0)	8.3 (7.0-14.0)	9.1 (7.0-14.0)	.57
Norepinephrine, No. (%)	103 (50)	89 (47)	192 (48)	.53
Dose, median (IQR), μg/kg per minute	0.2 (0.2-0.4)	0.2 (0.2-0.5)	0.2 (0.2-0.4)	.80
Epinephrine, No. (%)	42 (20)	23 (12)	65 (16)	.03
Dose, median (IQR), μg/kg per minute	0.2 (0.1-0.4)	0.2 (0.1-0.4)	0.2 (0.1-0.4)	.47
Phenylephrine, No. (%)	21 (10.2)	15 (7.9)	36 (9.1)	.43
Dose, median (IQR), μg/kg per minute	1.1 (0.6-1.9)	1.3 (0.4-2.0)	1.1 (0.6-1.9)	.95

Abbreviation: IQR, interquartile range.

Table 2. Baseline Vasopressor Use

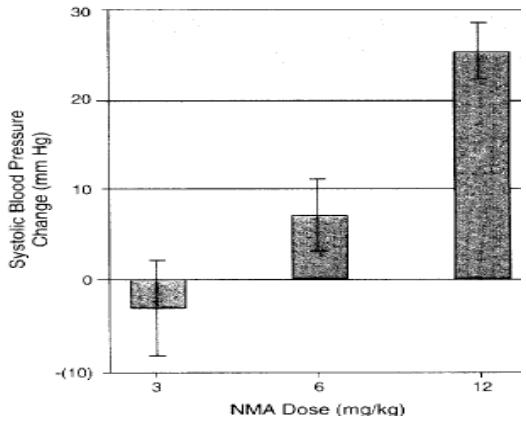
The median supported blood pressure just prior to study drug administration was 88/52 mm Hg. Most patients were supported with a single vasopressor at the time of study drug administration (TABLE 2). The majority of patients presented with anterior, ST-segment elevation MI with left anterior descending infarct artery location. Percutaneous coronary intervention was performed in nearly all patients to achieve the requirement for less than 70% infarct artery stenosis before study entry.

No significant differences in safety in these hundreds of were found, and the study concludes that Tilarginine, had no effect on mortality in patients with MI complicated by refractory cardiogenic shock.

Volunteer study

As LNMMA has been administered to thousands of patients, including a phase 3 clinical trial for cardiogenic shock (see above), the anticipated serious adverse events are known and documented (reviewed by Kilbourn *et al)*. In the cardiogenic shock trial, LNMMA proved to be safe and with few adverse events (AEs), other than transient **reversible** hypertension, i.e. the blood pressure reverted to normal within minutes of stopping LNMMA infusion (Fig 7). LNMMA was not associated with any abnormalities in hematologic, liver, or renal systems[45]. In normotensive patients, LNMMA was administered to metastatic renal cell carcinoma patient prior to infusion of interleukin-2 (IL-2), to differentiate the side effects of LNMMA from IL-2[45]. Transient reversible hypertension was observed with LNMMA. Bolus injections of LNMMA at 3, 6, and 12 mg/kg was administered to normotensive subjects (n=3 in each cohort). Doses of 3 and 6 mg/kg did not induce clinically apparent side effects, and blood pressure (BP) remained unchanged. **At a dose level of 12 mg/kg, patients experienced increase in systolic BP up to 25 mmHg, without any clinical symptoms, which normalized rapidly within a few minutes on stopping the LNMMA infusion** (Fig 10, personal communication from Dr. Kilbourn). In this phase 1 study, the initial dose level will be **7.5 mg/kg** in patients with no prior cardiac history and systolic BP< 150 mmHg. Additionally, all patients will be fully evaluated by a cardiologist before entry into this phase 1 study. This initial **human dose of 7.5 mg/kg is comparable to the mouse equivalent does tested in PDX models** in which LNMMA in combination with docetaxel resulted in significant tumor regression.

Fig 7. Systolic blood pressure change 15 minutes after administration of LNMMA in normotensive patients at 3, 6, 12 mg/kg.



These patients in these earlier clinical studies were not treated with anti-hypertensive like the calcium channel antagonist amlodipine, as is proposed here. **Amlodipine is expected to decrease systolic BP by**

at least 15 mmHg within 24 hours of the first dose. This drug produces peak plasma concentrations between 6 and 12 hours, and the absolute bioavailability has been estimated to be between 64 and 90%. In the hundreds of patients (n=800 on amlodipine and 538 on placebo) who received amlodipine in 15 double-blind, randomized trials, statistically significant reduction in systolic BP averaging 15 mmHg was observed 24 hours post first dose[54]. Amlodipine has been evaluated for safety in more than 11,000 patients in U.S. and foreign clinical trials. In general, treatment with amlodipine was well-tolerated at doses up to 10 mg daily. Most adverse reactions reported during therapy with amlodipine were of mild or moderate severity. In controlled clinical trials directly comparing amlodipine (N=1730) at doses up to 10 mg to placebo (N=1250), discontinuation of amlodipine due to adverse reactions was required in only about 1.5% of patients and was not significantly different from placebo (about 1%). As seen in our preliminary data, as expected with one of the most commonly prescribed antihypertensive, there was significant difference in any laboratory measurements including renal, liver and hematologic values in patients receiving docetaxel/amlodipine vs. docetaxel alone. In our preclinical xenograft experiments, we administered amlodipine which effectively reversed any elevation in blood pressure. **Additionally, given the short half-life of LNMMA, any elevation in BP will be reversed quickly by stopping the LNMMA infusion.**

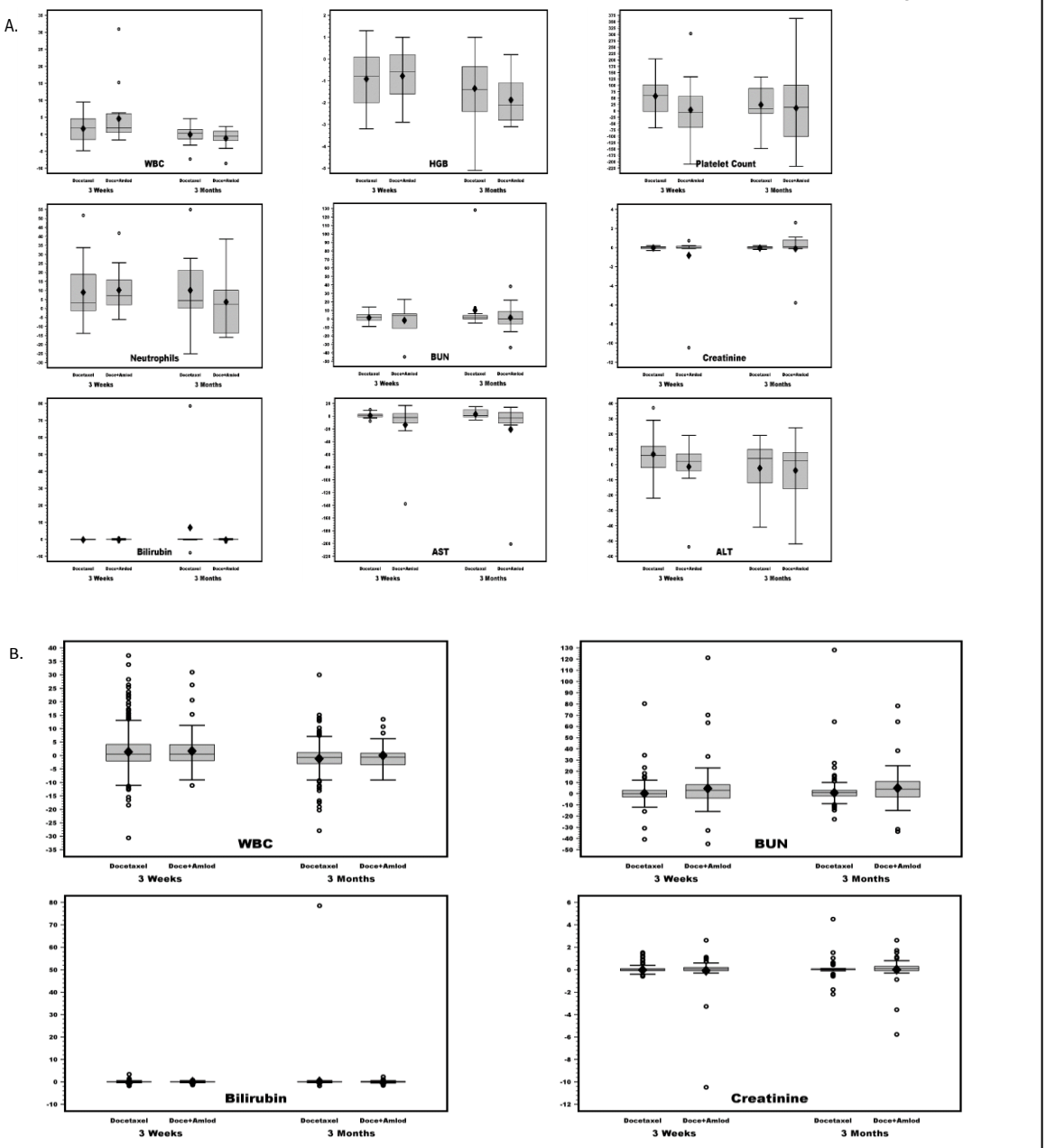
LNMMA has been found to be a safe and tolerable in the thousands of patients studied. Nonetheless, additional measures to ensure the safety of the patients in this phase 1b/II study will be undertaken. First, only patients without any cardiac history or known hypertension (defined as a systolic BP >150 mm Hg) will be included. Second, all patients will be evaluated by a cardiologist before entry into this study. Third, at each L-NMMA infusion, BP will be measured before infusion, 1 hour after infusion start, and at infusion completion. Automatic BP readings will be performed unless manual reading is needed. If systolic BP is >180 mmHg at the 1-hour check, recheck BP after 15−30 min. If BP is not <160 mmHg, recheck BP after another 15−30 min. If BP is still not <160 mmHg, the patient is to be discontinued from the study. Patients whose BP lowers to <160 mmHg at the first or second recheck will be rechallenged with the study drug. If systolic BP again increases beyond 180 mmHg, the patient is to be discontinued from the study. If systolic BP is >180 mmHg at infusion completion, recheck BP after 15−30 min. If BP is not <160 mmHg, recheck BP after another 15−30 min. If BP is still not <160 mmHg, the patient is to be discontinued from the study.

Safety of docetaxel and amlodipine

Additionally, we investigated the safety of amlodipine and docetaxel, to ensure there is no unanticipated interaction with this duet. As amlodipine is a very common antihypertensive, as is the use of docetaxel in cancer patients, we investigated if there would be significant differences in safety profile with the combination of amlodipine to docetaxel.

We identified 440 patients treated with docetaxel, and 65 patients treated with both docetaxel/amlodipine. Changes in total white cell count, urea, creatinine and bilirubin were examined in both groups at 3 weeks and 3 months after starting docetaxel chemotherapy. No statistically significant differences were identified in both groups (Fig 8A). In a smaller sample size of breast cancer patients, 40 in each group, again no statistically significant difference was noted in total white cell count, neutrophils, hemoglobin, platelet count, urea, creatinine, bilirubin, AST or ALT in docetaxel vs. docetaxel/amlodipine at 3 weeks and 3 months (Fig 8B). Docetaxel/amlodipine is commonly administered in cancer patients who are hypertensive and who receive docetaxel chemotherapy, and no black box warnings have been issued with this combination. All parameters for this dual combination showed no statistical difference compared to docetaxel alone.

Fig 8. Comparison between docetaxel/amlodipine vs. docetaxel in hematologic, renal and liver function tests in A) breast cancer patients and B) cancer patients at 3 weeks and 3 months of starting docetaxel



3. Hypothesis

L-NMMA plus taxane (docetaxel, paclitaxel, or nab-paclitaxel) will improve antitumor activity and drug tolerability in refractory locally advanced or metastatic TNBC patients.

Rationale: Robust evidence indicates that treatment resistance and metastasis arise from a subpopulation of cells with stem-like properties within a heterogeneous primary cancer[13-15]. The persistence of this unique subpopulation of chemoresistant cells can serve to reinitiate tumor growth and seed metastases. We have demonstrated that residual tumors after chemotherapy exposure are enriched for these stem-like cells, and we have characterized several regulatory pathways involved in treatment resistance, in particular the iNOS

signaling pathway. The overall scientific goal of this clinical trial is to target novel treatment resistance mechanisms in TNBC.

4. Objectives and endpoints

Primary Objectives:

- To assess the maximum tolerated dose (MTD) of L-NMMA when combined with docetaxel/amlodipine in the treatment of refractory locally advanced or metastatic TNBC patients.
- To determine the clinical benefit rate (CBR) of L-NMMA combined with taxane chemotherapy (docetaxel, paclitaxel, or nab-paclitaxel)/amlodipine, as assessed by the Response Evaluation Criteria in Solid Tumors (RECIST) 1.1.

Secondary Objectives:

- To describe the dose-limiting toxicities (DLTs) and other toxicities associated with L-NMMA when combined with docetaxel/amlodipine, as assessed by the Common Terminology Criteria for Adverse Events (CTCAE) v4.03.
- To determine the recommended phase 2 dose (RP2D) of L-NMMA when combined with docetaxel/amlodipine.
- To assess the antitumor activity of L-NMMA when combined with taxane chemotherapy (docetaxel, paclitaxel, or nab-paclitaxel)/amlodipine, as assessed by the RECIST 1.1.

Exploratory Objectives:

- To study the plasma pharmacokinetics (PK) and pharmacodynamics (PD) of L-NMMA when combined with docetaxel/amlodipine in patients with refractory locally advanced or metastatic TNBC.
- To determine potential predictive biomarkers such as iNOS (expression, phosphorylation), AKT, and the polycomb-regulated EZH2 pathway and markers related to treatment resistance, hypoxia and endoplasmic reticulum (ER) stress, survival, and autophagy.
- To measure serum levels of nitrite/nitrate and inflammatory biomarkers (interleukin [IL]-1β, IL-6, IL-8, tumor necrosis factor-α [TNF-α], and circulating vascular endothelial growth factor [VEGF]).
- To explore changes in angiogenesis by IHC analysis of cluster of differentiation 31 (CD31; platelet endothelial cell adhesion molecule [PECAM-1].
- To explore other blood-related biomarkers, including *RPL39*, *MLF2*, and *phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit alpha* (*PIK3CA*) mutations in cell-free DNA.

Primary Endpoints/Criteria for Evaluation

Primary Endpoints: The primary endpoints will be the MTD of L-NMMA when combined with docetaxel/amlodipine and the CBR of L-NMMA combined with taxane chemotherapy (docetaxel, paclitaxel, or nab-paclitaxel)/amlodipine. ORR will be defined as the percentage of patients with complete response (CR), partial response (PR), or stable disease (SD), as assessed by the RECIST 1.1.

Secondary Endpoints: Secondary endpoints include 1) to describe the DLTs and other toxicities of L-NMMA when combined with docetaxel/amlodipine; 2) to determine the RP2D of L-NMMA when combined with docetaxel/amlodipine; 3) to assess the antitumor activity of L-NMMA when combined with taxane chemotherapy (docetaxel, paclitaxel, or nab-paclitaxel)/amlodipine; 4) to assess PK and PD of L-NMMA when combined with docetaxel/amlodipine; and 5) to explore tissue and blood-based markers of treatment response and toxicity. DLTs will be defined as any treatment-related death or any ≥ Grade 3 AE unless there is clear alternative evidence that the AE was not caused by the study treatment (See Section 8 for a detailed list of DLTs). The DLT assessment window is defined as the duration required to complete one full cycle of treatment with the L-NMMA and docetaxel combination. If treatment is delayed, the timelines for dose escalation and MTD determinations will be adjusted to allow for the completion of the DLT assessment window.

Criteria for Evaluation of Subjects: Subjects will be considered evaluable for the purpose of establishing dose escalation/de-escalation decisions and the MTD if they satisfy one of the following criteria: receive at least one complete cycle of L-NMMA in combination with docetaxel and complete study assessments through

the DLT assessment window (through Day 21) without a DLT or experience a DLT within the DLT assessment window and are withdrawn from the study.

Tumor Assessments: Computed tomography (CT) scan of the chest, abdomen, and pelvis will be performed during the screening period. Repeat CT scans will be done at the end of cycles 2, 4 and 6 (+/- 5 days). Follow up scans will be done at the discretion of the physician. Disease status will be assessed using the RECIST 1.1.

Continuation of Treatment: Subjects will continue on study therapy (L-NMMA, docetaxel) if they have tolerated the study therapy without a DLT and their disease has not progressed. Subjects will be followed for approximately 3 months after the final treatment dose. The approximate length of the study from Cycle 1, Day 1 will be approximately seven (7) months (approximately four [4] months of treatment plus three [3] months of follow-up). We expect to recruit up to 48 patients to complete the clinical trial within 24 to 36 months.

Correlative Studies

Experimental Methods for Secondary Endpoints:

1). Blood samples

a) PK and PD studies for L-NMMA plus docetaxel.

Blood will be drawn to obtain plasma samples for PK and PD studies predose (10-30 minutes before L-NMMA infusion) on Days 1, 2, and 5 of Cycle 1 and Days 1 and 5 of Cycle 2. One 5-mL sample will be collected for PK assessment and one 5-mL sample wil be collected for PD assessment. **NOTE:** Patients should be fasted (nothing to eat or drink except water) for 12 hours prior to each collection time point. Patients should also keep a food diary the week before and the week of the L-NMMA infusion during Cycles 1 and 2.

b) Blood will be drawn to obtain serum samples for the determination of nitrite and nitrate levels and cytokine levels and other inflammatory biomarkers (IL-1β, IL-6, IL-8, TNF-α, circulating VEGF) at baseline, 1 hr (± 30 min) before every Day 1 L-NMMA infusion at each cycle, and 1 hr (± 30 min) after every Day 5 L-NMMA infusion at each cycle.

c) Blood samples for *RPL39*/*MLF2*/*PIK3CA* mutational analysis of plasma cell-free DNA will be collected before each cycle and at end of treatment (at progression or completion of 6 cycles).

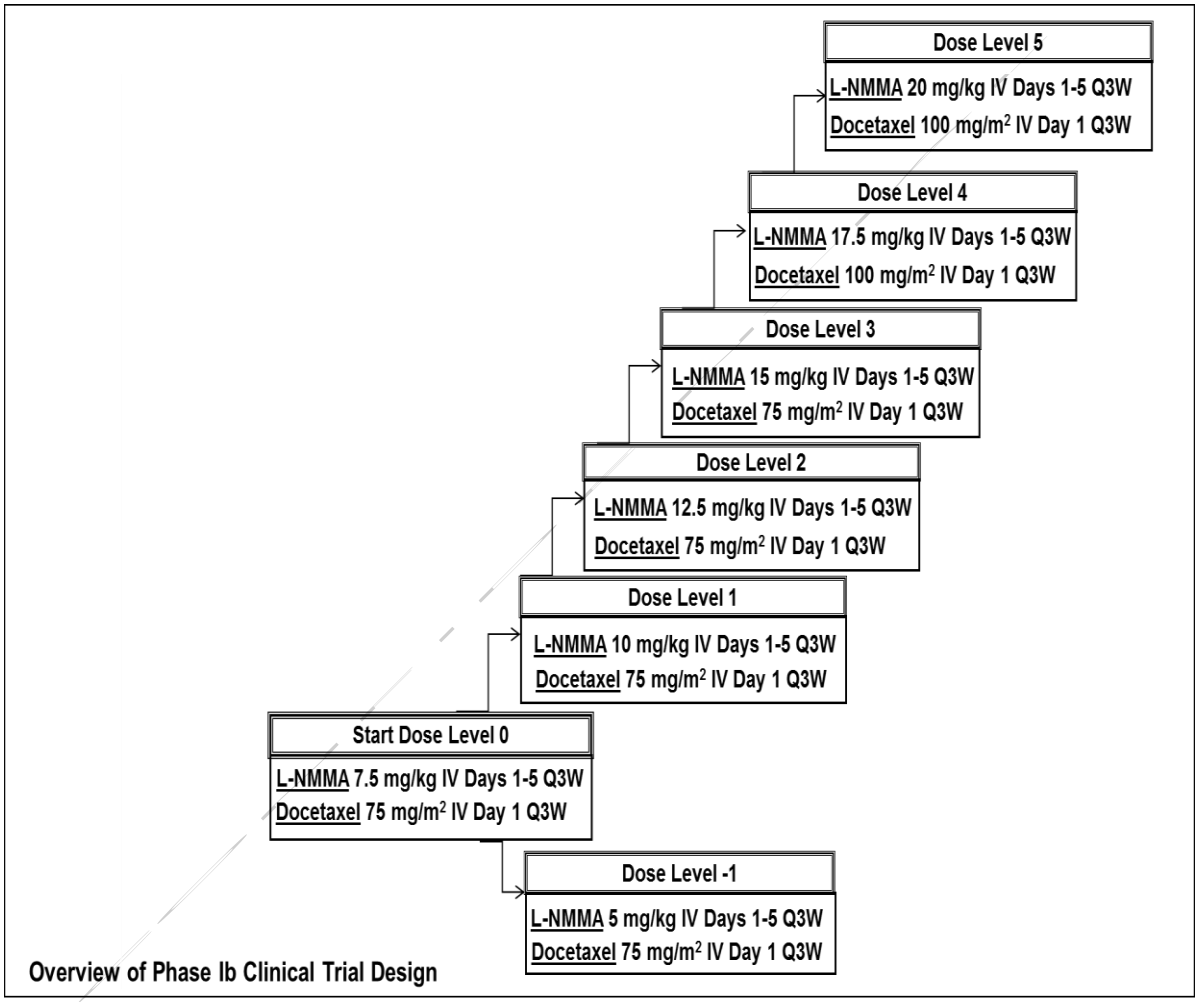
2). Tissue biopsies

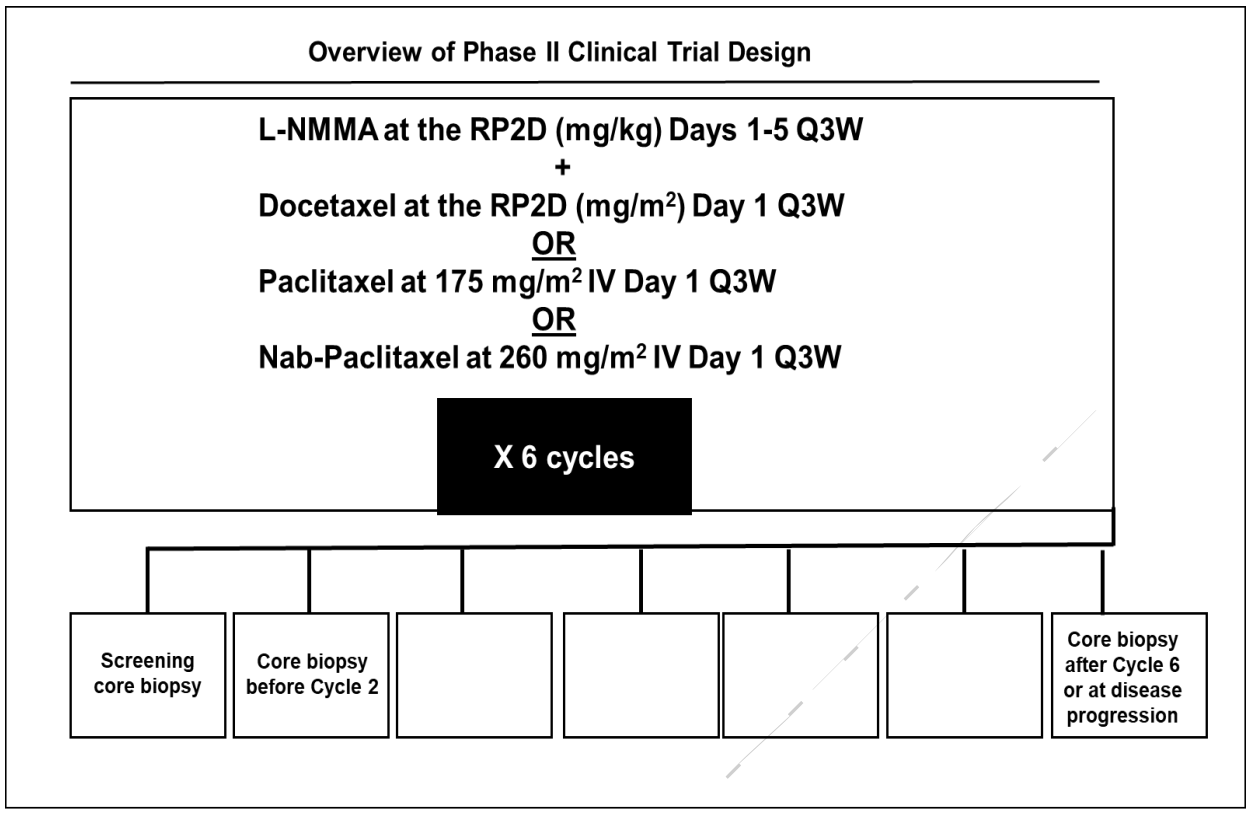
For patients with tumors amenable to biopsy, tumor tissue will be collected at screening, the end of Cycle 1, and end of treatment (at progression or completion of 6 cycles). Tumor tissue obtained as part of the patient's standard care and research biopsy tissues will be evaluated for biologic markers, including but not limited to polycomb-regulated EZH2 pathway, AKT, eNOS, and CD31.

5. Study design

This is a Phase Ib/II study assessing the MTD, DLTs, RP2D, and efficacy of L-NMMA when combined with taxane chemotherapy in refractory locally advanced or metastatic TNBC patients. The Phase Ib portion of the study is designed to investigate the combination at two dose levels of docetaxel (75 and 100 mg/m^2) and 7 dose levels of L-NMMA (5, 7.5, 10, 12.5, 15, 17.5, and 20 mg/kg). The starting dose will be L-NMMA at 7.5

mg/kg and docetaxel at 75 mg/m^2. As patients are accrued, a standard Bayesian model averaging continual reassessment method (CRM) approach will be used to determine the appropriate L-NMMA dosage. In the phase II portion of the study, patients will be treated with L-NMMA and taxane (docetaxel, paclitaxel, or nab-paclitaxel) per physician's choice. The L-NMMA and docetaxel combination will be administered at the RP2D determined in the phase Ib portion of the study. Paclitaxel will be administered per physician's choice at 175 mg/m^2 IV every 3 weeks (Q3W) or at 80 mg/m^2 IV weekly, and nab-paclitaxel will be administered at 260 mg/m^2 IV Q3W. All patients treated at the RP2D of L-NMMA and docetaxel in the phase Ib portion of the study will comprise the first patients enrolled into the phase II portion of the trial. The phase II portion of the trial will be conducted using a Simon's optimal two-stage design.





5.1 Phase Ib

L-NMMA and Docetaxel

Dose Level 5: On Days 1 through 5 of each cycle, patients will receive a 20 mg/kg two-hour IV infusion of L-NMMA. On Day 1 of each cycle, patients will receive a 100 mg/m^2 one-hour IV infusion of docetaxel approximately 15 minutes after the L-NMMA infusion.

Dose Level 4: On Days 1 through 5 of each cycle, patients will receive a 17.5 mg/kg two-hour IV infusion of L-NMMA. On Day 1 of each cycle, patients will receive a 100 mg/m^2 one-hour IV infusion of docetaxel approximately 15 minutes after the L-NMMA infusion.

Dose Level 3: On Days 1 through 5 of each cycle, patients will receive a 15 mg/kg two-hour IV infusion of L-NMMA. On Day 1 of each cycle, patients will receive a 75 mg/m^2 one-hour IV infusion of docetaxel approximately 15 minutes after the L-NMMA infusion.

Dose Level 2: On Days 1 through 5 of each cycle, patients will receive a 12.5 mg/kg two-hour IV infusion of L-NMMA. On Day 1 of each cycle, patients will receive a 75 mg/m^2 one-hour IV infusion of docetaxel approximately 15 minutes after the L-NMMA infusion.

Dose Level 1: On Days 1 through 5 of each cycle, patients will receive a 10 mg/kg two-hour IV infusion of L-NMMA. On Day 1 of each cycle, patients will receive a 75 mg/m^2 one-hour IV infusion of docetaxel approximately 15 minutes after the L-NMMA infusion.

Dose Level 0 (starting dose): On Days 1 through 5 of each cycle, patients will receive a 7.5 mg/kg two-hour IV infusion of L-NMMA. On Day 1 of each cycle, patients will receive a 75 mg/m^2 one-hour IV infusion of docetaxel approximately 15 minutes after the L-NMMA infusion.
Dose Level (-1): On Days 1 through 5 of each cycle, patients will receive a 5 mg/kg two-hour IV infusion of L-NMMA. On Day 1 of each cycle, patients will receive a 75 mg/m^2 one-hour IV infusion of docetaxel approximately 15 minutes after the L-NMMA infusion.

L-NMMA dosage will de-escalate/escalate based on the CRM.

Amlodipine will be administered at 10 mg orally (p.o.) QD for 6 days at each cycle. Amlodipine administration will start 24 hours before the first dose of L-NMMA. Dose may be adjusted by the treating physician if needed. Amlodipine dose will be held if systolic BP remains below 100 mmHg.

Pegfilgrastim (a colony-stimulating factor) will be administered as 6 mg subcutaneous (SQ) injection approximately 24 hours after every dose of docetaxel.

Enteric-coated aspirin will be administered as 81 mg QD during the 6 21-day cycles of L-NMMA and docetaxel.

5.2 Phase II

L-NMMA and Taxane Chemotherapy

Patients will be treated with L-NMMA and taxane chemotherapy (docetaxel, paclitaxel, or nab-paclitaxel) per physician's choice. L-NMMA will be administered on Days 1−5 and taxane chemotherapy on Day 1 Q3W or Day 1 Q1W. L-NMMA and docetaxel will be administered at the RP2D determined in the phase Ib portion of the study. Paclitaxel at 175 mg/m^2 will be IV infused over 3 hours or 80 mg/m^2 will be IV infused over 1 hour, and nab-paclitaxel at 260 mg/m^2 will be IV infused over 30 minutes.

Amlodipine, Pegfilgrastim, and **Enteric-coated aspirin** will be administered as described for the phase Ib portion of the study.

5.3 Biopsies

Study patients with tumors amenable to biopsy will be consented for core biopsies at three time points: screening, after Cycle 1, and at treatment discontinuation or completion of 6 cycles. We anticipate that the majority of biopsy tissues will be obtained from patients with treatment-refractory locally advanced breast cancer. Pre-treatment and post-treatment tumor samples will be used to evaluate iNOS; apoptosis and survival pathways; hypoxia and ER stress by phosphorylated extracellular signal-regulated kinase (pERK) expression; and the percentage of treatment-resistant cells by flow cytometry and MSFE. Pre-treatment and post-treatment biopsies will also be snap frozen for transcriptome analysis.

6. Population

The study is designed for locally advanced or metastatic TNBC patients. The investigator or designee must ensure that the patient meets all the following inclusion and none of the exclusion criteria before being offered enrollment in the study.
Study Duration: The study duration will be approximately seven (7) months (four [4] months of treatment and three [3] months of follow-up). *The maximum number of cycles will be six (6).*
Safety Criteria: All participants will be assessed by a physician for pre-existing medical conditions and baseline physical abnormalities prior to the initiation of investigational therapy. Patients presenting with any

medical history, physical exam, or laboratory abnormality that, in the opinion of the treating physician, would put their safety at risk will be excluded. Baseline signs and symptoms are to be recorded and monitored throughout the trial. Any increase in the severity or frequency of baseline signs and symptoms that occurs during treatment or the follow-up period will be recorded. Participants will be assessed for AEs by a physician or designated midlevel provider prior to each infusion. Vital signs including blood pressure, heart rate, and temperature will be measured at each physical exam. At each L-NMMA infusion, BP will be measured before infusion, 1 hour after infusion start, and at infusion completion. Automatic BP readings will be performed unless manual reading is needed. If systolic BP is >180 mmHg at the 1-hour check, recheck BP after 15−30 min. If BP is not <160 mmHg, recheck BP after another 15−30 min. If BP is still not <160 mmHg, the patient is to be discontinued from the study. Patients whose BP lowers to <160 mmHg at the first or second recheck will be rechallenged with the study drug. If systolic BP again increases beyond 180 mmHg, the patient is to be discontinued from the study. If systolic BP is >180 mmHg at infusion completion, recheck BP after 15−30 min. If BP is not <160 mmHg, recheck BP after another 15−30 min. If BP is still not <160 mmHg, the patient is to be discontinued from the study. Assessments may be performed more frequently if clinically indicated. In addition, blood samples for determination of hematology and serum chemistry profiles will be drawn before treatment initiation and every infusion to determine whether the study drug combination affects hematologic values, electrolytes, or liver function tests. Laboratory assessments will be performed more frequently if clinically indicated. This clinical and laboratory data will be used to determine the symptoms or side effects associated with the use of the study medications (L-NMMA plus taxane chemotherapy). Patients with abnormal laboratory or clinical findings that are believed to be treatment related will be followed every four weeks until the condition resolves to baseline or at least Grade II or lower according to CTCAE v4.03. CTCAE v4.03 will be used to grade toxicities. Laboratory tests may be done more frequently if medically indicated. After treatment initiation, for patients who experience a CTCAE v4.03 Grade 4 hematologic toxicity, a CBC with differential and platelets will be repeated in 7 days.

At suspected recurrence, CT scan of the chest, abdomen, and pelvis or breast ultrasound and additional directed evaluation, as appropriate, will be performed. Recurrence should be documented by biopsy and/or evidence of disease progression on radiologic studies. Abnormal blood studies alone (e.g., elevated liver function tests, CA 15-3, CEA, etc.) are not sufficient evidence of relapse.

7. Inclusion and Exclusion Criteria

7.1 Inclusion criteria:

Patient must meet all of the following criteria:

- Female patients with pathologically determined locally advanced TNBC refractory to at least 3 cycles of standard chemotherapy or metastatic (any line) TNBC. TNBC is defined as:
- Estrogen receptor (ER) negative and progesterone receptor (PR) negative (<10% staining by IHC).
- HER2 negative. HER2 negativity must be confirmed by one of the following:
  - Fluorescence *in situ* hybridization (FISH)-negative (FISH ratio <2), or
  - IHC 0-1+, or
  - IHC 2+ AND FISH-negative (FISH ratio <2).
- Eastern Cooperative Oncology Group performance status of ≤ 2
- Age ≥ 18 years
- Laboratory values within the following ranges:
  - Hemoglobin ≥9.0 g/dL (transfusions permitted)
  - Absolute neutrophil count (ANC) ≥1500/mm^3 (1.5 x 10^9/L)
  - Platelet count ≥100,000/mm^3 (100 x 10^9/L)
  - Total bilirubin <2 X upper limit of normal (ULN)
  - Creatinine (Cr) <2 X ULN and Cr clearance (CrCl) ≥30 by Cockcroft and Gault

- ALT and AST <2 X ULN; if liver metastases are present then ALT and AST must be <5 X ULN

- Have adequate organ function (cardiac ejection fraction of ≥ 45%)
- Negative serum pregnancy test within 7 days of the administration of the first treatment dose for women of childbearing potential (WOCBP). For WOCBP, adequate contraception must be used throughout the study. For this study, acceptable contraception methods are defined in Section 12.4.
- Patients or their legally authorized representative (LAR) must be able to understand the requirements of the study, provide written informed consent and authorization of use and disclosure of protected health information, and agree to abide by the study restrictions and return for the required assessments. *Use of a LAR is permissible and at both the LAR and investigator's discretion to determine if participation is in patient's best interest and according to institutional, local, state, and federal guidelines.
- Patient must be willing to undergo biopsies as required by the study protocol. Biopsies will be based on acceptable clinical risks as judged by investigator. Tissue from a previous biopsy will be accepted in the form of tissue slides.

7.2 Exclusion criteria:

- History of poorly controlled hypertension (defined as systolic BP >150 mmHg at baseline)
- Patients with metastatic disease who have received radiation therapy, chemotherapy, or non-cytotoxic investigational agents within 2 weeks of study treatment initiation. Patients may not receive any other antineoplastic treatment during the study or follow-up period.
- Patients who received taxane chemotherapy (docetaxel, paclitaxel, or nab-paclitaxel) at any line of treatment within the past 12 months
- Evidence of New York Heart Association class III or greater cardiac disease
- History of myocardial infarction, stroke, ventricular arrhythmia, or symptomatic conduction abnormality within the past 12 months
- History of congenital QT prolongation
- Absolute corrected QT interval of >480 msec in the presence of potassium >4.0 mEq/L and magnesium >1.8 mg/dL
- Any medical or psychiatric condition that would prevent informed consent or limit expected survival to less than 4 weeks
- Symptomatic central nervous system metastases
- Pregnant or nursing women
- Hypersensitivity or intolerance to L-NMMA, taxane chemotherapy (docetaxel, paclitaxel, or nab-paclitaxel), amlodipine, pegfilgrastim, or their components
- Use of amlodipine or another calcium channel blocker in the past 14 days
- Alcoholism or hepatic disease with the exception of liver metastases
- Severe renal insufficiency (CrCl <30 mL/min [Cockcroft and Gault])
- History of gastrointestinal bleeding, ulceration, or perforation
- Concurrent use of potent cytochrome P450 (CYP)3A4 inhibitors, such as ketoconazole, itraconazole, clarithromycin, atanazir, indinavir, nefazodone, neflinavir, ritonavir, saquinavir, telithromycin, and voriconazole
- Concurrent use of potent CYP3A4 inducers, such as dexamethasone, phenytoin, carbamazepine, rifampin, rifabutin, rifapentin, phenobarbitol, and St. John's wort.
- Concurrent use of substrates (e.g., repaglinide and rosiglitazone), inhibitors (e.g., gemfibrozil), or inducers (e.g., rifampin) of CYP2C8
- Concurrent use of medications that interact with nitrate/nitrites (See Appendix E)
- Use of an investigational drug within 14 days preceding the first dose of study medication.

- Concurrent use of any complementary or alternative medicines
- Patients with ≥ Grade 2 neuropathy
- Inability to take aspirin

8. Treatment

Intervention and Mode of Delivery:

Based on the safety profile of L-NMMA, we do not anticipate major AEs from the drug combination. A standard Bayesian model averaging CRM is proposed to determine the MTD. There are two proposed doses of docetaxel (75 and 100 mg/m^2). The starting dose of docetaxel will be 75 mg/m^2.On Day 1 of each cycle, patients will receive a one-hour IV infusion of docetaxel approximately 15 minutes after the L-NMMA infusion. Premedications for docetaxel will be given based on institutional standards. On Days 1 through 5 of each cycle, patients will receive a two-hour IV infusion of L-NMMA. The dose of L-NMMA will be escalated or de-escalated based on the CRM model and will depend on the absence or presence of Grade 3 or higher AEs/toxicities. Dose levels are given in Section 5.1. Amlodipine will be given as 10 mg p.o. QD for 6 days at each cycle. Amlodipine will start 24 hours prior to the Day 1 L-NMMA infusion. Amlodipine will be given at least 30 minutes before each L-NMMA infusion on Days 1 through 5. The dose will be adjusted by the treating physician if needed. An initial cohort of two (2) patients will be treated at dose level 0 **(DL0)**, where L-NMMA will be given Days 1 through 5 of each cycle at a dose of 7.5 mg/kg IV infused over two hours. For the phase II portion of the trial, patients will be treated with L-NMMA and taxane chemotherapy (docetaxel, paclitaxel, or nab-paclitaxel per physician's choice). L-NMMA will be administered on Days 1−5 and taxane chemotherapy on Day 1 Q3W. L-NMMA and docetaxel will be administered at the RP2D determined in the phase Ib portion of the trial. Paclitaxel at 175 mg/m^2 will be IV infused over 3 hours or 80 mg/m^2 will be IV infused over 1 hour, and nab-paclitaxel at 260 mg/m^2 will be IV infused over 30 minutes. Amlodipine, pegfilgrastim, and enteric-coated aspirin will be administered as described above for the phase Ib portion of the study.

For a dose level to be chosen as the MTD, at least 4 patients must have received said dose. A maximum of 34 subjects will be treated in the phase Ib portion of the trial. In deriving the RP2D, consideration will also be given to the rate and the nature of AEs observed beyond the first 3 weeks of treatment.

DLTs will be defined as any treatment-related patient death or any ≥ Grade 3 AE (NCI CTCAE v4.03) unless there is clear alternative evidence that the AE was not caused by the study treatment:

- Grade 4 neutropenia (ANC <500/mm^3) lasting ≥7 days
- Grade 3 febrile neutropenia lasting >24 hours (single temperature of >38.3ºC or a sustained temperature of >38ºC for >1 hour [non-axillary] with ANC <1000/mm^3)
- Grade 4 febrile neutropenia of any duration
- Grade 4 thrombocytopenia (platelets <25,000/mm^3) or grade 3 thrombocytopenia with significant bleeding
- Hematological toxicities can be managed for a period that will not exceed more than 7 days; hematological toxicity that persists for more than 7 days will be considered a DLT
- Grade 3 AST or ALT elevation
- Grade 3 electrolyte abnormality that is considered clinically significant or lasts >72 hours
- Grade 3 infusion-related reactions that recur despite appropriate medical management
- Any other drug-related ≥ Grade 3 non-hematologic AE except
  - alopecia and constitutional symptoms
  - hyperlipidemia in subjects not receiving maximum medical management
  - electrolyte abnormalities that may be managed with supplements.

The DLT assessment window is defined as the duration required for completing one full cycle (through Day 21) of treatment with L-NMMA and docetaxel. The incidence of DLT(s) during the first 3 weeks of treatment will be used by the CRM in dose escalation/de-escalation decisions and to define the MTD. Subjects must complete one full cycle of treatment during the DLT evaluation period (unless DLT has occurred) to be

considered evaluable by CRM in terms of dose escalation/de-escalation decisions. If treatment is delayed, the timelines for MTD determinations will be adjusted to allow for the completion of the DLT assessment window.

Table 3

Dose Level	L-NMMA 2-Hour Infusion Days 1 to 5 each cycle (mg/kg)	Docetaxel (mg/m^2) 1-Hour Infusion Day 1 each cycle
DL5	20	100
DL4	17.5	100
DL3	15	75
DL2	12.5	75
DL1	10	75
DL0*	7.5	75
DL-1	5	75

*Start dose level.

Specific guidelines for management of potential L-NMMA toxicities

L-NMMA has been administered to thousands of patients, including a Phase 3 clinical trial for cardiogenic shock, and the anticipated serious adverse events (SAEs) are known and documented. In the cardiogenic shock trial, L-NMMA proved to be safe with few AEs, other than transient reversible hypertension, i.e. the blood pressure reverted to normal within minutes of stopping L-NMMA infusion. L-NMMA was not associated with any hematologic, liver, or renal system abnormalities. In normotensive patients, L-NMMA was administered to metastatic renal cell carcinoma patients prior to IL-2 infusion to differentiate the side effects of L-NMMA and IL-2. Transient reversible hypertension was observed with L-NMMA. Bolus injections of L-NMMA at 3, 6, and 12 mg/kg were administered to normotensive subjects (n=3 in each cohort). Doses of 3 and 6 mg/kg did not induce clinically apparent side effects, and BP remained essentially unchanged. At a dose level of 12 mg/kg, patients experienced an increase in systolic BP up to 25 mmHg, without any clinical symptoms, which normalized rapidly in less than 5 minutes on stopping the L-NMMA infusion. In this Phase Ib/II study, the initial L-NMMA dose level will be 7.5 mg/kg in patients with no prior cardiac history and systolic BP <150 mmHg. This human dose is equivalent to the 200 mg/kg dose used in preclinical xenograft mouse studies. Additionally, all patients will be fully evaluated by a cardiologist before entry into this Phase Ib/II study. Patients in the abovementioned clinical studies were not treated with antihypertensive agents such as the calcium channel antagonist amlodipine, as is proposed here. Amlodipine is expected to decrease systolic BP by at least 15 mmHg within 24 hours of the first dose. As L-NMMA has a short half-life, any elevation in BP would be transient and reversed on stopping the infusion.

L-NMMA has been found to be a safe and tolerable in the thousands of patients studied. Nonetheless, additional measures to ensure the safety of the patients in this Phase 1b/II study will be undertaken. First, only patients without any significant cardiac history or uncontrolled hypertension (defined as a systolic BP >150 mm Hg) will be included. Second, all patients will be evaluated by a cardiologist before entry into this study. Third, at each L-NMMA infusion, BP will be measured before infusion start, 1 hour after infusion start, and at infusion completion. Automatic BP readings will be performed unless manual reading is needed. If systolic BP is >180 mmHg at the 1-hour check, recheck BP after 15−30 min. If BP is not <160 mmHg, recheck BP after another 15−30 min. If BP is still not <160 mmHg, the patient is to be discontinued from the study. Patients whose BP lowers to <160 mmHg at the first or second recheck will be rechallenged with the study drug. If systolic BP again increases beyond 180 mmHg, the patient is to be discontinued from the study. If systolic BP

is >180 mmHg at infusion completion, recheck BP after 15−30 min. If BP is not <160 mmHg, recheck BP after another 15−30 min. If BP is still not <160 mmHg, the patient is to be discontinued from the study.

Taxane chemotherapy dose modifications are allowed and are based on toxicities (please refer to Section 10)

L-NMMA and taxane chemotherapy (docetaxel, paclitaxel, or nab-paclitaxel) will be independently dose adjusted based on toxicity. For instance, if the docetaxel dose is modified, a subject can continue receiving the same dose of L-NMMA.
Note: Amlodipine dose will be held if systolic BP remains below 100 mmHg.

9. Visit schedule and assessments

9.1 Study flow and visit schedule.

Procedure	Screening[a]	Baseline[b]	Prior to Each Cycle*	Treatment Cycle[d*]	EOT
Informed Consent	X				
Inclusion/ Exclusion	X				
Demographics	X				
Medical History	X	X[b]			
Symptom-directed Medical History and Physical Exam[e]	X	X[b]	X		X
Height	X	X			
Weight	X	X	X		X
ECOG Performance Status	X	X[b]	X		X
Vital Signs[f, r]	X	X	X	X	X
12-Lead ECG and MUGA Scan or Echocardiogram[g]	X				X
Concomitant Therapies	Continuous from Screening period to study termination				
Hematology[h]	X	X[c]	X		X
Clinical Chemistry[i]	X	X[c]	X		X
Serum Pregnancy (β-hCG)[j]	X				
Related Biomarkers & serum Nitrite/Nitrate Levels [m]	X		X	X	
Tumor Assessments[k]	X			X	X
Tumor Biopsy[l]	X			X	X
Tumor Tissue[m]	X				X
Adverse Events and Serious Adverse Events	From informed consent signing up to and including 30 days after the last treatment dose[q]				
L-NMMA Administration[n]				Days 1-5 of each cycle	
Taxane Chemotherapy (Docetaxel, Paclitaxel, or Nab-Paclitaxel) Administration[n]				X	
Amlodipine Administration[n]			X	X	
Pegfilgrastim Administration[n]				X	
Enteric-coated aspirin[n]				X	
PK AND PD STUDIES					
Blood Samples for L-NMMA PK[o]	X				
Blood Samples for Docetaxel PK[o]	X				

Blood Samples for PDp	X

Abbreviations: ALT = alanine transaminase; ANC = absolute neutrophil count; AST = aspartate transaminase; β-hCG = beta-human chorionic gonadotropin; BP = blood pressure; BUN = blood urea nitrogen; CBC = complete blood count; CT = computed tomography; ECG = electrocardiogram; ECOG = Eastern Cooperative Oncology Group; eNOS = endothelial nitric oxide synthase; EOT = end of treatment; ER = endoplasmic reticulum; EZH2 = enhancer of Zeste 2 polycomb repressive complex 2 subunit; ; IL = interleukin; iNOS = inducible nitric oxide synthase; LDH = lactate dehydrogenase; MUGA = multigated acquisition; p.o. = daily; PD = pharmacodynamics; PK = pharmacokinetics; PI = principal investigator; SAE = serious adverse event; SQ = subcutaneously; TNF = tumor necrosis factor; VEGF = vascular endothelial growth factor; WBC = white blood cell. Each treatment cycle is 21 days in length. Tests and procedures should be done on schedule, but visit windows of ± 5 days are allowed (except as otherwise specified) occasionally for holidays, vacations, and other administrative reasons. If extenuating circumstances prevent a patient from beginning treatment or completing a scheduled assessment within this time frame, the patient may continue in the study only with written permission of the PI.

a Within 28 days prior to the Cycle 1, Day 1 (washout period of at least two [2] weeks before study treatment start)

b Within 7 days prior to the Cycle 1, Day 1

c Within 4 days prior to the Cycle 1, Day 1

d Treatment cycles are to be repeated every 21 days. The maximum number of cycles will be six (6).

e The baseline symptom-directed relevant medical history and physical examination are not required if the screening medical history and physical examination were conducted within 7 days prior to Cycle 1, Day 1. All patients will be evaluated by a cardiologist before entry into this study.

f Vital sign (**blood pressure**, heart rate, and oral temperature) measurements will be obtained during screening/baseline and before every infusion.

g A 12-lead ECG will be performed at screening and EOT. MUGA scan or echocardiogram will be performed at screening and when clinically indicated. The same test (MUGA scan or echocardiogram) must be used thoughout the duration of the study.

h A blood sample for CBC with platelet count and differential WBC count will be obtained during screening/baseline and before every cycle ([± 5 days). After treatment initiation, for patients who experience a CTCAE v4.03 Grade 4 hematologic toxicity, a CBC with differential and platelets will be repeated in 7 days. Patient will be followed until the ANC and platelet count have recovered to ≥1,000/mm^3 and ≥50,000/mm^3, respectively.

i A blood sample for the clinical chemistry panel (glucose, albumin, sodium, potassium, carbon dioxide, chloride, BUN, creatinine, total bilirubin, uric acid, alkaline phosphatase, AST [SGOT], ALT [SGPT], LDH, and magnesium) will be obtained at screening/ baseline, before every cycle, at EOT, and when clinically indicated. Labs can be obtained ± 4 days from schedule.

j For women of childbearing potential, the results of a serum β-hCG pregnancy test must be negative within 7 days of the administration of the first treatment dose. If the screening serum β-hCG pregnancy test is performed more than 7 days before dosing, it must be repeated at baseline, with results known to be negative prior to the administration of the first dose of the study drugs. β-hCG pregnancy testing is to be repeated as clinically indicated.

k CT scan of the chest, abdomen, and pelvis or breast ultrasound, as appropriate, is to be performed during screening. If the patient has had an appropriate CT scan or breast ultrasound performed within 28 days of the Cycle 1, Day 1 treatment dose, then that scan may be used for screening. CT scan or breast ultrasound will be repeated every 6 weeks (± 5 days) until completion of the protocol-specified study treatment and 3 months after the last dose of study treatment.

l For patients with tumors amenable to biopsy, biopsy will be completed during screening (within 28 days of Cycle 1, Day 1 is acceptable), before Cycle 2, and at treatment discontinuation or completion of 6 cycles. If the patient progresses while on treatment, a new tumor biopsy will be taken before the patient starts the new treatment.

m Tumor tissue obtained as part of the patient's standard care and additional biopsy tissues will be evaluated for candidate biomarkers of response and toxicity of the L-NMMA + docetaxel combination, including but not limited to iNOS, eNOS, polycomb-regulated EZH2 pathway, and AKT; markers related to treatment resistance, hypoxia and ER stress, survival, autophagy, and angiogenesis (i.e., CD31); and inflammatory biomarkers. Ten 5-µm slides will be obtained. Blood samples for *RPL39*/*MLF2*/*PIK3CA* mutational analysis of plasma cell-free DNA will be collected before each cycle and at EOT (at progression or completion of 6 cycles). Blood samples (5 mL) will be collected into heparinized tubes and plasma prepared by centrifugation at 1000g for 15 minutes. Plasma samples must be frozen at -70ºC until analysis. Blood samples for determination of serum nitrite/nitrate levels and inflammatory biomarkers (IL-1β, IL-6, IL-8, TNF-α, and VEGF) will be obtained at baseline, 1 hr (± 30 min) before every Day 1 L-NMMA infusion at each cycle, and 1 hr (± 30 min) after every Day 5 L-NMMA infusion at each cycle.

n Phase Ib: **L-NMMA** at a dose of 7.5 mg/kg (dose level 0) will be intravenously infused over two hours on Day 1; patients will continue on this same L-NMMA dose from Days 2 to 5. L-NMMA dose will be escalated in 2.5 mg/kg increments up to 20 mg/kg or de-escalated to 5 mg/kg. Cohorts will be studied until the dose-limiting toxicity of the combination is seen (See Section 8). At each L-NMMA infusion, BP will be measured before infusion, 1 hour after infusion start, and at infusion completion. Automatic BP readings will be performed unless manual reading is needed. If systolic BP is >180 mmHg at the 1-hour check, recheck BP after 15-30 min. If BP is not <160 mmHg, recheck BP after another 15-30 min. If BP is still not <160 mmHg, the patient is to be discontinued from the study. Patients whose BP lowers to <160 mmHg at the first or second recheck will be rechallenged with the study drug. If systolic BP again increases beyond 180 mmHg, the patient is to be discontinued from the study. If systolic BP is >180 mmHg at infusion completion, recheck BP after 15-30 min. If BP is not <160 mmHg, recheck BP after another 15-30 min. If BP is still not <160 mmHg, the patient is to be discontinued from the study.

Docetaxel at a dose of 75 mg/m^2 will be infused over one hour every 21 days. Docetaxel dose modifications are allowed and are based on toxicities (See Section 10.2).

Amlodipine will be given as 10 mg p.o. daily x 6 days at each cycle. Amlodipine administration will start 24 hours before the first dose of L-NMMA.

Pegfilgrastim at a dose of 6 mg will be injected SQ approximately 24 hours after every dose of docetaxel. **Enteric-coated aspirin** will be administered as 81 mg once daily during the 6 21-day cycles of L-NMMA and docetaxel.

Phase II: Patients will be treated with L-NMMA and taxane chemotherapy (docetaxel, paclitaxel, or nab-paclitaxel per physician's choice). L-NMMA will be administered on Days 1−5 and taxane chemotherapy on Day 1 Q3W. L-NMMA and docetaxel will be administered at the RP2D determined in the phase Ib portion of the study. Paclitaxel at 175 mg/m^2 will be IV infused over 3 hours or 80 mg/m2 will be IV infused over 1 hour, and nab-paclitaxel at 260 mg/m^2 will be IV infused over 30 minutes. Taxane chemotherapy dose modifications are allowed and are based on toxicities (see Section 10).

o Blood samples will be collected predose (10-30 minutes before L-NMMA infusion) on Days 1, 2, and 5 of Cycle 1 and Days 1 and 5 of Cycle 2 for determination of L-NMMA plus docetaxel plasma PK. Blood (5 mL) will be drawn into heparin-containing green top tubes (See Section 4 and Appendix F).

p For PD assessment, blood samples will be collected on Days 1, 2, and 5 of Cycle 1 and Days 1 and 5 of Cycle 2. Blood (5 mL) will be drawn into heparin-containing green top tubes. The 5-mL sample for PD assessment is in addition to the 5-mL sample for PK assessment. **NOTE:** For PK/PD assessment, patients should be fasted (nothing to eat or drink except for water) for 12 hours prior to each collection time point. Patients should keep a food diary the week before and the week of L-NMMA infusion during Cycles 1 and 2.

q SAEs will be captured from the time of informed consent signing up to and including 30 days after the last treatment dose. SAEs will be followed until resolution to grade 1 or baseline. Treatment-related SAEs occurring beyond 30 days from the last treatment dose and any study patient death

should also be reported. All patients will be followed for 3 months after the final dose of treatment and will be evaluated accordingly with the required standard of care labs and tests.

r At each L-NMMA infusion, BP will be measured before infusion, 1 hour after infusion start, and at infusion completion.

*Patients should be on a **low nitrite/nitrate diet** including avoidance of processed meats such hot dogs, ham, bacon, and sausage 1 week before study treatment start and for the duration of the study.*

10 Clinical Trial Materials

10.1 L-NMMA[70]

L-NMMA Structure

L-NMMA is a derivative of L-arginine, has one chiral center and is methylated on a terminal guanidino nitrogen.

Molecular Formula: C9H20N4O4
Molecular Weight: 248.28

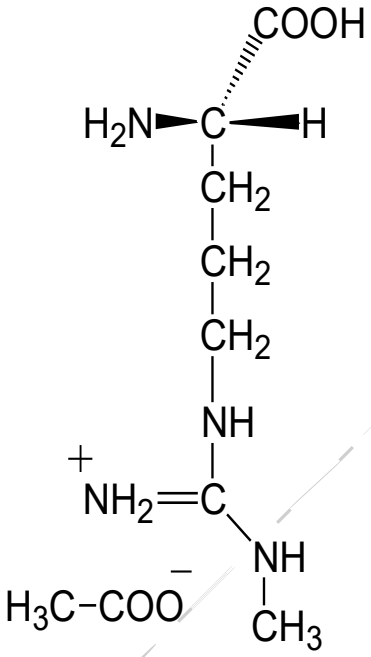


Figure 10 Structure of L-NMMA Drug Substance

L-NMMA Pharmacokinetics & Pharmacodynamics

Profile of systemic nitric oxidesynthase inhibition with L-NMMA in humans: It has been demonstrated that inhibition of endothelium derived nitric oxide with NG-monomethyl-l-arginine (L-NMMA) results in a different cardiac and peripheral vascular response. Pharmacokinetic-pharmacodynamic profile of L-NMMA and pharmacokinetic interactions with l-arginine was studied in healthy subjects. Plasma pharmacokinetics were analyzed from two different studies: In study 1, 3 mg kg−1 L-NMMA was administered i.v. over 5 min and systemic hemodynamics, cardiac output (CO), fundus pulsation amplitude (FPA), and NO-exhalation (exhNO) were measured at baseline and 15, 65, 95, 155, and 305 min after start of drug administration (n=7). In study 2, 17 mg kg−1 min−1 of the physiologic substrate for nitric oxide synthase, l-arginine, was coinfused i.v. over 30 min with a primed constant infusion of 50 mg kg−1 min−1 L-NMMA (n=8). Bolus infusion of L-NMMA resulted in a maximum plasma concentration of 12.9±3.4 mg ml−1 (mean±s.d.) with elimination half-life of 63.5±14.5 min and clearance of 12.2±3.5 ml min−1 kg−1 and caused a small hypertensive response, decreased CO by 13%, FPA by 26%, exhNO by 46% and increased systemic vascular resistance by 16% ($P<0.05$ each) 15 min after start of drug administration. Although only limited data points were available in the L-NMMA plasma concentration range between 0 and 4 mg ml−1, drug effects over time were in good agreement with an Emax model ($r2>0.98$ each), which also suggested that concentrations producing half-maximum effects were higher for FPA than for CO and exhNO. The coinfusion with l-arginine caused a nearly two-fold increase in plasma L-NMMA levels, indicating a pharmacokinetic interaction.

Table 5 Pharmacokinetic parameters of 3 mg kg−1 L-NMMA
i.v. over 5 min obtained from non-compartmental analysis. Results are presented as means±s.d., *n*=7.
Table 5

$t_{1/2}$ (min)	63.5±14.5
AUC (µg ml−1 min−1)	260.4±62.4
MRT (min)	48.9±10.5
C_{max} (µg ml−1)	12.9±3.4
t_{max} (min)	6
CL (ml min−1 kg−1)	12.2±3.5
V_c (ml kg−1)	257.6±116
Vd_{ss} (ml kg−1)	586.1±206.7

$t_{1/2}$, elimination half-life; AUC, area under the plasma concentration vs time curve; MRT, mean residence time; C_{max}, maximal plasma concentration; tmax, time to reach C_{max}, CL, total body clearance; V_c, initial volume of distribution; Vd_{ss}, volume of distribution at steady state.

Plasma samples were available from seven subjects in study 1 and eight subjects in study 2. The pharmacokinetic parameters calculated from study 1 are given in Table 8, plasma concentrations are illustrated in Figure 13. In the primed constant infusion experiments in study 2, plasma levels of L-NMMA increased to 9.5±5.2 µg ml−1 at the end of the 5 min bolus administration and to steady state plasma concentrations of #4.0±1.6 µg ml−1. During coinfusion with l-Arg, which resulted in a 66±6 fold increase in plasma l-Arg levels to 932±206 µg ml−1, a significant increase in mean plasma L-NMMA to 7.1±1.6 µg ml−1 (P<0.001, anova) was noted.

Plasma concentrations of L-NMMA remained elevated 25 min after the end of l-Arg infusion at 7.5±1.7 µg ml−1.

In study 1, L-NMMA had a transient and subtle effect on systemic hemodynamics: Mean arterial pressure increased slightly from 85.0±6.5–87.5±7.0 mmHg, pulse rate decreased from 69.5±9.6–65.2±10.6 beats min−1, and systemic vascular resistance significantly increased from 1013±295 dyn s cm−5 by 16%
(P<0.05) 15 min after start of drug administration. L-NMMA caused a maximum percentage reduction from the baseline value (E_{max}) in CO of 13%, in FPA of 26%, and in exhaled NO of 46% (P<0.01 each, Figure 1). The presented PD model allowed very good fits of the experimentally measured data although only limited points were available and an EC50 for CO, FPA, and exhaled NO of 0.02 µg ml− 1, 0.66 µg ml− 1 and 0.02 µg ml−1 was calculated, respectively.

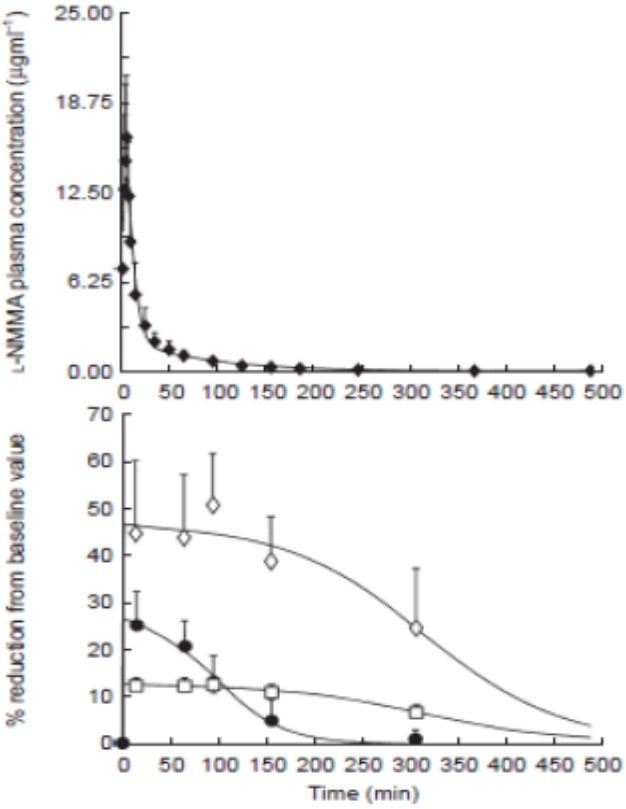


Figure 13 Plasma concentrations of l-NMMA following a single i.v. dose of 3 mg kg−1 over 5 min and pharmacodynamic effects on cardiac output (CO, %), fundus pulsation amplitude (FPA, $), and exhaled NO (exhNO, 1), which are presented as percentage reduction from the baseline values. Data are from study 1 and are presented as means±s.d., *n*=7.

The continuous infusion of L-NMMA in study 2 reduced exhaled NO by 69% (P<0.005), but no change in blood pressure and pulse rate was noted [10]. Figure 1 illustrates the effect of L-NMMA on the three dynamic parameters with respect to time. FPA and CO returned to near baseline values after 300 min. The relationship between the percentage reduction from the baseline values and the total plasma concentrations of L-NMMA is presented in Figure 14. The effect predicted by model was close to experimental measures as indicated by the good fit for the effect of the drug (model selection criteria >2.00 each).

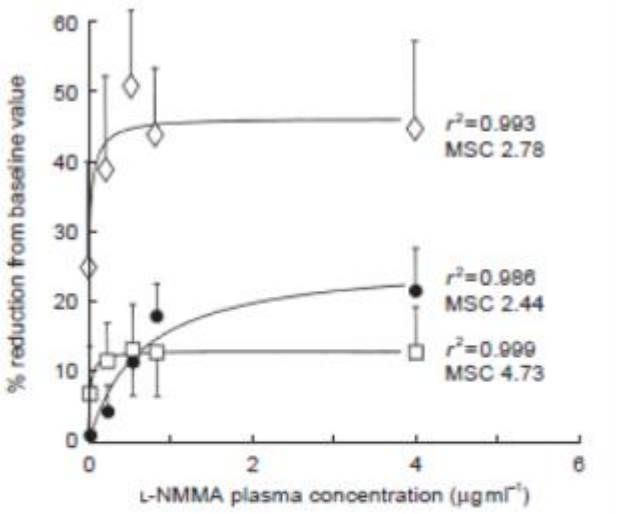


Figure 14 Relationship between the percentage reduction from the baseline values of cardiac output (CO, □), fundus pulsation amplitude (FPA, •), and exhaled NO (exhNO, 1), and the plasma concentrations of I-NMMA from study 1. Results are presented as means±s.d., n=7. The Emax model (solid lines) provided a good fit for the effect of the drug on all the three dynamic parameters as indicated by the high coefficients of correlation (r2) and model selection criteria (MSC).

General Properties

Table 6 **Physical Chemical Properties of L-NMMA Drug Substance**

Property	Result
Appearance	White to off-white powder
Solubility in organic solvents [1]	
Acetonitrile	Slightly soluble [2] mg/mL
Methanol	Slightly soluble [2] mg/mL
Acetone	Insoluble [2]
2-Propanol	Insoluble [2]
Aqueous Solubility [3]	
pH 0.7	At least freely soluble [2]
pH 2.4	At least freely soluble [2]
pH 5.8	At least freely soluble [2]
pH 6.7	At least freely soluble [2]
pH 8.0	At least freely soluble [2]
Hygroscopicity	Extremely hygroscopic
Optical rotation	α^{D}_{25} = +9.0° to +13.0°
pH 0.01M in water	5.5-7.5
Solid-state form	Under investigation

1 Determined by UV absorbance versus standard at 200 nm
2 USP definition:
very soluble = < 1 part solvent for 1 part solute
freely soluble = 1 to 10 parts solvent for 1 part solute
slightly soluble = 100 to 1000 parts solvent for 1 part solute
insoluble = ≥.10,000 parts solvent for 1 part solute
3 Determined visually. Aqueous solution adjusted with HCl or NaOH; solution at pH 6.7 was not adjusted

Other Characteristics

The L-configuration of the amino group substituent at the α-carbon of L-NMMA is the only asymmetric center present. The configuration of the α-amino group is determined by the configuration of the L-ornithine starting material and is unchanged during the synthesis of L-NMMA.

Impurities

Potential impurities in L-NMMA drug substance include organic impurities (process impurities and degradation products), residual solvents, and inorganic impurities, particularly copper, which is used in the synthesis. The potential organic impurities in L-NMMA, and their possible source(s), are outlined in Table 10 Potential residual solvents are listed in Table 11

Table7 **Potential Impurities in L-NMMA Drug Substance**

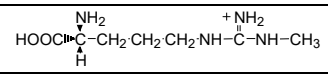
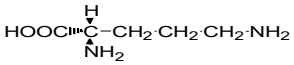
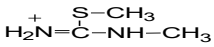
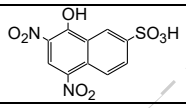
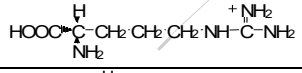
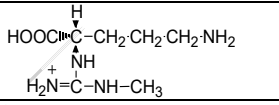
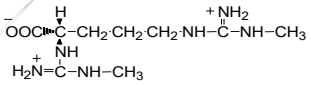
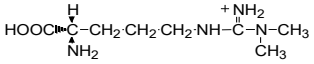
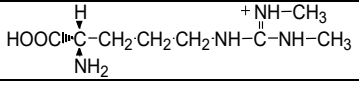
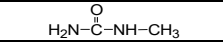
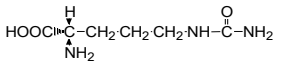
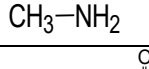
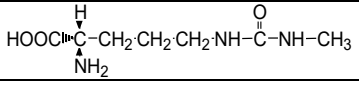
Name	Structure	Source
N^G-Monomethyl-D-arginine acetate (D-NMMA)		Process impurity (enantiomer)
L-Ornithine		Process impurity (starting material), degradation product
1,2-Dimethyl-2-thiopseudourea		Process impurity (starting material)
Flavianic acid		Process impurity (auxiliary material)
L-Arginine		Process impurity
α-N-Methylamidino-L-ornithine (α-NMMA)		Process impurity (substitution of α-amino group)
α,δ-Di-(N-methylamidino)-L-ornithine (α,δ-di NMMA)		Process impurity (disubstitution product)
Asymmetric N^G-Dimethyl-L-Arginine (L-ADMA)		Process impurity
Symmetric N^G-Dimethyl-L-Arginine(L-SDMS)		Process impurity
Ammonia		Process impurity
Methyl urea		Degradation product
L-Citrulline		Degradation product
Methyl amine		Degradation product
N^{ω}-Methyl-L-citrulline		Degradation product

Table 8 **Potential Residual Solvents in L-NMMA Drug Substance**

Solvent	Solvent Class [1]
2-Propanol	3
Methanol	2
1: ICH Q3C Impurities: Residual Solvents, December 1997	

Stability

Evaluation of the stability of L-NMMA drug substance batch 29020A, which was used to manufacture drug product for Phase 2 clinical studies, was initiated on October 8, 2003. The samples were evaluated under the ICH conditions recommended for product to be stored at room temperature, as shown in the protocol summarized in Table 9

L-NMMA drug substance was used to formulate L-NMMA drug product within 15 days of its manufacturing date.

Table 9 **Stability Protocol for L-NMMA Drug Substance**

Storage Condition[1]	Time Point (months)							
	Initial	1	3	6	9	12	18	24
25 ± 2°C/60 ± 5% RH	X	X	X	X	X	X	X	X
30 ± 2°C/60 ± 5% RH[2]	X	X	NT	X	X	X	NT	NT
40 ± 2°C/75 ± 5% RH	X	X	X	X	NT	NT	NT	NT
1: Packaged in double LDPE bags with desiccant between the bags 2: Tested only if there is a significant change at 40°C/75% RH								
X = Description, Assay , Impurities , Water content NT = Not Tested								

Drug Product

Description and Composition of the Drug Product

L-NMMA Injection was administered as a bolus injection followed by an infusion of L-NMMA Injection diluted in saline infusion bags. Four doses ranging from 0.15 to 1.5 mg/kg bolus followed by the same dose were administered each hour by continuous infusion for 5 hours was evaluated in the Phase 2 clinical study. For each patient, a kit was provided containing a vial of L-NMMA Injection for BOLUS Administration and a corresponding vial of L-NMMA Injection for INFUSION Administration. To allow for four doses, L-NMMA Injection was prepared in four bolus concentrations (1.2 to 12 mg/mL) and four infusion concentrations (7.5 to 75 mg/mL) that were diluted into 100-mL saline infusion bags prior to use. L-NMMA Injection is a clear colorless solution of N^G-monomethyl-L-arginine acetate in saline (bolus administration vials) or water (infusion administration vials). It is supplied in 20-mL clear glass vials with rubber stoppers and white (bolus administration vials) or grey (infusion administration vials) caps. Vials are filled to 16.6 ± 0.3 mL which represents a 0.6-mL overfill to allow withdrawal of up to 16 mL. An injection dosage form was chosen for ease of administration to the hospitalized patient population.

The compositions of L-NMMA Injection are provided in Table 10.

Table 10 **Composition of L-NMMA Injection**

			Injection for BOLUS Administration				Injection for INFUSION Administration			
Dose (mg/kg):			**0.15**	**0.50**	**1.0**	**1.5**	**0.15**	**0.50**	**1.0**	**1.5**
Formulation Number:			**001**	**002**	**003**	**004**	**005**	**006**	**007**	**008**
Concentration (mg/mL):			**1.2**	**4.0**	**8.0**	**12**	**7.5**	**25**	**50**	**75**
Component	**Quality Standard**	**Function**	**Composition**							
L-NMMA	In-house	Drug Substance	1.2 mg	4.0 mg	8.0 mg	12 mg	7.5 mg	25 mg	50 mg	75 mg
Sodium Chloride	USP	Osmolality adjustment	8.7 mg	8.0 mg	7.0 mg	5.9 mg	none	none	none	none
Water for Injection, q.s. ad	USP	Diluent	1 mL	1 mL	1 mL	1 mL	1 mL	1 mL	1 mL	1 mL
Acetic Acid NF and/or Sodium Hydroxide NF may be added for pH adjustment										

Stability Summary

L-NMMA Injection is stable as demonstrated by the results of analysis after autoclaving for 1 hour (Section Stress Studies). The stability of L-NMMA Injection Lots MA046 (1.2 mg/mL) and MA060 (75 mg/mL), the lowest and highest concentration of the product to be used in Phase 2 clinical studies, is currently being evaluated.

L-NMMA Injection 1.2 to 75 mg/mL, packaged in clear Type 1 glass and sealed with rubber stoppers, is assigned an expiration dating period of 6 months based on the results of the autoclaving study. This expiration dating period may be extended based on the results of real-time long-term.

Stress Studies

L-NMMA Injection is stable when exposed to high heat. L-NMMA Injection Lots MA046 (1.2 mg/mL) and MA060 (75 mg/mL) packaged in clear Type 1 glass and sealed with rubber stoppers were autoclaved at 121°C and 30 psi for 1 hour. The results presented in Table 11 show that the amount of L-NMMA remaining is essentially unchanged.

Table 11 **Stability of L-NMMA Injection Stored at 121°C and 30 psi for 1 Hour**

		1.2 mg/mL Lot MA046		75 mg/mL Lot MA060	
Test	**Acceptance Criteria**	**Initial**	**1 hour**	**Initial**	**1 hour**
Description	Clear, colorless solution free of foreign matter				
Assay anhydrous basis (% w/w)	90.0 – 110.0				
Impurities					
Each specified impurity					
L-Ornithine (% w/w)	NMT 0.5				
L-Citrulline (% w/w)	NMT 0.5				
Each unspecified impurity (% w/w)	NMT 0.2				
Total impurities (% w/w)	NMT 3.0				
pH	5.5 – 7.5				
NMT = Not more than					

Accelerated and Long-term Stability

The stability of L-NMMA Injection Lots MA046 (1.2 mg/mL) and MA060 (75 mg/mL), packaged in clear Type 1 glass and sealed with rubber stoppers, is being evaluated under the ICH conditions recommended

for product to be stored at room temperature. These lots are the lowest and highest concentration of the lots used in the Phase 2 clinical studies. The protocol is summarized in table 12.

Table 12 **Stability Protocol for L-NMMA Injection**

Storage Condition[1]	**Time Point (months)**						
	Initial	**3**	**6**	**9**	**12**	**18**	**24**
25 ± 2°C/60 ± 5% RH	XYZ	X	X	X	XY	X	XYZ
30 ± 2°C/60 ± 5% RH[2]	XYZ	NT	X	X	X	NT	NT
40 ± 2°C/75 ± 5% RH	XYZ	X	X	NT	NT	NT	NT
1: Samples are to be stored in both upright and inverted positions. Upright samples are tested only if there is a significant change in the inverted samples. 2: Tested only if there is a significant change at 40°C/75% RH							
X = Description, Assay, Impurities , pH Y = Sterility, Container closure integrity Z = Endotoxins, Particulate matter NT = Not Tested							

Infusion Solution Preparation

L-NMMA Injection for INFUSION Administration is administered by withdrawing 16 mL from the appropriate INFUSION vial and adding that volume to a 100-mL (nominal volume) IV infusion bag of saline infusion solution (actual volume 105 to 115 ml). The concentrations of L-NMMA after mixing with infusion solution (average final volume 126 mL) are:

- 0.952 mg/mL for the 0.15 mg/kg/hr dose
- 3.17 mg/mL for the 0.50 mg/kg/hr dose
- 6.35 mg/mL for the 1.0 mg/kg/hr dose
- 9.52 mg/mL for the 1.5 mg/kg/hr dose

The stability of the highest and lowest of these solutions in the infusions bags to be used in the clinical studies and stored at 25°C for 24 hours, which is the maximum time the infusion solution may be used in the clinical study, has been evaluated. Results presented in Table 13 and table 14, respectively, show the product is essentially unchanged.

Table 13 **Stability of L-NMMA Injection 7.5 mg/mL Diluted in Saline and Stored at Ambient Conditions for 24 Hours**

Test	**Acceptance Criteria**	**Time Point (hours)**					
		Initial	**3**	**6**	**9**	**12**	**24**
Description	Clear, colorless solution free of foreign matter						
Assay (% w/w)	90.0 – 110.0						
Impurities							
Each specified impurity							
L-Ornithine (% w/w)	NMT 0.5						
L-Citrulline (% w/w)	NMT 0.5						
Each unspecified impurity (% w/w)	NMT 0.2						
Total impurities (% w/w)	NMT 3.0						
pH	5.5 - 7.5						
Particulate matter							
Particles ≥10 µm/vial	NMT 6,000						
Particles ≥25 µm/vial	NMT 600						
Lot MA054 NMT = Not more than							

L-NMMA Previously Data Compared to Placebo

L-NMMA Placebo was 0.9% Sodium Chloride USP. It was filtered, filled into vials and sterilized at the same facility, using the same conditions and packaged in the same container closure system as L-NMMA Injection.

The specifications for L-NMMA Placebo and the analytical results for Lots XXX (white cap for bolus administration) and XXX (grey cap for infusion administration), which were used in the Phase 2 clinical studies Testing for endotoxins, particulate matter and sterility are in-progress. No material was used in any clinical study until testing is complete and results indicate that the lot has passed all acceptance criteria.

Table 14 **Stability of L-NMMA Injection 75 mg/mL Diluted into Saline and Stored at Ambient Conditions for 24 Hours**

Test	Acceptance Criteria	Time Point (hours)					
		Initial	3	6	9	12	24
Description	Clear, colorless solution free of foreign matter						
Assay (% w/w)	90.0 – 110.0						
Impurities							
Each specified impurity							
L-Ornithine (% w/w)	NMT 0.5						
L-Citrulline (% w/w)	NMT 0.5						
Each unspecified impurity (% w/w)	NMT 0.2						
Total impurities (% w/w)	NMT 3.0						
pH	5.5 - 7.5						
Particulate matter							
Particles ≥10 µm/vial	NMT 6,000						
Particles ≥25 µm/vial	NMT 600						
Lot MA060 NMT = Not more than							

Table 15 **Specifications and Batch Analysis Results for L-NMMA Placebo**

Lot No.:			**DALTON**	**DALTON**
Batch Size:			**DALTON**	**DALTON**
Manufacturing Site [1]:			**Dalton**	**Dalton**
Date of Manufacture:			**DALTON**	**DALTON**
Test	**Method**	**Acceptance Criteria**	**Result**	**Result**
Description	Visual	Clear, colorless solution free of foreign matter		
Identification	HPLC	Absence of L-NMMA		
pH	Potentiometer	5.5 to 7.5		
Fill volume (mL)	Gravimetric	NLT 16.3		
Particulate matter	USP <788> Light Obscuration	USP		
Particles ≥10 µm/vial		NMT 6000	In-prog	In-prog
Particles ≥25 µm/vial		NMT 600	In-prog	In-prog
Endotoxins (EU/mL)	USP	NMT 0.5	In-prog	In-prog
Sterility	Sterile	Sterile	In-prog	In-prog
1: Dalton = Dalton Chemical Laboratories, Inc., Toronto, Ontario, Canada				
NMT = Not more than NLT = Not less than In-prog = Testing is in-progress. No material will be used in any clinical study until testing is complete and results indicate that the lot has passed all acceptance criteria.				

Labeling

Copies of the following labels are presented in Fig 15 through Fig 18:

- Bolus vial label
- Infusion vial label
- Kit box label
- Label inside kit to differentiate between bolus and infusion vials

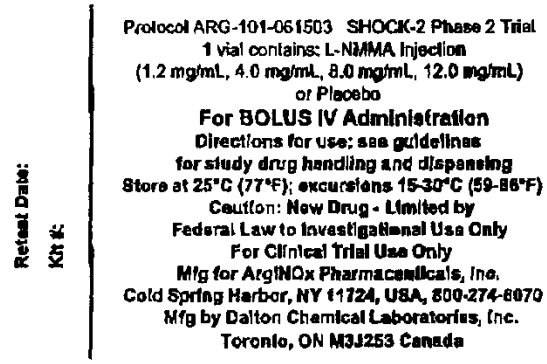


Figure 15 Label for L-NMMA Injection for BOLUS Administration Vial

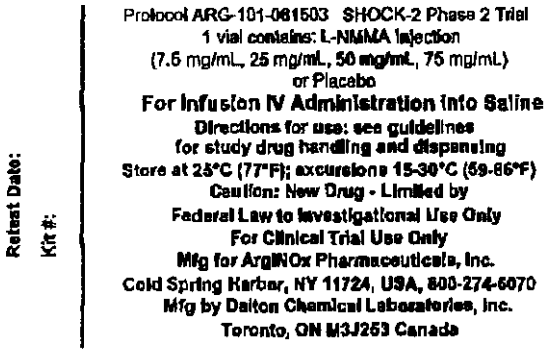


Figure 16 Label for L-NMMA Injection for INFUSION Administration Vial

Figure 17 Label for L-NMMA Injection Kit Box

For Dilution into Saline and IV Infusion Use Only

Figure 18 Label Inside L-NMMA Injection Kit to Differentiate Between Bolus and Infusion Vials

10.2 Docetaxel

DRUG NAME: Docetaxel
SYNONYM(S): **RP56976**
COMMON TRADE NAME(S): TAXOTERE®
CLASSIFICATION: Mitotic inhibitor, cytotoxic
Special pediatric considerations are noted when applicable, otherwise adult provisions apply.
MECHANISM OF ACTION:

Docetaxel is a semi-synthetic drug derived from precursor extracted from the needles of the European yew tree, Taxus baccata. It acts by disrupting the microtubular network that is essential for mitotic and interphase cellular functions. It promotes the assembly of tubulin into stable microtubules and inhibits their disassembly, causing inhibition of cell division and eventual cell death. Both docetaxel and paclitaxel bind to the same microtubule site, although the affinity of docetaxel is 1.9-fold higher. Cross-resistance between docetaxel and paclitaxel does not occur consistently. Docetaxel is a radiation-sensitizing agent. It is cell cycle phase-specific (G2/M phase). [71]

Table 16

PHARMACOKINETICS: Interpatient variability	wide interpatient variability, probably due to interpatient differences in cytochrome P450 3A4 (CYP3A4) activity.	
Distribution	distributed to all tissues and organs except the brain in animal studies	
	Cross blood brain barrier?	Very low levels were found in the brain in animal studies. In a single patient with leptomeningeal carcinomatosis, docetaxel was detected in CSF 2 hours after cessation of docetaxel infusion.
	volume of distribution	113 L
	plasma protein binding	> 95%
Metabolism	CYP3A involved in docetaxel metabolism in vitro	
	active metabolite(s)	none
	inactive metabolite(s)	one major and 3 minor
Excretion	primarily biliary/fecal elimination	
	urine	6% recovered in urine over 7 days
	feces	75% recovered in feces over 7 days, 80% of which was excreted during the first 2 days; < 8% was unchanged docetaxel.
	terminal half life	11.1 h
	clearance	21 L/h/m^2
Gender	no clinically significant difference	
Elderly	no clinically significant difference	

SPECIAL PRECAUTIONS:

Contraindicated in patients with a history of hypersensitivity reaction to docetaxel or to drugs formulated with polysorbate 80. Patients with prior severe hypersensitivity reactions should generally not be rechallenged with docetaxel. However, in patients with objective tumor responses and without other options to docetaxel therapy, re-treatment may be attempted with extreme caution and aggressive premedication by experienced practitioners. Contraindicated in patients with severe liver impairment. Patients hypersensitive to paclitaxel may also react to docetaxel.

Preexisting effusions: Patients with preexisting effusion should be closely monitored from the first dose for the possible exacerbation of the effusions.
Liver impairment: Patients treated with docetaxel 100 mg/m^2 are at a higher risk of developing severe adverse reactions if they have elevated transaminase (ALT and/or AST greater than 2 times the upper limit of normal [ULN]) and alkaline phosphatase (greater than 2 times ULN). Liver impairment reduces clearance and increases systemic exposure to docetaxel. Docetaxel should not be administered if AST/ALT >2x ULN or bilirubin >5x ULN unless liver metastases are present. Adverse reactions include life-threatening sepsis and gastrointestinal hemorrhage, febrile neutropenia, infections, thrombocytopenia, stomatitis and asthenia.
Alcohol abuse: When docetaxel is used in patients who abuse alcohol, or have abused alcohol, the risk of severe neurotoxic reactions may be increased.
Mutagenicity: Not mutagenic in Ames test or mammalian in vitro mutation test.
Clastogenic in mammalian in vitro and in vivo chromosome tests.
Pregnancy: FDA Pregnancy Category D. There is positive evidence of human fetal risk, but the benefits from use in pregnant women may be acceptable despite the risk (e.g., if the drug is needed in a life-threatening situation or for a serious disease for which safer drugs cannot be used or are ineffective).
Breastfeeding is not recommended due to the potential secretion into breast milk. [71] Table 17

SIDE EFFECTS: ORGAN SITE	9.1.1 SIDE EFFECT
allergy/immunology	hypersensitivity reaction (21%, severe 4%); moderate immunosuppression
blood/bone marrow febrile neutropenia	anemia (90%, severe 9%) , death, septic (1.7%) , death, non-septic (0.3%) , febrile neutropenia (11%) , infection with neutropenia (severe 6%) , leukocytopenia (96%, severe 75%) , ***neutropenia*** (96%, severe 32%) , nadir 8 days, duration of severe neutropenia 7 days , thrombocytopenia (8%)
cardiovascular (arrhythmia)	dysrhythmia (2%, severe 0.4%) , tachycardia (1%)
cardiovascular (general)	fluid retention, with premedication (52%), severe fluid retention, without premedication (82%, severe 22%); see paragraph , heart failure (0.3%) , hypertension (2%) , hypotension (3%, severe 0.5%)
constitutional symptoms	fatigue, 3-weekly schedule (62%, severe 13%) , ***fatigue, weekly schedule*** (72%, severe 14%), fever (32%, severe 2%)
dermatology/skin	*extravasation hazard:* irritant , alopecia (56-85%, severe 0.5%), injection site reactions (6%); , nail changes (19-51%, severe 2-22%) , hand-foot skin reaction (rare), rash/pruritus (48%, severe 5%)
gastrointestinal	*emetogenic potential:* low moderate, diarrhea (39%, severe <5%) , nausea (39%, severe <5%) , perforation (rare) , stomatitis (42%, severe 6%) , vomiting (22%, severe <5%)
hemorrhage	bleeding episode (1%) , bleeding episode with thrombocytopenia (0.1%)
infection	infection, including sepsis and pneumonia (22%)
metabolic/laboratory	elevated AST, ALT, bilirubin, and alkaline phosphatase (<2%)
neurology	neuropathy, motor (14%%, severe 4%), neuropathy, sensory (49%, severe 4%)
ocular/visual	tearing/watery eyes, weekly schedule (52%)
pain	arthralgia (9%, severe 0.6%) , myalgia (19%, severe 2%)

Pretreatment administration of dexamethasone is recommended to decrease the frequency and severity, and to delay the onset of docetaxel-induced fluid retention. Dexamethasone also reduces the severity of docetaxel-induced hypersensitivity reactions and cutaneous toxicity.
3-weekly regimen: A commonly used regimen consists of dexamethasone 8 mg PO twice a day for 3 consecutive days starting one day prior to each docetaxel infusion. This regimen has also been shown

to decrease the occurrence of severe stomatitis and infection.2 Patients must receive a minimum of 3 doses of dexamethasone prior to docetaxel treatment. If dexamethasone has not been taken prior to treatment, it should be started and the docetaxel infusion delayed until the following day. If treatment delay is not possible, diphenhydramine 50 mg IV and dexamethasone 10 mg IV may be given 30 minutes before starting docetaxel.35 Note that this premedication regimen has not been shown to reduce the incidence and severity of fluid retention, but is only an attempt to ameliorate hypersensitivity reactions. The patient should then be instructed to take dexamethasone 8 mg PO twice a day for two days.

Hypersensitivity reactions are most likely to occur during the first two cycles of docetaxel treatment, generally within the first few minutes after the infusion is started. Signs and symptoms usually abate within 15 minutes after the infusion is stopped. The most frequent minor manifestations were flushing, rash with or without pruritus, chest tightness, back pain, dyspnea, drug fever or chills. If minor reactions occur, therapy with docetaxel does not have to be discontinued. For severe reactions such as hypotension requiring treatment, bronchospasm, and generalized rash/erythema; stop the docetaxel, and have a physician assess the patient and order appropriate treatment.

Re-challenge after severe hypersensitivity reaction: Patients who have developed severe hypersensitivity reactions should generally not be rechallenged with docetaxel. However, in patients with objective tumor responses and without other options to docetaxel therapy, re-treatment may be attempted with extreme caution and aggressive premedication by experienced practitioners. It is recommended that a slower rate of infusion be used. One patient experienced major hypersensitivity symptoms during the first two cycles of docetaxel therapy despite prophylaxis with a corticosteroid and a histamine H1 blocking antagonist. Treatment was continued without further difficulty after sodium cromoglycate (400 mg PO four times a day, starting immediately after the second cycle) was added to the prophylactic regimen.

Fluid retention includes edema, and less frequently, pleural effusion, ascites, pericardial effusion and weight gain. Fluid retention occurs in 52% of patients receiving dexamethasone premedication, and in 82% of patients without premedication. It usually begins at the lower extremities and may become generalized with a weight gain of 3 kg or more. Fluid retention is not accompanied by acute episodes of dehydration, oliguria or hypotension. Fluid accumulation is due to increased capillary permeability rather than hypoalbuminemia or cardiac, hepatic or renal damage. It is slowly reversible after treatment is discontinued (median 29 [range, 0 to > 42] weeks). However, early, aggressive diuretic treatment may occasionally be required. Antihistamines have not been shown to be useful in controlling fluid retention.

Neuropathy: Rarely, neurologic effects result in moderate to severe neuropathy, leading to decreased dexterity and/or disturbances in gait, usually after cumulative doses of 600 mg/m^2.

Alopecia: Loss of hair, including on the head, eyebrows, eyelashes, pubic area, and underarm, occurs in most patients. Alopecia has a sudden onset, and occurs 14-21 days after treatment has begun. Hair should grow back once treatment has been completed; however cases of poor hair regrowth and/or persistent hair loss have been reported. Reports suggest some patients may experience prolonged hair loss lasting beyond 24 months, and possibly irreversibly.

Rash/pruritus: Cutaneous reactions are characterized by a rash, including localized eruptions mainly on feet and hands, but also on arms, face or thorax. These reactions are observed in 48% of patients. They are occasionally associated with pruritus. Eruptions generally occur within one week following the docetaxel infusion, resolve before the next infusion, and are not disabling.

Injection site reactions include skin sensitivities such as hyperpigmentation, inflammation, local erythema, dryness of the skin, or swelling of the vein. Injection site reactions occur in 6% of patients and are generally mild. Phlebitis or extravasations are observed less frequently. Leakage into surrounding tissue during intravenous administration (i.e., extravasation) may cause irritation, local tissue necrosis and/or thrombophlebitis.

Nail changes are characterized by hyperpigmentation, splinter hemorrhage, subungual hematoma and hyperkeratosis, orange discoloration, Beau-Reil lines (which indicate the cessation of nail growth), and acute paronychia. Some changes are cosmetic and asymptomatic, whereas others can be accompanied

by discomfort or pain. Changes are usually transient and disappear with treatment withdrawal, although there are reports of persistent changes. Loosening or loss of nails (onycholysis) occurs in 2-22%. Nail bed infections may be a complication and the application of topical antibiotics or antifungals may be necessary. Applying the principle of cold-induced vasoconstriction by wearing frozen gloves on the hands during treatment may reduce the incidence of nail and skin toxicity. In one study, overall incidence of nail toxicity was reduced with frozen glove treatment from 51% to 11%, and the incidence of grade 2 toxicity (onycholysis) was reduced from 22% to 0%. Additionally, median time of occurrence of nail toxicity was delayed to 106 days with frozen glove treatment as opposed to 58 days without.

Hand-foot skin reaction that occurs despite dexamethasone prophylaxis may respond to administration of pyridoxine 50 mg p.o. three times a day.

Tearing/watery eyes: An unexpected toxicity with the weekly schedule is excessive tearing, which was reported by half of the patients in a phase II study in women with metastatic breast cancer. Patients reported increased tearing and, in some cases, mild conjunctivitis or eye irritation. Formal ophthalmologic examination of several patients revealed no abnormalities. As with fluid retention, the onset of tearing seems to be related to cumulative dose and occurs after a median of 400 mg/m² (range, 120-960 mg/m²). Ten of 15 patients with tearing also developed some degree of fluid retention. Treatment with artificial tears or other ocular moisturizers ameliorated symptoms in some patients. Eye irritation led to a dose reduction in one patient.

INTERACTIONS:

Table 18

AGENT	EFFECT	MECHANISM	MANAGEMENT
dexamethasone	does not affect protein binding of docetaxel		
Doxorubicin	AUC of docetaxel increased, one hour interval between drugs does not change the results	doxorubicin may interfere at hepatic microsomal enzyme level	avoid concurrent therapy outside of clinical trial
Epirubicin	increased exposure to active metabolite of epirubicin	altered metabolism of epirubicin	avoid concurrent therapy outside of clinical trial
Etoposide	clearance of docetaxel decreased		avoid concurrent therapy outside of clinical trial
ifosfamide	when docetaxel given first it is associated with increased ifosfamide plasma clearance and decreased AUC	may be due to increased ifosfamide metabolism caused by corticosteroid premedication for docetaxel	avoid concurrent therapy outside of clinical trial

There have been no formal clinical studies to evaluate the drug interactions of docetaxel with other medications. In vitro studies have shown that the metabolism of docetaxel may be modified by the concomitant administration of compounds which induce, inhibit or are metabolized by (and thus may inhibit the enzyme competitively) cytochrome P450-3A such as cyclosporine, ketoconazole and erythromycin.

DOSAGE GUIDELINES:

Table 19

PLATELET COUNT (x 10^9/L)	ABSOLUTE NEUTROPHIL (ANC)* (x 10^9/L)			
	$\geq$ 1.8	1.5-1.8	1.0-1.5	<1.0
$\geq$ 100	75mg/m^2	60mg/m^2	50 mg/m^2	0%
70-100	60mg/m^2	60mg/m^2	50 mg/m^2	0%
50-70	50mg/m^2	50mg/m^2	50 mg/m^2	0%
<50	0%	0%	0%	0%

* ANC = WBC x (% polys + % stabs)

** 0% Indicates treatment should be postponed a week until the counts return to a level at which drugs may be given.

Guidelines for dosing also include consideration of ANC. Dosage may be reduced, delayed or discontinued in patients with bone marrow depression due to cytotoxic/radiation therapy or with other

toxicities.

Dosage in hepatic failure:

Table 20

Cycle length	Alkaline Phosphatase		AST +/or ALT	Dose
3 weeks:	< 2.5 x ULN	and	<1.5 x ULN	75mg/m^2
	2.5 – 5 x ULN	and	1.6 – 5 x ULN	60mg/m^2
	> 5 x ULN	or	> 5 ULN	discuss with contact physician

**Liver enzymes are recommended before cycle 1 and then prior to each treatment if clinically indicated (e.g., if liver enzymes are elevated, liver metastases are present or there is severe toxicity such as neutropenia). If liver enzymes are normal and there is no evidence of liver metastases or severe toxicity, check liver enzymes after 3 cycles (ie, at cycle 4).*

Table 21

Dosage in severe peripheral neuropathy:	Cycle length 3 weeks:	reduce by 25% to 60mg/m^2; further reduce by 15% to 50mg/m^2 if reactions continue; discontinue if patient experiences ≥ grade 3 peripheral neuropathy
Dosage in severe or cumulative cutaneous reactions:	Cycle length 3 weeks:	reduce by 25% to 60mg/m^2; further reduce by 15% to 50mg/m^2 if reactions continue ; discontinue if patient experiences ≥ grade 3 cutaneous reactions

Dosage in dialysis: Hemodialysis: no significant removal; no dose adjustment required, may be administered before or after hemodialysis

10.3 Paclitaxel

DRUG NAME:
Paclitaxel

COMMON TRADE NAME
Taxol

SIDE EFFECTS
Hypersensitivity reactions: Anaphylaxis and severe hypersensitivity reactions characterized by dyspnea and hypotension requiring treatment, angioedema, and generalized urticaria have occurred in 2 to 4% of patients receiving paclitaxel in clinical trials. Fatal reactions have occurred in patients despite premedication. All patients should be pretreated with corticosteroids, diphenhydramine, and H2 antagonists. Such premedication may consist of dexamethasone 20 mg p.o. administered approximately 12 and 6 hours before paclitaxel, diphenhydramine (or its equivalent) 50 mg IV 30 to 60 minutes before paclitaxel, and cimetidine (300 mg) or ranitidine (50 mg) IV 30 to 60 minutes before paclitaxel. Minor symptoms such as flushing, skin reactions, dyspnea, hypotension, or tachycardia do not require interruption of therapy. However, severe reactions, such as hypotension requiring treatment, dyspnea requiring bronchodilators, angioedema, or generalized urticaria require immediate discontinuation of paclitaxel and aggressive symptomatic therapy. Patients who have developed severe hypersensitivity reactions should not be re-challenged with paclitaxel.

Hematologic effects: Bone marrow suppression (primarily neutropenia) is dose-dependent and is the DLT. Neutrophil nadirs occur at a median of 11 days. Paclitaxel should not be administered to patients with baseline neutrophil counts of less than 1500 cells/mm^3. Patients should not be re-treated with subsequent cycles of paclitaxel until neutrophils recover to a level >1500 cells/mm^3 and platelets recover to a level >100,000 cells/mm^3.

Cardiovascular effects: Hypotension, bradycardia, and hypertension have been observed during administration of paclitaxel but generally do not require treatment. Occasionally paclitaxel infusions must

be interrupted or discontinued because of initial or recurrent hypertension. Frequent vital sign monitoring, particularly during the first hour of paclitaxel infusion, is recommended.

Nervous system: Although the occurrence of peripheral neuropathy is frequent, the development of severe symptomatology is unusual and requires a dose reduction of 20% for all subsequent courses of paclitaxel.

Injection site reaction: Injection site reactions, including reactions secondary to extravasation, are usually mild and consist of erythema, tenderness, skin discoloration, or swelling at the injection site. More severe events such as phlebitis, cellulitis, induration, skin exfoliation, necrosis, and fibrosis have been reported. In some cases the onset of the injection site reaction either occurred during a prolonged infusion or was delayed by a 7 to 10 days.

DRUG INTERACTIONS
The metabolism of paclitaxel is catalyzed by CYP isoenzymes CYP2C8 and CYP3A4. Caution should be exercised when paclitaxel is concomitantly administered with known substrates (e.g., midazolam, buspirone, felodipine, lovastatin, eletriptan, sildenafil, simvastatin, and triazolam), inhibitors (e.g., atazanavir, clarithromycin, indinavir, itraconazole, ketoconazole, nefazodone, nelfinavir, ritonavir, saquinavir, and telithromycin), or inducers (e.g., rifampin and carbamazepine) of CYP3A4. Caution should also be exercised when paclitaxel is concomitantly administered with known substrates (e.g., repaglinide and rosiglitazone), inhibitors (e.g., gemfibrozil), or inducers (e.g., rifampin) of CYP2C8.

DOSAGE AND ADMINISTRATION
Paclitaxel at 175 mg/m^2 will be IV infused over 3 hours or 80 mg/m2 will be IV infused weekly over 1 hour on Day 1 at each cycle. Paclitaxel will be administered 15 min after L-NMMA infusion on Day 1.

10.4 Nab-Paclitaxel

DRUG NAME
Nab-paclitaxel

COMMON TRADE NAME
Abraxane

SIDE EFFECTS

Hematologic effects: Bone marrow suppression (primarily neutropenia) is dose-dependent and a DLT of nab-paclitaxel. In clinical studies, Grade 3-4 neutropenia occurred in 34% of patients with metastatic breast cancer. Do not administer nab-paclitaxel to patients with baseline ANC of less than 1,500 cells/mm^3. In the case of severe neutropenia (<500 cells/mm^3 for seven days or more) during a course of nab-paclitaxel therapy, reduce the dose of nab-paclitaxel in subsequent courses. Resume treatment with nab-paclitaxel after ANC recovers to a level >1,500 cells/mm^3 and platelets recover to a level >100,000 cells/mm^3.

Nervous system: Sensory neuropathy is dose- and schedule-dependent. The occurrence of Grade 1 or 2 sensory neuropathy does not generally require dose modification. If ≥ Grade 3 sensory neuropathy develops, withhold nab-paclitaxel treatment until resolution to Grade 1 or 2.

Hypersensitivity reactions: Grade 1 or 2 hypersensitivity reactions consisting of dyspnea and flushing, hypotension, chest pain, and arrhythmia can occur on the day of nab-paclitaxel administration. Severe and sometimes fatal hypersensitivity reactions, including anaphylactic reactions, have also been reported. Patients who experience a severe hypersensitivity reaction to nab-paclitaxel should not be re-challenged with this drug.

Albumin (Human): Nab-paclitaxel contains albumin (human), a derivative of human blood. Based on effective donor screening and product manufacturing processes, it carries a remote risk for transmission of viral diseases. A theoretical risk for transmission of Creutzfeldt-Jakob disease also is considered extremely remote.

Infections: Infectious episodes were reported in 24% of patients treated with nab-paclitaxel. Oral candidiasis, respiratory tract infections, and pneumonia were the most frequently reported infectious complications.

Cardiovascular effects: Hypotension, during the 30-minute infusion, has occurred in 5% of patients. Bradycardia, during the 30-minute infusion, occurred in <1% of patients. These vital sign changes most often caused no symptoms and required neither specific therapy nor treatment discontinuation. Severe cardiovascular events possibly related to single-agent nab-paclitaxel have occurred in approximately 3% of patients. These events included cardiac ischemia/infarction, chest pain, cardiac arrest, supraventricular tachycardia, edema, thrombosis, pulmonary thromboembolism, pulmonary emboli, and hypertension. Cases of cerebrovascular attacks (strokes) and transient ischemic attacks have been reported. Electrocardiogram (ECG) abnormalities were common among patients at baseline. ECG abnormalities on study did not usually result in symptoms, were not dose-limiting, and required no intervention. ECG abnormalities were noted in 60% of patients. Among patients with a normal ECG prior to study entry, 35% of all patients developed an abnormal tracing while on study. The most frequently reported ECG modifications were non-specific repolarization abnormalities, sinus bradycardia, and sinus tachycardia.

Respiratory effects: Dyspnea (12%), cough (7%), and pneumothorax (<1%) have been reported after treatment with nab-paclitaxel.

Vision disorders: Ocular/visual disturbances have occurred in 13% of all patients treated with nab-paclitaxel and 1% were severe. The severe cases (keratitis and blurred vision) were reported in patients who received higher doses than those recommended (300 or 375 mg/m^2). These effects generally have been reversible.

Arthralgia/Myalgia: The symptoms were usually transient, occurred two or three days after nab-paclitaxel administration, and resolved within a few days.

Renal effects: Overall 11% of patients experienced creatinine elevation (1% severe). No discontinuations, dose reductions, or dose delays were caused by renal toxicities.

Other clinical events: Nail changes (changes in pigmentation or discoloration of the nail bed) have been reported. Edema occurred in 10% of patients; no patients had severe edema. Dehydration and pyrexia were also reported.

DRUG INTERACTIONS

The metabolism of paclitaxel is catalyzed by CYP2C8 and CYP3A4. Caution should be exercised when administering nab-paclitaxel concomitantly with medicines known to inhibit (e.g., ketoconazole and other

imidazole antifungals, erythromycin, fluoxetine, gemfibrozil, cimetidine, ritonavir, saquinavir, indinavir, and nelfinavir) or induce (e.g., rifampicin, carbamazepine, phenytoin, efavirenz, and nevirapine) either CYP2C8 or CYP3A4.

DOSAGE AND ADMINISTRATION
Nab-paclitaxel at 260 mg/m^2 will be IV infused over 30 minutes on Day 1 of each cycle. Nab-paclitaxel will be administered 15 min after L-NMMA infusion on Day 1.

10.5 Amlodipine[72]

DRUG NAME: Amlodipine
SYNONYM(S): besylate salt of amlodipine
COMMON TRADE NAME(S): NORVASC®
CLASSIFICATION: long-acting calcium channel blocker
Amlodipine besylate is chemically described as 3-Ethyl-5-methyl (±)-2-[(2-aminoethoxy)methyl]4-(2-chlorophenyl)-1,4-dihydro-6-methyl-3,5-pyridinedicarboxylate, monobenzenesulphonate. Its empirical formula is C20H25CIN2O5•C6H6O3S, and its structural formula is:

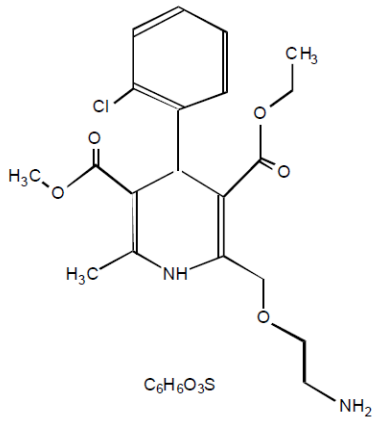


Figure 19: Amlodipine Molecular structure

Amlodipine besylate is a white crystalline powder with a molecular weight of 567.1. It is slightly soluble in water and sparingly soluble in ethanol. AMLODIPINE (amlodipine besylate) Tablets are formulated as white tablets equivalent to 2.5, 5, and 10 mg of amlodipine for oral administration. In addition to the active ingredient, amlodipine besylate, each tablet contains the following inactive ingredients: microcrystalline cellulose, dibasic calcium phosphate anhydrous, sodium starch glycolate, and magnesium stearate.
MECHANISM OF ACTION:
Amlodipine is a dihydropyridine calcium antagonist (calcium ion antagonist or slow-channel blocker) that inhibits the transmembrane influx of calcium ions into vascular smooth muscle and cardiac muscle. Experimental data suggest that amlodipine binds to both dihydropyridine and nondihydropyridine binding sites. The contractile processes of cardiac muscle and vascular smooth muscle are dependent upon the movement of extracellular calcium ions into these cells through specific ion channels. Amlodipine inhibits calcium ion influx across cell membranes selectively, with a greater effect on vascular been seen in intact animals at therapeutic doses. Serum calcium concentration is not affected by amlodipine. Within the physiologic pH range, amlodipine is an ionized compound (pKa=8.6), and its kinetic interaction with the calcium channel receptor is characterized by a gradual rate of association and dissociation with the receptor binding site, resulting in a gradual onset of effect.

Amlodipine is a peripheral arterial vasodilator that acts directly on vascular smooth muscle to cause a reduction in peripheral vascular resistance and reduction in blood pressure. The precise mechanisms by which amlodipine relieves angina have not been fully delineated, but are thought to include the following:
Exertional Angina: In patients with exertional angina, AMLODIPINE reduces the total peripheral resistance (afterload) against which the heart works and reduces the rate pressure product, and thus myocardial oxygen demand, at any given level of exercise.
Vasospastic Angina: AMLODIPINE has been demonstrated to block constriction and restore blood flow in coronary arteries and arterioles in response to calcium, potassium epinephrine, serotonin, and thromboxane A2 analog in experimental animal models and in human coronary vessels in vitro. This inhibition of coronary spasm is responsible for the effectiveness of AMLODIPINE in vasospastic (Prinzmetal's or variant) angina.

PHARMACODYNAMICS

Hemodynamics: Following administration of therapeutic doses to patients with hypertension, AMLODIPINE produces vasodilation resulting in a reduction of supine and standing blood pressures. These decreases in blood pressure are not accompanied by a significant change in heart rate or plasma catecholamine levels with chronic dosing. Although the acute intravenous administration of amlodipine decreases arterial blood pressure and increases heart rate in hemodynamic studies of patients with chronic stable angina, chronic oral administration of amlodipine in clinical trials did not lead to clinically significant changes in heart rate or blood pressures in normotensive patients with angina. With chronic once daily oral administration, antihypertensive effectiveness is maintained for at least 24 hours. Plasma concentrations correlate with effect in both young and elderly patients. The magnitude of reduction in blood pressure with AMLODIPINE is also correlated with the height of pretreatment elevation; thus, individuals with moderate hypertension (diastolic pressure 105–114 mmHg) had about a 50% greater response than patients with mild hypertension (diastolic pressure 90–104 mmHg). Normotensive subjects experienced no clinically significant change in blood pressures (+1/–2 mmHg).
In hypertensive patients with normal renal function, therapeutic doses of AMLODIPINE resulted in a decrease in renal vascular resistance and an increase in glomerular filtration rate and effective renal plasma flow without change in filtration fraction or proteinuria. As with other calcium channel blockers, hemodynamic measurements of cardiac function at rest and during exercise (or pacing) in patients with normal ventricular function treated with AMLODIPINE have generally demonstrated a small increase in cardiac index without significant influence on dP/dt or on left ventricular end diastolic pressure or volume. In hemodynamic studies, AMLODIPINE has not been associated with a negative inotropic effect when administered in the therapeutic dose range to intact animals and man, even when co-administered with beta-blockers to man. Similar findings, however, have been observed in normal or well-compensated patients with heart failure with agents possessing significant negative inotropic effects.
Electrophysiologic Effects: AMLODIPINE does not change sinoatrial nodal function or atrioventricular conduction in intact animals or man. In patients with chronic stable angina, intravenous administration of 10 mg did not significantly alter A-H and H-V conduction and sinus node recovery time after pacing. Similar results were obtained in patients receiving AMLODIPINE and concomitant beta-blockers. In clinical studies in which AMLODIPINE was administered in combination with beta-blockers to patients with either hypertension or angina, no adverse effects on electrocardiographic parameters were observed. In clinical trials with angina patients alone, AMLODIPINE therapy did not alter electrocardiographic intervals or produce higher degrees of AV blocks.

PHARMACOKINETICS AND METABOLISM

After oral administration of therapeutic doses of AMLODIPINE, absorption produces peak plasma concentrations between 6 and 12 hours. Absolute bioavailability has been estimated to be between 64 and 90%. The bioavailability of AMLODIPINE is not altered by the presence of food. Amlodipine is extensively (about 90%) converted to inactive metabolites via hepatic metabolism with 10% of the parent

compound and 60% of the metabolites excreted in the urine. Ex vivo studies have shown that approximately 93% of the circulating drug is bound to plasma proteins in hypertensive patients. Elimination from the plasma is biphasic with a terminal elimination half-life of about 30–50 hours. Steady-state plasma levels of amlodipine are reached after 7 to 8 days of consecutive daily dosing. The pharmacokinetics of amlodipine are not significantly influenced by renal impairment. Patients with renal failure may therefore receive the usual initial dose.

Elderly patients and patients with hepatic insufficiency have decreased clearance of amlodipine with a resulting increase in AUC of approximately 40–60%, and a lower initial dose may be required. A similar increase in AUC was observed in patients with moderate to severe heart failure.

CLINICAL STUDIES

The antihypertensive efficacy of AMLODIPINE has been demonstrated in a total of 15 double-blind, placebo-controlled, randomized studies involving 800 patients on AMLODIPINE and 538 on placebo. Once daily administration produced statistically significant placebo-corrected reductions in supine and standing blood pressures at 24 hours postdose, averaging about 12/6 mmHg in the standing position and 13/7 mmHg in the supine position in patients with mild to moderate hypertension. Maintenance of the blood pressure effect over the 24-hour dosing interval was observed, with little difference in peak and trough effect. Tolerance was not demonstrated in patients studied for up to 1 year. The 3 parallel, fixed doses, dose response studies showed that the reduction in supine and standing blood pressures was dose-related within the recommended dosing range. Effects on diastolic pressure were similar in young and older patients. The effect on systolic pressure was greater in older patients, perhaps because of greater baseline systolic pressure. Effects were similar in black patients and in white patients.

Effects in Chronic Stable Angina

The effectiveness of 5–10 mg/day of AMLODIPINE in exercise-induced angina has been evaluated in 8 placebo-controlled, double-blind clinical trials of up to 6 weeks duration involving 1038 patients (684 AMLODIPINE, 354 placebo) with chronic stable angina. In 5 of the 8 studies, significant increases in exercise time (bicycle or treadmill) were seen with the 10 mg dose. Increases in symptom-limited exercise time averaged 12.8% (63 sec) for AMLODIPINE 10 mg, and averaged 7.9% (38 sec) for AMLODIPINE 5 mg. AMLODIPINE 10 mg also increased time to 1 mm ST segment deviation in several studies and decreased angina attack rate. The sustained efficacy of AMLODIPINE in angina patients has been demonstrated over long-term dosing. In patients with angina, there were no clinically significant reductions in blood pressures (4/1 mmHg) or changes in heart rate (+0.3 bpm).

Effects in Vasospastic Angina

In a double-blind, placebo-controlled clinical trial of 4 weeks duration in 50 patients, AMLODIPINE therapy decreased attacks by approximately 4/week compared with a placebo decrease of approximately 1/week ($p<0.01$). Two of 23 AMLODIPINE and 7 of 27 placebo patients discontinued from the study due to lack of clinical improvement.

Effects in Documented Coronary Artery Disease

In PREVENT, 825 patients with angiographically documented coronary artery disease were randomized to AMLODIPINE (5–10 mg once daily) or placebo and followed for 3 years. Although the study did not show significance on the primary objective of change in coronary luminal diameter as assessed by quantitative coronary angiography, the data suggested a favorable outcome with respect to fewer hospitalizations for angina and revascularization procedures in patients with CAD. CAMELOT enrolled 1318 patients with CAD recently documented by angiography, without left main coronary disease and without heart failure or an ejection fraction <40%. Patients (76% males, 89% Caucasian, 93% enrolled at US sites, 89% with a history of angina, 52% without PCI, 4% with PCI and no stent, and 44% with a stent) were randomized to double-blind treatment with either AMLODIPINE (5–10 mg once daily) or placebo in addition to standard care that included aspirin (89%), statins (83%), beta-blockers (74%), nitroglycerin (50%), anti-coagulants (40%), and diuretics (32%), but excluded other calcium channel blockers. The mean duration of follow-up was 19 months. The primary endpoint was the time to first

occurrence of one of the following events: hospitalization for angina pectoris, coronary revascularization, myocardial infarction, cardiovascular death, resuscitated cardiac arrest, hospitalization for heart failure, stroke/TIA, or peripheral vascular disease. A total of 110 (16.6%) and 151 (23.1%) first events occurred in the AMLODIPINE and placebo groups, respectively, for a hazard ratio of 0.691 (95% CI: 0.540–0.884, p = 0.003). The outcome of this study was largely derived from the prevention of hospitalizations for angina and the prevention of revascularization procedures. In an angiographic substudy (n=274) conducted within CAMELOT, there was no significant difference between amlodipine and placebo on the change of atheroma volume in the coronary artery as assessed by intravascular ultrasound.

Studies in Patients with Heart Failure

AMLODIPINE has been compared to placebo in four 8–12 week studies of patients with NYHA Class II/III heart failure, involving a total of 697 patients. In these studies, there was no evidence of worsened heart failure based on measures of exercise tolerance, NYHA classification, symptoms, or left ventricular ejection fraction. In a long-term (follow-up at least 6 months, mean 13.8 months) placebo-controlled mortality/morbidity study of AMLODIPINE 5–10 mg in 1153 patients with NYHA Classes III (n=931) or IV (n=222) heart failure on stable doses of diuretics, digoxin, and ACE inhibitors, AMLODIPINE had no effect on the primary endpoint of the study which was the combined endpoint of all-cause mortality and cardiac morbidity (as defined by life-threatening arrhythmia, acute myocardial infarction, or hospitalization for worsened heart failure), or on NYHA classification, or symptoms of heart failure. Total combined all-cause mortality and cardiac morbidity events were 222/571 (39%) for patients on AMLODIPINE and 246/583 (42%) for patients on placebo; the cardiac morbid events represented about 25% of the endpoints in the study. Another study (PRAISE-2) randomized patients with NYHA Class III (80%) or IV (20%) heart failure without clinical symptoms or objective evidence of underlying ischemic disease, on stable doses of ACE inhibitors (99%), digitalis (99%), and diuretics (99%), to placebo (n=827) or AMLODIPINE (n=827) and followed them for a mean of 33 months. There was no statistically significant difference between AMLODIPINE and placebo in the primary endpoint of all-cause mortality (95% confidence limits from 8% reduction to 29% increase on AMLODIPINE). With AMLODIPINE there were more reports of pulmonary edema.

Clinical Trials Experience

Because clinical trials are conducted under widely varying conditions, adverse reaction rates observed in the clinical trials of a drug cannot be directly compared to rates in the clinical trials of another drug and may not reflect the rates observed in practice. AMLODIPINE has been evaluated for safety in more than 11,000 patients in U.S. and foreign clinical trials. In general, treatment with AMLODIPINE was well-tolerated at doses up to 10 mg daily. Most adverse reactions reported during therapy with AMLODIPINE were of mild or moderate severity. In controlled clinical trials directly comparing AMLODIPINE (N=1730) at doses up to 10 mg to placebo (N=1250), discontinuation of AMLODIPINE due to adverse reactions was required in only about 1.5% of patients and was not significantly different from placebo (about 1%). The most common side effects are headache and edema. The incidence (%) of side effects that occurred in a dose related manners are as follows: Table 22

Adverse Event	2.5 mg	5.0 mg	10.0 mg	Placebo
	N=275	N=296	N=268	N=520
Edema	1.8	3.0	10.8	0.6
Dizziness	1.1	3.4	3.4	1.5
Flushing	0.7	1.4	2.6	0.0
Palpitation	0.7	1.4	4.5	0.6

Other adverse experiences that were not clearly dose related but were reported with an incidence greater than 1.0% in placebo-controlled clinical trials include the following:

Table 23

Placebo-Controlled Studies			

	AMLODIPINE (%)	Placebo (%)			
	(N=1730)	(N=1250)			
Headache	7.3	7.8			
Fatigue	4.5	2.8			
Nausea	2.9	1.9			
Abdominal Pain	1.6	0.3			
Somnolence	1.4	0.6			

For several adverse experiences that appear to be drug and dose related, there was a greater incidence in women than men associated with amlodipine treatment as shown in the following table:

Table 24

	AMLODIPINE		Placebo	
Adverse Event	Male=%	Female=%	Male=%	Female=%
	(N=1218)	(N=512)	(N=914)	(N=336)
Edema	5.6	14.6	1.4	5.1
Flushing	1.5	4.5	0.3	0.9
Palpitations	1.4	3.3	0.9	0.9
Somnolence	1.3	1.6	0.8	0.3

The following events occurred in <1% but >0.1% of patients in controlled clinical trials or under conditions of open trials or marketing experience where a causal relationship is uncertain; they are listed to alert the physician to a possible relationship:

Cardiovascular: arrhythmia (including ventricular tachycardia and atrial fibrillation), bradycardia, chest pain, hypotension, peripheral ischemia, syncope, tachycardia, postural dizziness, postural hypotension, vasculitis.

Central and Peripheral Nervous System: hypoesthesia, neuropathy peripheral, paresthesia, tremor, vertigo.

Gastrointestinal: anorexia, constipation, dyspepsia, dysphagia, diarrhea, flatulence, pancreatitis, vomiting, gingival hyperplasia.

General: allergic reaction, asthenia, back pain, hot flushes, malaise, pain, rigors, weight gain, weight decrease.

Musculoskeletal System: arthralgia, arthrosis, muscle cramps, myalgia.

Psychiatric: sexual dysfunction (male and female), insomnia, nervousness, depression, abnormal dreams, anxiety, depersonalization.

Respiratory System: dyspnea, epistaxis.

Skin and Appendages: angioedema, erythema multiforme, pruritus, rash, rash erythematous, rash maculopapular.

Special Senses: abnormal vision, conjunctivitis, diplopia, eye pain, tinnitus.

Urinary System: micturition frequency, micturition disorder, nocturia.

Autonomic Nervous System: dry mouth, sweating increased.

Metabolic and Nutritional: hyperglycemia, thirst.

Hemopoietic: leukopenia, purpura, thrombocytopenia.

These events occurred in less than 1% in placebo-controlled trials, but the incidence of these side effects was between 1% and 2% in all multiple dose studies. The following events occurred in <0.1% of patients: cardiac failure, pulse irregularity, extrasystoles, skin discoloration, urticaria, skin dryness, alopecia, dermatitis, muscle weakness, twitching, ataxia, hypertonia, migraine, cold and clammy skin, apathy, agitation, amnesia, gastritis, increased appetite, loose stools, coughing, rhinitis, dysuria, polyuria, parosmia, taste perversion, abnormal visual accommodation, and xerophthalmia. Other reactions occurred sporadically and cannot be distinguished from medications or concurrent disease states such as myocardial infarction and angina.

AMLODIPINE therapy has not been associated with clinically significant changes in routine laboratory tests. No clinically relevant changes were noted in serum potassium, serum glucose, total triglycerides, total cholesterol, HDL cholesterol, uric acid, blood urea nitrogen, or creatinine.

DRUG INTERACTIONS

In Vitro Data, indicate that AMLODIPINE has no effect on the human plasma protein binding of digoxin, phenytoin, warfarin, and indomethacin.

Co-administration of AMLODIPINE with cimetidine did not alter the pharmacokinetics of AMLODIPINE.

Co-administration of 240 mL of grapefruit juice with a single oral dose of amlodipine 10 mg in 20 healthy volunteers had no significant effect on the pharmacokinetics of amlodipine.

Co-administration of a magnesium and aluminum hydroxide antacid with a single dose of AMLODIPINE had no significant effect on the pharmacokinetics of AMLODIPINE.

A single 100 mg dose of sildenafil in subjects with essential hypertension had no effect on the pharmacokinetic parameters of AMLODIPINE. When AMLODIPINE and sildenafil were used in combination, each agent independently exerted its own blood pressure lowering effect.

Co-administration of multiple 10 mg doses of AMLODIPINE with 80 mg of atorvastatin resulted in no significant change in the steady-state pharmacokinetic parameters of atorvastatin.

Co-administration of AMLODIPINE with digoxin did not change serum digoxin levels or digoxin renal clearance in normal volunteers.

Single and multiple 10 mg doses of AMLODIPINE had no significant effect on the pharmacokinetics of ethanol.

Co-administration of AMLODIPINE with warfarin did not change the warfarin prothrombin response time.

Co-administration of a 180 mg daily dose of diltiazem with 5 mg amlodipine in elderly hypertensive patients resulted in a 60% increase in amlodipine systemic exposure. Erythromycin co-administration in healthy volunteers did not significantly change amlodipine systemic exposure. However, strong inhibitors of CYP3A4 (e.g., ketoconazole, itraconazole, ritonavir) may increase the plasma concentrations of amlodipine to a greater extent. Monitor for symptoms of hypotension and edema when amlodipine is co-administered with CYP3A4 inhibitors.

No information is available on the quantitative effects of CYP3A4 inducers on amlodipine. Blood pressure should be closely monitored when Amlodipine is co-administered with CYP3A4 inducers.

OVERDOSAGE

Overdosage might be expected to cause excessive peripheral vasodilation with marked hypotension and possibly a reflex tachycardia. In humans, experience with intentional overdosage of AMLODIPINE is limited.

Single oral doses of amlodipine maleate equivalent to 40 mg amlodipine/kg and 100 mg amlodipine/kg in mice and rats, respectively, caused deaths. Single oral amlodipine maleate doses equivalent to 4 or more mg amlodipine/kg or higher in dogs (11 or more times the maximum recommended human dose on a mg/m^2 basis) caused a marked peripheral vasodilation and hypotension.

If massive overdose should occur, initiate active cardiac and respiratory monitoring. Frequent blood pressure measurements are essential. Should hypotension occur, provide cardiovascular support including elevation of the extremities and the judicious administration of fluids. If hypotension remains unresponsive to these conservative measures, consider administration of vasopressors (such as phenylephrine) with attention to circulating volume and urine output. As AMLODIPINE is highly protein bound, hemodialysis is not likely to be of benefit.

DOSAGE AND ADMINISTRATION [72]

The initial antihypertensive oral dose of AMLODIPINE is 10 mg once daily starting the day before of C1D1.

Dose may be adjusted by the treating physician if needed. Amlodipine dose will be held if systolic BP remains below 100 mmHg.

10.6 Enteric-Coated Aspirin 81 mg
DRUG NAME: Acetylsalicylic acid

SIDE EFFECTS
Many adverse reactions due to acetylsalicylic acid ingestion are dose-related.

Gastrointestinal (the frequency and severity of these adverse effects are dose-related): nausea, vomiting, diarrhea, gastrointestinal bleeding and/or ulceration, dyspepsia, heartburn, hematemesis, melena, abdominal pain, and rarely gastrointestinal inflammation.

Bleeding: Due to platelet inhibition, bleedings e.g. perioperative hemorrhage, hematomas, epistaxis, urogenital bleedings, and gingival bleedings may occur. Serious bleedings, such as gastrointestinal tract hemorrhages, and cerebral hemorrhages are rare. Isolated cases of potentially life-threatening bleedings have been reported, especially in patients with uncontrolled hypertension and/or concomitant antihemostatic agents.

Ear: dizziness, tinnitus, vertigo, hearing loss. Dizziness and tinnitus have been reported, which may be indicative of an overdose.

Hematologic: leukopenia, thrombocytopenia, purpura, anemia. Anemia with respective laboratory and clinical signs and symptoms, such as asthenia, pallor, and hypoperfusion is generally caused by bleeding (e.g., occult microbleeding, acute or chronic bleeding). Hemolysis and hemolytic anemia in patients with severe forms of glucose-6-phosphate dehydrogenase deficiency have been reported.

Dermatologic and hypersensitivity: urticaria, pruritus, skin eruptions, asthma, anaphylaxis, edema, nasal congestion, and rhinitus. Severe allergic reactions, including anaphylactic shock are very rarely reported.

DRUG INTERACTIONS
Methotrexate, used at 15 mg/week or less: Salicylates may retard the elimination of methotrexate by decreasing renal clearance of methotrexate, displacing methotrexate from protein binding sites, and thereby increasing its hematological toxicity.

Anticoagulants, thrombolytics / other inhibitors of platelet aggregation/hemostasis, e.g. warfarin, heparin: Caution is necessary when salicylates and anticoagulants, thrombolytics /other inhibitors of platelet aggregation / hemostasis prescribed concurrently, as salicylates can depress the concentration of prothrombin in the plasma, leading to an increased risk of bleeding.

Oral hypoglycemics, e.g. insulin, sulfonylureas: Large doses of salicylates have a hypoglycemic action and may enhance the effect of oral hypoglycemic agents. Diabetics receiving concurrent salicylate and hypoglycemic therapy should be monitored closely: reduction of the sulfonylurea hypoglycemic drug dosage may be necessary.

Diuretics: Diuretics in combination with acetylsalicylic acid at higher doses leads to decreased glomerular filtration via decreased prostaglandin synthesis. As a result, sodium excretion may be decreased by salicylate administration.

Uricosuric Agents: Salicylates in large doses are uricosuric agents; smaller amounts may depress uric acid clearance and thus decrease the uricosuric effects of other drugs.

Valproic Acid: Salicylates may alter valproic acid (VPA) metabolism and may displace VPA from protein binding sites, possibly intensifying the effects of VPA. Caution is recommended when VPA is administered concomitantly with salicylates.

Glucocorticoids (systemic), except hydrocortisone used as replacement therapy in Addison's disease: Decreased blood salicylate levels during corticosteroid treatment and risk of salicylate overdose after this treatment is stopped via increased elimination of salicylates by corticosteroids.

Angiotensin Converting Enzyme (ACE) Inhibitors: The hyponatremic and hypotensive effects of ACE inhibitors *may* be diminished by the concomitant administration of acetylsalicylic acid due to its indirect effect on the renin-angiotensin conversion pathway (i.e., inhibition of vasodilatory prostaglandins leading to decreased glomerular filtration). The potential interaction may be related to the dose of acetylsalicylic acid (≥3 g/day).

Selective Serotonin Re-uptake Inhibitors: Increased risk of upper gastrointestinal bleeding due to possibly synergistic effect.

Digoxin: Plasma concentrations of digoxin are increased due to a decrease in renal excretion.

Non-steroidal anti-inflammatory drugs (NSAIDs): The use of other NSAIDs with salicylates at high doses (≥3 g/day) may increase the risk of ulcers and gastrointestinal bleeding due to a synergistic effect. Ibuprofen can interfere with the anti-platelet effect of low-dose acetylsalicylic acid (81-325 mg QD). Long-term daily use of ibuprofen may render acetylsalicylic acid less effective when used for cardioprotection and stroke prevention. To minimize this interaction, regular users of ibuprofen and of low-dose, immediate-release acetylsalicylic acid should take the ibuprofen at least one hour after and 11 hours before the daily acetylsalicylic acid dose. The use of delayed-release (e.g. enteric-coated) acetylsalicylic acid is not recommended when using ibuprofen regularly. Naproxen may attenuate the irreversible platelet inhibition induced by acetylsalicylic acid. Clinical pharmacodynamic data suggest that concurrent (same day) naproxen sodium usage for more than one day consecutively inhibits the effect of low-dose acetylsalicylic acid on platelet activity and this inhibition may persist for up to several days after stopping naproxen sodium therapy. The clinical relevance of this interaction is not known. Treatment with naproxen, in patients with increased cardiovascular risk may limit the cardiovascular protection of acetylsalicylic acid.

DOSAGE AND ADMINISTRATION
Enteric-coated daily low-dose aspirin should preferably be taken after meals, with plenty of liquid**.** Enteric-coated aspirin will be administered as 81 mg QD during the 6 21-day cycles of L-NMMA and docetaxel.

11 Prohibited concomitant therapy and dietary restrictions

The following medications and procedures are prohibited during the study:

- Alternative therapy, including palliative radiotherapy, for treatment of the patient's malignancy
- Any investigational therapy other than L-NMMA, amlodipine, and taxane chemotherapy.
- Medications Listed in Appendix E
- Any complementary or alternative medications
- Enzyme inducers, such as the enzyme-inducing antiepileptic drugs phenytoin, carbamazepine or phenobarbital, or rifampin, rifabutin, rifapentine or St. John's Wort within 14 days prior to the first dose of the study drugs and during the period of treatment with L-NMMA in this study.
- Use of the following medications and procedures should be considered with caution:

- Interaction with medicinal products metabolized through CYP3A4 and CYP2C8 route:
  - Docetaxel is a CYP3A4 substrate. In vitro studies have shown that the metabolism of docetaxel may be modified by the concomitant administration of compounds that induce, inhibit, or are metabolized by cytochrome P450 3A4.
  - In vivo studies showed that the exposure of docetaxel increased 2.2-fold when it was coadministered with ketoconazole, a potent inhibitor of CYP3A4. Protease inhibitors, particularly ritonavir, may increase the exposure of docetaxel. Concomitant use of docetaxel and drugs that inhibit CYP3A4 may increase exposure to docetaxel and should be avoided.
  - The metabolism of paclitaxel is catalyzed by CYP2C8 and CYP3A4. Caution should be exercised when administering paclitaxel and nab-paclitaxel concomitantly with medicines known to inhibit (e.g., ketoconazole and other imidazole antifungals, erythromycin, fluoxetine, gemfibrozil, cimetidine, ritonavir, saquinavir, indinavir, and nelfinavir) or induce (e.g., rifampicin, carbamazepine, phenytoin, efavirenz, and nevirapine) either CYP2C8 or CYP3A4.

11.1 Anti-neoplastic therapies

Other anti-neoplastic therapies must be avoided during the length of the trial

11.2 Dietary restrictions

Patients should be on a low nitrite/nitrate diet including avoidance of processed meats such as hot dogs, ham, bacon, and sausage 1 week before study treatment start and for the duration of the study. Patients should also keep a food diary the week before and the week of the L-NMMA infusion during Cycles 1 and 2.

12 Assessment types

12.1 CRITERIA FOR RESPONSE

The main endpoint in this study is to determine the MTD of the L-NMMA plus docetaxel combination and the CBR of the L-NMMA plus taxane chemotherapy (docetaxel, paclitaxel, or nab-paclitaxel) combination in patients with refractory locally advanced or metastatic triple negative breast cancer. Tumor response will be evaluated using the RECIST 1.1

12.2 RECIST 1.1 Criteria

The clinical tumor response will be assessed using RECIST criteria at baseline and before surgery. RECIST criteria offer a simplified, conservative, extraction of imaging data for wide application in clinical trials. They presume that linear measures are an adequate substitute for 2-D methods and register four response categories[73]

- CR (complete response) = disappearance of all target lesions
- PR (partial response) = 30% decrease in the sum of the longest diameter of target lesions
- PD (progressive disease) = 20% increase in the sum of the longest diameter of target lesions
- SD (stable disease) = small changes that do not meet above criteria
-

12.3 Withdrawal of subjects from study

Withdrawal

Subjects **must be withdrawn from the trial** (treatment and procedures) for the following reasons:

- Subject withdraws consent from study treatment and study procedures. A subject must be removed from the trial at her own request or at the request of her legally acceptable

representative. At any time during the trial and without giving reasons, a subject may decline to participate further. The subject will not suffer any disadvantage as a result.

- Blood pressure increases beyond 180 mmHg after L-NMMA infusion and does not return to <160 mmHg after two rechecks spaced 15-30 min apart.
- Subject is lost to follow-up.
- Death.

Subjects **may be** withdrawn from the study for the following reasons:

- The subject is non-compliant with study drug, trial procedures, or both; including the use of anti-cancer therapy not prescribed by the study protocol. Non-compliant will be considered if a subject takes ≤ 75% of study drug (L-NMMA or taxane chemotherapy).
- Pregnancy. Pregnancy will be reported as an SAE. (Note: subjects who have been withdrawn from treatment with study drug because of pregnancy should not undergo any scans while pregnant, except ultrasound).
- If, in the investigator's opinion, continuation of the trial would be harmful to the subject's well-being.
- Severe allergic reaction to study drug (L-NMMA, taxane chemotherapy) (such as stomatitis or Grade 3 or 4 hypersensitivity reaction).
- The development of a second cancer.
- Development of an intercurrent illness or situation which would, in the judgment of the investigator, significantly affect assessments of clinical status and trial endpoints.
- Deterioration of ECOG performance status to 4.
- Use of illicit drugs or other substances that may, in the opinion of the investigator, have a reasonable chance of contributing to toxicity or otherwise skewing trial result.

Any subject removed from the trial will remain under medical supervision until discharge or transfer is medically acceptable and for 30 days following last dose of study treatment. In all cases, the reason for withdrawal must be recorded in the CRF and in the subject's medical records.

12.4 Pregnancy and assessments of fertility

Pregnancy testing is required at screening or whenever pregnancy is suspected. Serum pregnancy testing should be performed at screening and at the end of the study or if clinically indicated.

Women of child-bearing potential, defined as all women physiologically capable of becoming pregnant as defined in Appendix D, must use highly effective contraception during the study and for 60 days after stopping L-NMMA plus taxane chemotherapy. Highly effective contraception is defined as either:

- Total abstinence: When this is in line with the preferred and usual lifestyle of the subject. [Periodic abstinence (e.g., calendar, ovulation, symptothermal, post-ovulation methods) and withdrawal are not acceptable methods of contraception]
- Sterilization: have had surgical bilateral oophorectomy (with or without hysterectomy) or tubal ligation at least six weeks before taking study treatment. In case of oophorectomy alone, only when the reproductive status of the woman has been confirmed by follow up hormone level assessment
- Male partner sterilization (with the appropriate post-vasectomy documentation of the absence of sperm in the ejaculate). [For female subjects on the study, the vasectomised male partner should be the sole partner for that subject].

  or
- Use of a combination of any two of the following (a+b or a+c or b+c):
  a. Use of oral, injected, implanted or other hormonal methods of contraception
  b. Placement of an intrauterine device (IUD) or intrauterine system (IUS)
  c. Barrier methods of contraception: Condom or Occlusive cap (diaphragm or cervical/vault caps) with spermicidal foam/gel/film/cream/vaginal suppository

- In case of use of oral contraception, women should have been stable on the oral agent before taking study treatment.

13. Safety monitoring and reporting

Information about all adverse events, whether volunteered by the subject, discovered by investigator questioning, or detected through physical examination, laboratory test or other means, will be collected and recorded and followed as appropriate.

13.1 Adverse events

13.1.1 Definitions and reporting

Adverse events that begin or worsen after informed consent should be recorded in the Adverse Events CRF. Conditions that were already present at the time of informed consent should be recorded in the Medical History page of the patient's CRF. Adverse event monitoring should be continued for at least 30 days or until adverse event is resolved to baseline or at least Grade II according to CTCAE v4.03 following the last dose of study treatment. Adverse events (including lab abnormalities that constitute AEs) should be described using a diagnosis whenever possible, rather than individual underlying signs and symptoms. When a clear diagnosis cannot be identified, each sign or symptom should be reported as a separate Adverse Event.

The occurrence of adverse events should be sought by non-directive questioning of the patient at each visit during the study. Adverse events also may be detected when they are volunteered by the patient during or between visits or through physical examination, laboratory test, or other assessments. As far as possible, each adverse event should be evaluated to determine:

1. The severity grade (CTCAE Grade 1-4)
2. Its duration (Start and end dates or if continuing at the Safety Follow-up Visit)
3. Its relationship to the study treatment (Reasonable possibility that AE is related: No, Yes)
4. Action taken with respect to study or investigational treatment (none, dose adjusted, temporarily interrupted, permanently discontinued, hospitalized, unknown, not applicable)
5. Whether medication or therapy was given (no concomitant medication/non-drug therapy, concomitant medication/non-drug therapy)
6. Outcome (not recovered/not resolved, recovered/resolved, recovering/resolving, recovered/resolved with sequelae, fatal, unknown)
7. Whether it is serious, where a serious adverse event (SAE) is defined as in Section 14.

All adverse events should be treated appropriately. Such treatment may include changes in study drug treatment including possible interruption or discontinuation, starting or stopping concomitant treatments, changes in the frequency or nature of assessments, hospitalization, or any other medically required intervention. Once an adverse event is detected, it should be followed until its resolution to baseline or at least Grade II according to CTCAE v4.03, and assessment should be made at each visit (or more frequently, if necessary) of any changes in severity, the suspected relationship to the study drug, the interventions required to treat it, and the outcome. Information about common side effects already known about the investigational drug can be found in the Investigators' Brochure. This information should be included in the patient informed consent and should be discussed with the patient during the study as needed. Adverse event monitoring should be continued for at least 30 days following the last dose of study treatment

13.1.2 Laboratory test abnormalities

Laboratory abnormalities that constitute an Adverse event in their own right (are considered clinically significant, induce clinical signs or symptoms, require concomitant therapy or require changes in study treatment), should be recorded on the Adverse Events CRF. Whenever possible, a diagnosis, rather than

a symptom should be provided (e.g. anemia instead of low hemoglobin). Laboratory abnormalities that meet the criteria for Adverse Events should be followed until they have returned to normal or an adequate explanation of the abnormality is found. When an abnormal laboratory or test result corresponds to a sign/symptom of an already reported adverse event, it is not necessary to separately record the lab/test result as an additional event. Laboratory abnormalities, that do not meet the definition of an adverse event, should not be reported as adverse events. A Grade 3 or 4 event (severe) as per CTCAE does not automatically indicate a SAE unless it meets the definition of serious as defined below and/or as per investigator's discretion. A dose hold or medication for the lab abnormality may be required by the protocol and is still, by definition, an adverse event.

14 Serious Adverse Events

14.1 Definitions

A serious adverse event is an undesirable sign, symptom or medical condition which:

- is fatal or life-threatening
- results in persistent or significant disability/incapacity
- constitutes a congenital anomaly/birth defect
- requires inpatient hospitalization or prolongation of existing hospitalization, unless hospitalization is for:
    - routine treatment or monitoring of the studied indication, not associated with any deterioration in condition (specify what this includes)
    - elective or pre-planned treatment for a pre-existing condition that is unrelated to the indication under study and has not worsened since the start of study drug
    - treatment on an emergency outpatient basis for an event not fulfilling any of the definitions of a SAE given above and not resulting in hospital admission
    - social reasons and respite care in the absence of any deterioration in the patient's general condition
- is medically significant, i.e., defined as an event that jeopardizes the patient or may require medical or surgical intervention to prevent one of the outcomes listed above

14.1.1 Reporting

The principal investigator has the obligation to report all related and unexpected adverse events to the local IRB. To ensure patient safety, every SAE, regardless of suspected causality, following local IRB policy, occurring:

- after the patient has provided informed consent and until at least 30 days after the patient has stopped study treatment/participation
- after protocol-specified procedures begin (e.g., placebo run-in, washout period, double-blind treatment, etc.) and 30 days after the patient has stopped study treatment
- after the start of any period in which the study protocol interferes with the standard medical treatment given to a patient (e.g., treatment withdrawal during washout period, change in treatment to a fixed dose of concomitant medication) and until 30 days after the patient has stopped study treatment must be reported to the institutional IRB within 24 hours of learning of its occurrence ; This includes serious, related, labeled (expected) and serious, related, unlabeled (unexpected) adverse experiences. All deaths during treatment or within 30 days following completion of active protocol therapy must be reported within 5 working days.

Recurrent episodes, complications, or progression of the initial SAE must be reported as follow-up to the original episode within 24 hours of the investigator receiving the follow-up information. A SAE occurring at a different time interval or otherwise considered completely unrelated to a previously reported one should be reported separately as a new event. The end date of the first event must be provided.

Each re-occurrence, complication, or progression of the original event should be reported as a follow-up to that event regardless of when it occurs. The follow-up information should describe whether the event has resolved or continues, if and how it was treated, whether the blind was broken or not (if applicable), and whether the patient continued or withdrew from study participation.

If the SAE is not previously documented in the Investigator Brochure or Package Insert (new occurrence) and is thought to be related to the study drug, may urgently require further information from the investigator to the local IRB. The Investigator and the local IRB may need to issue an Investigator Notification (IN), to inform all investigators involved in any study with the same drug that this SAE has been reported. Suspected Unexpected Serious Adverse Reactions (SUSARs) will be collected and reported to the competent authorities. For Comparator Drugs/Secondary Suspects (Concomitant Medications), all serious adverse experiences will be forwarded to Investigational Drug Services Department by the investigator. The following categories and definitions of causal relationship to study drug should be considered for use for this clinical study.

- Certain: There is a known causal relationship between the study drug and the SAE. The event responds to withdrawal of study drug (dechallenge), and recurs with rechallenge when clinically feasible. (>95% certainty)
- Probable: There is reasonable causal relationship between the study drug and the SAE. The event responds to dechallenge. Rechallenge is not required. (65%-95% probability)
- Possible: There is reasonable causal relationship between the study drug and the SAE. Dechallenge information is lacking or unclear. (35%-65% probability of relatedness)
- Not likely: There is temporal relationship to study drug administration, but there is not a reasonable causal relationship between the study drug and the SAE. (5-35% probability of relatedness)
- Not related: There is not a temporal relationship to study drug administration (too early, or late, or study drug not taken), or there is known causal relationship between the SAE and another drug, concurrent disease, or other circumstance. (<5% chance of relatedness)
- Adverse events classified as “serious” require expeditious handling and reporting to Houston Methodist Cancer Center (HMCC) and local IRB to comply with regulatory requirements.
- All related or unexpected AEs of any of the study drug must be immediately reported to local IRB by the investigator or designee within 24 hours of becoming aware of the event. If only limited information is initially available, follow-up reports are required. The original SAE form must be kept on file at the study site.

  All SAEs should be faxed or emailed to

  Houston Methodist Cancer Center at:
  hmccsaereports@houstonmethodist.org
  Fax Number: 713-790-5106
- For studies conducted under an Investigator IND, any event that is both related and unexpected must be reported to the local IRB as soon as possible and, in no event, later than 7 days (death or life-threatening event) or 15 days (all other SAEs) after the investigator’s or institution’s initial receipt of the information.

14.1.2 Pregnancy

Preclinical data regarding reproductive toxicity is described in the most recent Investigator Brochure. The potential reproductive risk for humans is unknown. Women of childbearing potential should be advised to use highly effective contraception methods while they are receiving L-NMMA plus Docetaxel combination and up to 8 weeks after treatment has been stopped.

To ensure patient safety, each pregnancy occurring while the patient is on study treatment must be reported to the local HMRI&HMCC IRB within 24 hours of learning of its occurrence. The pregnancy should be followed up to determine outcome, including spontaneous or voluntary termination, details of

the birth, and the presence or absence of any birth defects, congenital abnormalities, or maternal and/or newborn complications. The newborn will be followed for at least 12 months.
Pregnancy should be recorded on a Clinical Trial Pregnancy Form and reported by the investigator to the local HMRI&HMCC IRB Medication Safety office. Pregnancy follow-up should be recorded on the same form and should include an assessment of the possible relationship to the study treatment and any pregnancy outcome. Any SAE experienced during pregnancy must be reported on the SAE Report Form.

15 Statistical methods

15.1 Sample Size.

Statistical Considerations

This phase Ib/II study is designed to determine the DLTs, MTD, and RP2D of an L-NMMA plus docetaxel combination as well as to investigate the efficacy of the L-NMMA and taxane chemotherapy (docetaxel, paclitaxel, or nab-paclitaxel) combination. The phase Ib portion of the study will utilize the standard Bayesian CRM. A method based entirely on a Bayesian decision framework. This portion of the trial is designed to investigate the combination at two dose levels of docetaxel (75 and 100 mg/m^2) and 7 dose levels of L-NMMA (5, 7.5, 10, 12.5, 15, 17.5, and 100 mg/kg). Table 25 presents the proposed dose levels of the combination. The starting dose will be docetaxel at 75 mg/m^2 and L-NMMA at 7.5 mg/kg. The phase Ib portion of the trial will be comprised of 34 patients. Once the MTD and RP2D for L-NMMA in combination with docetaxel has been determined, the phase II portion of the trial will proceed.

The phase Ib portion of the study was designed and will be conducted using Bayesian Model Averaging Continual Reassessment Method software developed by the Biostatistics Department of the University of Texas M. D. Anderson Cancer Center (https://biostatistics.mdanderson.org/SoftwareDownload/) [74, 75]. The software assumes the probability of toxicity at dose i (π_i) is modeled as $\pi_i = p_i \times \exp(\alpha)$ where p_i is a constant and α is distributed a priori as a normal random variable with mean 0 and variance 2. The software requires the investigator to specify the prior median probability of toxicity at each of the 6 doses of L-NMMA under consideration (s_i, i = -1, 0, 1, 2, 3, 4). The CRM model assumes that toxicity is a monotonic increasing function with dose, and thus, the s_i values may not decrease as i increases. The values p_i in the probability model are selected so that $E[p_i \times \exp(\alpha)] = s_i$. After the first cohort, each successive cohort is given the dose whose posterior probability of toxicity given the data collected thus far is closest to the target toxicity (the software does not allow an untried dose to be skipped). Thus as information accumulates, the model is continually updated *posteriorly* and decisions are made on the *posterior* distribution. As a result, no data is lost and there is the assurance that the decision to increase or decrease the dose level is consistently reassessed, as more information becomes available. For a dose level to be chosen as the MTD, at least 4 patients must have received said dose.

Table 25. Phase Ib Dose Levels

Dose Level	L-NMMA (mg/Kg)	Docetaxel (mg/m^2)
-1	5	75
0	7.5	75
1	10	75
2	12.5	75
3	15	75
4	17.5	100
5	20	100

The target toxicity probability will be 0.25 to 0.30 and a maximum of 34 patients will be treated in the phase Ib portion of the trial. The first cohort of patients will be treated at dose level 0. As an extra measure of safety, the trial will be stopped early if the lowest dose level is unacceptably toxic, formally if

$$P_r\{p_{-1} \times e^{\alpha} > 0.27 | data\} > 0.85$$

The Bayesian model averaging CRM will be implemented using the following three toxicity probability sets: 1) (p_{-1}, p_0, p_1, p_2, p_3, p_4, p_5) = (0.01, 0.04, 0.07, 0.10, 0.13, 0.16, 0.19); 2) (p_{-1}, p_0, p_1, p_2, p_3, p_4, p_5) = (0.96, 0.96, 0.96, 0.96, 0.96, 0.96, 0.96); and 3) (p_{-1}, p_0, p_1, p_2, p_3, p_4, p_5) = (0.30, 0.30, 0.30, 0.30, 0.30, 0.30, 0.30). Operating characteristics of the design under six dose-toxicity scenarios are tabulated below.

Table 26. Phase Ib Design Operating Characteristics. Optimal decisions are given in boldface type.

Scenario	Study Parameter	Dose Level							None
		-1	0	1	2	3	4	5	
1 High Toxicity	Prob[toxicity]	0.45	0.55	0.65	0.75	0.85	0.90	0.95	
	Prob[Selected]	0.03	0.00	0.00	0.00	0.00	0.00	0.00	**0.96**
	# treated	5.3	2.7	0.6	0.1	0.00	0.00	0.00	
2 Target @ 0	Prob[toxicity]	0.14	**0.28**	0.50	0.60	0.70	0.80	0.90	
	Prob[Selected]	0.20	**0.54**	0.09	0.00	0.00	0.00	0.00	0.17
	# treated	9.7	**13.1**	5.0	1.1	0.2	0.0	0.00	
3 Target @ 1	Prob[toxicity]	0.08	0.16	**0.28**	0.50	0.65	0.80	0.95	
	Prob[Selected]	0.02	0.33	**0.48**	0.12	0.01	0.00	0.00	0.04
	# treated	4.0	10.8	**11.1**	5.3	1.2	0.2	0.0	
4 Target @ 2	Prob[toxicity]	0.04	0.08	0.16	**0.28**	0.50	0.70	0.90	
	Prob[Selected]	0.00	0.06	0.33	**0.47**	0.13	0.01	0.00	0.01
	# treated	1.4	5.3	9.5	**10.4**	5.7	1.3	0.1	
5 Target @ 3	Prob[toxicity]	0.02	0.04	0.08	0.16	**0.28**	0.50	0.75	
	Prob[Selected]	0.00	0.00	0.06	0.33	**0.45**	0.15	0.01	0.00
	# treated	0.5	2.9	4.7	8.5	**10.1**	5.8	1.5	
6 Target @ 4	Prob[toxicity]	0.01	0.02	0.04	0.08	0.16	**0.28**	0.50	
	Prob[Selected]	0.00	0.00	0.01	0.07	0.30	**0.40**	0.22	0.00
	# treated	0.2	2.3	2.5	4.0	7.3	**8.9**	8.7	
7 Target @ 5	Prob[toxicity]	0.01	0.02	0.04	0.08	0.12	0.16	**0.28**	
	Prob[Selected]	0.00	0.00	0.00	0.01	0.05	0.13	**0.80**	0.00
	# treated	0.2	2.3	2.3	2.7	3.7	4.7	**18.1**	
8 Low Toxicity	Prob[toxicity]	0.01	0.02	0.03	0.04	0.06	0.08	**0.10**	
	Prob[Selected]	0.00	0.00	0.00	0.00	0.00	0.00	**1.00**	0.00
	# treated	0.2	2.2	2.2	2.2	2.4	2.6	**22.2**	

Under Scenario 7 in none of the dose levels are too toxic, the estimated probability of finding the highest dose as the RP2D is 1.00. Scenario 1 represents a setting in all doses are too toxic. For this scenario, the probability of finding no acceptable doses is 0.96. If 15 patients are treated at an individual dose, the phase Ib portion of the trial will end and these 15 patients will be considered the first patients of the phase II portion of the trial.

The primary endpoint of the phase II portion of this study will be CBR (CR + PR + SD). All patients treated at the RP2D in the phase Ib portion of the study will comprise the first patients enrolled into the phase II portion of the trial, which will be conducted using a Simon's optimal two-stage design. A null response rate of 15% is assumed and the target response rate for the combination will be 35%. When the probability of accepting a "bad" regimen (i.e. response rate $\leq$ 15%) is 0.10 (i.e., alpha) and the probability of rejecting a "good" regimen (i.e. response rate $\geq$ 35%) is 0.20 (i.e., beta or 1 – power), Simon's optimal design requires 15 patients in the first stage. If three or fewer patients respond to the combination, the trial will be stopped and the regimen will be declared as ineffective. If at least four of the first 15 patients respond, 9 additional patients will be entered on the study to reach a total of 24 patients. The new regimen will be rejected if the response rate is less than or equal to 5 out of 24 patients and will be accepted otherwise. This design yields a type I error rate of a = 0.098 (targeted rate of 0.10) and power of 80.1% (targeted power of 80%) when the true response rate is 0.35. The operating characteristics of the phase II portion of the trial are as follows: 1) When the true response rate is 15% (i.e., null hypothesis is true), the probability of stopping the trial early (i.e., after the first stage) is 82.3%. On the other hand, if the true response rate is 35%, the probability of stopping the trial early is only 17.3%. The expected sample size is 16.6 if the response rate is 15% and 22.5 if the true response rate is 35%. If there are 3 or fewer responders in the first 15 patients the probability of achieving a significant result at the end of stage 2 is only 0.046 (the futility rate) assuming the observed data in stage 1 provides a reasonable estimate of the response rate. At the end of the study, the CBR and its associated 95% Wilson score confidence interval will be reported. With 24 patients, the CBR confidence interval will be no wider than 37.1 percentage points. If a total of 6 patients out of 24 respond such that the combination will be considered successful and warrant further investigation, the confidence the estimated CBR will be 25% with a confidence interval of (12.0%, 44.9%). This trial was designed using PASS 13.0.8. Based on the Phase Ib operating characteristics given in Table 26, it is anticipated that total accrual for this phase Ib/II trial will be 36 patients with a maximum of 48 patients.

Statistical Analysis Plan

Safety Analysis of DLT and Adverse Event Data: All patients who received study drug will be included in the safety analysis of this combination regimen. Dose-limiting toxicity rate, adverse event data and corresponding toxicity grades will be summarized in each dose level and in the overall patient population. Incidence tables will be generated to summarize incidence of patients reporting at least one episode of each specific adverse event, incidence of adverse events causing withdrawal and incidence of serious adverse events. The total number of episodes for each event reported (Frequency Table), the severity and attribution to study therapy of each episode reported (Severity Table and Attribution Table) will also be displayed.

Listings of adverse events by patients will include the time to onset, the duration of each event, the severity of each event, and the relationship of the event to study therapy, whether it was a serious event, and whether it caused withdrawal. Safety data will be summarized for the overall patient group and by dose levels. Toxicities will be graded according to Common Toxicity Criteria (CTCAE) v4.03 .

The safety profiles of the study will be assessed through summaries of adverse events (AEs), serious adverse events (SAEs), AEs leading to treatment discontinuation, and treatment-related death. All patients who receive at least 1 dose of treatment will be included in the analysis for safety. The safety analysis will report the frequency of all AEs and laboratory abnormalities, as well as the frequency of dose interruptions, dose reductions, and treatment discontinuation for toxicity. Toxicity rates will be presented using the worst NCI CTCAE V4.03 grade per patient.

Several exploratory analyses of biomarkers will be perfomed in this aim. Analysis will primarily be based on correlations between biomarkers using correlation coefficients and comparison of changes in biomarker levels from baseline to post-treatment levels using paired t-test or nonparametric analogs as well as linear mixed models for repeated measurements. Although the correlative results will be largely

hypothesis generating, they will likely provide useful information for the design of future studies. We would be able to evaluate impact of L-NMMA on iNOS levels, as a measurement of target engagement.
For transcriptional analysis, we will align RNA-Seq short reads to the mm9 genome using STAR and will extract read counts mapping to ENSEMBL genes using HTseq. Then DEseq2 will be used in pre/post paired mode to identify a gene expression signature of treatment response using FDR=5% and fold-change >2. Heat maps will be generated for visual exploration. We will perform pathway analyses of the treatment response signature using DAVID, network analysis using IPA Ingenuity and rank-based pathway analysis using GSEA and gene sets in MSigDB. The goal of these analyses is to identify mechanisms and pathways that are mobilized by treatment. In parallel, we will perform mutational analysis as described in to identify point mutations and indels in expressed genes whose abundance differs between pre and post treatment. [75-77] These analyses will only be performed in the context of well-expressed genes in both conditions, using Fisher exact test p-values adjusted for multiple testing. The objective of these analyses is to nominate genes and pathways where mutations may be either associated with sensitivity (mutation abundance decreases in post vs. pre) or resistance (mutation abundance decreases in post vs. pre). These analyses will be performed tumor by tumor and the gene lists will be pooled and frequently altered genes will be identified as described in [77,78]. Dr. Elemento and his group including Ms. Verma have extensive experience performing these analyses as demonstrated by their extensive publication record.

16 Protocol amendments, or changes in study conduct

Any change or addition to this protocol requires a written protocol amendment that must be reviewed by the local IRB and the investigator before implementation. Amendments significantly affecting the safety of subjects, the scope of the investigation or the scientific quality of the study require additional approval by the IRB at each study center. Examples of amendments requiring such approval are:

1. increases in drug dose or duration of exposure of subjects,
2. significant changes in the study design (e.g. addition or deletion of a control group),
3. increases in the number of invasive procedures,
4. addition or deletions of a test procedure required for monitoring of safety.

These requirements for approval should in no way prevent any immediate action from being taken by the investigator in the interests of preserving the safety of all patients included in the trial. If an immediate change to the protocol is felt to be necessary by the investigator and is implemented for safety reasons must be notified to the IRB and other centers must be informed immediately. Amendments affecting administrative aspects of the study do not require formal protocol amendments. Amendments affecting administrative aspects of the study will require IRB approval.

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18 Appendices

Appendix A: ECOG/ Karnofsky Performance Status Criteria

ECOG Performance Status Scale		Karnofsky Performance Scale	
Grade	**Descriptions**	**Percent**	**Description**
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.	100	Normal, no complaints, no evidence of disease.
		90	Able to carry on normal activity; minor signs or symptoms of disease.
1	Symptoms, but ambulatory. Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (e.g., light housework, office work).	80	Normal activity with effort; some signs or symptoms of disease.
		70	Cares for self, unable to carry on normal activity or to do active work.
2	In bed < 50% of the time. Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours.	60	Requires occasional assistance, but is able to care for most of his/her needs.
		50	Requires considerable assistance and frequent medical care.
3	In bed > 50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.	40	Disabled, requires special care and assistance
		30	Severely disabled, hospitalization indicated. Death no imminent.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.	20	Very sick, hospitalization indicated. Death not imminent.
		10	Moribund, fatal processes progressing rapidly.
5	Dead	0	Dead

Appendix B: New York Heart Association (NYHA) Classifications

Class	Description
I	Patients with cardiac disease but without resulting limitations of physical activity. Ordinary physical activity does not cause undue fatigue, palpitation, dyspnea, or anginal pain.
II	Patients with cardiac disease resulting in slight limitation of physical activity. They are comfortable at rest. Ordinary physical activity results in fatigue, palpitation, dyspnea, or anginal pain.
III	Patients with cardiac disease resulting in marked limitation of physical activity. They are comfortable at rest. Less than ordinary physical activity causes fatigue, palpitation, dyspnea, or anginal pain.
IV	Patients with cardiac disease resulting in inability to carry on physical activity without discomfort. Symptoms of cardiac insufficiency or of the anginal syndrome may be present event at rest. If any physical activity is undertaken, discomfort is increased.

Appendix C: Version 4.03 (dated June-14-2010)

CTCAE Files:
NCI Common Terminology Criteria for Adverse Events (CTCAE) v.4 data files and related documents are published here. The most current release files appear in this directory:

Files: Booklet	Content
CTCAE_4.03_2010-06-14_QuickReference_5x7.pdf	Most recent release of core terminology: PDF document, traditional small booklet format.

http://evs.nci.nih.gov/ftp1/CTCAE/About.html

WOCBP

Women of childbearing potential and men must agree to use adequate contraception (one of the following listed below) prior to study entry, for the duration of study participation and up to 60 days following completion of therapy. Additionally, male subjects (including those who are vasectomized) whose partners are pregnant or might be pregnant must agree to use condoms for the duration of the study and for 90 days following completion of therapy.

- Total abstinence from sexual intercourse (minimum one complete menstrual cycle).
- Vasectomized male subjects or vasectomized partner of female subjects.
- Hormonal contraceptives (oral, parenteral or transdermal) for at least 3 months prior to study drug administration.
- Double-barrier method (condoms, contraceptive sponge, diaphragm or vaginal ring with spermicidal jellies or cream).
- Intrauterine device (IUD).

The following criteria will be used to define postmenopausal:

- Age 56 or older with no spontaneous menses for at least 12 months prior to study entry;

Or

- Age 55 or younger with no spontaneous menses for at least 12 months prior to study entry (e.g., spontaneous or secondary to hysterectomy) and with a documented estradiol level in the postmenopausal range according to local institutional/laboratory standard;

Or

- Documented bilateral oophorectomy.

Women failing to meet one of these criteria will be classified as pre-menopausal.

Appendix E : **Nitrate/Nitrite Drug Interactions**

Drug Class	Medications
Phosphodiesterase inhibitors	sildenafil, tadalafil, vardenafil, avanafil
Nitrates	isosorbide dinitrate, isosorbide mononitrate, nitroglycerin,
Nitrites	amyl nitrite, sodium nitrite
General anesthetics	Nitrous oxide

** *Patients should be on a low nitrite/nitrate diet including avoidance of processed meats such hot dogs, ham, bacon, and sausage.*

Appendix F: **Pharmacokinetic (PK) and Pharmacodynamic (PD) Sample Collection and Processing Guidelines**

Sampling Schedule for L-NMMA and Docetaxel PK and PD Evaluation

Patients will have PK and PD sampling (plasma) performed predose (10-30 minutes before L-NMMA infusion) on Days 1, 2, and 5 of Cycle 1 and Days 1 and 5 of Cycle 2.
One 5-mL blood sample is required for each PK sample time point and each PD sample time point.

*The actual times and date of samples drawn must be recorded.

Sample Preparation Guidelines

Samples should be labeled with the protocol number, patient identification (ID) number, date, time of collection (recorded as 24-hour clock time) and sample type (PK or PD)

PLASMA SAMPLE PREPARATION FOR L-NMMA & DOCETAXEL

1. Draw at least 5mL blood into one 6-mL green top heparinized (Na or Lithium) tube. Mix the tube by gently inverting the tube 2-3 times. To avoid hemolysis, do not mix vigorously.
2. Centrifuge the blood using 1,100 to 1,300 relative centrifugal force (RCF) (g) for 10 minutes for a swinging bucket rotor or for 15 minutes for a fixed-angle bucket rotor. If ideal equipment not available, the minimum requirement are 1,000 RCF at room temperature for 15 minutes.
3. Using a transfer pipet, withdrawn the plasma from the vacutainer and dispense the plasma into a cryovial. Securely cap the cryovial.
4. Affix label designated as L-NMMA onto the vials. Document the patient ID #, date, time of blood collection on the label.
5. Freeze and store the plasma in an upright position at -20^0 C or colder and ship when directed by the principle investigator.

BIOMARKER SAMPLE PREPARATION

1. Draw at least 5 mL blood into one 6 mL Lavender EDTA tube. Mix the tube by gently inverting the tube 2-3 times. To avoid hemolysis, do not mix vigorously
2. Centrifuge the blood using 1,100 to 1,300 relative centrifugal force (RCF) (g) for 10 minutes for a swinging bucket rotor or for 15 minutes for a fixed-angle bucket rotor. If ideal equipment not available, the minimum requirement are 1,000 RCF at room temperature for 15 minutes.
3. Using a transfer pipet, withdrawn the plasma from the vacutainer and dispense the plasma into a cryovial. Securely cap the cryovial.
4. Affix label designated as L-NMMA onto the vials. Document the patient ID #, date, time of blood collection on the label.
5. Freeze and store the plasma in an upright position at -20^0 C or colder and ship when directed by the principle investigator.

PK and PD Sample Shipping Guidelines

1. Shipments should be performed Monday through Thursday ONLY
2. Place the frozen samples into a biohazard bag containing absorbent material, seal the bag and place it into Styrofoam container.
3. Add a minimum of 2 Kg of Dry-Ice into the Styrofoam shipping container.
4. Affix IATA labels (UN33733, Biohazard and Dry-Ice Label) to the cardboard box
5. Seal the cardboard box.
6. Notify the laboratory by email of the shipment prior to shipping. Include the air bill number, the name of the site Shipping the package, and a contact person at the site.

Ship to: TBD