Protocol ID: 160178

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Study of Cirmtuzumab and Paclitaxel for Metastatic or Locally Advanced, Unresectable Breast Cancer

UC San Diego
MOORES CANCER CENTER

A Phase 1b pilot clinical trial of Cirmtuzumab, an anti-ROR1 monoclonal antibody, in combination with paclitaxel (TAXOL) for the treatment of patients with metastatic, or locally advanced, unresectable breast cancer

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Signature Page

The signature below constitutes the approval of this protocol and the attachments, and provides the necessary assurances that this trial will be conducted according to all stipulations of the protocol, including all statements regarding confidentiality, and according to local legal and regulatory requirements and applicable U.S. federal regulations and ICH guidelines.

Principal Investigator

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_____ _____
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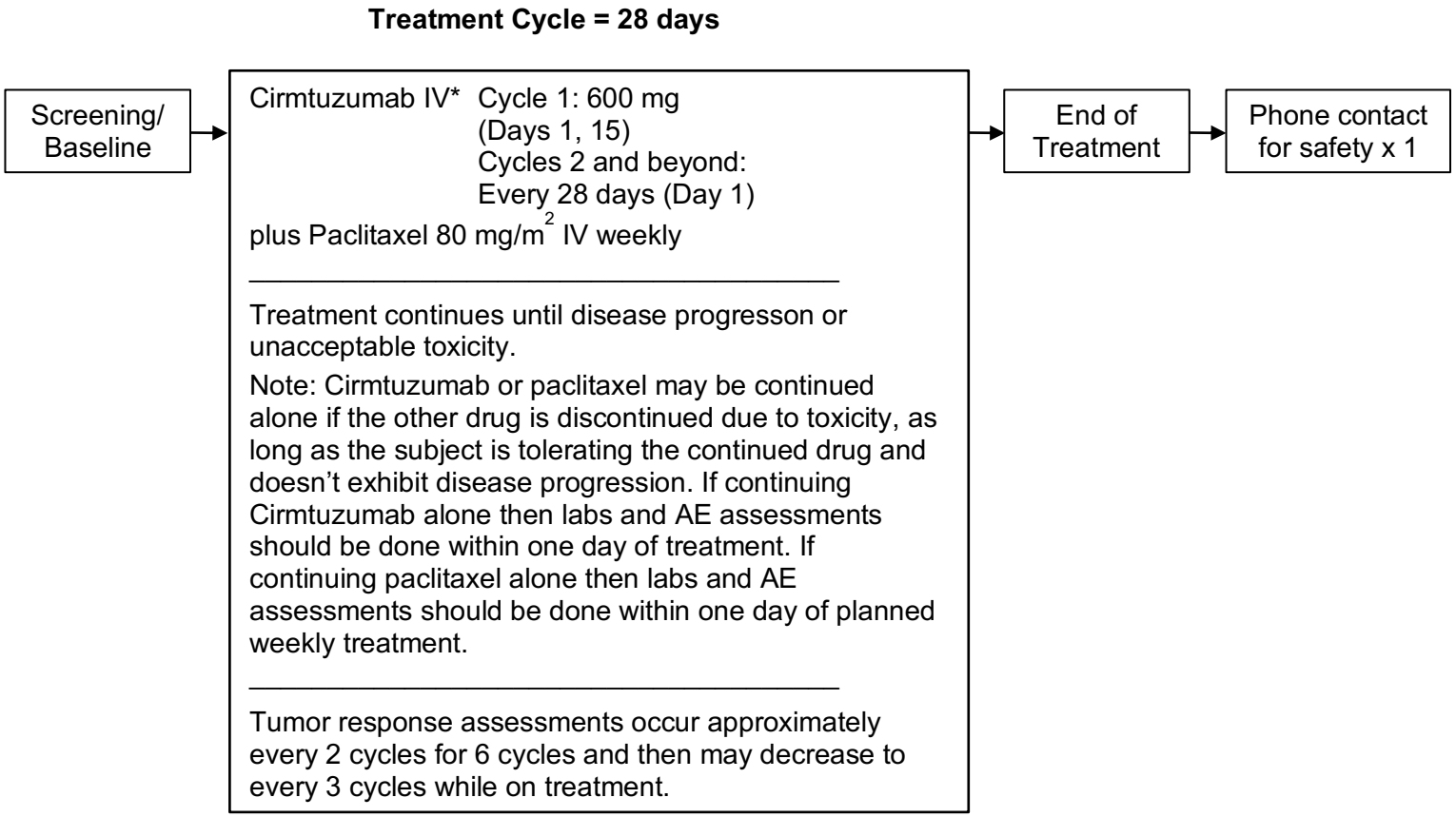
LIST OF ABBREVIATIONS

AE	Adverse Event
ALT	Alanine Aminotransferase
aPTT	Activated Partial Thromboplastin Time
ASCO	American Society of Clinical Oncology
AST	Aspartate Aminotransferase
BID	Bis in die/ twice a day
BUN	Blood Urea Nitrogen
CAP	College of American Pathologists
CBC	Complete Blood Count
CLL	Chronic Lymphocytic Leukemia
CMP	Comprehensive Metabolic Panel
CNS	Central Nervous System
CR	Complete Response
CSC	Cancer Stem Cells
CT	Computed Tomography
CTCAE	Common Terminology Criteria for Adverse Events
DLT	Dose Limiting Toxicity
DSMB	Data and Safety Monitoring Board
ECOG	Eastern Cooperative Oncology Group
FDA	Food and Drug Administration
GCP	Good Clinical Practice
GLP	Good Laboratory Practice
HIV	Human Immunodeficiency Virus
HIPAA	Health Insurance Portability and Accountability Act
HRPP	Human Research Protections Program
ICH	International Conference on Harmonization
IHC	Immunohistochemistry
IRB	Institutional Review Board
I.V.	Intravenous
LDH	Lactate Dehydrogenase
mAb	Monoclonal Antibody
MRI	Magnetic Resonance Imaging
MTD	Maximum Tolerated Dose
NCI	National Cancer Institute
ORR	Overall Response Rate
OS	Overall Survival
PD	Progressive Disease
PK	Pharmacokinetic
PDX	Patient Derived Xenograft
PET	Positron Emission Tomography
p.o.	per os/by mouth/orally

PR	Partial Response
PT	Prothrombin Time
RECIST	Response Evaluation Criteria in Solid Tumors
ROR1	Receptor-tyrosine-kinase-like Orphan Receptor 1
RP2D	Recommended phase II dose
SAE	Serious Adverse Event
SD	Stable Disease
ULN	Upper Limit of Normal
UPR	Unanticipated Problems involving Risk to subjects or others

STUDY SCHEMA



* Cirmtuzumab dose will be 600 mg. Patients will be enrolled in cohorts of 5. If two or more of the 5 patients in a cohort experience dose-limiting toxicity, then further patients will be enrolled at a 50% dose reduction (300 mg fixed dose level).

STUDY SUMMARY

Title	A phase 1b pilot clinical trial to determine the safety and tolerability of cirmtuzumab, an anti-ROR1 monoclonal antibody, in combination with paclitaxel for the treatment of patients with metastatic, or locally advanced, unresectable breast cancer.
Short Title	Cirmtuzumab treatment for metastatic breast cancer
Protocol Number	HRPP #160178
Phase	Phase 1b
Methodology	Single arm, open label
Study Duration	Subjects will continue study treatment until disease progression or unacceptable toxicity. Subjects will have a follow-up 56 days after study treatment discontinuation unless patient has started new anti-cancer therapy.
Study Center	Single center: UC San Diego
Objectives	*Primary Objectives:* 1. To determine the safety of fixed dose cirmtuzumab during the first 4 weeks when administered in combination with weekly, standard of care paclitaxel to patients with metastatic, or locally advanced, unresectable breast cancer. *Secondary Objectives:* 1. To determine the safety and tolerability of cirmtuzumab in combination with paclitaxel by ongoing evaluation of adverse events (AE's) during treatment and follow-up. 2. To assess clinical activity by evaluating objective tumor response according to RECIST v1.1 and time to progression. 3. To measure ROR1 density on tumor and on the putative cancer stem cell population, and to correlate these with response. *Exploratory Objectives:* 1. To assess the mechanism of action through pharmacokinetic, safety and pharmacodynamic studies including: • Circulating cirmtuzumab levels in peripheral blood • Level of circulating antibodies against cirmtuzumab • As available, we will perform pharmacodynamic, biochemical and molecular studies on tissue samples of treated patients, depending on sample availability. 2. To assess imaging correlation of optional PET/CT with standard cross-sectional imaging
Number of Subjects	Up to 30 patients will be consented with a goal of 15 evaluable patients who complete at least 4 weeks of combination therapy or experience DLT
Diagnosis and Main Inclusion Criteria	• Women or men with biopsy proven (most recent biopsy), Her2/neu negative, metastatic or locally advanced, unresectable breast cancer who have not received paclitaxel in the metastatic setting. • There is no limit to prior therapies received. • CNS metastases are allowed, as long as the metastases are asymptomatic, have been treated with radiation, and have been stable for > 6 weeks off steroids. • Patients must have no greater than grade 1 neuropathy

	• ECOG ≤ 2. • Patients must have adequate hematologic, renal, hepatic and coagulation function. • No concurrent antibody therapy is allowed with the exception of denosumab for use in bone metastasis.
Study Product(s), Dose, Route, Regimen	Each treatment cycle is 28 days. Cirmtuzumab is administered by intravenous infusion every other week for two doses (i.e., Days 1 and 15 of Cycle 1) then every 28 days for subsequent cycles (i.e., Day 1 of each cycle). Paclitaxel 80 mg/m^2 is administered weekly by intravenous infusion on Days 1, 8, 15, and 22 of each 28-day cycle. On Days when both paclitaxel and cirmtuzumab are administered, paclitaxel will be given at least 30 minutes following cirmtuzumab infusion.
Duration of Administration	Cirmtuzumab and paclitaxel are administered until the subject experiences disease progression or unacceptable toxicity. Cirmtuzumab or paclitaxel may be continued alone if the other drug is discontinued due to toxicity, as long as the subject is tolerating the drug and does not exhibit disease progression.
Reference Therapy	Paclitaxel 80 mg/m^2 weekly.

SCHEDULE OF EVENTS

Treatment Cycle	**Screening**	**Cycle 1**				**Cycle 2 and beyond (– (Combionation Therapy phase).**				**Mono-Therapy phase[12]**	**End of Treatment**	**Follow-up**
Visit Day		D1	D8	D15	D22	D1	D8	D15	D22	D1	28 days after last dose of cirmtuzumab or paclitaxel (whichever is stopped last) 14	56 days after last dose of cirmtuzumab or paclitaxel (whichever is stopped last) 15
Visit window	≤ 14 days	±1d[13]	±1d[13]	±1d[13]	±1d[13]	±1d[13]	±1d[13]	±1d[13]	±1d[13]	±1d[12,13]	±3d	±7d
Informed Consent	≤ 30 days of C1D1											
Eligibility Checklist	X											
History and Symptoms	X	X		X		X		X		X	X	
Vital signs (Height: screening only)	X	X	X	X	X	X	X[12]	X[12]	X[12]	X[12]	X	
Comprehensive PE	X	X		X		X[1]		X[1]		X	X	
ECOG Performance Status	X	X				X				X	X	
CMP[2]	X	X	X	X	X	X	X[12]	X[12]	X[12]	X[12]	X	
CBC and differential[3]	X	X	X	X	X	X	X[12]	X[12]	X[12]	X[12]	X	
INR or PT	X											
Serum hCG (female of child bearing potential)	X											
Adverse Events Screen		X	X	X	X	X	X[12]	X[12]	X[12]	X[12]	X[14]	X[15]
Concomitant Medication	X	X	X	X	X	X	X[12]	X[12]	X[12]	X	X	X[4]
Cirmtuzumab administration		X		X		X				X[12]		
Paclitaxel administration		X	X	X	X	X	X[12]	X[12]	X[12]	X[12]		
Tumor assessment by physical exam[5]	X	X				X				X[12]	X	
CT/MRI scan Chest/ Abdomen/ Pelvis (optional PET scan)[6]	≤ 30 days of C1D1	X[6]									X[7]	
Bone Scan[8]	≤ 30 days of C1D1	X[8]										
Blood collection for pharmacokinetics and correlative studies[9]	≤30 days of C1D1	X	X	X	X	X				X[12]	X	
Tumor biopsy and/or fluid collection samples for correlative studies[10, 11]	≤ 30 days of C1D1	X[10,11]									X	

1. Comprehensive PE will not be performed at Day 15 for Cycles 2 and beyond.
2. Comprehensive metabolic panel (CMP) to include: Na+, Cl-, K+, CO2, BUN, Creatinine, Glucose, Ca2+, total protein, albumin, total bilirubin, SGPT, SGOT, alkaline phosphatase.
3. If grade 4 neutropenia is documented (ANC <500/mcl), obtain twice per week until resolved to grade 2.
4. Record if patient is receiving concomitant medication for cancer treatment.
5. For those patients whose disease can be evaluated by physical examination according to RECIST v1.1.
6. CT (or MRI) should be should be performed within 7 days of Day 1 of Cycles 3, 5, and 7. Beyond Cycle 7, scans may be decreased to every 3 cycles (i.e., within 7 days of Day 1 of Cycles 10, 13, etc.). Scans should be performed at the same frequency but within 7 days of Day 1 of the cycle.. Optional PET/CT imaging will be performed at the same time as CT (or MRI).
7. Assess response at disease progression.
8. Bone scans may be performed at baseline (recommended within 30 days of C1D1 if possible) and whenclinically indicated. Bone scans will not be used to determine progression of disease.
9. Blood samples are collected for PK analysis and correlative studies according to Protocol Table 5-1.
10. If the patient consents to biopsy, a core needle biopsy (3 samples) of tumor tissue should be performed or tumor cells should be collected from body fluid within 30 days prior to starting study treatment on Cycle 1 Day1. If feasible, a new sample of tumor tissue may be obtained between Day 1 and Day 15 of Cycle 3 and at the time of progression. Patients can still participate in study whether or not biopsies are performed. Biopsies will be obtained either using imaging-guided needle biopsy, skin biopsy, lymph node biopsy, or by recovering tumor cells from malignant ascites, pleural effusion,or other body fluid.
11. In addition, samples from pleural and peritoneal fluid may be obtained from patients who have repeated pleural taps, pleural drainage, or peritoneal taps for palliation at other timepoints while the patient is on study or in follow-up per the protocol.
12. If continuing Cirmtuzumab alone then labs and AE assessments should be done within one day of treatment (typically every four weeks). If continuing paclitaxel alone then labs and AE assessments should be done weekly within one day of treatment.
13. The intent of the visit window is within 1 Day of each planned treatment; however, the visit window of +3d allows for an unanticipated delay in treatment.
14. End of Treatment (EOT) adverse event monitoring and visit will occur at 28 days +/- 3 days or at the initiation of new cancer therapy whichever occurs earlier. PK blood specimen may be drawn at 28 days or, if not feasible, at the start of new anti-cancer therapy.
15. Followup Visit will occur 56 days +/- 7 days or at the initiation of new cancer therapy whichever occurs earlier. In the event that the start of a different anti-cancer therapy occurs earlier than 28 +/- 3 days, the End of Treatment visit and the Followup Visit will be the same visit.

1.0 BACKGROUND AND RATIONALE

1.1 Disease Background

Breast cancer is the most commonly diagnosed cancer, and the second leading cause of cancer-related mortality in women. There are approximately 232,000 new cases of invasive breast cancer every year in the US, and an estimated 40,000 breast cancer deaths every year.[1] Despite recent advances, the projected 5 year overall survival for a female diagnosed with metastatic breast cancer is still only 25.9%.[2] Also, although there are a variety of agents available for the treatment of metastatic breast cancer many of these agents are associated with significant toxicity and poor tolerability.

In addition, highly proliferative hormone receptor positive (Luminal B) or triple negative metastatic breast cancer are aggressive subtypes of breast cancer with a poor overall prognoses. Currently, the only FDA approved targeted agent for triple negative breast cancer is olaparib for the small minority of patients who have BRCA1 or BRCA2 associated breast cancer. The backbone of treatment for endocrine-refractory or triple negative breast cancer is cytotoxic chemotherapy with significant toxicity and clinical benefit that last months not years. The success of agents like trastuzumab and second-generation HER2 targeted agents in breast cancer over the past 20 years has illustrated that the goal of treating breast cancer with less toxic, targeted therapies is very effective. However, for Her2-negative aggressive breast cancers, an unmet medical need exists for new breast cancer therapies that can target rapidly proliferating cancer cells and target cancer stem cells, with minimal damage to normal tissues.

1.2 ROR1 and Cancer Stem Cells

Promising translational research from the lab of Dr. Thomas Kipps and several others have recently demonstrated that the highly conserved transmembrane protein ROR1 (receptor tyrosine kinase-like orphan receptor 1) is expressed on many high-grade cancers including breast cancer. ROR1 is not expressed on the surface of normal adult tissues, with the possible exception of a rare subset of precursor B cells, called hematogones, which may be observed in the marrow of pediatric patients or patients recovering from myeloablative therapy.[3] ROR1 plays a key role in fetal development, but based on exhaustive profiling studies in human hematopoietic stem cells, it is no longer detectable by the 2nd trimester fetal liver stage, and is not on marrow stem cells. Due to a potential lack of cross-reactivity with normal adult tissues it represents an ideal target for immunotherapy.

Adult chronic lymphocytic leukemia (CLL) cells express ROR1, and patients immunized with CLL cells can make auto-antibodies against this protein.[4-6] Some breast cancer cells also express ROR1. Several groups have discovered that high-level expression of ROR1 is associated with shorter progression-free survival after therapy and shorter median overall survival in some datasets. Expression of ROR1 may also be associated with more aggressive growth as well as with activation of PI3K, AKT, and cAMP-response-element-binding protein (CREB). ROR1 may interact with EGFR, and play a pivotal role in epithelial-mesenchymal transition and metastasis.[7-9]

Zhang et. al recently demonstrated, both *in vitro* and in triple negative, patient-derived-xenograft (PDX) murine models that ROR1 expressed in breast cancer is associated with shorter progression-free survival after therapy and shorter overall median survival.[7,8] Our recent studies have shown that some tumors that express ROR1 also express the putative cancer stem cell

(CSC) markers ALDH1, CD44 and CD24. In addition, in both cell culture and in murine models, these cells revealed aggressive growth patterns characteristic of CSC.[10]

1.3 Cirmtuzumab and Paclitaxel

Cirmtuzumab (CLL Interacting Ror1 Molecule TUmor targeting Z(enotropic) hUmanized Monoclonal AntiBody) is a fully-humanized monoclonal antibody designed to bind the extracellular immunoglobulin-like domain of ROR1 with high-affinity.

Initial preclinical studies show that cirmtuzumab is an ideal therapy due to the following features:

- High affinity for cell-surface ROR1
- Activity against cancer cells that express ROR1, which include cancer-stem cells.
- Lack of cross reactivity with normal adult tissues
- Reduced immunosuppressive potential with lack of targeting most normal lymphoid or myeloid cells
- Low immunogenic potential due to substantial humanization of the antibody framework

The anti-microtubule agent paclitaxel is well established as an effective therapy for metastatic breast cancer both in the first line and heavily pretreated population. Expected overall response rates for single agent paclitaxel are between 20-45%.[11-14] When given weekly at a dose of 80mg/m^2, paclitaxel is both effective and generally well tolerated.[15]

In this study we propose to combine the monoclonal antibody (mAb) cirmtuzumab with weekly paclitaxel. The rationale for this combination comes from recent pre-clinical data shown in **Figure 1**, which displays the activity of cirmtuzumab with and without paclitaxel (Taxol) in immune-deficient mice bearing tumors following engraftment with PDX5 cells. For this, tumor-bearing mice received intravenous (i.v.) control IgG or cirmtuzumab (at 10 mg/kg) with and without paclitaxel (Taxol, at 13.4 mg/kg). The tumors grew significantly more slowly in cirmtuzumab or paclitaxel treated mice compared to control IgG treated mice. Moreover, the inhibitory effect of cirmtuzumab in combination with paclitaxel on the growth of PDX5 cells was greater than that of either cirmtuzumab or paclitaxel alone.[8] Perhaps most importantly, when cells were harvested from tumors treated with both agents and implanted in fresh host animals, no tumors formed, suggesting that the tumor initiating stem cells had been eliminated. These data demonstrate that cirmtuzumab has activity against human breast cancer and that this effect is augmented when used in combination with paclitaxel.

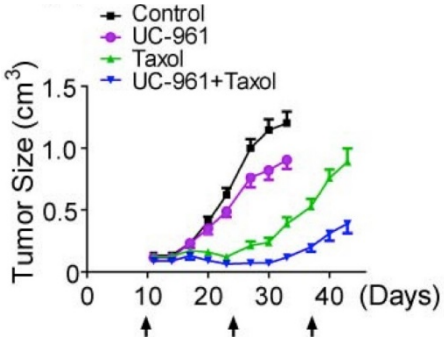


Fig. 1 The effect of ROR1mAb in combination with Taxol on breast cancer cells. 1X10^6 single cells from PDX 5 were engrafted in mammary pads of immune-deficient mice. UC-961 at 10mg/kg or paclitaxel (Taxol) at 13.4mg/kg were injected iv. at the times indicated by the arrows on the bottom. Tumor growth was monitored over time. The average sizes ± SEM of the tumors that formed in mice in each treatment group (N=10) are shown. The line colors depict the treatment groups, as indicated in the legend.

1.4 Cirmtuzumab Pre-clinical Pharmacology and Toxicology

To support the use of cirmtuzumab in clinical trials, a comprehensive single-species pharmacology/toxicology study was conducted in rats. In this study, groups of animals (15 of each sex) received cirmtuzumab at doses of 40, 120 and 400 mg/kg given weekly for 5 doses over 28 days. Three days following the final product administration, 20 animals (10 of each sex) in each dosing cohort were sacrificed and the remaining animals in the recovery phase sacrificed on day 56. During the study, parameters measured included twice-daily clinical signs, food consumption, weekly body weights, ophthalmic examinations (pretreatment, after last dose and last week of drug-free period) urinalysis (prior to sacrifice) and clinical pathology indices including serum chemistries, hematology and coagulation studies (prior to sacrifice). Safety pharmacology measurements including functional observation batteries were conducted weekly. Finally, full necropsy and a complete microscopic analysis was performed on all animals in all groups and a select panel of organs including the liver, spleen, heart, kidneys, brain and ovaries were weighed.

In all dosing cohorts, the cirmtuzumab drug product was well tolerated by the treated rats. No adverse events were noted during the conduct of the study. At terminal sacrifice, gross pathologic exams were normal and no untoward effects of product administration were noted.

1.5 Cirmtuzumab Clinical Experience - Phase 1 CLL Trial

The single agent safety of cirmtuzumab was evaluated in a Phase 1, open label clinical trial that enrolled patients with relapsed/refractory chronic lymphocytic leukemia.

Between September 2014 and September 2017, 26 patients enrolled and received Cirmtuzumab at doses between 15 mcg/kg and 20 mg/kg every 14 days for a maximum of four infusions. All patients had relapsed or refractory disease with objective signs of disease progression and an indication for therapy according to international working group criteria (Hallek et al., 2008). The majority of patients had at least one factor associated with poor prognosis, including unmutated immunoglobulin heavy-chain variable region gene (IGHV) or deletion of chromosome 17p (Del 17p). All patients were previously treated with anti-CD20 monoclonal antibodies, and 27% and 23% previously received inhibitors of BCL-2 and B-cell receptor signaling (including ibrutinib), respectively. All patients had leukemic cells that expressed ROR1 (MFI range 11.6 to 113.9), with half of the patients having a mean fluorescence intensity (MFI) greater than 32, which was previously associated with a poor prognosis (Cui et al., 2016).

Cirmtuzumab infusions were safe and well-tolerated. There were no infusion-related reactions, though all patients received pre-medications for the first infusion with corticosteroids, acetaminophen, and antihistamine. The maximum rate of infusion was 800 mg/hr. There was also no dose-limiting toxicities (DLT) and there were no serious adverse events up to a dose of 20 mg/kg. The main recurrent laboratory abnormalities included anemia, thrombocytopenia, and neutropenia, which were primarily attributed to underlying CLL. There were two instances of grade 3 lipase elevation (greater than two-times the upper limit of normal). Neither was accompanied by abdominal pain or other symptoms of pancreatitis. Both resolved spontaneously without intervention, including one case resolving within the same day upon a repeat blood draw. There was one instance of grade 3 ALT elevation, which coincided with a patient having a gallstone. Table 1 lists all recurrent AEs, regardless of attribution.

Table 1: Adverse Events

Adverse Events (AE) by Type, Incidence, and Intensity, % (Total N=26)					
AE (Regardless of Attribution)	CTCAE (Ver 4.03) or iwCLL Grade				Total
	1	2	3	4	
Anemia	58%	19%	8%		85%
Thrombocytopenia	38%	23%	4%		65%
Neutropenia	23%	15%	4%		42%
Upper Resp. Infection		27%			27%
Fatigue	23%	4%			27%
Diarrhea	27%				27%
Cough	23%				23%
Cough	23%				23%
Headache	23%				23%
Dyspnea	19%				19%
Lipase Increased	4%	4%	8%		16%
Nausea	16%				16%
Constipation	16%				16%
Urinary Tract Infection		12%			12%
Akathisia	12%				12%
Dizziness	12%				12%
Bloating	12%				12%
Insomnia	12%				12%
Urinary Frequency	12%				12%
Creatinine Increased	12%				12%
Hyperuricemia	12%				12%
Skin Infection		4%	4%		8%
Amylase Increased		4%	4%		8%
ALT Increased	4%		4%		8%
Conjunctivitis	4%	4%			8%
Anorexia	4%	4%			8%
Maculopapular Rash	4%	4%			8%
Hypokalemia	8%				8%
Vomiting	8%				8%
Flushing	8%				8%
Hyperkalemia	8%				8%
Urinary Urgency	8%				8%
Hyperhidrosis	8%				8%
GERD	8%				8%

Overall, no patient stopped treatment due to an adverse event. Five patients discontinued cirmtuzumab prior to completing the four planned infusions. One patient in the first cohort stopped due to progressive disease with worsening thrombocytopenia following 1 dose of 15 mcg/kg. The other patients met criteria for stable disease at the time of discontinuation, but stopped to pursue other treatment options that became available to them (eg: approval of venetoclax by the US Food and Drug Administration in 2016).

Pharmacokinetic studies performed using an ELISA assay generated for human IgG binding to immobilized ROR1 detected cirmtuzumab at levels above background, with peak concentrations that were highest in samples collected within 60 minutes of the completion of each infusion, and increased with each subsequent infusion of cirmtuzumab. Peak levels increased proportionally

with the dosage administered, with peak cirmtuzumab concentrations above 400 mcg/mL for patients who received doses of 20 mg/kg. There was evidence of in vivo antibody stability. Plasma collected approximately 3 months after the last infusion still had significant levels of cirmtuzumab (> 40 ug/ml). The half-life of cirmtuzumab is calculated to be 32.4 days, similar to other monoclonal antibodies.

Pharmacodynamic studies confirmed sustained and dose-dependent target inhibition. Starting at doses of 2 mg/kg or higher, there was inhibition of leukemia-cell Rho-GTPase activation within 24 hours of the initial cirmtuzumab dose. Phosphorylation of HS-1 was also inhibited upon initiation of cirmtuzumab. In keeping with plasma pharmacokinetics of cirmtuzumab, inhibition of leukemia-cell activation of pHS-1 was sustained four to six months after the final infusion of cirmtuzumab.

Following Cirmtuzumab treatment, 16 of 20 evaluable patients, including 9 of 10 evaluable patients treated at dose levels of 2 mg/kg or greater, had stable disease, despite having progressive disease upon study entry. Patients that discontinued cirmtuzumab early without meeting criteria for progressive disease were considered not evaluable, although they met criteria for stable disease at the time of discontinuation. While 4 patients met criteria for progressive disease, three received cirmtuzumab doses of 240 mcg/kg or less and 1 patient received 20 mg/kg. Patients did not require subsequent therapy for prolonged periods. The median time to requiring next treatment due to progressive disease was 259 days, which is similar to the time at which cirmtuzumab plasma levels become undetectable.

1.6 Rationale

Currently, the standard of care for patients with metastatic breast cancer is palliative anti-estrogen therapy for patients with ER-positive or PR-positive and Her2-negative breast cancer until the patient becomes refractory, at which time serial monotherapy chemotherapy agents are administered, as long as performance status remains adequate. For triple negative breast cancer patients, serial monotherapy chemotherapy, or combination chemotherapy regimens are administered depending upon how rapidly the disease is progressing. For all patients with metastatic breast cancer approximately 75% die within 5 years (40,000 patients annually) due to refractory disease[2]; therefore, new therapeutic approaches are needed. Paclitaxel's relatively low toxicity profile when given on a weekly basis makes it an ideal agent for combination drug studies like the one proposed. Based on the biological rationale behind cirmtuzumab and preclinical activity with paclitaxel, an open label, pilot phase Ib clinical trial is proposed to evaluate the safety of cirmtuzumab in combination with paclitaxel for the treatment of metastatic, or locally advanced, unresectable breast cancer.

Given the observation of clinical benefit and inhibition of ROR1 signaling at cirmtuzumab dose levels of 2 mg/kg or higher in a majority of chronic lymphocytic leukemia patients, the prolonged time to progression after discontinuation of 4 doses of cirmtuzumab, and the half-life of 32.4 days, the recommended fixed dose of 600 mg would translate to 8 mg/kg in an average woman and with dose reduction 4 mg/kg – dose levels above those for which disease stability were observed in a majority of chronic lymphocytic leukemia patients. Therefore 2 mg/kg is felt to be the minimum Biologically Active Dose, and is the lowest dose planned for evaluation in subsequent Cirmtuzumab trials. The 600 mg dose is therefore justified based on safety and biologic activity.

Standard imaging to assess therapeutic response to treatment for patients with metastatic breast cancer is by cross-sectional imaging of target lesions by CT or MRI. PET/CT imaging offers the potential to monitor for changes in the metabolic activity of metastatic foci. Currently it is not certain whether this information will have any predictive value for assessing outcome. As such, PET/CT cannot be used for tumor response assessment for investigational agents. However, by providing the option of Fluoro-deoxy-glucose PET/CT imaging at baseline and at 8-week intervals to patients treated on protocol, we can collect such information and assess how it relates to outcome in order to generate hypotheses for future prospective trials.

2.0 STUDY OBJECTIVES

2.1 Primary Objectives

1. To determine the safety and tolerability during the first 4 weeks of fixed dose cirmtuzumab when administered in combination with weekly standard of care paclitaxel to patients with metastatic, or locally advanced, unresectable breast cancer.

2.2 Secondary Objectives

1. To determine the safety and tolerability of cirmtuzumab in combination with paclitaxel by ongoing evaluation of adverse events (AE's) during treatment and follow up.
2. To assess clinical activity by evaluating objective tumor response and time to progression as defined by RECIST v1.1.
3. To measure ROR1 receptor density on primary tumor or metastatic tumor specimen and on putative cancer stem cell population and to correlate this with anti-tumor activity.

2.3 Exploratory Objectives

1. To assess the mechanism of action through pharmacokinetic and safety studies including
   - Circulating cirmtuzumab levels in peripheral blood
   - Level of circulating antibodies against cirmtuzumab
   - As available, we will perform pharmacodynamic, biochemical and molecular studies on tissue samples of treated patients, depending on sample availability.
   - Compare PET/CT imaging to standard cross-sectional imaging

2.4 Endpoints

Primary Endpoint:

- Safety as determined by the rate of dose-limiting toxicities during the first 4 weeks of treatment at a fixed dose of cirmtuzumab in combination with paclitaxel by recording adverse events (description, CTCAE v 4.03 grade, seriousness) at least possibly related to cirmtuzumab or to cirmtuzumab with paclitaxel.

Secondary Endpoints:

- Safety and tolerability of the combination therapy since the start of any study treatment.
- Objective tumor response rate, best tumor response rate, and time to progression as assessed by RECIST v1.1.
- ROR1 expression levels and putative breast cancer stem cell markers in tumor

pre-treatment tumor specimen measured by immunohistochemistry or flow cytometry

Exploratory Endpoints:

- Plasma pharmacokinetics of cirmtuzumab.
- Level of circulating antibody formation against cirmtuzumab.
- As available, we will perform pharmacodynamic biochemical and molecular studies on tissue samples of treated patients, depending on sample availability.
- Compare PET/CT imaging to standard cross-sectional imaging

3.0 PATIENT ELIGIBILITY

The protocol-specified eligibility criteria are designed to select subjects for whom study participation is considered appropriate. All relevant medical and non-medical conditions should be taken into consideration when deciding whether this clinical trial is suitable as a potential study subject. Prior to treatment, the Principal Investigator and the treating physician must agree that participation in this clinical trial is in the best interest of patient.

3.1 Inclusion Criteria

Subjects must meet all of the inclusion criteria to participate in this study.

1. Patient has the ability to understand and the willingness to sign a written informed consent.

2. Patients must have biopsy-confirmed, metastatic or locally advanced surgically unresectable, Her2 negative breast cancer. Her2 status should reflect the most recent biopsy results.

   Note: HER2 negative breast cancer is defined according to the American Society of Clinical Oncology (ASCO)/College of American Pathologists (CAP) guidelines 2013 for HER2 testing performed in a CLIA-certified laboratory.[16]

3. Patient should have ER/PR negative (<10% of cells staining for ER or PR) breast cancer or have ER/PR positive (≥10% of cells staining for ER or PR) breast cancer that has exhausted standard endocrine therapy and/or in the opinion of the treating oncologist, warrants cytotoxic chemotherapy.

4. Patient must have measurable disease as defined by RECIST v1.1. Measurable lesions will be confirmed by radiographic imaging (CT or MRI). Patients with bone only disease will be eligible if disease is considered measurable and a soft tissue component is present and can be biopsied.

5. Allowable types and amount of prior therapy:
   There is no limit to prior lines of therapy, but patients must not have received prior taxane chemotherapy in the metastatic setting.

6. Patient must be ≥ 18 years of age.

7. All genders, races and ethnic groups are eligible for this trial.

8. Patients must have ECOG Performance Status ≤ 2.

9. Patient has adequate organ function as defined below:

- Absolute Neutrophil Count ≥ 1.0 x 10^9/L
- Platelet count ≥ 100,000 /µl
- Hemoglobin ≥ 8.0 g/dL
- Total bilirubin ≤ 1.5 x upper limit of normal
- AST and ALT ≤ 3 x upper limit of normal
- Serum creatinine ≤ 2 x upper limit of normal OR
- Creatinine clearance > 40 ml/min/1.73 m^2

10. Women of child-bearing potential and male subjects who are sexually active with a woman of childbearing potential must agree to use adequate contraception (hormonal or barrier method of birth control; abstinence) prior to study entry, for the duration of study participation, and for at least 6 months following last infusion of cirmtuzumab. Should a woman become pregnant or suspect she is pregnant while participating in this study, she should inform her treating physician immediately.

11. Women of childbearing potential must have a negative pregnancy test prior to initiating study drug dosing.

12. Existing neuropathy must be no greater than Grade 1.

13. No concurrent antibody therapy can be planned with the exception of denosumab for use in bone metastasis.

14. CNS metastases are allowed as long as the metastases are asymptomatic, have been treated with radiation, and have been stable for > 6 weeks off steroids.

3.2 Exclusion Criteria

Subjects meeting any of the exclusion criteria at baseline will be excluded from study participation.

1. Patient is currently receiving chemotherapy or has received another chemotherapy within 5 half-lives, radiotherapy or immunotherapy within 2 weeks prior to study treatment initiation.

2. Patient has known, untreated and/or symptomatic central nervous system (CNS) metastases and/or carcinomatous meningitis.

3. Patient had disease that was refractory to paclitaxel in the neoadjuvant setting and/or developed metastatic breast cancer within 6 months of neoadjuvant or adjuvant taxane chemotherapy.

4. Patient has had major surgery within 3 weeks prior to enrollment.

5. Patient has severe and/or uncontrolled medical disease(s) (i.e., myocardial infarction within 6 months of study, CKD stage IV or above, severe chronic pulmonary disease or active infection).

6. The patient has known acute or chronic hepatitis B or C.

7. The patient has a history of allergic reactions attributed to compounds of similar chemical or biologic composition to paclitaxel.

8. The patient has a history of another malignancy within 2 years prior to study entry, except curatively treated non-melanotic skin cancer, cervical carcinoma in situ or stage I colon cancer.

9. Patient has a history of non-compliance or other medical illness that would preclude compliance with study procedures.

10. Patient has a known diagnosis of human immunodeficiency virus (HIV) infection.

11. Patient has severe cardiac insufficiency (NYHA III or IV) with uncontrolled and/or unstable cardiac or coronary artery disease

12. Patient is pregnant or nursing. There is a potential for congenital abnormalities and for this regimen to harm nursing infants.

4.0 TREATMENT PLAN

4.1 Study Drugs Dose Assignment

This is an open label, single institution, Phase Ib, study of fixed dose cirmtuzumab in combination with weekly paclitaxel in patients with metastatic, or locally advanced, unresectable breast cancer.

During the treatment period, cirmtuzumab and paclitaxel will be administered by intravenous (IV) infusion on an outpatient basis. Treatment cycles are 28 days in length. Cirmtuzumab will be administered IV with acetaminophen and cetirizine pre-medication (see below infusion plan) on day 1 and 15 of cycle 1, and on day 1 of each subsequent cycle. Paclitaxel will be administered after cirmtuzumab with diphenhydramine, dexamethasone, famotidine and anti-emetic medication as indicated on days 1, 8, 15 and 22 of each cycle per institutional standard and/or physician discretion.

The dose of cirmtuzumab will be 600 mg fixed dose (the full contents of two vials). Paclitaxel will be given at a dose of 80 mg/m^2.

REGIMEN DESCRIPTION					
Agent	**Pre-medications**	**Dose**	**Route**	**Schedule**	**Cycle Length**
Cirmtuzumab	acetaminophen 650 mg p.o.;cetirizine 10 mg p.o.	600mg	IV prior to paclitaxel	Cycle 1: Day 1, 15 Cycles 2 and beyond: Day 1	28 days

Paclitaxel	Per physician discretion or institutional standard, may include dexamethasone* 10 mg IV at least 30 minutes prior to paclitaxel; famotidine 20 mg IV; diphenhydramine 25 mg IV; and anti-emetic medication as indicated.	80 mg/m^2	IV at least 30 minutes after completion of cirmtuzumab	Days 1,8,15, 22	

**Note: additional dexamethasone may be given up to 24 hours prior to paclitaxel if indicated according to institutional practice.*

Replacement patients may be enrolled for the following:
Patients who withdraw prior to completing 2 cycles of study treatment for a reason that is definitely unrelated to toxicity of cirmtuzumab (*e.g.,* disease progression). Replacement patients will ensure enough safety data is obtained from patients having received 8 weeks of combination treatment in order for cirmtuzumab to potentially meet entry criteria as an agent for the I-SPY 2 clinical trial (NCT01042379).

4.2 Definition of Dose-Limiting Toxicity

For the purposes of defining the recommended phase 2 dose (RP2D), dose-limiting toxicities are based on events occurring during the first 28 days of treatment. The recommended dose for further study will be equal to or less than the MTD and will take into account any cumulative or delayed toxicity.

DLTs will be graded for severity based according to NCI Common Terminology Criteria for Adverse Events version 4.03 (NCI CTCAE v4.03).[17] If two of the first 5 patients experience dose-limiting toxicity, then further patients will be enrolled at a 50% dose reduction or 300 mg flat dose level.

Dose limiting toxicity is defined as a clinically significant adverse event that is considered by the investigator to be possibly, probably or definitely related to cirmtuzumab or the combination of cirmtuzumab with paclitaxel within 28 days of investigational treatment initiation:

- Grade 4 hematologic toxicity lasting > 7 days.
- Grade 3 thrombocytopenia lasting > 7 days or with bleeding requiring transfusion.
- Grade 3 neutropenia (ANC <1000 per mm^3) and documented infection or febrile neutropenia lasting > 7 days.
- Grade 3 diarrhea that lasts longer than 48 hours (despite intensive loperamide therapy).
- Grade 3 fatigue that lasts more than 1 week.
- Rash associated with pain not relieved with analgesia or rash causing disfigurement.
- Other Grade ≥ 3 non-hematologic toxicity

Exceptions:

- Grade 4 neutropenia lasting less than 8 days.
- Grade 3 infusion reaction.

4.3 Dose Reduction and safety stopping rule

Subjects will be enrolled in cohorts of five. If two or more of the first five patients experience dose-limiting toxicity (DLT) attributed to cirmtuzumab, then further patients will be enrolled at a 50% dose reduction (300 mg flat dose level); if two or more of the next five patients experience dose-limiting toxicity attributed to cirmtuzumab, then the study will be stopped. If fewer than two

of the first five patients experience dose-limiting toxicity attributed to cirmtuzumab, but two or more of the next five patients experience dose-limiting toxicity again, then the last five patients will also undergo dose reduction and be treated at the 300 mg dose level.

4.4 Pre-Medications

Patients will be pre-medicated with acetaminophen 650 mg p.o. and cetirizine 10 mg p.o. prior to cirmtuzumab infusion. Prior to paclitaxel infusion, patients be pre-medicated with dexamethasone 10 mg IV, diphenhydramine 25 mg IV, famotidine 20 mg IV, and an anti-emetic medication as indicated. Pre-medication may be changed according to institutional standards, patient allergies/intolerances, and/or physician discretion. Dexamethasone injection will be given at least 30 minutes prior to start of paclitaxel infusion.

4.5 Study Drugs Infusion

4.5.1 Cirmtuzumab infusion plan

- A qualified person (eg, nurse with experience in monitoring the administration of therapeutic agents used in patients with cancer) will be responsible for infusing cirmtuzumab.
- Using an infusion pump, cirmtuzumab should be administered via a polyvinyl chloride (PVC) administration set and through a 0.2 µm in-line filter. The IV line should be flushed with 0.9% Sodium Chloride Injection, USP, before and after each dose.
- The initial cirmtuzumab infusion will be administered over a planned infusion time of ~90 minutes, with 20% of the total dose to be administered in the first ~30 minutes and the remaining 80% of the total protein dose to be administered in the subsequent ~60 minutes. For subjects who prove able to tolerate infusions without infusion-related toxicity, subsequent infusions may be administered over a minimum time of ~30 minutes. Infusion times may be extended as necessary to accommodate individual subject tolerance of treatment.

4.5.2 Paclitaxel infusion

- If cirmtuzumab is given on that day, once cirmtuzumab infusion has completed, the IV line must be flushed and then pre-medication for paclitaxel can begin.
- Patients must be pre-medicated with dexamethasone 10 mg IV at least 30 minutes prior to start of paclitaxel infusion. Patients might have received additional dexamethasone within 24 hours priors to paclitaxel if indicated according to institutional practice.
- Patients will also receive diphenhydramine 25 mg IV, famotidine 20 mg IV, and anti-emetic medication as indicated.
- Paclitaxel 80 mg/m^2 in 250cc D5W will be run over one hour (+/-10 minutes) or as tolerated according to institutional standards.

4.6 Permitted Concomitant Therapy

- All concomitant medications and blood products, as well as interventions received by patients from the first dose of study drugs until the end of study visit should be recorded.

- Palliative and supportive care for disease-related symptoms, including pain medications and antiemetics, are permitted. Hematopoietic white blood cell growth factors such as filgrastim are allowed, if clinically indicated, according to institutional standards.

- Patients may continue bone anti-resorptive agents if started prior to study treatment.

4.7 Prohibited Concomitant Therapy

- Use of anti-neoplastic or anti-tumor agents not part of the study therapy, including chemotherapy, radiation therapy, immunotherapy, and hormonal anticancer therapy, are not permitted while participating in this study.

- Use of concurrent investigational agents is not permitted.

4.8 Toxicities and Dosing Delays/Dose Modifications

Any patient who receives treatment on this protocol will be evaluable for toxicity. Each patient will be assessed for the development of toxicity according to the NCI Common Toxicity Criteria for Adverse Events (CTCAE), version 4.03. Dose adjustments should be made according to the system showing the greatest degree of toxicity.

Dose reduction or deferral of cirmtuzumab and/or paclitaxel administration for toxicity may take place at any time during the study. Dose reductions for paclitaxel will be made according to the product label and institutional standards. Patients requiring dose reductions of cirmtuzumab will be dose reduced according to.Table 4-1.

Table 4-1. Cirmtuzumab dose reduction scheme

Cirmtuzumab Dose-Reduction		
	Dose	**Dose Reduction**
	600 mg	300 mg

Patients experiencing a DLT attributed to cirmtuzumab will have their dose lowered unless, in the interest of the patient, the treating investigator recommends study discontinuation.

Patients who discontinue paclitaxel due to unacceptable toxicity attributed to paclitaxel may continue receiving cirmtuzumab in the absence of disease progression and cirmtuzumab-related unacceptable toxicity.

Patients who discontinue cirmtuzumab due to unacceptable toxicity attributed to cirmtuzumab may continue receiving paclitaxel in the absence of disease progression and paclitaxel-related unacceptable toxicity.

4.8.1 Dosage Modification Criteria and Guidelines for Management of Cirmtuzumab Related Toxicities

NCI-CTCAE (v4.03) Grade	Cirmtuzumab Dose Modification	Guideline for Management
Diarrhea		
Grade 1	None	Consider loperamide (4 mg at first onset, followed by 2 mg q 2–4 hours until free of diarrhea for 12 hours)

NCI-CTCAE (v4.03) Grade	Cirmtuzumab Dose Modification	Guideline for Management
Grade 2	None (Dose reduction of cirmtuzumab is necessary if diarrhea persists over 48–72 hours despite optimal medical management)	Loperamide (4 mg at first onset, followed by 2 mg q 2–4 hours until diarrhea free for 12 hours)
Grade 3	• Defer then dose reduce cirmtuzumab. Cirmtuzumab should not be re-escalated. • If diarrhea persists over 48 hours despite optimal medical management during the first 28 days, discontinue study treatment.	Defer cirmtuzumab and paclitaxel administration until resolution to Grade ≤1, and restart at reduced cirmtuzumab dose
Grade 4	Discontinue study treatment.	
Pulmonary Events (if possibly Interstitial Lung Disease)		
All Grades	Temporarily defer cirmtuzumab and paclitaxel administration pending diagnostic evaluation. If the pulmonary adverse event is assessed as possibly, probably, or definitely related to cirmtuzumab, discontinue the patient from study treatment.	Unexplained dyspnea, either new or progressive, should be aggressively evaluated.
Rash		
Grade 1 and 2 Tolerable rash	None	Any of the following: oral antibiotics (tetracycline, minocycline, doxycycline) topical clindamycin, diphenhydramine, topical or oral corticosteroids at discretion of investigator
Grade 3 Intolerable rash	Consider deferral and/or dose reduction if unresponsive to symptomatic management. Re-escalation is allowed.	Manage as described above
Grade 4	Discontinue study treatment.	
Hepatotoxicity		
AST/ALT Grade 3 (>5.0 – 10.0 x ULN)	Defer cirmtuzumab and paclitaxel then dose reduce cirmtuzumab; cirmtuzumab should not be re-escalated.	Defer cirmtuzumab and paclitaxel until resolution to Grade ≤1, and restart at reduced dose

NCI-CTCAE (v4.03) Grade	Cirmtuzumab Dose Modification	Guideline for Management
Bilirubin > 1.5 X ULN	Defer cirmtuzumab and paclitaxel, unless patient has history of Gilbert's syndrome in which case defer cirmtuzumab and paclitaxel if bilirubin >2.0 X ULN.Reduce dose of cirmtuzumab for all other patients.	Defer cirmtuzumab and paclitaxel until resolution to < 1.25 X ULN and restart cirmtuzumab at reduced dose. If patient has history of Gilbert's syndrome, defer cirmtuzumab and paclitaxel until resolution to ≤2.0 X ULN.
Hematologic		
ANC <1000/mm^3 Grade 3; Hemoglobin < 8 gm/dL Grade 3;	Defer cirmtuzumab and paclitaxel administration until resolution to Grade ≤2 (filgrastim may be used per investigator discretion), and restart at same dose.	Defer cirmtuzumab and paclitaxel administration until resolution to Grade ≤2, and restart at same dose cirmtuzumab. If occurs a second time, cirmtuzumab will be reduced to reduced dose level.
Platelet count < 75,000/uL Grade 2	Defer cirmtuzumab and paclitaxel administration until resolution to ≤ Grade 1 (≥75,000/uL); restart cirmtuzumab without dose modification	Defer cirmtuzumab and paclitaxel administration until resolution to ≤ Grade 1, restart cirmtuzumab at full dose. When paclitaxel only is administered and platelet count is <75,000/uL, defer paclitaxel and resume the following week if platelet count is ≥75,000/uL
Other Non-hematologic		
Grade ≥ 3	First occurrence: Defer cirmtuzumab and paclitaxel then dose reduce cirmtuzumab; Re-escalation per discretion of Principal Investigator. Second occurrence: discontinue treatment.	Defer cirmtuzumab and paclitaxel administration until resolution to Grade ≤1, and restart at reduced dose. Re-escalation per discretion of Principal Investigator. Second occurrence: Discontinue treatment.

Notes:

1. If any of the above are noted, the patient will be re-assessed within 4 days by the treating physician or co-investigator. Isolated laboratory abnormalities may be re-evaluated by laboratory draw only, or based on physician or investigator discretion.
2. Cirmtuzumab infusions can be delayed for a maximum of 28 days; if not restarted in that time-span, cirmtuzumab study treatment will be permanently discontinued.
3. If dose is decreased for Grade 3 or 4 toxicity, dose re-escalation is not planned except as noted above.
4. For unanticipated invasive procedures, cirmtuzumab infusions can be delayed for a maximum of 28 days; if not restarted in that time-span, cirmtuzuamb study treatment will be permanently discontinued.

5. Doses of cirmtuzumab and paclitaxel will be deferred until improvement in toxicity is seen to allow patient to receive all treatment. Study procedures will be deferred and repeated the following week to allow patients to complete the full treatment regimen.
6. If doses of cirmtuzumab are delayed by 8 days for issues other than toxicity due to study treatment, the same cirmtuzumab dose will be repeated 2 weeks after resumption of cirmtuzumab and then continued every 4 weeks. The purpose of the additional two-week “loading dose” is to optimize pharmacokinetic levels of cirmtuzumab.

4.8.2 Dosage Modification Criteria and Guidelines for Management of Paclitaxel Related Toxicities

Dose modification for paclitaxel will occur according to the product label and institutional standards.

For Grade ≥ 2 rash, allergy, or infusion reaction, ABRAXANE® for Injectable Suspension (paclitaxel protein-bound particles for injectable suspension) (albumin-bound) 100 mg/m2 per week may be substituted for paclitaxel according to investigator discretion. Any premedications are at the discretion of the investigator.

4.8.3 Modifications for Infusion Reactions

Patients will be monitored for the presence of infusion-related reactions. Treatment for infusion-related reactions will be managed according to the following guidelines:

Infusion Reaction	Occurrence	Management Guidelines
Grade ≤ 2	any	Institute supportive measures. See section 4.8.2 for option to switch to Abraxane per investigator discretion. When resolved, resume study treatment at same dose level.
Grade 3	1st	Institute supportive measures. See section 4.8.2 for option to switch to Abraxane per investigator discretion. When resolved, resume study treatment at the same dose level. Pre-medications listed in protocol are mandatory for re-challenge.
	2nd	Institute supportive measures. Permanently discontinue study treatment.
Grade 4	any	Institute institutional procedures for adult medication reaction/anaphylactic response. Permanently discontinue study treatment.

4.9 Duration of Study Treatment

In the absence of treatment delays due to adverse events, treatment with cirmtuzumab and

paclitaxel combination therapy may continue until one of the following, whichever occurs first:

- Disease progression unless the patient is receiving clinical benefit based on physician judgement,
- Inter-current illness that prevents further administration of treatment,
- Unacceptable adverse event(s),
- Patient decides to withdraw from study treatment, OR
- General or specific changes in the patient's condition render the patient unacceptable for further treatment in the judgment of the investigator.

Note: During the study treatment period, if one treatment agent is discontinued due to unacceptable toxicity, the patient may continue receiving the other treatment agent in the absence of disease progression. The patient is considered on study treatment until both drugs are discontinued for toxicity or disease progression. If continuing Cirmtuzumab alone then labs and AE assessments should be done within one day of treatment. If continuing paclitaxel alone then labs and AE assessments should be done within one day of the planned weekly treatment.

4.10 Duration of Follow Up

Patients will be contacted to document any adverse events 28 days (+/- 7 days) after the End of Treatment visit (56 days after the last dose of cirmtuzumab or paclitaxel treatment) or at the time of initiation of a different anti-cancer therapy, whichever occurs first. In the event that the start of a different anti-cancer therapy occurs earlier than 28 +/- 3 days, the End of Treatment visit and the Followup Visit will be the same visit. Patients removed from treatment for unacceptable adverse events will be followed until resolution or stabilization of the adverse event.

4.11 Discontinuation from Study Participation

Patients may be removed from study participation for the following reasons:

- The patient or legal representative withdraws consent for treatment.
- The patient or legal representative withdraws consent (termination of treatment and follow-up).
- The patient has documented disease progression.
- The patient experiences clinically significant deterioration of their condition prior to treatment discontinuation.
- The patient is non-compliant.
- The patient is lost to follow-up.
- The patient dies.
- The investigator determines that it is not in the patient's best interest to continue participation.
- The patient becomes pregnant.
- The patient develops a second malignancy (except for basal cell carcinoma or squamous cell carcinoma of the skin) that requires treatment which would interfere with the study

5.0 STUDY PROCEDURES

Refer to the study Schedule of Events for procedures.

All patients will be closely monitored for safety and tolerability during all cycles of therapy, at study treatment completion/early study treatment discontinuation, and during the follow-up period.

5.1 Definitions of Study Assessments

5.1.1 Medical history and symptoms

Medical/Oncology history should include smoking history, surgeries, clinically significant diseases within the last 5 years, history of malignancy (date of first diagnosis, disease course, and prior therapies), and all medications per 5.1.4.

5.1.2 Demographics

Demographics consist of age, gender, race, and ethnicity.

5.1.3 Review subject eligibility criteria

Review of eligibility criteria as described in Section 3 to ensure subject qualification for study entry.

5.1.4 Concomitant medications

All concomitant therapy, including anesthetic agents, vitamins, homeopathic/herbal remedies, nutritional supplements, received by patients from five days prior to the first day of study treatment until 28 days after the last study dose (or until the start of a new treatment, whichever comes first) will be recorded in the patient's medical record. If a reportable adverse event deemed related to study intervention (see Section 7) occurs within 28 days after last study dose and the patient has not started a new treatment, recording of concomitant medications related to the treatment of that adverse event should continue until resolution of the adverse event.

5.1.5 Physical exam

A complete physical examination should include the evaluation of general appearance; evaluation of head, eyes, ears, nose, and throat (HEENT); and cardiovascular, pulmonary, abdominal, musculoskeletal, skin, lymph nodes, neurological, and genitourinary systems. Subsequent exams may be targeted as appropriate.

Changes from baseline abnormalities should be recorded at each subsequent physical examination. New or worsened abnormalities should be recorded as adverse events if clinically significant.

5.1.6 Vital signs and height

Vital signs should include temperature, pulse, and blood pressure and weight. Height will only be collected at screening.

5.1.7 Performance status

Performance status will be evaluated according the ECOG criteria listed in Appendix A.

5.1.8 Adverse event assessment

Baseline assessment of subject status for determining adverse events. See Section 7 for Adverse Event monitoring and reporting.

5.1.9 CBC and differential

Complete Blood Count (CBC) including hemoglobin, hematocrit, platelet count, white blood cell count, and percentage or absolute differential count.

5.1.10 CMP

Serum chemistry panel includes comprehensive metabolic panel (sodium, potassium, chloride, bicarbonate, BUN, creatinine, glucose, calcium, total protein, albumin, AST, ALT, total bilirubin, and alkaline phosphatase).

5.1.11 Coagulation

Coagulation profile includes International Normalized Ratio (INR) or prothrombin time (PT).

5.1.12 Blood draw for pharmacokinetic (PK) and correlative studies

Blood samples (up to 20 ml) for pharmacokinetic evaluation will be collected in the appropriate tubes.

See Section 5.5 for additional details.

5.1.13 Pregnancy test (for females of child bearing potential)

All women of childbearing potential will have a serum or urine pregnancy test at screening.

5.1.14 Tumor assessment

Assessment of radiographic response will occur according to the Response Evaluation Criteria in Solid Tumors (RECIST) v1.1 from the National Cancer Institute listed in Appendix B.

5.1.15 Tumor tissue or fluid sample collection

Tumor tissue will be collected by an image-directed core needle biopsy that is not collected from a site close to vital structures. Tumor cells from malignant (peritoneal) ascites, pleural effusion, or other body fluid may also be collected.

See Section 5.5 for additional details.

5.2 Screening/Baseline Procedures

Assessments performed exclusively to determine eligibility for this study will be done only after obtaining informed consent. Assessments performed for clinical indications (not exclusively to determine study eligibility) may be used for baseline values even if the studies were done before informed consent was obtained.

The study population for this trial will consist of men and women with biopsy proven Her2 negative, locally advanced, unresectable or metastatic breast cancer. Measurable lesions will be confirmed by radiological scans (CT or MRI). All patients who meet the inclusion and exclusion criteria, and the Principal Investigator and treating physician determines the trial to be in the best interest of patient, will be offered enrollment into this study. To confirm patient eligibility for study participation, the following assessments and procedures will be completed during the screening phase.

All screening procedures must be performed within 14 days prior to registration unless otherwise stated. The screening procedures include:

- Written informed consent *(within 30 days prior to registration)*.
- Review of inclusion and exclusion criteria.

- Complete medical/oncology history.
- Documentation of concomitant medications.
- Demographics.
- Complete physical examination.
- Vital signs and height.
- ECOG performance status assessment.
- CBC with differential, CMP, and INR or PT.
  Note: if blood samples are taken within 5 days of the first dose of study treatment, Cycle 1 Day 1 samples do not need to be re-collected.
- Pregnancy test.
- Documentation of tumor status per RECIST *(tumor assessment within 30 days prior to Cycle 1 Day 1)*.
- Tumor assessment by physical exam, for patients whose disease can be evaluated by physical exam.
- Chest/abdomen/pelvis imaging with CT or MRI *(within 30 days prior to Cycle 1 Day 1).*
- Bone scan if clinically indicated *(recommended within 30 days prior to Cycle 1 Day 1 if possible).*
- Blood draw for correlative studies, and if patient consents, tumor biopsy *(within 30 days prior to Cycle 1 Day 1)*.
- Optional PET/CT for correlation with cross-sectional imaging

5.3 Procedures During Treatment

During the treatment period, a window of +/- 1 day will apply to all visits and assessments, unless otherwise specified. Not all procedures need to be completed on the same day provided they are completed within the specified window. Assessments scheduled on the day of study drug administration should be performed prior to study drug administration, unless otherwise noted. Cirmtuzumab and paclitaxel administration may be delayed due to unforeseen scheduling issues but should not be delayed more than 5 days.

Cycle 1 Day 1 (+/- 1 day)

- Physical exam, vital signs
- History and symptoms
- ECOG performance status
- Blood collection for CBC, CMP and PK/correlative studies
- Tumor assessment by physical exam, for patients whose disease can be evaluated by physical exam.
- Cirmtuzumab and paclitaxel administration
- Concomitant medication recording
- Adverse events screening

Cycle 1 Day 8 (+/- 1 day)

- Vital signs
- Blood collection for CBC,CMP and PK/correlative studies
- Paclitaxel administration
- Concomitant medication recording
- Adverse events screening

Cycle 1 Day 15 (+/- 1 day)

- Physical exam, vital signs
- History and symptoms
- Blood collection for CBC, CMP and PK/correlative studies
- Cirmtuzumab and paclitaxel administration
- Concomitant medications recording
- Adverse events screening

Cycle 1 Day 22 (+/- 1 day)

- Vital signs
- Blood collection for CBC, CMP and PK/correlative studies
- Paclitaxel administration
- Concomitant medication recording
- Adverse events screening

Cycles 2 and beyond (Combination Therapy phase) , Day 1 (+/- 1 day)

- Physical exam, vital signs
- History and symptoms
- ECOG performance status
- Blood collection for CBC, CMP and PK/correlative studiesTumor assessment by physical exam, for patients whose disease can be evaluated by physical exam.
- Cirmtuzumab and paclitaxel administration
- Concomitant medication recording
- Adverse events screening

Cycles 2 and beyond, Day 8 (+/- 1 day)

- Vital signs
- Blood collection for CBC and CMP
- Paclitaxel administration
- Concomitant medication recordingAdverse events screening

Cycles 2 and beyond, Day 15 (+/- 1 day)

- Vital signs
- History and symptoms
- Blood collection for CBC and CMP
- Paclitaxel administration
- Concomitant medication recording
- Adverse events screening

(NOTE: Physical exam will not be performed at Day 15 for Cycles 7 and beyond.)

Cycles 2 and beyond, Day 22 (+/- 1 day)

- Vital signs
- Blood collection for CBC and CMP
- Paclitaxel administration
- Concomitant medication recording
- Adverse events screening

Assess Response [Within 7 days of Day 1 of Cycles 3, 5, and 7. Beyond Cycle 7, scans may be decreased to every 3 cycles (i.e., within 7 days of Day 1 of Cycles 10, 13, etc.)]

- Chest/abdomen/pelvis imaging by CT or MRI
- Bone scan if clinically indicated
- Optional PET/CT scan

Monotherapy phase Day 1 (+/- 1 day)

- Physical exam, vital signs
- History and symptoms
- ECOG performance status
- Blood collection for CBC, CMP and PK/correlative studies. Tumor assessment by physical exam, for patients whose disease can be evaluated by physical exam.
- Cirmtuzumab and paclitaxel administration
- Concomitant medication recording
- Adverse events screening

Monotherapy phase Day 8 (+/- 1 day)

- Vital signs
- Blood collection for CBC and CMP
- Paclitaxel administration
- Concomitant medication recording
- Adverse events screening

(NOTE: For patients taking Cirmtuzumab monotherapy there will be no Day 8 visit.)

Monotherapy phase Day 15 (+/- 1 day)

- Vital signs
- History and symptoms
- Blood collection for CBC and CMP
- Paclitaxel administration
- Concomitant medication recording
- Adverse events screening

(NOTE: Physical exam will not be performed at Day 15 during the monotherapy phase . For patients taking Cirmtuzumab monotherapy there will be no Day 15 visit.)

Monotherapy phase Day 22 (+/- 1 day)

- Vital signs
- Blood collection for CBC and CMP
- Paclitaxel administration
- Concomitant medication recording

Adverse events screening(NOTE: For patients taking Cirmtuzumab monotherapy there will be no Day 22 visit.)

End of Treatment Visit ***(28 days (+/-1) after last dose of cirmtuzumab or paclitaxel) or at the time of initiation of a different anti-cancer therapy, whichever occurs earlier. PK/correlative studies blood sample may be drawn at 28 days or, if not feasible, at the start of new anti-cancer therapy if new therapy starts before 28 days***

- Physical exam, vital signs
- ECOG performance status
- Blood collection for CBC, CMP and PK/correlative studies

- Blood collection for correlative studies
- Repeat tumor sample collection at the time of progression, if safe and patient consents.
- Tumor assessment by physical exam, for patients whose disease can be evaluated by physical exam.
- Concomitant medication recording
- Adverse events screening

5.4 Follow-up Procedures *(56 days (+/-7) after last dose of cirmtuzumab or paclitaxel)*

Patients will be contacted by telephone 28 days (+/- 7 days) after their End of Treatment Visit to determine if any serious or non-serious adverse events have occurred, and to document any concomitant cancer therapy. Follow up procedures will occur at 56 days +/- 7 days after last dose of cirmtuzumab or paclitaxel or at the time of starting different anti-cancer therapy, whichever occurs first. In the event that the start of a different anti-cancer therapy occurs earlier than 28 days, the End of Treatment visit and the Followup Visit will be the same visit.

5.5 Correlative Studies

5.5.1 Blood Sample Collection Guidelines for PK and Correlative Studies

Blood Sampling Schedule

Serum samples for pharmacokinetic analyses and research will be collected as outlined in **Table 5-1.**

Table 5-1. Blood PK and Correlative sampling schedule

Cycle	Day	Time point
1	**1**	• Prior to cirmtuzumab infusion • 30 minutes (+/- 5 minutes) post completion of cirmtuzumab infusion • immediately post completion of paclitaxel infusion
	8	• Prior to paclitaxel infusion
	15	• Prior to cirmtuzumab infusion • 30 minutes (+/- 5 minutes) post completion of cirmtuzumab infusion • immediately post completion of paclitaxel infusion
	22	• Prior to paclitaxel infusion
2 and beyond		• Prior to any cirmtuzumab infusion
End of Treatment and/or Progression visit		28 (+/- 3) days after last dose of cirmtuzumab

Blood Collection and Handling Instructions

Blood will be collected using the appropriate tubes (up to 10ml). The exact time that the sample is drawn along with the exact time that the drug is administered should be recorded.

Sample Processing

Blood samples will be processed in the translational lab of Dr. Thomas Kipps.

Sample Labeling
Each tube must be labeled with the patient's study number and the date and time the sample was drawn. Data should be recorded on the Correlative Study Form, which must accompany the sample(s).

5.5.2 Tumor and Malignant Ascites / Pleural Effusion Sample Collection Guidelines

When feasible, tumor samples will be collected by image-directed core needle biopsy, or by recovering tumor cells from malignant ascites, pleural effusion or body fluid at the following time points:

- Within 30 days prior to Cycle 1 Day 1
- Between Cycle 3, Day 1 and Day 15
- At the time of progression

In addition, samples from pleural or peritoneal fluid may be obtained from patients who have repeated pleural taps, pleural drainage, or peritoneal taps for palliation while receiving protocol therapy and who do not meet progressive disease criteria. These sample collections may occur at timepoints other than the three timepoints stated above and while the patient is on study or in follow-up per the protocol.

If baseline biopsy not feasible, paraffin block material from the patient's prior metastatic biopsy (or biopsies) and/or from the primary tumor at diagnosis may be obtained for ROR1 correlative studies as in Section 5.5.5.

When biopsies are performed, at least three cores should be obtained. One core will be placed in formalin, and the other two cores will be placed in shipping material without fixation. These samples are to be sent to the Pathology Core at UCSD, 3855 Health Sciences Drive, #5314, at room temperature.

5.5.3 Specimen Banking

Patient samples collected for this study will be retained at the UCSD School of Medicine (Kipps Laboratory). Specimens will be stored indefinitely or until they are exhausted. If future use is denied or withdrawn by the patient, best efforts will be made to stop any additional studies and to destroy the specimens. Samples will be labeled with the subject's de-identified study number and collection date. The link between study number and medical record number will be viewed over a password secured encrypted server-client.

Drs. Parker and Shatsky, in collaboration with Dr. Kipps, will be responsible for reviewing and approving requests for research specimens from potential research collaborators outside of UCSD. Collaborators will be required to complete an agreement (a Material Transfer Agreement or recharge agreement) that states specimens will only be released for use in disclosed research. Any data obtained from the use of clinical specimens will be the property of UCSD for publication and any licensing agreement will be strictly adhered to.

The study research coordinator will review the subject's medical record for demographic and clinical information pertaining to the subject's general medical history, diagnosis, and outcomes of any treatments received. Samples and data extracted from the subject's medical record will be coded with a de-identified study number, and the subject's name and identifying information will be removed. A log that links the subject's name and identifiers to the study number will be

maintained in a secure database distinct from the secure database into which the subject's clinical information will be entered

The specimens and their derivatives may have significant therapeutic or commercial value. The Informed Consent form contains this information and informs the subject that there is the potential for financial gain by UCSD, the investigator or a collaborating researcher or entity.

The following information obtained from the subject's medical record may be provided to research collaborators when specimens are made available:

- Diagnosis
- Collection time in relation to study treatment
- Clinical outcome – if available
- Demographic data

5.5.4 Assay methodologies

1. Cirmtuzumab and anti-cirmtuzumab antibody levels in peripheral blood will be monitored.

2. We will examine tumor cells for expression of ROR1 and other stem cell markers, such as ALDH and CD133 by immunohistochemistry or flow cytometry.

3. Functional assays will be performed if enough tumor specimens are available. These include assays on the relative capacity of tumor cells to form spheroids or engraft immune deficient mice.

5.6 Patient Discontinuation

Patients may discontinue study treatment at any time. Any patient who discontinues treatment will be encouraged to return to the study center to undergo treatment discontinuation assessments. The primary reason for discontinuation should be recorded. Reasons for discontinuation of a patient by the investigator include, but are not limited to, the following:

- Voluntary withdrawal from treatment (follow-up permitted)
- Voluntary withdrawal of consent (termination of treatment and follow-up)
- Documented disease progression
- Clinically significant deterioration of the patient’s condition prior to treatment discontinuation
- Patient noncompliance
- Investigator determination that it is not in the patient’s best interest to continue participation
- Pregnancy
- Development of second malignancy (except for basal cell carcinoma or squamous cell carcinoma of the skin) that requires treatment which would interfere with the study
- Lost to follow-up
- Death

6.0 MEASUREMENT OF EFFECT

6.1 Safety/tolerability

Analyses will be performed for all patients having received at least one dose of study drug. The study will use the CTCAE version 4.03 for reporting of adverse events.[16]

6.2 Antitumor Effect- Solid Tumors

Response and progression will be evaluated using Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1 guidelines.[18]

6.2.1 Best Overall Response

The best overall response is the best response recorded from the start of the study treatment until the end of treatment taking into account any requirement for confirmation.

6.2.2 Progression-Free Survival

Progression-free survival (PFS) is defined as the duration of time from start of study treatment until objective tumor progression or death.

6.2.3 Time to Progression

Time to progression is defined as the duration of time from start of study treatment until objective tumor progression.

7.0 ADVERSE EVENTS

An adverse event (AE) is any untoward medical occurrence in a patient receiving study treatment and which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including a clinically significant abnormal laboratory finding), symptom, or disease temporally associated with the use of an experimental intervention, whether or not related to the intervention.

Progression of the cancer under study or events, which are unequivocally due to disease progression should not be reported as an AE during the study (unless it is considered to be drug related by the investigator).

7.1 Adverse Event Monitoring

Adverse event data collection and reporting, which are required as part of every clinical trial, are done to ensure the safety of subjects enrolled in the studies as well as those who will enroll in future studies using similar agents. Adverse events are reported in a routine manner at scheduled times during a trial. Additionally, certain adverse events must be reported in an expedited manner to allow for optimal monitoring of patient safety and care.

As far as possible, each adverse event should be evaluated to determine:

- duration (start and end dates)
- severity (grade)
- seriousness
- relationship to study agent
- action taken (i.e., none, study agent modification, medical intervention)
- outcome (i.e., resolved without sequelae, resolved with sequelae, ongoing)

Adverse events monitoring begins after initiation of study procedures and ends 30 days following the last administration of study treatment or start of new anti-cancer therapy, whichever occurs earlier.

All patients experiencing an adverse event, regardless of its relationship to study drug or at least possibly related to the drug, will be monitored until:

- the adverse event resolves or the symptoms or signs that constitute the adverse event return to baseline;
- any clinically significant abnormal laboratory values have returned to baseline;
- there is a satisfactory explanation other than the study drug for the changes observed; or
- death.

7.2 Severity

All adverse events will be graded according to the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.03.[16] The CTCAE v4.03 is available at http://ctep.cancer.gov/reporting/ctc.html

If no CTCAE grading is available, the severity of an AE is graded as follows:

Mild (grade 1): the event causes discomfort without disruption of normal daily activities.
Moderate (grade 2): the event causes discomfort that affects normal daily activities.
Severe (grade 3): the event makes the patient unable to perform normal daily activities or significantly affects his/her clinical status.
Life-threatening (grade 4): the patient was at risk of death at the time of the event.
Fatal (grade 5): the event caused death.

7.3 Seriousness

A “serious” adverse event is defined in regulatory terminology as any untoward medical occurrence that:

1. **Results in death.**
   If death results from (progression of) the disease, the disease should be reported as event (SAE) itself.
2. **Is life-threatening.**
   The patient was at risk of death at the time of the event; it does not refer to an event that hypothetically might have caused death if it were more severe.
3. **Requires in-patient hospitalization or prolongation of existing hospitalization.**
   Note: Hospitalization (including hospitalization for an elective procedure) for a pre-existing condition, which has not worsened does not constitute a serious adverse event.
4. **Results in persistent or significant disability or incapacity.**
5. **Is a congenital anomaly/birth defect**
6. **Is an important medical event**
   Any event that does not meet the above criteria, but that in the judgment of the investigator jeopardizes the patient, may be considered for reporting as a serious adverse event. The event may require medical or surgical intervention to prevent one of the outcomes listed in the definition of “Serious Adverse Event“.
   For example: allergic bronchospasm requiring intensive treatment in an emergency room or at home; convulsions that may not result in hospitalization; development of drug abuse or drug dependency.

7.4 Relationship

Attribution categories for adverse events in relationship to protocol therapy are as follows:
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.

7.5 Prior experience

Expected events are those that have been previously identified as resulting from administration of the agent. An adverse event is considered unexpected, for expedited reporting purposes only, when either the type of event or the severity of the event is not listed in the current known adverse events listed in the agent clinical experience section of this protocol or the current Investigator's Brochure.

7.6 Reporting Requirements for Adverse Events

7.6.1 Expedited Reporting

A. The **Principal Investigator** must be notified within 24 hours of learning of any serious adverse events, regardless of attribution, occurring during the study or within 30 days of the last administration of the study drug.

B. The **UCSD Human Research Protections Program (HRPP)** and **Moores Cancer Center Data and Safety Monitoring Board (DSMB)** must be notified within 10 business days of "any unanticipated problems involving risk to subjects or others" (UPR).

The following events meet the definition of UPR:

1. Any serious event (injuries, side effects, deaths or other problems), which in the opinion of the Principal Investigator was unanticipated, involved risk to subjects or others, and was possibly related to the research procedures.
2. Any serious accidental or unintentional change to the IRB-approved protocol that alters the level of risk.
3. Any deviation from the protocol taken without prior IRB review to eliminate apparent immediate hazard to a research subject.
4. Any new information (e.g., publication, safety monitoring report, updated sponsor safety report), interim result or other finding that indicates an unexpected change to the risk/benefit ratio for the research.
5. Any breach in confidentiality that may involve risk to the subject or others.
6. Any complaint of a subject that indicates an unanticipated risk or that cannot be resolved by the Principal Investigator.

C. The **FDA** must be notified according to the following timelines:

- Within 7 calendar days of any unexpected fatal or life-threatening adverse event with possible relationship to study drug, and
- Within 15 calendar days of any event that is considered: 1) serious, 2) unexpected, and 3) at least possibly related to study participation.

7.6.2 Routine Reporting Requirements

A. The **UCSD HRPP** must be notified of any adverse events that are not unanticipated problems involving risk to subjects or others (non-UPRs) at the time of the annual Continuing Review.

B. The **FDA** must be notified of all non-serious adverse events annually at the time of the annual report.

8.0 AGENT INFORMATION

8.1 Cirmtuzumab

Please refer to Investigator's Brochure for more comprehensive information.

Other names for the drug: UC-961

Structure: Monoclonal antibody

Mechanism of action (or Product description):
Cirmtuzumab is a fully-humanized monoclonal antibody designed to bind the extracellular immunoglobulin-like domain of ROR1 with high-affinity.

Supplied by: Cirmtuzumab has been produced at Pacific GMP (San Diego, CA) and Oncternal Therapeutics, Inc. in compliance with cGMP standards.

Formulation:
Cirmtuzumab supplied by Pacific GMP is a liquid formulation with a concentration of 44 mg/ml protein, with 7.2 to 7.8 mL/vial. Each ml of the formulation contains 40 mg cirmtuzumab, 14.7 mg sodium citrate, 10 mg trehalose, 216 µg Polysorbate 80, 15 µg sodium EDTA and adjusted to pH 5.2 ± 0.2. Each vial is considered to deliver approximately 300mg of cirmtuzumab.

Cirmtuzumab manufactured by Oncternal Therapeutics, Inc. is supplied in vials nominally containing 300 mg of drug substance in 7.5 mL of diluent (40 mg/mL). Each milliliter of drug product contains: 14.7 mg of sodium citrate, 10 mg of trehalose, 216 µg of polysorbate 80, and 15 µg of sodium EDTA.

Cirmtuzumab is filled into 10ml clear borosilicate Type 1 glass vials with 20 mm grey butyl siliconized rubber stopper and 20 mm aluminum royal blue flip off seals.

Storage:
Cirmtuzumab is to be kept refrigerated (2-8°C) until use, in single use vials.

Solution Preparation:
To prepare the agent for administration, it will be dissolved in normal saline to a final concentration of 1 mg/mL.

Administration:
The drug will be administered by intravenous infusion per infusion plan outlined in Section 4.1.

Side effects:
Common adverse events associated with cirmtuzumab administration have not yet been determined. In the phase I trial of cirmtuzumab in CLL, no adverse events have been probably or definitely related to cirmtuzumab at doses between 15 µg/kg and 20 mg/kg.

Potential risks are based on class effects of monoclonal antibody therapies, including infusional toxicity. Patients will receive premedication with antihistamine and acetaminophen, and infusion

rates increased based on vital sign and nursing monitoring every 15-20 minutes. Grade 1 or higher infusion reactions will prompt discontinuation of infusion until resolution of symptoms; grade 2 or higher reactions will be treated with additional corticosteroid or symptom-directed therapies.

Toxicities:
Toxicity associated with cirmtuzumab administration has not yet been determined. In the phase I trial of cirmtuzumab in CLL, no adverse events have been probably or definitely related to cirmtuzumab at doses between 15 µg/kg and 20 mg/kg. There was no off-target binding identified in initial preclinical assays. Infusion reaction, fluid retention, and immunogenicity are possible class effects of monoclonal antibody therapy. Depletion of precursor B cells and B cell lymphopenia are possible as well. Other ROR1 antibodies, distinct from cirmtuzumab have demonstrated binding to pancreatic islet cells and adipose tissue.[19] Glucose levels and body weight will therefore be monitored.

8.1.1 Return and Retention of Study Drug

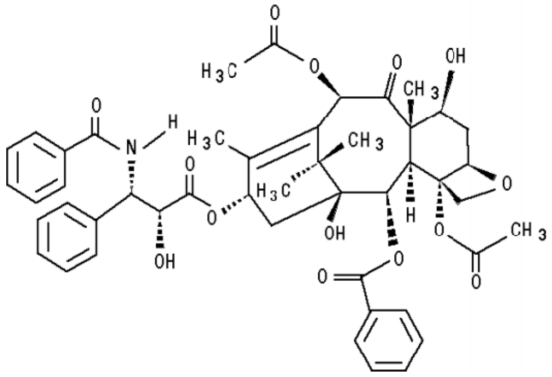
Remaining drug is to be destroyed, according to Moores Cancer Center Investigational Drug Services destruction policy.

8.2 Paclitaxel

Please refer to the TAXOL product Label[19] for more comprehensive information.

Other names for the drug:
TAXOL

Structure:



Mechanism of action (or Product description):
Paclitaxel is an anti-microtubule agent that promotes assembly of microtubules from tubulin dimers and stabilizes microtubules by preventing depolymerization. This stability results in the inhibition of the normal dynamic reorganization of the microtubule network that is essential for vital interphase and mitotic cellular functions. In addition, paclitaxel induces abnormal arrays or “bundles” of microtubules throughout the cell cycle and multiple asters of microtubules during mitosis.

Supplied by: commercially supplied.

Formulation:
Paclitaxel injection is a clear, colorless to slightly yellow viscous solution. It is supplied as a non-aqueous solution intended for dilution with a suitable parenteral fluid prior to intravenous infusion. Paclitaxel is available in 30 mg (5 mL), 100 mg (16.7 mL), and 300 mg (50 mL) multidose vials. Each mL of sterile nonpyrogenic solution contains 6 mg paclitaxel, 527 mg of purified Cremophor® EL* (polyoxyethylated castor oil) and 49.7% (v/v) dehydrated alcohol, USP.

Storage:
Store the vials in original cartons between 20°–25° C (68°–77° F). Retain in the original package to protect from light.

Solution Preparation:
Paclitaxel will be prepared in accordance with UCSD institutional standard practice.

Administration: Intravenous.

Side effects:
The most common side effects of paclitaxel include but are not limited to:

- Neutropenia, leucopenia,
- Thrombocytopenia and anemia resulting in red blood cell and platelet transfusions
- Infection
- Bleeding
- Hypersensitivity reactions
- Bradycardia
- Hypotension
- Peripheral neuropathy
- Myalgias/arthralgias
- Nausea and vomiting
- Diarrhea
- Mucositis
- Alopecia
- Hepatic toxicity
- Injection site reactions

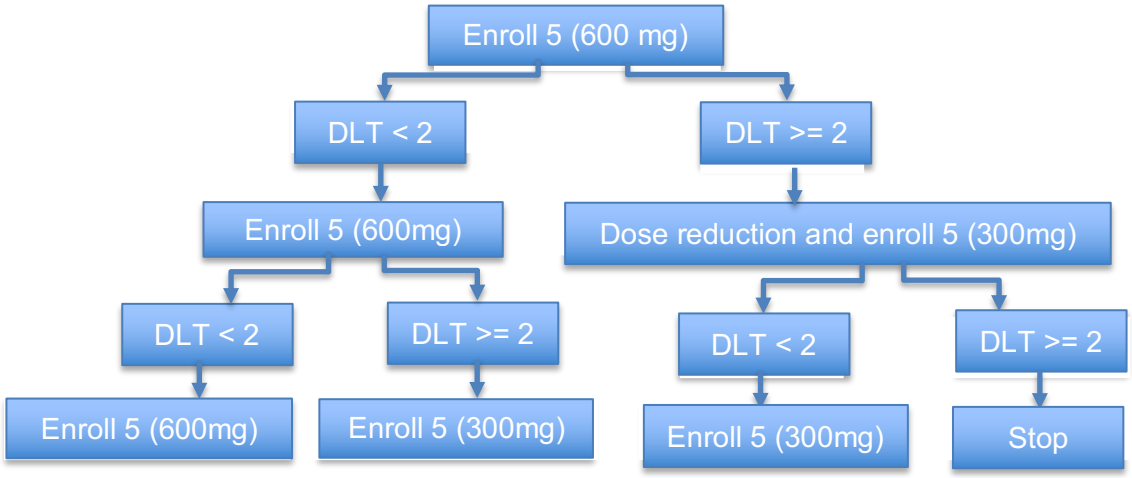
9.0 STATISTICAL CONSIDERATIONS

9.1 Study Design/Study Endpoints

This is an open label, single institution, Phase Ib pilot study of the safety and tolerability of cirmtuzumab at fixed dose administered with paclitaxel in patients with metastatic, or locally advanced, unresectable breast cancer. Patients will be enrolled in cohorts of five. Each cohort will be assessed for DLT's prior to enrolling the next cohort.

Patients will start with cirmtuzumab 600mg (2 cirmtuzumab vials) on Day 1 and Day 15 of Cycle 1. If two or more of the first 5 patients experience dose-limiting toxicity, further patients will be enrolled at a 50% dose reduction, 300 mg flat dose level(1 cirmtuzumab vial).The DLT rate is expected to be low, but if there are two or more DLTs occurring in any cohort of 5 patients, the study team may request a DSMB review before proceeding to enroll next cohort

- 1): Five patients are initially enrolled into the 600 mg cirmtuzumab and 80 mg/m^2 paclitaxel cohort. If there are fewer than 2 DLTs observed out of these 5 patients, proceed to 2), otherwise, reduce the dose level of cirmtuzumab to 300 mg for further patients and proceed to 3).
- 2): The trial proceeds to enroll an additional five subjects. If there are fewer than 2 DLTs observed out of these 5 patients, proceed to 4), otherwise reduce the dose level of cirmtuzumab to 300 mg and proceed to 4)
- 3): The trial proceeds to enroll an additional 5 subjects. If there are 2 or more DLTs observed out of these 5 patients, the study would be stopped, otherwise proceed to 4).
- 4): The trial proceeds to enroll the last additional five subjects.



The primary endpoint is the rate of dose-limiting toxicities during the first 4 weeks of treatment, assessed to be related to cirmtuzumab, or to the combination of cirmtuzumab with paclitaxel.

Secondary endpoints include:

- Safety and tolerability of the combination therapy since the start of any study treatment.
- Objective tumor response rate, best tumor response rate and time to progression as assessed by RECIST v1.1.
- ROR1 expression levels and putative breast cancer stem cell markers in tumor specimen measured by immunohistochemistry.

Exploratory endpoints include:

- Plasma pharmacokinetics of cirmtuzumab.
- Level of circulating antibody formation against cirmtuzumab.
- As available, we will perform pharmacodynamic, biochemical and molecular studies on tissue samples of treated patients, depending on sample availability.
- Comparison of PET/CT imaging to standard cross-sectional imaging using Spearman Rank Correlation.

9.1.1 Study Stopping Rules

The study will also be placed on hold if any of the following occur that are at least possibly

attributable to cirmtuzumab or the combination of cirmtuzumab and paclitaxel:

- Any death
- 2 or more non-hematological Grade 4 adverse events not resolving with standard therapy within 7 days. Grade 4 hematologic events will not stop the study, but do meet protocol-defined criteria for Dose Limiting Toxicity (with the exception of Grade 4 neutropenia lasting less than 8 days).

All events resulting in the stoppage of the clinical trial will be reported to the appropriate regulatory agencies. The Principal Investigator will be required to report to the UCSD IRB and the DSMB chair. UCSD will be responsible for reporting serious adverse events to the FDA.

9.1.1.1 Treatment-related DLT

Protocol-defined criteria for Dose Limiting Toxicity will be monitored in every five patients to evaluate whether the study should be stopped early. Two or more DLT's in the first five patients would lead to dose reduction. Two or more DLT's in an additional five patients would lead to study termination. The table below gives the probability of observing two or more DLT'S in each group of 5 patients, under assumed DLT rates of 10%, 20%, and 30%.

Probability of DLT at 600 mg cirmtuzumab level	Probability of DLT at 300 mg cirmtuzumab level	Probability of early stopping (first 5 patients)	Probability of early stopping (middle 5 patients)	Probability of early stopping (last 5 patients)	Probability of early stopping (total)
0.1	0.05	0	0.002	0.003	0.005
0.2	0.1	0	0.021	0.035	0.056
0.3	0.15	0	0.078	0.106	0.184

This computation assumes that the probability of a DLT drops by half when the dose is reduced.

9.2 Sample Size and Accrual

The planned sample size is 15 evaluable patients defined as undergoing combination therapy for at least 2 cycles. Replacement patients will be enrolled, up to a maximum of 30 patients to achieve 15 evaluable patients. Currently, we estimate enrolling 1 patient per month. Given the maximum number of study subjects is 30, the longest time period to complete enrollment would be approximately 30 months.

9.2.1 Evaluable Subjects and Subject Replacement

A subject will be considered evaluable for assessment of DLTs if the subject receives at least 2 doses of cirmtuzumab and completes the safety follow-up through the DLT evaluation period of 28 days, or the subject experiences a DLT at any time during the DLT evaluation period.

The safety population will include all subjects who receive any treatment of cirmtuzumab. The safety population will be used to evaluate baseline characteristics as well as all descriptive endpoints for safety.

The intent-to-treat efficacy population will include all subjects who receive at least one dose of cirmtuzumab.

The per-protocol efficacy population will include all subjects who receive at least 2 cycles of

cirmtuzumab treatment. These efficacy populations will be used to evaluate the efficacy related endpoints, such as the objective response rate.

9.3 Data Analyses Plans

9.3.1 Primary Analyses

The rate of DLTs or RP2D will be calculated along with its 95% confidence interval using the exact (Clopper-Pearson) method, appropriately adjusted for the 5+5+5 stopping rule.

9.3.2 Secondary Analyses

Safety will be assessed through summaries of adverse events. All subjects who receive at least one dose of study medication will be considered evaluable for safety.

Adverse events from treatment beginning to six months after treatment completion, will be listed, documenting the dose level, course, outcome, severity, and relationship to the study treatment. AEs will be summarized by the event type, grade, relatedness, outcome, and organ system. Incidence rates of AEs and the proportion of subjects prematurely withdrawn from the study due to AEs will be reported. Specifically, the rates of infusion reactions, major infections and grade 3 or higher non-infection adverse events will be reported.

Tumor response and time to progression will be assessed by RECIST v1.1. The objective tumor response rate (CR, PR) and best tumor response rate (stable disease, or better) will be reported with 95% confidence intervals. Median progression-free survival will be estimated using the Kaplan-Meier method. These analyses will be done by overall and dose cohorts.

The expression levels of ROR1 and other cancer stem cell markers (ALDH, CD133) on the primary pre-treatment and after-treatment tumor specimens will be measured by immunohistochemistry. The changes for each patient will be tested by the Wilcoxon signed rank test. Fisher's exact test will be used to test the association of the baseline levels with tumor responses.

All statistical analyses will be at the 5% significance level using 2-sided tests.

9.3.3 Exploratory Analyses

Exploratory endpoints to be analyzed include the following mechanism of action studies, by dose cohort:

1. Plasma pharmacokinetics of cirmtuzumab
2. Level of circulating antibody formation against cirmtuzumab.
3. As available, we will perform pharmacodynamic, biochemical and molecular studies on tissue samples of treated patients, depending on sample availability.
4. Compare PET/CT imaging to standard cross-sectional imaging using Spearman Rank Correlation

The serum concentrations of cirmtuzumab will be evaluated via descriptive summary statistics (N, mean, standard deviation, median, quartiles) and graphical measures (boxplots, time-course graphs, etc.).

10.0 STUDY MANAGEMENT

10.1 Conflict of Interest

Any investigator who has a conflict of interest with this study (patent ownership, royalties, or financial gain greater than the minimum allowable by their institution, etc.) must have the conflict reviewed according to UCSD conflict of interest policy.

10.2 Institutional Review Board (IRB) Approval and Consent

The IRB should approve the consent form and protocol prior to any study-related activities. It is expected that the IRB will have the proper representation and function in accordance with federally mandated regulations.

In obtaining and documenting informed consent, the investigator should comply with the applicable regulatory requirement(s), and should adhere to Good Clinical Practice (GCP) and to ethical principles that have their origin in the Declaration of Helsinki.

Before recruitment and enrollment onto this study, the patient will be given a full explanation of the study and will be given the opportunity to review the consent form. Each consent form must include all the relevant elements currently required by the FDA Regulations and local or state regulations. Once this essential information has been provided to the patient and the investigator is assured that the patient understands the implications of participating in the study, the patient will be asked to give consent to participate in the study by signing an IRB-approved consent form.

Prior to a patient's participation in the trial, the written informed consent form should be signed and personally dated by the patient and by the person who conducted the informed consent discussion.

10.3 Subject Data Protection

In accordance with the Health Information Portability and Accountability Act (HIPAA), subjects who have provided written informed consent must also sign a subject authorization to release medical information to the study Sponsor and allow a regulatory authority, or Institutional Review Board access to subject's medical information relevant to the study.

10.4 Data and Safety Monitoring/Auditing

In addition to adverse event monitoring and clinical oversight by the principal investigator and co-investigators, this study will also use the UCSD Moores Cancer Center Data Safety and Monitoring Board (DSMB) to provide oversight in the event that this treatment approach leads to unforeseen toxicities. Data from this study will be reported annually and will include:

1) the protocol title, IRB protocol number, and the activation date of the study.
2) the number of patients enrolled to date
3) the dates of patient enrollment
4) a summary of all adverse events regardless of grade and attribution
5) a response evaluation for evaluable patients when available
6) a summary of any recent literature that may affect the ethics of the study.

10.5 Adherence to the Protocol

Except for an emergency situation in which proper care for the protection, safety, and well-being of the study patient requires alternative treatment, investigators are required to conduct their research according to the plans reviewed and approved by the IRB.

10.6 Amendments to the Protocol

Should amendments to the protocol be required, the amendments will be originated and documented by the Study Chair. It should also be noted that when an amendment to the protocol substantially alters the study design or the potential risk to the patient, a revised consent form might be required.

The written amendment, and if required the amended consent form, must be sent to the IRBs for approval prior to implementation.

10.7 Record Retention

Study documentation includes all Case Report Forms, data correction forms or queries, source documents, Sponsor-Investigator correspondence, monitoring logs/letters, and regulatory documents (e.g., protocol and amendments, IRB correspondence and approval, signed patient consent forms).

Source documents include all recordings of observations or notations of clinical activities and all reports and records necessary for the evaluation and reconstruction of the clinical research study.

Government agency regulations and directives require that the study investigator must retain all study documentation pertaining to the conduct of a clinical trial. In the case of a study with a drug seeking regulatory approval and marketing, these documents shall be retained for at least two years after the last approval of marketing application in an International Conference on Harmonization (ICH) region. In all other cases, study documents should be kept on file until three years after the completion and final study report of this investigational study.

10.8 Obligations of Investigators

The Principal Investigator is responsible for the conduct of the clinical trial at the site in accordance with Title 21 of the Code of Federal Regulations and/or the Declaration of Helsinki. The Principal Investigator is responsible for personally overseeing the treatment of all study patients. The Principal Investigator must assure that all study site personnel, including sub-investigators and other study staff members, adhere to the study protocol and all FDA/GCP/NCI regulations and guidelines regarding clinical trials both during and after study completion.

The Principal Investigator at each institution or site will be responsible for assuring that all the required data will be collected and entered onto the Case Report Forms. Periodically, monitoring visits will be conducted and the Principal Investigator will provide access to his/her original records to permit verification of proper entry of data. At the completion of the study, all case report forms will be reviewed by the Principal Investigator and will require his/her final signature to verify the accuracy of the data.

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12.0 APPENDICES

Appendix A. Performance Status

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

Appendix B. Response evaluation criteria in solid tumors (RECIST)

Tumor assessments will be made according to the schedule of assessments. Response and progression will be evaluated using Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1 guidelines [Eisenhauer et al. 2009].

B1 MEASURABILITY OF TUMOR AT BASELINE

B1.1 Definitions

At baseline, tumor lesions will be categorized as follows:

B1.1.1 Measurable

Tumor lesions: must be accurately measured in at least one dimension (longest diameter in the plane of measurements is to be recorded) with a minimum size of:

- 10 mm by CT scan (CT scan slice thickness no greater than 5 mm).
- 10 mm caliper measurement by clinical exam (lesions which cannot be accurately measured with calipers should be recorded as non-measurable).
- 20 mm by chest X-ray.

Malignant lymph nodes: to be considered pathologically enlarged and measurable, a lymph node must be >= 15 mm in *short* axis when assessed by CT scan. At baseline and in follow-up, only the *short* axis will be measured and followed.

B1.1.2 Non-measurable

All other lesions, including small lesions (longest diameter < 10 mm or pathological lymph nodes with >= 10 to < 15 mm short axis) as well as truly non-measurable lesions include: leptomeningeal disease, ascites, pleural or pericardial effusion, inflammatory breast disease, lymphangitic involvement of skin or lung, abdominal masses/abdominal organomegaly identified by physical exam that is not measurable by reproducible imaging techniques.

B1.1.3 Special considerations regarding lesion measurability

Bone lesions, cystic lesions, and lesions previously treated with local therapy require particular comment:

Bone lesions:

- Bone scan, PET scan or plain films are not considered adequate imaging techniques to measure bone lesions. However, these techniques can be used to confirm the presence or disappearance of bone lesions.
- Lytic bone lesions or mixed lytic-blastic lesions, with *identifiable soft tissue components*, that can be evaluated by cross sectional imaging techniques such as CT or MRI can be considered as measurable lesions if the soft tissue component meets the definition of measurability described above.
- Blastic bone lesions are non-measurable.

Cystic lesions:

- Lesions that meet the criteria for radiographically defined simple cysts should not be considered as malignant lesions (neither measurable nor non-measurable) since they are, by definition, simple cysts.
- 'Cystic lesions' thought to represent cystic metastases can be considered as measurable lesions, if they meet the definition of measurability described above. However, if non-cystic lesions are present in the same patient, these are preferred for selection as target lesions.

Lesions with prior local treatment:

- Tumor lesions situated in a previously irradiated area, or in an area subjected to other loco-regional therapy, are usually not considered measurable unless there has been demonstrated progression in the lesion. Study protocols should detail the conditions under which such lesions would be considered measurable.

B1.2 Specifications by methods of measurements

B1.2.1 Measurement of lesions

All measurements should be recorded in metric notation, using calipers if clinically assessed. All baseline evaluations should be performed as close as possible to the treatment start and never more than 4 weeks before the beginning of the treatment.

B1.2.2 Method of assessment

The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during follow-up. Imaging based evaluation should always be done rather than clinical examination unless the lesion(s) being followed cannot be imaged but are assessable by clinical exam.

Clinical lesions: Clinical lesions will only be considered measurable when they are superficial and 10mm diameter as assessed using calipers (e.g. skin nodules). For the case of skin lesions, documentation by color photography including a ruler to estimate the size of the lesion is suggested. As noted above, when lesions can be evaluated by both clinical exam and imaging, imaging evaluation should be undertaken since it is more objective and may also be reviewed at the end of the study.

Chest X-ray: Chest CT is preferred over chest X-ray, particularly when progression is an important endpoint, since CT is more sensitive than X-ray, particularly in identifying new lesions. However, lesions on chest X-ray may be considered measurable if they are clearly defined and surrounded by aerated lung.

CT, MRI: CT is the best currently available and reproducible method to measure lesions selected for response assessment. This guideline has defined measurability of lesions on CT scan based on the assumption that CT slice thickness is 5mm or less. When CT scans have slice thickness greater than 5 mm, the minimum size for a measurable lesion should be twice the slice thickness. MRI is also acceptable in certain situations (e.g. for body scans).

Ultrasound: Ultrasound is not useful in assessment of lesion size and should not be used as a method of measurement. Ultrasound examinations cannot be reproduced in their entirety for independent review at a later date and, because they are operator dependent, it cannot be guaranteed that the same technique and measurements will be taken from one assessment to the next. If new lesions are identified by ultrasound in the course of the study, confirmation by CT or MRI is advised. If there is concern about radiation exposure at CT, MRI may be used instead of CT in selected instances.

Endoscopy, laparoscopy: The utilization of these techniques for objective tumor evaluation is not advised. However, they can be useful to confirm complete pathological response when biopsies are obtained or to determine relapse in trials where recurrence following complete response or surgical resection is an endpoint.

Tumor markers: Tumor markers alone cannot be used to assess objective tumor response. If markers are initially above the upper normal limit, however, they must normalize for a patient to be considered in complete response. Because tumor markers are disease specific, instructions for their measurement should be incorporated into protocols on a disease specific basis.

Cytology, histology: These techniques can be used to differentiate between PR and CR in rare cases if required by protocol (for example, residual lesions in tumor types such as germ cell tumors, where known residual benign tumors can remain). When effusions are known to be a potential adverse effect of treatment (e.g. with certain taxane compounds or angiogenesis inhibitors), the cytological confirmation of the neoplastic origin of any effusion that appears or worsens during treatment can be considered if the measurable tumor has met criteria for response or stable disease in order to differentiate between response (or stable disease) and progressive disease.

B2 TUMOR RESPONSE EVALUATION

B2.1 Baseline documentation of 'target' and 'non-target' lesions

When more than one measurable lesion is present at baseline all lesions up to a maximum of five lesions total (and a maximum of two lesions per organ) representative of all involved organs should be identified as target lesions and will be recorded and measured at baseline (this means in instances where patients have only one or two organ sites involved a maximum of two and four lesions respectively will be recorded).
Target lesions should be selected on the basis of their size (lesions with the longest diameter), be representative of all involved organs, but in addition should be those that lend themselves to reproducible repeated measurements. It may be the case that, on occasion, the largest lesion does not lend itself to reproducible measurement in which circumstance the next largest lesion which can be measured reproducibly should be selected.

Lymph nodes merit special mention since they are normal anatomical structures which may be visible by imaging even if not involved by tumor. Pathological nodes which are defined as measurable and may be identified as target lesions must meet the criterion of a short axis of P15mm by CT scan. Only the short axis of these nodes will contribute to the baseline sum. The short axis of the node is the diameter normally used by radiologists to judge if a node is involved by solid tumor. Nodal size is normally reported as two dimensions in the plane in which the image is obtained (for CT scan this is almost always the axial plane; for MRI the plane of acquisition may be axial, sagittal or coronal). The smaller of these measures is the short axis. For example, an abdominal node which is reported as being 20mm· 30mm has a short axis of 20mm and qualifies as a malignant, measurable node. In this example, 20mm should be recorded as the node measurement. All other pathological nodes (those with short axis P10mm but <15 mm) should be considered non-target lesions. Nodes that have a short axis <10mm are considered non-pathological and should not be recorded or followed.

A *sum of the diameters* (longest for non-nodal lesions, short axis for nodal lesions) for all target lesions will be calculated and reported as the baseline sum diameters. If lymph nodes are to be included in the sum, then as noted above, only the short axis is added into the sum. The baseline sum diameters will be used as reference to further characterize any objective tumor regression in the measurable dimension of the disease.

All other lesions (or sites of disease) including pathological lymph nodes should be identified as non-target lesions and should also be recorded at baseline. Measurements are not required and

these lesions should be followed as 'present', 'absent', or in rare cases 'unequivocal progression' (more details to follow). In addition, it is possible to record multiple non-target lesions involving the same organ as a single item on the case record form (e.g. 'multiple enlarged pelvic lymph nodes' or 'multiple liver metastases').

B2.2 Response criteria

This section provides the definitions of the criteria used to determine objective tumor response for target lesions.

B2.2.1 Evaluation of target lesions

Complete Response (CR):	Disappearance of all target lesions. Any pathological lymph nodes (whether target or non-target) must have reduction in short axis to <10 mm.
Partial Response (PR):	At least a 30% decrease in the sum of diameters of target lesions, taking as reference the baseline sum diameters.
Progressive Disease (PD):	At least a 20% increase in the sum of diameters of target lesions, taking as reference the smallest sum on study (this includes the baseline sum if that is the smallest on study). In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5 mm. (Note: the appearance of one or more new lesions is also considered progression).
Stable Disease (SD):	Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum diameters while on study.

B2.2.2 Special notes on the assessment of target lesions

Lymph nodes. Lymph nodes identified as target lesions should always have the actual short axis measurement recorded (measured in the same anatomical plane as the baseline examination), even if the nodes regress to below 10mm on study. This means that when lymph nodes are included as target lesions, the 'sum' of lesions may not be zero even if complete response criteria are met, since a normal lymph node is defined as having a short axis of <10mm. Case report forms or other data collection methods may therefore be designed to have target nodal lesions recorded in a separate section where, in order to qualify for CR, each node must achieve a short axis <10mm. For PR, SD and PD, the actual short axis measurement of the nodes is to be included in the sum of target lesions.

Target lesions that become 'too small to measure'. While on study, all lesions (nodal and non-nodal) recorded at baseline should have their actual measurements recorded at each subsequent evaluation, even when very small (e.g. 2mm). However, sometimes lesions or lymph nodes which are recorded as target lesions at baseline become so faint on CT scan that the radiologist may not feel comfortable assigning an exact measure and may report them as being 'too small to measure'. When this occurs, it is important that a value be recorded on the case report form. If it is the opinion of the radiologist that the lesion has likely disappeared, the measurement should be recorded as 0mm. If the lesion is believed to be present and is faintly seen but too small to measure, a default value of 5mm should be assigned (Note: It is less likely that this rule will be used for lymph nodes since they usually have a definable size when normal and are frequently surrounded by fat such as in the retroperitoneum; however, if a lymph node is believed to be present and is faintly seen but too small to measure, a default value of 5mm

should be assigned in this circumstance as well). This default value is derived from the 5mm CT slice thickness (but should not be changed with varying CT slice thickness). The measurement of these lesions is potentially non-reproducible; therefore providing this default value will prevent false responses or progressions based upon measurement error. To reiterate, however, if the radiologist is able to provide an actual measure, that should be recorded, even if it is below 5mm.

Lesions that split or coalesce on treatment. When non-nodal lesions 'fragment', the longest diameters of the fragmented portions should be added together to calculate the target lesion sum. Similarly, as lesions coalesce, a plane between them may be maintained that would aid in obtaining maximal diameter measurements of each individual lesion. If the lesions have truly coalesced such that they are no longer separable, the vector of the longest diameter in this instance should be the maximal longest diameter for the 'coalesced lesion'.

B2.2.3 Evaluation of non-target lesions

This section provides the definitions of the criteria used to determine the tumor response for the group of non-target lesions. While some non-target lesions may actually be measurable, they need not be measured and instead should be assessed only qualitatively at the time points specified in the protocol.

Complete Response (CR):	Disappearance of all non-target lesions and normalization of tumor marker level. All lymph nodes must be non-pathological in size (<10mm short axis).
Non-CR/Non-PD:	Persistence of one or more non-target lesion(s) and/or maintenance of tumor marker level above the normal limits.
Progressive Disease (PD):	Unequivocal progression (see comments below) of existing non-target lesions. (Note: the appearance of one or more new lesions is also considered progression).

B2.2.4 Special notes on assessment of progression of non-target disease

The concept of progression of non-target disease requires additional explanation as follows:

When the patient also has measurable disease. In this setting, to achieve 'unequivocal progression' on the basis of the non-target disease, there must be an overall level of substantial worsening in non-target disease such that, even in presence of SD or PR in target disease, the overall tumor burden has increased sufficiently to merit discontinuation of therapy. A modest 'increase' in the size of one or more non-target lesions is usually not sufficient to quality for unequivocal progression status. The designation of overall progression solely on the basis of change in non-target disease in the face of SD or PR of target disease will therefore be extremely rare.

When the patient has only non-measurable disease. This circumstance arises in some phase III trials when it is not a criterion of study entry to have measurable disease. The same general concepts apply here as noted above, however, in this instance there is no measurable disease assessment to factor into the interpretation of an increase in non-measurable disease burden. Because worsening in non-target disease cannot be easily quantified (by definition: if all lesions are truly non-measurable) a useful test that can be applied when assessing patients for unequivocal progression is to consider if the increase in overall disease burden based on the change in non-measurable disease is comparable in magnitude to the increase that would be

required to declare PD for measurable disease: i.e. an increase in tumor burden representing an additional 73% increase in 'volume' (which is equivalent to a 20% increase diameter in a measurable lesion). Examples include an increase in a pleural effusion from 'trace' to 'large', an increase in lymphangitic disease from localized to widespread, or may be described in protocols as 'sufficient to require a change in therapy'. If 'unequivocal progression' is seen, the patient should be considered to have had overall PD at that point. While it would be ideal to have objective criteria to apply to non-measurable disease, the very nature of that disease makes it impossible to do so, therefore the increase must be substantial.

B2.2.5 New lesions

The appearance of new malignant lesions denotes disease progression; therefore, some comments on detection of new lesions are important. There are no specific criteria for the identification of new radiographic lesions; however, the finding of a new lesion should be unequivocal: i.e. not attributable to differences in scanning technique, change in imaging modality or findings thought to represent something other than tumor (for example, some 'new' bone lesions may be simply healing or flare of pre-existing lesions). This is particularly important when the patient's baseline lesions show partial or complete response. For example, necrosis of a liver lesion may be reported on a CT scan report as a 'new' cystic lesion, which it is not.

A lesion identified on a follow-up study in an anatomical location that was not scanned at baseline is considered a new lesion and will indicate disease progression. An example of this is the patient who has visceral disease at baseline and while on study has a CT or MRI brain ordered which reveals metastases. The patient's brain metastases are considered to be evidence of PD even if he/she did not have brain imaging at baseline.

If a new lesion is equivocal, for example because of its small size, continued therapy and follow-up evaluation will clarify if it represents truly new disease. If repeat scans confirm there is definitely a new lesion, then progression should be declared using the date of the initial scan.

While FDG-PET response assessments need additional study, it is sometimes reasonable to incorporate the use of FDG-PET scanning to complement CT scanning in assessment of progression (particularly possible 'new' disease). New lesions on the basis of FDG-PET imaging can be identified according to the following algorithm:

a. Negative FDG-PET at baseline, with a positive FDG-PET at follow-up is a sign of PD based on a new lesion.
b. No FDG-PET at baseline and a positive FDG-PET at follow- up:
   If the positive FDG-PET at follow-up corresponds to a new site of disease confirmed by CT, this is PD. If the positive FDG-PET at follow-up is not confirmed as a new site of disease on CT, additional follow-up CT scans are needed to determine if there is truly progression occurring at that site (if so, the date of PD will be the date of the initial abnormal FDG-PET scan). If the positive FDG-PET at follow-up corresponds to a pre-existing site of disease on CT that is not progressing on the basis of the anatomic images, this is not PD.

B2.3 Evaluation of best overall response

The best overall response is the best response recorded from the start of the study treatment until the end of treatment taking into account any requirement for confirmation. On occasion a response may not be documented until after the end of therapy so protocols should be clear if post-treatment assessments are to be considered in determination of best overall response. Protocols must specify how any new therapy introduced before progression will affect best response designation. The patient's best overall response assignment will depend on the

findings of both target and non-target disease and will also take into consideration the appearance of new lesions. Furthermore, depending on the nature of the study and the protocol requirements, it may also require confirmatory measurement. Specifically, in non-randomized trials where response is the primary endpoint, confirmation of PR or CR is needed to deem either one the 'best overall response'. This is described further below.

B2.3.1 Time point response

It is assumed that at each protocol specified time point, a response assessment occurs. Table 1 provides a summary of the overall response status calculation at each time point for patients who have measurable disease at baseline.
When patients have non-measurable (therefore non-target) disease only, Table 2 is to be used.

Table 1. Time point response: patients with target (+/- non-target) disease.

Target Lesions	Non-target Lesions	New Lesions	Overall Response
CR	CR	No	CR
CR	Non-CR/non-PD	No	PR
CR	Not evaluated	No	PR
PR	Non-PD or not all evaluated	No	PR
SD	Non-PD or not all evaluated	No	SD
Not all evaluated	Non-PD	No	Inevaluable
PD	Any	Yes or No	PD
Any	PD	Yes or No	PD
Any	Any	Yes	PD

Table 2. Time point response: patients with non-target disease only.

Non-target Lesions	New Lesions	Overall Response
CR	No	CR
Non-CR/non-PD	No	Non-CR/non-PD
Not all evaluated	No	Inevaluable
Unequivocal PD	Yes or No	PD
Any	Yes	PD

B2.3.2 Missing assessments and inevaluable designation

When no imaging/measurement is done at all at a particular time point, the patient is not evaluable (NE) at that time point. If only a subset of lesion measurements are made at an assessment, usually the case is also considered NE at that time point, unless a convincing argument can be made that the contribution of the individual missing lesion(s) would not change the assigned time point response. This would be most likely to happen in the case of PD. For example, if a patient had a baseline sum of 50mm with three measured lesions and at follow-up

only two lesions were assessed, but those gave a sum of 80 mm, the patient will have achieved PD status, regardless of the contribution of the missing lesion.

B2.3.3 Best overall response: all time points

The best overall response is determined once all the data for the patient is known.

Best response determination in trials where confirmation of complete or partial response IS NOT required: Best response in these trials is defined as the best response across all time points (for example, a patient who has SD at first assessment, PR at second assessment, and PD on last assessment has a best overall response of PR). When SD is believed to be best response, it must also meet the protocol specified minimum time from baseline. If the minimum time is not met when SD is otherwise the best time point response, the patient's best response depends on the subsequent assessments. For example, a patient who has SD at first assessment, PD at second and does not meet minimum duration for SD, will have a best response of PD. The same patient lost to follow-up after the first SD assessment would be considered inevaluable.

Best response determination in trials where confirmation of complete or partial response IS required: Complete or partial responses may be claimed only if the criteria for each are met at a subsequent time point as specified in the protocol (generally 4 weeks later). In this circumstance, the best overall response can be interpreted as in Table 3.

Table 3. Best overall response when confirmation of CR and PR required.

Overall response First time point	**Overall response Subsequent time point**	**BEST overall response**
CR	CR	CR
CR	PR	SD, PD OR PR
CR	SD	SD provided minimum criteria for SD duration met, otherwise PD
CR	PD	SD provided minimum criteria for SD duration met, otherwise PD
CR	Inevaluable	SD provided minimum criteria for SD duration met, otherwise inevaluable
PR	CR	PR
PR	PR	PR
PR	SD	SD
PR	PD	SD provided minimum criteria for SD duration met, otherwise PD
PR	Inevaluable	SD provided minimum criteria for SD duration met, otherwise inevaluable
Inevaluable	Inevaluable	Inevaluable

B2.3.4 Special notes on response assessment

When nodal disease is included in the sum of target lesions and the nodes decrease to 'normal' size (<10 mm), they may still have a measurement reported on scans. This measurement should be recorded even though the nodes are normal in order not to overstate progression should it be based on increase in size of the nodes. As noted earlier, this means that patients with CR may not have a total sum of 'zero' on the case report form (CRF).

In trials where confirmation of response is required, repeated 'NE' time point assessments may complicate best response determination. The analysis plan for the trial must address how missing data/assessments will be addressed in determination of response and progression. For example, in most trials it is reasonable to consider a patient with time point responses of PR-NE-PR as a confirmed response.

Patients with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be reported as 'symptomatic deterioration'. Every effort should be made to document objective progression even after discontinuation of treatment. Symptomatic deterioration is not a descriptor of an objective response: it is a reason for stopping study therapy. The objective response status of such patients is to be determined by evaluation of target and non-target disease as shown in Tables 1–3.

Conditions that define 'early progression, early death and inevaluability' are study specific and should be clearly described in each protocol (depending on treatment duration, treatment periodicity).

In some circumstances it may be difficult to distinguish residual disease from normal tissue. When the evaluation of complete response depends upon this determination, it is recommended that the residual lesion be investigated (fine needle aspirate/biopsy) before assigning a status of complete response. FDG-PET may be used to upgrade a response to a CR in a manner similar to a biopsy in cases where a residual radiographic abnormality is thought to represent fibrosis or scarring. The use of FDG-PET in this circumstance should be prospectively described in the protocol and supported by disease specific medical literature for the indication. However, it must be acknowledged that both approaches may lead to false positive CR due to limitations of FDG-PET and biopsy resolution/sensitivity.

For equivocal findings of progression (e.g. very small and uncertain new lesions; cystic changes or necrosis in existing lesions), treatment may continue until the next scheduled assessment. If at the next scheduled assessment, progression is confirmed, the date of progression should be the earlier date when progression was suspected.

B2.4 Confirmatory measurement/duration of response

B2.4.1 Confirmation

In non-randomized trials where response is the primary endpoint, confirmation of PR and CR is required to ensure responses identified are not the result of measurement error. This will also permit appropriate interpretation of results in the context of historical data where response has traditionally required confirmation in such trials. However, in all other circumstances, i.e. in randomized trials (phase II or III) or studies where stable disease or progression are the primary endpoints, confirmation of response is not required since it will not add value to the interpretation of trial results. However, elimination of the requirement for response confirmation may increase the importance of central review to protect against bias, in particular in studies which are not blinded.

In the case of SD, measurements must have met the SD criteria at least once after study entry at a minimum interval (in general not less than 6–8 weeks) that is defined in the study protocol.

B2.4.2 Duration of overall response

The duration of overall response is measured from the time measurement criteria are first met for CR/PR (whichever is first recorded) until the first date that recurrent or progressive disease

is objectively documented (taking as reference for progressive disease the smallest measurements recorded on study).

The duration of overall complete response is measured from the time measurement criteria are first met for CR until the first date that recurrent disease is objectively documented.

B2.4.3 Duration of stable disease
Stable disease is measured from the start of the treatment (in randomized trials, from date of randomization) until the criteria for progression are met, taking as reference the smallest sum on study (if the baseline sum is the smallest, this is the reference for calculation of PD).

The clinical relevance of the duration of stable disease varies in different studies and diseases. If the proportion of patients achieving stable disease for a minimum period of time is an endpoint of importance in a particular trial, the protocol should specify the minimal time interval required between two measurements for determination of stable disease.

Note: The duration of response and stable disease as well as the progression-free survival are influenced by the frequency of follow-up after baseline evaluation. It is not in the scope of this guideline to define a standard follow-up frequency. The frequency should take into account many parameters including disease types and stages, treatment periodicity and standard practice. However, these limitations of the precision of the measured endpoint should be considered if comparisons between trials are to be made.