A Phase I-II Study of Interferon-gamma Plus Weekly Paclitaxel, Trastuzumab and Pertuzumab in Patients with HER-2 Positive Breast Cancer

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TREATMENT SCHEMA

Phase I
HER2 positive metastatic breast cancer

IFN-γ 50 or 75 mcg/m^2 SQ x3 days/week

plus

Paclitaxel 80 mg/m^2/week
Trastuzumab 8mg/kg IV loading dose on C1D1, followed by 6mg/kg on subsequent cycles every 3 weeks
Pertuzumab 840 mg IV loading dose on C1D1, followed by 420 mg on subsequent cycles every 3 weeks

x 12 weeks

Phase II – clinical stage 1-3 HER2 positive early stage breast cancer

IFN-γ RP2D SQ x3 days/week

plus

Paclitaxel 80 mg/m^2/week
Trastuzumab 8mg/kg IV loading dose on C1D1, followed by 6mg/kg on subsequent cycles every 3 weeks
Pertuzumab 840 mg IV loading dose on C1D1, followed by 420 mg on subsequent cycles every 3 weeks

x 12 weeks

Phase II Surgery

1. OBJECTIVES

1.1 Primary Objectives

- Phase I
  To evaluate the safety and tolerability of the combination of IFN-γ with paclitaxel, trastuzumab and pertuzumab for 12 weeks and determine the recommended phase II dose (RP2D) in patients with metastatic breast cancer
- Phase II
  To evaluate the pathologic complete response rate (pCR) after combination of IFN-γ with paclitaxel, trastuzumab and pertuzumab for 12 weeks of neoadjuvant therapy in patients with clinical stage 1-3(primary tumor size ≥10mm)) HER2 positive breast cancer

1.2 Secondary Objectives:

- Phase I
  To evaluate overall response rate (OR, CR+PR) using standard response evaluation criteria in solid tumors (RECIST) version 1.1
  To evaluate median progression free survival (PFS)
- Phase II
  To evaluate safety and tolerability of the combination therapy
  To evaluate the PFS

1.3 Exploratory Objectives:

(A) Determine whether IFN-γ combination therapies restore anti-HER-2 CD4 Th1 response,
(B) Change the tumor associated immune response, or
(C) Change the known tumor biomarkers that predict response in the HER-2 positive breast cancer

2. INTRODUCTION

2.1 Background

About 500,000 patients worldwide will die each year from breast cancer (1). This comes with considerable cost burdens to all nations, not to mention associated morbidities to individual breast cancer survivors. Patients with HER-2-expressing (HER-2^{pos}) breast cancer have significant risk of developing systemic disease (2, 3). HER-2 targeted therapies have improved survival in many patients (4-6) but resistance often develops, resulting in relapse. There are also increased central nervous system failures (7). Additional therapies are therefore needed to complete the eradication of HER-2^{pos}breast cancer. Addition of immune based therapies against HER-2^{pos} breast cancer offers an alternative approach from antibody and kinase-inhibitor approaches. Our goal is to place this disease in the category of Hodgkin's Lymphoma, anal carcinoma, and testicular cancer; diseases once causing significant mortalities that today are

extremely curable. We have made a series of observations that support the hypothesis that restoring a deficit in Th1 immunity will render HER-2 targeted therapies more effective, increase pathologic complete responses (pCR), and reduce mortality in patients with HER-2^{pos} breast cancer.

2.2 Preclinical data

Evidence for Loss of anti-HER-2 Th1 Response during Tumorigenesis: We have made the observation that healthy adult women possess unusually high, pre-existing Th1 immunity against HER-2 in the peripheral blood, and that these Th1 cells are progressively lost during HER-2^{pos} tumorigenesis (8). This deficit is first detectable at the ductal carcinoma in situ (DCIS) stage, and becomes profoundly suppressed in early invasion, even by stage I (**Fig 1**). There is no such loss of HER-2 Th1 immune responses during HER-2^{neg} tumorigenesis, neither are there losses of responsiveness against other (non-tumor) control antigens, suggesting this suppression is antigen-selective and not a product of global anergy (**Fig 1**). Standard therapy of surgery, radiation, and chemotherapy with trastuzumab does not routinely correct this anti-HER-2 Th1 loss, but vaccination with HER-2-pulsed type I dendritic cells (DC1) can dramatically increase peripheral anti-HER-2-CD4 Th1, suggesting this is not a fixed deficit, but instead one that can be corrected by appropriate immunization (8).

The anti-HER-2 Th1 Response Correlates with Clinical Outcomes: HER-2^{pos} Breast cancer patients achieving a pCR to neoadjuvant chemotherapy demonstrate improved survival while those with residual disease at the time of surgery demonstrate increased risk of recurrence (9). Although as a group, invasive HER-2^{pos} breast cancer patients show depressed anti-HER-2 Th1 immunity, some individuals are profoundly suppressed while others retain moderate responsiveness. Those patients that regain or partially retain anti-HER-2 Th1 demonstrate higher pCR rates to neoadjuvant chemo/trastuzumab therapy, while those with residual disease display the lowest anti-HER-2 CD4 Th1 responses (**Fig 2A**). Shown is the loss of repertoire but there is also significant difference in overall and cumulative responses (9). The anti-HER-2 CD4 immune response represents an increase interferon gamma (IFN-γ) production in the $Tbet^{pos}$ Th1 population and not GATA 3^{pos} Th2 population (**Fig 2B**), suggesting the Th1 immune response may be critical to mediating anti-HER-2 breast cancer responses. Indeed, recent studies suggest elevated immune gene expression in the tumor is associated with improved outcomes (10). We have also investigated the relationship between anti-HER-2 immunity and disease-free survival. Patients with most diminished anti-HER-2 Th1 immune responses show decreased disease free survival (DFS) (**Fig 2C**) while those with retained anti-HER-2 Th1 responses have substantially greater DFS (**Fig 2C**) (11). This deficit of anti-HER-2 CD4 fits with the clinical picture of those with metastatic breast cancers who initially respond for a period of time to HER-2 targeted therapies but later fail, become resistant to therapy and recur. Correcting this response is paramount to preventing this clinical scenario. These results also suggest that the anti-HER-2 Th1 response can be used to determine if pCR is achieved before and after neoadjuvant immunologic therapies proposed**.** Since the oncodriver is the critical tumor antigen involved in tumor growth and invasion, the anti-HER-2 CD4 Th1 response can be both a novel monitor of

risk as well as a therapeutic rescue. The current gold standard is MRI, which is neither sensitive nor specific enough to personalize therapy. We have submitted a preliminary manuscript demonstrating anti-HER-2 CD4 Th1 response has 98% accuracy rate in predicting pCR to neoadjuvant therapy.

Correcting the Anti-HER-2 CD4 Th1 may be Essential for Clinical Response in HER-2^{pos} Breast Cancer: We and others have shown that there is a significant loss of MHC class I expression with increasing HER-2 expression (12). Hence CD8 T cells do not readily recognize these tumor cells. Interestingly the MHC class I peptide HER-2 vaccine Neuvax (E75) had its greatest activity in low HER-2 1+ and 0 expressing patients, which aligns with our findings of MHC class I expression being highest in HER-2 low expressing breast cells vs HER-2 high and intermediate cells with low Class I expression (12, 13). However, IFN- γ and TNF-α treatment of HER-2 expressing breast cancer cells increases MHC class I expression and also enhances CD8 T cell recognition (12). Hence, as we have shown *ex vivo* that correction of the anti-HER-2 CD4 Th1 response is critical to allowing anti-HER-2 CD8 T cells to recognize and kill, or release cytokines in HER-2 intermediate and high expressing cells (HER-2 2+ and HER-2 3+). Restoring this anti-HER-2 CD4 Th1 response or providing Th1 cytokines may be critical for effective CD8 T cell activity, which has been shown to be critical for response to HER-2 targeted therapies, chemotherapy and radiation (14-16). With the increase in MHC class I caused by IFN-γ is a simultaneous increase in PD1L on the breast cancer cells, which may afford the opportunity to add checkpoint inhibitors to therapy when restoring anti-HER-2 CD4 Th1 (12).

Th1 Cytokines Cause Induction of Tumor Senescence and Apoptosis in HER-2 Expressing Breast Cancer: Since there is a specific loss of anti-HER-2 CD4 Th1 during breast tumorigenesis, and the Th1 cytokines increase MHC class I expression on HER-2^{pos} breast cancer cells, we further explored whether Th1 cytokines had other effects on HER-2 expressing tumor cells. We have investigated the role of Th1 cytokines IFN-γ and TNF-α and found the combination of these two cytokines induce tumor senescence and apoptosis in breast cancer cells expressing HER-2 (**Fig 3**) as measure by β gal or p15 expression. There is a dose dependent increase in tumor senescence with these cytokines (**Fig 3C),** as well as a sensitivity of HER-2 expressing cells, with high HER-2 expressing cells being more sensitive to lower concentrations of Th1 cytokines than intermediate expressing cells (**Fig 3 D**). These two cytokines work in concert and only impact HER-2 expressing cell lines, as there is no significant impact in triple negative breast cancer (TNBC) (**Fig 3D**). More recently, we have investigated the use of combination therapy against HER-2 using trastuzumab and pertuzumab with Th1 cytokines IFN-γ and TNF-α (**Fig 4**), and noted a synergistic effect on the induction of apoptosis and tumor senescence in HER-2 expressing breast cancer, suggesting IFN-γ and TNF-α in combination with HER-2 targeted therapies can have dramatic impact on HER-2 expressing breast cancer.

We have also investigated the impact of single agent IFN-γ with trastuzumab and pertuzumab, and identified that at relatively low concentrations, there is an increase in tumor senescence of HER-2 expressing breast cancer cells (**Fig 5**). In addition, in HER-2 expressing cells that have become resistant to trastuzumab, IFN-γ and trastuzumab and pertuzumab dramatically induce tumor senescence (**Fig 5)**. Shown is an example of the HCC 1419 cell line that is resistant to the

effects of trastuzumab, however the combination of IFN-γ, trastuzumab, and pertuzumab induces tumor senescence and increased tumor apoptosis. Other resistant cell lines have not shown have similar effects.

Pre-clinical Data: IFN-γ synergizes with Taxanes and anti-HER-2 Blockade: Mark Green's group at University of Pennsylvania has documented that combining IFN-γ, HER-2 targeted therapy, and taxane chemotherapy results in enhanced response of HER-2 expressing breast cancers (**Fig 6**) (17). We propose that combinations of systemic IFN-γ combined with trastuzumab and pertuzumab, alone and in combination with single agent taxanes, will enhance sensitivity of HER-2 expressing breast cancer cells to HER-2 directed therapies, inducing more complete responses and paving the way for correcting the anti-HER-2 Th1 response for long term cures and persistent disease free survival. Since IFN-γ is already approved and a well-tolerated therapy, we propose to conduct a Phase I study in metastatic HER-2 expressing breast cancer receiving taxanes with trastuzumab and pertuzumab. These patients generally become resistant to HER-2 targeted therapies and fail therapy, often dying of disease. This trial would serve an unmet need in this group of patients that have no good alternatives except salvage palliative chemotherapy. If IFN-γ in combination with taxanes with trastuzumab and pertuzumab is well tolerated in phase 1 clinical trial, a subsequent phase 2 neoadjuvant study of this combination will be conducted to assess the efficacy and safety.

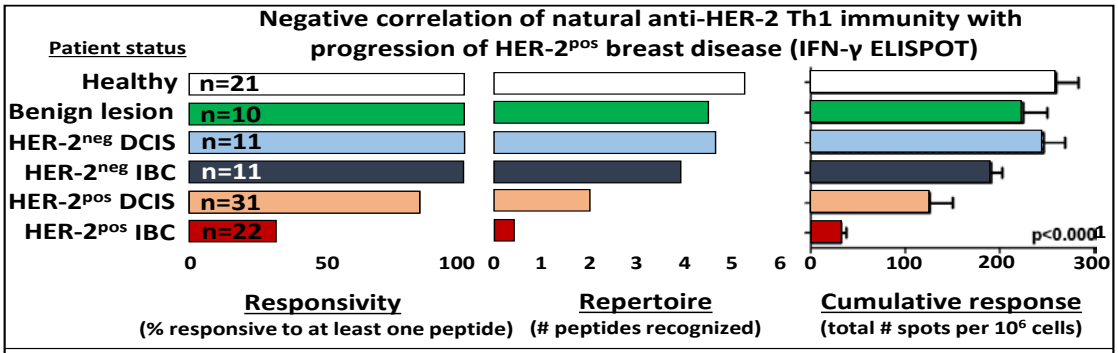

Figure 1. Fresh PBMCs from unvaccinated subjects (either healthy or experiencing a variety of defined breast lesions) were individually stimulated with each of 6 MHC class II promiscuous-binding peptides based on the HER-2 primary sequence and subjected to IFN-γ ELISPOT analysis. Results are displayed as the percentage of subjects responding to at least one of the 6 peptides ("responsivity; left panel), the absolute number of peptides responded to ("repertoire"; center panel) and the total number of IFN-γ spots from all 6 peptides ("cumulative response; right panel). In each instance it was noted that healthy individuals had unusually high natural immunity to HER-2 peptides that waned significantly with the development of HER-2^{pos} DCIS and further diminished with HER-2^{pos} invasive cancer (IBC). Significant suppression of anti-HER-2 Th1 responses were not observed for any HER-2^{neg} disease state.

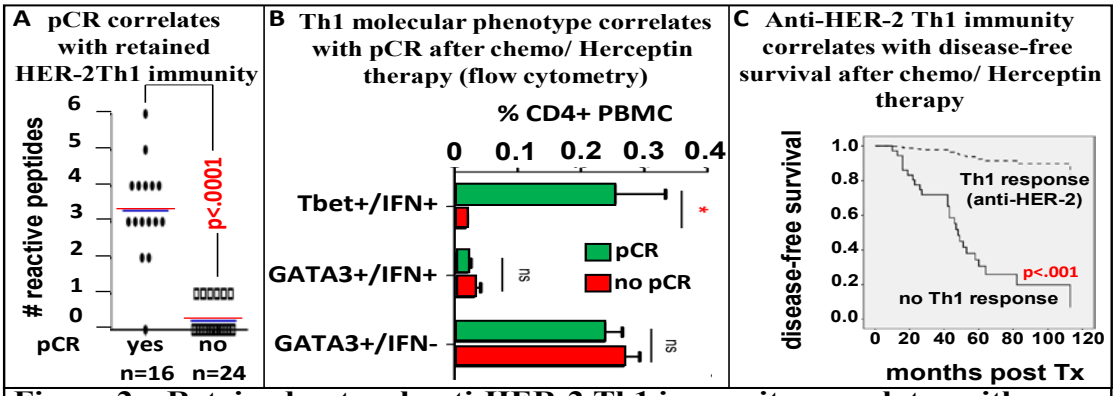

Figure 2. Retained natural anti-HER-2 Th1 immunity correlates with complete pathologic response (pCR) to chemo/herceptin therapy. A) Fresh unexpanded PBMCs from HER-2^{pos} breast cancer patients that received standard chemo- herceptin therapy were stimulated individually by 6 HER-2 peptide epitopes in IFN-γ ELISPOT assay. Plot displays number of epitopes recognized by each individual tested. B) Flow cytometry analysis of anti-HER-2 T cells shows disparity between pCR and non-pCR subjects lies in the Tbet^{pos}/IFN-γ compartment and not with GATA3^{pos} Th2 cells. C) Plot of disease-free survival of HER-2^{pos} breast cancer patients with retained (upper trace) versus lost (lower trace) anti-HER-2 TH1 immunity suggests strong advantage for retained anti-HER-2 Th1 immunity.

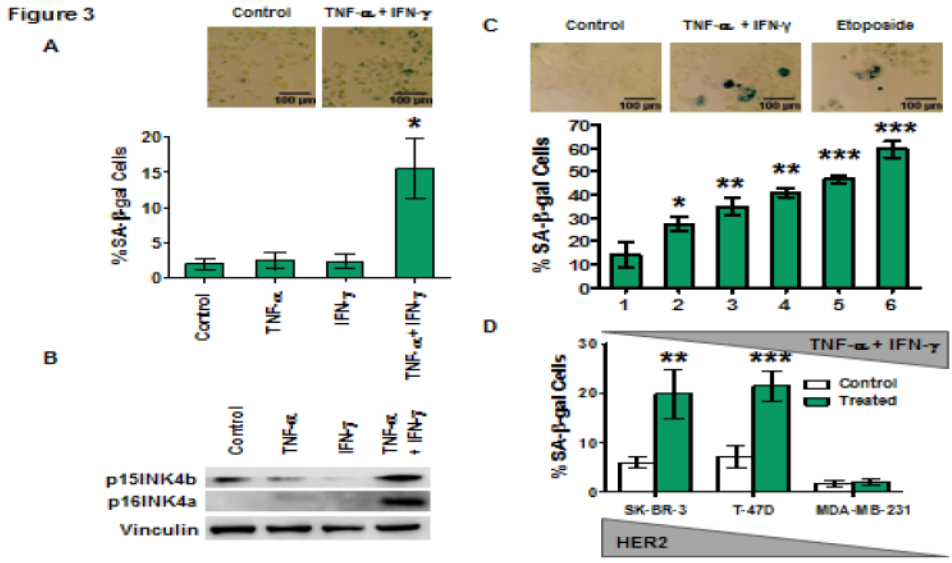

Figure 3. Th1 cytokines, TNF-α and IFN-γ synergize to induce senescence in breast cancer cells in a dose dependent manner inversely correlated with HER2 expression. A. SK-BR-3 breast cancer line untreated or incubated with 10 ng/ml TNF-αalone, 100 U/ml IFN-γ alone, or TNFαand IFN-γ in combination. Only paired cytokines induced senescence. Top panel: representative data from 1 of 3 independent experiments. Bottom panel: densitometric analysis presented as % of SA-□-gal-positive cells, mean ± SD (n=3), *p < 0.05. **B.** p15INKb and p16INK4a expression of cells described in A were analyzed by western blot. Vinculin was used as loading control. **C.** T-47D breast cancer cells were untreated (1), treated with etoposide (6), or incubated with increasing concentrations of TNF-□ and IFN-□: 10 ng/ml and 100 U/ml (2), 50 ng/ml and 500 U/ml (3), 75 ng/ml and 750 U/ml (5), 100 ng/ml and 1000 U/ml (5). *Top panel*: representative data from 1 of 3 independent experiments. *Bottom panel*: densitometric analysis presented as % of SA-□-gal-positive cells, mean ± SD (n=3), *p < 0.05, **p < 0.01, ***p < 0.001. **D.** Combination treatment with Th1 cytokines, TNF-α and IFN-γ, resulted in greater senescence in SK-BR-3 (10 ng/ml TNF-α + 100 U/ml IFN-γ) and T-47D (100 ng/ml TNF-α + 1000 U/ml IFN-γ) cells, compared with untreated controls; MDA-MB-231 cells (200 ng/ml TNF-α + 2000 U/ml IFN-γ) remained largely unaffected by dual TNF-α + IFN-γ treatment. Data presented as % of SA-□-gal-positive cells, mean ± SD (n=3), **p < 0.01, ***p < 0.001.

Figure 4

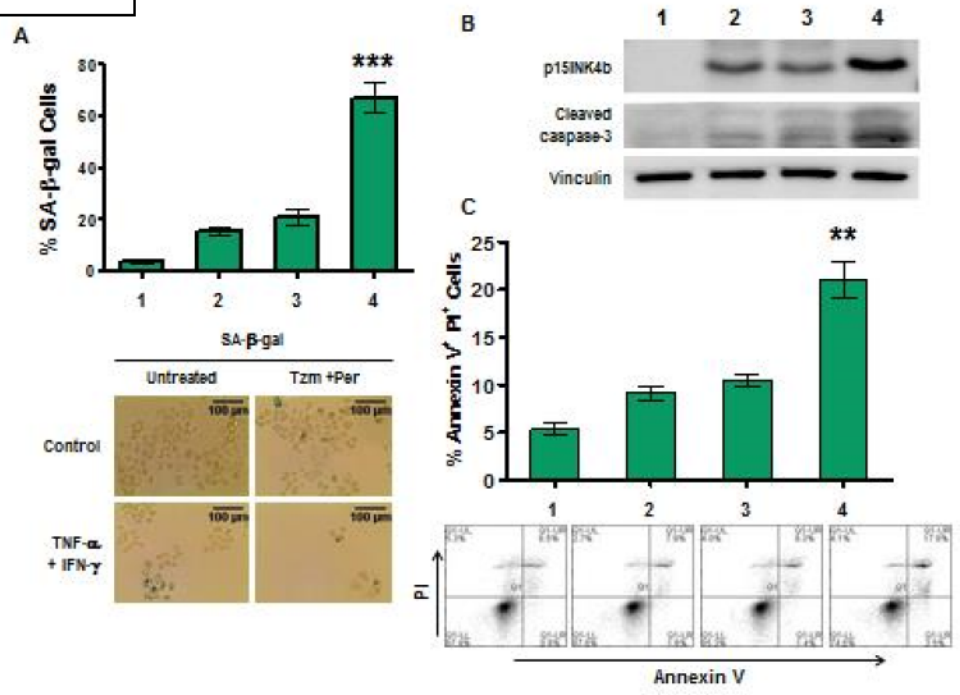

Figure 4. Combined HER2 inhibition and HER2 dimerization inhibition enhances Th1 cytokine-mediated senescence and apoptosis in breast cancer cells. A. SK-BR-3 cells untreated (1), treated with 10 ng/ml TNF-□ and 100U/ml IFN-□ (2), treated with 10 ug/ml of trastuzumab (Tzm), and pertuzumab (Per) (3), or treated with the combination of both TNF-□ and IFN-□ and Tzm and Per treatments (4) *Top panel:* densitometric analysis presented as % of SA-□-gal-positive cells, mean ± SD (n=3), ***p < 0.001. *Bottom panel:* representative data from 1 of 3 independent experiments. B. p15INKb or cleaved caspase-3 expression of cells described in A. Vinculin was used as loading control. Similar results were observed in 3 independent experiments. C. Induction of apoptosis of SK-BR-3 cells treated as described above was performed by staining for annexin V and PI expression of cells described in A and analyzed by flow cytometry. *Top panel:* densitometric analysis presented as % of annexin V^+ PI^+ cells, mean ± SEM (n=3), **p < 0.01. *Bottom panel*: plots are representative data from 1 of 3 independent experiments.

Figure 5

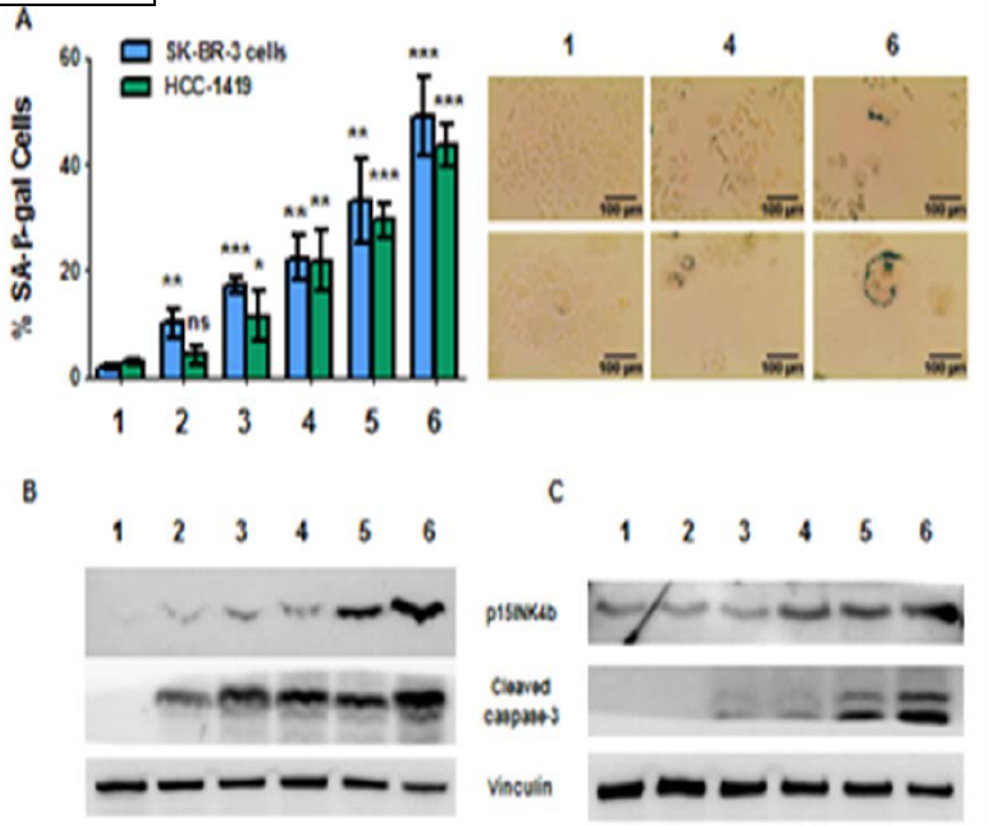

Figure 3. IFN-γ with trastuzumab and pertuzumab induce senescence and apoptosis in breast cancer cells. A. SK-BR-3 and HCC-1419 cells, untreated (1), or treated with 10 ug/ml of trastuzumab (Tzm) and pertuzumab (Per) (2-6), and incubated with increasing concentrations IFN-□□ for SKBR-3 and HCC-1419 respectively: 50 and 250 U/ml (3), 100 and 500 U/ml (4), 250 and 750 U/ml (5), 500 and 1000 U/ml (6). *Left panel*, densitometric analysis presented as % of SA-□-gal-positive cells, mean ± SD (n=3), *p < 0.05, **p < 0.01, ***p < 0.001; ns, not significant. *Right panel*: representative data from 1 of 3 independent experiments in SK-BR-3 cells (*top panel*) and HCC-1419 cells (*bottom panel*). **B and C.** p15INKb and cleaved caspase-3 expression of SK-BR-3 (**B**) and HCC-1419 (**C**) cells described in A. Vinculin was used as loading control. Similar results were observed in 3 independent experiments.

Figure 6

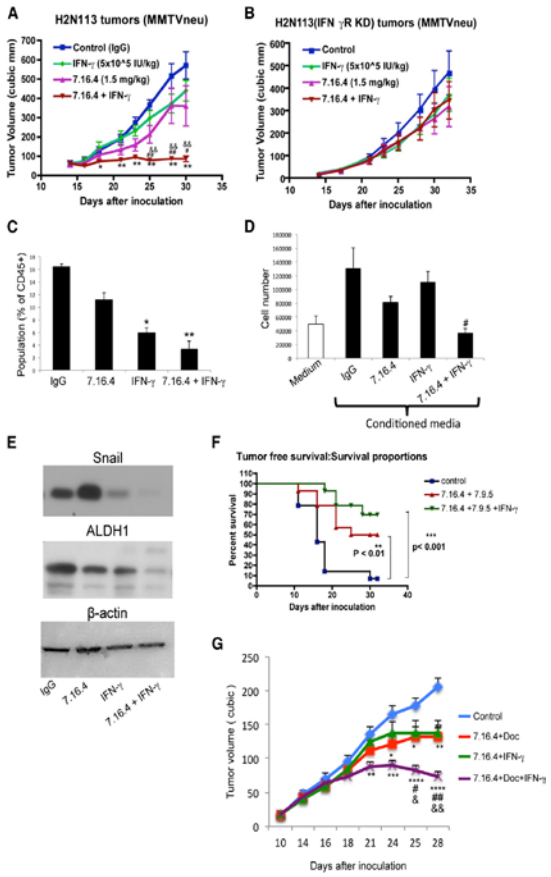

Figure 6. Murine models of HER-2 expressing breast cancer inhibited by combinations of anti-HER-2 and INF-γ and taxanes.
(A) Implanted H2N113 tumors were treated with control IgG, IFN- γ (three times per week), 7.16.4 (twice per week), or the combination of IFN- γ and 7.16.4.
(B) IFN- γ receptor was knocked down by shRNA in H2N113. The resulting tumor cells ($1x10^6$) were injected subcutaneously into MMTV-neu mice and treated similarly as in (A). Data represent mean + SEM. A Student's t test was performed to compare the difference in the tumor size of different treatment groups. *p < 0.05 and **p < 0.01 compared with control; #p < 0.05 and ##p < 0.01 compared with the 7.16.4 group; &p < 0.05 and &&p < 0.01 compared with the IFN-g group.
(C) H2N113 tumors from each group of mice treated as indicated were obtained after treatment for Figure 4A was finished. Tumor-infiltrated MDSCs were isolated and compared using CD11b, Gr-1, and CD45 antibody by FACS. *p < 0.05 and **p < 0.01 as compared with the IgG treated group.
(D) In vitro migration assay. H2N113 cells were seeded on 12-well plate and cultured until subconfluent. Cells were then treated with control IgG (10 mg/ml), 7.16.4 (10 mg/ml), IFN- γ (10 IU/ml), and 7.16.4 and IFN- γ, and conditioned media were collected at day 3 of culture. Migration of MDSC was measured by the Transwell system (pore size: 4 mm). MDSCs were isolated from spleens of tumor-bearing mice and then seeded in the apical chamber. Conditioned media were then placed in the basolateral chamber and incubated for 3 hr. The cells that migrated to the bottom chamber were collected and counted. Fresh medium was used as control (medium). #p < 0.05 (compared with either 7.16.4- or IFN-g-treated group).
(E) In vivo co-treatment significantly reduced the expression of ALDH1 in the tumor. Tumors from each group of mice treated as indicated were obtained after treatment for Figure 4A was finished. Each lysate was adjusted to 10 mg/lane and examined for Snail and ALDH1 expression by western blot. b-actin was used as the loading control. Shown are representative western blots of typical experiments.
(F) H2N113 tumor cells (0.25×10^6) were injected subcutaneously into both sides of the back of 6- to 10-week-old MMTV-neu mice at day 0. Mice were treated with control PBS, 7.9.5, and 7.16.4 (0.625 mg/kg each) or the combination of IFN- γ, 7.9.5, and 7.16.4 twice per week from day 1.
(G) H2N113 tumor cells (1×10^6) were injected subcutaneously into MMTV-neu mice similarly as in (A). Mice were treated with control PBS, 7.16.4 (1.5 mg/kg twice per week), and docetaxel (5.5 mg/kg twice per week), 7.16.4 and IFN- γ (5×10^5 IU/kg three times per week), or the combination of IFN- γ, 7.16.4, and docetaxel. Data represent mean + SEM. A Student's t test was performed to compare the difference in the tumor size of different treatment groups. *p < 0.05, **p < 0.01, ***p < 0.001, and ****p < 0.0001 compared with control; #p < 0.05 and ##p < 0.01 compared with the 7.16.4 and docetaxel group; &p < 0.05 and &&p < 0.01 compared with the 7.16.4 and IFN-g group.

2.3 Summary

There is an identified deficit in the anti-HER-2 CD4 Th1 response that occurs early in HER-2 breast tumorigenesis. The loss of anti-HER-2 Th1 response is predictive of an incomplete response to neoadjuvant therapy and shortened DFS. The Th1 cytokine IFN-γ has tremendous impact on HER-2 expressing breast cancer cells and can make them more susceptible to taxane chemotherapy and HER-2 directed therapy. We will leverage these findings and translate this into clinical trials to determine the effects of combining IFN-γ with taxane and HER-2 directed therapies, first in those with metastatic HER-2 positive breast cancer, and then in a neoadjuvant

trial with the same combination therapy to increase the pCR rates of patients with HER-2 expressing locally advanced breast cancer. The significance of enhanced pCR in HER-2 expressing breast cancer would translate into increased survival and decreased morbidity. In addition, we would develop the anti-HER-2 CD4 Th1 response as a biomarker to predict response to neoadjuvant therapy, systemic therapy in metastatic breast cancer, and ultimately to identify those at risk of recurrence, since we will collect blood specimens in all patients in these studies. This would be a significant advance to personalizing therapy and a useful tool for both patients and oncologists treating these patients.

2.4 Agents under Investigation

2.4.1 Interferon-gamma 1b (IFN-γ 1b, Actimmune®)

IFN-γ is a cytokine and is the only type II interferon. It is naturally produced mainly by natural killer cells and T-lymphocytes and works as an immunomodulator in various capacities. In its role as an immunomodulator it is 100-10,000 times more potent than the type I interferons. IFN-γ has many functions physiologically, including but not limited to regulating major histocompatibility complex (MHC) expression, activating the differentiation and function of phagocytes, augments interactions between macrophages and T-cells, and plays a key role in regulating T-cell subsets to determine the type of immune effector function during a specific immune response. (18)

Recombinant IFN-γ 1b (Actimmune®) is commercially available and approved for the treatment of the rare pediatric conditions chronic granulomatous disease (CGD) and severe malignant osteopetrosis (SMO). (18) It has, however, previously been evaluated in patients with advanced malignancies and thus has a known and proven safety and toxicity profile. Previous phase III trials of IFN-γ as a single agent in advanced RCC and melanoma did not improve outcomes compared to their respective comparator arms, but it was safe and reasonably well tolerated. (19, 20) Additionally, Devane and colleagues performed a dose titration study in healthy volunteers to establish the best tolerated dosing and schedule in adults in regards to the onset of flu-like symptoms. (21)

IFN-γ will be self-administered as a subcutaneous (SQ) injection at a starting dose of 50 mcg/m^2 on a three times weekly basis. This starting dose was selected based on data from a phase I dose-finding study of patients with resected melanomas performed by Maluish and colleagues which demonstrated evidence of enhanced immunologic activity (as determined by repeated measurements of hydrogen peroxide levels from monocytes and natural killer cell activity) at various doses and routes of administration.(22) Based on their results and conclusions, IFN-γ dosing from 10-100 mcg/m^2 achieved consistently high immunologic effects with a tolerable side effect profile. The 50 mcg/m^2 is the approved dose in CGD and SMO and was deemed the appropriate starting dose level.

The recombinant form of IFN-γ is rapidly cleared after intravenous administration, but is absorbed more slowly by the SQ route. (23) Following SQ injection, greater than 89% of the dose given is absorbed. As measured at a 100 mcg/m^2 dose, the mean elimination half-life is 5.9 hours. Peak plasma concentration is achieved at seven hours following SQ dosing. No drug accumulation has been reported after twelve consecutive daily injections using a 100 mcg/m^2 dose. While studies in healthy volunteers have failed to document detection of IFN-γ in the

urine of healthy subjects, animal studies have suggested some urinary excretion may be present, but predominant elimination is via the liver.

2.4.2 Paclitaxel, Trastuzumab and Pertuzumab

Paclitaxel promotes the assembly of microtubule formation and stabilizes them by preventing depolymerization. It is insoluble in water and, therefore, is formulated in cremophor. Paclitaxel is approved by the FDA for the treatment of breast cancer and is widely used in the adjuvant, neoadjuvant and metastatic setting. It is administered as an intravenous (IV) infusion and can be used either on a 3-weekly schedule, weekly or every 2 week schedule. Main toxicities associated with the use of paclitaxel are myelosuppression and neuropathy. Hypersensitivity reactions requiring treatment have occurred in 2% to 4% of patients receiving paclitaxel in clinical trials; thus, patients should be premedicated with corticosteroids, diphenhydramine, and H_2 antagonists. Combination of taxanes (docetaxel or paclitaxel) with HER2 targeted agents of trastuzumab and pertuzumab is routinely used for the treatment of patients with HER2 positive breast cancer as neoadjuvant therapy for locally advanced stage (24) and upfront line of therapy for metastatic stage (25). Paclitaxel 80 mg/m^2 weekly regimen will be used in combination with trastuzumab (8mg/kg loading dose, then 6mg/m^2 q 21 days) and pertuzumab (840 mg loading dose, then 420 mg q 21 days) for this proposed clinical trial. The study protocol is looking at the safety/efficacy of the combination of standard therapy (paclitaxel, trastuzumab and pertuzumab) and the study drug, interferon-gamma. Known potential adverse effects from the standard therapy (paclitaxel, trastuzumab and pertuzumab) include but are not limited to neutropenia, anemia, thrombocytopenia, neutropenic fever, sepsis, nausea, vomiting, diarrhea, mucositis, allergic reaction, neuropathy, alopecia and liver enzyme elevation and ejection fraction decline/ congestive heart failure. In addition, known adverse reactions from Interferon-gamma include flu-like symptoms such as fever/chills, headache, nausea, vomiting, diarrhea, rash, myalgia and arthralgia and less commonly increased liver enzymes, neutropenia, thrombocytopenia, allergic reactions.

3. PATIENT SELECTION

1.1 Inclusion Criteria

3.1.1 Patients must have a histologically confirmed HER2 positive breast cancer (by IHC 3+ or FISH ratio $\geq$ 2.0)
Phase I: unresectable locally advanced or metastatic breast cancer
Phase II: clinical stage 1-3 early stage breast cancer with primary tumor is at least 1 cm measured by clinical exam or by radiologic breast imaging tests

3.1.2 Prior therapy
Phase I: patients must be candidates to receive paclitaxel chemotherapy in combination with trastuzumab and pertuzumab

Phase II: No prior chemotherapy, radiation, or definitive therapeutic surgery (e.g., mastectomy, lumpectomy or axillary dissection) for this malignancy. Patients who have had a prior sentinel lymph node biopsy for this malignancy are eligible.

Note: Patients who receive equal to or less than 1 cycle of therapy (up to 4 weeks) will be allowed to enroll on this trial.

3.1.3 Patients who received tamoxifen or another selective estrogen receptor modulator (SERM) for the prevention or treatment of breast cancer or for other indications (e.g., osteoporosis, prior ductal carcinoma in situ [DCIS]), or who receive aromatase inhibitors for prevention or treatment of breast cancer, are eligible. Patients who are hormone-receptor positive and who have received other hormonal agents for the treatment of breast cancer (e.g., Fulvestrant®) are also eligible. Tamoxifen therapy or other hormonal agents should be discontinued at least 1 week before the patient is started on study therapy.

3.1.4 Age ≥ 18 years.

3.1.5 ECOG performance status 0 or 1.

3.1.6 Patients must have normal organ and marrow function as defined below within 2 weeks of registration (except where specified otherwise):

- Absolute neutrophil count (ANC) ≥ 1,500/ µL
- Platelets ≥ 100,000/ µL
- Total bilirubin ≤ 1.5 x institutional upper limit of normal (ULN), except patients with Gilbert's syndrome in whom total bilirubin must be <3.0 mg/dL
- AST/ALT ≤ 3 x institutional upper limit of normal
- Left ventricular ejection fraction (LVEF) EF ≥ of 50% (by echocardiogram or MUGA scan within 12 weeks of registration)
- Creatinine ≤ 1.5 x institutional upper limit of normal (ULN)
- ECG - QTc **<** 480 msec

3.1.7 Women of childbearing potential must agree to use adequate contraception (hormonal or barrier method of birth control) prior to study entry and for the duration of study participation. Should a woman become pregnant or suspect she is pregnant while participating in this study, she should inform her treating physician immediately.

3.1.8 Ability to understand and willingness to sign a written informed consent document.

3.2 Exclusion Criteria

3.2.1 Patients may not be receiving any other investigational agents during protocol therapy, or up to 14 days or 5 half-lives (whichever is longer) prior to beginning protocol therapy. There should be a least a 1-week interval between last dose of endocrine therapy and protocol therapy.

3.2.2 Patients who have had chemotherapy or radiation therapy within 2 weeks prior to beginning protocol therapy.

3.2.3 Uncontrolled intercurrent illness including, but not limited to, ongoing or active infection, congenital prolonged QT syndrome, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situations that would limit compliance with study requirements.

3.2.4 Current use of corticosteroid therapy > 5 mg/day of prednisone or equivalent doses of other corticosteroids (topical, intranasal, and inhaled corticosteroids in standard doses and physiologic replacement for subjects with adrenal insufficiency are allowed).

3.2.5 Patients with known active or symptomatic central nervous system (CNS) metastases and/or carcinomatous meningitis. Asymptomatic, treated, and/or stable brain metastases, as measured by subsequent radiologic evaluations at least two months apart, are permitted.

3.2.6 Pregnant or breast feeding.

3.2.7 Known HIV-positive

3.2.8 Known current or a history of hepatitis B or C virus, including chronic and dormant states, unless disease has been treated and confirmed cleared.

3.2.9 Major surgery within 4 weeks of initiation of study drug

3.2.10 Second invasive malignancy requiring active treatment

3.3 Inclusion of Women and Minorities

Both men and women of all races and ethnic groups are eligible for this trial.

3.4 Patient Registration

Once clinical eligibility is confirmed, patients must sign an informed consent prior to registration indicating awareness of the investigative nature of the study and its inherent risks in keeping with the policies of the hospital and federal regulations.

4. TREATMENT PLAN

4.1 Agent Administration

Treatment will be administered on an outpatient basis. Reported adverse events and appropriate dose modifications for all agents are described in Section 5.1. No investigational or commercial agents or therapies other than those described below may be administered with the intent to treat the patient's malignancy.

The portions of the treatment regimen that must be administered as per protocol guidelines include the following. Each cycle will be consisted of 21 days.

- Cycles 1 to 4: IFN-γ + paclitaxel + trastuzumab + pertuzumab (phase I and II portions of study)
- Biopsies and/or tissue acquisition (phase I or II portion)
- Surgery (phase II portion only)

4.1.1 IFN-γ + Paclitaxel + Trastuzumab + Pertuzumab

- IFN-γ (50, 75 mcg/m^2), three times weekly for 12 weeks. Patients and/or caregivers will be trained on self-administration of subcutaneous IFN-γ on Cycle 1 Day 1 (C1D1).. Patients will document each dose of administration in a diary provided to them by the study coordinator.

- On C1D1, patients will perform supervised self-administration of IFN-γ at Moffitt Cancer Center (MCC) clinical research unit (CRU) and will receive their first dose of paclitaxel at least 1 hour after the IFN-γ injection.

- Paclitaxel 80 mg/m^2 IV infusion over 1 hour weekly x 12 weeks (days 1, 8, 15, 22, 29, 36, 43, 50, 57, 64, 71, and 79)

- Trastuzumab will be given using an 8 mg/kg loading dose on C1D1, followed by 6 mg/kg IV on subsequent cycles q21 days.

- Pertuzumab will be given using an 840 mg IV loading dose on C1D1, followed by 420 mg IV on subsequent cycles q21 days.

- Repeat paclitaxel weekly x 12 doses if: (a) ANC ≥ 1000/μL, platelets ≥ 50,000/μL, (b) recovered from non-hematologic toxicity to grade 1 or less (except alopecia), or grade 2 or less neuropathy. G-CSF may be added if there is febrile neutropenia or neutropenia preventing treatment on schedule (see Sections 5.2.1 and 5.2.2 for guidelines). If treatment cycles are delayed, a minimum of 12 paclitaxel ± IFN-γ injection cycles must be

completed within 16 weeks.

- Patients will be assessed for DLTs during the first 3 week period of cycle 1 (Phase I).
- Tumor assessments with computed tomography of the chest, abdomen, and pelvis (CT CAP), as well as optional nuclear medicine bone scan per physician discretion, will occur after four cycles (at the conclusion of the study treatment) on or about C5D1, then per standard of therapy thereafter at the treating physician's discretion(Phase I).
- Study treatment will conclude after four 21-day cycles at which point IFN-γ will be stopped and patients who are clinically benefitting will continue to receive the standard therapy per the treating physician's discretion.
- Tumor assessments with clinical breast examination will occur on each cycle during neoadjuvant therapy (a total of 4 cycles) and patients will go for definitive surgery. Adjuvant therapy will be determined further per treating physician's discretion. (Phase II)

4.1.2 Prophylactic or Supportive Medications

IFN-γ

- Acetaminophen 325 mg orally PRN can be taken 30-60 minutes prior to injection for fevers and myalgias and may be used supportively at a dose of 325-650 mg orally Q6H PRN (≤ 3 gm/day).
- Anti-depressant medication in the form of a selective serotonin re-uptake inhibitor (SSRI) or next generation anti-depressant medication may be taken orally by patient to start up to two weeks prior or at any point during therapy to treat or protect against the development of depression. This should be decided between the patient and treating clinician.
- Ibuprofen 200 mg orally to 400 mg orally Q4-6H PRN (≤ 1200 mg/day) may be taken prophylactically or for supportive purposes for fevers and myalgias assuming adequate renal function as viewed by the treating clinician
- Diphenhydramine 25 to 50 mg orally Q4-6H prn (</= 300 mg/day)

Paclitaxel

- Premedication for paclitaxel: administer dexamethasone 10 mg, 30-60 minutes prior to C1D1 paclitaxel infusion. Dexamethasone should be reduced to 4 mg dose on C1D8 then omitted on subsequent cycles if there are no hypersensitivity reactions in the previous cycle. This is due to the potential concern of steroid inducing reduced immune response.

Trastuzumab and Pertuzumab

- Premedication for pertuzumab: administer diphenhydramine 25-50 mg by IVP over 2-5 minutes/PO, and an H2 blocker (e.g. ranitidine 50 mg or equivalent) 30-60 minutes prior to pertuzumab infusion.
- If the patient is on weekly trastuzumab, pre-medications will be given prior to pertuzumab on Day 1 (i.e. Week 1) and prior to trastuzumab on Days 8 and 15 (i.e. Weeks 2 and 3) of each cycle.
- Otherwise, follow institution's guidelines. Additional pre-medications may be added per protocol at the discretion of the physician.

4.1.3 Dose Escalation Schema and Definition of Dose Limiting Toxicity (DLT) (Phase I)

The phase I will use a 3+3 design with two dose levels of IFN-γ. The first dose level will be 50 mcg/m^2 and the second dose level will be 75 mcg/m^2. The DLT evaluation period for dose escalation will be during the first three weeks. The MTD dose level is defined as the highest dose level with ≤1 out of 6 patients experiencing a DLT. If the first dose level experience two or more DLTs, then dose de-escalation will occur to 30 mcg/m^2 dose (see table 2).

Dose-limiting toxicity (DLT) during cycle one (C1) is defined as follows:

- Non-hematologic or hematologic toxicities that are ≥ grade 3 in severity and probably or definitely related to study therapy which leads to chemotherapy treatment delays > 14 days are considered DLT.

Table 1: Dose Escalation Schema

Cycle 1 DLT	Action
0 of 3	Escalate to next dose level
1 of 3	Treat up to 3 additional patients at same dose level
1 of 6	Escalate to next dose level
≥ 2 of 3 *or* ≥ 2 of 6	Consider this dose unacceptable. De-escalate dose level per below.

Table 2: Dose Levels of IFN-γ

Dose Level	Dose (TIW)
-2	15 mcg/m^2
-1	30 mcg/m^2
1 (starting dose)	**50 mcg/m^2**

2	75 mcg/m^2

4.1.4 Recommended Phase II Dose (RP2D) Definition

The final dose level during the dose escalation phase that yields ≤ 1 DLTs will define the RP2D selection for phase II study. Optimum dosing level can be decided further with input from scientific advisory board.

4.1.5 Surgery (Phase II)

Patients with early breast cancer will be assessed for surgery following the fourth cycle of study therapy (or earlier if treatment is discontinued due to toxicity).

4.1.6 Post-Protocol Therapy

The following portions of the treatment are recommended after surgery in accordance with the standard of care:

- Adjuvant chest wall/breast irradiation if standard indications met
- Adjuvant chemotherapy if indicated
- Adjuvant hormonal therapy for patients with ER and/or PR-positive disease

4.1.7 Determination of the Recommended Phase II Dose

As of January 2, 2018, a total of 9 patients have been enrolled (3 patients on dose level (DL) 1 and 6 patients on DL2). No DLT has been observed out of all 9 patients enrolled. For DL1, no SAE or significant AEs have been observed for 3 patients who completed 12 weeks of treatment. However, for DL2, there have been 2 out of 6 patients had SAEs including grade 3 pneumonitis (at week 8 therapy and treatment was discontinued subsequently) and grade 3 non-neutropenic fever (at week 6 therapy) respectively, possibly related to study treatment. These toxicities however did not meet the definition of dose limiting toxicities. Based on these findings suggesting an improved tolerability of DL1, the investigators have selected DL1 (50 mcg/m2) as the recommended phase 2 dose.

4.2 Duration of Therapy

Patients should continue protocol treatment unless one of the following criteria applies, at which time protocol therapy should be discontinued:

- disease progression
- intercurrent illness that prevents further administration of treatment
- unacceptable adverse events(s)

- patient decides to withdraw from the study
- general or specific changes in the patient's condition render the patient unacceptable for further treatment in the judgment of the investigator
- completion of protocol therapy (12 weeks)
- patients who require a delay in the administration of paclitaxel for more than 3 weeks, or more than one dose reduction of paclitaxel

4.2.1 Monitoring of Discontinued Patients

Patients who discontinue IFN-γ due to adverse events related to the study drug must be followed until resolution or stabilization of the event. This condition applies to both the phase I and phase II portions of the trial.

4.3 Stopping Rules for Phase II Portion of the Trial

For efficacy, we will use the stopping rules based on a Simon's two stage design, where the study will seek to determine whether there is an increase in complete pathologic response (pCR, defined by RCB score 0). Since the enrollment of patients involves both ER+/HER2+ and ER-/HER2+ patients, with the final proportion of ER+ and ER - status patients unknown until the completion of enrollment, we cannot determine ahead of time the pCR rates to test. After 23 patients have been enrolled in stage 1, we will compute the proportion of ER+ and ER- subjects and compute the pCR under the null and alternative hypothesis as outlined in **Section 12.1.** Using $pCR1_{baseline}$ and $pCR1_{treatment}$ rates determined from the stage 1 enrollment of ER+ and ER- patients, we will determine the needed number of pCRs to determine if the study should stop or proceed to Stage 2.

For safety, there is an interim safety analysis upon completion of phase I and the first stage of phase II. For phase II, after the enrollment of the first 6 patients in stage 1 of the phase II study, if at any time more than 50% of the treated patients experience a G3-4 toxicity related to IFN-γ which leads to chemotherapy treatment delays > 14 days or unplanned cessation of neoadjuvant treatment then accrual to the trial will be held until discussions with the sponsor and protocol monitoring committee are arranged to determine if accrual can resume. Any deaths on study treatment will trigger an immediate safety review and accrual hold until the event is fully investigated and a determination is made by the study sponsor and protocol monitoring committee.

4.4 Duration of Follow up

- After all therapy has been completed, patients will be followed for recurrence and survival for up to 2 years after registration. (Every 6 months per either medical records or phone calls)

- Blood samples will be collected for biomarkers at baseline, 3 month, 6 month and 12 month follow up visits.
- Any patients who experience adverse events related to the study drug should be followed until one of the following occurs:
    - Resolution of the adverse event to ≤ Grade 1 or baseline severity
    - Determination that the adverse event is stable and is unlikely to resolve
    - Initiation of another anti-cancer treatment

5. REPORTED ADVERSE EVENTS/DOSE MODIFICATIONS

5.1 Reported Adverse Events

5.1.1 Paclitaxel

- Hematologic: Myelosuppression (neutropenia, leukopenia, thrombocytopenia, anemia).

- Hypersensitivity: Thought to be caused by the Cremophor vehicle. Minor symptoms include hypotension, flushing, chest pain, abdominal or extremity pain, skin reactions, pruritus, dyspnea, and tachycardia. More severe reactions include hypotension requiring treatment, dyspnea with bronchospasm, generalized urticaria, and angioedema. The majority (53%) of the reported reactions occurred within 2-3 minutes of initiation of treatment and 78% occurred within the first 10 minutes. Reactions usually occurred with the first and second doses.

- Cardiovascular: Atrial arrhythmia (sinus bradycardia [usually transient and asymptomatic], sinus tachycardia, and premature beats); significant events include syncope, hypotension, other rhythm abnormalities (including ventricular tachycardia, bigeminy, and complete heart block requiring pacemaker placement), and myocardial infarction. Hypertension (possibly related to concomitant medication - dexamethasone) may also occur.

- Neurologic: Sensory (taste changes); peripheral neuropathy; arthralgia and myalgia (dose-related, more common when colony-stimulating factors are also administered); seizures; mood alterations; neuroencephalopathy; hepatic encephalopathy; motor neuropathy; and autonomic neuropathy (paralytic ileus and symptomatic hypotension).

- Dermatologic: Alopecia (universal, complete and often sudden, between days 14 and 21); injection site reactions (erythema, induration, tenderness, skin discoloration); infiltration (phlebitis, cellulitis, ulceration, and necrosis, rare); radiation recall; and rash.

- Gastrointestinal: Nausea, vomiting, diarrhea, stomatitis, mucositis, pharyngitis, typhlitis (neutropenic enterocolitis), ischemic colitis, and pancreatitis.

- Hepatic: Increased AST, ALT, bilirubin, alkaline phosphatase; hepatic failure, and hepatic necrosis.

- Other: Fatigue, headache, light-headedness, myopathy, elevated serum creatinine, elevated serum triglycerides, and visual abnormalities (sensation of flashing lights, blurred vision).

5.1.2 Trastuzumab

Experience with trastuzumab administration has shown that the drug is relatively safe. The most significant safety signal observed during clinical trials was cardiac dysfunction (principally clinically significant heart failure [CHF]), particularly when trastuzumab was given in combination with an anthracycline-containing regimen. Much of the cardiac dysfunction was reversible on discontinuation of trastuzumab.

In addition, during the first infusion with trastuzumab, a symptom complex most commonly consisting of fever and/or chills was observed in approximately 40% of patients. The symptoms were usually mild to moderate in severity and controlled with acetaminophen, diphenhydramine, or meperidine. These symptoms were uncommon with subsequent infusions. However, in the post-approval setting, more severe adverse reactions to trastuzumab have been reported. These have been categorized as hypersensitivity reactions (including anaphylaxis), infusion reactions, and pulmonary events. Rarely, these severe reactions culminated in a fatal outcome.

Trastuzumab appears to be relatively nonimmunogenic. Only 1 of 903 patients evaluated developed neutralizing antibodies to trastuzumab. The development of anti-trastuzumab antibodies in this patient was not associated with clinical signs or symptoms.

5.1.3 Pertuzumab

Pertuzumab is well-tolerated as monotherapy and that it can be given in combination with trastuzumab and a range of other therapeutic agents with manageable additional toxicity. No new or unexpected toxicities were encountered other than those that are known for agents that target the HER family of receptors. Serious or severe infusion-associated symptoms have been rarely observed in patients receiving pertuzumab. A low level of cardiac toxicities, predominantly asymptomatic declines in left ventricular ejection fraction (LVEF), has been reported. In the pivotal Phase III trial WO20698/TOC4129g the rates of symptomatic and asymptomatic left ventricular systolic dysfunction (LVSD) were not higher in patients receiving pertuzumab, trastuzumab and docetaxel than in those receiving placebo, trastuzumab and docetaxel.

No fetal studies in humans have been performed but pertuzumab caused oligohydramnios, delayed renal development and embryo-fetal deaths in pregnant cynomolgus monkeys. Moreover, in the post-marketing setting, cases of oligohydramnios, some associated with fatal pulmonary hypoplasia of the fetus,

have been reported in pregnant women receiving trastuzumab (for further details, see trastuzumab prescribing information). Therefore, pertuzumab should not be used in pregnant women. Protocols for ongoing pertuzumab studies indicate that highly effective contraceptive measures must be used; continuous pregnancy monitoring must be performed during the trials and for six months after the last dose of study drug is administered. Because of the long half-life of pertuzumab women should be warned not to become pregnant for at least six months after completion of treatment.

5.1.4 IFN-γ

Interferon gamma -1b (IFN-g 1b, IFN-γ) is a biologic response modifier with diverse immunomodulatory properties. As with other interferons, the main risk of IFN-g 1b therapy is the development of "flu-like" or constitutional symptoms including fever, headache, chills, myalgia, and fatigue. These events may decrease in severity as treatment continues and may be minimized by bedtime administration of IFN-g 1b. Additionally, the severity of flu-like symptoms may be lessened with initial dose titration. Acetaminophen administration may be useful in the prevention/alleviation of fever and headache.

In addition to constitutional symptoms, patients may experience injection site reactions, which are usually mild and transient. While IFN-g 1b therapy is generally well tolerated, reversible neutropenia and thrombocytopenia, as well as, elevations in liver function tests (aspartate aminotransferase (AST) and/or alanine aminotransferase (ALT)) have been observed. Caution should be exercised when administering IFN-g 1b to patients with myelosuppression or in combination with other potentially myelosuppressive agents. IFN-g 1b does not appear to be mutagenic and does not appear to provoke neutralizing antibodies.

5.2 Dosing Delays/Dose Modifications

5.2.1 Criteria for Administering Chemotherapy Paclitaxel and IFN-γ

See Section 4.1.1 for criteria for administering therapy

- If treatment is delayed due to neutropenia, delay treatment until satisfactory ANC (as per Section 4.1.1) and resume at 100% dose; filgrastim (5 µg/kg rounded to 300 or 480 µg SC daily on days 2-6 of each cycle) may be used if neutropenia prevents administration of weekly paclitaxel on schedule. Filgrastim may also be used with weekly paclitaxel if a cycle is complicated by grade 3 or 4 febrile neutropenia.
- If the platelet counts recovery is delayed due to thrombocytopenia, delay treatment until satisfactory plate count (as per Section 4.1.1) and resume with one dose level reduction in paclitaxel.
- If treatment is delayed > 3 weeks due to protocol associated toxicity, the patient will be removed from study.

5.2.2 Dose Modifications for Toxicity

Pertuzumab – fixed dose, no modification of dose. If the patient is unable to tolerate the standard dose, they will be discontinued from study.

Trastuzumab - After consulting with the Principal Investigator, if grade 2 or higher infusion reaction occurs and is attributed to Trastuzumab, it may be given on weekly dosing of 2mg/kg.

Paclitaxel – After consulting with the Principal Investigator, if grade 2 or higher infusion reaction occurs and is attributed to Paclitaxel, nab-Paclitaxel may be given on weekly 80mg/m^2 dosing.

Dose modifications will be made using the schema outlined in the **Table 3** below.

The initial dose level of paclitaxel given during the first cycle is dose level 1. Patients who require a dose reduction to dose level -2 will be removed from study.

The following toxicities will require reduction of paclitaxel by one dose level:

- Grade 3 or 4 febrile neutropenia (fever $\geq$ 38.5°C and ANC < 1,000/mm^3) between courses. Filgrastim may also be used with weekly paclitaxel if a cycle is complicated by grade 3 or 4 febrile neutropenia (see Section 5.2.1 above for dosing guidelines)
- Platelet nadir $\leq$ 20,000/mm^3.
- Failure to recover adequate platelet count on the planned day of therapy.
- Grade 3-4 non-hematologic toxicity (excluding neuropathy)
- Grade 3-4 neuropathy (hold paclitaxel until $\leq$ grade 2 neuropathy, reduce paclitaxel dose one dose level; keep IFN-γ dose stable)

Table 3 Dose Modifications for Toxicity

Dose Level	Paclitaxel
1	80 mg/m^2 day 1
-1	60 mg/m^2 day 1
-2	Remove from study

IFN-γ **Dose**	**Reduced Dose**
15 mcg/m^2	Discontinue
30 mcg/m^2	15 mg/m^2
50 mcg/m^2	30 mcg/m^2
75 mcg/m^2	50 mcg/m^2

6. AGENT FORMULATION AND PROCUREMENT

6.1 Paclitaxel

Other Names: Taxol, Onxol, Nov-Onxol, Paclitaxel Novaplus, NSC# 125973.
Classification: Antimicrotubule agent.
Mode of Action: Promotes microtubule assembly and stabilizes tubulin polymers by preventing their depolarization, resulting in the formation of extremely stable and nonfunctional microtubules, and consequently inhibition of many cell functions.
Storage and Stability: The intact vials may be stored under refrigeration or at room temperature. Freezing does not adversely affect the product. Solutions diluted to a concentration of 0.3 to 1.2 mg/mL in normal saline, 5% dextrose, 5% dextrose and normal saline, or 5% dextrose in Ringer's solution are stable for up to 27 hours when stored at room temperature and normal room light.
Dose Specifics: Refer to Section 4.1.1.
Preparation: Preparation of standard regimens should follow site standards.
Administration: Administered as an IV infusion over 1 hour with an in-line 0.22 micron filter.
Incompatibilities: Avoid the use of PVC bags and infusion sets due to leaching of DEHP (plasticizer).
Drug Interactions: Ketoconazole may inhibit paclitaxel metabolism, based on in vitro data.
Availability: A concentrated solution of 6 mg/mL in polyoxyethylated castor oil (Cremophor EL) 50% and dehydrated alcohol 50% is commercially available in 5 mL vials. 100 mg/16.7 mL and 300 mg/50 mL vials are also available. See package insert for further information.

6.2 Trastuzumab

Formulation

Trastuzumab is a sterile, white to pale yellow, preservative-free lyophilized powder for intravenous (IV) administration. Each vial of trastuzumab contains 440 mg of trastuzumab, 9.9 mg of L-histidine HCl, 6.4 mg of L-histadine, 400 mg of α,α-trehalose dihydrate, and 1.8 mg of polysorbate 20, USP. Reconstitution with 20 mL of the supplied Bacteriostatic Water for Injection (BWFI) USP, containing 1.1% benzyl alcohol as a preservative, yields 21 mL of a multidose solution containing 21 mg/mL trastuzumab, at a pH of ~6.

Dosage

The recommended initial loading dose is 8 mg/kg trastuzumab administered as a 90-minute infusion. The recommended maintenance trastuzumab dose is 6 mg/kg q3wk and can be administered as a 30-minute infusion if the initial loading dose was well tolerated. Trastuzumab may be administered in an outpatient setting. If trastuzumab 6mg/kg q 3 wk is not tolerated, trastuzumab 2mg/kg q week can be administered.

Preparation

Use appropriate aseptic technique. Each vial of trastuzumab should be reconstituted with 20 mL of BWFI, USP, 1.1% benzyl alcohol preserved, as supplied, to yield a multidose solution containing 21 mg/mL trastuzumab. Immediately upon reconstitution with BWFI, the vial of trastuzumab must be labeled in the area marked "Do not use after" with the future date that is 28 days from the date of reconstitution. If the patient has known hypersensitivity to benzyl alcohol, trastuzumab must be reconstituted with sterile water for injection. Trastuzumab that has been reconstituted with SWFI must be used immediately and any unused portion discarded. Use of other reconstitution diluents should be avoided. Determine the dose of trastuzumab needed, based on a loading dose of 8 mg trastuzumab/kg for q3wk dosing schedules or a maintenance dose of 6 mg/kg trastuzumab/kg for q3w dosing schedules. Calculate the correct dose using 21 mg/mL trastuzumab solution. Withdraw this amount from the vial and add it to an infusion bag containing 250 mL of 0.9% sodium chloride, USP. DEXTROSE (5%) SOLUTION SHOULD NOT BE USED. Gently invert the bag to mix the solution. The reconstituted preparation results in a colorless to pale yellow transparent solution. Parenteral drug products should be inspected visually for particulates and discoloration prior to administration.

No incompatibilities between trastuzumab and polyvinylchloride or polyethylene bags have been observed.

Storage

Vials of trastuzumab are stable at 2C–8C (36F–46F) prior to reconstitution. Do not use beyond the expiration date stamped on the vial. A vial of trastuzumab reconstituted with BWFI, as supplied, is stable for 28 days after reconstitution when stored refrigerated at 2C–8C (36F–46F), and the solution is preserved for multiple use. Discard any remaining multi-dose reconstituted solution after 28 days.

6.3 Pertuzumab

Formulation

Pertuzumab drug product is provided as a single use formulation containing 30 mg/mL pertuzumab in 20 mM L-histidine acetate (pH 6.0), 120 mM sucrose and 0.02% polysorbate 20. Each 20 mL vial contains 420 mg of Pertuzumab (14.0 mL/vial). Upon receipt, pertuzumab vials are to be refrigerated at 2°C–8°C (36°F–46°F) until use. Pertuzumab vials should not be used beyond the expiration date provided by the manufacturer. Because the formulation does not contain a preservative, the vial seal may only be punctured once. Any remaining solution should be discarded. Vial contents should be protected from light, and should not be frozen. The solution of pertuzumab for infusion, diluted in PVC or non-PVC polyolefin bags containing 0.9% Sodium Chloride Injection, USP, may be stored for up to 24 hours prior to use. Diluted pertuzumab has been shown to be stable for up to 24 hours at a temperature range of 2°C–25°C. However, since diluted pertuzumab contains no preservative, the diluted solution should be stored refrigerated (2°C–8°C).

Dosage

The initial dose of pertuzumab is 840 mg administered as a 60 minute intravenous

infusion, followed every 3 weeks thereafter by a dose of 420 mg administered over a period of 30 - 60 minutes.

Preparation
Prepare the solution for infusion, using aseptic technique, as follows:

Parenteral drug products should be inspected visually for particulates and discoloration prior to administration.

Withdraw the appropriate volume of pertuzumab liquid concentrate from the vial(s).

Dilute into the 250 mL 0.9% sodium chloride PVC or non-PVC polyolefin infusion bags.

Mix diluted solution by gentle inversion. Do not shake.

Administer immediately once prepared.
Dextrose (5%) solution should not be used to dilute *pertuzumab.*

6.4 nab-Paclitaxel

Other Names: Abraxane

Classification: Microtubule inhibitor

Mode of Action: Microtubule inhibitor that promotes the assembly of microtubules from tubulin dimers and stabilizes microtubules by preventing depolymerization.

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

Dose Specifics: Refer to Section 5.2.2

Preparation: Preparation of standard regimens should follow site standards.

Administration: 80 mg/m2 intravenously over 30 minutes every week.

Drug Interactions: Use caution when concomitantly administering with inhibitors or inducers of either CYP2C8 or CYP3A4.

Availability: For injectable suspension: lyophilized powder containing 100 mg of paclitaxel formulated as albumin-bound particles in single-use vial for reconstitution. See package insert for further information.

6.5 IFN-γ

Product description

- IFN-γ is a cytokine or biologic response modifier that exists as a single-chain polypeptide containing 140 amino acids. Production of this agent is achieved via fermentation of a genetically engineered *Escherichia coli* bacterium containing the DNA which encodes for the human protein. The agent is a sterile, clear, colorless solution pre-filled in a single-use vial for subcutaneous injection.
- Each 0.5 mL vial contains 100 mcg of IFN-γ formulated in 20 mg of mannitol, 0.37 mg disodium succinate hexahydrate, 0.14 mg succinic acid, 0.05 mg polysorbate 20 and sterile water.

Availability

- Will be supplied by Horizon Pharma, LLC

Solution preparation

- Available as described above and per product label. Distributed as single use vials and designated amount per dose to be administered by patient

Storage requirements and Stability

- Vials should be refrigerated at 2-8°C (36-46°F)
- If exposed to room temperature for greater than 12 hours, vials should be discarded
- Do not freeze
- Avoid shaking or excessive vigorous activity

Route of administration

- Will be self-administered SQ by patients or representative
- Note: Patients will be trained on appropriate administration technique and appropriate documentation of self-administration in the patient diary

7. CORRELATIVE/SPECIAL STUDIES

Blood will be collected at the time of screening, 3 months, 6 months and 12 months from the start of therapy. For phase II clinical trial, tissue samples from pre-therapy and post-therapy (surgical specimen) will be collected.

7.1 Determine whether IFN-γ combination therapies restore anti-HER-2 CD4 Th1 response

Immune Monitoring: Systemic anti-HER2 $CD4^{pos}$ T-cell responses will be generated from autologous peripheral blood mononuclear cells (PBMC) pulsed with HER2 peptides. PBMC will be collected at least three time points about 30 cc blood each draw. IFN-ɣ production will be measured by enzyme-linked immunosorbent spot (ELISPOT) assays or by in vitro sensitization assays, as previously described (26, 27).

Briefly, ELISPOT PVDF membrane plates will be coated overnight with anti-IFN-ɣ capture antibody (Mabtech Inc). The following day, after the plates will be washed with PBS (Mediatech Inc) and blocked with 10% human serum/DMEM, $2x10^5$ PBMCs or SLN cells will be plated in each well either unstimulated, pulsed with HER2-derived Class II peptide (4 μg) (42-56, 98-114, 328-345, 776-790, 927-941, 1166-1180), or pulsed with anti-human CD3/CD28 antibodies (0.5 μg/mL) (positive control, BD Pharmingen), and incubated at 37°C with 5% CO_2 for 24-36 hours. After the plates will be washed with PBS, 100 μL of detection antibody (1 mg/mL; 7-B6-1-biotin) will add to each well and the plates will be incubated for 2 hours. The plates will be washed again with PBS, 100 μL of 1:1000 diluted streptavidin-HRP will added to each well and the plates will be incubated for another hour. TMB substrate solution (Mabtech Inc) will added to reveal spot formation. Spot forming cells (SFC) were counted using an automated reader (ImmunoSpot CTL).

A positive response to an individual HER2 Class II peptide will be defined as a minimum of 20 SFC/$2x10^5$ cells after subtracting the unstimulated background. Three metrics will be used to quantify the $CD4^{pos}$ Th1 response: (1) responsivity (the proportion of patients responding to ≥ 1 peptide), (2) response repertoire (the number of peptides to which a patient responds), and (3) cumulative response (the sum of the SFCs across all 6 Class II HER2 peptides) (12). We will also assess whether there is a change in anti-HER-2 IL-10, IL-4, granzyme B by multicolor imaging of a percentage of the samples. Controls will be utilized of normal response proteins such as candida, tetanus and anergy will be assessed by CD3/CD28 antibody stimulation.

We will assess the CD8 T cell response against HER-2 as well as validate ELISPOT assays using in vitro sensitization, $CD8^{pos}$ T-cells will be selected from the cryopreserved lymphocyte cell fractions via negative selection (StemCell Technologies). Autologous DC1 will be suspended in SFM with GM-CSF (10 ng/ml), pulsed with one of the six Class II HER2 peptides, and co-cultured with the $CD4^{pos}$ T-cells at a ratio of 10:1. IL-2 (30 IU/m; ThermoFisher) will be added on day 2. On day 10, T-cells will be harvested and tested against T2 target cells pulsed with one of the six class II HER2 peptide or irrelevant controls (p53 and colon cancer peptide). After 24 hours, the supernatant will be harvested and analyzed by enzyme-linked immunosorbent assay (ELISA). A positive response to the HER2 peptide will be defined as a two-fold increase in CD8 or $CD4^{pos}$ T-cell IFN-ɣ production compared to the irrelevant peptide controls. Again, responsivity and response repertoire metrics will be used to quantify the $CD4^{pos}$ immune response.

7.2 Determine whether IFN-γ combination therapies change in immune infiltrates following therapy

Rationale: There is data to suggest that pCR to HER-2 targeted regimens requires immune response (10); we will therefore assess the quality and quantity of the immune infiltrates prior to and following therapy to determine predictors of responses to therapy. It will be important to know if IFN-γ is more responsive in patients with significant pre-existing immune infiltrate in the tumor or not. Being able to identify patients that are likely to respond to immune therapies is critical. In our neoadjuvant DCIS vaccine studies there is a trend toward more complete

responses to vaccines in patients with higher pre-existing anti-CD4 Th1 cells (manuscript submitted). It will be important to search for clues to determining response.

Approach: Using immunohistochemistry, we will assess the change in the quality and quantity of the immune infiltrate in subjects receiving the neoadjuvant IFN-γ regimen compared to a control matched group not receiving IFN-γ. We will analyze sections from regions of residual tumor. Sections of the pre-treatment biopsy and of the residual tumor/tumor bed will be stained using CD4, CD8, CD20, CD1c, CD11b, CD14,CD141, pan MHC class I, II, FOXP3, CD56, CD16, antibodies to study recruitment of TIL, as well as other populations, including NK, B cell macrophage and DC. We will also perform IHC for IFN-γ on the tumor cells, macrophages (M1 and M2) on surrounding tissues. PD1 and PD1-L expression also will be measured. In those with sufficient tissue we will perform exploratory analysis examining immune gene signatures using standard Affymetrix.

7.3 Determine whether IFN-γ combination therapies changes known tumor biomarkers that predict response in the HER-2^{pos} IBC

Rationale: IFN-γ combination therapy may be effective in many but not all patients. We would like develop assays to determine whether this therapy can prevent recurrence, sparing patients from additional therapies. A serum HER-2 assay that measures cleaved or circulating HER-2 protein in the serum will be assessed to correlate with response.

Rationale for Analysis of Serum HER-2: Recent data suggests that soluble serum HER-2 levels can predict those at risk to fail therapy (28). Since HER-2 is the target antigen and the oncodriver it will be useful to determine whether this can serve as a biomarker of response. We will analyze serum HER-2 levels in patients prior to following completion of therapy. We will also check serum HER-2 at 3 months, 6 months, and 12 months from therapy. .

Approach: Dr. Frank Pass and Martell Co have developed several high-sensitivity HER-2 serum assays that will be utilized to detect decreases in serum HER-2 as another potential biomarker of response; we will evaluate these biomarkers in the context of the proposed therapy regimens. These assays will be run on pre and post plasma specimens from patients on clinical trials. Samples of serum or plasma will be sent to Martell for analysis. The assays are based off ELISA technology that has been modified to improve sensitivity.

7.4 Anti-drug (IFN-γ) neutralizing antibody assay will be done at baseline and at the end of treatment (3 months) to evaluate whether IFN-γ stimulates a detectable host antibody response

8. STUDY CALENDAR

a) Pre-study assessments obtained during standard of care is acceptable for use in this study. Pre-study complete blood count (CBC, with differential and platelet count), serum creatinine, and liver function tests must be obtained within 14 days prior to registration.
b) CT scan of the chest/abdomen/pelvis and bone scan (or PET/CT) should be performed within 30 days of registration.
c) MUGA scan or echocardiogram to determine left ventricular ejection fraction must be obtained within 30 days of registration.
d) A negative serum or urine beta HCG is required for women with childbearing potential within 7 days of registration.
e) All the study procedures including labs, visits, scans and treatment (during study therapy) have windows +/- 3 days.

	Pre-Study	Week 1	2	3	4	5	6	7	8	9	10	11	12	After therapy completed (a)
Informed consent	x													
Eligibility checklist	x													
History and Physical examination	x	x	x		x			x			x			X (a)
Concurrent meds	x													
Adverse event evaluation (b)	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Vital signs	x	x	x		x			x			x			x
Height	x													
Weight	x				x			x			x			x
CBC w/diff, plts	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Serum chemistry (c)	x	x	x	x	x			x			x			x
Serum b-HCG	x													
ECHO or MUGA(d)	x						x							x
EKG	x	x	x	x	x									x
Tumor measurements by clinical exam (phase II only)	x													x
CT of chest/abdomen /pelvis (bone scan if clinically indicated) (e)	x (k)													x (for phase I only)
IFN-γ (f)		x	x	x	x	x	x	x	x	x	x	x	x	
Paclitaxel (g)		x	x	x	x	x	x	x	x	x	x	x	x	
Trastuzumab (h)		x			x			x			x			
Pertuzumab(h)		x			x			x			x			
Pathology submission (i)	x													x

Blood (biomarkers)	x	x												X (j)

(a) Off-study treatment evaluation should be completed within 30 days after last chemotherapy treatment. After all therapy has been completed, patients will be followed for recurrence and survival for up to 2 years after registration. (every 6 months per either medical records or phone calls)
(b) Adverse event evaluation (by study physician and/or research nurse, physician assistant) should be performed per study calendar. All adverse events must be documented and graded using CTCAE version 4.03.
(c) Serum chemistry- Albumin, alkaline phosphatase, total bilirubin, bicarbonate, BUN, calcium, chloride, creatinine, glucose, potassium, total protein, SGOT [AST], SGPT [ALT], sodium.
(d) MUGA or ECHO should be performed at baseline and after completion of therapy in all patients in the phase I and phase II portions of the trial. The same modality should be used at each assessment.
(e) Repeat CT chest/abdomen /pelvis after completion of all study therapy in patients with metastatic breast cancer. Bone scan may be repeated if performed at baseline and if clinically indicated.
(f) IFN-γ will be administered by patient or representative under supervision for first dose on cycle 1 day 1. Subsequent dosing will be self-administered by patient or representative at home on three times weekly basis through end of combination phase unless schedule change coordinated by clinician and study coordinator, except on C1D1 when self-administered at MCC. The clinician or designee will train patient or representative on appropriate administration technique and appropriate documentation of self-administration in the diary.
(g) Paclitaxel 80mg/m2 IV weekly x 12
(h) Trastuzumab and Pertuzumab per the standard of care every 3 weeks IV x 4
(i) Pretreatment paraffin specimens should be submitted within one month of registration (phase II) if available; representative paraffin specimens from the mastectomy/lumpectomy specimen should be submitted within one month following surgery (Phase II) if available.
(j) Blood sample will be collected for biomarker at baseline (either pre-study or pre-treatment on week 1), 3 months, 6 months, and 12 months (window +/- 4 weeks). Eight, 10mL green top heparin tubes and 3, 10mL red top tubes will be collected at baseline, 3 months, 6 months and 12 months
(k) Baseline CT scan is required for phase I subjects only. For phase II subjects, CT scan is only if clinically indicated.

9. MEASUREMENT OF EFFECT

9.1 Definitions

9.1.1 Pathological Response

For patients with early breast cancer (phase II), the primary endpoint of this study is pathologic complete response in the breast at definitive surgery after completion of protocol therapy. The pathologic response to treatment will be assessed by the institutional pathologist (preferably a single pathologist for all patients treated at that institution). The institutional pathologist (Laila Khazai, MD; H. Lee Moffitt Cancer Center) will evaluate response by the "Residual Cancer Burden"(RCB) for each patient as described in the online calculator (http://www3.mdanderson.org/app/medcalc/index.cfm?pagename=jsconvert3).

9.1.2 Clinical Response

For patients with early breast cancer (phase II), a secondary endpoint will be clinical complete response and partial response based upon tumor measurements obtained on physical examination at baseline, after completion of 4 cycles of study therapy. Tumor measurements obtained by physical exam should be performed using a caliper, using metric notation, preferably by the same examiner with the patient lying in the supine position with the ipsilateral arm placed behind the head (or in the same position on each exam), and will be recorded in study specific case report forms. Factors that will be evaluated include:

- Breast mass(es) - size (longest dimension)
- Axillary lymph node(s) - size (longest dimension)
- Skin edema of the breast - present worse, present unchanged, present improved, or absent
- Skin erythema of the breast - present worse, present unchanged, present improved, or absent

9.1.3 Radiologic Response

For patients with metastatic breast cancer (phase I), response and progression will be evaluated in this study using the Response Evaluation Criteria in Solid Tumors (RECIST) criteria, version 1.1. RECIST criteria will be used for assessing response in patients with metastatic disease using the baseline and post treatment imaging studies, plus physical examination when appropriate. For patients with locally advanced disease, physician examination will be used and measurements recorded at baseline, after completion of study therapy.

10. REGULATORY AND REPORTING REQUIREMENTS

10.1 Adverse Event

An adverse event (AE) is defined as the appearance of (or worsening of any pre-existing) undesirable sign(s), symptom(s), or medical condition(s) after starting study drug (or therapy) even if the event is not considered to be related to study drug (or therapy). An AE will be graded using the National Cancer Institute CTCAE version 4.03. AEs will be followed until the safety follow up visit (30 days after last dose). A laboratory test abnormality considered clinically relevant, e.g., causing the subject to withdraw from the study, requiring treatment or causing apparent clinical manifestations, or judged relevant by the investigator, should be reported as an AE.

All AEs will be recorded on the appropriate case report forms (CRF and in the subject's medical records starting from the first treatment. The Investigator will also identify the date of onset, date of resolution, seriousness, outcome, and the relationship to study drug. Every effort should be made to determine the cause of each AE and whether or not it is related to the study drug. The relationship of the AE to the study drug must be rated and recorded following the guidelines outlined in the CTCAE v4.03. All the AEs will be reviewed by the Principal Investigator of the study. The 3 categories for AE grading are:

Unrelated	The lack of a temporal relationship of the event to study treatment makes a causal relationship not reasonably possible, or by any other drugs, therapeutic interventions or underlying conditions that provide a sufficient explanation

Possible	The temporal relationship of the event to study treatment makes a causal relationship reasonably possible, and the event is more likely explained by exposure to the study treatment than by any other drugs, therapeutic interventions or underlying conditions
Definite	The temporal relationship of the event to study treatment makes a definitive relationship, and the event is more likely explained by exposure to the study treatment than by any other drugs, therapeutic interventions or underlying conditions

10.2 Serious Adverse Event

AEs are classified as serious or non-serious. A serious adverse event (SAE) is any AE that is:

1. is fatal or life-threatening
2. requires in-patient hospitalization or prolonged hospitalization
3. results in persistent or significant disability/incapacity
4. constitutes a congenital anomaly or a birth defect
5. is medically significant, may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed above.

Pregnancy, although not itself a serious adverse event, should also be reported on a serious adverse event form or pregnancy form and be followed up to determine outcome, including spontaneous or voluntary termination, details of birth, and the presence or absence of any birth defects or congenital abnormalities.

Events not considered to be serious adverse events are hospitalizations for the:

1. routine treatment or monitoring of the studied indication, not associated with any deterioration in condition.
2. treatment, which was elective or pre-planned (e.g. surgery as per Phase II of this study) for a pre-existing condition that did not worsen
3. treatment on an emergency, outpatient basis for an event not fulfilling any of the definitions of serious given above and not resulting in hospital admission.

The sponsor is responsible for submitting reports of AEs that are associated with the use of the drug that are both serious and unexpected to the FDA. Any serious adverse event occurring after the patient started taking the study medication, and until 30 days after the patient has stopped study participation must be reported. Information about all serious adverse events will be collected and recorded on the FDA MedWatch 3500a form within 48 hours of the notification. SAEs and pregnancy reports will be reported to Horizon Pharma as well.

In addition, all SAEs should be reported to H. Lee Moffitt Cancer Center & Research Institute through Oncore for review by our Protocol Review & Monitoring Committee. They will be submitted to IRB per institution policy.

SAE reporting begins after the patient has signed the ICF and has received study treatment. However, if an SAE occurs after signing the ICF, but prior to receiving study treatment, it needs to be reported ONLY if it is considered reasonably possibly related to study procedure. SAE reporting forms will be completed within 24 hours of the event and e-mailed to:

Email AdverseEvents@horizonpharma.com

FAX for SAEs: 1-800-860-7836

The drug provider will medically review all SAEs.

The following detailed information must be recorded for each serious adverse event in the SAE report form:

- A description of the AE in medical terms
- The severity grade as assessed by the investigator according to the definitions in NCI-CTC Version 4.03
- The date of becoming serious and the date of becoming known (if different)
- The reason for seriousness
- The outcome of the SAE at the time of the report
- Information on administration of the study drug and chemotherapy and any action taken
- Information on any treatment procedures necessary for the SAE, concomitant medications, relevant lab tests and relevant medical history

If in any one subject the same SAE occurs on several occasions, then the SAE in question must be documented and assessed anew each time.

The investigator is required to submit SAE Follow-up reports until the SAE has resolved or stabilized and all queries have been answered.

10.3 Laboratory Test Abnormalities

Laboratory test results will be recorded on the laboratory results pages of the CRF. In the event of unexplained abnormal laboratory test values, the tests should be repeated immediately and followed up until they have returned to the normal range and/or an adequate explanation of the abnormality is found.

Laboratory test value abnormalities as such should not be reported on the AE page of the CRF as adverse events unless they are treatment-emergent and they satisfy one or more of the following conditions for clinical significance:

1. Accompanied by clinical symptoms
2. Leading to a change in study medication (e.g. dose modification, interruption or permanent discontinuation)
3. Requiring a change in concomitant therapy (e.g. addition of, interruption of, discontinuation of, or any other change in a concomitant medication, therapy or treatment)

Please note: Any laboratory result abnormality fulfilling the criteria for a serious adverse event (SAE) should be reported as such, in addition to being recorded as an adverse event in the CRF.

10.4 Pregnancy

Female patients must be instructed to immediately inform the investigator if they become pregnant during the study. The study treatment must immediately be stopped and the patient must be withdrawn from the study. Pregnancies occurring up to 3 months after the completion of the last treatment cycle must also be reported to the investigator. The investigator must report all pregnancies within 24 hours to the sponsor and drug provider within 24 hours. The investigator should counsel the patient; discuss the risks of continuing the pregnancy and the possible effects on the fetus. The patient should be monitored until the conclusion of the pregnancy.

Pregnancy occurring in the partner of a patient participating in the study should also be reported to the investigator, the sponsor and the drug provider. The partner should be counseled and followed as described above.

10.5 Adverse Drug Reactions with Concomitant Medication

The investigators must be aware that for all concomitant medications the regulations of post-marketing reporting for suspected adverse drug reactions apply, i.e. reporting to the marketing authorization holder or the local regulatory bodies.

10.6 Adverse Events Updates / IND Safety Reports

Horizon Pharma shall notify the Investigator via an IND Safety Report of the following information:

- Any AE associated with the use of study drug in other studies that is both serious and unexpected.
- Any finding from tests in laboratory animals that suggests a significant risk for human subjects including reports of mutagenicity, teratogenicity, or carcinogenicity.

The Investigator shall notify his/her IRB/EC promptly of these new serious and unexpected AE(s) or significant risks to subjects.

11. DATA MANAGEMENT

11.1 Data Collection

The Clinical Research Coordinators and Investigators of each site will be responsible for the recording of the site's data into the electronic data capture system, ONCORE.

11.2 Protocol Monitoring Committee

The Protocol monitoring committee (PMC) will be composed of medical and statistical independent reviewers and will meet to review the efficacy and safety data and determine a risk/benefit analysis in this subject population. The purpose of the PMC is to advise on serious safety considerations, lack of efficacy and any other considerations within the charge to the Committee. The PMC may request additional meetings or safety reports as deemed necessary upon discussion with the principal investigator. The PMC may stop the study following review of results from each interim analysis. The PMC meets once a month.

11.3 Study Monitoring and Auditing

11.3.1 Investigator Responsibilities

Investigator responsibilities are set out in the ICH guideline for Good Clinical Practice (GCP) and in the US Code of Federal Regulations.

Investigators, or a designated member, must enter study data onto CRFs or other data collection system. The Investigator will permit study-related monitoring visits and audits by Horizon Pharma or its representatives, IRB/EC review, and regulatory inspection(s) (e.g., FDA, EMEA, TPP), providing direct access to the facilities where the study took place, to source documents, to CRFs, and to all other study documents.

The Investigator, or a designated member of the Investigator's staff, must be available at some time during monitoring visits to review data and resolve any queries and to allow direct access to the subject's records (e.g., medical records, office charts, hospital charts, and study related charts) for source data verification. The data collection must be completed prior to each visit and be made available to the Horizon Pharma representative so that the accuracy and completeness may be checked.

11.3.2 Site Responsibilities

A conference call/study meeting will be held monthly to review patient enrollment and accrual, safety and toxicity data, and treatment results, as available.

11.3.3 Monitoring

Data will be captured in Oncore, Moffitt's Clinical Trials Database. Regulatory documents and case report forms will be monitored internally according to Moffitt Cancer Center Monitoring Policies. The monitoring will include source data verification, utilizing research subjects' medical records.

The Sponsor maintains a robust pharmacovigilance system comprising a governance framework and standard operating procedures supporting a systematic process for review, evaluation, and management of accumulating safety data from clinical trials and other sources to identify a potential new safety signal and ensure that an investigational product's risks are adequately assessed and communicated to investigators, IRBs/IECs, and regulatory bodies during clinical development.

For this study, safety monitoring activities will include but are not limited to review and evaluation of single serious adverse event (SAE) occurrences in real-time as reported through the SAE reporting process outlined in this section of the protocol, and review and evaluation, in real-time, of one or more occurrences of an uncommon SAE that is not commonly associated with product exposure.

Findings and/or safety data obtained during this study will provide information for the overall review of safety that is conducted by the Sponsor on a routine basis. The Sponsor will report expeditiously any findings from clinical trials (ongoing or completed), epidemiological studies, pooled analysis of multiple studies, and findings from animal or *in vitro* testing that suggest a significant risk in humans exposed to the study product.

Safety data collection for this study begins at the time of the subject's receiving the first dose of study medication. The investigator is responsible for the detection and documentation of events that meet the definition of an unanticipated problem (refer to protocol Section 10.1), AE or SAE.

11.4 Interim Analyses

Efficacy interim analysis (Phase II): The first analysis will take place after the first 23 patients are accrued and stopping criteria are outlined in the statistical section.

Safety interim analysis: The safety data will be continually assessed as patients are enrolled on the study. After the enrollment of the first 6 patients in stage 1 of the phase II study, if at any time more than 50% of the treated patients experience a G3-4 toxicity related to IFN-γ which leads to chemotherapy treatment delays > 14 days or unplanned cessation of neoadjuvant treatment then accrual to the trial will be held until discussions with the sponsor and protocol monitoring committee are arranged to determine if accrual can resume. Any deaths on study treatment should trigger an immediate safety review and accrual hold until the event is fully investigated and a determination is made by the study sponsor and protocol monitoring committee.

12. STATISTICAL CONSIDERATIONS

12.1 Sample Size Determination

Phase I (metastatic disease): There are 2 dose levels, with 3 to 6 patients treated at each dose level. Therefore, a minimum of 6 and maximum of 12 patients will be treated in the phase I portion. Adverse effects will be summarized using frequency tables. The proportion of patients who completed all 12 cycles of paclitaxel (at full dose or with dose modification) will be tabulated. Dose escalation, AEs, overall safety, and efficacy will be monitored by the Moffitt Cancer Center Data Safety Monitoring Committee.

Phase II (early stage breast cancer): The study will seek to determine whether there is an increase in complete pathologic response (pCR, defined by RCB score 0).

We will use a Simon's two stage design with 90% power and a type I error rate of 0.10. The choice of this power and type I error rate will ensure an appropriate stopping rule for stage 1 and to ensure adequate power after stage 2 to determine if the treatment is promising. Since the enrollment of patients involves both ER+/HER2+ and ER-/HER2+ patients, with the final proportion of ER+ and ER - status patients unknown until the completion of enrollment, we cannot determine ahead of time the pCR rates to test. **Table 4** presents the designs for the two extreme cases, the scenarios in which all the patients enrolled are either all ER+ or all ER- patients. In both extremes, the number of stage 1 and total number of patients are the same. Thus, we will enroll 23 patients into stage 1. After 23 patients have been enrolled, we will compute the proportion of ER+ and ER- subjects and compute the pCR under the null and alternative hypothesis based on the following formulas.

- Will assume baseline/standard of care and study drug rates of 50% ($p_{0.ER-}$) and 70% ($p_{1.ER-}$) for ER- patients.
- Will assume baseline/standard of care and study drug rates of 25% ($p_{0.ER+}$) and 45% ($p_{1.ER+}$) for ER+ patients.
- Let $N1_{ER+}$ and $N1_{ER-}$ represent the number of ER+ and ER- patients in the study at the conclusion of stage 1.
- Stage 1 baseline pCR rate will be computed as $pCR1_{baseline} = \{(N1_{ER+})(p_{0.ER+}) + (N1_{ER-})(p_{0.ER-})\} / \{N1_{ER+} + N1_{ER-}\}$.
- Stage 1 treatment pCR rate will be computed as $pCR1_{treatment} = \{(N1_{ER+})(p_{1.ER+}) + (N1_{ER-})(p_{1.ER-})\} / \{N1_{ER+} + N1_{ER-}\}$.
- Using $pCR1_{baseline}$ and $pCR1_{treatment}$ we will determine the needed number of pCRs to determine if the study should stop or proceed to Stage 2.

If the study proceeds to stage 2, following the enrollment of all patients, the final rates ($pCR_{baseline}$ and $pCR_{treatment}$) to be tested will be based on a weighted average as follows.

- Let N_{ER+} and N_{ER-} represent the final number of ER+ and ER- patients in the study (stage 1 + stage 2).
- Final baseline pCR rate will be computed as $pCR_{baseline} = \{(N_{ER+})(p_{0.ER+}) + (N_{ER-})(p_{0.ER-})\} / \{N_{ER+} + N_{ER-}\}$.
- Final treatment pCR rate will be computed as $pCR_{treatment} = \{(N_{ER+})(p_{1.ER+}) + (N_{ER-})(p_{1.ER-})\} / \{N_{ER+} + N_{ER-}\}$.
- Using $pCR_{baseline}$ and $pCR_{treatment}$ we will determine the needed number of pCRs to determine if the treatment is promising and warrants additional future studies.

Table 4 Simon's 2-stage designs for the two extreme cases (90% power, 10% type I error rate)

Design	Stage 1 N1	Stage 1 R1	Stage 2 N2	Total N	R	Ave N	PET	Constraint
25% vs 45% (if all were ER+)	23	5	16	39	13	31.5	0.468	Mimimax
50% vs 70% (if all were ER-)	23	11	16	39	23	31	0.500	Mimimax

N1 & N2 are the sample size in the first and second stage.
R1 is the drug rejection number in the first stage.
PET is the probability of early termination of the study.
N is the combined sample size of both stages.
R is the combined drug rejection number after both stages.
Ave N is the average sample size if this design is repeated many times.

Stop rules for the Simon's two-stage design will be completed using the current version of *PASS* software. The sample sizes the scenarios with all ER+ or all ER- patients, presented in Table 2, was computed using *PASS* version 15.0.1.

Adverse effects will be summarized using frequency tables. The estimated pCR along with exact confidence interval will be presented. The statistical analysis corresponding to the correlative study will be descriptive and exploratory in nature. Therefore, results will be summarized using descriptive statistics and associations will be evaluated using Fisher exact test.

12.2 Reporting and Exclusions

Evaluation of toxicity: All patients will be evaluable for toxicity from the time of their first treatment with protocol therapy.

Evaluation of clinical and pathologic response (Phase II): All patients included in the study must be assessed for clinical and pathologic response to treatment, even if there are major protocol treatment deviations or if they are ineligible. The definitions of clinical and pathologic responses are provided in Section 9. Each patient will be assigned one of the following clinical response categories: 1) complete response, 2) partial response, 3) stable disease, 4) progressive disease, 5) early death from malignant disease, 6) early death from toxicity, 7) early death because of other cause, or 9) unknown (not assessable, insufficient data). [Note: By arbitrary convention, category 9 usually designates the "unknown" status of any type of data in a clinical database.] A patient with unknown pathologic response due to refusal of surgery for non-medical reasons will be replaced with another patient.

All of the patients who met the eligibility criteria should be included in the main analysis of the response rate. Patients in response categories 4 to 9 should be considered as failing to respond to treatment (disease progression). Thus, an incorrect treatment schedule or drug administration does not result in exclusion from the analysis of the response rate. Precise definitions for categories 4 to 9 will be protocol-specific.

All conclusions should be based on all eligible patients. Subanalyses may then be performed on the basis of a subset of patients, excluding those for whom major protocol deviations have been identified (e.g., early death due to other reasons, early discontinuation of treatment, major protocol violations). However, these subanalyses may not serve as the basis for drawing conclusions concerning treatment efficacy, and the reasons for excluding patients from the analysis should be clearly reported. The 95% confidence intervals should also be provided.

13. REFERENCES

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APPENDIX A - Page 1 of 2
Correlative Studies: Paraffin Tissue

Formalin fixed, paraffin-embedded specimens must be forwarded to Dr. Brian Czerniecki at the Moffitt Cancer Center:

Patient study ID #: _____

_____

Date of shipment of specimens: _____

Name of study coordinator sending specimens: _____

Contact information of study coordinator:

Telephone: _____

Fax : _____

Email: _____

Specimen	Date	Pathology Specimen No.
Pretreatment diagnostic biopsy specimen(s).		
Mastectomy or lumpectomy specimens. Representative sections should be selected as outlined on next page		

If specimen not available, please indicate N.A. in table above.

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APPENDIX A - Page 2 of 2
Pathologic Response Form – Submission of Pathologic Materials for Review

All patients with early stage breast cancer treated per phase II study who undergo mastectomy/lumpectomy will be reviewed by a single pathologist. Procedures are indicated below. Indicate identification numbers in tables below. Attach copy of pathology report with identifiers removed and study sequence number indicated on report.

Patient sequence number: _____ Pathology specimen number: _____
Dates of specimen: _____

Mastectomy/lumpectomy with residual primary tumor (including those with DCIS) with/without positive nodes:

	Date	**Block identification number(s)**
At least one representative tumor block with the least necrosis		
At least one representative block of a positive lymph node		
One representative block of histologically normal breast		

Mastectomy/lumpectomy with no residual primary tumor with negative nodes:

	Date	**Block identification number(s)**
At least one representative block from the tumor area		
One representative block of histologically normal breast		

Mastectomy/lumpectomy with no residual primary tumor but with positive nodes:

	Date	**Block identification number(s)**
At least one representative block from the tumor area		
At least one representative block of a positive lymph node		
One representative block of histologically normal breast		