CITY OF HOPE NATIONAL MEDICAL CENTER
1500 E. DUARTE ROAD, DUARTE, CA 91010

DEPARTMENT OF MEDICAL ONCOLOGY

TITLE: PHASEII TRIAL OF NEOADJUVANT CHEMOTHERAPY WITH CARBOPLATIN ANDNAB-PACLITAXEL IN PATIENTS WITH LOCALLY ADVANCED AND INFLAMMATORY TRIPLE NEGATIVE BREAST CANCER

CITY OF HOPE PROTOCOL NUMBER: 11174 PROTOCOL DATED: 06/09/2020

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COH Amendment 10	(Protocol Dated 7/26/13)	Version 10
COH Amendment 11	(Title Page Dated 10/08/13)	Version 11
COH Amendment 12	(Protocol dated 10/11/13 Registration &DSMP)	Version 12
COH Amendment 13	(Title Page Change dated 12/17/13)	Version 13
COH Amendment 14	(Title Page dated 7/1/14)	Version 14
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COH Amendment 16	(Protocol dated 3/18/15)	Version 16
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SPONSOR/IND NUMBER: City of Hope/N/A

DISEASE SITE: Breast
HISTOLOGY: Adenocarcinoma
STAGE: Stage II/III
MODALITY: Chemotherapy

TYPE: Neoadjuvant therapy

PHASE: Phase II

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STUDY SPONSOR AND MONITOR:	City of Hope National Medical Center

CITY OF HOPE, 1500 E. DUARTE ROAD, DUARTE, CA 91010

DEPARTMENT OF MEDICAL ONCOLOGY

TITLE: PHASE II TRIAL OF NEOADJUVANT CHEMOTHERAPY WITH CARBOPLATIN AND NAB-PACLITAXEL IN PATIENTS WITH LOCALLY ADVANCED AND INFLAMMATORY TRIPLE NEGATIVE BREAST CANCER

City of Hope #: 11174 Protocol Version: 06/09/20

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PARTICIPATING CLINICAL SITES: City of Hope, Duarte, CA
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STUDY SPONSOR AND MONITOR: City of Hope

Experimental Design Schema

- Identification of patients with locally advanced breast cancer/inflammatory breast cancer
- Consent
- Work-up, including validation of triple negative tumor status, and, if feasible, additional biopsy, procurement of blood samples, and imaging
- Eligibility criteria met : registration for treatment
- Therapy with 4 cycles of carboplatin (AUC 6) given every 4 weeks, nab-paclitaxel 100 mg/m^2 weekly. Total planed duration: 16 weeks
- Imaging of the primary tumor, and sampling of the tumor (optional) and blood (optional), after 2 cycles
- Re-imaging, followed by either breast conserving surgery (only for locally advanced breast cancer and not for inflammatory breast cancer) or mastectomy, and lymph node assessment/node dissection, as per standard clinical practice
- Analysis of clinical/correlative data, plan for follow-up randomized trial.

Protocol Synopsis

Protocol Title:

PHASE II TRIAL OF NEOADJUVANT CHEMOTHERAPY WITH CARBOPLATIN AND NAB-PACLITAXEL IN PATIENTS WITH LOCALLY ADVANCED AND INFLAMMATORY TRIPLE NEGATIVE BREAST CANCER

Brief Protocol Title for the Lay Public (if applicable):

N/A

Study Phase:

Phase II

Participating Sites:

City of Hope

Rationale for this Study:

Despite a decrease in breast cancer-related mortality, primarily due to screening/detection at an earlier stage, thousands of patients still present with locally advanced (LABC) and inflammatory breast cancer (IBC), conditions associated with high rates of recurrence and poor survival. Neoadjuvant chemotherapy (NT) is applied in the majority of such cases to allow for optimal surgical intervention, and also aiming for complete/near-complete pathologic response (pCR), a surrogate marker/predictor for longer relapse-free and overall survival, especially in estrogen and progesterone receptor negative patients.

Standard neoadjuvant therapy for locally advanced/inflammatory breast cancer results in pathological CR (pCR) in ~ 20-25 % of patients. We have previously conducted a neoadjuvant trial comparing a novel regimen of dose-dense doxorubicin and cyclophosphamide followed by carboplatin and nab-paclitaxel versus docetaxel, doxorubicin, and cyclophosphamide (TAC), in patients with locally advanced and inflammatory HER2- breast cancer. We documented the safety of combining carboplatin and nab-paclitaxel, but found no difference between the 2 regimens, and the pCR rate was somewhat disappointing at < 15%, with Symmans residual cancer burden (RCB) scores of 0-1 approaching 30% with either regimen. The combination of carboplatin and paclitaxel has been reported to be effective in triple negative breast cancer by others, with pCR rates of ~ 60% in the neoadjuvant setting, and without the use of anthracyclines. Nab-paclitaxel as a single agent results in over 60% response rate in the metastatic setting.

We hypothesize, that nab-paclitaxel, based on its favorable toxicity profile and efficacy when compared to cremophor-based paclitaxel in a randomized phase III trial in metastatic breast cancer, and given on a weekly schedule, may further improve efficacy in combinations including carboplatin. The proposed trial will therefore include carboplatin and nab-paclitaxel in the treatment of triple negative disease to further improve pCR rate, and to avoid anthracycline-associated side effects including myelodysplasia and secondary hematological malignancies.

Objectives: Primary Objective

- To test the hypothesis that carboplatin + nab-paclitaxel therapy will demonstrate a promising neoadjuvant pCR rate for eligible patients.
- To test the hypothesis that carboplatin + nab-paclitaxel therapy will demonstrate a promising Symmans 0-1 pathological response rate for eligible patients..

Secondary Objectives

- To evaluate the overall survival and event-free survival of eligible patients treated with carboplatin + nab-paclitaxel neoadjuvant chemotherapy.
- To evaluate the toxicities and tolerance of carboplatin + nab-paclitaxel therapy in this patient population.
- To evaluate the role of laboratory correlates in response, toxicity and survival endpoints.

1. To procure tissue and perform analysis of gene and protein expression profiles of pre-treatment primary tumor (estimated success rate: 80%) and residual tumors (25%) and lymph nodes (50%), including sequential assessment of cellular characteristics and gene and protein expression profiles and assessment of metastatic niche in lymph nodes.
2. To identify specific mutations in tumor DNA in comparison to adjacent tissue and germ line DNA procured prior to, during, and subsequent to neoadjuvant chemotherapy; and to detect/measure, as feasible, the presence of such mutations in fragmented, circulating DNA from plasma; and to correlate these mutations with the presence/characteristics of circulating tumor cells in order to identify prognostic and predictive indicators of persisting/relapsed disease and targets for therapy.
3. To assess RNA ((using Mammaprint/Blueprint and 44,000 Agilent 3platform gene array), miRNA and exosome and protein profiles in tumor, adjacent tissue and plasma prior to, during, and at completion of neoadjuvant chemotherapy in order to establish prognostic and predictive indicators of outcome, markers of persistent/relapsed disease, and targets for therapy.
4. To analyze tumor DNA and genomic DNA from plasma by microarray and RT-PCR analysis to assess copy numbers/SNP/genomic polymorphisms in genes for the purposes of establishing prognostic and predictive indicators of outcomes; markers of persistence/relapse disease, drug resistance, and drug metabolism; and targets of therapy.
5. To assess the prognostic and predictive value of conventional pathological features (stage, estrogen and progesterone receptor and HER-2 status, presence of lymphovascular invasion, high grade tumor status) in comparison to such values derived from the molecular approaches.
6. To procure tumor from the primary and definitive surgical specimen for the purpose of establishing breast cancer stem cell lines.
7. To procure blood samples for the purpose of identifying and characterizing circulating tumor cells.

Study Design:

Phase II Neoadjuvant Chemotherapy Trial; Single-site; type of study: safety, efficacy, exploratory correlative marker analysis. To permit early termination for a discouraging result, we will employ a two-stage design, with first stage accrual to 22 evaluable patients and final accrual to 45 evaluable patients (49 total patients). An expanded cohort of 20 patients will be added at the end, given a promising result, to better explore the response rate in specific breast cancer subtypes.

Endpoints:

Pathological complete response (pCR) and Residual Cancer Burden (RCB) score (Symmans)

Sample Size:

The sample size is to be 69 total patients. Forty-five (45) evaluable and 49 total in two-stage design to evaluate if the combination holds promise, and an additional 20 (given promising results) in an expanded cohort to gain experience in specific breast cancer subsets. All patients are to receive all study medications, and patients treated will be included in any reported analysis.

Summary of Subject Eligibility Criteria:

Inclusion Criteria:

Disease Status

Locally advanced (T2 and higher with or without lymph node involvement), and/or inflammatory breast cancer; triple negative biology only.

Age Criteria and Life Expectancy

Greater than 18 years of age, female patients

Child Bearing Potential

The effects of the proposed therapeutic agents (carboplatin, nab-paclitaxel) on the developing fetus are unknown. For this reason, women of child-bearing potential and men must agree to use adequate contraception (hormonal or barrier method of birth control or abstinence} prior to study entry and for six months following duration of study participation. Should a woman become pregnant or suspect that she is pregnant while participating on the trial, she should inform her treating physical immediately.

Protocol-Specific Criteria

1) Tumor negative for expression of hormone receptors (IHC < 10%) and not overexpressing HER2 by IHC (0-1), or, in case of IHC of 2, negative by FISH or by alternative gene testing.
2) ECOG ≤ 1
3) Organ function:

   Liver enzymes < 2 x upper limit of institutional normal

   Calculated or measured creatinine clearance of > 50 ml/min

   Left ventricular ejection fraction > 50%

   Absolute neutrophil count $\geq$ 1,500/μl; platelets $\geq$ 100,000/μl

Informed Consent/Assent

All subjects must have the ability to understand and the willingness to sign a written informed consent.

Prior Therapy

No prior therapies are allowed for the treatment of the newly diagnosed breast cancer. Patients with a prior diagnosis of malignancy treated $\geq$ 5 years ago are eligible, provided that they have not received prior taxanes or carboplatin as part of their prior treatment regimen, and that they meet all eligibility criteria.

Exclusion Criteria:

Study-Specific Exclusions

1) Known active Hepatitis B, or C

2) Known active HIV (necessitating therapy)
3) Prior breast cancer or other invasive malignancy treated within 5 years
4) Pregnancy
5) Neuropathy > grade 1
6) Any other intercurrent medical/psychological problem deemed exclusionary by the treating physician or investigators/PI

Non-Compliance

Subjects will be excluded who, in the opinion of the investigator, may not be able to comply with the safety monitoring requirements of the study.

Investigational Product Dosage and Administration:

Nab-paclitaxel 100 mg/m^2 weekly x 16 weeks

Clinical Observations and Tests to be Performed:

Laboratory studies will be required to be performed within a week prior to initiating therapy. Other diagnostic assessments should be performed within 28 days prior to initiating therapy.

Statistical Considerations:

A two stage design is proposed based on detecting a promising pCR rate (using the MD Anderson criteria of lack of evidence of any residual invasive tumor in breast and/or regional lymph nodes) or a promising Symmans 0-1 pathological response. In the first stage, accrual will continue until 22 patients are enrolled, with second stage accrual to an evaluable 45 and total of 49 patients. The design selected will meet our objectives and permits the early termination of the trial in the event that the therapy appears inferior to other neoadjuvant regimens.

An expanded cohort of 20 patients (given the promising results in the first 45) will be added to better evaluate the response rates in specific subsets: basal, androgen receptor positive, immunomodulatory subtype, mesenchymal/stem cell like. This will result in a total of 69 total patients.

Study size is based on our therapeutic target of achieving with acceptable toxicity either a promising pCR rate or a promising Symmans 0-1 pathological response rate.

We have chosen a design that simultaneously discriminates between pCR rate of 20% vs 38% and a Symmans response rate of 25% and 45%.

In particular:

If no more than four (4) Symmans 0-1 responses are observed and no more than three (3) pCRs are observed in the first 22 patients, the study will be terminated early and declared negative. If five (5) or more Symmans 0-1 responses or four (4) or more pCRs are observed in the first 22 patients, an additional 23 patients will be accrued during the second stage of the study. If at least 17 Symmans 0-1 responses (38%) or at least 14 pCRs are observed in the 45 evaluable patients (31%), this combination would be considered worthy of further testing in this setting.

This design yields at least 87% power to detect a true Symmans response rate of at least 45%. It yields at least 86% power to detect a true pCR rate of at least 38%. It yields at least .91 probability of a negative result if the true pCR response rate is no more than 20% and the Symmans 0-1 response rate is no more than 25%. This last probability is calculated assuming that pCR rate and the Symmans 0-1 rate are uncorrelated. As they are positively correlated, the probability will be

higher (type I error less than 9%).

Given the promising results at 45 evaluable patients, the expanded cohort of 20 patients will result in a total of 69 patients (~65 evaluable). This will allow a better exploratory analysis of the response rate based on phenotypic and gene array profiling subsets: Approximately 40% are expected to be of the basal subtypes (~27 patients), androgen subtype 11% (~7 patients expected), tumor infiltrating lymphocyte/immunomodulatory subtype 25% (~17 patients), and mesenchymal/mesenchymal/stem cell like ~25% (~17 patients). With 69 patients, there is an 89% chance of having 5 or more patients in even the smallest subset, and as a result, we should observe a pCR in all above subsets (e.g. 97% chance of observing at least one pCR with 5 patients if the true pCR response rate is 50%). Dramatic reduction/lack of pCR in any subset will help guide future development of subset specific treatment additions to the backbone of nab-paclitaxel and carboplatin. For the more common subsets, such as the basal subtype, the 95% CI for the pCR can be estimated with a half-width of at most 12% assuming 27 patients in that group. The confidence interval will be larger or smaller depending on the exact number of patients in that group enrolled, and these estimates will be made for the pCR rate for all patients who initiated treatment and are eligible patients, along with all patients who were treated per protocol.

Sponsor

City of Hope

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Abbreviations

Abbreviation	Meaning
AE	Adverse Event
CFR	Code of Federal Regulations
COH	City of Hope
CR	Complete Response
CRA	Clinical Research Associate
CRF	Case Report Form
CTCAE	Common Terminology Criteria for Adverse Events
CTEP	Cancer Therapy Evaluation Program
DLT	Dose Limiting Toxicity
DSMB	Data Safety Monitoring Board
FDA	Food and Drug Administration
GCP	Good Clinical Practice
IB	Investigator Brochure
ICF	Informed Consent Form
IDS	Investigational Drug Services
IND	Investigational New Drug
IRB	Institutional Review Board
MTD	Maximum Tolerated Dose
NCI	National Cancer Institute
PD	Progressive Disease
PI	Principal Investigator
PMT	Protocol Monitoring Team
PR	Partial Response
SAE	Serious Adverse Event
SD	Stable Disease

1.0 Goals and Objectives (Scientific Aims)

1.1 Primary Objective

- To test the hypothesis that carboplatin + nab-paclitaxel therapy will demonstrate a promising neoadjuvant pCR rate for eligible patients.
- To test the hypothesis that carboplatin + nab-paclitaxel therapy will demonstrate a promising Symmans 0-1 pathological response rate for eligible patients.

1.2 Secondary Objectives

- To evaluate the overall survival and event-free survival of eligible patients treated with carboplatin + nab-paclitaxel neoadjuvant chemotherapy.
- To evaluate the toxicities and tolerance of carboplatin + nab-paclitaxel therapy in this patient population.
- To evaluate the role of laboratory correlates in response, toxicity and survival endpoints.
    1. To procure tissue and perform analysis of gene and protein expression profiles of pre-treatment primary tumor **(**estimated success rate**:** 80%) and residual tumors (25%) and lymph nodes including the study of tumor niche (50%), studying sequential assessment of cellular characteristics and gene and protein expression profiles.
    2. To identify specific mutations in tumor DNA in comparison to adjacent tissue and germ line DNA procured prior to, during, and subsequent to neoadjuvant chemotherapy, and to detect/measure, as feasible, the presence of such mutations in fragmented circulating DNA from plasma, and to correlate these mutations with the presence/characteristics of circulating tumor cells in order to identify prognostic and predictive indicators of persisting/relapsed disease and targets for therapy.
    3. To assess RNA (using Mammaprint/Blueprint and 44,000 Agilent 3platform gene array) , miRNA and exosome and protein profiles in tumor, adjacent tissue and plasma prior to, during, and at completion of neoadjuvant chemotherapy in order to establish prognostic and predictive indicators of outcome, markers of persistent/relapsed disease, and targets for therapy.32
    4. To analyze tumor DNA and genomic DNA from plasma by microarray and RT-PCR analysis to assess copy numbers/SNP/genomic polymorphisms in genes for the purposes of establishing prognostic and predictive indicators of outcomes; markers of persistence/relapse disease, drug resistance, and drug metabolism; and targets of therapy.
    5. To assess the prognostic and predictive value of conventional pathological features (stage, estrogen and progesterone receptor and HER-2 status, presence of lymphovascular invasion, high grade tumor status) in comparison to such values derived from the molecular approaches.
    6. To procure tumor from the primary and definitive surgical specimen for the purpose of establishing breast cancer stem cell lines.

7. To procure blood samples for the purpose of identifying and characterizing circulating tumor cells.

2.0 Background

2.1 Introduction/Rationale for Development

Despite a decrease in breast cancer-related mortality, primarily due to screening/detection at an earlier stage, thousands of patients still present with locally advanced (LABC) and inflammatory breast cancer (IBC), conditions associated with high rates of recurrence and poor survival. Neoadjuvant chemotherapy (NT) is applied in the majority of such cases to allow for optimal surgical intervention, and also aiming for complete/near-complete pathologic response (pCR), a surrogate marker/predictor for longer relapse-free and overall survival **(1)**, especially in estrogen and progesterone receptor negative patients.

Standard neoadjuvant therapy for locally advanced/inflammatory breast cancer results in pathological CR (pCR) in ~ 20-25 % of patients. We have previously conducted a neoadjuvant trial comparing a novel regimen of dose-dense doxorubicin and cyclophosphamide followed by carboplatin and nab-paclitaxel versus docetaxel, doxorubicin, and cyclophosphamide (TAC), in patients with locally advanced and inflammatory HER2- breast cancer. We documented the safety of combining carboplatin and nab-paclitaxel, but found no difference between the two regimens, and the pCR rate was somewhat disappointing at < 15%, with Symmans residual cancer burden (RCB) scores of 0-1 approaching 30% with either regimen **(2, 3)**. Platinum compounds are currently being tested as potentially useful therapeutic agents in the treatment of triple negative breast cancer **(4-6)**. The combination of carboplatin and paclitaxel has been reported to be effective in triple negative breast cancer with pCR rates of ~ 60% in the neoadjuvant setting, and without the use of anthracyclines **(7)**. Nab-paclitaxel, a formulation of paclitaxel within an albumin-nanoparticle may be associated with preferential tumor uptake **(8-11)**. It has been successfully evaluated in the metastatic setting on an variety of different schedules **(12, 13)** and, when given as a single agent on a weekly schedule, it resulted in an even higher ~ 60% response rate **(14)**. In the neoadjuvant setting nab-paclitaxel has been evaluated as sequential therapy, and as part of combination with carboplatin **(15, 2)**. Hence, the proposed trial aimed at triple negative breast cancer will include carboplatin and nab-paclitaxel to further improve pCR rate, and to avoid anthracycline-associated side effects including myelodysplasia and secondary hematological malignancies.

2.2 Completed phase II randomized/stratified neoadjuvant trial

We have rapidly completed a single institution phase II randomized/stratified neoadjuvant trial **(2)** in 121 patients with LABC/IBC who received 6 cycles of docetaxel 75 mg/m^2, doxorubicin 50 mg/m^2, cyclophosphamide 500 mg/m^2 with filgrastim support (TAC, arm A) versus a novel regimen of A 60 mg/m^2 and C 600 mg/m^2 given every 2 weeks x 4, followed by 3 weekly doses of carboplatin (AUC 2) and nab-paclitaxel 100 mg/m^2 repeated as 28 day cycles x 3 (arm B). Patients with HER2 + BC received NT similar to arm B, but with the addition of 12 weekly doses of trastuzumab given together with carboplatin and nab-paclitaxel (arm C). Patients treated for HER2+ disease experienced a 48% pCR, and 62% responded with Symmans residual cancer burden (RCB) scores of 0-1 **(3)**. When tumors were assessed by the 70-gene risk-profiling tool, Mammaprint **(16)**, and the 80-gene profiling Blueprint (allowing for classification of tumors into basal [overlapping with the triple negative

phenotype], luminal, and HER2+ genotypes), pCR of 20% and 56% were observed in patients with basal and HER2+ LABC/IBCs. Mammaprint classified 100% of the basal, and 94% of the HER2+ molecular types as high risk. There was a significant correlation between the high-risk subtype, and pCR, suggesting that patients whose tumors were characterized by HER2+ and basal molecular profiles benefit most from these specific NTs. An alternative regimen developed for stage IV metastatic breast cancer (MBC) by Brown University investigators, consisting 4 cycles of carboplatin at an AUC of 6 given every 4 weeks and weekly paclitaxel 80 mg/m^2, had been tested in triple negative disease, and (in combination with trastuzumab) in HER2+ disease, with pCR rates of 67% and 76%, respectively, although the sample size was small **(7)**. Early data with carboplatin and nab-paclitaxel in combination with trastuzumab in the neoadjuvant setting suggests that such a schedule is feasible (Dr. William Sikov, verbal communication).

2.3 Carboplatin and nab-paclitaxel

Carboplatin and paclitaxel may provide superior pCR to previously tested standard regimens, and our own anthracycline-containing regimen **(2)**, with acceptable levels of short and long term toxicity, possibly including a decreased risk of secondary leukemias and cardiac problems **(17)**. ABRAXANE (nab-paclitaxel) is a biologically interactive albumin-bound paclitaxel combining a protein with a chemotherapeutic agent in the particle form. This composition allows for a novel approach of increasing intra-tumoral concentrations of the drug by a receptor-mediated transport process allowing transcytosis across the endothelial cell. This albumin-specific receptor mediated process involves the binding of albumin to a specific receptor (gp60) on the intralumminal endothelial cell membrane, resulting in activation of a protein (caveolin-1), which initiates an internalization process in the endothelial cell through the formation of caveolae, with transport of the intact albumin-bound chemotherapeutic complex via these caveolae to the underlying tumor interstitium. A protein specifically secreted by the tumor (SPARC) binds albumin, allowing release of the hydrophobic drug to the tumor cell membrane **(8-10)**.

2.4 Molecular characterization of biopsy specimens

Molecular characterization of biopsy specimens of primary tumors procured prior to exposure to neoadjuvant chemotherapy (NT) from different varieties of breast cancer subtypes, and of subsequent mid-treatment and intraoperative (procured during definitive surgery following completion of neoadjuvant therapy) samples should help to assess the predictive value of the pre-treatment and post-treatment gene and protein expression profile for complete and near complete response, as a surrogate marker for survival. Similarly, patterns of *de novo* and acquired resistance may emerge when assessment of pre- and post-treatment gene expression profiles are analyzed in a supervised manner of classification, using pathologic response as a classifier. Specimens from biopsies or surgical specimens will be preserved fresh-frozen, in RNAlater, and as FFPE, and when feasible, will also be subjected to cell sorting and inoculation in mouse fat pad to generate xenografts. DNA, RNA, and miRNA extraction, sequencing/characterization will be carried out in well-established collaboration with the City of Hope core laboratories and in collaboration with the Shiuan Chen, Karineh Petrossian laboratories, Peter Lee, Hua Yu, and Xiaochun Yu COH laboratories. External (MTA-covered or grant-supported) collaborators include TGEN (J. Trent), the Jackson Laboratory (Drs. Liu and Mengi), and and Agendia, Inc**(16)**. Additional collaboration includes (MTA-covered) NantOmics, for proteomics analysis. Circulating tumor cells (CTCs) will be assessed in collaboration with Translational Research Core or Pathology Core pending resources.

In addition, as an exploratory and hypothesis-generating investigation, tumor samples will ne assessed by immunohistochemistry and by H&E for expression of AR, tumor infiltrating lymphocytes, and

markers of mesenchymal/stem-cell like (vimentin, ALDH staining through the anatomic pathology core) properties. (23-25).

2.5 Overview of proposed study

We hypothesize, that nab-paclitaxel, based on its favorable toxicity profile and efficacy when compared to cremophor-based paclitaxel in a randomized phase III trial in metastatic breast cancer, may further improve efficacy combinations including carboplatin. A combination of carboplatin, given on day one of a 28-day cycle, and cremophor-based weekly paclitaxel reported to be quite effective when used in locally advanced and metastatic triple negative disease. The weekly schedule of nab-paclitaxel (when compared to either docetaxel, cremophor-based paclitaxel) yielded favorable response rates at doses 100 and 100 mg/m2, as a single agent. Our recently completed neoadjuvant trial demonstrated the feasibility of combining carboplatin weekly, and nab-paclitaxel weekly **(2)**. Reducing the frequency of carboplatin-administration, while giving nab-paclitaxel weekly, is likely to decrease the potential for dose-frequency-related, well-described risks of developing carboplatin-related allergy, while maintaining/impoving efficacy.

This study will be conducted in compliance with the protocol, Good Clinical Practice (GCP) and the applicable regulatory requirements.

3.0 Patient Eligibility

3.1 Inclusion Criteria: To be eligible, patients must meet all of these criteria.

3.1.1 Disease Status

Patients must be diagnosed with locally advanced (T2 and higher with or without lymph node involvement), and/or inflammatory triple negative breast cancer.

3.1.2 Age Criteria and Life Expectancy

Greater than 18 years of age

3.1.3 Child Bearing Potential

The effects of the proposed therapeutic agents (carboplatin, nab-paclitaxel) on the developing fetus are unknown. For this reason, women of child-bearing potential and men must agree to use adequate contraception (hormonal or barrier method of birth control or abstinence} prior to study entry and for six months following duration of study participation. Should a woman become pregnant or suspect that she is pregnant while participating on the trial, she should inform her treating physical immediately.

3.1.4 Protocol-Specific Criteria

- Tumor negative for expression of hormone receptors (< 10%) and not overexpressing HER2 by IHC (0-1), or, in case of IHC of 2, negative by FISH or by alternative gene testing.
- Bilirubin $\leq$ 1.5 mg/dL

- Patients must have adequate liver function: AST and ALT ≤ 2 X upper limit of normal, alkaline phosphatase ≤ 2 X upper limit of normal
- Patients must have adequate bone marrow function: Platelets ≥100,000 cells/mm3, Hemoglobin > 9.0g/dL and ANC ≥ 1,500 cells/mm^3
- Patients must have adequate renal function: creatinine ≤1.5 mg/dL is recommended; however, institutional norms are acceptable.
- Left ventricular ejection fraction >50%
- Women of childbearing potential and sexually active males must use an effective contraception method during treatment and for three months after completing treatment
- Negative serum or urine β-hCG pregnancy test at screening for patients of childbearing potential

3.1.5 Informed Consent/Assent

All subjects must have the ability to understand and the willingness to sign a written informed consent.

3.1.6 Prior Therapy

No prior therapies are allowed for the treatment of the newly diagnosed breast cancer. Patients with a prior diagnosis of malignancy treated ≥ 5 years ago are eligible, provided that they have not received prior taxanes or carboplatin as part of their prior treatment regimen, and that they meet all eligibility criteria.

3.2 Exclusion Criteria: A patient is rendered ineligible if any of these criteria applies.

3.2.1 Study-Specific Exclusions

1) Known active Hepatitis B or C (due to the potential for disease/treatment-related pharmacological and liver function-specific interactions).
2) Known active HIV (due to the complexity and potential pharmacological interactions between the standard neoadjuvant therapeutic agents, and HAART).
3) Prior breast cancer or other invasive malignancy treated within 5 years.
4) Pregnancy
5) Neuropathy > grade 1
6) Any other intercurrent medical/psychological problem deemed exclusionary by the treating physician or investigators/PI.

3.2.2 Non-Compliance

Subjects will be excluded who, in the opinion of the investigator, may not be able to comply with the safety monitoring requirements of the study.

3.3 Inclusion of Women and Minorities

The study is open to anyone regardless of ethnicity; however, breast cancer is rare in men compared to its incidence in women. Female subjects of all racial/ethnic groups are eligible for this study if they

meet the eligibility criteria specified in sections 3.1 and 3.2. To address disparities in healthcare and breast cancer treatment among women from underserved populations, all efforts will be made to accrue subjects from ethnically diverse, underserved and minority populations. While efforts will be made to extend the accrual to a representative population, in a trial which will accrue 40 fully evaluable subjects, a balance must be struck between subject safety considerations and limitations on the number of individuals exposed to potentially toxic or ineffective treatments on the one hand and the need to explore gender, racial, and ethnic aspects of clinical research on the other. If differences in outcome that correlate to racial or ethnic identity are noted, accrual may be expanded or additional studies may be performed to investigate those differences more fully.

City of Hope Breast Patients , 2009								
		By Sex		**By Ethnicity**				
Site	Total	Female	Male	White	Hispanic	Black	Asian	Other
Breast	477	476(99%)	1 (1%)	255 (53%)	120 (25%)	38 (8%)	63 (13%)	1 (1%)

Accrual Goal for Women and Minorities on this Study								
		By Sex		**By Ethnicity**				
Site	Accrual Goal	Female	Male	White	Hispanic	African-American	Asian	Other
Breast	45	45 (100%)	0 (0%)	20 (44%)	12 (27%)	4 (9%)	8 (18%)	1 (2%)

4.0 Registration Procedures

4.1 To register a patient, the treating physician should contact the protocol nurse or the responsible Clinical Research Associate (CRA) in Clinical Trial Office (CTO) to complete the eligibility/registration form. The protocol nurse or CRA will contact the Data Coordinating Center at the City of Hope (626-256-4673, ext. 64267 or e-mail dcc@coh.org), EMAIL a copy of the completed eligibility checklist, required pre-study tests (per protocol – and may include laboratory, CT and pathology reports), signed Informed Consent, signed Patients' Bill of Rights and HIPAA authorization form to dcc@coh.org.

The patient registration process will be handled by the Department of Clinical Research Information Support (CRIS) Data Coordinating Center (DCC) at City of Hope. Documentation of current IRB approval must be on file with the DCC prior to registration of patients on this study for participating institution.

The steps below are to be taken when registering a **patient:**

- The research staff must assure they have the most current and updated version of the protocol and informed consent prior to enrolling a patient. If a question arises, please contact the Data Coordinating Center at 626-256-4673 extension 64267 or via email at dcc@coh.org.
- The study staff must assure that all prestudy laboratory tests, scans and x-rays have been completed prior to registration according to the study calendar
- The study staff must assure that the patient has signed an approved informed consent prior to registration/randomization, including the Experimental Subject Bill of Rights and appropriate HIPAA authorization.
- The study staff must confirm that the patient meets all inclusion and exclusion eligibility criteria for the protocol. The eligibility checklist (provided by the COH DCC) must be completed in its entirety.
- Patients must be registered prior to initiation of treatment but no more than 5 working days prior to planned start of treatment. A patient failing to meet all protocol requirements may not be registered.
- Once a patient is eligible, all the pre-study requirements have been fulfilled, and the informed consent obtained, the research nurse or the data manager (study coordinator) will inform the COH Data Coordinating Center at (626) 256-4673, extension 64267; email dcc@coh.org and FAX (fax number 626 256-8794) a copy of the patient's signed informed consent, , completed eligibility checklist and corresponding source documentation confirming eligibility (including pathology reports, lab reports, x-ray reports, etc.).

The City of Hope Data Coordinating Center will:

- Review all materials/source documentation to ensure the patient is eligible.
- Ensure the consent form is valid and is signed correctly by all parties. If additional information is needed or should there be any questions, the Data Coordinating Center will immediately contact the study staff and registration will not occur until all issues are resolved.
- If there are questions regarding exceptions to the eligibility criteria, please contact he study Principal Investigator, as well as the COH DCC. Documentation of IRB approval of exception will need to be submitted as well as the COH DCC.
- Confirmation of Registration will be emailed/faxed to the study staff noting the patient's study number within 24 hours post receipt of a complete eligibility packet.
- The COH DCC will call the research nurse or data manager (study coordinator) and verbally confirm the registration (if needed).
- If the patient does not receive protocol therapy following registration, the patient's registration on the study may be cancelled. The COH DCC should be notified of cancellations as soon as possible.

The COH DCC will log into the Electronic Data Capture (EDC) system and enter the patient's study number.

4.2 Informed Consent

Diagnostic or laboratory studies performed exclusively to determine eligibility for this trial will be done only after obtaining written informed consent. Studies or procedures that were for clinical indications (not exclusively to determine study eligibility but consistent with good medical practice [GMP]) may be used for baseline values, even if the studies were done before informed consent was obtained. Reference is made to Section 10.0 – Study Calendar.

The investigational nature and objectives of the trial, the procedures and treatments involved and their attendant risks and discomforts, and potential alternative therapies will be carefully explained to the subject and a signed informed consent will be obtained. Documentation of informed consent for screening will be maintained in the subject's research chart and medical record.

4.3 Dose Assignment

Patients will receive carboplatin AUC of 6 on day 1 of a 28-day cycle, nab-paclitaxel, 100 mg/m2 weekly.

Dose reductions can occur as medically necessary as described below in section 6.0.

5.0 Treatment Program

5.1 Treatment Overview

Treatments are planned to be delivered in the outpatient setting.

Management and dose modification associated with adverse events are outlined in **Section 6**.

Off label: This proposal includes a commercial agent for use in an investigational setting/trial.

5.1.1 Dose summary

Carboplatin at AUC at 6 every 4 weeks (28 day cycles x 4).

Nab-Paclitaxel 100 mg/m^2 weekly x 16 weeks (28 day cycles x 4).

5.1.2 Details

For details of nab-paclitaxel dosing and administration, see Agent Information, section 8.

Carboplatin will be administered as an intravenous infusion over 30 minutes.

Carboplatin dose (mg) = 6 X (GFR + 25)

(Calculated total dose is in mg -not mg/m^2)

The Creatinine Clearance (to replace GFR) will be calculated for each treatment course using the formula:

For Females:

***Important: The serum creatinine level to be used in the following calculations must be greater than or equal to 0.7. If the measured serum creatinine level is less than 0.7, use 0.7 as the value in this calculation.**

$$\text{CrCI} = \frac{(140\text{-age}) \times \text{wt. in kg.} \times 0.85}{72 \times \text{serum creatinine*}}$$

Use calculated creatinine clearance for GFR in Calvert formula.

Note: Remember to re-calculate the dose for each treatment cycle. The actual body weight should be used for all calculations. **If** the actual weight is greater than 1.2 times the ideal body weight, use 1.2 times the ideal body weight as the value in this calculation.

Note: The GFR (calculated by Cockcroft-Gault or any other means using creatinine) used in the Calvert formula to calculate AUC-based dosing should not exceed 125 mL/min under any circumstance.

By definition, this results in the following upper limits on the dose to be administered, by AUC target:

AUC target (mg•min/mL)	Maximum carboplatin dose (mg)
2	300
3	450
4	600
5	750
6	900

5.1.3 Criteria for Starting Subsequent Cycles

Patients should meet the laboratory parameters and the performance status as outlined in section 3.1. before initiation of each cycle of therapy. All toxicities (except alopecia and lymphopenia, hyperglycemia, hypoalbuminemia, elevated serum alkaline phosphatase) should have resolved to grade 1 or lesser severity before initiation of the next cycle of therapy.

Qualifying laboratory tests and procedures can be obtained up to 72 hours before planned initiation of therapy from the 2nd cycle onwards.

5.2 Planned Duration of Therapy

Four cycles, approximately 16 weeks.

5.3 Criteria for Removal from Treatment

5.3.1 Criteria for Removal

Removal of a patient from treatment will be based on the following: unacceptable toxicities as assessed by the treatment team based on the grade, duration, need for dose adjustment/modification of treatment; patient desire; or disease progression.

5.3.2 Subject Follow-Up

All patients enrolled and having received at least 1 dose of therapy will be evaluated for toxicity. Patients will be followed for an indefinite period of time after enrollment. Subsequent to the 5-year mark, standard follow-up measures per tumor registry guidelines will apply.

5.4 Supportive Care and Other Concomitant Therapy

Supportive care will be at the treating physician's discretion, and in line with standard practice. For usage of antiemetics, granulocyte and erythrocyte growth factors, physicians are advised to follow ASCO and/or NCCN guidelines, as appropriate.

5.5 Laboratory Studies

See Section 10. Study Calendar

.

5.6 Additional Studies

Reference is made to Section 9.0 for any correlative studies to be conducted under this protocol.

6.0 Dose Delays/Modifications for Adverse Events

6.1 Modifications due to toxicity during second and subsequent cycles

Dose adjustments are to be made according to the organ system showing the greatest degree of toxicity. The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 4.0. A copy of the CTCAE version 4.0 can be downloaded from the CTEP web site:

http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm#ctc_40

Therapy can be held until initiation of the next cycle of therapy, which may be delayed no more than two weeks to allow recovery from toxicity. Treatment delay of > 2 weeks due to toxicity will lead to removal of the patient from the study.

6.2 Carboplatin dose modifications for hematological toxicity

6.2.1 Neutrophils

The following dose adjustments are based on the neutrophil nadir of the preceding treatment course.

Absolute Granulocyte Count Nadir	**Carboplatin**
Grade 3 (without fever)	No change
Grade 4 (without fever) 1st episode	Reduce AUC by 1 level and consider G-CSF support
2nd episode	Reduce AUC by 2 level And use G-CSF
3rd episode	Reduce AUC by 2 level And use G-CSF
4th episode	Off study
Grade 3/4 (with fever) 1st episode	Reduce AUC by 1 level And use G-CSF
2nd episode	Reduce AUC by 2 level And use G-CSF
3rd episode	Off study

6.2.2 Platelets

Nadir Platelet count	**Carboplatin**
Grade 3/4 1st episode	Reduce AUC by 1 level
2ndepisode	Reduce AUC by 2 levels
3rd episode	Reduce AUC by 2 levels
4rth episode	Off study

6.2.3 Dose Delay according to hematologic recovery:

The hematologic parameters must meet the criteria specified in section 3.1.4 of the protocol on day 1 of each treatment cycle. Treatment of both drugs should be delayed until recovery of the counts to the specified eligibility levels. Delay in treatment of more than 2 weeks due to toxicity will result in removal of the patient from the study.

6.2.4 Colony Stimulating Factors

Patients should not routinely receive prophylactic colony stimulating factors (e.g., G-CSF; GM-CSF) during cycle 1, unless the use is following the recommendations in section 6.1.1. Subsequent use will be at the discretion of the treating physician.

6.3 Carboplatin dose modifications for non-hematological toxicity

6.3.1 Gastrointestinal Toxicity

Nausea and/or vomiting should be controlled with adequate antiemetic therapy. Prophylactic anti-emetic therapy can be used at the discretion of the treating physician. Patients are encouraged to take plenty of oral fluids. If symptoms persist despite maximal anti-emetic therapy, treatment should be withheld until recovery to ≤ grade 1.

Diarrhea should be managed with appropriate anti-diarrheal therapy. Patients should be encouraged to take plenty of oral fluids. If symptoms do not decrease to grade 1 or less with adequate anti-diarrheal therapy, treatment should be held until recovery from symptoms to ≤ grade 1.

6.3.2 Hypersensitivity Reactions

Caution: Patients who had a mild to moderate hypersensitivity reaction have been successfully rechallenged, but careful attention to prophylaxis and bedside monitoring of vital signs is recommended.

Mild symptoms

(e.g., mild flushing, rash, pruritus) - **Complete infusion.** Supervise at bedside. No treatment required.

Moderate symptoms

(e.g., moderate rash, flushing, mild dyspnea, chest discomfort) - **Stop infusion.** Give intravenous diphenhydramine 25 mg and intravenous dexamethasone 10 mg. Resume infusion after recovery of symptoms at a low rate, 20 mg/hr. For 15 minutes, then, if no further symptoms, at full dose rate until infusion is complete. If symptoms recur, stop infusion. Record toxicity on flow sheets.

Severe life threatening symptoms

(e.g., hypotension requiring pressor therapy, angioedema, respiratory distress requiring bronchodilation therapy, generalized urticaria) - **Stop infusion**. Give intravenous diphenhydramine and dexamethasone as above. Add epinephrine or bronchodialtors if indicated. If wheezing is present, that is not responsive to bronchodilators, epinephrine is recommended. Patient should be removed from further protocol therapy. **Report as an adverse event.**

6.3.3 Other Toxicities

For any grade 3 or 4 toxicity not mentioned above, withhold both drugs until the patient recovers to grade 1 or less toxicity. The treatment should then be resumed at one lower dose level for carboplatin and same dose for Abraxane. For grade 2 toxicities, withhold treatment until the patient recovers, then resume treatment at a one dose-level reduction for carboplatin (permanent dose reduction) and same dose for Abraxane. For grade 1 toxicities, no dose reduction should be made.

6.4 Nab-paclitaxel (Abraxane) dose modifications

Refer to section 8.2 for dose calculation of nab-paclitaxel

ABRAXANE dosing should not be administered at the start of each cycle until the absolute neutrophil count returns to ≥1.5 x 10^9 cells/L and the platelet count returns to ≥100 x 10^9 cells/L. For patients receiving weekly ABRAXANE, for each subsequent dose of ABRAXANE within a cycle (Days 8, 15, 22), patients must have an ANC ≥ 1.0 x 10^9 cells/L and platelets ≥ 75 x 10^9 cells/L. If the ANC and platelets are not adequate for treatment on Day 8, 15, and/or 22, the dose will be omitted and the total cycle length remains the same.

6.4.1 Administration of Study Drug to Patients with Abnormal Hepatic Function

Study drug should only be administered if hepatic function is within the parameters established in the eligibility criteria. Hepatic toxicity from taxanes may occur but it is uncommon. Therefore, hepatic dysfunction that occurs while the patient is on study should prompt an evaluation to determine the cause, including the possibility of progressive metastatic disease and hepatotoxicity from concurrent medications.

6.4.2 Dose Modification Table

Use this table as a guideline to determine any necessary dose modifications. The modification is dependent on the starting dose for the study.

Table 2: Dose Modification

Dose Level	ABRAXANE (mg/m^2)
0	100
-1	80
-2	60

Dose Reductions and guidelines for optional use of Growth Factors for Hematologic Toxicity

The table below provides a guideline for implementing dose reductions and optional use of growth factor treatment for hematologic toxicity:

Table 3: Use of G-CSF and Dose reductions for Hematologic Toxicity

Adverse Event	Occurrence	Action to be Taken

ANC < 500 cells/mm^3 (nadir count) with neutropenic fever > 38° OR Delay of next cycle due to persistent neutropenia (ANC < 1500 cells/mm^3) OR For patients on weekly treatment whose next treatment within the cycle (Day 8 or Day 15) is omitted due to persistent neutropenia (ANC < 1000 cells/mm^3). OR Neutropenia < 500 cells/mm^3 for > 1 week	Any Occurrence	At the first occurrence of a hematological toxicity (as outlined in the Adverse Event column), the same dose is maintained and G-CSF is given as outlined below. In the event that a hematological toxicity re-occurs in the face of G-CSF, dose reduction to the next lower level will be required for subsequent cycles once ANC is ≥ 1500 cells/mm^3. If G-CSF is given concurrently with weekly ABRAXANE, administration may begin the day after ABRAXANE is given and should stop at least 48 hours prior to when ABRAXANE is given the following week. If a delay of more than 2 weeks is necessary even with the use of neutrophil growth factors and after dose adjustment to dose level minus 2, the patients will be taken off study
Thrombocytopenia Grade 3 or Grade 4*	1st Occurrence	Dose reduction to next lower level
	Recurrence	Dose reduction to next lower level

*See NCI Toxicity Criteria Scale for definition of Grade 3 and Grade 4 events.

G-CSF Administration

For QW study drug administration administer G-CSF 5 mcg/kg/day (rounded to the nearest vial size per investigator/institution's standard of care) 24 hours after chemotherapy and hold 48 hours prior to the next dose

For Q3W study drug administration, administer G-CSF 5 mcg/kg/day (rounded to the nearest vial size per investigator/institution's standard of care) 24 hours after chemotherapy until recovery to the predetermined neutrophil count.

Sensory Neuropathy

ABRAXANE should be withheld in patients who experience ≥ Grade 3 sensory neuropathy. Treatment may be resumed at the next lower dose level (see Table 2) in subsequent cycles after the sensory neuropathy improves to ≤ Grade 1. The time to resolution to Grade ≤ 1 should be the adverse event duration used for adverse event reporting. In those patients who experience Grade 4 sensory neuropathy, study drug should be withheld, and treatment resumed at a reduction of 2 dose levels (Dose Level -2; see Table 2) in subsequent cycles after the sensory neuropathy improves to ≤ Grade 1. Note: the investigator may elect to dose modify for Grade 3 sensory neuropathy.

Hypersensitivity Reactions

Hypersensitivity reactions rarely occur. If they do occur, minor symptoms such as flushing, skin reactions, dyspnea, lower back pain, hypotension, or tachycardia may require temporary interruption of the infusion. However, severe reactions, such as hypotension requiring treatment, dyspnea requiring bronchodilators, angioedema or generalized urticaria require immediate discontinuation of study drug administration and aggressive symptomatic therapy. Patients who experience a severe hypersensitivity reaction to ABRAXANE should not be re-challenged. It is not recommended to administer ABRAXANE to patients with prior hypersensitivity to a taxane.

Other Toxicities

If toxicities are ≥ grade 3, except for anemia, treatment should be withheld until resolution to ≤ grade 1 or baseline if baseline was greater than grade 1, then reinstituted, if medically appropriate, at the next lower dose level (see Table 2).

7.0 Data and Safety Monitoring

7.1 Definition of Risk Level

This is a Risk Level 3 study, as defined in the "City of Hope Data and Safety Monitoring Plan", http://www.coh.org/dsmc/Pages/forms-and -procedures.aspx because it is a Phase II clinical trial where the risks are at least balanced by the potential benefit to subjects and the importance of the knowledge that may result.

7.2 Monitoring and Personnel Responsible for Monitoring

The Protocol Management Team (PMT) consisting of the PI, at least two Collaborating Investigators, CRA, protocol nurse, and statistician is responsible for monitoring the data and safety of this study, including implementation of any stopping rules for safety and efficacy.

Data and safety will be reported to the COH DSMC using the PMT report and submitted according to the timelines in Table 1 below. Protocol specific data collection will include the following items: summary of accrual, adverse events and treatment related mortality.

Table 1: City of Hope PMT Reporting Timelines for the DSMC

Risk Level	Phase	Standard Reporting Requirement
RL 1, RL2, and Expanded Access Studies	No reports required	
3	I	Every 3 months from activation date, as indicated in MIDAS
3	Pilot, Feasibility, II-IV	Every 6 months from activation date, as indicated in MIDAS
4	Pilot, Feasibility, I-IV	Every 3 months from activation date, as indicated in MIDAS

7.3 Definitions

Adverse event (AE) - An adverse event is any untoward medical experience or change of an existing condition that occurs during or after treatment, whether or not it is considered to be related to the protocol intervention.

Unexpected Adverse Event [21 CFR 312.32 (a)] - An adverse event is unexpected if it is not listed in the investigator's brochure and/or package insert; is not listed at the specificity or severity that has been observed; is not consistent with the risk information described in the protocol and/or consent; is not an expected natural progression of any underlying disease, disorder, condition, or predisposed risk factor of the research participant experiencing the adverse event.

Expected Adverse Event - Any event that does not meet the criteria for an unexpected event OR is an expected natural progression of any underlying disease, disorder, condition, or predisposed risk factor of the research participant experiencing the adverse event.

Serious Adverse Event (SAE) [21 CFR 312.32] - defined as any expected or unexpected adverse event that results in any of the following outcomes:

- Death
- Is life-threatening experience (places the subject at immediate risk of death from the event as it occurred)
- Unplanned hospitalization (equal to or greater than 24 hours) or prolongation of existing hospitalization
- A persistent or significant disability/incapacity
- A congenital anomaly/birth defect
- Secondary malignancy
- Any other adverse event that, based upon appropriate medical judgment, may jeopardize the subject's health and may require medical or surgical intervention to prevent one of the outcomes listed above (examples of such events include allergic bronchospasm requiring intensive treatment in the emergency room or at home, blood dyscrasias of convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse).

Unanticipated problem (UP) – Any incident, experience, or outcome that **meets all three** of the following criteria:

1. Unexpected (in term nature, severity, or frequency) given the following: a) the research procedures described in the protocol-related documents such as the IRB approved research protocol, informed consent document or Investigator Brochure (IB); and b) the characteristics of the subject population being studied; **AND**
2. Related or possibly related to participation in the research (possibly related means there is a reasonable possibility that the incident, experience, or outcomes may have been caused by the drugs, devices or procedures involved in the research); **AND**
3. Suggests that the research places subjects or others at greater risk of harm (including physical, psychological, economic, or social harm) than previously known or recognized.

7.4 Reporting of Unanticipated Problems and Adverse Events

Unanticipated Problems - Most unanticipated problems must be reported to the COH DSMC and IRB **within 5 calendar days** according to definitions and guidelines at http://www.coh.org/policy/Policies%20and%20Procedures/REVIEWING_AND_REPORTING_UNANTICIPATED_PROBLEMS.pdf. Any unanticipated problem that occurs during the study conduct will be reported to the DSMC and IRB by submitting electronically in iRIS (http://iris.coh.org).

Serious Adverse Events - All SAEs occurring during this study, whether observed by the physician, nurse, or reported by the patient, will be reported according to definitions and guidelines at http://www.coh.org/policy/Policies%20and%20Procedures/REVIEWING_AND_REPORTING_UNANTICIPATED_PROBLEMS.pdf and Table 2 below. Those SAEs that require expedited reporting will be submitted electronically in iRIS (http://iris.coh.org).

Adverse Events - Adverse events will be monitored by the PMT. Adverse events that do not meet the criteria of *serious* OR are not unanticipated problems will be reported only in the protocol continuation reports and PMT report (see Table 2 below).

Table 2: City of Hope Adverse Event and Unanticipated Problem Reporting Timelines for the DSMC and IRB

DSMC Risk Level 3 and Risk Level 4 Protocol Reporting Timelines

Required Reporting Timeframe to the DSMC		
Attribution	**Unexpected**	**Expected**
Death while on active treatment or within 30 days of last day of treatment		
Possibly, Probably, Definitely	5 calendar days	
Unlikely, Unrelated		
Death after 30 days of last active treatment/therapy		
Possibly, Probably, Definitely	5 calendar days	No reporting required
Unlikely, Unrelated	No reporting required	No reporting required
Within 30 days of last active treatment/therapy		
	Grades 3 and 4 AND meeting the definition of "serious"	
Possibly, Probably, Definitely	5 calendar days	10 calendar days

Required Reporting Timeframe to the DSMC		
Attribution	**Unexpected**	**Expected**
Unlikely, Unrelated	5 calendar days	10 calendar days
	Grades 1 and 2 AND resulting in "hospitalization"	
Possibly, Probably, Definitely	10 calendar days	10 calendar days
Unlikely, Unrelated	10 calendar days	10 calendar days
After 30 days of last active treatment/therapy		
	Grades 3 and 4 AND meeting the definition of "serious"	
Possibly, Probably, Definitely	10 calendar days	10 calendar days
Unlikely, Unrelated	No reporting required	No reporting required
	Grades 1 and 2 AND resulting in "hospitalization"	
Possibly, Probably, Definitely	10 calendar days	10 calendar days
Unlikely, Unrelated	No reporting required	No reporting required

DSMC Risk Level 1 and Risk Level 2 Protocol Reporting Timelines

Required Reporting Timeframe to DSMC		
Attribution	**Unexpected**	**Expected**
	Death while on active treatment or within 30 days of last day of treatment	
Possibly, Probably, Definitely	5 calendar days	5 calendar days
Unlikely, Unrelated	No reporting required	No reporting required
	Death after 30 days of last active treatment/therapy	
Possibly, Probably, Definitely	5 calendar days	No reporting required
Unlikely, Unrelated	No reporting required	No reporting required
	Grades 3 and 4 AND meeting the definition of "serious"	
Possibly, Probably, Definitely	5 calendar days	10 calendar days
Unlikely, Unrelated	No reporting required	No reporting required
	Grades 1 and 2 AND resulting in "hospitalization"	
Possibly, Probably, Definitely	5 calendar days	10 calendar days
Unlikely, Unrelated	No reporting required	No reporting required

COH IRB Adverse Event Reporting Timelines

Required Reporting Timeframe to COH IRB		
Attribution	**Unexpected**	**Expected**
	Death while on active treatment/therapy or within 30 days of the last day of active treatment/therapy	
Possibly, Probably, Definitely	5 calendar days[1]	Annual
Unlikely, Unrelated	Annual	Annual
	Grades 3 and 4	
Possibly, Probably, Definitely	5 calendar days[1]	Annual
Unlikely, Unrelated	Annual	Annual
	Grade 1 and 2	
Possibly, Probably, Definitely	5 calendar days[1]	Annual[2]
Unlikely, Unrelated	Annual[2]	Annual[2]

[1] These events must be reported in the time frame if they meet the definition of an unanticipated problem.

[2] For studies that are not first in human, Phase I and first in pediatric trials, only grades 3-5 must be reported at annual review.

7.4.1 Abnormal Laboratory Values

An abnormal laboratory value is considered to be an AE if the abnormality:

- results in discontinuation from the study;
- requires treatment, modification/ interruption of IP dose, or any other therapeutic intervention; or
- Is judged to be of significant clinical importance.

Regardless of severity grade, only laboratory abnormalities that fulfill a seriousness criterion need to be documented as a serious adverse event.

If a laboratory abnormality is one component of a diagnosis or syndrome, then only the diagnosis or syndrome should be recorded on the AE page/screen of the CRF. If the abnormality was not a part of a diagnosis or syndrome, then the laboratory abnormality should be recorded as the AE.

7.4.2 Pregnancy

Females of Childbearing Potential:

Pregnancies and suspected pregnancies (including a positive pregnancy test regardless of age or disease state) of a female subject occurring while the subject is on IP, or within 28 days of the subject's last dose of IP, are considered immediately reportable events. IP is to be discontinued immediately. The pregnancy, suspected pregnancy, or positive pregnancy test must be reported to

Celgene Drug Safety immediately by facsimile, or other appropriate method, using the Pregnancy Initial Report Form, or approved equivalent form.

The female subject may be referred to an obstetrician-gynecologist (not necessarily one with reproductive toxicity experience) or another appropriate healthcare professional for further evaluation.

The Investigator will follow the female subject until completion of the pregnancy, and must notify Celgene Drug Safety immediately about the outcome of the pregnancy (either normal or abnormal outcome) using the Pregnancy Follow-up Report Form, or approved equivalent form.

If the outcome of the pregnancy was abnormal (e.g., spontaneous or therapeutic abortion), the Investigator should report the abnormal outcome as an AE. If the abnormal outcome meets any of the serious criteria, it must be reported as an SAE to Celgene Drug Safety immediately by facsimile, or other appropriate method, within 24 hours of the Investigator's knowledge of the event using the SAE Report Form, or approved equivalent form.

All neonatal deaths that occur within 28 days of birth should be reported, without regard to causality, as SAEs. In addition, any infant death after 28 days that the Investigator suspects is related to the in utero exposure to the IP should also be reported to Celgene Drug Safety immediately by facsimile, or other appropriate method, within 24 hours of the Investigator's knowledge of the event using the SAE Report Form, or approved equivalent form.

7.5Additional Reporting Guideline for Celgene

The Sponsor-Investigator will utilize the FDA MedWatch program for the reporting of adverse events and follow up information to those events. Full information regarding these procedures is described on the FDA website. (http://www.fda.gov/medwatch/).

The Sponsor-Investigator will also utilize the Celgene SAE Completion Form for the reporting of adverse events and follow up information to those events.

All serious adverse events regardless of severity or relationship must be reported to Celgene Corporation within 24 hours of the investigational staff's knowledge.

Celgene Corporation

Drug Safety Department

86 Morris Avenue

Summit, NJ 07901

Fax: (908) 673-9115

E-mail: drugsafety@celgene.com

and

Industry Contact:

Norma Powers

Director, Medical Operations

Celgene Corporation

86 Morris Avenue

Summit, NJ 07901

Mobile: 267-337-2720

Fax: 908-673-2779

Email: npowers@celgene.com

Any AE that meets any criterion for an SAE requires the completion of an SAE Report Form in addition to being recorded on the AE page/screen of the CRF. All SAEs must be reported to Celgene Drug Safety within 24 hours of the Investigator's knowledge of the event by facsimile, or other appropriate method, using the SAE Report Form, or approved equivalent form. This instruction pertains to initial SAE reports as well as any follow-up reports.

The Investigator is required to ensure that the data on these forms is accurate and consistent. This requirement applies to all SAEs (regardless of relationship to IP) that occur during the study (SAE's will be reported from the time the patient starts treatment to at least 28 days after the last dose of investigational product.) , and those made known to the Investigator at anytime thereafter that are suspected of being related to IP. SAEs occurring prior to treatment will be captured.

The SAE report should provide a detailed description of the SAE and include summaries of hospital records and other relevant documents. If a subject died and an autopsy has been performed, copies of the autopsy report and death certificate are to be sent to Celgene Drug Safety as soon as these become available. Any follow-up data will be detailed in a subsequent SAE Report Form, or approved equivalent form, and sent to Celgene Drug Safety.

Where required by local legislation, the Investigator is responsible for informing the IRB/EC of the SAE and providing them with all relevant initial and follow-up information about the event. The Investigator must keep copies of all SAE information on file including correspondence with Celgene and the IRB/EC.

Safety Queries

Queries pertaining to SAEs will be communicated from Celgene Drug Safety to the site via facsimile or electronic mail. The response time is expected to be no more than five (5) business days. Urgent queries (e.g., missing causality assessment) may be handled by phone.

Expedited Reporting of Adverse Events

For the purpose of regulatory reporting, Celgene Drug Safety will determine the expectedness of events suspected of being related to the IP based on the Investigator Brochure.

Celgene or its authorized representative shall notify the Investigator of the following information:

- Any AE suspected of being related to the use of IP in this study or in other studies that is both serious and unexpected (i.e., SUSAR);
- Any finding from tests in laboratory animals that suggests a significant risk for human subjects including reports of mutagenicity, teratogenicity, or carcinogenicity.

- In Japan, measures taken in foreign countries to ensure patient safety, study reports that indicates potential risk of cancer, etc., or biannual SAE report according to the local regulations.

Where required by local legislation, the Investigator shall notify his/her IRB/EC promptly of these new serious and unexpected AE(s) or significant risks to subjects.

The Investigator must keep copies of all pertinent safety information on file including correspondence with Celgene and the IRB/EC.

Celgene Drug Safety Contact Information:

Celgene Corporation

Drug Safety
86 Morris Avenue
Summit, N.J. 07901
Toll Free: (800)-640-7854
Phone: (908) 673-9667
Fax: (908) 673-9115
E-mail: drugsafety@celgene.com

8.0 Agent Information

8.1 Carboplatin (Paraplatin®) (NSC-241240) (CBDCA)

Carboplatin (CBDCA) is a hydrophilic platinum coordination compound and is an analog of cisplatin, producing intrastrand DNA cross-links.

8.1.1 Risks and Contraindications

Human Toxicology: Side effects of carboplatin (CBDCA) include myelosuppression, nausea, vomiting, abdominal pain, diarrhea and constipation. Other toxicities include allergic reaction (including hypersensitivity, i.e., rash, urticaria, erythema, pruritus, bronchospasm and hypotension), peripheral neuropathy, paresthesia, loss of hair, hearing loss, visual disturbances and change in taste. Serum creatinine elevations and blood urea elevations have occurred, as well as abnormal liver function tests and decreased serum electrolyte values. Although rare, pain, asthenia, cardiovascular, respiratory, genitourinary and mucosal side effects have occurred in some patients. Cancer-associated hemolytic uremic syndrome has been reported rarely.

Carboplatin may cause fetal harm; therefore women of childbearing potential should be advised to avoid becoming pregnant.

Contraindications:

Carboplatin is contraindicated in patients with a history of severe allergic reactions to cisplatin or other platinum-containing compounds or mannitol.

This drug should not be used in patients with severe bone marrow depression or significant bleeding.

8.1.2 Concomitant Medications/Precautions

The renal effects of nephrotoxic compounds may be potentiated by carboplatin.

The occurrence of acute leukemia has been reported rarely in patients treated with anthracycline/alkylator combination chemotherapy.

8.1.3 Dose and Administration

Administration of Carboplatin is intravenous.

See section 5.1

8.1.4 Storage and Stability

Unopened vials of carboplatin for injection are stable for the life indicated on the package when stored at controlled room temperature 15–30 ˚C, and protected from light. When reconstituted as directed, the solution of carboplatin exhibits no decomposition for 8 hours at room temperature (25 ˚C).

Like cisplatin, this drug should not be given through aluminum needles.

Caution: The single-use lyophilized dosage form contains no antibacterial preservatives. Therefore, it is advised that the reconstituted product be discarded eight hours after dilution.

8.1.5 Structure and Molecular Weight

Chemical name: platinum, diammine [1,1-cyclobutane-dicarboxylato(2-)-0,0']-, (SP-4-2).

Molecular formula: C6H12N2O4Pt

Molecular weight: 371.25

8.1.6 Formulation/Agent Preparation

Carboplatin is supplied as a sterile lyophilized powder available in single-dose vials containing 50 mg, 150 mg and 450 mg of carboplatin for administration by intravenous injection. Each vial contains equal parts by weight of carboplatin and mannitol. Immediately before use, the content of each vial must be reconstituted with either Sterile Water for Injection, USP, 5% Dextrose in Water, or 0.9% Sodium Chloride Injection, USP, according to the following schedule:

Vial Strength	**Diluent Volume**
50 mg	5 mL
150 mg	15 mL
450 mg	45 mL

These dilutions all produce a carboplatin concentration of 10 mg/ml. Carboplatin can be further diluted to concentrations as low as 0.5 mg/ml with 5% Dextrose in Water or 0.9% Sodium Chloride Injection, USP (NS).

8.1.7 Supplier

Carboplatin is commercially available.

8.2 Nab-Paclitaxel (ABI-007; tradename: Abraxane®)

ABRAXANE for Injectable Suspension (also known as ABI-007, nab-paclitaxel, paclitaxel protein-bound particles for injectable suspension) is an albumin-bound form of paclitaxel with a mean particle size of approximately 130 nanometers. Paclitaxel exists in the particles in a non-crystalline, amorphous state. ABRAXANE is supplied as a white to yellow, sterile, lyophilized powder for reconstitution with 20 mL of 0.9% Sodium Chloride Injection, USP prior to intravenous infusion. Each single-use vial contains 100 mg of paclitaxel and approximately 900 mg of human albumin. Each milliliter (mL) of reconstituted suspension contains 5 mg paclitaxel. ABRAXANE is free of solvents. The active agent in ABRAXANE is paclitaxel.

In the United States, ABRAXANE for Injectable Suspension (paclitaxel protein-bound particles for injectable suspension) is indicated for the treatment of breast cancer after failure of combination chemotherapy for metastatic disease or relapse within 6 months of adjuvant chemotherapy. Prior therapy should have included an anthracycline unless clinically contraindicated.

8.2.1 Risks and Contraindications

Preclinical Studies with ABRAXANE

Preclinical studies comparing ABRAXANE to Taxol® (paclitaxel ® EL solvent-based, BMS) demonstrated lower toxicities, with an MTD approximately 50% higher for ABRAXANE compared to Taxol. At equal doses there was less myelosuppression and improved efficacy in a xenograft tumor model of human mammary adenocarcinoma. At equitoxic doses of paclitaxel, ABRAXANE treated groups showed more complete regressions, longer time to recurrence, longer doubling time, and prolonged survival. At equal dose, tumor paclitaxel area under the curve was 33% higher for ABRAXANE versus solvent based paclitaxel, indicating more effective intratumoral accumulation of ABRAXANE **(21)**.

Clinical Studies with Abraxane®

Weekly for 3 Weeks, Every 4 Weeks Schedule

Thirty-nine patients were enrolled into a Phase I study of ABRAXANE administered QW for 3 weeks followed by a 1 week rest in patients with advanced solid tumors **(11)**. The MTDs for heavily and lightly pre-treated patients were 100 and 150 mg/m^2 respectively. Dose limiting toxicities included grade 4 neutropenia and grade 3 sensory neuropathy. Premedication was not required, and unexpected, non-taxane associated toxicities were not observed.

In a Phase II trial in heavily pretreated patients with taxane-refractory metastatic breast cancer, objective antitumor responses occurred in 14% of women treated with ABRAXANE 100 mg/m^2 QW schedule. ABRAXANE weekly regimen was well tolerated; 91% of 106 patients were treated at the full dose of 100 mg/m^2 of ABRAXANE without dose reductions. Based on the activity and low toxicity documented with this schedule, the study was expanded to evaluate the efficacy and safety/tolerability of a higher dose of ABRAXANE 125 mg/m^2 weekly regimen in 75 additional patients. Results of this dose-finding study confirm the dose of ABRAXANE 100 mg/m^2 as the appropriate dose for further study in this patient population **(12)**.

In an open-label, randomized, multicenter phase II study comparing the antitumor response and toxicity of in two QW dosing regimens, ABRAXANE dosed Q3W, and Taxotere® (polysorbate solvent-based docetaxel Sanofi-Aventis] Q3W for the first-line treatment of metastatic breast cancer (**14**), a total of 300 patients were randomized to one of four treatment arms: (A) ABRAXANE 300 mg/m^2 IV Q3W, (n=76); (B) ABRAXANE 100 mg/m^2 (n=76); (C) ABRAXANE 150 mg/m^2 QW (n=74) QW every 28 days; or (D) Taxotere 100 mg/m^2 Q3W (n=74). The primary objective of the trial was to evaluate the antitumor activity and safety of three different ABRAXANE regimens to determine the optimal dose and frequency to be used. Secondary objectives included the comparisons of each treatment group with respect to efficacy and safety, specifically: ABRAXANE to Taxotere; ABRAXANE QW regimens to ABRAXANE Q3W regimen; and the two dose levels of QW ABRAXANE. Patients received ABRAXANE as a 30-minute IV infusion without premedication; Taxotere was administered as a 60-minute infusion with corticosteroid premedication. Total of 75% of all patients were post-menopausal with a mean age of 53.9 years at randomization.

Both ORRs and total response rates were higher in all ABRAXANE arms compared to the Taxotere arm. The investigator-reported ORRs were 46%, 63%, 74%, and 39%, for arms A, B, C, and D, respectively. This difference was statistically significant for both QW dosing arms of ABRAXANE compared with Taxotere, (P = 0.002 for arm B *v* D, and P <0.001 for arm *v* D). The corresponding investigator-reported total response rates were 72%, 83%, 91% and 69% for the four arms, respectively. This difference reached statistical significance for arms B and C compared to Taxotere (P = 0.009 for arm B *v* D, and p=0.005 for arm C *v*. D). No significant difference in ORR was noted between the two weekly dosing arms (arm B vs. C, P = 0.24). A significant increase in PFS was observed in the 150 mg/m^2 QW arm compared to the Taxotere arm (14.6 *v* 7.8 months, respectively, P = 0.012, hazard ratio 0.57). No significant difference in PFS was found between the ABRAXANE 300 mg/m^2 Q3W arm and Taxotere arm (A and D). Similarly, PFS was not significantly different between arms A and C, or arms B and D.

All three ABRAXANE arms demonstrated a favorable safety profile when compared with the Taxotere arm. The most frequent hematologic adverse event was neutropenia, with significantly lower rates of Grade 3/4 neutropenia in all ABRAXANE arms (Grade 4, 5%, 5%, 9%, 75% for arms A, B, C, D, respectively). ABRAXANE also had lower rates of febrile neutropenia (1%, 1%, 1%, 8% for arms A, B, C, D, respectively) and fatigue (Grade 3, 5%, 0%, 3%, 19% for arms A, B, C, D, respectively) compared to Taxotere. While the incidence of sensory neuropathy was similar in the ABRAXANE and Taxotere arms, the median time to improvement in patients with Grade 3 neuropathy was shorter in all three ABRAXANE arms (22, 22, 19 and 37 days in arms A, B, C and D, respectively).The ABRAXANE arms demonstrated improved safety and increased efficacy compared with Taxotere. All three ABRAXANE regimens produced lower rates of neutropenia, febrile neutropenia, and fatigue than Taxotere.

<u>Continuous Weekly (QW) Schedule in Neoadjuvant Breast Cancer</u>

The NSABP studied the administration of ABRAXANE in a neoadjuvant setting to patients with locally advanced breast cancer at a dose of 100 mg/m^2 QW for 12 weeks, with no break **(15)**. Four cycles of FEC were administered sequentially based on patients' HER2 status: HER2 negative patients received FEC-100 (F: 500 mg/m^2, E: 100 mg/m^2, C: 500 mg/m^2 Q3 weeks) and HER2 positive patients received weekly trastuzumab in addition to FEC-75 (F: 500 mg/m^2, E: 75 mg/m^2, C: 500 mg/m^2 Q3 weeks). Weekly trastuzumab was permitted during ABRAXANE and FEC-75 treatment at the discretion of the investigator. The primary objective of the trial was to determine the pathologic complete response rate (pCR) in the breast. At the time of initial report at SABCS 2006, 65 patients had been entered on study and were evaluable for cCR and safety. Following 12 weeks of ABRAXANE, a clinical complete response rate (cCR) of 32% was noted. The therapy was well tolerated, with 48/65 patients receiving 12 doses in 12 weeks and 13/65 receiving 12

doses in 13-14 weeks. The incidence of peripheral (sensory) neuropathy was low (11% grade 2, 5% grade 3) as was neutropenia (3% grade 3 and no grade 4). The authors concluded that the administration of ABRAXANE 100 mg/m^2 QW x 12 was both effective and tolerable.

At the time of this update, more than 20 abstracts and publications have been presented at major oncology conferences or published in medical journals related to ABRAXANE QW schedule in breast cancer, including completed and ongoing studies.

Human Toxicity:

Please refer to the Clinical Investigator's Brochure for details of the Adverse Reactions in the overall Safety Database for Abraxane® **(21)**. The following have been observed: myelosuppression, nausea and vomiting, diarrhea, mucositis, infections, hypotension, abnormal ECG changes, cough, dyspnea, edema, sensory neuropathy, bilirubin/liver enzyme elevations, allergic reactions, alopecia, asthenia, arthralgia, and myalgia. During post marketing surveillance, rare cases of severe hypersensitivity reactions have occurred.

Pregnancy

Abraxane® may cause fetal harm when administered to a pregnant woman. A developmental toxicity study in rats showed that no gross external, soft tissue or skeletal fetal alterations were caused by Abraxane® at doses of 0.5 mg/kg/day. Higher doses of Abraxane® resulted in significant maternal toxicity. This was evidenced by increased mortality, reduction in body weight gain, reduced terminal body weight, reduced food intake, and embryo-fetal lethality. Dose-related increases in malformations, variations, fetal deaths and/or resorptions occurred in pregnant rats that were administered intravenous doses of 1 or 2 mg/kg/day of Abraxane®.

There are no adequate and well-controlled studies of Abraxane® in pregnant women. If Abraxane® is used during pregnancy, or if the patient becomes pregnant while receiving this drug, the patient should be apprised of the potential hazard to the fetus. Women of childbearing potential should be advised to avoid becoming pregnant.

Nursing Mothers

It is not known whether paclitaxel (and therefore Abraxane®) is excreted in human milk. It has been reported that following IV administration of 14C- paclitaxel to rats on days 9 to 10 postpartum, concentrations of radioactivity in milk were higher than in plasma and declined in parallel with the plasma concentrations. Because many drugs are excreted in human milk and because of the potential for serious adverse reactions in nursing infants, it is recommended that nursing be discontinued when receiving Abraxane® therapy.

8.2.2 Concomitant Medications/Precautions

Supportive care, including but not limited to anti-emetic medications, may be administered at the discretion of the Investigator. Concurrent treatment with bisphosphonates is allowed. Erythropoietin and G-CSF may be administered at the discretion of the investigator, consistent with institutional guidelines.

8.2.3 Storage and Stability

Storage: Store the vials in original cartons at 20º C to 25º C (68º F to 77ºF). Retain in the original package to protect from bright light.

Stability: Unopened vials of ABRAXANE are stable until the date indicated on the package when stored between 20ºC to 25ºC (68ºF to 77ºF), in the original package. Neither freezing nor refrigeration adversely affects the stability of the product.

Stability of Reconstituted Suspension in the Vial

Reconstituted ABRAXANE should be used immediately, but may be refrigerated at 2ºC to 8ºC (36ºF to 46ºF) for a maximum of 8 hours if necessary. If not used immediately, each vial of reconstituted suspension should be replaced in the original carton to protect it from bright light. Discard any unused portion.

Stability of Reconstituted Suspension in the Infusion Bag

The suspension for infusion prepared as recommended in an infusion bag should be used immediately, but may be stored at ambient temperature (approximately 25º C) and lighting conditions for up to 8 hours.

8.2.4 Formulation/Agent Preparation

Nab-paclitaxel, Abraxane®, is a -free formulation of paclitaxel albumin for injectable suspension. Each 50 mL vial contains 100 mg of paclitaxel, and human albumin, as a white to off-white sterile lyophilized powder for reconstitution with 20 mL of 0.9% Sodium Chloride Injection USP.

8.2.5 Dose and Administration

Administration: Abraxane® is injected into a vein [intravenous (I.V.) infusion] over 30 minutes. The use of an in-line liter is not recommended.

NOTE: It is not a requirement to use filter needles in the preparation of, or in-line filters during the administration of Abraxane®. In any event, filters of pore-size less than 15 micrometers must not be used.

Dose: Abraxane® will be reconstituted by appropriate study personnel and administered to the patient in the study site setting at 1-week intervals. The investigator will calculate the body surface area (BSA) of the patient in order to determine the total amount of paclitaxel to be administered.

Reconstitution and use of Abraxane®:

1. Calculate the patient's body surface area at the beginning of the study and if the weight changes by >10%.
2. Calculate the total dose (in mg) to be administered by:

   Total Dose (mg) = BSA x (study dose mg/m^2)

   Actual body weight will be used for dosing calculation;

   Patients with body weight ≥ 1.2 x ideal body weight, maximum 1.2 x bodyweight will be used for dose calculation.

3. Calculate the total number of vials required by:

   Total Number of Vials = $\frac{\text{Total Dose (mg)}}{\text{100 (mg/vial)}}$

   Round up the number of vials to be reconstituted to the next higher whole number when a fractional number of vials is obtained by the above formula

(eg, if the total number of vials = 4.05 or 4.5, then 5 vials would be reconstituted).

4. Using sterile technique, prepare the vials for reconstitution.

5. Swab the rubber stoppers with alcohol.

6. Reconstitute each Abraxane® vial by using a sterile syringe to inject 20 mL of 0.9% Sodium Chloride Injection, USP or equivalent into each vial over a period of not less than 1 minute (Note: Change the syringes after reconstituting every 3 vials).
   - **Slowly** inject the 20mL of 0.9% Sodium Chloride Injection, USP,, using the sterile syringe directing the solution flow onto the **inside wall** of the vial.
   - **DO NOT INJECT** the 0.9% Sodium Chloride Injection, USP solution directly onto the lyophilized cake as this will result in foaming.
   - Once the injection is complete, allow the vial to sit for a **minimum of 5 minutes** to ensure proper wetting of the lyophilized cake/power.
   - **Gently** swirl and/or invert the vial **slowly** for at least **2 minutes** until complete dissolution of any cake/powder occurs. **Avoid** generation of foam.
   - If foaming or clumping occurs, stand solution for at least 15 minutes until foam subsides.
   - Each mL of reconstituted product will contain 5 mg of paclitaxel.

7. Calculate the exact total dosing volume (to the nearest mL) of 5 mg/mL suspension required for the patient:
   Dosing volume (mL) = Total dose (mg)/5 (mg/mL)

8. The reconstituted sample should be milky and homogeneous without visible particulates. If unsuspended powder is visible, the vial should be **gently** inverted again to ensure complete resuspension, prior to use.

9. Once the exact volume of reconstituted Abraxane® has been withdrawn from the vials, discard any excess solution left over in accordance with standard operating procedures.

10. Inject the calculated dosing volume of reconstituted Abraxane® suspension into an empty sterile, standard PVC IV bag using an injection port. Inject perpendicularly into the center of the injection port to avoid dislodging plastic material into the IV bag. Repeat steps 10 and 11 until the patient's entire required dose is injected into the IV bag.

11. Administer the calculated dosing volume of reconstituted Abraxane® suspension by IV infusion over 30 minutes. The use of in-line filters is not necessary. If used, in-line filters with pore sizes of <15μ should not be used.

8.2.6 Supplier

Abraxane® will be supplied by Celgene Corporation. Each single-use vial contains 100 mg of paclitaxel and approximately 900 mg of human albumin. Each milliliter (mL) of reconstituted suspension contains 5 mg paclitaxel.

Celgene Corporation
86 Morris Avenue
Summit, N.J. 07901
Toll Free: (800)-640-7854
Phone: (908) 673-9667
FAX: (908) 673-9115

Drug Distribution and Destruction

a. Drug Distribution

ABRAXANE® will be distributed by Abraxis BioScience, LLC. No supplies will be shipped to any site until regulatory approval has been obtained. Investigational sites will be supplied with ABRAXANE® upon identification and screening of a potential trial subject.

Upon identification of a potential subject, sites must fax a completed Drug Request Form to Abraxis BioScience, LLC. Allow at least 5 working days for drug shipment. There are no shipments on Fridays or holidays.

b. Drug Return and Destruction

If the investigational site does not have a policy, procedure or SOP detailing the process to follow for study drug destruction, the study drug must then be returned to Abraxis using the Drug Return Form provided in the package containing the study drug. The following information must be recorded on the site's pharmacy drug accountability log: quantity of vials to be returned, expiration date and lot number. A copy of the Drug Return Form and the study drug should be returned to Abraxis Clinical Supplies Dept. using the mailing address on the packaging slip that came with the original study drug order. A copy of the Drug Return Form should be retained at the clinical site. In the event of study completion or termination, a copy of all pharmacy records (drug dispensing log, drug accountability log and any destruction memos) must be mailed to Abraxis Medical Operations.

If the investigational site has a policy, procedure or SOP detailing the process to follow for study drug destruction, the pharmacist or designee can choose to destroy the study drug on site. The following information must be recorded on the site's pharmacy drug accountability log: quantity of vials destroyed, expiration date and lot number. The pharmacist must document that the study drug was destroyed in accordance with their institution's drug destruction policy or SOP. A drug destruction memo and the site's drug destruction SOP/policy should be sent to Abraxis Medical Operations Dept. A copy of the drug destruction memo should be retained at the clinical site. In the event of study completion or termination, a copy of all pharmacy records (drug dispensing log, drug accountability log and any destruction memos) must be mailed to Abraxis Medical Operations.

9.0 Correlative/Special Studies

Tissue and body fluid samples will be collected under this IRB approved clinical protocol in accordance with the IRB-approved HIPPA compliant and signed consent form.

9.1 Tissue Specimen Processing and Designation

9.1.1 Fresh core and other tissue specimens

Biopsies and lumpectomy/mastectomy specimens and, if feasible, lymph nodes will be fresh frozen according to standard procedure by the Department of Anatomic Pathology under Drs. Schmolze and P. Chu and in conjunction with the Translational Research Core and Anatomic Pathology Core, under the direction of Drs. Wu, Lee and Chen. Core biopsies (estimated 6-12 specimens from biopsy), and larger tissue samples from biopsy or resections of subsequent post-chemotherapy specimens will be stored fresh frozen. In case of abundance, some specimens will also be embedded. H&E and immunohistochemical analysis will be carried out by or under the direction of Drs. Schmolze and P. Chu.

Additional specimens (as feasible) will be processed fresh frozen, or RNA later and will be evaluated and explored for molecular characteristics in collaboration with internal (Dr. Hua Yu, Peter Lee, Shiuan Chen and laboratories, and external collaborators (TGEN-Dr. Jeff Trent; Jackson Laboratory: F. Mengi and Ed Liu), E. Wang (UC San Diego). Fresh samples as feasible from pre-treatment and post-neoadjuvant surgical specimens will be processed for generating cell lines, xenografts under collaborative agreements or on house (Jun Wu, and above listed collaborators.) An attempt will be made to obtain core biopsy specimens halfway through the neoadjuvant chemotherapy course, but this biopsy will be optional and patients may elect to opt out.

All specimens will be logged in and out using a master logbook, and stored according to standards developed by the Department of Anatomic Pathology and in conjunction with the Translational Research Core. A unique patient identifier (specific for this study) will be assigned to each patient and subclassification.

9.1.2. Fixed sample collections

Core biopsies of primary tumors (an estimated 4-6 [as feasible] specimens from biopsy) and larger tissue samples from biopsy or resections of subsequent post-chemotherapy specimens will be procured in the following preservatives:

- 2-3 (or more if feasible) specimens in formalin for Anatomic Pathology
- 2-3 specimens in RNAlater to be sent to the translational research lab for further distribution

Core and other biopsy specimens will be preserved according to standard procedure by the Department of Anatomic Pathology and the Translational Research Core. Tissue collected in RNAlater (RNAlater is stored at room temperature) will be submerged in ten times the volume of the tissue and the will be less than 0.5 cm in at least one dimension. The tissue will be submerged immediately after it is extracted from the patient and the vial kept upright so the tissue stays submerged in transit. Vials will be delivered to the translational research laboratory for further distribution to other collaborators listed on the face page of the protocol, as appropriate, pending availability of funding to perform the appropriate studies.

9.1.3. RT-PCR and DNA microarray analysis

Gene expression analysis, pending sufficient quantity of fresh frozen tissue specimens (9.1.1), will also be carried out in collaboration with Agendia Corporation under a City of Hope-approved material transfer agreement (MTA). This goal is in line with the original objectives and goal of this study. The statistical analysis will be carried out as a collaboration between the study

statistician, Paul Frankel, PhD, and the designated bioinformatics personnel (to be named) at Agendia, at the NCI of the Netherlands.

MiRNA and DNA assessment will be carried out through the Sequencing Core and Translational Research Core and in collaboration with Xiwei Wu Ph.D, Emily Wang PhD, at UC San Diego. To assess RT-PCR and protein expression, and immunohistochemistry (from formalin-fixed material, 9.1.2) as feasible in addition to work performed by Dr. Daniel Schmolze, MD (City of Hope Anatomic Pathology). Potential collaborations include Agendia (MTA in place), TGEN, Jackson Laboratory, NantOmics (all with MTAs in place).(**21**) Standard operating procedures according to the City of Hope standards are in place to keep samples de-identified for the outside investigators.

9.1.4. Clarke Lab Protocol for breast tumor dissociation & implantation for xenografts

Xenographs will be generated in the Clarke laboratory at Stanford University **(22)**, as well as on campus in collaboration with Jun Wu, PhD.

Specimens (either mastectomy or large segmentectomy) will be removed in OR using sterile instruments and sent for inking by pathologist. Tissue sections are cut with sterile instruments and placed into 50-ml conical tubes containing RPMI/10% HICS/Ceftazidime/PSA on wet ice. Samples are kept at 4^{o}C and shipped same day to Jun Wu on campus, or using overnight shipping to:

Dalong Qian

Stanford University

Institute for Stem Cell and Regenerative Medicine

1050 Arastradero Road

Palo Alto, CA 94304

Lab phone: (650) 724-0574

Cell: (734) 330-3585

Total time on ice will therefore be less than 24 hours.

Keep cells on ice or at 4°C for entire protocol

1) Make sure to clean and autoclave all surgical instruments before processing tissue. We commonly employ the following equipment: razor blades or scalpels, tissue forceps, surgical scissors. Tools may be cleaned during the dissection by rinsing with 10% bleach followed by 70% EtOH.
2) Put 5ml of Medium 199/H_2CO_3/Earl's/25mM HEPES/pH 7.3 supplemented with PSA into 10mm or 15mm plastic petri dish (cells will not adhere as much as with tissue culture dishes). Transfer tumor piece(s) to petri dish with tissue forceps.

We routinely perform two separate procedures for human tumors: xenotransplantation and flow cytometry. This protocol will mainly deal with processing the tumor tissue for flow cytometry.

3) **Xenotransplantation**: Using "L"-shaped forceps, cut 1-2mm cubes. Transfer all pieces to a sterile eppendorf tube containing medium and keep on ice. Generally, we implant 2 pieces per mouse at different sites. We use a trocar to deliver the tissue into the upper fat pads of NOD/SCID mice. We also place an estrogen pellet into the back of the mouse if dealing with an ER+ breast tumor. All tumor chunks are coated with matrigel before engraftment.

The following is based on our procedures for processing human breast tumors. Other tumor types may require alternate dissociation enzymes, digestion times, etc.

4) **If leaving tissue overnight**: Put entire piece in RPMI w/ 10% HICS and ceftazidime and Pen/Strep/Ampho and leave on ice O/N. In the morning, wash 1x with cold PBS, then proceed as follows.
5) **Make single cell suspension**: If specimen contains a significant amount of adipose tissue, trim this first. Then cut tissue into slivers by holding with forceps and sliding prongs around razor blade. Next mince using slicing motion. Once adequately disaggregated, bring total volume to 27 ml with media and add 3 ml of 2000 unit/ml collagenase type 3 (10x) [Can adjust total vol based on size of specimen]. Add DNase to the digestion (5 Kunitz units/ml). Scrape cells off bottom of plate with razor blade before placing into incubator (37°C, 5% CO_2) and mechanically dissociate by pipetting every 20 minutes. Incubate for 2-4 hours, or until tissue is disaggregated. Often we need to spike the digestion with an additional 1.5 ml Collagenase Type III at ~1.5 hrs to achieve complete dissociation.
6) **Neutralizing:** Add 10 mls of neutralizing media (RPMI 1640/10% HICS/PSA) per 20 mls of digestion media. Mix by pipetting.
7) **Filtering**: First pipette media over nylon mesh held over 50ml conical. May need to change nylon mesh several times due to clogs. Next pipette through 70 µm filter into a fresh 50ml conical. Tilt filter slightly to increase flow speed if it slows down. Finally, pipette through 40 µm filter into a fresh 50ml conical. Pellet cells for 5 min at 1000 RPM at 4°C.
8) **RBC lysis:** Resuspend pellet in 5 ml of ACK buffer and incubate on ice for 2 minutes. Bring up volume to 50 ml with HBSS w/ 2% HICS to stop lysis. Pellet cells for 5 min at 1000 RPM at 4°C.
   Optional: can repeat 40 µm filtering to remove debris from RBC lysis if desired.
9) **Count cells:** Bring up cells in an appropriate volume of HBSS w/ 2% HICS. Cells are now ready for staining.

Optional: Freezing Tumor Samples

1) Resuspend cells in 10% DMSO/80% DMEM/10%FBS at a concentration of 13 million cells/ml in cyrovials.
2) Place in freezing chamber O/N in $^{-}$80C.
3) Transfer to liquid nitrogen for long term storage.

Once City of Hope capabilities allow, cell line development and stem cell line identification will be carried out in the laboratories of Drs. Xiwei Wu, Peter Lee, Shiuan Chen, and other COH collaborators as feasible. Xenografts as feasible will also be generated – using the above technique or modifications as necessary), in the core at COH, by Jun Wu, or by the Lee, or Chen Laboratories.

9.1.5 Non-coding RNA-based prognostic biomarkers in TNBC

Long noncoding RNA (lncRNA) are cellular RNAs larger than 200 nucleotides in length that are overexpressed in various cancers, including breast cancer. Non-coding RNAs regulate cellular processes by transcriptional and epigenetic regulations, post-translational modification, signaling pathway, and intercellular communication via exosomes. In this study, lncRNA analysis will be carried out to identify biomarkers using available RNA-sequencing databases with associated outcomes of interest. Integrative RNA-seq analysis will be used to identify 1) differentially expressed lncRNA and 2) presence of lncRNA

mutations in the following comparison groups: good vs poor prognosis; pathologic complete response vs. minimal response following neoadjuvant therapy; primary vs. metastatic tumors; low vs. high tumor infiltrating lymphocytes (starting with 30% cut-off); and BRCA vs. non-BRCA patients. Future work will include whole transcriptomic RNA-seq data from neoadjuvant responders vs. non-responders, and orthogonal validation of bioinformatic findings by *in situ* hybridization to analyze key lncRNAs, in a separate validation cohort.

9.2. Blood Specimen Processing and Designation

1.2.1 Blood Samples

All blood specimens will be drawn using the following vacutainer tubes:

Tubes #1-3 – Three 7 ml EDTA (purple-top) tube

Tube #4 – One 7 ml Paxgene tube

Tubes #5-6 – Two - 10ml tubes containing EDTA anticoagulant and Cytochex preservative

Timing:

Pre-chemotherapy:	Tubes #: 1-6
Mid-chemotherapy:	Tubes #: 1-6
Prior to definitive surgery:	Tubes #: 1-6
Every 6 months x 5 years:	Tubes #: 1-6

9.2.2. Specimen Handling:

All EDTA tubes (purple top) will be kept on ice. After centrifugation at 1100 rpm for 10 minutes at 4 degrees Celsius, the plasma will be transferred to Eppendorf tubes and centrifuged at 14000 rpm for 10 minutes at 4 degrees Celsius to separate the WBC fraction. The plasma will be divided into 500 microliter aliquots and stored at $^{-}$80 degrees Celsius. The WBC will be washed with a red cell lysis buffer, pelleted and stored at $^{-}$80 degrees Celsius.

The EDTA tubes will be delivered to the translational research core lab within 1 hour of procurement and centrifuged to separate the buffy coat and plasma fractions.

Tube #4 is a PAXgene tube for stabilization of whole blood and subsequent isolation of cellular RNA. After incubation for 2 hours at ambient temperature, the tube will be stored in a loose wire rack at $^{-}$20 degrees Celsius for up to one year. The RNA will be extracted following the protocol in the PAXgene Blood RNA Kit sold by QIAGEN. The RNA will be analyzed by real-time PCR or micro array. This tube will be procured for RNA extraction from PMN cells in Dr. Yen's laboratory.

Tubes # 5-6 will be procured and shipped to the Yen lab, or other collaborator's laboratory, in order to quantitate, isolate, and characterize circulating tumor cells.

After venipuncture, the initial 1/2 cc of blood will be discarded (or drawn into any pediatric tube, which is then discarded) so as not to contaminate the subsequent blood sample with skin epithelial cells.

To test for the presence of tumor-like cells, the blood will be labeled with fluorescent molecules marking for Cytokeratin (CK), CD-45 and DAPI antibodies with subsequent processing for markers for ER, HER2, and possibly for EGFR and ERCC-1 or other suitable markers.

Tubes # 1-3: Blood will be collected in the 10ml tubes containing EDTA anticoagulant and Cytochex preservative,The blood samples will be split between the involved research laboratories (Dr. Wang and others at COH).

9.2.3. Specimen Tracking:

The date of blood draw will be recorded. For tracking purposes, the following will also be recorded upon receipt to the processing and receiving laboratories:

a. Specimen ID number (all specimens will be anonymized by assigning a unique identifier, similarly to the process described under "Tissue specimen handling", with coding provided by Biostatistics.
b. Number and types of tubes received and date of receipt.
c. Number of aliquots of plasma and serum (5-6 aliquots per tube expected), freezer location(s) stored, and date(s) of initial storage.

Liquid nitrogen tank location of stored PBMC and date of initial storage will be tracked.

Upon transfer of specimens to any designated labs, tracking will include:

a. Specimen ID number
b. Lab to which specimen was sent
c. Date of transfer

Upon return of data from the designated labs, the following will be recorded:

a. Specimen ID number
b. Date of run and run number (all specimens will be run in replicate for at least two runs in order to evaluate intra-and inter-assay reliability)
c. Type of assay
d. Lab performing assay
e. Assay results

10. Study Calendar

Baseline evaluations are to be conducted within 1 week prior to administration of protocol therapy. Scans must be done within 4 weeks prior to the start of therapy. Tests/procedures indicated for the following weeks maybe performed within ± three days of the indicated dates. Brief interruptions and delays in the 28 day cycle may occasionally be required due to travel delays, airport closure, inclement weather, family responsibilities, security alerts, government holidays, etc. This can also extend to complications of disease or unrelated medical illness not related to disease progression. These delays will not be considered protocol violations. Therapy can be delayed (after cycle 1) up to 3 days due to holidays/personal reasons.

	Pre-Study	Pre Cycle 1 Day 1	Cycle 1 Wk 1	Wk 2	Wk 3	Wk 4	Cycle 2 Wk 1	Wk 2	Wk 3	Wk 4	Mid-Tx	Cycle 3 Wk 1	Wk 2	Wk 3	Wk 4	Cycle 4 = same as Cycles 1 & 2	Defi-nitive Surgery Visit	Follow-up[e]
carboplatin			Day (D) 1				D 1					D 1						
nab-paclitaxel			D 1	D 8	D 15	D 22	D 1	D 8	D 15	D 22		D 1	D 8	D 15	D 22			
Informed consent	X																	
Medical history	X																	
Concurrent meds				X----------X														
Physical exam, Height, weight	X		X			X	X			X		X			X		X	X
Vital signs	X		X			X	X			X		X			X		X	X
Performance Status	X		X			X	X			X		X			X		X	X
CBC w/diff, plts	X		X	X	X	X	X	X	X	X		X	X	X	X		X	X
Serum chemistry[a]	X		X	X	X	X	X	X	X	X		X	X	X	X		X	X
EKG (as indicated)	X																	
Adverse event evaluation				X----------X													X	
Tumor measurements as applicable (clinical)	X			Tumor assessments (clinical) are repeated every 4 weeks. Documentation (radiologic or clinical) must be provided for patients removed from study for progressive disease.													X	
Radiologic evaluation	X			Radiological assessment will be carried out at the conclusion of 16 weeks of therapy, as applicable (Mammogram, US, MRI, as deemed appropriate by the treatment team).													X	X[h]
MUGA/ECHO	X																	
B-HCG	X[b]																	
Correlatives - Tissue: Core Biopsies/Tumor Specimens		X[c]									X[c]						X[c]	
Correlatives - Blood [d]:		X									X						X	X

a:Albumin, alkaline phosphatase, total bilirubin, bicarbonate, BUN, calcium, chloride, creatinine, glucose, phosphorus, potassium, total protein, SGOT[AST], SGPT[ALT], sodium.

b:Serum pregnancy test (women of childbearing potential).

c: Procurement of pretreatment tumor, core biopsies, and (if feasible) lymph node sample pre-treatment ; the mid-treatment (prior to cycle 3) procurement is optional. See Section 9.1 for complete Tissue instruction regarding preparation/submission

d: Procurement of blood specimens; to occur prior to treatment on Cycle 1, 3, and Day of Definitive Surgery, prior to surgery., and each follow up visit. Please see Section 9.2 for complete blood instruction regarding preparation/submission

e: Follow up to be done every 6 months x5 years or till disease progression. After 5 years, follow up should be as per standard of clinical practice.
f:Requested as per Standard of Care

11. Evaluation Criteria/Measurement of Effect

11.1. Response Criteria

The study endpoint is pathological complete response (pCR), and this will be assessed after completion of study therapy, based on assessment of the surgical specimen, by Dr. Sean Lau, non-treating pathologist/collaborating investigator. Dr. Lau will also assess response scored for residual cancer burden (RCB) by applying the Symmans criteria **(3)**.

12. Data Reporting/Protocol Deviations

12.1. Data Reporting

12.1.2. Confidentiality of Records

The original data collection forms will be stored in the Department Clinical Trials Office, in a secure location. When results of this study are reported in medical journals or at meetings, identification of those taking part will not be disclosed. Medical records of subjects will be securely maintained in the strictest confidence, according to current legal requirements. They will be made available for review, as required by the FDA, HHS, or other authorized users such as the NCI, and the sponsor City of Hope under the guidelines established by the Federal Privacy Act and rules for the protection of human subjects.

12.1.3. Subject Consent Form

At the time of registration, the original signed and dated Informed Consent form, HIPAA research authorization form, and the California Experimental Subject's Bill of Rights (for the medical record) and three copies (for the subject, the research record, and the Coordinating Center) must be available. All Institutional, NCI, Federal, and State of California requirements and requirements by the sponsor, City of Hope, will be fulfilled.

12.1.4. Data Collection Forms and Submission Schedule

All data will be collected as per standard institutional timeline using data collection forms and electronic data entry as per COH standard. Data will be stored in a secure location/and in secure format.

12.1.4.1. Eligibility Checklist

The Eligibility Checklist must be completed by a protocol nurse or clinical research associate and signed by an authorized investigator prior to registering the subject. See Section 4.3 for the registration procedure.

12.1.4.2. Prior Therapy and On-Study Data

Within a week of registration, the clinical research associate will submit on-study checklist form.

12.1.4.3 Treatment Data

Within four (4) weeks of completion of each course the following data should be recorded in the EDC:

12.1.4.3.1 Treatment and Adverse Events

12.1.4.3.2 Supplemental Data

12.1.4.3.3 Flow Sheets

12.1.4.4 Response/Off-Study/Follow-up

Each time a patient is evaluated for response and/or new follow-up information is obtained, the CRA should record this in the appropriate section of the EDC.

12.1.4.5 Results Reporting

Preliminary and full results of this clinical and translational research trial may be reported in abstract form(s) at national meeting(s) and in full manuscript form(s) in appropriately selected journal(s).

12.2. Protocol Deviations

12.2.2. Deviation Policy

Planned deviations may be permitted in accordance with the COH policy on "Clinical Research Protocol Planned Deviations and Single Subject Exception." These planned deviations, considered Single Subject Exceptions, are considered an Amendment to the Protocol. In addition, if contractually obligated, the sponsor must also approve any planned deviations.

12.2.3. Reporting of Unplanned Deviations

All unplanned deviations will be reported to the COH DSMB who will forward to the IRB following review.

12.2.4. Resolving Disputes

If there is a dispute among the persons involved in the provision of research treatment, in regard to whether a treatment deviates from the protocol, resolution will be resolved in accordance with the Clinical Research Protocol Planned Deviations and Single Subject Exceptions policy.

13. Statistical Considerations

13.1. Study Design/Endpoints

13.1.1 Study Design

This is a Phase II study of neoadjuvant therapy with carboplatin + nab-paclitaxel for triple negative locally advanced or inflammatory breast cancer.

A two stage design followed by an expanded cohort is proposed based on detecting a promising pCR rate (using the MD Anderson criteria of lack of evidence of any residual invasive tumor in breast and/or regional lymph nodes) or a promising Symmans 0-1 pathological response. The design selected will meet our objectives and permits the early termination of the trial in the event that the therapy appears inferior to other neoadjuvant regimens.

13.2 Sample Size/Accrual Rate

13.2.1 Study Size **(49 total in two-stage design, 20 in expanded cohort for 69 total study patients maximum)**

Study size for the two stage design is based on our therapeutic target of achieving with acceptable toxicity either a promising pCR rate or a promising Symmans 0-1 pathological response rate. To permit early termination for a discouraging result, we have employed a two-stage design. In the first stage, accrual will continue until 22 patients are enrolled, with second stage accrual to 45 patients during the two-stage design evaluation.

An expanded cohort of 20 patients (given the promising results in the first 45) will be added to the 49 patients (see note below) to better evaluate the response rates in specific subsets: basal, androgen receptor positive, immunomodulatory subtype, mesenchymal/stem cell like. This will result in a total of 69 total patients.

Two-stage design considerations:

Our institutional experience with two neoadjuvant regimens suggests a pCR rate in her2- patients of 11-14% (N=75), and in the basal subtype (N=20) of approximately 20%. Other investigators have observed higher pCR rates in triple negative patients, although with differing definitions of pCR. As a result, a true 20% pCR rate in triple negative patients treated with this regimen would be considered a disappointing result, not superior to current approaches to neoadjuvant chemotherapy. A true encouraging pCR rate (where we want a small probability of declaring it discouraging) is considered 38%.

Similarly, we observed a Symmans 0-1 response rate of 27-28% in our institutional experience (75pts) in her2- patients, and a Symmans 0-1 response rate of 35% in the basal subtype (N=20). Due to the paucity of other data on the Symmans response rate in basal subtype, and our limited prior experience, we consider a 25% Symmans 0-1 response rate to be discouraging and a 45% Symmans response rate to be encouraging.

Based on these considerations, we have chosen a design that simultaneously discriminates between pCR rate of 20% vs 38% and a Symmans response rate of 25% and 45%.

In particular:

If no more than four (4) Symmans 0-1 responses are observed and no more than three (3) pCRs are observed in the first 22 patients, the study will be terminated early and declared negative. If five (5) or more Symmans 0-1 responses or four (4) or more pCRs are observed in the first 22 patients, an additional 23 patients will be accrued during the second stage of the study. If at least 17 Symmans 0-1 responses (38%) or at least 14 pCRs are observed in the 45 patients (31%), this combination would be considered worthy of further testing in this setting.

This design yields at least 87% power to detect a true Symmans response rate of at least 45%. It yields at least 86% power to detect a true pCR rate of at least 38%. It yields at least .91 probability of a negative result if the true pCR response rate is no more than 20% and the Symmans 0-1 response rate is no more than 25%. This last probability is calculated assuming that pCR rate and the Symmans 0-1 rate are uncorrelated. As they are positively correlated, the probability will be higher (type I error less than 9%).

Note: the initial 45 patients was based on eligible patients starting treatment, but as the response rate clearly exceeded the threshold for promising (20 of the first 38 had a pCR) accrual continued to 49 to achieve 45 who were treated per protocol for a more thorough evaluable as part of the two-stage design. The added cohort of 20 patients will result in a total of 69 patients.

Accrual will be suspended at the end of the first stage pending the interim analysis decision, based on the response of the previously accrued patients.

Expanded Cohort Considerations:

Given the promising results at 45 evaluable patients (49 total), the expanded cohort of 20 patients will result in a total of 69 patients (~65 evaluable). This will allow a better exploratory analysis of the response rate based on phenotypic and gene array profiling subsets: Approximately 40% are expected to be of the basal subtypes (~27 patients), androgen subtype 11% (~7 patients expected), tumor infiltrating lymphocyte/immunomodulatory subtype 25% (~17 patients), and mesenchymal/mesenchymal/stem cell like ~25% (~17 patients). With 69 patients, there is a 89% chance of having 5 or more patients in even the smallest subset, and as a result, we should observe a pCR in all subsets (e.g. 97% chance of observing at least one pCR with 5 patients if the true pCR response rate is 50%). Dramatic reduction/lack of pCR in any subset will help guide future development of subset specific treatment additions to the backbone of nab-paclitaxel and carboplatin. For the more common subsets, such as the basal subtype, the 95% CI for the pCR can be estimated with a half-width of at most 12% assuming 27 patients in that group. The confidence interval will be larger or smaller depending on the exact number of patients in that group enrolled, and these estimates will be made for the pCR rate for all patients who initiated treatment and are eligible, along with all patients who were treated per protocol. Symmans response rate will also be evaluated similarly.

Toxicity:

Previous experience with carboplatin/paclitaxel suggests this is a well-tolerated regimen, and prior experience also suggests that carboplatin/nab-paclitaxel should be better tolerated. However, toxicity will be monitored and reported to the COH/DSMB every 6 months as specified in section 7.

13.2.2 Accrual Rate

Estimated monthly accrual rate is 1-3 patients per month. With a maximum target accrual of 69 patients, we expect accrual to be completed in approximately 54 months.

13.3 Stratification Factors

NA

13.4 Analysis of Secondary Endpoints

The outcome status in terms of toxicity, reason off study, overall survival and laboratory correlates of all eligible patients will be reported. Subset specific analysis is also a secondary endpoint as discussed above. Additionally:

13.4.1 Overall Survival

Patients' survival times will be measured from the initial date of treatment to the recorded date of death.

Survival (both overall and progression-free) will be estimated by the Kaplan-Meier method. The corresponding median survival times (with 90% confidence limits) will be determined.

13.4.2 Safety Profile

Using NCI CTCAE (v4.0), the number of patients experiencing SAEs (serious adverse events) in each cycle of treatment, and for 30 days beyond the last protocol treatment administered, will be characterized by type of adverse event and grade, and by the time of onset in relation to the first day of therapy for each cycle. Attribution of SAEs to treatment (unrelated, unlikely, possible, probable, or definite) will also be reported. We will also report the cumulative percentage (%) of patients experiencing treatment-related SAEs and its relationship to treatment duration.

13.5 Reporting and Exclusions

13.5.1 Evaluation of Toxicity

All patients will be evaluable for toxicity from the time of their first treatment with carboplatin + nab-paclitaxel.

13.5.2 Evaluation of Response

All patients included in the study must be assessed for response to treatment, even if there are major protocol treatment deviations or if they are ineligible. Each patient will be assigned a Symmans score, and Yes/No on pCR. In addition, patient response could be categorized in one of the following categories which would be considered a non-response: stable disease, progressive disease, early death from malignant disease, early death from toxicity, early death because of other cause, refused surgery, or unknown (not assessable, withdrew consent, insufficient data).

All of the patients who met the eligibility criteria (with the exception of those who received no study medication) should be included in the main analysis of the response rates. An incorrect treatment schedule or drug administration does not result in exclusion from the analysis of the response rate.

All conclusions should be based on all eligible patients who receive any of the study drug. Subanalyses may then be performed on the basis of a subset of patients, excluding those for whom major protocol deviations have been identified (inevaluable patients, e.g., early death due to other reasons, early discontinuation of treatment, major protocol violations, etc.). However, these subanalyses may not serve as the main basis for drawing conclusions concerning treatment efficacy, and the reasons for excluding patients from the analysis should be clearly reported.

14. Human Subject Issues

14.1. Institutional Review Board

In accordance with City of Hope policies, an Institutional Review Board (IRB) that complies with the federal regulations at 45 CFR 46 and 21 CFR 50, 56 and State of California Health and Safety code, Title 17, must review and approve this protocol and the informed consent form prior to initiation of the study. All institutional, NCI, Federal, and State of California regulations must be fulfilled.

14.2. Recruitment of Subjects

Participants for this study will be recruited from among patients undergoing treatment at City of Hope Cancer Center for stage II/III –including inflammatory - breast adenocarcinoma.

Patients will be recruited through encounters by the Breast Oncologists in the Department of Medical Oncology and/or Breast Surgical Oncologists of the Department of General Oncological Surgery.

They will be indentified, screened, consented, eligibility criteria established by members of a designated research team within the Breast Cancer Program, under the guidance and together with the PI and co-investigators and participating clinical investigators listed on the protocol. Once all pretreatment evaluations have been performed, patients will be entered on study after review of patient eligibility by a member of the Department of Clinical Trials Office. Patients may be screened for registration by calling the City of Hope Department of Clinical Trials Office, ext. 62468.

This study will be performed at COH, and will be listed at www.clinicaltrials.gov.

14.3. Confidentiality

This research will be conducted in compliance with federal and state of California requirements relating to protected health information (PHI). The study will record clinical characteristics, response to treatment by clinical, imaging, and pathological means, in addition to toxicities, and other outcomes, as appropriate (recurrence-free/overall survival). These findings, as well as data generated from the translational research investigations of procured biological specimens, will be linked to the subject's identity using a coded study number designated by the statistician/Department of Bioinformatics and in conjunction with the Translational Research Core of COHCC. The principal investigator and the statistician, and laboratory technicians will have access to this information, but all information will be treated confidentially. No identifiers will be used in any subsequent publication of these results.

Financial Obligations and Compensation

The study drug, Abraxane (nab-paclitaxel), will be provided by the manufacturer (Celgene Corporation) free of charge to patients on this study research. Research lab draws, processing, and results will be free of charge as a part of research. Routine standard of care clinic visits, laboratory tests, and tumor imaging will be billed to the patient and/or the patient's insurance. Carboplatin, the infusion center time, and routine nursing care involved in administering of these drugs and supportive medications during infusion will be billed to the patient and/or the patient's insurance. Medication and/or treatment needed for side effects of either study drug will be billed to the patient and/or the patient's insurance.

If there is a serious medical complication of the research, treatment will be available at City of Hope, but there will be no compensation to the subject for this injury.

14.4. Informed Consent Processes

The Principal Investigator or IRB approved named designate will explain the nature, duration, purpose of the study, potential risks, alternatives and potential benefits, and all other information contained in the informed consent document. In addition, they will review the experimental subject's bill of rights and the HIPAA research authorization form. Research subjects will be informed that they may withdraw from the study at any time and for any reason without prejudice, including as applicable, their current or future care or employment at City of Hope or any relationship they have with City of Hope.

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