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Title: LY2157299 Monohydrate (LY2157299) and Radiotherapy in Metastatic Breast Cancer

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Document History

Amendments	Version	Version Date
Protocol Amendment 4	**4.0**	**04.21.2017**
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Protocol Amendment 2	2.1	07.18.2016
Protocol Amendment 1	2.0	09.29.2015
Initial Protocol	1.0	07.10.2015

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Confidentiality Statement

This document is confidential and is to be distributed for review only to investigators, potential investigators, consultants, study staff, and applicable independent ethics committees or institutional review boards. The contents of this document shall not be disclosed to others without written authorization from WCM.

List of Abbreviations

All abbreviations used throughout the protocol must be defined.

1D11	Murine monoclonal antibody against TGFB
4T1 murine model	murine breast tumor model
ADL	Activities of daily living
AE	Adverse event
AE	Adverse Event
Akt	Agammaglobulinemia Tyrosine Kinase)
ATM	Ataxia Telangectasia Mutated
AUC	Area Under the Curve - a measure of total drug concentration in blood plasma over a period of time
BCC	Basal Cell Carcinomas
B-HCG	Beta Human Chorionic Gonadotrophin
BUN	Blood Urea Nitrogen
CAT	Cambridge Antibody Technology
CBC	Complete blood count
CHF	Congestive Heart Failure
CI	Confidence interval
CIN	Cervical Intraepithelial Neoplasia
Cmax	Maximum concentration of drug
CNS	Central nervous system
CR	Complete response
CRF	Case report/Record form
CTCAE	Common Terminology Criteria for Adverse Events
DC	Dendritic Cells
DDR	DNA Damage Response
DLT	Dose Limiting Toxicity
DSMB	Data Safety Monitoring Board
DTC	Disseminated Tumor Cells
ECG	Electrocardiogram
ECM	Extra Cellular Matrix
EGFR	Epidermal Growth Factor Receptor
ELISA	Enzyme-Linked Immunosorbent Assay

EMT	Epithelial-Mesenchymal Transdifferentiation
ER	Estrogen Receptor
ERK	Extracellular Signal-Regulated Kinase
FSGS	Focal Segmented Glomerulosclerosis
GC1008	Fresolimumab, human IgG4 kappa monoclonal antibody against TGFB
GI	Gastrointestinal
Hct	Hematocrit
Hgb	Hemoglobin
HIV	Human Immunodeficiency Virus
HLA-A2	MHC Class I, A cell surface antigen: Human Leukocyte Antigen serotype within HLA-A "A" serotype group
HPF	High-power field
HTN	Hypertensions
IL-6	Interleukin-6
IPF	Idiopathic Pulmonary Fibrosis
IRB	Institutional Review Board
irRC	Immune response criteria
IV	Intravenous
JNK	c-Jun N-terminal protein Kinase
KA	Keratoacanthoma
Kd	Dissociation Constant
LAP	Latency-Associated Protein
LY2157299	LY2157299 Monohydrate
MAPK	Mitogen-Activated Protein Kinase
MDA-MB-231	human breast cancer cell line
MHC-I	Major Histocompatibility Complex class I
NCI	National Cancer Institute
NK	Natural Killer Lymphocytes
nM	Nanomolar
NOAEL	No-Observed-Adverse-Effect Level
OS	Overall survival
PBMC	Peripheral Blood Mononuclear Cells
PD	Pharmacodynamic
PFS	Progression free survival

PI3K	Phosphoinositide 3-Kinase
PLT	Platelet
PO	Per Os – orally
PR	Partial response
PSA	Prostate-Specific Antigen
PTHrP	Parathyroid Hormone-related Peptide
RCC (RENCA)	murine renal cell carcinoma
RECIST	Response evaluation criteria in solid tumors
RT	Radiation Therapy
SAE	Serious adverse event
SC	subcutaneous
SCC	Squamous Cell Carcinomas
SD	Stable disease
Smad	Sma- [small body size] and Mad-related protein
sur1M2	synthesized analogue of survivin in which a better anchor residue (methionine) replaces the natural threonine at position 2 so as to better bind HLA-A2
TβRII:Fc	soluble type II TGFB receptor:Fc fusion protein
TBRS	TGFβ gene Response Signature
TGFB	Transforming Growth Factor-beta
TGFBR	Transforming Growth Factor-beta Receptor
TNFR	Tumor Necrosis Factor Receptor
T-reg	Regulatory T cells
TTP	Time to progression
ULN	Upper limit of normal
USP	United States Pharmacopoeia
CFR	Code of Federal Regulations
CRF	Case Report Form
DSMB	Data Safety Monitoring Board
DSMP	Data Safety Monitoring Plan
FDA	Food and Drug Administration
GCP	Good Clinical Practice
HRBAF	Human Research Billing Analysis Form
ICF	Informed Consent Form
IND	Investigational New Drug

IRB	Institutional Review Board
PHI	Protected Health Information
PI	Principal Investigator
REDC	Research Electronic Data Capture
SAE	Serious Adverse Event
SUSAR	Suspected Unexpected Serious Adverse Reaction
WCM	Weill Cornell Medicine
UAP	Unanticipated Problem
HIPAA	Health Insurance Portability and Accountability Act of 1996

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Protocol Amendment 3	3.0	11.22.2016
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Summary of changes: Protocol Amendment 4 Version 4.0 dated 04.21.2017

1. Removal of the following cardiotoxicity testing; hsCRP, Cystatin C and BNP. Due to a change in WCM processing procedures, hsCRP, cystatin C and BNP testing are no long being processed at WCM and are now being sent to an outside lab which takes 7-10 days to be resulted. Due to the delay in receiving the results we are removing these labs so as to not delay the enrollment of metastatic breast cancer patients onto this study.

2. Per DSMB recommendation, the Data Safety Monitoring Plan section 12.1 has been revised to reflect the study will be reviewed on an annual basis.

3. Pre study and On study evaluations have been amended to include the following:
   A. Patients must have an MRI of the brain. A Brain CT with contrast is also acceptable
   B. Patients must also have a PET/CT or a CT of the Chest/Abdomen/ Pelvis
   C. Patients can begin two weeks from prior treatment if they are asymptomatic from previous therapy

Summary of changes: Protocol Amendment 3 version 3.0 dated 11.22.2016

1. DoD requested changes: Research Monitor responsibilities – Section 12.

2. WCM DSMB recommendation for semi-annual review – letter dated 10.20.2016.

Summary of changes: Amendment 2 dated 07.18.2016

1. Data safety Monitoring board recommendations from 03.24.2016 were added to the protocol section 12.1.
   "The WCM DSMB is to perform a data and safety analysis every three months (quarterly) following the accrual of the first subject".

Summary of Changes: Amendment 1 09.29.2015

1. Adding Maria Fenton-Kerimian as the Research Nurse

2. Data Safety Monitoring board review modifications

PROTOCOL SUMMARY

TGFβ blockade will enhance response of irradiated tumors and improve the function of Dendritic and T cells.

Full Title:	*LY2157299 Monohydrate (LY2157299) and Radiotherapy in Metastatic Breast Cancer*
Short Title:	*LY2157299 and Radiotherapy in Metastatic Breast Cancer*
Clinical Phase:	*Phase II*
Principal Investigator:	*Dr. Silvia C. Formenti, M.D.*
Sample Size:	*N= 28* (WCM will enroll a total of 18 patients and UCLA will enroll 10 patients)
Accrual Ceiling:	*35*
Study Population:	*Patients must have biopsy proven metastatic breast cancer and have stable disease or progressed after at least one course of chemotherapy or hormonal therapy. This study will not enroll vulnerable population. Women > 18 years and < 90 years.*
Accrual Period:	3 *years*
Study Design:	TGFB blockade will enhance response of irradiated tumors and improve the function of Dendritic and T cells. Patients will receive 300 mg/day of study drug administered via oral drug tablet every day for 14 days on and 14 days off (=28 day cycle). Radiation to a metastatic site will be delivered at a dose of 7.5 Gy, given consecutively on days 1-3-5.
Study Duration:	S*tudy participation will involve 15 visits over the course of 16 weeks (approx.4 months). Patients will have an annual follow-up up to 5 years.*
Study Agent/ Intervention Description:	*LY2157299 will be administered oral drug tablet: 300mg/day. Patients will receive 300 mg/day of study drug administered via oral drug tablet every day for 14 days on and 14 days off (=28 day cycle) .*
Primary Objective:	1**.** *to assess safety and feasibility of combining TGFβ receptor I kinase inhibitor LY2157299 and local radiotherapy* *2. To determine if treatment with TGFB receptor I kinase inhibitor LY2157299 and localized RT achieves an abscopal tumor regression;*
Secondary Objectives:	1**.** *to estimate the local response rate of combining TGFB receptor I kinase inhibitor LY2157299 and local radiotherapy* *2. To determine if treatment with TGFB receptor I kinase inhibitor LY2157299 and localized RT enhances tumor-specific immunity in*

patients with metastatic breast cancer, and if this is associated with abscopal tumor regression.
3. To determine if treatment with TGFB receptor I kinase inhibitor LY2157299 and localized RT alters the numbers and function of T-reg cells in patients with metastatic breast cancer.

Endpoints:

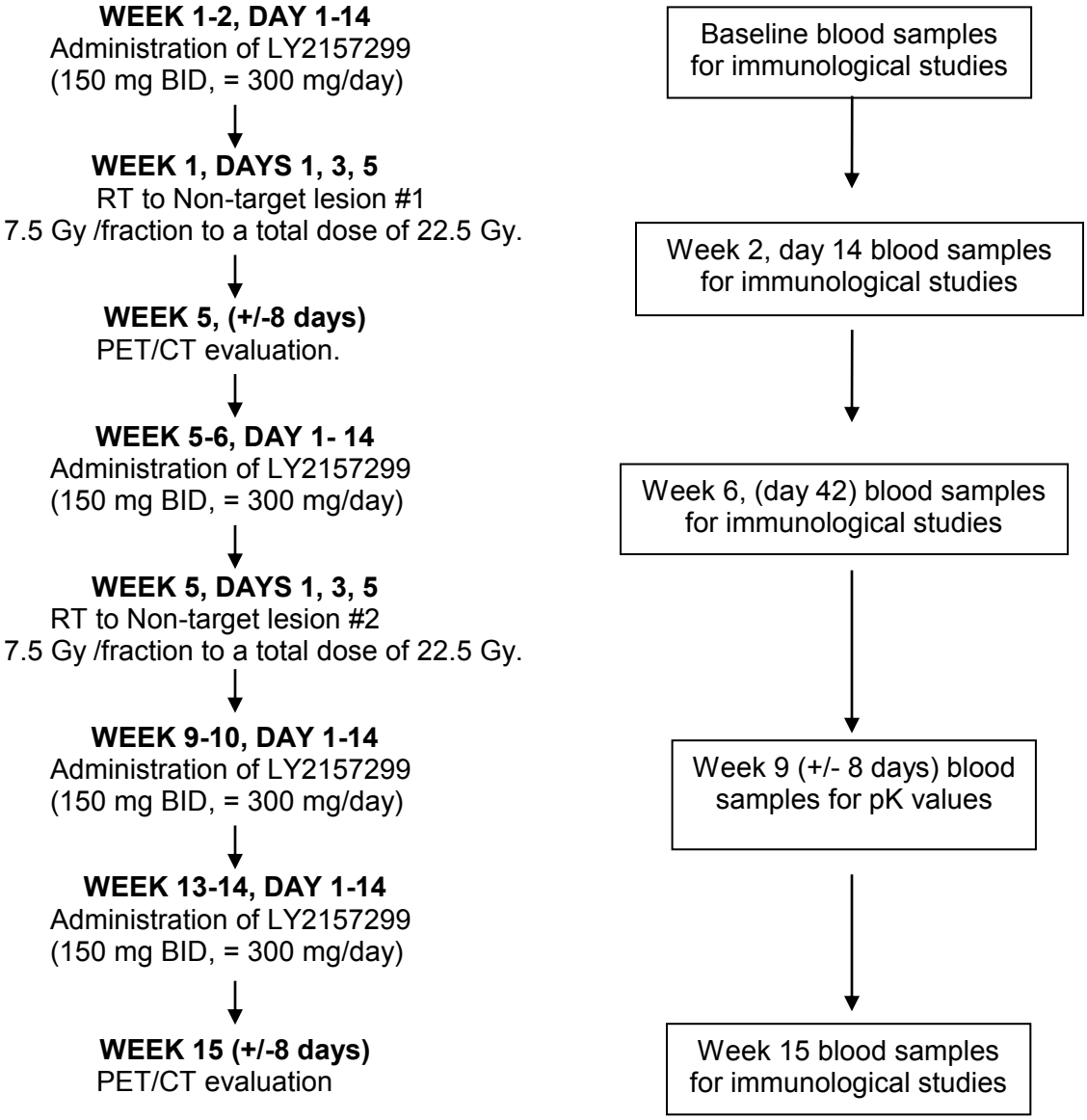
Primary Endpoint: A. To assess safety and feasibility of combining TGFβ receptor I kinase inhibitor LY2157299 and local radiotherapy
B. To determine if treatment with TGFB receptor I kinase inhibitor LY2157299 and localized RT achieves an abscopal tumor regression
Secondary Endpoint: *A. to estimate the local response rate of combining TGFβ receptor I kinase inhibitor LY2157299 and local radiotherapy,*
B. to determine if treatment with TGFβ receptor I kinase inhibitor LY2157299 and localized RT enhances tumor-specific immunity in patients with metastatic breast cancer, and if this is associated with abscopal tumor regression.
C. to determine if treatment with TGFβ receptor I kinase inhibitor LY2157299 and localized RT alters the numbers and function of T-reg cells in patients with metastatic breast cancer.

SCHEMA

Patients with metastatic breast cancer receiving at least one single agent chemotherapy and demonstrating stable disease or disease progression at two consecutive clinical/radiological assessments (at an interval of at least 2 weeks). Stable disease is defined as: < 30% decrease in the sum of the longest diameter (LD) of target lesions, taking as reference the baseline sum LD or < 20% increase in the sum of the LD of target lesions without the appearance of one or more new lesions. After obtaining informed consent:

Baseline CT and PET scan

Definition of target and non-target lesions: **Non-target** lesion is treated by radiation while **Target** lesions are followed to assess possible abscopal effect



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1.1. Primary Objectives

1.1.1 To assess safety and feasibility of combining TGFβ receptor I kinase Inhibitor LY2157299 and local radiotherapy

1.1.2 To determine if treatment with TGFβ receptor I kinase inhibitor LY2157299 and localized RT achieves an abscopal tumor regression;

1.2. Secondary Objectives

1.2.1 To estimate the local response rate of combining TGFβ Receptor I kinase inhibitor LY2157299 and local radiotherapy

1.2.2 To determine if treatment with TGFβ receptor I kinase inhibitor LY2157299 and localized RT enhances tumor-specific immunity in patients with metastatic breast cancer, and if this is associated with abscopal tumor regression.

1.2.3. To determine if treatment with TGFβ receptor I kinase inhibitor LY2157299 and localized RT alters the numbers and function of T-reg cells in patients with metastatic breast cancer.

2. BACKGROUND

2.1 TGF β

Transforming growth factor-beta (TGFβ) is a pleiotropic cytokine which belongs to a superfamily of ligands, including bone morphogenetic proteins and activins [1-5]. Under normal conditions, members of the TGFβ family maintain homeostasis in many organ systems. In normal and non-cancerous cells, TGFβ limits the growth of epithelial, endothelial, neuronal, and hematopoietic cell lineages through anti-proliferative and apoptotic responses. In addition, TGFβ exerts potent effects that influence immune function, cell proliferation/ functional differentiation, cell adhesion, extracellular matrix production, cell motility, angiogenesis, and cytokine production.

TGFβ exists in 3 isoforms: TGFβ 1, β2, and β3. Each isoform is encoded by distinct, highly conserved genes and is a 25 kilodalton homodimeric, disulphide-bonded protein. TGFβ members

are expressed in a tissue-specific and developmentally regulated fashion. TGFβ 1 is expressed most commonly and is found in endothelial, hematopoietic, and connective tissues. TGFβ 2 is found primarily in epithelial and neuronal tissues, and TGFβ 3 resides in mesenchymal tissues. In vitro, each has similar activities. However, data from knockout mice suggest that each may be associated with distinct phenotypes.

TGFβ is secreted by cells in a biologically inactive "latent form" by virtue of its association with latency-associated proteins (LAPs). Much of the TGFβ /LAP "pro-drug" is stored in the extracellular matrix as a complex. However, other notable sites exist, including platelet granules and the surface of certain cells such as regulatory T cells. The mechanism of release of active TGFβ may allow for local control. Activation can occur either under acidic conditions or through the action of proteases such as thrombospondin-1, plasmin, and prostate-specific antigen (PSA).

TGFβ binds to cells via 3 major receptors: TGFβ RI, TGFβ RII, and TGFβ RIII (transforming growth factor-beta receptor type I, type II, and type III). The binding of TGFβ to receptors occurs in a specific sequence and results in a cascade of events leading to the formation of a receptor complex and the phosphorylation and activation of TβRI. The receptor complex has serine/threonine kinase activity and can activate the Smad (Sma- [small body size] and Mad related protein) pathway by phosphorylation of Smad2 or 3. A key event is the formation of an activated Smad2(3)/4 complex, which is then transported to the nucleus where it induces gene transcription. This leads to a variety of effects on cell differentiation and growth.

Although TGFβ/TGFβR/Smad is an essential pathway, TGFβ's influence on cellular activities appears to be much more complex. TGFβ also binds to other receptors, such as endoglin, a cell-surface glycoprotein associated with proliferation of human endothelial cells and angiogenesis. Other signaling pathways, such as ERK (extracellular signal-regulated kinase), JNK (c-Jun N-terminal protein kinase), MAPK (mitogen-activated protein kinase), PI3K (phosphoinositide 3-kinase), Rho-kinase, Akt (agammaglobulinemia tyrosine kinase), and GTPases (guanosine triphosphatases) may also be involved. In addition, other receptor/signal pathways may intersect with the Smad pathway – including estrogen receptor (ER), androgen receptor (AR), steroid, epidermal growth factor receptor (EGFR), and other TGFβ family members such as the activins. Because of these interactions, the overall effects of TGFβ cannot always be predicted based on examination of any single pathway such as Smad.

2.1.1 TGF β in Oncology: Rationale for Blocking TGF β signaling Therapy

TGFβ has been implicated as an important factor in the growth, progression, and metastatic potential of advanced cancers. Although TGFβ has been shown to suppress the growth of epithelial cells in the early stages of tumor development (premalignant conditions), the effect on advanced cancers is more complex [1, 5-6]. Increased production of TGFβ has been found in many neoplasms such as breast, prostate, gastric, renal, and epidermal carcinomas, and elevated plasma TGFβ levels in patients have been correlated with advanced disease, metastases, and lower survival rates [7-13]. In these later stage cancers, TGFβ induced growth suppression is lost, and instead, TGFβ promotes tumor growth and metastasis.

TGFβ can influence many aspects of cell physiology. In the later stages of tumor development, TGFβ acts to promote tumor cell motility, migration, invasiveness, and metastasis through autocrine and paracrine effects. TGFβ has direct actions on tumor cells and can induce an aggressive appearing, morphologic cell change to occur [14]. In fact, investigators have found that transformed epidermal cells grown in the presence of TGFβ acquire the ability to form spindle cell carcinomas when transplanted into animals [14]. These phenotypic changes following exposure to TGFβ are referred to as an epithelial-mesenchymal transition (EMT) and are characterized by a “fibroblast-like”/spindle cell morphology, down regulation of E-cadherin and cytokeratin, loss of cell-cell junctions, and remodeling of the cytoskeleton. As a consequence of these changes, TGFβ causes the cells to become more motile and to migrate. These TGFβ-induced changes have been described in many different cancer models [6, 14-16].

In addition to the direct effects above, TGFβ can promote tumor growth and progression through paracrine functions. TGFβ alters the tumor microenvironment to fulfill the space and nutrient requirements needed for the growth of primary and metastatic cancers. This remodeling of the tumor stroma and environment occurs through the induction of angiogenesis, by increasing ECM deposition, and by inducing the production of factors such as parathyroid hormone-related peptide (PTHrP), which stimulates bone resorption by increased osteoclastic activity [5, 17-19]. Importantly, TGFβ can also deactivate the anti-tumor defenses of the hosts by suppressing the immune system. With broad activity over natural killer (NK) cells, T cells, monocytes/ macrophages, and dendritic cells, TGFβ can affect the initiation and stimulation of both primary and secondary immune responses as well as suppress anti-tumor effector cells [20-25]. These autocrine and paracrine effects combine to make TGFβ a key factor in the promotion of tumor growth and metastasis.

For these reasons, neutralizing or blocking TGFβ represents a unique method of intervening and

disrupting a key promoter of tumor growth and may provide a new therapeutic opportunity. Investigators have examined the anti-tumor activity of inhibiting anti-TGFβ soluble receptors. These agents have demonstrated direct anti-tumor activity and have increased the survival of animals bearing a wide variety of neoplasms, including breast cancer, prostate cancer, melanoma, hepatic cancer, and pancreatic cancer [5, 20, 26-36]. Inhibition of soluble receptors can act through a variety of mechanisms including increased apoptosis of primary tumors and the prevention of tumor cell migration, ECM deposition, angiogenesis, bone remodeling, and interleukin-6 (IL-6) and PTHrP secretion. Receptor kinase inhibitors can also reverse TGFβ induced immune suppression and can activate important NK and T cell mediated anti-tumor responses [20, 22-23, 25, 37]. All of these effects contribute to the anti-tumor activity of blocking TGFβ.

2.2 Investigational Agent or Device

Description of LY2157299

Eli Lilly has developed and produced a Transforming Growth Factor-beta (TGF-β) receptor type-1 kinase inhibitor. LY2157299 monohydrate (LY2157299) is a small molecule that inhibits the TGF-β receptor type 1 kinase activity. LY2157299 mechanism of action is determined by changes in phosphorylated SMAD (pSMAD) levels, with subsequent anti-tumor activity demonstrated in 3 in-vivo tumor animal models: 2 breast cancer (MX1, 4T1) and one non-small cell lung cancer (NSCLC) model (CALU6).

LY2157299 was developed to investigate its activity in patients with glioblastoma where TGF-β has been demonstrated to play a specific role in tumor progression. In addition, LY2157299 was investigated in other patient populations, either as a stand-alone therapy or in combination with standard anti-tumor treatment regimens for indications including hepatocellular carcinoma and pancreatic cancer. Future investigations include indications with likely TGF-β associated pathway activation, such as melanoma, breast and prostate cancer as well as hematologic malignancies.

2.2.1 [redacted]

[redacted]

[redacted]

2.2.2 Preclinical Studies

Pharmacokinetic and toxicology exposure and metabolism studies were conducted in Fischer 344 rats and beagle dogs utilizing LY2157299. The compound is well absorbed after oral administration and is dose linear up to 250 mg/kg in rats and 20 mg/kg in dogs. In rats, exposure was higher in females than those in males, but no discernable difference in exposure between male/female dogs. LY2157299 is extensively metabolized, with 2 oxidative pathways accounting for most of the metabolic activity. The parent compound is the major circulating material. Protein binding is not high, 56 – 68% in mouse, rat and dog plasma, 66% in human plasma. In [^{14}C]-LY2157299, the majority of the radioactivity was present in feces or bile of the rats or dogs, with <25% of the dose recovered in urine including <10% of the parent compound.

2.2.3 Toxicology Studies

Eli Lilly and Company has performed toxicology studies in rodents and dogs. Details of each of these studies can be found in the Investigator Brochure.

In 6-month long-term nonclinical toxicity studies, daily dosing of LY2157299 resulted in dose-progressive incidence of vascular degeneration to the base of the aorta in dogs and rats. Vascular changes led to transmural aneurysms and mortality in mid-high dose-treated rats. Due to the possibility of aneurysm formation, patients with a history of or predisposition of developing aneurysms should not receive LY2157299. Subsequent cardiovascular function studies carried

out in dogs revealed that LY2157299 can cause a decrease in blood pressure and increases in heart rate and arterial pulse pressure, all reversible. There were no compound-attributable changes in QT intervals, arrhythmias, or altered waveforms noted. Because of the associated cardiac events in animal studies, patients on LY215799 undergo cardiac monitoring, including assessment of heart rate, blood pressure and cardiac functions. Regular monitoring of biomarkers for cardiac events, including troponin-I, B type natriuretic peptide and high sensitivity C-reactive protein is required..TGF-β is an important immunomodulator, hence, careful monitoring of immune functions and infection rates is important. Because LY2157299 inhibits TGF-β receptor type-1 kinase, an autoimmune reaction may occur. To date, no clinically significant immune reactions have been reported or observed up to doses of 300 mg/day in a 14 days-on / 14 days-off treatment regimen.

LY2157299 should not be administered to pregnant patients or all efforts should be made to prevent pregnancy due to TGF-β's role in embryogenesis. Literature searches as well as treatment with LY2157299 in pregnant rats showed increased resorptions and alterations in fetal skeletal morphology at dose equivalents of 300 mg/day in humans.

LY2157299 does not demonstrate any direct bone marrow toxicity in either animal studies with continuous dosing for 6 months, nor in human colony-forming unit studies with human bone marrow progenitor cells. There have been 2 cases with drug-related severe thrombocytopenia and associated pan-cytopenia observed, but they cannot be explained at this juncture.

Thromboembolic events are common events in cancer, present in up to 70% of glioblastoma cases. Whenever they occurred, embolic events detected during administration of LY2157299 in glioblastoma patients were successfully treated with low-molecular heparins, with LY2157299 not affecting treatment interventions for embolic events.

2.2.4 Clinical Experience with LY2157299

To date, approximately 406 patients have been treated with LY2157299, with no definite drug-related toxicity profile emerging. The potential risks outlined in the Investigator's Brochure provided by Lilly are based upon the assumed mechanism of action for LY2157299 and the reported cases where LY2157299-associated toxicity was found to likely be the related cause due to lack of other contributing factors.

2.3 Rationale

Five years ago, Drs. Formenti, Demaria and McBride argued for a revival of the notion of combining RT and IT in the treatment of cancer [45], the basic concept of this trial. The rationale is based on findings that radiation generates “danger” signals in tissues [46] that, under certain conditions, enhance immune presentation of tumor antigen liberated from radiation-damaged cells, thus working as a vaccine. This hypothesis stems from the ideas of Matzinger that the immune system is more concerned with responding to damage than to “foreignness” [47]. Radiation elicits damage signals, by up-regulating pro-inflammatory cytokines that are involved in DC maturation, immunomodulatory molecules such as major histocompatibility complex class I (MHC-I), co-stimulatory and adhesion molecules, death receptors of the tumor necrosis factor receptor (TNFR) family, and heat shock proteins (reviewed in [46]). McBride’s lab showed that irradiation of DC greatly enhances their ability to cross-present tumor antigen, a relevant immunological pathway during RT [48].

Increased production of TGFβ has been found in many neoplasms such as breast, prostate, gastric, renal, and epidermal carcinomas, and elevated plasma TGFβ levels in patients have been correlated with advanced disease, metastases, and lower survival rates [7-13]. TGFβ can deactivate the anti-tumor defenses of the host by suppressing the immune system. With broad activity over natural killer (NK) cells, T cells, monocytes/macrophages, and dendritic cells, TGFβ can affect the initiation and stimulation of both primary and secondary immune responses as well as suppress anti-tumor effector cells [20-25].

Barcellos-Hoff’s lab and other groups have shown that ionizing radiation induces TGFβ activation in vitro and in vivo in normal and cancer cells [49-55], which is at least in part occurring through a redox mechanism that acts directly on the secreted latent protein [56-57]. Moreover, TGFβ is actually required for execution of the epithelial damage response to radiation [58-59]. Dr. Barcellos-Hoff’s group made the surprising discovery that either genetic loss or pharmaceutical inhibition of TGFβ inhibits ataxia telangiectasia mutated (ATM) kinase activity, thereby impeding the DNA damage response (DDR) and as a consequence, increasing radiation cell kill [59]. In addition, LY2109761, a similar compound to LY2157299 was evaluated in radiation-induced fibrosis models and showed a reversal of TGF-β-associated pro-inflammatory responses. This suggests that small molecule inhibitors of the TGF-bRI kinase may have a beneficial impact on TGF-β-mediated inflammation.

Recent studies underscore the growing concern that high TGFβ levels in tumors drive cancer cells towards a more aggressive behavior and support their survival, while simultaneously limiting immune-responses by the host and, possibly, co-opting normal tissue functions like angiogenesis. Together with the recognition that radiation triggers activation of TGFβ, which in turn promotes DNA damage repair, and mediates EMT [60] these results suggest a novel therapeutic advantage for TGFβ inhibition in the context of radiotherapy.

2.4 RT Dose Rationale

RT to a measurable metastatic site will be delivered at a dose of 7.5 Gy X 3 (22.5 Gy total dose), given consecutively on days 1-3-5, by intensity modulated radiotherapy (IMRT) and/or image guided radiotherapy (IGRT) techniques, to best spare the normal tissue. The choice of this fractionation regimen of RT is based on the NYU preclinical experience of radiation and ipilimumab [61], that suggests superiority of the 8 Gy x 3 fractions regimen (for a total dose of 24 Gy) to induce abscopal effects in murine cancer models. In humans, the biological equivalent can be calculated by applying the linear quadratic model [62] to determine which dose delivered in 3 fractions would results in comparable tumor control to that of the standard regimen of 3 Gy x 10 fractions (total dose 30 Gy) commonly used in the clinic to palliate metastatic disease. The α/β is a tissue and effect-specific parameter associated with this conversion model, which α/β is taken to be 10 Gy for cancer metastases [63-65]. As demonstrated in Table 1, the closest equivalence is achieved by 7.5 Gy x 3, which explains the choice for this trial. Importantly a 3 fraction regimen is very user-friendly (only 3 visits to radiation oncology). Both UCLA and WCM are equipped to provide Intensity Modulated RT (IMRT) and, if necessary Image guided RT (IGRT), if the site targeted is close to vital organs.

Table 1. Biologically effective dose (BED) for different fractionation regimens of radiotherapy

	8 Gy x 3	6 Gy x 5	7 Gy x 3	7.25 Gy x 3	7.5 Gy x 3	3 Gy x 10 standard dose
(α/β = 10 Gy)	43.2	48	35.7	37.5	**39.4**	**39**

2.5 Risk/Benefit Assessment

The currently available information on the toxicology profile of LY2157299 is quite favorable, and the dose and fractionation of radiotherapy used in this study is unlikely to be associated with severe side effects. Thus the combination represents a viable alternative for metastatic breast cancer patients interested in investigational immunotherapy approaches.

2.6 Correlative Studies Background

2.6.1 TGF β gene response signature

A well-defined and robust TGFβ gene response signature (TBRS) [66] was strongly associated with three specific subsets of human breast cancer [67]. These findings suggest that the TGFβ pathway is activated in particular breast cancer subgroups and may predict for response to TGFβ neutralization, a concept that will be tested in this study. We plan to assess TGFβ gene response signature (TBRS) positivity in primary tumors (from blood cells and formalin-fixed and paraffin-embedded archival tissue samples) as predictive biomarker of response to LY2157299 plus radiation therapy combination therapy. Based on past experience, we assume that approximately 50% of subjects will have TBRS-positive tumors. Subjects who have TBRS-positive tumor and derive clinical benefit will be considered true positive. Sensitivity, specificity, positive and negative predictive value will be estimated.

2.6.2 Circulating levels of TGF β

As a biomarker, many studies have suggested that circulating TGFβ levels provide an index of tumor response to RT and may predict normal tissue toxicity. We will monitor circulating levels of TGFβ prior to, during and after radiation therapy in patients with metastatic cancer before and after LY2157299 treatment. Measuring TGFβ presents several challenges. First, TGFβ is produced and circulates in a latent form that is abundant, but its activity is highly regulated and short-lived. Second, the low levels of active TGFβ present in serum can easily be obscured by the potentially high background of TGFβ unintentionally released by platelet degranulation in specimens. Lastly, most methods require exogenous latent TGFβ activation by acid or heat, which compromises the simultaneous detection of other cytokines. These factors contribute to erratic measurements by most commonly used assays, including the widely used ELISA. We will measure TGF-β1 levels in EDTA and/or CDTA from collected plasma utilizing an ELISA design developed by Lilly Research Laboratories.

2.6.3 Tumor-specific immune responses

Tumor-specific responses will be assessed initially in HLA-A2+ cells that will be re-stimulated ex vivo with sur1M2 survivin peptide and additionally, for HER2neu positive tumors, the HER: p369-377 peptide. Non-HLA-A2 patients will be tested using peptides immunodominant for their

known HLA type. Quite a range of known epitopes is available [68-69]. While it is unrealistic to expect that all responses will be detected with equal sensitivity as this is influenced by the binding avidities, positive longitudinal responses to any peptide with time after treatment would indicate significant responses. In a follow-up assay, if sufficient PBMCs are available, the ability of LY2157299 plus RT to alter the hierarchy of the response and to determine whether responses to all epitopes are equally affected will be tested using HLA-A2+ PBMCs that respond to sur1M2 survivin but with 4 distinct survivin peptides [70]. We anticipate that LY2157299 plus RT will enhance tumor-specific immune responses and cause epitope spreading in the majority of patients.

2.6.4 Levels of circulating Tregs

The levels of circulating Tregs will be evaluated in patients. Tregs are especially relevant for the study because of their relative radiation resistance and because of the importance of TGF-β in their generation and function. Because the composition of PBMCs changes during cancer treatment [71], it is important that Treg levels are assessed both as a % of CD4 cells, as a % total lymphocytes, % total PBMCs, and as absolute counts. The assay can be performed at the same time as tetramer staining and on the same sample if necessary (7), enabling the assessment of lymphocyte representation in one sample and the determination of CD4:CD8 T cell ratios. Since Tregs are radiation resistant, if significant differences in response are found between patients, efforts could also be made to correlate changes with dose volume histograms obtained during RT. It is anticipated that Treg numbers and function will decrease in patients receiving LY2157299 plus RT, in particular in those who show enhanced tumor immunity and demonstrate abscopal anti-tumor effects. In general, RT increases Treg activity, but it is expected that LY2157299 will counter this effect allowing immune activation.

3. SUBJECT SELECTION

3.1 Study Population

Patients must have biopsy proven metastatic breast cancer and who have stable disease or progressed after at least one course of chemotherapy or hormonal therapy. In addition, patients

must have at least 3 metastases with at least one measurable metastatic lesion and who meet the inclusion and exclusion criteria will be eligible for study participation.

3.2 Inclusion Criteria

3.2.1 Biopsy proven breast carcinoma which is persistent and metastatic or recurrent and metastatic.

3.2.2 Patients must have failed at least one line of chemotherapy for metastatic disease.

3.2.3 Patients who are HER2+ as defined by ASCO CAP guidelines must have failed all prior therapy known to confer clinical benefit

3.2.4 Patients must have at least 3 distinct metastatic sites with at least one measurable lesion which is at least 1 cm or larger in largest diameter.

3.2.5 At the time of enrollment, patients must be ≥ 4 weeks‡ since all of the following treatments (and recovered from the toxicity of prior treatment to ≤ Grade 1, exclusive of alopecia):

- major surgery;
- radiotherapy;
- chemotherapy (note: must be ≥ 6 weeks since therapy if treated with a nitrosourea, mitomycin, or monoclonal antibodies such as bevacizumab);
- immunotherapy;
- Biotherapy/targeted therapies.

(‡ - Patients can begin two weeks from prior treatment if they are asymptomatic from previous therapy)

3.2.6 Patient ≥ 18 years of age.

3.2.7 Patient life expectancy > 6 months.

3.2.8 ECOG status of 0 or 1

3.2.9 Adequate organ function including:

a) Marrow: Hemoglobin ≥ 10.0 g/dL, absolute neutrophil count (ANC) ≥ 1,500/mm3, and platelets ≥ 100,000/mm3.

b) Hepatic: Serum total bilirubin ≤ 1.5 x upper limit of normal (ULN) (Patients with Gilbert's Disease may be included if their total bilirubin is ≤ 3.0 mg/dL), alanine aminotransferase (ALT), and aspartate aminotransferase (AST) ≤ 2.5 x ULN. If the patient has known liver metastases, an ALT and/or AST ≤ 5 x ULN are allowed.

c) Renal: Estimated or measured creatinine clearance ≥ 60 mL/min.

d) Other: Prothrombin time (PT) and partial thromboplastin time (PTT) < ULN.

3.2.10 Patients must have negative tests (antibody and/or antigen) for hepatitis

viruses B and C unless the result is consistent with prior vaccination or prior infection with full recovery.

3.2.11 Male and female patients of child-producing potential must agree to use effective contraception while enrolled on study and receiving the experimental drug, and for at least 3 months after the last treatment.

3.2.12 Ability to understand and the willingness to sign a written informed consent document. Patients may not be consented by a durable power of attorney.

3.3 Exclusion Criteria

3.3.1 Patients diagnosed with another malignancy – unless following curative intent therapy, the patient has been disease free for at least 2 years and the probability of recurrence of the prior malignancy is $< 5\%$. Patients with curatively treated early-stage squamous cell carcinoma of the skin, basal cell carcinoma of the skin, or cervical intraepithelial neoplasia (CIN) are eligible for this study.

3.3.2 Concurrent cancer therapy is not permitted.

3.3.3 Uncontrolled central nervous system (CNS) metastases, meningeal carcinomatosis, malignant seizures, or a disease that either causes or threatens neurologic compromise (e.g., unstable vertebral metastases).

3.3.4 History of ascites or pleural effusions, unless successfully treated.

3.3.5 Patients with an organ transplant, including those that have received an allogeneic bone marrow transplant.

3.3.6 Patients on immunosuppressive therapy including:

a) Systemic corticosteroid therapy for any reason, including replacement therapy for hypoadrenalism. Patients receiving inhaled or topical corticosteroids may participate (if therapy is < 5 days and is limited to systemic steroids as antiemetics).

b) Patients receiving cyclosporine A, tacrolimus, or sirolimus are not eligible for this study.

3.3.7 Use of investigational agents within 4 weeks prior to study enrollment (within 6 weeks if the treatment was with a long-acting agent such as a monoclonal antibody).

3.3.8 Patients with moderate or severe cardiac disease:

a) have the presence of cardiac disease, including a myocardial infarction within 6 months prior to study entry, unstable angina pectoris, New York Heart Association Class III/IV congestive heart failure, or uncontrolled hypertension.

b) have documented major electrocardiogram (ECG) abnormalities (not responding to medical treatments) at the investigator's discretion (for example, symptomatic or sustained atrial or ventricular arrhythmias, second- or third-degree atrio ventricular block, complete bundle branch block, ventricular hypertrophy, or recent myocardial infarction).

c) have major abnormalities documented by echocardiography (ECHO) with Doppler (for example, moderate or severe heart valve function defect and/or left ventricular ejection fraction <50%, evaluation based on the institutional lower limit of normal). For additional details, refer to ECHO protocol.

d) have predisposing conditions that are consistent with development of aneurysms of the ascending aorta or aortic stress (for example, family history of aneurysms, Marfan-Syndrome, bicuspid aortic valve, evidence of damage to the large vessels of the heart documented by computed tomography (CT) scan with contrast).

3.3.9 Troponin I above ULN,

3.3.10 Patients with a remote history of asthma or active mild asthma may participate.

3.3.11 Active infection, including unexplained fever (temperature > 38.5°C).

3.3.12 Systemic autoimmune disease (e.g., systemic lupus erythematosus, active rheumatoid arthritis, Marfan Syndrome, etc.).

3.3.13 A known allergy to any component of LY2157299.

3.3.14 Patients who, in the opinion of the Investigator, have significant medical or psychosocial problems that warrant exclusion. Examples of significant problems include, but are not limited to:

a) Other serious non-malignancy-associated medical conditions that may be expected to limit life expectancy or significantly increase the risk of SAEs.

b) Any condition, psychiatric, substance abuse, or otherwise, that, in the opinion of the Investigator, would preclude informed consent, consistent follow-up, or compliance with any aspect of the study

3.3.15 Pregnant or nursing women, due to the unknown effects of LY2157299 on the developing fetus or newborn infant.

4 REGISTRATION PROCEDURES

4.1 General Guidelines

All eligible patients who are referred to the Radiation Oncology or Medical Oncology Department at Weill Cornell Medicine for radiation will be offered the opportunity to participate in this experimental protocol. Treatment may commence once all mandatory pretreatment studies are documented, informed consent is obtained and the patient is approved according to the eligibility checklist. Eligibility will be determined by the investigators of the study.

4.2 Patient Registration Process

Before any protocol specific procedures can be carried out, investigators/staff will fully explain the details of the protocol, the study procedures and the aspects of patient privacy regarding research information. Patients will be provided a comprehensive explanation of the proposed treatment including the type of therapy, the rationale for treatment on the protocol, alternative treatments that are available, any known adverse events, the investigational nature of the study and the potential risks and benefits of the treatment. The informed consent document will meet all requirements of the Institutional Review Board (IRB). All subjects/patients are informed in the consent that participation or refusal to participate in the research study will not affect any of the clinical treatment or services to which they would otherwise be entitled.

The physicians who may obtain informed consent are listed on the title page of this protocol. The informed consent form will be signed by the participant and the registering physician. Once signed, a copy will be given to the patient and one will be maintained with the patient's medical record. Once eligibility is confirmed and informed consent is documented, the patient will be registered by the study coordinator/data manager.

Patients will be centrally registered with the Office of Billing Compliance. To register a patient, submit the following documents via the JIRA Registration Process:

1. **Legible copy of the HRBAF**
2. **First and last page of signed informed consent form**

Registration must be completed within 24 hours of the signing of informed consent.

5. TREATMENT PLAN

5.1 Investigational Agent or Device Administration

Enrolled patients will receive 300 mg/day of LY2157299. LY2157299 will be administered as an oral drug tablet.

The study drug will be administered orally on a 28-day cycle (1 cycle=28 days), every 2 weeks, or 14 days on / 14 days off. Blood samples will be obtained at baseline, and weeks 2, 6 and 15 for immune monitoring. Imaging by PET/CT or CT(Chest/Abdomen/Pelvis) will be performed at baseline, 5 weeks and 15 weeks. The chosen metastatic sites will receive conformal external beam radiation 7.5 Gy/fraction x 3, to a total of 22.5 Gy over the course of one week (on alternating days):

- lesion 1 will be irradiated at week 1 (RT starts 1 day after 1st dose of LY2157299),
- lesion 2 will be irradiated at week 5 (RT starts after 1stdose of the second cycle of LY2157299)

5.2 Pretreatment Evaluations

Screening and Baseline Evaluations/Procedures

These evaluations will be carefully reviewed to determine the patient's eligibility for the study (see study calendar, section 5.10).

1. Signed, written informed consent: Consent must be completed prior to performing any study-related procedures
2. Complete physical examination including height, weight and full skin exam.

3. Medical history: Detailed documentation of demographics, disease and treatment history with outcomes.
4. ECOG performance status assessment.
5. Concurrent medications.
6. Echo / Doppler.
7. Hematology: CBC with differential and platelet count and peripheral blood smear. Coagulation (PT/INR, PTT); Serum chemistries: Electrolytes (sodium, potassium, chloride, and bicarbonate), blood urea nitrogen (BUN), creatinine, glucose, and liver function tests (aspartate aminotransferase (AST) and/or alanine aminotransferase (ALT), alkaline phosphatase (ALP), total bilirubin, lactate dehydrogenase (LDH), and Troponin I..
8. Tumor measurement at baseline by PET/CT or CT(chest/abdomen/pelvis).
9. MRI or Brain CT with contrast is required at baseline
10. Stool fecal occult blood test of 2 consecutive samples: if patients have positive fecal occult blood, full GI workup will be conducted and patients will be excluded if active bleeding is detected.
11. LY2157299 Serum pregnancy test for women of childbearing age.

5.3 On-Study Evaluations

(See study calendar, section 5.10)

1. Physical exam: including complete skin evaluation and evaluation of oral mucosa prior to start of **each** RT cycle and **prior** to each LY2157299 administration.
2. Hematology: CBC with differential and platelet count and peripheral blood smear. PT/INR,PTT should be evaluated following multiple doses of LY2157299; Serum chemistries: Electrolytes (sodium, potassium, chloride, and bicarbonate), BUN, creatinine, glucose, and liver function tests (AST, ALT, ALP, total bilirubin, LDH, and Troponin I C). These will be carried out prior to start of each RT cycle and prior to each cycle of 2 weeks of BID LY2157299 administration.
3. ECHO/Doppler : Week 8 within 5 days prior to starting week 9 LY2157299 administration and Week 15
4. Study Special Tests: TGFβ levels (at wk 1 day -1 and week 15 visit), immune evaluation at weeks 1 day -1, 2, 5 and 15 LY2157299 PK (pre- & after completion of drug cycle day 14 & at day 42).
5. Tumor measurement by PET/CT/ CT(chest/abdomen/pelvis) at weeks 5 and 15.
6. Adverse events evaluated and recorded using the NCI CTCAE version 4.0throughout the treatment period (weeks 0-15).

To date, LY2157299 at the planned dose for this study is well tolerated in patients. If a toxicity is observed, a dose reduction may be considered based on the aggregate safety information of LY2157299, if other plausible reasons for a toxicity have been excluded.

Specifically, no bone marrow toxicities were detected in nonclinical toxicology studies; 1 toxicity was reported in a patient with glioblastoma in whom a Grade 4 thrombocytopenia was observed and this toxicity was potentially associated with administration of LY2157299 monotherapy. If a similar case occurs, the drug should be discontinued until platelet counts either return to Grade 1 or baseline.

If a patient experiences any of the following events that are considered possibly related to LY2157299 and not to study disease, LY2157299 will be omitted until the event resolves:

- Grade 4 Thrombocytopenia
- Grade 3 thrombocytopenia associated with bleeding
- Grade 4 Anemia
- absolute neutrophil count (ANC) <0.5 x 10^9/L for longer than 7 days, or ANC <1.0 x 10^9/L with a single temperature of >101ºF/38.3ºC or a sustained temperature of >100.4ºF/38ºC for more than 1 hour, or platelet count <25,000 x 10^9/L
- Common Terminology Criteria for Adverse Events Grade 3 or 4 non-hematologic toxicity

Non-hematologic and hematologic toxicity must resolve to CTCAE Grade 0, 1, or baseline (with the exception of alopecia, fatigue, skin rash, nausea, vomiting, constipation, or diarrhea that can be controlled with treatment) before start of next cycle. If recovery is >29 days (Day 1of the next cycle), patient must be withdrawn from study treatment.

If moderate or severe heart valve toxicities are observed, or the patient develops aneurysms of the great vessels of the heart, the patient must immediately be discontinued from the treatment and the toxicity reported to sponsor within 24 hours (see references on the ECHO assessment based on the Guidelines of the American and European Societies of Cardiac Echocardiography). If the patient develops new cardiovascular lesions in the chest, an MRI or CT with contrast of the abdomen should be performed to evaluate for development of abdominal vascular lesions. Exceptions to this rule must be approved by the IRBs and sponsor.

5.4 General Concomitant Medication and Supportive Care Guidelines

No concomitant cancer therapies, immunosuppressive agents or anti-coagulation therapies are permitted during the study unless specified below. Inhaled or topical corticosteroids are allowed.

Use of alternative medications (herbals, botanicals, etc.) is strongly discouraged during the entire study period. In addition, starting new medications other than anti-cancer agents may be associated with side effects; therefore, medications should not be started for the first time during or around the time of LY2157299 administration.

5.5 Duration of Therapy

Patients completing the week 15 visit will have completed the study treatment.

5.6 Duration of Follow Up

Patients will be followed with follow-up visits monthly for the first three months after completing therapy then annually for 5 years with physical exam, performance status evaluation, labs and Echocardiography and Doppler (see study calendar, section 5.10).

5.7 Criteria for Removal from Study

Patients may withdraw consent and discontinue participation in the study at any time, without prejudice to further treatment. A patient's participation in the study may also be discontinued at any time at the discretion of the Investigator.

The following are reasons why the Investigator may remove a patient from study treatment and further follow-up:

- The patient withdraws consent;
- The patient is found to be not eligible after enrollment;
- The patient is non-compliant with study requirements;
- The Study is terminated

The following are reasons why the Investigator or Sponsor may remove a patient from study treatment but continue follow-up:

- General or specific changes in the patient's condition render the patient
- unacceptable for further treatment per the investigator's judgment;

- The patient becomes pregnant during the study (note: patients who become pregnant during the study must be followed throughout their pregnancy; it will also be important to follow any infants exposed to LY2157299 during fetal development);
- The patient suffers an intolerable AE;
- The patient develops progressive disease (progression at week 5 PET/CT or CT(chest/abdomen/pelvis) does not warrant study discontinuation if clinically stable);
- The patient requires a prohibited medication;
- There is new information to suggest a significant change in risk/benefit for LY2157299;

5.8 Alternatives

Standard of care treatment will be offered to all eligible patients.

5.9 Compensation

Patients will receive no compensation for participating in the trial.

5.10 Study Calendar

	Pre-Study	Wk 1 (Day -1) 0	Wk 1 (Day +1) (+/- 3days)	Wk 2 (+/- 3 days)	Wk 3 (+/- 3 days)	Wk 4 (+/- 8 days)	Wk 5 (+/- 3 days)	Wk 6 (+/- 3 days)	Wk 7 (+/- 3days)	Wk 8 (+/- 3 days)	Wk 9 (+/- 8 days)	Wk 10 (+/- 3 days)	Wk 11 (+/- 3 days)	Wk 12 (+/- 3 days)	Wk 13 (+/- 3 days)	Wk 14 (+/- 3 days)	Wk 15 (+/- 8 days)	Follow-up
Investigational Agent ***LY2157299*** [a]			X	X[a,d]			X	X[a,d]			X[a,d]	X[a,d]			X[a,d]	X[a,d]		
RT			X[d]				X[d]											
Informed consent	X																	
Demographics	X																	
Medical history	X																	
EKG[b]	X[b]																	
Concurrent meds	X	X----------																X
B-HCG[c]	X[c]																	
Performance status	X	X		X		X		X						X			X[d]	X
Physical exam[d] (Vital signs)	X[d]	X[d]		X[d]		X[d]		X[d]		X[d]		X[d]		X[d]			X[d]	X
CBC w/diff, plts, Serum chemistry[e], CEA/CA 27.29[f]	X[e, f]	X[e]		X[e]	X[e]		X[f]	X[e]		X[e]	X[e]			X[e]			X[e f.]	
PT/INR/PTT	X	X		X	X			X		X	X			X			X	
Hepatitis B and C HbSAg, HCV Ab (antibody and/or antigen)	X																	

Stool fecal occult blood test (2 consecutive)	X																	
Adverse event evaluation		X----------X																
PET/CT/CT(CAP)	X						X										X	
MRI or Brain CT with contrast	X																	
Tumor tissue for TBRS signature	X[g]																	
Blood draw for VEGF		X		X				X									X	
Blood draw for immunological studies		X		X				X									X	
Blood draw (pre and post) for LY2157299 pK		X									X							
Blood draw for LY2157299 cardiotoxicity (Troponin I)	X	X				X				X				X				X
Echo/Doppler	X									X							X	X

a: *LY2157299* Administration of investigational drug is given 14 days on and 14 off.
B: Before 1st RT & repeated during the study if indicated.
C: Serum pregnancy test (women of childbearing potential).
D: PE must include full vital signs, skin and oral mucosa exam and prior to each LY2157299 administration and each RT cycle. PE continue monthly for the first 3 months following therapy then yearly for 5 years.
E: Albumin, alkaline phosphatase, total bilirubin, bicarbonate, BUN, calcium, chloride, creatinine, glucose, LDH, phosphorus, potassium, total protein, SGOT[AST], SGPT[ALT], sodium
f. CEA/CA 27.29 if elevated at baseline will be repeated at weeks 5 and 15.
g. Archival tissue.

6 PHARMACEUTICAL INFORMATION

6.1 Investigational Agent

LY2157299 is a receptor kinase inhibitor of TGF receptor type I (ALK5).

Packaging and Labeling

LY2157299 will be supplied in open-label blister packs or other appropriate packaging of film-coated, paracapsule tablets that are light yellow/yellow in appearance.

Drug Shipment and Storage

Eli Lilly & Co. will provide the clinical material for this study.

6.2 Availability

Full records must be maintained to account for the study drug supplied to the Investigators, the disposition of the study drug, and the return or destruction of unused supplies.

6.3 Agent Accountability

All investigational drugs are stored with the Investigational Pharmacy. A separate binder is issued to each study as well as a bin for storage. The bins and binder are labeled with the protocol number and drug name. At WCM, the Investigational Pharmacy is located in a separate room within the Inpatient Pharmacy. There is limited access, and only the Pharmacists and support personnel working in the Research Pharmacy have access. The hours of operation are Monday- Friday from 8-5pm. At the end of the day the room is locked. The research Pharmacist and staff can open the room using magnetic ID badges. Only the Research staff and management can enter the research area. Initial shipment of the investigational agent (LY2157299) will be delivered to WCM Investigational Pharmacy, which will disburse the agent within the WCM research group and to all collaborating institutions.

At UCLA, the Investigational Pharmacy is located in the Ronald Regan Medical Center. There is limited access, and only the Pharmacists and support personnel working in the Research Pharmacy have access. The hours of operation are Monday- Friday from 8-5pm. This area is locked at all times.

7 DOSING DELAYS/DOSE MODIFICATIONS

LY2157299 will be administered as a flat dose of 300 mg/day, divided in a BID administration of 150 mg each, as an oral tablet.

8 ADVERSE EVENTS: LIST AND REPORTING REQUIREMENTS

8.1 Adverse Events and Potential Risks List

LY2157299 has no reported history of Adverse Events or Serious Adverse Events.

CTCAE term (AE description) and grade: The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for AE reporting. A copy of the CTCAE version 4.0 can be downloaded from the CTEP web site (http://ctep.cancer.gov).

Attribution of the AE:

Definite – The AE *is clearly related* to the study treatment.

Probable – The AE *is likely related* to the study treatment.

Possible – The AE *may be related* to the study treatment.

Unlikely – The AE *is doubtfully related* to the study treatment.

Unrelated – The AE *is clearly NOT related* to the study treatment

8.1.1 Recording of Adverse Events

All adverse events will be recorded on a patient specific AE log. The AE log will be maintained by the research staff and kept in the patient's research chart.

8.1.2 Reporting of AE to WCM IRB

All Aes occurring on this study will be reported to the IRB according to the IRB policy, which can be accessed via the following link: http://researchintegrity.weill.cornell.edu/forms_and_policies/forms/Immediate_Reporting_Policy.pdf.

8.2 Expedited Adverse Event Reporting

The principal investigator is responsible for monitoring the safety of patients who enroll in the study. All AEs occurring after any administration of the study drug will be followed until resolution. The descriptions and grading scales found in the revised NCI CTCAE version 4.0 will be used for adverse event reporting. A copy of the CTCAE version 4.0 can be downloaded from the CTEP web site (http://ctep.cancer.gov/reporting/ctc.html).

A serious adverse event (SAE) is any adverse drug experience that occurs at any dose that results in any of the following outcomes:

- Death.
- Life-threatening adverse drug experience.
- Requires inpatient hospitalization or prolongation of existing hospitalization.

- For the purpose of this study, hospitalizations for protocol-scheduled procedures, blood product transfusions, or for social reasons (i.e., awaiting transport home) will not be considered SAEs.
- Persistent or significant disability/incapacity.
- A congenital anomaly/birth defect.
- Important medical events: Defined as AEs that, based upon appropriate medical judgment, may jeopardize the patient and may require medical or surgical intervention to prevent 1 of the outcomes listed above, even though these events may not be immediately life-threatening or result in death or hospitalization.

All SAEs occurring on this study will be reported to the IRB according to the IRB policy, which can be accessed via the following link: http://researchintegrity.weill.cornell.edu/forms_and_policies/forms/Immediate_Reporting_Policy.pdf.

8.3 Reporting Serious Adverse Events

All new SAEs and Medical Events of Interest occurring during the study or within 45 days of the last administration of LY2157299 will be reported as long as the patient has not withdrawn consent. The principal investigators are responsible for reporting SAEs to the IRB and the FDA (21 CFR §312.32] or other applicable regulatory authority. The principal investigator is responsible for submitting follow-up reports for all SAEs regarding the patient's subsequent course until the SAE has resolved or until the patient's condition stabilizes (in the case of persistent impairment), or the patient dies.

Institution and Investigator understand and agree that Investigator and Institution are obligated under applicable law and regulations to report any serious and related adverse event, if any that occurs during treatment with the Product to the Institution's IRB/Ethics Committee and to the governing regulatory authority in accordance with applicable filing timelines promptly after any such event occurs. Investigator, within 24 hours (US) or one business day (EU) of first knowledge of such serious and related adverse event, will notify Lilly's assigned medical Monitor.

Prior to or at the time of filing any such report with the governing regulatory authority, the Investigator will also transmit an information copy of the report as sent to the authorities to Lilly.

The Investigator shall make available to Eli Lilly promptly such records as may be necessary and pertinent to investigate any such expedited adverse event, if specifically requested by Lilly.

Furthermore, the Investigator will inform Eli Lilly of the following:

- any events that result in protocol amendments for safety reasons, as well as any safety related regulatory action such as a clinical hold of the Research
- any pregnancies occurring in patients who are exposed to the Product in connection with the Research.
- In addition, the Investigator shall notify Lilly within 24 hours (US) or one business day (EU) of first knowledge of any Product complaints (communication of dissatisfaction that alleges deficiencies related to the identity, quality, durability, effectiveness, safety, labeling, purity, stability, and appearance).

The Investigator will also inform Eli Lilly within 1 business day of becoming aware of any actions from any authority that may affect the performance of the Research

Medical Events of Interest

For reporting purposes, the Investigator will make note of any medical events of interest that occur during the treatment period.

8.4 Routine Adverse Event Reporting Guidelines

The descriptions and grading scales found in the revised NCI CTCAE version 4.0 will be used for adverse event reporting to the institutional IRB, DSMC, and sponsor. The IRB Reportable Events Forms (http://researchintegrity.weill.cornell.edu/forms_and_policies/forms/Immediate_Reporting_Policy.pdf.) should be used for all reportable adverse events.

8.5 Safety Profile of LY2157299

Mild to Moderate (Related to LY2157299):

Diarrhea (12%), Fatigue (26%), Anemia (14%), Nausea (15%), Vomiting (10%), Abdominal pain (14%), Headache (13%), Neutropenia (5%), Anorexia (4%), Pruritus (4%), Constipation (3%).

Possible Side Effects related to LY2157299:

Neutropenia, Thrombocytopenia, Lymphopenia, Leukopenia, Dysgeusia, Anorexia, Abdominal Discomfort, Dyspepsia, Pruritus, Dehydration, Asthenia, elevated liver enzymes, elevated creatinine.

Rare but Serious side effects related to LY2157299:

Pneumonia/Lobar Pneumonia, Pulmonary embolism, Deep vein Thrombosis, Sepsis, Neutropenia, Ascites, Anemia, Neoplasms, Pancreatitis, Asthenia, Retroperitoneal Hematoma, Heart attack, Stroke, Atrial Thrombosis, Heart Failure.

Death resulting from side effects considered related to LY2157299 in less than 1% of subjects have been reported.

9 CORRELATIVES AND SPECIAL STUDIES

9.1 Laboratory Correlatives Studies

Immune-monitoring studies will be conducted at UCLA under the supervision of Dr. McBride as well as at WCM under the direction of Dr. Demaria and Mary Helen Barcellos-Hoff. Blood samples will be obtained at baseline, and weeks 2, 5 and 15 for immune monitoring. All samples obtained for immune-monitoring studies will be processed for assay testing at the respective sites of collection. The following analyses will be performed:

9.1.1 TGF β gene response signature

Assessment of TGFβ gene response signature (TBRS) positivity in primary tumors or metastases as predictive biomarker of response to palliative LY2157299 plus radiation therapy combination therapy will be conducted.

We will use the 153-gene TGFβ response signature (TBRS) developed by Massague` and colleagues. TBRS identified TGFβ activity in 38/67 of metastatic lesions extracted from bones, lungs and other sites representing the natural metastatic spectrum of human breast cancer samples [72]. The resulting data will be processed by the WCM Core to ascertain the degree of TBRS in each patient; the study Biostatistician and staff will evaluate the associations with

outcomes. We predict that the presence of TBRS in the primary or metastatic cancer will predict tumor response to RT+LY2157299. Based on past experience, we assume that approximately 50% of subjects will have TBRS-positive tumors. Subjects who have TBRS-positive tumor and derive clinical benefit will be considered true positive.

9.1.2 Circulating Levels of TGF β

Monitoring of circulating levels of TGFβ prior to, during and after radiation therapy in patients with metastatic breast cancer before and after LY2157299 treatment. We recognize that TGFβ measurements are complicated by the presence of the neutralizing antibody. We will develop a new ELISA design based on LAP antibodies to overcome this limitation, in collaboration with Dr. Daniel Rifkin at NYU. Plasma TGFβ levels will also be measured.

9.1.3 Anti-angiogenic Activity of LY2157299 in vivo

Exploration of whether LY2157299 has anti-angiogenic activity *in vivo* by comparing circulating VEGF levels in plasma before and after treatment (ELISA). Plasma VEGF levels will be measured.

9.1.4 Tumor specific Immune responses

The most accepted technology for assessing T-cell responses to tumor antigen, namely ELISPOT and tetramer assays will be used. ELISPOT assays measure local release of cytokines by activated T cells following *ex vivo* re-stimulation by antigen. IFNγ production is most often used as a surrogate marker of T cell-mediated anti-tumor immunity. ELISPOT can be as sensitive as the tetramer assay and can be adapted to measure T cell avidity, although this will not be performed in this study, as it is not relevant to its aims. However, ELISPOT will be used to measure the hierarchy of epitope-specific responses for survival, since this might increase following radiation-induced tumor cell death, and indicate epitope spreading which we believe is an important correlate of clinical response [73].

The MHC tetramer assay uses flow cytometry to enumerate individual CD8 T lymphocytes that specifically recognize a peptide antigen of choice. Tetramers specific for CD4 or CD8 T cells are available, though MHC-I tetramers only will be used as they are more advanced. The tetramer assay is very able to detect expected increased responses in PBMCs from patients receiving RT. A detailed statistical analysis on data obtained with repeated sampling of over 100 patient

samples at UCLA showed that if antigen-specific T cells started below the lower limit of detection of 0.038%, they could be considered to have been boosted if they had an increase to over 0.066%, representing the mean + 3 standard deviations (unpublished). The assay does not require *ex vivo* T cell clone expansion, minimizing possible artifacts. It is chosen primarily because it directly quantifies tumor-specific CD8 T cells. However, it does not assess the functional status of cells; which is why both tetramer staining and ELISPOT assays will be performed, but with the expectation of a direct correlation. A lack of correlation would indicate involvement of divergent mechanisms that would be investigated further, depending on the results.

All 4 longitudinal samples from 5 HLA-A2 patients will be tested at one time using one vial of cells, in parallel with PBMCs from controls. Initially, PBMCs from HLA-A2+ patients, who should be 45% of the cohort, will be tested for CD8+ T cells that bind tetramers containing the (LMLGEFLKL) survivin peptide, which has a high binding affinity for HLA-A2 [69] and, for patients whose tumors are also HER2/neu positive, also the HER:p369-377 peptide [74]. The usual controls will be included, including a nonsense tetramer and PBMCs from controls, as described previously [71]. If ELISPOT assays indicate utility, non-HLA-A2 patients will be tested by tetramer assay using peptides immunodominant for their known HLA type [68-69].

9.1.5 Levels of circulating Tregs

Tregs will be detected primarily by staining PBMCs with antibodies for CD25, CD4, and intracellular FoxP3 (FACS analysis). Additional markers will be CD127, CD45RA/RO, and CTLA4. We include an examination of CTLA-4 because CTLA-4 signaling seems to be required for Treg function, possibly through TGF-β induction [75-76]. In addition, the NYU team has shown in preclinical models that anti-CTLA-4 synergizes with RT. CD45RA/RO and CD127 will be included because of recent reports that these mark subsets of Tregs with distinct proliferative and migratory properties that are relevant to cancer [77]. CD25, CD4, and intracellular FoxP3 staining will be performed at the same time as tetramer staining (see above) but another vial of each sample will be used for additional evaluation of Treg function, performed as previously described [71].

9.2 Collection of Specimen(s)

Peripheral blood for immune monitoring will be obtained from all trial participants at 5 time points: 1) baseline, 2) day 14; 3) day 42 infusion and 4) time of disease evaluation, at week 15. Blood will be drawn prior to radiation since radiation may increase TGFβ levels as shown in animal models. Up to an average of 60 ml of peripheral blood for research purposes will be collected at each time point into EDTA Lavender top tubes from consented patients enrolled in the trial, for analysis of plasma and PBMCs.

Tissue biopsy is not mandatory for participation in the trial, but archived tumor samples (slides and blocks, where available) will be obtained from consented patients. This tissue may be utilized to study TGFβ response expression signatures.

Sample collection is described in more detail in Appendix 1.

9.3 Handling of Specimens

PBMCs will be isolated within 24 hours of blood draw. Plasma will be stored for measurements of circulating TGFβ. An aliquot of $2x10^6$ cells will be removed for HLA typing. DNA isolated with the QIAamp DNA Blood Mini prep kit (Qiagen) will be submitted to the UCLA Immunogenetics Center (UIC) for PCR-based HLA typing that allows accurate and reliable results in resolving allele-level differences. The UIC is internationally recognized as a pioneer in HLA typing and crossmatch testing for tissue and organ transplants and a World Health Organization reference laboratory for HLA typing. Remaining cells will be frozen in 4 x 1 ml aliquots in 10% DMSO in human AB serum and plasma in 2.5 ml cryovials. Samples will be stored initially at -80C and then liquid N2 until used. All freezers will have alarm systems to avoid sample loss. Batches of PBMCs will be sent on dry ice overnight to UCLA from the other centers and plasma samples similarly shipped to WCM, one vial set at a time to prevent complete loss of samples due to unlikely shipping delays. All samples will be coded for laboratory analysis. A master list with the patient's name and an identification number will be kept in a locked cabinet.

9.4 Storage of Samples

Serial blood samples for immune monitoring collected at baseline, and at selected time points outlined in section 5.10.

All blood and tissue samples will be stored in a -80°C locked freezer in the WCM Bio repository for an indefinitely for research purposes only. These specimens will not be linked to any clinical data and will be de-identified in the clinical research database - only the data manager will have access to the master list with the patient name and an identification number. This master list will be secured in a locked cabinet at the WCM. Only the investigators listed on this protocol will have access to these samples as is described in sections 9.1.1-9.1.5.

The storage of your blood and tissue is optional and you may withdraw your consent for the banking of these specimens at any time. You may make this request by writing to the Principal Investigator Silvia C. Formenti, M.D. at Weill Cornell Medicine, 525 East 68th Street, New York, NY 10065.

9.5 Shipping of specimens

Frozen blood specimens from WCM will be de-identified and shipped to UCLA at -80°C.

9.6 Site(s) Performing Correlative Study

Correlative studies (as described in sections 9.1.1-9.1.5) will be performed at UCLA.

9.7 Coding of Specimens for privacy protection

All patients enrolled will be given a unique identifier (study ID number). Only the data manager will know the code linking patient and study ID number. Patients will be assigned a unique code number. All specimens collected will be de-identified and assigned the same unique study number of the corresponding patient and will also be marked with the collection time point. Clinical information regarding toxicities and response will likewise be stored in a de-identified database using only the unique identifier (study ID number).

10 INVESTIGATOR RESOURCES

10.1 Qualifications

At Cornell Dr. Formenti, will be responsible for the accrual and care of study patients. Research Nurse will be in charge of study screening, eligibility checklist and will participate in the process of acquisition of an informed consent, after the faculty has discussed the trial with the patient.

Dr. Paul Christos will participate in the design of the study and oversee the statistical analysis and interpretation of the data.

At UCLA Dr. Steinberg, Dr. Steve Lee, Dr. Phillip Beron, Dr. Kupelian, Dr. Glaspy, Dr. McCloskey, and Dr. Hurvitz will be responsible for the accrual and care of study patients. Dr. McBride will be responsible for overseeing the laboratory correlative studies. The research component at UCLA will be conducted by the Radiation Clinical Oncology Research Team. .

10.2 Location and Use of WCM and UCLA Facilities

At WCM therapy will be administered in the Department of Radiation Oncology at the Stich Radiation Center. LY2157299 will be administered at the infusion center at 525 East 68th street, Starr 3, New York, NY – 10065.

At UCLA therapy will be administered in the Department of Radiation Oncology, 200 UCLA Medical Plaza, Suite B265, Los Angeles, CA. 90095. LY2157299 will be administered at the CTSI infusion center at UCLA Santa Monica, CA, 2825 Santa Monica, Ste. 200 Santa Monica, CA 90404.

10.3 Conflict of Interest

There are no conflicts of interest to declare.

11 MEASUREMENT OF EFFECT

Abscopal responses will be assessed at 15 weeks based on [78] (Table 2). This choice of this interval is to enable a sufficient length of study to detect abscopal responses. In fact, the experience in the clinical trials of Ipilimumab in melanoma demonstrated occasional initial progression of disease with appearance of new metastatic sites when compared to the baseline imaging assessment. “Flares” at the first PET/CT or CT(Chest/Abdomen/Pelvis) assessment were often followed by clinical responses to the antibody. While the mechanism of action of

Ipilimumab is different from that of LY2157299, the experience with TGFβ inhibition in malignant glioma reported by Hau et al [79] confirms the lesson from the melanoma trials, i.e. that whenever the response is mediated by the immune system the following course is likely to manifest: 1) achievement of a clinical and radiological measurable response is slower than that of a typical response to cytotoxic therapy (RT or chemotherapy); 2) it can be heralded by an initial "flare" or emergence of new metastatic sites and, 3) with time it may be dramatic and would have been missed if the drug was discontinued too soon [80] [81].

Table 2: Immune response criteria (irRC)

	Summary of irRC
Note:	New, measurable lesions are incorporated into tumor burden
CR	Disappearance of all index and non-index lesions in two consecutive observations not less than 4 weeks apart, without the development of any new lesions.
PR	≥ 50% decrease in tumor burden compared with baseline in two observations at least 4 weeks apart
SD	50% decrease in tumor burden compared with baseline cannot be established nor 25% increase compared with nadir
PD	At least 25% increase in tumor burden compared with nadir (at any single time point) in two consecutive observations at least 4 weeks apart

11.1 Anti-tumor Effect

11.1.1 Definitions

Patients will be evaluated by PET/CTor CT(chest/abdomen/pelvis) at baseline, week 5 and week 15. However, systemic anti-tumor response will be by PET/CT or CT(chest/abdomen/pelvis) as defined by irRC at 15 weeks (primary endpoint).

Patients who have received at least one 14 days cycle of the study drug will be followed for toxicity (lack of grade 4 toxicity– primary safety end point).Patients will receive a total of 14 doses of LY2157299 (one dose daily for 14 consecutive days), per cycle. Patients who have received any treatment will also be evaluated for abscopal response to the metastatic sites using the immunologic response criteria based on the 5 and 15 week PET/CT or CT(chest/abdomen/pelvis) scans as outlined above in Table 2.

11.1.2 Disease Parameters

Measurable lesions are defined as those that can be accurately measured in at least one dimension (longest diameter to be recorded) as ≥ 20 mm with conventional techniques (CT, MRI, x-ray) or as ≥ 10 mm with CT scan. All tumor measurements must be recorded in millimeters (or decimal fractions of centimeters).

All other lesions (or sites of disease), including small lesions (longest diameter < 20 mm with conventional techniques) are considered non-measurable disease. Bone lesions, leptomeningeal disease, ascites, pleural/pericardial effusions, lymphangitis cutis/pulmonis, inflammatory breast disease, abdominal masses (not followed by CT or MRI) and cystic lesions are all non-measurable.

11.1.3 Methods for Evaluation of Measurable Disease

All measurable lesions that are not irradiated are identified as target lesions and recorded and measured by PET/CT or CT (chest/abdomen/pelvis) at baseline and at weeks 5 and 15. The abscopal response based on target lesions is defined in Table 2.

A sum of the longest diameter (LD) for all target lesions will be calculated and reported as the baseline sum LD. The baseline sum LD will be used as reference by which to characterize the objective tumor clinical response (definition in Table 2).

In parallel, assessment at PET scanning will be conducted. After at least a 4 hour fast, blood glucose will be checked. Patients will be injected with 10-15 mCi of F-18 FDG (fluoro-deoxy-glucose) in a resting state. After 45 minutes, whole body PET scans with attenuation correction will be performed from the cerebellum to the mid-thigh. Images will be corrected to provide standardized uptake values in g/ml. The Full Width Half Maximum of the scanner is approximately 4.6 mm. Therefore, we expect to be able to accurately assess lesions > or equal to 1 cm in size. A qualitative assessment of lesions will be performed, i.e., visibility of lesion on F-18 FDG PET. In addition, the maximum standardized uptake value will be determined for each lesion. Criteria as described by the EORTC PET study group will be used to assess response based on standard uptake value (SUV) [82]:

Progressive metabolic disease:

Increase in SUV of more than 25%
Visible increase in the extent of FDG uptake > 20% in longest dimension
New metastatic lesions
Stable metabolic disease:
Increase in SUV of less than 25% or decrease of less than 15%

No visible increase in the extent of FDG uptake
Partial metabolic response:
Reduction in SUV between 15% and 25% after one cycle of treatment
Reduction in SUV of more than 25% after more than one cycle of treatment
Complete metabolic response:
Complete resolution of FDG uptake

11.1.4 Response Criteria

11.1.4.1 Evaluation of Target Lesions

Criteria for evaluation of response are reported in Table 2.

11.1.4.2 Evaluation of Non-Target Lesions (irradiated Lesions)

Complete Response (CR):	Disappearance of all non-target lesions and normalization of tumor marker level
Incomplete Response/ Stable Disease (SD):	Persistence of one or more non-target lesion(s) or/and maintenance of tumor marker level above the normal limits
Progressive Disease (PD):	Appearance of one or more new lesions and/or unequivocal progression of existing non-target lesions

Although a clear progression of “non-target” lesions only is exceptional, in such circumstances, the opinion of the treating physician should prevail, and the progression status should be confirmed at a later time by the review panel (or study chair).

Note: If tumor markers are initially above the upper normal limit, they must normalize for a patient to be considered in complete clinical response.

11.1.4.3. Evaluation of Best Overall Response

The best overall clinical response is the best clinical response recorded from the start of the treatment until disease progression/recurrence (taking as reference for progression disease the smallest measurements recorded since the treatment started). The patient’s best response assignment will depend on the achievement of both measurement and confirmation criteria (see section 12.1.4.4 below).

Notes: Patients with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be classified as having

"symptomatic deterioration". Every effort should be made to document the objective progression, even after discontinuation of treatment.

In some circumstances, it may be difficult to distinguish residual disease from normal tissue. When the evaluation of complete response depends on this determination, it is recommended that the residual lesion be investigated (fine needle aspirate/biopsy) before confirming the complete response status.

11.1.4.4 Confirmation Criteria

To be assigned a status of PR or CR, changes in tumor measurements must be confirmed by repeat assessments that should be performed after a minimum of 4 weeks, no less, after the criteria for response are first met.

11.1.5 Response Review

All responses will be reviewed by an expert(s) independent of the study at the study's completion.

12 DATA REPORTING/ REGULATORY CONSIDERATIONS

The WCM Data and Safety Monitoring Committee (DSMC) is the central monitoring board for this study. The UCLA Cancer Institute Data and Safety Monitoring Committee (DSMC) is the local monitoring board for UCLA patients. Peter Martin, M.D. is the Co-Chairman of the Cancer Clinical Trials at the WCM Data Safety Monitoring Committee. He will also serve in the capacity of the Independent Research Monitor.

The Research Monitor, Peter Martin, MD, is responsible to oversee the safety of the research and report observations/findings to the IRB or a designated institutional official. The Research Monitor will review all unanticipated problems involving risks to subjects or others associated with the protocol and provide an independent report of the event to the IRB. The Research Monitor may discuss the research protocol with the investigators; shall have authority to stop a research protocol in progress, remove individual human subjects from a research protocol, and take whatever steps are necessary to protect the safety and well-being of human subjects until the IRB can assess the monitor's report; and shall have the

responsibility to promptly report their observations and findings to the IRB or other designated official and the HRPO.

12.1 Monitoring plan

This study will be conducted in accordance with the guidelines in the 2001 NCI approved data Safety and Monitoring plan for the WCM Cancer Institute and with the UCLA approved data Safety and Monitoring plan for the UCLA Cancer Institute. Reports to the Data Safety and Monitoring Committee will include the following information: accruals, targets, responses, adverse events and evidence of reporting to appropriate review committees. The WCM Data and Safety Monitoring Board (DSMB) will review the IRB approved protocol, the data and safety monitoring plan and any stopping guidelines during protocol initiation. The WCM DSMB is to perform a data and safety analysis annually (as per DSMB letter dated 04.20.2017) following the accrual of the first subject. . The WCM DSMB may also convene as needed if stopping criteria are met or other safety issues arise that the Principal Investigator and/or IRB would like the WCM DSMB to address.

As per WCM DSMB recommendations, the safety analysis will be performed annually, at which point the protocol and safety analysis should be submitted to the DSMB for review.

12.2 Stopping rules (for the individual patient and for the study as a whole)

Patients will be discontinued from study therapy when any clinical adverse event greater than Grade 3 or intercurrent illness in the opinion of the Principal Investigator, indicates that continued treatment with the study therapy is not in the best interest of the subject. Patients will be monitored until the resolution of the adverse event. Based on PI's discretion, patients will resume or will be withdrawn from the study therapy.

If five or more non-hematological toxicities, Grade 3 and above, are reported over a period of 3 months, will result in temporary discontinuation of combination therapy.

12.3 Data Management

All patient data will be entered and maintained using,REDCAP, CTSC data management system. These data include clinical data and all patient safety data. The REDCap provides audit trails that track creation and modification of records that include user id and timestamp. Depending on the database account privileges, the data managers may be able to correct a discrepancy or if not, route it to the project data manager at WCM who can take appropriate action to correct the problem. Data clarification forms can also be printed out when necessary to be sent to the project data manager at JCTO.

The Nurse practitioner will also be documenting adverse events in EPIC during every follow up visit, All Adverse Events are captured in REDCap database and a report will be sent to Data safety monitoring board during their quarterly review meetings.

All key end points will be source verified by a second person at each site and errors will be corrected. Once the data is verified and all discrepancies are closed, the data can be locked/frozen. Locking and freezing can be done at different granular levels and will follow institutional SOPs and any specific requirements for the project.

Security measures that will be taken in order to protect patient data will include firewall technology and database level security which will be achieved by assigning roles and privileges to different levels of users and by requiring that the users authenticate themselves using user id and password. Additional security for data transfer between remote clients and servers will be achieved by using digital certificates/SSL. All data will be backed-up to tape periodically according to the Institutional SOPs. All data will be stored for at least 5 years following the termination of this study.

12.4 Confidentiality

The medical, hospital and research records associated with this study are considered confidential. Members of the treating team and designated study assistants will have access to the records as required to administer treatment and comply with the protocol. Neither the name nor any other identifying information for an individual will be used for reporting or publication regarding this study. All laboratory and baseline data will be de-identified and transferred via secure links to the database at WCM. Patient records will be made available for inspection to auditing agencies to satisfy regulatory requirements. Privacy will be maintained throughout the consent process and protocol treatment.

13 STATISTICAL CONSIDERATIONS

The characteristics of patients and their disease will be described for the study as a whole and by study site using summary statistics (including means, standard deviations, quantiles) and graphical displays (e.g., boxplots) for quantitative variables and frequency distributions and proportions for qualitative data. Clinical and immune response rates will be estimated along with exact 95% confidence limits. Similarly, for each immunological parameter, summary statistics and graphical displays will be provided at baseline, before radiation, at week 5, and at week 15. Tests for normality will be used (eg Kolmogorov-Smirnov tests at alpha = 0.05); transformations (eg, logarithmic) will be used to meet the normality assumptions. The patterns of change over time for each of the immunologic parameters will be evaluated using mixed effects regression models that incorporate time as a fixed effect and subject as a random effect, and take into account different numbers of observations for each patient (missing data, [83]). Exploratory analyses will also be undertaken to describe the association of these parameters and changes in parameters with response recognizing that the numbers of subjects will be small. Additionally, the TGFβ gene suppressor signature (see 9.1.1.) will be estimated for each subject and the AUC (area under the Receiver Operating Characteristic Curve) will be estimated. The sensitivity and specificity of the defined cut point will be also be provided along with 95% confidence intervals.

14.1 Endpoints

14.1.1 Primary endpoint

1. To assess safety and feasibility of combining TGF β receptor I kinase inhibitor LY2157299 and local radiotherapy
2. To determine if treatment with TGFβ receptor I kinase inhibitor LY2157299 and localized RT achieves an abscopal tumor regression

14.1.2 Secondary endpoints

1. To estimate the local response rate of combining TGFβ receptor I kinase inhibitor LY2157299 and local radiotherapy
2. To determine if treatment with TGFβ receptor I kinase inhibitor LY2157299 and

localized RT enhances tumor-specific immunity in patients with metastatic breast cancer, and if this is associated with abscopal tumor regression.

3. To determine if treatment with TGFβ receptor I kinase inhibitor LY2157299 and localized RT alters the numbers and function of T-reg cells in patients with metastatic breast cancer.

A. Analysis Populations

Efficacy analysis will be conducted on all patients who are entered into the study and received any treatment. Additional supportive efficacy analyses will be conducted for those patients who have completed week 15 visit.

B. Sample Size

i. Accrual estimates

Expected number of eligible patients will be 4 per month.

ii. Sample size justification

In this trial, a total of 28 patients with metastatic breast cancer will be accrued both at WCM and at UCLA (18 patients at WCM and 10 patients at UCLA) to treatment with radiotherapy + 300 mg/daily LY2157299 for 14 days on / 14 days off, and followed for evaluation of abscopal responses based on the immune response criteria at 15 weeks. With 28 patients enrolled in this study, we can test the null hypothesis that the abscopal response rate at 15 weeks is ≤0.05 versus the alternative that the response rate is 0.19 with α= 0.05 and power of 80%. If there are 3 or fewer responses in the 28 patients, then the combination will be rejected for further study in this single stage design (calculations from PASS 2008, J. Hintze, NCSS, Kaysville, Ut). The trial is designed to allow for up to 56 patients to be enrolled to ensure that 28 patients will be evaluable at 15 weeks. Response rates will also be estimated for all enrolled patients.

APPENDIX 1 SPECIMEN COLLECTION PROCEDURES

NOTE regarding samples for TGF-β assessment:

It is important to follow special steps to reduce the release of TGF-β into the blood sample – particularly, using a straight 20 or 21 gauge needle instead of a butterfly, minimizing tourniquet

stasis, directly drawing blood into Vacutainer tubes instead of using a manual syringe and transfer, and avoiding contact EDTA. In addition, CTAD tubes are sensitive to light, so they should be kept inside the kit boxes when not in use.
The suggested method of phlebotomy is to minimize the breakage of platelets and thus the release of TGFβ from platelet granules. The order of blood tubes was designed to avoid potential cross-contamination of the plasma CTAD tube with any potential anticoagulants, EDTA and heparin, as these agents may activate platelets and/or interfere with the tests.

If the sites need to draw their standard hematology and chemistry labs before any research tests, it is recommended that:

1. Standard blood tubes containing anticoagulant be drawn first (e.g. CBC, PT/INR, and PTT) followed by collection tubes which do not contain anticoagulants (e.g. serum chemistries).
2. The biomarkers should then be drawn in the order specified below in order to adequately separate the anti-coagulant tubes from the CTAD tube.

Blood draws should be performed in the following order in order to minimize cross contamination of the TGFβ plasma collection:

1. SST tube (Gold hemoguard)
2. CTAD tube (blue top)

1.2 Plasma, FROZEN (TGFβ1, VEGF)

Note: CTAD tubes are sensitive to light, so they should be kept inside the kit boxes when not in use.

- Collect blood into two 4.5 mL blue top Vacutainer® (CTAD) tubes using a 20 or 21-gauge straight needle and the following special instructions:
  - Tourniquet stasis should be minimized. ***If possible*, do not use a tourniquet when drawing the sample for this tube**. If a tourniquet is needed, minimize the constriction strength and try to remove it at the start of the venipuncture
  - It is recommended that blood is drawn directly into CTAD tubes in order to avoid platelet shearing/destruction. Do not use a standard syringe, manual pull, and transfer to tubes method to draw blood.
  - A **central line should not be used** for blood draws the day of infusion. In the case where it is not at all possible to draw the blood peripherally, then the central line must have been flushed with 2-5 ml of normal saline to clear any heparin
- **Invert tubes 3-4 times and immediately place in ice water bath for no more than 30 minutes**
- **Remove from bath and centrifuge whole blood at 2,500 RCF (xg) for 30 minutes at 4°C. Turn the centrifuge brake OFF.**

- **Immediately after centrifugation, pipette** ~0.5 mL plasma into 7-8 cryovials, **Use only the top 2/3 of the plasma volume.**
- Label the cryovials with the Patient Initials, Patient ID #, sample collection date (dd/mmm/yyyy format), sample collection time (24 hour clock) and sample type as serum for the specific biomarkers.

Immediately freeze all cryovials in the upright position at or below -70ºC (-20ºC is NOT acceptable) in a **non-defrosting** freezer until shipped. **Keep all samples frozen until ready to ship.**

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