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STUDY PROTOCOL

Official Study Title: A Phase II Study of Adjuvant Therapy Using a Regimen of Cyclophosphamide, Paclitaxel with Trastuzumab in Stage I-II HER2/neu Positive Breast Cancer Patients

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A Phase II Study of Adjuvant Therapy Using a Regimen of Cyclophosphamide, Paclitaxel with Trastuzumab in Stage I-II HER2/neu Positive Breast Cancer Patients

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UNMC Investigator Initiated Project (No Sponsor) IND EXEMPT *Per IND regulations [21 CFR 312.2(b)] this study meets all of the requirements for exemption from the IND regulations and, therefore, an IND is not required to conduct the investigation. In accordance with 21 CFR 312.2(b)(4) of the regulations, the FDA will not accept the application*

This protocol is being conducted as a translational clinical research project as part of the UNMC Fred & Pamela Buffett (NCI) Cancer Center Support Grant # P30 CA036727.

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A Phase II Study of Adjuvant Therapy Using a Regimen of Cyclophosphamide, Paclitaxel with Trastuzumab in Stage I-II HER2/neu Positive Breast Cancer Patients

Abstract: This is a Phase II study of adjuvant therapy using a dose-dense regimen of cyclophosphamide and paclitaxel with trastuzumab in patients with newly diagnosed stage I-II HER2/neu positive breast cancer. This will be a non-randomized study; all subjects will receive the combination chemotherapy.

Objectives:

1. To determine the toxicities and ability to complete the planned treatment of a dose-dense regimen of cyclophosphamide and paclitaxel with trastuzumab in subjects with newly diagnosed stage I-II HER2/neu positive breast cancer.
2. To estimate recurrence free survival of a dose-dense regimen of cyclophosphamide and paclitaxel with trastuzumab in subjects with newly diagnosed stage I-II HER2/neu positive breast cancer.

Eligibility: Histologically confirmed newly diagnosed Stage I-II HER2/neu positive breast cancer, women with no evidence of metastatic cancer and normal cardiac function as defined by an ejection fraction of >50% by echocardiogram.

Intervention:

Adjuvant (post surgery) treatment naïve subjects with newly diagnosed stage I-II HER2/neu positive breast cancer will receive therapy with growth factor support as follows:

Systemic Therapy:

- Cyclophosphamide 600mg/m^2 in normal saline IV over 1 hour Day 1 cycled every 14 days (2 weeks) for 6 cycles
- Paclitaxel 175mg/m^2 in normal saline IV over 3 hours Day 1 cycled every 14 days (2 weeks) for 6 cycles
- All cycles are with pegfilgrastim support, 1-6 mg SQ on day 1 after PC dose adjust as medically indicated or hold for ANC >12,000 or bone pain (SOC). (Pegfilgrastim support is optional following cycle 6)
- Loading dose trastuzumab 6mg/kg in normal saline IV over 30 to 90 minutes per institutional protocol Day 1, Cycle 1.

THEN

- Subsequent cycles (Cycles 2-5) trastuzumab 4mg/kg in normal saline IV over 30 to 60 minutes per institutional protocol Day 1 every 14 days (2 weeks), Cycles 2-5.

THEN

- Maintenance therapy beginning with Cycle 6 trastuzumab 6mg/kg in normal saline IV over 30 to 60 minutes per institutional protocol Day 1 every 21 days (3 weeks) to complete a total of 52 weeks, Cycles 6- 19.

Radiation Therapy:

Radiation will be given by the radiation oncologist and timing determined by the radiation and medical oncologist as per standard NCCN guidelines, either concurrent with chemotherapy or following chemotherapy unless the patient has received radiation to the area in the past.

Hormonal Therapy:

Subjects with estrogen/progesterone receptor positive tumors will receive hormonal therapy following adjuvant chemotherapy, and hormonal therapy will be determined by the medical oncologist as per standard NCCN guidelines.

Evaluation: Subjects will have standard hematological and cardiac evaluations. Subjects will be clinically evaluated by an oncologist for toxicities every 2 weeks during chemotherapy, then at least every 12 weeks after completing chemotherapy (until they complete 52 weeks of trastuzumab), and then every 3 months for 2 years. Subjects will have a repeat echocardiogram every 12 weeks until completion of trastuzumab to monitor their cardiac function.

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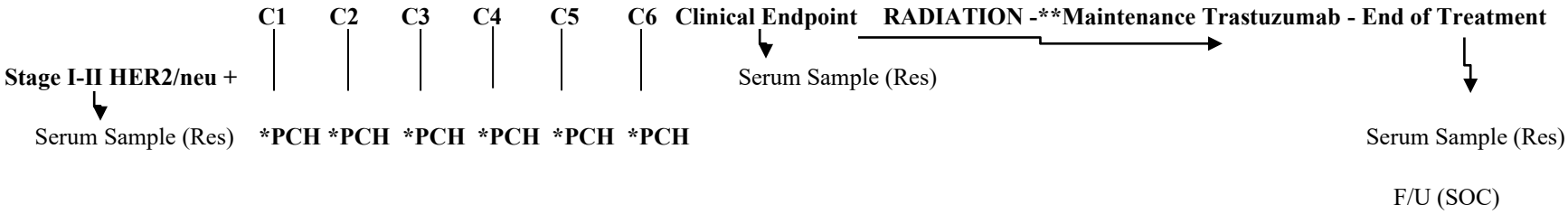
ADJUVANT SCHEMA

(HER2/neu Positive Breast Cancer)

Post-SURGERY (SOC)



***PCH Paclitaxel** 175mg/m^2 in normal saline IV over 3 hrs / **Cyclophosphamide** 600mg/m^2 in normal saline IV over 1 hr REPEAT Day 1 Q 2wks x 6 cycles (Cycles 1-6), **Pegfilgrastim** support 1-6 mg SQ on day 1 after PC dose adjust as medically indicated or hold for ANC >12,000 or bone pain (SOC).

(Pegfilgrastim support is optional following cycle 6)

Herceptin® (Trastuzumab) loading dose 6mg/kg Day 1, Cycle 1THEN subsequent dosing 4mg/kg Day 1 Q2wks Cycles 2-5, (SOC)

Research Serum Samples collected at baseline Clinical Endpoint and End of treatment (Res)

****Maintenance Herceptin®** 6mg/kg Day 1 Q21 Days for a total of 52 weeks Cycles 6-19. (SOC)

RADIATION Clinical Decision per standard NCCN guidelines, following surgery and chemotherapy. (SOC)

SUBJECTS WITH ER/PR + TUMOR = HORMONAL THERAPY Subjects with **estrogen/progesterone receptor positive** tumors will also receive Hormonal Therapy to be determined by the medical oncologist as per standard NCCN guidelines. (SOC)

End of Treatment Follow-up (F/U)

Echocardiogram at completion of CT and then every 12 weeks until completion of trastuzumab.

Subjects will be monitored Q3 months until 2 years from date of treatment. (SOC)

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1.0 Objective(s)

1. To determine the toxicities and ability to complete the planned treatment of a dose-dense regimen of cyclophosphamide and paclitaxel with trastuzumab in subjects with newly diagnosed stage I-II HER2/neu positive breast cancer.

2. To estimate recurrence free survival of a dose-dense regimen of cyclophosphamide and paclitaxel with trastuzumab in subjects with newly diagnosed stage I-II HER2/neu positive breast cancer.

2.0 Background

Breast cancer is the most common cancer diagnosed in American women and the second most common cause of death. It is estimated that 232,670 women will be diagnosed with breast cancer and 40,000 would die of the disease in 2014[1]. The mainstay of treatment for early breast cancer (EBC) is surgical removal of the tumor followed by adjuvant systemic treatment (chemotherapy +/- hormonal therapy) and/or radiation therapy to reduce the recurrence. SEER Summary Stage 2000 shows the stage of distribution at diagnosis for localized breast cancers (those diagnosed while the cancer is still confined to the primary site) is 60% (Stage I); while 33% are diagnosed after the cancer has spread to regional lymph nodes or directly beyond the primary site (Stage II). The overall survival rates are 98.3% for localized stage I and 83.5% for regional stage II disease[2]. The relative overall survival, as well as, the risk of recurrence is improved after adjuvant therapy. It is estimated that the risk of recurrence for patients after adjuvant therapy with stage I breast cancer is 5% and patients with stage II disease is 11%[3].

Evolution of adjuvant chemotherapy in EBC:

Until 1998, the regimen of cyclophosphamide, methotrexate and 5-fluorouracil (5-FU) (CMF) remained the standard adjuvant therapy of choice for women with node positive EBC[4]. CMF is a six month long regimen in which chemotherapy is administered intravenously twice every four weeks along with a daily oral chemotherapy, two of every four weeks. A newer and simpler regimen, adriamycin and cyclophosphamide (AC), given every three weeks for four cycles (total duration-12 weeks) has been compared to the CMF regimen with the goal to shorten the treatment duration. Two National Surgical Adjuvant Breast and Bowel Project (NSABP) trials (NSABP B15 in node positive and B23 in node negative patients) did demonstrate that four cycles of AC are equivalent to six cycles of CMF in the disease free and overall survival of EBC[5].

Later on, a meta-analysis reported on by the Early Breast Cancer Trialists Collaborative Group (EBCTCG) showed that the doublet regimen of AC was also associated with a small but

significant decrease in the recurrence and mortality rates of breast cancer when compared to the triplet CMF regimen[6]. Based on this observation, the National Institutes of Health Consensus Development Conference on Adjuvant Therapy for Breast Cancer recommended anthracycline-based regimens in the adjuvant treatment of breast cancer.

With the evolution of taxanes in 1998 and studies demonstrating or documenting taxanes excellent activity in the treatment of metastatic breast cancer (MBC)[7], the addition of a taxane to the conventional adjuvant therapy regimen for node positive and high risk node negative EBC was studied. The results of these trials showed an improvement of the recurrence free and overall survival rates of EBC's. A systematic review of taxane versus non-taxane containing chemotherapy regimens for adjuvant and neoadjuvant (before surgery) treatment of operable breast cancer was published by the EBCTCG in 2004[8]. The review included data on more than 12,000 women and strongly supported the addition of four cycles of the taxane paclitaxel to the standard four cycles of the AC regimen (PAC), or the substitution of the six cycle regimen of 5-FU, adriamycin and cyclophosphamide (FAC) with a six cycle regimen of the taxane docetaxel in combination with adriamycin and cyclophosphamide (TAC).

The largest study in the 2004 EBCTCG systematic review was the CALGB 9344 trial, which showed that the sequential addition of 4 cycles of every 3 weeks paclitaxel to the 4 cycles of every 3 week AC in the adjuvant setting is beneficial in achieving better disease free and overall survival in node positive breast cancer[9].

Single agent paclitaxel has been compared to FAC in the neo adjuvant setting in a small study by Buzdar *etal*. In this study, 174 patients were randomized to receive, pre-operatively, either 4 cycles of FAC or paclitaxel. In this setting, paclitaxel was as effective as FAC, as shown in this table [10].

FAC vs paclitaxel as neo adjuvant therapy in pts with operable breast cancer

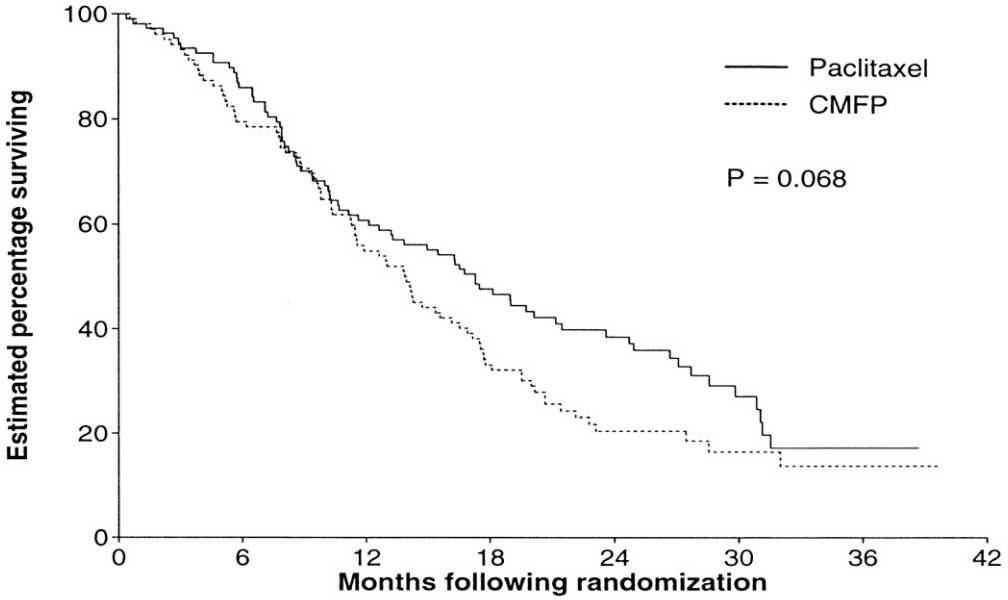
	FAC	Paclitaxel
Clinical CR	24%	27%
Clinical PR	55%	53%
Path CR	18%	6%
Path in situ	5%	8%
Path min. (<1cm)	12%	27%

* Results not statistically significant. *Buzdar et al. JCO 1999; 17:3412*

Since the FAC regimen has been considered an acceptable standard adjuvant chemotherapy regimen, it raised the question of the possibility of the equivalent efficacy of the single agent paclitaxel in the adjuvant setting. This hypothesis led to the inception of an Alliance trial, CALGB 40101in the adjuvant setting . The objective was to evaluate four cycles of single agent paclitaxel given every two weeks compared to four cycles of AC given every two weeks with growth factor support. The trial comparison did not show non inferiority (means equivalence) of the single agent paclitaxel to AC however, paclitaxel was shown to be less toxic than AC[11].

Paclitaxel alone has been compared with the CMF plus prednisone (CMFP) regimen as a first-line therapy for MBC and was found to have similar response rates and also significantly

improved overall survival as shown below[12]. Paclitaxel alone has also been compared with single agent adriamycin in MBC and was found to have equivalent efficacy and disease free survival[7].



Number at risk

Paclit. 107	92	65	47	30	13	2	0
CMFP 102	81	56	33	15	8	2	0

Cardiotoxicity of anthracyclines:

Significant cardiac toxicity has been observed with anthracyclines when used in the adjuvant setting for breast cancer patients. This observation rendered the evaluation of non-anthracycline based regimen particularly interesting.

In order to get the same efficacy but less cardio toxicity, docetaxel was combined with cyclophosphamide (TC regimen) and was compared with standard regimen AC. Stage I – III breast cancer patients were randomized to receive TC every 3 weeks for 4 cycles vs AC every 3 weeks for 4 cycles. At 5 years TC had comparable efficacy with slightly better disease free survival and less cardiac toxicity[13]. In an update of the above trial, at a median follow up of 7years,TC was shown to be significantly better than AC in terms of disease free survival (81% Vs 75%) and overall survival (87% Vs 82%)[14].

Docetaxel has been compared to paclitaxel in a large randomized trial (ECOG 1199) involving 4,950 women with EBC. This study consisted of 4 arms that compared weekly and every 3week docetaxel vs weekly and every 3 week paclitaxel in a 2 × 2 factorial design. These regimens were given sequentially after standard AC (AC every 3 weeks). Similar efficacy and disease free survival was noticed when paclitaxel was given weekly and docetaxel was given every 3 weeks compared to the other arms[15].
Considering the high level of anti-neoplastic activity of paclitaxel in metastatic and locally advanced breast cancer (given pre-operatively), it is suggested that there is a high likelihood of the paclitaxel and cyclophosphamide regimen (PC) being at least as effective as other standard adjuvant regimens for breast cancer. We hypothesize that this regimen would have a comparable

efficacy and adverse event profile to standard adjuvant adriamycin based regimens. The reason to choose paclitaxel instead of docetaxel in our study will be explained later in this discussion.

Dose dense hypothesis & reasons to choose paclitaxel instead of docetaxel:

Based on tumor growth, shortening the interval between treatment cycles from every 3 weeks to every 2 weeks(in a dose-dense fashion) improved the outcome for patients with node-positive EBC[16]. Dose-dense regimens limit the opportunity of re-growth of cancer cells between the chemotherapy cycles and improve overall and disease free survival.

Using anthracyclines and taxanes in a dose dense schedule has been shown to be more beneficial than the conventional every 3 week chemotherapy. The CALGB 9741 trial tested both sequential versus concurrent chemotherapy administration and a conventional dosing interval versus a dose-dense interval. In this trial, 2,005 women were randomly assigned to one of four arms in a 2 × 2 factorial design.

- Arm 1: Ax4→Tx4→Cx4 q 3 weeks (conventional interval, sequential administration)
- Arm 2: Ax4→Tx4→Cx4 q 2 weeks with filgrastim (dose-dense interval, sequential administration)
- Arm 3: ACx4→Tx4 q 3 weeks (conventional interval, concurrent administration)
- Arm 4: ACx4→Tx4 q 2 weeks with filgrastim (dose-dense interval, sequential administration).

A = adriamycin 60mg/m^2, T = paclitaxel 175 mg/m^2 over 3 hours, and C = cyclophosphamide 600mg/m^2. Tamoxifen was given after chemotherapy for hormone receptor positive (about 70 %) patients.

The dose-dense treatment improved both disease free and overall survival. There was no difference between concurrent and sequential scheduling of taxanes. Surprisingly there was less frequent severe neutropenia in the patients receiving dose-dense regimens. Grade 3 and 4 neutropenia was 11%, but this did not translate to significantly increased febrile episodes (<5%).

Based on the above study, dose-dense AC followed by dose-dense paclitaxel is now one of the standard treatment options for node-positive women as defined by the National Comprehensive Cancer Network (NCCN).

Also, paclitaxel given in a dose-dense fashion (every 2 weeks) was proven superior to once weekly paclitaxel in a SWOG (S0221) trial published last year[17]. The study design was an open-label 2 × 2 factorial design with equal probability of receiving each treatment combination. The first factor compared dose-dense AC for six cycles (total 12 weeks) versus a novel continuous schedule of adriamycin 24 mg/m^2 IV once per week, cyclophosphamide 60 mg/m^2 orally once per day, and filgrastim 5 micrograms/kg subcutaneously daily for 15 weeks. The second factor was subsequent dose-dense paclitaxel 175 mg/m^2 IV day 1 once every 2 weeks for six cycles versus paclitaxel 80 mg/m^2 IV once per week for 12 weeks. After October 2005, patients with human epidermal growth factor receptor 2 (HER2) positive tumors were allowed to receive trastuzumab concurrently with or after treatment with paclitaxel for a total of 1 year. It should be noted that the paclitaxel once a week was proven superior to paclitaxel given every 3 weeks in the ECOG 1199 trial discussed previously. This was the reason paclitaxel once per week (rather than paclitaxel given every three weeks) was taken as the control arm in the S0221 study.

Trial S0221 also showed that the patients achieved a similar DFS with any of these regimens. However, examination of relevant biologic subsets revealed that the advantage for dose-dense schedule observed only in patients with hormone receptor negative/HER2-negative disease. This observation is the result of a subset analysis that was not protocol specified and should be regarded as hypothesis generating only.

Based on the above discussion, we can conclude that the paclitaxel weekly and every two week schedules has equal efficacy and better than paclitaxel given every 3 weeks. Docetaxel given every 3 weeks has equal efficacy to paclitaxel given weekly (as per ECOG 1199) but it was never tested in a phase III trial in a dose-dense (ever 2 week) setting.

Of the taxanes, paclitaxel and docetaxel, paclitaxel has less hematological toxicity and has been used full dose every 2 weeks (dose-dense) with growth factor support in the CALGB 9741 and S0221 studies as discussed above. No similar dose-dense dosing has been established for docetaxel in phase III clinical trials.

Both paclitaxel and docetaxel include hair loss, peripheral neuropathy, myopathy (mainly muscle pains), nausea, vomiting, diarrhea, mucositis and generalized weakness as their main non-hematological toxicities.

Docetaxel, unlike paclitaxel, is associated with significant nail changes in up to 40 % of patients which include nail loosening or separation from the nail bed, nail discoloration, hyper-pigmentation and dislodgement. Also it was associated with lacrimal canal (tear canal) stenosis causing overflow of tears. These side effects were dose dependent and were reported in up to 75 % of patients with weekly administration and in less than 1% with every 3 week administrations.

For many young women with breast cancer, these toxicities were associated with psychological distress, pain and functional impairment. There is no curative treatment for nail toxicity associated with docetaxel and it takes up to 6 months for finger nails and up to a year for toe nails to become normal after treatment discontinuation[18].

As docetaxel was not studied in a dose-dense fashion in a phase III clinical trial and due to its significant non-hematological and hematological side effects as described above, we chose paclitaxel as our taxane of choice in combination with cyclophosphamide.

In our protocol, patients will have the opportunity to receive chemotherapy in a dose-dense fashion with growth factor support and will be able to complete their treatment earlier than the conventional standard regimens without exposure to anthracyclines and the associated cardiac risks.

<u>Evolution of adjuvant therapy in Her 2 positive EBC</u>:

Her2 receptor (protein) overexpression or Her2 gene amplification occurs in 20-30% of breast cancers and is considered as one of the poor prognostic markers in EBC. In 1996, Baselga *et al* at Memorial Sloan Kettering Cancer Center showed an overall response rate of 11.6% with weekly trastuzumab in a small phase II study of extensively treated Her2 positive women[19].In the pivotal

phase III trial for trastuzumab in MBC, the addition of trastuzumab to chemotherapy was associated with a significantly longer time to disease progression (median 7.4 Vs 4.6 months), higher objective response rate (50 vs 32 %) and longer survival (25.1 Vs 20.3 months). Based on this phase III trial, FDA approved trastuzumab in combination with paclitaxel for the first line treatment of women with Her2 positive MBC in 1998. This success motivated investigators to evaluate its role in the adjuvant setting.

Two trials NSABP-B31 and NCCTG (North Central Cancer Treatment Group) N 9831 were analyzed jointly in view of their similarities between the control arms. Patients with Her-2 positive tumors were treated with four cycles of AC every 3 weeks followed by 12 weekly doses of paclitaxel given concurrently or sequentially with 52 weeks of trastuzumab. The updated data (median follow up of 8 years) showed that the addition of adjuvant trastuzumab to chemotherapy increased the ten year overall survival from 75% to 84%[20].

Cardiac safety from these joint analyses, reviewed by an independent board of cardiologists and oncologists, concluded that the rate of symptomatic congestive heart failure was 0.5% with chemotherapy alone and 2.0% with AC followed by paclitaxel and trastuzumab. Moreover, 86% of patients in the trastuzumab arms had either complete or partial resolution of the cardiac events.

The concern over cardio toxicity associated with adjuvant trastuzumab and anthracycline treatment emphasizes more to evaluate non-anthracycline based regimens in Her2 positive disease. This has led to the Breast Cancer International Research Group, BCIRG 006 trial.

The docetaxel, carboplatin and trastuzumab (TCH) combination was compared with standard anthracycline based chemotherapy in the BCIRG 006 trial[21]. In this three arm study, TCH every 3 weeks for 6 cycles was compared to AC every 3 weeks for 4 cycles followed by docetaxel every 3 weeks for 4 cycles (AC->T) and the same (AC -> T) with trastuzumab (AC-TH). After a median follow-up of 65 months, risk of recurrence was reduced to nearly 50% in both the trastuzumab containing arms. The rates of CHF were significantly higher in the group receiving AC-TH than in the TCH (2.0% Vs 0.4%). Eight cases of leukemia were reported and out of which seven were in anthracycline based regimens. This study established TCH ever 3 weeks for 6 cycles (total duration 18 weeks) as the standard non anthracycline regimen for the Her 2 positive disease in the adjuvant setting.

Duration of Adjuvant Trastuzumab (6 months Vs 12 months Vs 24 months)

The Finland Herceptin (Fin Her) study randomized 3 cycles of either every 3 weeks docetaxel or weekly vinorelbine followed by 3 cycles of FEC (5FU, epirubicin and cyclophosphamide). The subset of Her2 positive patients were further randomized to either observation or weekly trastuzumab for nine treatments given concurrently with docetaxel or vinorelbine[22]. The final analysis of this trial showed that the docetaxel arm had a better distant disease free survival than the vinorelbine arm. In the Her 2 positive subgroup, adjuvant trastuzumab for 9 weeks in combination with docetaxel improved the distant disease free survival.

The HERA trial allowed the use of any chemotherapy regimen prior to trastuzumab, and patients received either one or two years of trastuzumab. The study reported 50% reduction in relapse in patients receiving at least one year of trastuzumab. In this trial 94% patients received anthracyclines and 26% had a taxane. Clinical cardiac toxicity was a major side effect and was reported in 5% of patients and trastuzumab was discontinued[23]. It also showed that two years of adjuvant trastuzumab was *not* more effective than one year of trastuzumab.

The PHARE study showed that the adjuvant trastuzumab for a shorter duration for 6 months was not non inferior (means equivalence) to standard 12 months of adjuvant trastuzumab[24].

The above three trials established one year of trastuzumab as the standard of care in the adjuvant setting. Based on these resuls, the NCCN recommends one year of adjuvant trastuzumab as the standard of care for Her 2 positive breast cancer.

The evidence from the above large randomized controlled trials in the adjuvant setting, along with some smaller trials in the neoadjuvant setting (NOAH), firmly established trastuzumab (for one year) in combination with various anthracycline and non anthracycline based chemotherapy regimens as the standard of care for the Her 2 positive EBC[25]. However, the TCH (where "C" stands for carboplatin and not cyclophosphamide) regimen was the only non anthracycline based combination chemotherapy regimen tested in a phase III clinical setting and it was administered every 3 weeks. So far, no dose-dense (every 2 weeks) non anthracycline regimens were studied in the Her2 positive EBC.

We were hoping to test the feasibility of giving trastuzumab with the above mentioned PC regimen [The "PC-H" regimen] in a dose dense fashion (every 2 weeks) with growth factor support for 6 cycles. This non anthracycline based regimen, if maintains efficacy, will not only reduce the cardio-toxicity but also shorten the duration of chemotherapy for Her 2 positive patients by 6 weeks compared to the now standard non anthracycline regimen TCH.

3.0: Eligibility Criteria

3.1 Inclusion

1. Histologically confirmed newly diagnosed Stage I-II HER2/neu positive breast cancer.
2. Women above 19 years of age will be included.
3. Women of reproductive potential must be non-pregnant and non-nursing and must agree to employ an effective barrier method of birth control throughout the study and for up to 6 months following treatment.
4. Women of child-bearing potential must have a negative pregnancy test within 7 days of initiating study. *(No childbearing potential is defined as age 55 years or older and no menses for two years or any age with surgical removal of the uterus and/or both ovaries).*
5. ECOG performance status of 0 or 1.
6. Adequate laboratory parameters within 30 days prior to enrollment defined as:
    - Absolute neutrophil count greater than or equal to 1,500/mcl
    - Platelet count equal to or greater than 150,000/mcl
    - Hemoglobin >11gm/dl
    - Alkaline phosphatase equal or less than 1.5 times the ULN
    - Total bilirubin equal to or less than 1.5 times the ULN
    - AST and ALT no greater than 1.5 times the ULN
    - Creatinine less than 1.5 times the ULN
    - Able to give informed consent.
7. All included subjects must have normal cardiac function as defined by an ejection fraction of >50% by echocardiogram.
8. Able to return for treatment and follow-up on the specified days.

3.2 Exclusion

1. Prior malignancy; except for adequately treated basal cell or squamous cell skin cancer or noninvasive carcinomas.
2. Subjects with pre-existing Grade II peripheral neuropathy.
3. History of previous chemotherapy.
4. Stage IV or metastatic breast cancer.
5. Pregnant or nursing women.
6. Inability to cooperate with treatment protocol.
7. No active serious infections or other conditions precluding chemotherapy
8. Any comorbidity or condition which, in the opinion of the investigator, may interfere with the assessments and procedures of this protocol e.g. unstable angina, myocardial infarction within 6 months, severe infection, etc.
9. Known hypersensitivity to any component of required drugs in the study.
10. Known positive for HIV or infectious hepatitis, type A,B or C or active hepatitis.
11. Any serious medical condition, laboratory abnormality, or psychiatric illness that would prevent the subject from signing the informed consent form.
12. Myocardial infarction within 6 months prior to enrollment or has New York Heart Association (NYHA) Class III or IV heart failure uncontrolled angina, severe uncontrolled ventricular arrhythmias, or electrocardiographic evidence of acute ischemia or active

conduction system abnormalities. (Appendix A) Prior to study entry, any ECG abnormality at screening has to be documented by the investigator as not medically relevant.

NOTE: All questions regarding eligibility for **UNMC potential subjects** should be directed to the UNMC Coordinator .

All questions regarding eligibility for **Participating/Collaborating Site** potential subjects should be directed to the Sponsor Principal Investigator by contacting the UNMC Fred & Pamela Buffett Cancer Center Multi-site Project Manager at 402-559-4596.

NOTE: UNMC and Participating/Collaborating Sites may use the Eligibility Checklist (Appendix B) as source documentation if it has been reviewed, signed, and dated prior to registration by the treating physician. The eligibility checklist must be accompanied by all other source documents that evidence the subject's eligibility (i.e., dictation, pathology, radiology, laboratory, etc.)

4.0: Registration Procedures

4.1 Recruitment

Subjects who are referred to the Nebraska Medicine (NM) / UNMC; Faith Regional Carson Cancer Center (FRCCC); or other IRB approved participating/collaborating sites, with newly diagnosed Stage I-II HER2/neu positive breast cancer may be eligible for this trial.

Screening eligibility, based on standard clinical care, will be performed by the treating physician at the time of encounter. On initial presentation, a history and physical examination are performed, laboratory data obtained, assessment of Performance Status, and echocardiogram. Radiologic/Imaging evaluations for staging will be performed as clinically indicated and is at the discretion of the treating physician. Any pathologic specimens obtained at referring Institutions will be reviewed for accuracy.

The patient's primary Oncologist will make the decision as to screened eligibility of the candidate based on the eligibility criteria listed above, prior to offering consent. (Section 3.0)

If the patient is screened as potentially eligible, she will then be offered the option to participate. An informed consent will be signed by the patient after thorough review of the study is completed by the physician and his/her designee.

Some Insurance carrier's may decline to cover the costs of usual medical care if the patient is participating in a clinical trial. The patient will be provided assistance by the research nurse coordinator or designated staff in determining if the insurance carrier will decline coverage. Insurance carriers may or may not pay for study related expenses. The patient can then decide if they wish to participate.

NOTE: Problems related to insurance coverage for UNMC potential subjects or enrolled subjects will be reported to the IRB as they are encountered.

4.2 Eligibility Verification/Registration

Before Subjects are registered to the study, an Eligibility Checklist (Appendix B) must be completed to verify the subject meets the eligibility criteria. Informed consent must be obtained by following procedures defined in section 12.6 entitled Process of Informed Consent.

Subjects will be registered through the Sponsor Principal Investigator by contacting the UNMC Fred & Pamela Buffett Cancer Center Research Multi-site Project Manager.

All Study personnel from UNMC and non-UNMC IRB approved sites will contact the UNMC Multi-site Project Manager if a subject appears to meet the eligibility criteria. They will submit the completed Eligibility Checklist (Appendix B) and relevant de-identified source documents to email to the UNMC Multi-site Project Manager to verify the subject meets the eligibility criteria. The Eligibility Check list will be maintained in a study file. If the UNMC Multi-site Project Manager confirms that the subject meets criteria and target

accrual has not been met, approval for the subject will be given. A confirmation of Registration will be forwarded by the UNMC Multi-site Project Manager.

For **UNMC subjects:**
Study personnel will provide the UNMC Fred & Pamela Buffett Cancer Center PRMS office and the UNMC Multi-site project manager a copy of the signed and dated consent form for each UNMC subject registered to the protocol within one (1) week that includes the following information:

- Protocol Number
- Subject Identification: Subject's name/initials, Study ID number
- Subject Demographics: gender, birth date (mm/dd/yyyy), race, ethnicity (See Subject Registration Request Form located in the Study Manual)
- Additional CTRP reporting requirements: Subject zip code/country (if not USA) and primary method of payment information

Participating/Collaborating Sites:
Participating/Collaborating sites must have both local *and* UNMC IRB approval, and have met all other UNMC criteria to enroll. Study personnel from non-UNMC IRB approved sites will provide the UNMC Multi-site Project Manager with the following information:

- Demographics cover sheet (located in the Study Manual)
- Copy of the signed and dated consent form (subject signature obliterated with signature line and subject initials visible)
- Signed Eligibility Checklist
- De-identified source documents with assigned subject I.D. (if known on each page or subject identification) to support eligibility (i.e., H&P, Medical/Surgical Hx, Lab, Pathology, scans, etc.).

Registration Date: eligibility verification and notification of assigned subject number (by UNMC) will be known as the Registration date.

Date of enrollment: is defined as the date of the start of study treatment / first protocol related intervention.

The listed documents/ information for subjects enrolled will be provided to the PRMS office within one (1) week of enrollment as applicable:

- UNMC and Participating Site Protocol Numbers
- Investigator/Participating Site Identifier (ID)
- Subject ID: Assigned by UNMC [Site ID followed by subject number (##-###)]
- Consent Date: Date subject signed consent
- Patient demographics: gender, birth date (mm/dd/yyyy), race, ethnicity
- Re-consent Date: (If applicable)
- Ineligibility Status: (If known)

- Off Study Date: (If applicable)

4.3 Instructions for Subjects Who Do Not Start Assigned Protocol Treatment

If a subject does not receive any assigned protocol treatment after consenting, baseline and follow-up data will still need to be collected and must be submitted according to the instructions in the protocol. The reason he/she did not receive any treatment and the date and type of the first non-protocol treatment that the subject receives must also be reported.

4.4 Requirements for Non-UNMC Sites/Participating/Collaborating Institutions Submitting Regulatory Documents

Before an Institution may enroll Subjects, all site activation criteria must be met by submitting required protocol specific regulatory documents to the UNMC Multi-site Project Manager via email.

4.5 Required Protocol Specific Regulatory Documents

1. Confirmation that the UNMC Team Designee(s) conducted an Initial Site Teleconference prior to opening the protocol to accrual at each participating/collaborating site.

5.0: Research Design

This is a Phase II study of adjuvant therapy using a dose-dense regimen of cyclophosphamide and paclitaxel with trastuzumab in subjects with newly diagnosed stage I-II breast cancer. This will be a non-randomized study; all subjects will receive the combination chemotherapy. All subjects will receive the same doses of the active compound.

5.1 *Standard of Care (SOC)* **Pre-treatment Evaluations within 30 days** prior to the Date of Enrollment (unless otherwise specified below). No tests or procedures are conducted solely for the purposes of research to determine subject eligibility.

- History and physical
- Current medications
- Documentation of baseline events (adverse events) if applicable
- CBC, diff, Platelet count
- Comprehensive Metabolic Panel (CMET) – electrolytes (sodium, potassium, chloride, bicarbonate, creatinine, BUN, AST, ALT, T. protein, albumin, bilirubin, alkaline phosphatase, calcium, glucose)
- Serum or urine pregnancy test (if applicable) **within 7 days** of first study-related treatment date.
- Height and weight
- Vital signs, including blood pressure
- ECOG Performance Status Assessment
- Echocardiogram
- The need for further radiologic evaluations for staging as clinically indicated and is at the discretion of the treating physician.
- Any pathologic specimens obtained at referring institutions will be reviewed for accuracy.

Research Blood Samples will be drawn at baseline, at the clinical endpoint (after cycle 6) and at the end of treatment (after year one or end of therapy). At each time point a blood collection of 30 ml including two – 10ml EDTA lavender top tubes and one – 10ml red top tube will be drawn to store for future research purposes including DNA sequencing.

Specimens submitted, by patients who consent to the additional specimen collection, will be processed to maximize their utility for future research projects and may include, but not limited to, extraction of plasma, serum, DNA and RNA.

The appropriate materials will be distributed to investigators for the diagnostic reviews and research studies.

Specimens from patients who consented to allow their specimens to be used for future approved research studies, including residuals from the currently defined reviews and research studies, will be retained in an UNMC-designated central repository.

If the patient agrees to let the samples be kept for future research, the samples will be kept until they are used up or destroyed. Most future research studies will focus on cancer, some research projects may also include other diseases, such as heart disease, diabetes or Alzheimer's disease. This may also include research on inherited traits.

5.2 Administration Schedule

Premedication: All subjects will receive premedication for nausea and vomiting with appropriate anti-emetic regimens based on recommendations made by the NCCN Guidelines (SOC), pre and post medications as prescribed by the treating physician.

(See http://www.nccn.org/professionals/physician_gls/PDF/antiemesis.pdf)

Subjects will receive diphenhydramine, ranitidine (or Pepcid if ranitidine is not on formulary) and dexamethasone prior to paclitaxel (SOC).

Systemic Therapy (Medicare Qualifying research related combination chemotherapy):

- Cyclophosphamide 600mg/m^2 in normal saline IV over 1 hour Day 1 cycled every 14 days (2 weeks) for 6 cycles

- Paclitaxel 175mg/m^2 in normal saline IV over 3 hrs Day 1 cycled every 14 days (2 weeks) for 6 cycles

- All cycles are with pegfilgrastim support, 1-6 mg SQ on day 1 after PC dose adjust as medically indicated or hold for ANC >12,000 or bone pain (SOC). (Pegfilgrastim support is optional following cycle 6)

  **NOTE: May use Neulasta On Body Injector per MD discretion.

- Loading Dose trastuzumab 6mg/kg in normal saline IV over 30 to 90 minutes per institutional protocol Day 1, Cycle 1

- THEN for Subsequent Cycles (Cycles 2-5), trastuzumab 4mg/kg in normal saline IV over 30 to 60 minutes per institutional protocol Day 1 every 14 days (2 weeks) Cycles 2-5.

- THEN for Maintenance Therapy beginning with Cycle 6, trastuzumab 6mg/kg in normal saline IV over 30 to 60 minutes per institutional protocol Day 1 every 21 days (3 weeks) to complete a total of 52 weeks after completing chemotherapy (Cycles 6- 19).

All subjects will receive a maximum of 6 cycles of chemotherapy and a maximum of 52 weeks of trastuzumab.

Radiation Therapy will be given by the radiation oncologist and timing determined by the radiation and medical oncologist as per standard NCCN guidelines, either concurrent with chemotherapy or following chemotherapy.
See NCCN guidelines. http://www.nccn.org/professionals/physician_gls/PDF/breast.pdf

Hormonal Therapy will be determined by the medical oncologist as per standard NCCN guidelines. See NCCN guidelines. Subjects with estrogen/progesterone receptor positive tumors will receive Hormonal Therapy following adjuvant chemotherapy.
See NCCN guidelines.http://www.nccn.org/professionals/physician_gls/PDF/breast.pdf.

5.3 Evaluations during therapy *(SOC)*:

- Subjects will have standard hematological and cardiac evaluations.

- Subjects will be clinically evaluated by an oncologist during chemotherapy every 2 weeks for toxicities, then at least every 12 weeks after completing chemotherapy until they complete 52 weeks of trastuzumab and then every 3 months for 2 years with the following at each visit:

    - CBC, platelet count
    - Comprehensive metabolic panel (CMET) – electrolytes (sodium, potassium, chloride, bicarbonate, creatinine, BUN, AST, ALT, T. protein, albumin, bilirubin, alkaline phosphatase, calcium, glucose)
    - Weight
    - Performance Status Assessment
    - Vital signs, including blood pressure

- Subjects will have repeat echocardiogram every 12 weeks until completion of trastuzumab to monitor their cardiac function. Normal cardiac function as defined by an ejection fraction of >50% by echocardiogram.

- The need for further radiologic evaluations for staging as clinically indicated and is at the discretion of the treating physician

- Research Blood Samples will be drawn at the clinical endpoint (after cycle 6) and at the end of treatment (after year one or end of therapy). At each time point a blood collection of 30 ml including two – 10ml EDTA lavender top tubes and one – 10ml red top tube will be drawn to store for future research purposes including DNA sequencing.

- Data will be collected on dose delays/reductions, hospitalizations, treatment discontinuation, deaths, and hematologic and cardiac toxicity.

5.4 Criteria for holding and resuming treatment

Treatment should be held until any non-hematologic grade 3-4 toxicities resolve to less than or equal to a grade 1 with dose modifications as per instructions below.

5.5 Dose Modifications

5.5.1 General instructions for dose modifications and delays

- Adverse events (AEs) will be graded according to the National Cancer Institute Common Terminology, Criteria for Adverse Events (NCI-CTCAE), version 4.03 June 14, 2010which can be accessed at the following URL. http://evs.nci.nih.gov/ftp1/CTCAE/CTCAE_4.03_2010-06-14_QuickReference_8.5x11.pdf
- All doses should be based on the AE requiring the greatest modification.

- Chemotherapy should be held for at least 1 week until any AE requiring dose modification returns to ≤ grade 1. The exceptions are neutropenia and bilirubin elevation (see Tables 3 and 4). If recovery to ≤ grade 1 has not occurred after 3 weeks of delay, chemotherapy must be discontinued.

- Doses that have been reduced may not be escalated.

- Proceeding with study therapy after grade 4 events, other than neutropenia/granulocytopenia and thrombocytopenia, is at the discretion of the physician.

5.5.2 Dose modifications and delays for cyclophosphamide and paclitaxel

All dose modifications for cyclophosphamide are based on the dose level changes in Table1.

TABLE 1. Dose level changes for cyclophosphamide

	Dose Level-0 ***Starting Dose*** **(mg/m^2)**	**Dose Level-1 (mg/m^2)**	**Dose Level-2 (mg/m^2)**	**Dose Level-3**
cyclophosphamide	600	500	400	Discontinue

All dose modifications and delays for paclitaxel are based on the dose level changes in Table 2

TABLE 2. Dose level changes for paclitaxel

	Dose Level-0 Starting Dose (mg/m^2)	**Dose Level-1 (mg/m^2)**	**Dose Level-2 (mg/m^2)**	**Dose Level-3 (mg/m^2)**	**Dose Level-4 (mg/m^2)**
paclitaxel	175	175	135	135	100

Instructions for all other toxicities are listed on Table 3. Note: Dose modifications must be based on AE requiring the greatest modification; AEs observed during the cycle; ***and*** AEs present on the scheduled Cycle Day 1.

TABLE 3. Dose modifications & delays for cyclophosphamide & paclitaxel

NCI CTCAE v4.03 Category/Grade	**Modifications for AEs that occurred during a cycle but DID NOT REQUIRE DELAY IN TREATMENT** *(a)*	**Modifications for AEs that REQUIRED TREATMENT DELAY** (*b*)
Blood/bone marrow: **Neutrophils/granulocytes** Grades 3, 4	Maintain dose	**ANC:** Hold until ≥ 1000/mm^3. If recovery takes: 1 week – maintain dose 2 to 3 weeks – ↓ one dose level > 3 weeks – D/C study therapy
Platelets Grades 2, 3 Grade 4	Maintain dose ↓ one dose level	**Platelets**: Hold until ≥ 75,000/mm^3 If grade 2 or 3 and recovery takes: ≤ 1 week – maintain dose 2 to 3 weeks - ↓ one dose level > 3 weeks – D/C study therapy If grade 4, ↓ one dose level

		If recovery takes> 3 weeks, D/C study therapy.
GI (if related to chemotherapy)		
Diarrhea		
Grade 2	Maintain dose	↓ one dose level
Grade 3	↓ one dose level	↓ one dose level
Grade 4	↓ two dose levels or D/C	↓ two dose levels or D/C
Mucositis/stomatitis		
Grade 2	Maintain dose	↓ one dose level
Grade 3	↓ one dose level	↓ one dose level
Grade 4	↓ two dose levels or D/C	↓ two dose levels or D/C
Vomiting (*despite antiemetics*)		
Grade 2	↓ one dose level (opt)	↓ one dose level (opt)
Grades 3,4	↓ two dose levels or D/C	↓ two dose levels or D/C
Hepatic function:		Hold until bilirubin returns to the baseline grade and AST and alk phos have returned to ≤ grade 1.
Bilirubin or AST or alk phos		
Grade 2	↓ one dose level	↓ one dose level
Grade 3	↓ two dose levels	↓ two dose levels
Grade 4	D/C	D/C
Infection:		
Febrile neutropenia		
Grade 3	↓ one dose level	↓ one dose level
Grade 4	↓ two dose levels or D/C	↓ two dose levels or D/C
Infection with grade 3 or 4 ANC		
Grade 3	↓ one dose level	↓ one dose level
Grade 4	↓ two dose levels or D/C	↓ two dose levels or D/C
Infection with normal ANC		
Grade 3	Maintain dose	Maintain dose
Grade 4	↓ two dose levels or D/C	↓ two dose levels or D/C
Other clinically significant AEs *(c):*		
Grade 3	↓ one dose level	↓ one dose level
Grade 4	↓ two dose levels or D/C	↓ two dose levels or D/C

(a) -Treatment may not proceed until toxicity is ≤ grade 1, except for neutrophils/ granulocytes, which must be ≥ 1000/mm^3 and bilirubin, which must be at or below the baseline grade.
(b) -Hold and check weekly. With exception of neutrophils and bilirubin, resume treatment when toxicity is ≤ grade 1. If toxicity has not resolved to ≤ grade 1 after 3 weeks of delay, discontinue chemotherapy.
(c) -Determination of "clinically significant" AEs is at the discretion of the investigator.

5.5.3 Management of paclitaxel-related hypersensitivity reactions

Management of paclitaxel-related hypersensitivity reactions is at the investigator's discretion.

5.5.4 Management of paclitaxel-related Neurosensory Toxicity and Musculoskeletal pain

Dose modifications for paclitaxel related neurosensory toxicity are outlined on Table 4.
Dose modifications for paclitaxel related musculoskeletal pain are outlined on Table 5.

TABLE 4. Dose modifications for neurosensory toxicity related to the paclitaxel

Paresthesias/Dysesthesias	1-7 Day Duration	Persistent for > 7 Days *or* Caused the Next Cycle to be Delayed
Grade 1 Paresthesias/dysesthesias of short duration that resolve and do not interfere with function	Maintain dose	Maintain dose
Grade 2 Paresthesias/dysesthesias interfering with function, but not activities of daily living	Maintain dose (*a)*	Decrease the ***paclitaxel*** to the next lower dose. (*b),(c)*
Grade 3 Paresthesias/dysesthesias with pain or with function impairment that also interfere with activities of daily living	**First episode:** Decrease the ***paclitaxel*** to the next lower dose	

	(*a),(c)* **Second episode:** Stop the ***paclitaxel***	Stop the ***paclitaxel***
Grade 4 Persistent paresthesias/dysesthesias that are disabling or life-threatening	Stop the ***paclitaxel***	Stop the ***paclitaxel***

(a) -Use of narcotics and NSAIDs is encouraged to maintain dose of paclitaxel if possible.
(b) -Consider modifying G-CSF.
(c) -Decrease the paclitaxel to the *next lower dose*, e.g., for paclitaxel, from 175 mg/m^2 to 135 mg/m^2 or from135 mg/m^2 to 100 mg/m^2. (Note that, the paclitaxel dose does not always decrease by one dose level; therefore, reducing the paclitaxel dose may mean adjusting by more than one dose level increment. Refer to Table 2.)

TABLE 5. Dose modifications for musculoskeletal pain attributed to the paclitaxel and *not controlled by analgesics* *(a)*

Musculoskeletal Pain	1-7 Day Duration	Persistent for > 7 Days *or* Caused the Next Cycle to be Delayed
Grade 1	Maintain dose	Maintain dose
Grade 2	Maintain dose	Maintain dose OR Decrease the ***paclitaxel*** to the next lower dose (*b),(c)*
Grade 3	**First episode:** Decrease the ***paclitaxel*** to the next dose (*b),(c)* **Second episode:** Stop the ***paclitaxel***	**First episode:** Decrease the ***paclitaxel*** to the next lower dose (*b),(c)* OR Stop the ***paclitaxel*** **Second episode:** Stop the ***paclitaxel***
Grade 4	Stop the ***paclitaxel***	Stop the ***paclitaxel***

(a)- Use of narcotics and NSAIDs is encouraged to maintain dose of paclitaxel if possible.
(b)- Consider modifying G-CSF.
(c)- Decrease the paclitaxel to the *next lower dose*, e.g., for paclitaxel, from 175 mg/m^2 to 135 mg/m^2 or from135 mg/m^2 to 100 mg/m^2. (Note that, the paclitaxel dose does not always decrease by one dose level; therefore, reducing the paclitaxel dose may mean adjusting by more than one dose level increment. Refer to Table 2.)

5.6 Correlative Studies Related to the Research -

Research Blood Samples will be drawn at baseline, clinical endpoint (after cycle 6) and end of treatment (after year one or end of therapy)-30 ml including two – 10ml EDTA lavender top tubes and one – 10ml red top tube to store for future research purposes including DNA sequencing. **SEE APPENDIX C** for instructions.

Send blood samples overnight at room temperature. Monday through Thursday only; do not send on Friday or the day before a holiday.

Shipping Address:

David L. Kelly PhD
Assistant Professor
Eppley Institute for Research in Cancer and Allied Diseases
University of Nebraska Medical Center
601 S. Saddle Creek Rd.

F&PBCC Rm. 5.12.428
Omaha, Nebraska 68106
Tel: (402) 559-9157
Cell: (402) 699-1132
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dkelly@unmc.edu

And / Or

Amy Wells, MS
Research Technologist
Eppley Institute for Research in Cancer and Allied Diseases
University of Nebraska Medical Center
601 S. Saddle Creek Rd.
F&PBCC Rm. 5.12.428
Omaha, Nebraska 68106
Tel: (402) 559-6015
awells@unmc.edu

6.0: Measurement of Effect

This trial will assess the safety and toxicity, particularly regarding cardiac function, of administering a dose dense regimen of cyclophosphamide, paclitaxel with trastuzumab for 6 cycles with growth factor support in newly diagnosed Stage I-II HER2/neu breast cancer subjects by measuring adherence to the intended dosing schedule and accrual, as well as by documenting and reporting toxicities, and evaluating echocardiogram results from baseline, during and after trastuzumab. Normal cardiac function as defined by an ejection fraction of >50% by echocardiogram.

Toxicity Criteria: The NCI Common Toxicity Criteria Adverse Events version 4.03 June 14, 2010 will be used to grade toxicity; it is available at the following internet site http://evs.nci.nih.gov/ftp1/CTCAE/CTCAE_4.03_2010-06-14_QuickReference_8.5x11.pdf .

Survival: Subjects will be analyzed with respect to overall and recurrence free survival; however there will be no comparative group.

Overall survival is defined as time from the first chemotherapy administered on the trial until death from any cause. For subjects who are still alive at the time of the study analysis or are lost

to follow-up, survival will be censored at the last recorded date that the subject was known to be alive.

Recurrence-free survival is defined as from the first date of therapy until the first notation of clinical progression, relapse or death from any cause. For subjects who are still recurrence-free at the time of the study analysis or are lost to follow-up, recurrence-free will be censored at the last recorded date that the subject was known to be recurrence-free. Response will be determined by the principal investigator or the co-principal investigators.

7.0: Study Parameters

Table 6:

Study	Within 30 days Rx start	Cycle 1 (Week 1-2, Cycles are Q2wks)	Cycles 2-6 (Week 3-12, Cycles are Q2wks)	Maintenance Cycles 7-19 (52 weeks) /Follow up
Informed consent	X			
History	X			
Meds	X	X	X	X
VS Wt & ECOG PS	X	X	X	X [c]
Ht	X			
Serum or urine Pregnancy test (if applicable)	X (within 7 days of tx)			
CBC, diff, plt	X	X	X	X [c]
CMET	X	X	X	X [c]
Research Blood Sample	X		X [g]	X [g]
Echocardiogram [a]	X [a]		X [a]	X [a]
Adverse events	X	X	X	X [e]
Radiologic evaluations [f]	X [f]		X [f]	X [f]
All SUBJECTS:				
N= pegfilgrastim		Day1 [d]	Day1 [d]	
CT=cyclophosphamide paclitaxel		Day 1	Day 1 Q Cycle 2-6	
Trastuzumab		Loading dose 6mg/kg Day 1 Cycle 1	Subsequent dose 4mg/kg Day 1 Cycles 2-5	Maintenance dose 6mg/kg/q **3** weeks ***(begin with cycle 6 & continue thru cycle 19 (52 weeks)*** [c]**.**
Radiation and hormone TRX [b] SOC (NCCN Guidelines)				

X = Prior to each cycle

a- Repeat echocardiogram every 12 weeks until completion of trastuzumab to monitor cardiac function.

b- Radiation Therapy will be given by the radiation oncologist and timing determined by the radiation and medical oncologist as per standard NCCN guidelines, either concurrent with chemotherapy or following chemotherapy unless the patient has received radiation to the area in the past. Subjects with estrogen/progesterone receptor positive tumors will receive Hormonal Therapy following adjuvant chemotherapy, and Hormonal Therapy will be determined by the medical oncologist as per standard NCCN guidelines.

c- Subjects will have standard hematological and cardiac evaluations. Subjects will be clinically evaluated by an oncologist during chemotherapy every 2 weeks for toxicities, then at least every 12 weeks after completing chemotherapy until they complete 52 weeks of trastuzumab and then every 3 months for 2 years.

d- Administered 1-6 mg SQ on day 1 after PC dose adjust as medically indicated or hold for ANC >12,000 or bone pain (SOC). (Pegfilgrastim support is optional following cycle 6)

****Note**: May use Neulasta On Body Injector per MD discretion.

e- All AEs will be followed until resolution or a cause is identified. AEs judged by the investigator as not related or probably not related to the treatment will not be followed beyond the 4 weeks after the final chemotherapy (including trastuzumab). However, subjects will be monitored for neuro and cardio toxicities for up to 3 months after final chemotherapy treatment (including trastuzumab). Concomitant medications will be collected up to 3 months after final chemotherapy treatment (including trastuzumab) to facilitate AE attribution assessment.

f- The need for further radiologic evaluations for staging as clinically indicated and is at the discretion of the treating physician

g- Research Blood Samples collected at Baseline, Clinical Endpoint (after cycle 6 and End of treatment (after year one or end of therapy). (Res)

8.0: Drug Formulation and Procurement

8.1 Trastuzumab (Herceptin)

8.1.1 Form

Herceptin for injection

8.1.2 Preparation

Supplied as 440mg lyophilized Powder.

8.1.3 Dilution

Reconstitute with 20ml SWFI (sterile water for injection) or BWFI (bacteriostatic water for injection); dilute with NS to a concentration of 21mg/ml. DO NOT USE D5W.

8.1.4 Storage and Stability

Refrigerate vials, do not freeze.

8.1.5 Method of Administration

Loading dose: 6 mg/kg IV C1. Subsequent dose: 4 mg/kg IV C2-5. Maintenance dose: 6mg/kg IV weekly C6-19.

8.1.6 Precautions

Benzyl alcohol hypersensitivity: use SWFI for reconstitution. Extreme caution should be exercised in treating Subjects with pre-existing cardiac dysfunction, congestive heart failure/ventricular dysfunction. Treatment discontinuation should be considered. Prior/concurrent AC or radiation to the chest may enhance the risk of cardiotoxicity.

8.1.7 Clinical Toxicities

COMMON

Hematologic: may exacerbate chemotherapy induced neutropenia or anemia
Gastrointestinal: Diarrhea, nausea, and vomiting
Other: May cause "flu-like symptoms" with generalized aches and pains, headache, fever, chills, nausea, vomiting and diarrhea

SERIOUS

Hypersensitivity reactions, including fatal anaphylaxis
Infusion reactions, including some with a fatal outcome
Respiratory: Acute respiratory distress syndrome (ARDS), bronchospasm, pneumonitis
Other: cardiomyopathy, infusion associated symptoms, hypersensitivity reactions, changes in kidney function

8.1.8 Commercial Availability

This drug is commercially available.

8.2 Pegfilgrastim (per Micromedex®) Neulasta

8.2.1Classification

Colony stimulating factor, hematopoietic

8.2.2 Mode of Action

Systemic: Pegfilgrastim is a Colony Stimulating Factor that acts on hematopoietic cells by binding to specific cell surface receptors thereby stimulating proliferation, differentiation, commitment, and end cell functional activation.

8.2.3 Storage and Stability

1) The manufacturer recommends storage of Neulasta(TM) syringes at 2 to 8 degrees C (36 to 46 degrees F), avoidance of freezing or shaking, and leaving syringes in the carton provided until time of use to protect from light (Prod Info Neulasta(TM), 2000).

2) Syringes of Neulasta(TM) can reach room temperature for up to 48 hours prior to use; during this time, they should be protected from light. Syringes left at room temperature for longer than 48 hours should be discarded (Prod Info Neulasta(TM), 2000).

3) If syringes are accidentally frozen, thawing under refrigeration is acceptable prior to use. However, syringes should be discarded if accidentally frozen a second time (Prod Info Neulasta(TM), 2000).

8.2.4 Known Clinical Toxicities

COMMON

Gastrointestinal: Nausea and vomiting
Musculoskeletal: Bone pain
Other: Influenza-like illness

SERIOUS

Hematologic: Hemoglobin SS disease with crisis
Respiratory: Acute respiratory distress syndrome
Other: Rupture of spleen (rare)

8.2.5 Supplier

AMGEN

8.2.6 Commercial Availability

This drug is commercially available

8.3 Cyclophosphamide per Micromedex®) Cytoxan, Cytoxan Lyophilized

8.3.1 Classification

Alkylating Agent, Antineoplastic Agent, Nitrogen Mustard

8.3.2 Mode of Action

Cyclophosphamide is classed as an alkylating agent of the nitrogen mustard type. An activated form of cyclophosphamide, phosphoramide mustard, alkylates or binds with many intracellular molecular structures, including nucleic acids. Its cytotoxic action is primarily due to cross-linking of strands of DNA and RNA, as well as to inhibition of protein synthesis. Cyclophosphamide is a potent immunosuppressant. It also causes marked and persistent inhibition of cholinesterase activity.

8.3.3 Storage and Stability

1) Intravenous Powder for Solution: 1 GM, 2 GM, 100 MG, 200 MG, 500 MG

2) Reconstituted solutions of lyophilized cyclophosphamide are chemically and physically stable for 24 hours at room temperature or for 6 days in the refrigerator. Specific temperatures are not provided by the manufacturer (Prod Info Cytoxan(R), 2000a; Brooke et al, 1973).

3**)** The rate of dissolution is occasionally slow (10 to 15 minutes) due to various crystal forms. Warming the solution to 50 or 60 degrees Celsius for 15 minutes may improve dissolution with negligible decomposition. However, heating to 70 to 80 degrees Celsius for 15 minutes has resulted in 10% to 23% loss of potency. Lyophilized cyclophosphamide for injection has a rapid dissolution rate with no warming needed (Brooke et al, 1975).

4) Reconstitution of cyclophosphamide with bacteriostatic water containing benzyl alcohol preservative may result in decomposition (Brooke et al, 1973).

8.3.4 Known Clinical Toxicities

COMMON

Dermatologic: Alopecia
Gastrointestinal: Nausea and vomiting
Hematologic: Leukopenia
Reproductive: Amenorrhea

SERIOUS

Cardiovascular: Cardiomyopathy
Dermatologic: Stevens-Johnson syndrome, Toxic epidermal necrolysis (rare)
Renal: Hemorrhagic cystitis
Reproductive: Azoospermia, Oligozoospermia
Respiratory: Interstitial pneumonia
Other: Infectious disease

8.3.5 Supplier

Bristol-Myers Squibb

8.3.6 Commercial Availability

This drug is commercially available

8.4 Paclitaxel (per Micromedex®) Taxol, Onxol, Nov-Onxol, Paclitaxel Novaplus

8.4.1 Classification

Antineoplastic Agent, Mitotic Inhibitor

8.4.2 Mode of Action

Paclitaxel belongs to the class of medications known as antimicrotubule agents. It promotes the assembly of microtubules from tubulin dimers and stabilizes microtubules by preventing depolymerization. This stability results in the inhibition of the normal dynamic reorganization of the microtubule network that is essential for vital interphase and mitotic cellular functions. In addition, paclitaxel induces abnormal arrays or 'bundles' of microtubules throughout the cell cycle and multiple asters of microtubules during mitosis. Paclitaxel enhances the cytotoxic effects of ionizing radiation in vitro.

8.4.3 Storage and Stability

1) Intravenous Solution: 6 MG/ML

2) Paclitaxel vials should be stored between 20 to 25 degrees Celsius (68 to 77 degrees Fahrenheit)) in the original containers protected from light. Refrigeration or freezing will not adversely affect the product. If a precipitate forms upon refrigeration, allow the vial to come to room temperature and the precipitate will dissolve with little or no agitation (Prod Info Taxol(R), 2000c).

3**)** Paclitaxel solutions prepared in 5% dextrose or 0.9% sodium chloride in concentrations of 1.2 mg/mL, leached diethylhexyl phthalate (DEHP) from polyvinyl chloride (PVC) bags at 1, 2, 4, and 8 hours (DEHP range of 50 to 208 micrograms/mL). Leaching also occurred after simulated three-hour infusions. In all tested conditions, leaching was primarily due to the lipophilic vehicle. The highest amount of DEHP leached by the vehicle was 83 mcg/mL at 8 hours, with a 1.2 mg/mL 0.9% sodium chloride solution, stored at 20 to 22 degrees Celsius. Leaching of DEHP, a commonly used plasticizer in PVC bags increased with time and was independent of the brand of PVC infusion materials. When compared to docetaxel, paclitaxel leached two to eight times more DEHP from the PVC infusion materials (Mazzo et al, 1997).

4) Extended-stability paclitaxel admixtures containing 1 mg/mL paclitaxel in 5% dextrose injection with the addition of sterile, dehydrated alcohol injection to yield a 20% or 25% ethanol concentration was found to be physically stable for at least 7 days as compared to conventional paclitaxel admixtures. Extended-stability paclitaxel 1 mg/mL containing 25% ethanol was also able to maintain a low concentration of bacteria, yeast and molds throughout the 28-day antimicrobial preservatives effectiveness test period demonstrating its low potential for contamination during long-term continuous infusion (Trissel et al, 1997a).

5) Stability studies have demonstrated that paclitaxel, in concentrations of 0.3 to 1.2 milligrams/milliliter is stable for 24 hours at room temperature in Dextrose 5% in water or Sodium chloride 0.9% (Waugh et al, 1991).

8.4.4 Black Box Warning

Anaphylaxis and severe hypersensitivity reactions characterized by dyspnea and hypotension requiring treatment, angioedema, and generalized urticaria have occurred in 2% to 4% of patients receiving paclitaxel in clinical trials. Fatal reactions have occurred in patients despite premedication. All patients should be pretreated with corticosteroids, diphenhydramine, and H(2) antagonists. Patients who experience severe hypersensitivity reactions to paclitaxel should not be rechallenged with the drug.

Paclitaxel therapy should not be given to patients with solid tumors who have baseline neutrophil counts of less than 1,500 cells/mm(3). In order to monitor the occurrence of bone marrow suppression, primarily neutropenia, which may be severe and result in infection, it is recommended that frequent peripheral blood cell counts be performed on all patients receiving paclitaxel.

8.4.5 Known Clinical Toxicities

COMMON

Dermatologic: Alopecia
Gastrointestinal: Nausea and vomiting
Hepatic: Liver function tests abnormal
Musculoskeletal: Arthralgia, Myalgia
Neurologic: Peripheral neuropathy

SERIOUS

Cardiovascular: Coronary artery stent thrombosis (late (> 1 year), 0.7%), Hypotension (12%) bradycardia
Hematologic: Myelosuppression, Neutropenic disorder
Immunologic: Anaphylaxis (2-4%), Immune hypersensitivity reaction

8.4.6 Supplier

Abbott Laboratories

8.4.7 Commercial Availability

This drug is commercially available

9.0: Toxicity Reporting Guidelines

The chemotherapeutic agents used in the protocol are commercially available agents with well-characterized toxicity profiles.

This protocol will comply with monitoring and adverse event reporting requirements of the UNMC/Fred & Pamela Buffett Cancer Center Data Monitoring plan. The protocol will adhere to the institutional, and FDA Guidelines for Toxicity Reporting.

Adverse events (AEs) will be graded according to the National Cancer Institute Common Terminology, Criteria for Adverse Events (NCI-CTCAE), Version 4.03 June 14, 2010 which can be accessed at the following URL.

http://evs.nci.nih.gov/ftp1/CTCAE/CTCAE_4.03_2010-06-14_QuickReference_8.5x11.pdf

All subjects will be closely followed for toxicity/adverse events (AEs). Adverse events will be assessed by reports from subjects to their physician/Investigator and by physical examinations. All AEs will be followed until resolution or a cause is identified.

AEs considered by the Investigator as not related or probably not related to the treatment will not be followed beyond 4 weeks (30 days) after the final dose of study drug is administered (including trastuzumab). However, subjects will be monitored for neuro and cardio toxicities for a minimum of 3 months after final dose of study drug is administered (including trastuzumab). Concomitant medications will be collected up to 3 months after final dose of study drug is administered (including trastuzumab) to facilitate AE attribution assessment

Deaths occurring within 30 days of study treatment regardless of relationship will be reported to the Fred & Pamela Buffett Cancer Center Data and Safety Monitoring Committee (DSMC).

Adverse Experiences Definitions

Adverse Event

An adverse event (AE) is defined as any untoward medical occurrence in a clinical trial subject administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment. An adverse event can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not the event is considered causally related to the use of the product.

An elective surgery or procedure that is scheduled to occur during a study will not be considered an adverse event if the surgery or procedure is being performed for a pre-existing condition and the surgery or procedure has been planned before study entry. However, if the pre-existing condition deteriorates unexpectedly during the study (*i.e.,* the surgery is performed earlier than

planned), then the deterioration of the condition for which the elective surgery or procedure is being done will be considered an adverse event.

Any worsening of a pre-existing condition or illness is considered an adverse event. Laboratory abnormalities and changes in vital signs are considered to be adverse events if they result in discontinuation from the study, necessitate therapeutic medical intervention, meet protocol specific criteria (see Section 5.0, Treatment Plan) and/or if the Investigator considers them to be adverse events.

Unexpected Adverse Event
An unexpected adverse event is any adverse drug event that is not listed in the current labeling/Investigator's Brochure. "Unexpected," as used in this definition, refers to an adverse drug experience that has not been previously observed (i.e., included in the labeling) rather than from the perspective of such experience not being anticipated from the pharmacological properties of the pharmaceutical product.

Serious Adverse Event
UNMC IRB SAE DEFINITION: A serious adverse event is one that at any dose (including overdose) and regardless of causality that:

Results in death

Is a serious threat to life, health, safety or welfare-fare of subject [1]

Requires inpatient hospitalization or prolongation of existing hospitalization

Required intervention to prevent permanent impairment or damage

Persistent or significant disability or incapacity[2]

Is a congenital anomaly or birth defect

Another serious important medical event[3]

Any medical event in an investigational drug study that requires treatment to prevent one of the outcomes listed above

The rights, safety, or welfare of subjects is seriously jeopardized

[1]"Life-threatening" means that the subject was at immediate risk of death at the time of the serious adverse event; it does not refer to a serious adverse event that hypothetically might have caused death if it were more severe.

[2]"Persistent or significant disability or incapacity" means that there is a substantial disruption of a person's ability to carry out normal life functions.

[3]Medical and scientific judgment should be exercised in deciding whether expedited reporting is appropriate in situations where none of the outcomes listed above occurred. Important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the subject or may require intervention to prevent one of the other outcomes listed in the definition above should also usually be considered serious. Examples of such events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in subject hospitalization, or the development of drug dependency or drug abuse. A new diagnosis of cancer during the course of a treatment should be considered as medically important.

9.1 Adverse Event Reporting Per the University of Nebraska Medical Center, Institutional Review Board (IRB) and Fred & Pamela Buffett Cancer Center Data and Safety Monitoring Committee (DSMC)

This protocol will adhere to all institutional guidelines for adverse event reporting.

9.1.1 IRB REPORTING

All internal serious adverse events (SAEs) must be reported to the local IRB promptly per Institutional Policy and in no case later than two (2) business days following PI notification that the event occurred. *If* the PI determines that conditions A, B, and C are met:

A. **The AE is unexpected, *AND***
B. **The AE is related to, or possibly related to, the drug, biologic, device, or other research intervention**

All *unexpected*, internal, fatal AEs must be reported promptly to the local IRB per Institutional Policy, but no later than *24 hours* following PI notification that the event occurred. If documentation is still pending, the IRB office must be notified by a telephone call or e-mail.

All *expected*, internal, fatal AEs (i.e., due to progressive disease or which reflect a risk currently found in the consent form) must be reported to the local IRB per Institutional Policy no later than ten (10) business days following PI notification that the event occurred.

9.1.2 FRED & PAMELA BUFFETT CANCER CENTER DATA AND SAFETY MONITORING COMMITTEE (DSMC) REPORTING

All adverse events greater or equal to grade 3 (expected and unexpected, regardless of attribution) and any Internal AE that meets one or more of the definitions of SAE outlined in the DSMC SAE definition above regardless of whether or not it meets the UNMC IRB's requirements for reporting, must be fully reported to the University of Nebraska Medical Center, Fred & Pamela Buffett Cancer Center Data and Safety Monitoring Committee (DSMC) in accordance with DSMC guidelines (DSMC Policies and Procedures and DSMC Plan http://www.unmc.edu/cancercenter/clinical/prms.html).

SAEs should be fully reported to the DSMC at the same time that they are reported to the UNMC IRB. In general, reporting of SAEs to the DSMC is expected within seven (7) business days of the occurrence of the event. If additional information about the SAE is still being collected, a preliminary report should be filed within seven (7) business days with final reporting when additional information is available. Failure to fully report an SAE as required will be considered a study violation and may be reported to the Associate Director for Clinical Research.

Attribution of AE: The likelihood of relationship of the AE to the study drugs will be determined by the Investigator based on the following definitions:

Not related: The subject was not exposed to the study treatment or another cause is obvious (AE related to underlying or concurrent illness).

Probably not related: The AE is most likely explained by another cause, and the time of occurrence of the AE is not reasonably related to the study treatment (AE has no temporal relationship to the study drug and/or a more likely etiology exists).

Possibly related: Study treatment administration and AE occurrence reasonably related in time, and the AE is explained equally well by causes other than study treatment, or treatment administration and AE occurrence are not reasonably related in time, but the AE is not obviously a result of other causes (AE has strong temporal relationship to study drug, alternative etiology is equal or less likely).

Probably related: Study treatment administration and AE occurrence are reasonably related in time, and the AE is more likely explained by study treatment than by other mechanisms (AE has strong temporal relationship to the study drug or recurs on re-challenge, another etiology is unlikely or significantly less likely).

Definitely related: There occurrence and timing of the AE are clearly attributable to the study treatment.

Severity Grade of AE. The severity of events reported on the AE case report form will be determined by the principal investigator according the NCI Common Toxicity Criteria (CTC version 4.03).

AEs will be collected from the time the subject starts the study drugs and ending 30 days following the final chemotherapy. All AEs will be followed until resolution or a cause is identified. Prescription medication taken to relieve symptoms of the AE will be recorded in addition to the outcome.

AEs judged by the Investigator as not related or probably not related to the study drugs will NOT be followed beyond the 30 days after the final chemotherapy.

ALL internal/external Adverse events (AEs) will be reported to the UNMC Fred & Pamela Buffett Cancer Center Data and Safety Monitoring Committee (DSMC). The DSMC will also review the protocol on at least a quarterly basis.

Copies of the AE report will be submitted to the IRB as indicated in Section 9.1.1.

A copy of the Policy and Procedures for this section may be reviewed at: http://www.unmc.edu/cancercenter/clinical/prms.html.

9.1.3 FOOD AND DRUG ADMINISTRATION (FDA) REPORTING

It is the responsibility of the UNMC Sponsor-Investigator, Amulya Yellala, MBBS, to submit to the FDA Post-marketing Safety Reports in accordance with 21 CFR 314.80.

SAEs not meeting Post-marketing 15-day "Alert" will not be made available to FDA by the sponsor-investigator pursuant to 21 CFR 314.80 (c)(1)(i).

All sites will utilize the FDA MedWatch Form (See Appendix D) for the reporting of serious adverse events (SAEs) and follow up information to those events. The form can be found at the following URL: http://www.fda.gov/Safety/MedWatch/default.htm

It is the responsibility of ALL sites to submit the completed MedWatch Form, SAE PI Assessment Form and SAE fax cover sheet (located in your Study Manual) to the UNMC Multi-site Project Manager within 24 hours of his/her knowledge of the SAE.

The SAE information will be routed to the UNMC PI who will further evaluate the SAE. The SAE will be submitted to FDA if the PI determines 21 CFR 314.80 (c) criteria are met.

SAE reporting instructions are reiterated and further outlined in the Participating Site Study Manual.

Additionally, serious adverse events will be reported to the IRB, SRC and the Data Safety Monitoring Committee by the Investigator.

9.2 Auditing

The UNMC Fred & Pamela Buffett Cancer Center Scientific Review Committee (SRC) will review this protocol on at least an annual basis.

This study will undergo audit on at least a quarterly basis by the UNMC Fred & Pamela Buffett Cancer Center Audit Committee.

The UNMC Fred & Pamela Buffett Cancer Center Protocol Review & Monitoring System (PRMS) Office Audit Committee defines a *Participating/Collaborating Site* as: a hospital clinic, or other provider of medical services who has agreed to participate in a therapeutic trial that has been designed and developed by a University of Nebraska Medical Center/The Nebraska Medical Center (UNMC/TNMC) Investigator and is Sponsored by UNMC.

For participating/collaborating site(s) that are NCI Cancer Centers, the protocol specific finding of the site's Audit Committee will be submitted to the UNMC Audit Committee for review on a schedule to be determined by the UNMC Audit Committee.

For participating /collaborating site(s) that are not NCI Cancer Centers, the Audit process will be established by the UNMC Audit Committee on a site-by-site basis.

9.3 Monitoring

9.3.1 Various methods will be implemented by the Sponsor (UNMC) to exchange information with participating/collaborating sites. Methods of communication will include:

- Site Initiation/Orientation
- Regular Teleconferences including group wide progress within the agenda
- Investigator meetings as feasible (remote or TBA, possibly in conjunction with larger meetings)
- Email distributions/reports as needed

9.3.2 Ongoing safety monitoring for all the subjects in this study:

All Participating/Collaborating sites are required to execute a Data Compliance Policy agreement. UNMC will monitor the data of participating sites in adherence to applicable Research regulations, the protocol, and the Policy Agreement. De-identified source documents which support data entered must be provided to the Sponsor by mail, fax, or electronic means for centralized compliance monitoring.

9.3.3 Data and Safety Monitoring Plan

The UNMC Fred & Pamela Buffett Cancer Center Data and Safety Monitoring Plan is designed to ensure the safety of participants in clinical trials conducted by the UNMC Fred & Pamela Buffett Cancer Center members and to comply with National Cancer Institute (NCI) and National Institutes of Health (NIH) requirements. This protocol will comply with all Monitoring Policies and Procedures outlined in the Monitoring Plan.

A copy of the Policy and Procedures for this section may be reviewed at:

http://www.unmc.edu/cancercenter/clinical/prms.html.

10.0: Statistical considerations

Statistical design
This is a Phase II study of adjuvant therapy using a dose-dense regimen of cyclophosphamide and paclitaxel with trastuzumab in subjects with stage I-II HER2/neu positive breast cancer. This will be a non-randomized study; all subjects will receive the same doses of the active compound. Dose modifications for adverse events are described in Tables 1-5.

Patient Accrual and Expected Duration of Trial
We plan to accrue a total of 50 subjects. For analysis, all subjects from this trial and Herceptin treated subjects from trial IRB#264-12 will be used.

A total of 50 evaluable subjects are expected over a 6-year period. Evaluable patient defined as one that remains on study for 2 years. Target accrual is 50 to account for ineligible or withdrawn subjects.

Definition of Analysis Sets
Full analysis set: All patients who received any dose of paclitaxel and cyclophosphamide with trastuzumab f.

Safety analysis set: All patients who received any dose of paclitaxel and cyclophosphamide with trastuzumab from the current study.
Inability to complete treatment will be defined as a patient that a) requires a lower dose of therapy (defined as dose lowered by 50%) or b) a postponement of scheduled treatment of longer than 28 days, or c) discontinuation of treatment for any reason.

Sample size justification
This study is a non-randomized Phase II trial to estimate the toxicity profile for paclitaxel and cyclophosphamide with trastuzumab combination. The primary toxicity for the sample size justification is neutropenia. The secondary toxicities are paclitaxel-related neuropathy, grade 3/4 cardiotoxicity and grade 3/4 nausea/vomiting. A fixed expected toxicity rate of 10% will be used for grade 3/4 neutropenia and grade 3/4 paclitaxel-related neuropathy and a rate of 6% will be used for Grade 3/4 cardiotoxicity and grade 3/4 nausea/vomiting.

Neutropenia and paclitaxel-related neuropathy: 10% of subjects experience neutropenia or paclitaxel-related neuropathy with standard treatment. A sample size of 50 evaluable subjects will produce a 97% confidence interval equal to the sample proportion plus or minus 0.09 when the estimated proportion is 0.10.

Grade 3/4 cardiotoxicity and grade 3/4 nausea/vomiting: < 6% of subjects should experience grade 3/4 cardiotoxicity or grade 3/4 nausea/vomiting with standard treatment, with herceptin addition, the cardiac toxicity should not be more than 6%. We anticipate fewer grade 3/4

cardiotoxicity and grade 3/4 nausea/vomiting with this regimen when compared to standard regimens. A sample size of 50 evaluable subjects will produce a 95% confidence interval equal to the sample proportion plus or minus 0.07 (precision) when the estimated proportion is 0.06.

Recurrence free survival: Under the null hypothesis, recurrence-free survival at three-years is 85%. Because outcome for these subjects is favorable, it is important to protect against the possibility of recurrence-free survival (RFS) inferior to 85%. A reduction in RFS at 3 years to 75% will be considered unacceptable. Assuming accrual of 48 subjects over 6 years, with a minimum of 2 years of follow-up, the study will have 80% power at the 0.10 level of significance (one-sided) to detect a decrease in RFS at 3 years to 75%. Two interim monitoring analyses will be conducted, at 33% and 66% of the expected information. An Obrien and Fleming adjustment of 1.027 results in a sample size of 50 evaluable subjects. Therefore, the sample size of 50 subjects is planned to allow for the two interim looks.

Statistical analysis plan

Descriptive statistics will be computed for all variables to examine data quality. Variable distributions will be examined using stem-and-leaf plots, means, medians, standard deviations, ranges and frequencies and 90% confidence intervals.

Inability to complete treatment will be described using frequencies and proportions and 90% confidence intervals.

Since this is a single arm study, the data analyses are descriptive. The incidence rates of neutropenia and paclitaxel-related neuropathy, grade 3/4 cardiotoxicity and grade 3/4 nausea/vomiting and adverse events will be summarized using frequencies, percentages and 90% confidence intervals. Adverse events will be described by cycle. The frequency of toxicity, categorized by toxicity grades, will be summarized.

RFS and survival curves will be plotted following the method of Kaplan and Meier using the full analysis set. (11)

Monitoring Plan

The safety analysis set will be used for interim monitoring. Therapy will be discontinued at any time if the data suggest this combination is found to be inferior to standard adjuvant therapy, regardless of whether the formal stopping rules are satisfied.

Neutropenia and paclitaxel-related neuropathy: One interim analysis will be performed after 25 subjects have been off of their last cycle of chemotherapy for 2 weeks. Follow up after the completion of therapy should be, as described earlier, every 3 months for 2 years and then every six months for total of 5 years. If 5 of 25 subjects develop grade 3/4 neutropenia or develop grade 3/4 paclitaxel-related neuropathy, accrual will be terminated. If the true toxicity rate is 10%, the probability of stopping accrual early is 10%. If the true rate is 40%, the probability of stopping accrual early is 99%.

Nausea/vomiting/cardiac toxicity for Her-2 neu negative subjects: One interim analysis will be performed after the accruals of 25 subjects have been off of their last cycle of chemotherapy for

2 weeks. Follow up after the completion of therapy should be as described earlier, every 3 months for 2 years and then every six months for total of 5 years. If 3 of 25 subjects develop grade 3/4 cardiotoxicity or develop grade 3/4 nausea/vomiting, accrual will be terminated. If the true toxicity rate is 6%, the probability of stopping accrual early is 19%. If the true rate is 20%, the probability of stopping accrual early is 90%. Cardiac toxicity will be monitored for Her-2 positive subjects with an echocardiogram every 12 weeks until completion of trastuzumab. One interim analysis will be performed after 10 subjects have received trastuzumab for 26 weeks. If 1 of 10 Her-2 positive subjects develop grade 3/4, accrual will be terminated.

Relapse-free survival: Chemotherapy with cyclophosphamide and paclitaxel with trastuzumab is expected to produce recurrence free survival comparable to standard therapy. Because the outcome for these subjects is favorable, it is important to protect against the possibility that cyclophosphamide and paclitaxel with trastuzumab will produce a RFS result inferior to 75%. A one sample one-sided test will be conducted to compare RFS against a fixed expected null hypothesis of 0.85. Two interim monitoring analyses will be conducted at 33% and 66% of the expected information. The monitoring p-values to be used for the 2 interim looks are p=0.003 and p=0.036 with the final analysis at p=0.087. Separate analyses will also be conducted for subjects with Stage 1 and Stage 2 disease.

11.0 Records to be kept

Information regarding the actual treatments, adverse effects, radiographic and laboratory information, and pathology are to be recorded on appropriate forms. See attached Data Forms. De-identified source documents which support data entered must be provided to the sponsor by mail, fax, or electronic means for centralized compliance monitoring. Serious adverse events, when noted, will be recorded on site via the standard Serious Adverse Event (SAE) form.

11.1.1 Quality assurance:
Complete records must be maintained in a research chart on each patient treated on the protocol. These records should include primary documentation (i.e., lab report slips, X-ray reports, scan reports, pathology reports, physician notes, etc.) which confirm that:

- The patient met the eligibility criteria.
- Signed informed consent was obtained prior to treatment.
- Treatment was given according to protocol (i.e., dated notes about doses given and reasons for any dose modifications).
- Toxicity was assessed according to protocol (i.e., laboratory report slips, etc.).
- Response was assessed according to protocol (i.e., x-ray, scan, lab reports, dated notes on measurements and clinical assessment, as appropriate).

11.1.2 Forte Electronic Data Capturing (EDC) System
Data will be stored electronically for this study in the Forte EDC system contained on the Forte secure server. Data forms will not differ from the paper versions with the exception of an electronic format containing the UNMC Fred & Pamela Buffett Cancer Center and Forte logo.

Forte provides for remote data collection that meets FDA 21 CFR Part 11 requirements as well as HIPAA and other regulatory requirements designed to enhance data security and protect patient confidentiality. Authorized users log into Forte through a secure connection and must provide a valid username, password, and database ID. This data may be made available to the public at large.

12.0 Patient Consent

12.1 Human Subjects Research Protection Training
All personnel involved in this research project will have completed the OHRP-approved computer based training course on the Protection of Human Research Subjects. All clinical and correlative research included in this application will have approval by the institutional review board.

12.2 Study Population

Subjects are from all socio-economic groups and will be entered into the study without bias with respect to gender or race. Attempts will be made to recruit minorities. No vulnerable subjects will be included in the study.

12.3 Sources of Material

Pathology material will be reviewed, and the diagnosis confirmed by the University Nebraska Medical Center Pathology Department as outlined in the protocol.

12.4 Recruitment and Informed Consent

Subjects with an initial diagnosis of cancer seen and evaluated at TNMC/UNMC and participating IRB approved sites will be available for recruitment. These Subjects will be informed of the nature of this study, and will be asked to participate on a voluntary basis after informing them of the possible risks and benefits of the study. A number of public registries may be accessible to health care providers and prospective subjects as listed below.

National Library of Medicine –http://clinicaltrials.gov (NCT02654119)
National Cancer Institute – http://www.cancer.gov (NCI-2015-01879)

12.5 Subject Competency

Subjects will be eligible to participate in the study only if they are competent to give informed consent. A subject that the investigators judges to be incompetent will not be enrolled.

12.6 Process of Informed Consent

If the patient chooses to be a participant in this study informed consent will be obtained by the investigators. The study and procedures involved including the risks will be explained in detail to each subject. It will be clearly explained to the subject that this is a research study and that participation is entirely on a voluntary basis. Subjects will be given the option to discuss the study with a family member, friend, counselor or, another physician. The participating investigators will be available to discuss the study with them.

12.7 Subject/Representative Comprehension

When the process of informed consent is completed, the subject will be asked to state in his/her own words the purpose of the study, the procedures that will be carried out, potential risk, potential benefits to the subject, the alternatives and the right to withdraw from the study. If there are any indications that a given subject's comprehension is anything less than accurate, the points of confusion will be discussed and clarified.

12.8 Information Purposely Withheld.

The results of the tests done solely for research purposes will not be disclosed to the subject. No other information will be purposely withheld from the subject.

12.9 Potential Benefits of the Proposed Research to the Subjects

It is anticipated that the use of the protocol chemotherapy in this patient population represents a reasonable treatment option. There are risks associated with chemotherapy, but the risk to benefit ratio is considered acceptable for patients with cancer.

12.10 Potential Benefits to Society.
Information obtained from this study may help other patients by contributing to the knowledge of the biology of cancers, and to understand the potential clinical benefit of this regimen.

12.11 Potential Risks
The use of cytototoxic chemotherapy are associated with numerous potential risks. Combined chemotherapy is considered a valid treatment option for patients with cancer. It is believed the treatment option outlined in the study will not pose significant additional risks compared to conventional treatment.

12.12 Therapeutic Alternatives
If Subjects choose not to participate in this study they may elect to receive standard therapy as per their primary oncologist, which may include other chemotherapy drugs, radiation, surgery, or a combination of these approaches. The treatment recommendations may or may not be similar to treatment as described in this protocol.

12.13 Risk/Benefit Relationship
Although there are inherent risks involved because of the use of chemotherapy, we anticipate that Subjects who receive the treatment phase of the protocol will do no worse than expected with standard therapy, and may experience an improved outcome. The risk is considered to be acceptable in the setting of cancer.

12.14 Consent Form Documents
The consent document used in this study will include the adult consent document.

13.0: References

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14.0 Data Collection Forms

Attached

APPENDIX A

NYHA Classification

Excerpted from Oxford Textbook of Medicine. Vol 2, p.2228. Oxford Press.1997.

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

Appendix B – ELIGIBILITY CHECKLIST

IRB# 318-15 Title: A Phase II Study of Adjuvant Therapy Using a Regimen of Cyclophosphamide, Paclitaxel with Trastuzumab in Stage I-II HER2/neu Positive Breast Cancer Patients

Date Completed:	Institution:	Subject ID:	Checklist #: V4.0 dated 01-03-2018
PI: Amulya Yellala, MBBS	Last Name:	First Name:	Date of Birth: (MM/DD/YYYY)
Gender: [ ] M [ ] F	Race: [ ] White [ ] Black [ ] Hispanic [ ] Asian	[ ] Native American [ ] Other [ ] Unknown	MRN:
Zip Code/Country (if not USA):	Primary method of payment information:		

Inclusion Criteria:	Yes	No	N/A
1. Histologically confirmed newly diagnosed Stage I-II HER2/neu positive breast cancer.	[ ]	[ ]	1.
2. Women above 19 years of age. Enter Age:___	[ ]	[ ]	2.
3. Women of reproductive potential must be non-pregnant and non-nursing and must agree to employ an effective barrier method of birth control throughout the study and for up to 6 months following treatment.	[ ]	[ ]	[ ] 3.
4. Women of child-bearing potential must have a negative pregnancy test within 7 days of initiating study. *(No childbearing potential is defined as age 55 years or older and no menses for two years or any age with surgical removal of the uterus and/or both ovaries)*. Enter date: __/___/____ and Result _____	[ ]	[ ]	[ ] 4.
5. ECOG Performance Status of 0 or 1. Enter PS: _____	[ ]	[ ]	5.
6. Adequate laboratory parameters within 30 days prior to enrollment defined as: Enter date: __/___/____			
• Absolute neutrophil count greater than or equal to 1,500/mcl Enter ANC: _____			
• Platelet count equal to or greater than 150,000/mcl Enter Platelet count: _____	[ ]	[ ]	6.
• Hemoglobin >11gm/dl Enter Hgb count: _____			
• Alkaline phosphatase equal or less than 1.5 times the ULN Enter Alk Phos: ____			
• Total bilirubin equal to or less than 1.5 times the ULN Enter Total Bili:____			
• AST and ALT no greater than 1.5 times the ULN Enter AST: ____ Enter ALT: ____			
• Creatinine less than 1.5 times the ULN Enter creatinine: _____			
7. Able to give informed consent.	[ ]	[ ]	7.
8. Normal cardiac function as defined by an ejection fraction of >50% by echocardiogram.	[ ]	[ ]	8.
9. Able to return for treatment and follow-up on the specified days.	[ ]	[ ]	9.
*All of the above must be **yes** to be eligible*			
Exclusion Criteria:			

1. Prior malignancy; except for adequately treated basal cell or squamous cell skin cancer or noninvasive carcinomas.	[ ] [ ]1.
2. Pre-existing Grade II peripheral neuropathy.	[ ] [ ]2.
3. History of previous chemotherapy.	[ ] [ ]3.
4. Stage IV or metastatic breast cancer.	[ ] [ ]4.
5. Pregnant or nursing women.	[ ] [ ]5.
6. Inability to cooperate with treatment protocol.	[ ] [ ]6.
7. Active serious infections or other conditions precluding chemotherapy.	[ ] [ ]7.
8. Any comorbidity or condition which, in the opinion of the investigator, may interfere with the assessments and procedures of this protocol e.g. unstable angina, myocardial infarction within 6 months, severe infection, etc.	[ ] [ ]8.
9. Known hypersensitivity to any component of required drugs in the study.	[ ] [ ]9.
10. Known positive for HIV or infectious hepatitis, type A,B or C or active hepatitis.	[ ] [ ]10.
11. Any serious medical condition, laboratory abnormality, or psychiatric illness that would prevent the subject from signing the informed consent form.	[ ] [ ]11.
12. Myocardial infarction within 6 months prior to enrollment or has New York Heart Association (NYHA) Class III or IV heart failure uncontrolled angina, severe uncontrolled ventricular arrhythmias, or electrocardiographic evidence of acute ischemia or active conduction system abnormalities. (Appendix A) Prior to study entry, any ECG abnormality at screening has to be documented by the investigator as not medically relevant.	[ ] [ ]12.
*All of the above must be **no** to be eligible.*	

NOTE: All questions regarding eligibility for **UNMC potential subjects** should be directed to the UNMC Coordinator at 402-559-5582 or UNMC Research Support Nurse Manager at 402-559-5286.

All questions regarding eligibility for **Participating/Collaborating Site** potential subjects should be directed to the Sponsor PI by contacting the UNMC Fred & Pamela Buffett Cancer Center Multi-site Project Manager at 402-559-4596 or the Research Support Nurse Manager at 402-559-5286.

Patient Initials: _____MRN or Study ID#_____DOB _____

Eligibility: [ ] Subject satisfies all criteria.
[ ] Subject not formally eligible, but admitted to study because (state reason);

ELIGIBILITY reviewed and confirmed
Site Investigator Signature _____Date_____

_____

ELIGIBILITY reviewed and confirmed.

Sponsor Investigator Signature _____ Date_____

Printed Name of Sponsor Investigator: _____

Appendix C:

HBM Sample Processing and Shipping (for research purposes only)

Sample Collection: Source and human biologic material (HBM) to be used: We will use two 10 ml EDTA (lavender top tubes) and one- 10ml red top tube blood sample from the patient to store for future research purposes including DNA sequencing. (See protocol section 5.6 for details) *No therapeutic intervention will be undertaken and the results of these studies will not have any influence on the medical management of the subjects.*

Sample	Contact Person(s)	Date and Time Points
Blood: (30 ml total) two- 10*ml EDTA (lavender top) and one- 10ml red top* blood collection deliver or ship same day at room temperature	**Facility**: Laboratory of David L. Kelly, PhD Fred & Pamela Buffett Cancer Center 986805 Nebraska Medical Center Omaha, Nebraska 68198-6805 **Personnel:** David L. Kelly PhD Tel: (402) 559-9157 Cell: (402) 699-1132 Fax: (402) 559-4651 dkelly@unmc.edu Amy Wells, MS Research Technologist Tel: (402) 559-6015 awells@unmc.edu **Shipping Address:** Attn: Amy Wells, MS University of Nebraska Medical Center 601 S. Saddle Creek Rd. F&PBCC 4.12.428 Omaha, Nebraska 68106	☐ Baseline, Blood Sample Date: ___/___/____ ☐ Clinical endpoint (after cycle 6), Blood Sample Date: ___/___/____ ☐ End of study (one year or end of tx) Blood Sample Date: ___/___/____

Please contact Amy Wells above to advise of planned shipments, and to discuss appropriate procedures and/or the UNMC Fred & Pamela Buffett Cancer Center Multi-site Project Manager at 402-559-4596.

APPENDIX D
FDA 3500A MEDWATCH Form

Available on-line at http://www.fda.gov/medwatch/SAFETY/3500.pdf