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Inhibiting fatty acid synthase to improve efficacy of neoadjuvant chemotherapy

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Inhibiting fatty acid synthase to improve efficacy of neoadjuvant chemotherapy.

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I confirm I have read this protocol, I understand it, and I will work according to this protocol and to the ethical principles stated in the latest version of the Declaration of Helsinki, the applicable guidelines for good clinical practices, or the applicable laws and regulations of the country of the study site for which I am responsible, whichever provides the greater protection of the individual. I will accept the monitor's overseeing of the study. I will promptly submit the protocol to applicable ethical review board(s).

Instructions to the investigator: Please **SIGN** and **DATE** this signature page. **PRINT** your name and title, the name and location of the facility in which the study will be conducted, and the expected IRB approval date. Scan and email the completed form to Indiana University Simon Cancer Center and keep a record for your files.

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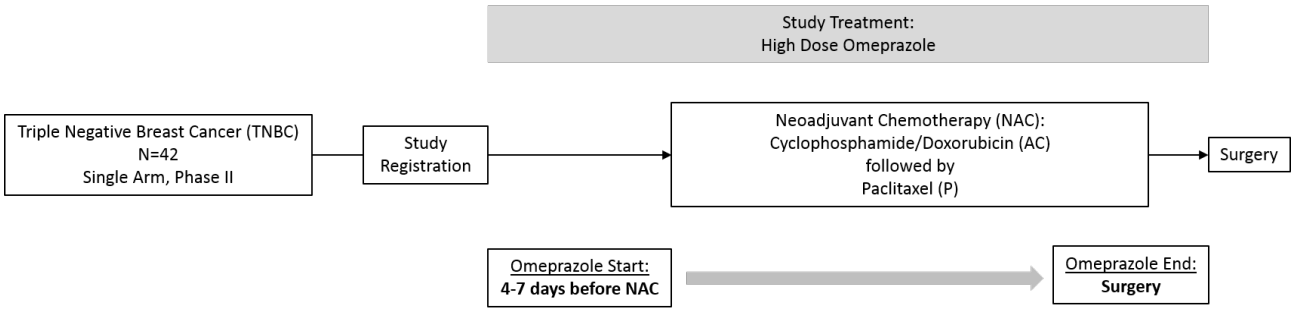
1. SCHEMA



2. BACKGROUND & RATIONALE

2.1 Overview

Human fatty acid synthase (FASN) is the sole cytosolic enzyme responsible for *de novo* synthesis of palmitate (1-3). It has seven catalytic domains (β-ketoacyl synthase, malonyl/acetyltransferase, dehydrogenase, enoyl reductase, β-ketoacyl reductase, acyl carrier protein, and thioesterase) and synthesizes palmitate by condensing acetyl-CoA and malonyl-CoA (1). Breast cancers with high levels of FASN are much more likely to recur and metastasize with significantly shorter disease free and overall survival (2-5). FASN induces resistance to multiple anticancer drugs including doxorubicin and cisplatin (6-8). The typical Western diet contains sufficient free fatty acids so that FASN is not required for normal cell function and, thus, its expression is very low in normal cells except lactating breast, cycling endometrium, and fatty tissues. In contrast, breast cancer cells require *de novo* fatty acid synthesis for survival. Inhibition of FASN induces apoptosis selectively in cancer cells both *in vitro* and *in vivo* (9-12) with minimal effect on normal cells (6, 13, 14). Thus, FASN drives breast cancer survival and is an ideal therapeutic target. In this application, we will target FASN to stop breast cancer growth.

Having identified FASN as an important therapeutic target, we began searching for an inhibitor. In preliminary studies, we found that the proton pump inhibitors (PPIs) effectively inhibit FASN, reduce breast cancer cell survival, and restore sensitivity to chemotherapy (15). PPIs are FDA-approved for treatment of a variety of acid-related diseases that plague the digestive system, are widely used, and are well tolerated without major adverse effects (16-18). Interestingly, a recent retrospective study in head and neck cancer patients reported an increased overall survival associated with PPI use (19). Chinese investigators reported intriguing results of a phase II trial of intermittent high dose esomeprazole in combination with docetaxel and cisplatin in patients with metastatic breast cancer (NCT01069081, 20). Median PFS was 7.5 months in the control group compared to 9.5 months in PPI treated patients (p=0.030); impact as greatest in the subset of patients with triple negative disease

(n=17, 3.3 vs. 9.5 months, p=0.014). Though small, the results of this trial are consistent with our hypothesis and support further exploration. Thus, we hypothesize that the PPIs may be repositioned as safe and effective drugs to augment the effect of chemotherapy without adding significantly to the cost or toxicity of therapy.

2.2 The Role of FASN Function in Drug Resistance

FASN up-regulation in doxorubicin-resistant breast cancer cells. While comparing protein profiles between the parental MCF7 and its doxorubicin-selected and resistant derivative MCF7/AdVp3000 (M3K) cells, we found a 270-kDa protein with increased expression in the drug resistant M3K cells (Fig. 1A). This protein was identified to be human FASN by MALDI-TOF mass spectrometry. Functional analysis showed that FASN activity was dramatically (5 fold) up-regulated in M3K cells (Fig. 1B). FASN up-regulation in M3K cells was validated using Western blot analysis (Fig. 1C). During the stepwise selection of M3K cells, cell lines (e.g., M10 and M100) with low and intermediate doxorubicin resistance levels were also generated (21-23). Interestingly, these cells also showed increased FASN level but to a less extent than M3K cells (Fig. 1C).

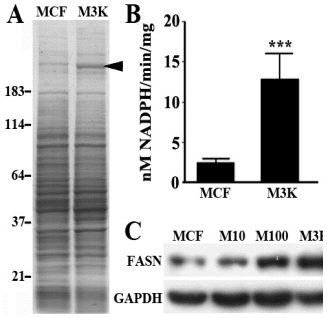


Figure 1. Over-expression of FASN in drug resistant M3K cells. **A,** SDS-PAGE profile of cell lysates from parental MCF7 (MCF) and its drug resistant derivative MCF7/AdVp3000 (M3K) cells. The arrowhead indicates the protein, which was identified as FASN. **B,** FASN activity in MCF7 and M3K cells as determined using coupled enzyme assay. **C,** Western blot analysis of FASN expression in MCF7 (MCF) and drug resistant derivative cell lines, MCF7/AdVp10 (M10), MCF7/AdVp100 (M100), and MCF7/AdVp3000 (M3K) cells, which were generated by stepwise selection of MCF7 in the presence of 10 ng/ml, 100 ng/ml, and 3000 ng/ml Adriamycin (doxorubicin), respectively.

FASN over-expression causes resistance to multiple DNA-damaging agents. To investigate whether FASN up-regulation contributes to drug resistance in M3K cells, we first performed stable FASN knockdown using shRNA and tested cellular response to doxorubicin and mitoxantrone. As shown in Fig. 2A-B, FASN knockdown significantly reduced resistance of M3K cells to both doxorubicin and mitoxantrone. We next performed the reverse experiment to over-express ectopic FASN in the parental MCF7 cells. A stable MCF7 clone with FASN over-expression (FASN) was generated, and as predicted, it was significantly more resistant to doxorubicin and mitoxantrone than the control Vec clone (Fig. 2C-D). The FASN-mediated doxorubicin resistance

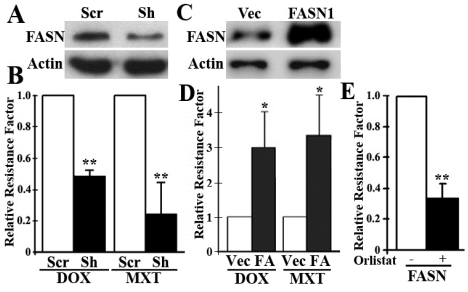


Figure 2. Effect of FASN over-expression on drug resistance. A & C, Western blot analysis of FASN in M3K cells stably transfected with FASN shRNA (Sh) or scrambled control shRNA (Scr) (**A**) and in MCF7 cells with stable FASN overexpression (FASN) or vector-transfected control (Vec) cells (**C**). **B & D,** Relative drug resistance of M3K cells with FASN knockdown (**B**) and MCF7 cells with FASN over-expression (**D**) as determined using SRB assay. **E,** Orlistat sensitization of FASN-mediated doxorubicin resistance. Dose response of SRB assay was analyzed by GraphPad Prism to generate IC50. Relative resistance factor=IC50(FASN or Sh)/IC50(Vec or Scr). DOX=doxorubicin; MXT=mitoxantrone. (* p<0.05; **p<0.01).

was also dramatically reduced using the known FASN inhibitor, orlistat (Fig. 2E). FASN over-expression also significantly increased resistance to etopside, cisplatin, and ionizing radiation (IR) but had no effect on cellular response to paclitaxel and vinblastine [data not shown but see (7)]. Thus, FASN up-regulation likely causes resistance to DNA-damaging therapy but not anti-mitotic drugs. To ensure that the impact of FASN was not unique to MCF-7 cells, we performed FASN knockdown and survival analysis of another breast cancer cell line, MDA-MB-468. Fig. 3A shows effective knockdown of FASN and Fig. 3B shows that FASN knockdown is accompanied by significant reduction in cellular resistance to both doxorubicin and mitoxantrone. Similar results were observed with MDA-MB-231 cells (data not shown).

FASN silencing does not increase chemo-sensitivity in non-malignant cells. We had hypothesized that non-malignant cells would be less dependent on FASN expression, and thus would not be affected by FASN inhibition. To test this hypothesis we performed a similar knockdown experiment using human mammary epithelia cell line MCF10A1. Fig. 3A shows effective FASN knockdown in MCF10A1 cells. However, FASN knockdown had no significant effect on cellular response to doxorubicin or mitoxantrone in MCF10A1 cells (Fig. 3C).

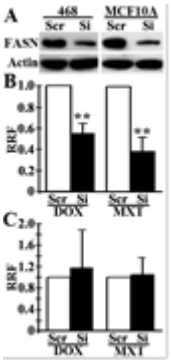


Figure 3. Effect of FASN knockdown on drug response of MDA-MB-468 and MCF10A1 cells. **A**, Western blot analysis of FASN expression in MDA-MB-468 (468) and MCF10A1 cells following transient transfection with FASN (Si) or scrambled (Scr) siRNAs. **B & C,** Survival analysis of MDA-MB-468 (**B**) and MCF10A1 (**C**) cells following transfection with FASN siRNA (Si) or control scrambled (Scr) siRNAs in the absence or presence of doxorubicin (DOX) or mitoxantrone (MXT). RRF=relative resistance factor=IC50(Si)/IC50(Scr). (**p<0.01).

2.3 Targeting FASN to Reverse Chemotherapy Resistance

Identification of PPIs as FASN TE inhibitors. To identify FDA-approved drugs that target FASN TE, we first performed *in-silico* screening of a library of 2,417 FDA-approved drugs using DOCK programs and the crystal structure of FASN TE (ID: 3TJM) (24). The 200 top-scoring compounds were clustered based on their chemical structure and 25 representative drugs from different clusters were tested using the 4-MUH fluorogenic assay. We found that one of these drugs, pantoprazole, dose-dependently inhibited FASN TE activity (Fig. 4A) with a Ki of 4.1 μM (Table 1). Interestingly, the remaining drugs in the cluster containing pantoprazole were other PPIs including omeprazole, lansoprazole and rabeprazole. As shown in Fig. 4A and Table 1, all these PPIs similarly inhibited TE in a dose-dependent manner with Ki values of 3.4-5.9 μM and an activity ranking of omeprazole>pantoprazole>lansoprazole>rabeprazole. Hence, PPIs are effective FASN TE inhibitors. Analysis of the structure and activity of PPIs suggests that the size of either 2- pyridylmethyl or the benzimidazole moiety in PPIs may affect the ability of PPIs to inhibit FASN TE.

Figure 4. PPIs inhibit FASN TE activity. A, Dose dependent inhibition of TE activity by PPIs. Each plot represents the average of three independent experiments. **B,** Average simulated structures of PPIs bound to TE. TE is shown in gold ribbon. Omeprazole, pantoprazole, lansoprazole, and rabeprazole are shown as ball and stick in green, blue, pink, and orange, respectively. In each panel, the catalytic triad residues and the residues predicted to interact with each PPI are

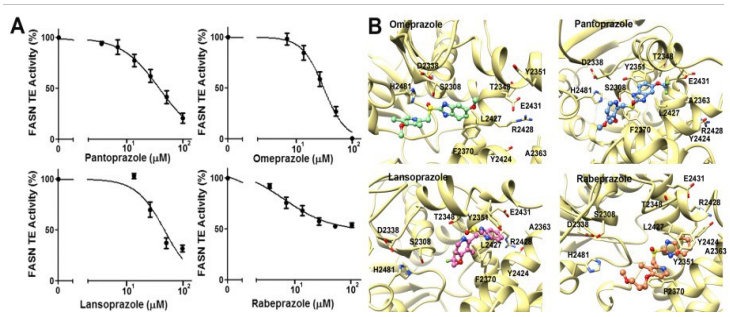


Table 1. Structure, Ki and ΔGBind of PPIs

PPIs	Structure	K_i (μM)	ΔG_{Bind} (kcal/mol)
Omeprazole		3.4±0.9	-26.0±3.3
Pantoprazole		4.1±0.8	-21.6±4.5
Lansoprazole		5.3±1.3	-24.3±5.5
Rabeprazole		5.9±2.0	-20.4±4.2

We also performed molecular dynamics (MD) simulations of each PPI docked in the active site of TE and calculated the binding free energy (ΔGbind) using Poisson Boltzman surface area (PBSA) analyses. Table 1 shows that the ΔGbind is favorable and that omeprazole has the highest while rabeprazole has the lowest ΔGbind, similar to the ranking of their experimental Ki's. Fig. 4B shows the simulated average structure of each PPI in FASN TE. Omeprazole, with the most favorable ΔGbind and Ki, shows potential formation of a strong hydrogen bond between serine residue (Ser2308) of the catalytic triad of TE and the sulfoxide moiety of omeprazole, which may prevent Ser2308 from nucleophilically attacking a substrate with an ester moiety. The hydrophobic benzamidazole moiety of PPIs may interact with residues of the "specificity channel," which is predicted to accommodate the growing carbon chain during fatty acid synthesis (30), and may block access of the carbon chain to the channel. These findings provide theoretical support for PPI inhibition of TE.

PPIs inhibit and bind to cellular FASN. To determine if PPIs inhibit cellular FASN, we performed FASN activity assay in the absence or presence of PPIs using MCF7 cell lysate as we previously described (6, 7). Fig. 5A shows that 10 μM PPIs reduce >75% while 50 μM reduces >95% FASN activity. To investigate if PPI inhibits cellular FASN by binding to FASN, we performed a probe binding displacement experiment using the ActivX Desthiobiotin-fluorophosphonate (FP) serine hydrolase probe, which covalently binds to the Ser residue in the catalytic triad of serine hydrolases such as FASN TE (25). For this experiment, we tested lansoprazole. Briefly, cell lysate was incubated with the FP probe in the absence or presence of different concentrations of lansoprazole and subjected to Western blot analysis probed with streptavidin conjugated HRP. As shown in Fig. 5B, lansoprazole inhibited FP probe labeling of FASN in a dose-dependent manner. However, it had no effect on the binding of the FP probe to other Ser hydrolases, suggesting that lansoprazole selectively inhibits FASN TE. Lansoprazole also had no effect on the total FASN level (Fig. 5C).

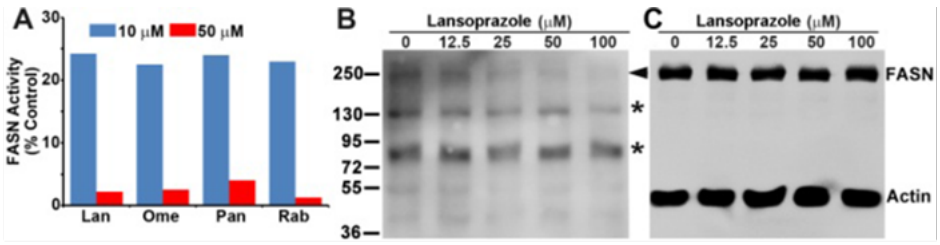


Figure 5. Dose-dependent inhibition of cellular FANS by PPIs. A, FASN activity. Lysate from MCF7 cells were used for coupled FASN activity assay in the absence or presence of 10 or 50 μM lansoprazole (Lan), omeprazole (Ome), pantoprazole (Pan), or rabeprazole (Rab). Data shown were average of duplicated experiments. **B,** FP serine hydrolase probe labeling (**B**) and expression (**C**) of FASN in cell lysate by lasoprazole. Arrow head indicates FP probe-labeled FASN. Asterisks indicate FP probe labeled other serine hydrolases. Actin was used as a loading control for FASN.

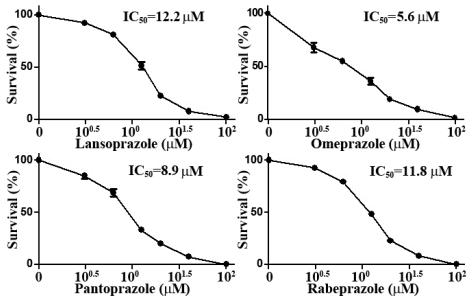
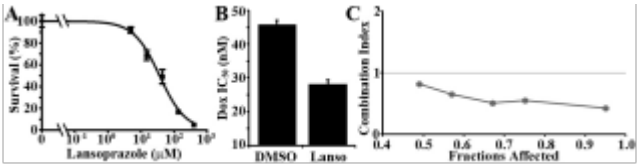


Figure 6. Effect of PPIs on survival of breast cancer MDA-MB-231 cells as determined by colony formation assay.

PPIs inhibit breast cancer cell proliferation and sensitize doxorubicin resistance. To determine if PPIs have any anti-breast cancer activity, we performed a colony formation assay using the TNBC cell line MDA-MB-231 in the absence or presence of PPIs. All PPIs effectively inhibited proliferation of MDA-MB-231 cells with IC50 of 5.6-12.2 μM (Fig. 6). Omeprazole appears to be the most effective with an IC50 of 5.6 μM, consistent with the observation that it is most effective in inhibiting FASN TE activity (Table 1). Importantly the IC50 is near the plasma concentration (Cmax=4.2 μM and AUC=9.7 μM·h) achieved in patients (40 mg dose). Furthermore, PPI treatments induced apoptosis in a dose dependent manner (data not shown). Next, we tested the drug-resistant M3K cells using lansoprazole, which has the highest IC50 at 12.2 μM for MDA-MB-231 cells with the idea that if lansoprazole is effective, the other PPIs may be better. We found that M3K cells are more resistant to lansoprazole with a higher IC50 of ~37 μM (Fig. 7A) than its IC50 of 12.2 μM in MDA-MB-231 cells, presumably due to high FASN level in M3K cells. However, lansoprazole at 5 μM, which causes <10% cell death

(Fig. 7A), was able to reduce the doxorubicin IC50 from 46 to 28 nM in M3K cells (Fig. 7B). Further study of lansoprazole-doxorubicin combination using the combination assay as described previously (26) showed that lansoprazole and doxorubicin have a synergistic effect on M3K cells with combination index around 0.3-0.7 (Fig. 7C). These findings suggest that PPIs not only have independent anticancer activity but also sensitize resistant breast cancer cells to doxorubicin.

Figure 7. Effect of PPI on breast cancer cell drug resistance. A, Lansoprazole IC50 in drug resistant M3K cells by MTT assay. **B,** Effect of 5 μM lansoprazole on doxorubicin IC50 in M3K cells by MTT assay. **C,** Combination index of lansoprazole and doxorubicin in M3K cells.



2.4 Pharmaco-epidemiologic Support for Use of PPIs

Many breast cancer patients use PPIs for management of concurrent gastrointestinal reflux or ulcer disease. Thus, we performed a preliminary retrospective analysis of de-identified electronic medical records (EMR) of 6,323 breast cancer patients from Indiana Network for Patient Care (INPC). The average age at diagnosis was 58.9 years (range 19-102) and average age at death or last visit is 61.5 (range 21-104). Of this cohort, the EMR documented PPI use after diagnosis in 534 patients. There are 488 triple negative breast cancer (TNBC) patients, among them 35 used PPIs. Using the Cox proportional hazard regression model, overall survival was analyzed against PPI usage. Breast cancer patients who took PPIs had significantly increased overall survival than patients who did not (Fig. 8A). Analysis of the TNBC subgroup showed that PPI usage also had significant but perhaps more protective effect (Fig. 8B). The protective effect of PPIs on patient survival was adjusted for age, tumor stage, and time of first diagnosis.

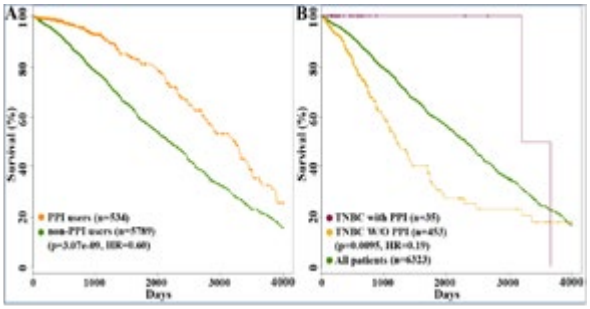


Figure 8. Retrospective survival analysis of breast cancer patients with or without use of PPIs. De-identified EMR of 6,323 breast cancer patients were retrieved from the database of INPC and subjected to Kaplan-Meier survival analysis in association with PPI usage in all patients (**A**) or in TNBC subgroup (**B**). The p-value and hazard ratio (HR) were calculated from Cox proportional hazard models, which were adjusted for age, tumor stage, and time of first diagnosis.

2.5 Neoadjuvant Therapy in Breast Cancer

Neoadjuvant chemotherapy has a well-established role in the management of both early-stage and locally advanced breast cancer. Providing treatment prior to definitive surgery not only improves the ability to achieve breast conservation, but also allows determination of *in vivo* sensitivity to therapy and offers an ideal platform for clinical research. Although many will experience shrinkage in tumor volume with neoadjuvant therapy, at the time of surgery only about ~35% of patients with triple negative disease will experience a pathologic complete response (pCR), indicating the absence of invasive tumor tissue in the surgical specimen. Long-term follow-up of neoadjuvant studies consistently demonstrates significantly improved survival in individuals with pCR, with comparatively inferior outcomes in those with residual disease at surgery (27-29).

We propose a single arm phase II trial of omeprazole + standard neoadjuvant chemotherapy as an obligate first step toward the definitive trial we envision. This study will provide safety, pharmacokinetic, biologic, and preliminary efficacy data that is needed for marshal support of an adequately designed, definitive randomized trial.

3. OBJECTIVES

3.1 Primary Objective

- Estimate the rate of pCR in patients with triple negative breast cancer and FASN expression treated with standard neoadjuvant chemotherapy in combination with high dose omeprazole.

3.2 Secondary Objectives

- Quantify the number of patients with newly diagnosed TNBC with tumors that express FASN.
- Estimate the rate of pCR in patients with triple negative breast cancer (irrespective of FASN status) treated with standard neoadjuvant chemotherapy in combination with high dose omeprazole.
- Describe the safety of incorporating high dose omeprazole with standard neoadjuvant chemotherapy.
- Estimate the biologic activity of high dose omeprazole in modulating FASN expression and activity.

3.3 Tertiary/ Exploratory/ Correlative Objectives

- Describe omeprazole exposure
- Compare peak omeprazole concentration to level associated activity based on preclinical testing (~5.6 μM based on IC_{50})
- Using PK-PD models, use omeprazole concentration and change in lipid levels to predict clinical outcome
- Explore changes in lipid profile over time

3.4 Primary Outcome Measure

- pCR is defined as no invasive disease in the breast or axilla at the time of definitive surgery

3.5 Secondary Outcome Measures

- FASN expression at baseline
- Toxicity based on NCI CTC v 4.0
- Change in FASN expression from baseline to after omeprazole monotherapy (Day 4-7 biopsy) and after all therapy (surgery sample).
- Change in FASN activity from baseline to after omeprazole monotherapy (Day 4-7 biopsy) and after all therapy (surgery sample).
- Change in FASN downstream target gene expression from baseline to after omeprazole monotherapy (Day 4-7 biopsy) and after all therapy (surgery sample).

3.6 Tertiary/ Exploratory/ Correlative Outcome Measures

- Population PK analyses based on a limited sampling strategy
- Lipid profile including total cholesterol, triglycerides, high-density lipoprotein, low-density lipoprotein, and non-esterified free fatty acids (NEFA) at baseline, after omeprazole monotherapy (NEFA only), the mid-point of chemotherapy and prior to surgery.

4. ELIGIBILITY CRITERIA

4.1 Inclusion Criteria

1. Newly diagnosed triple negative breast cancer (TNBC) clinical stage I (must be T1c), II, or III
   - ER and PR < 10%
   - HER2 negative based on one of the following:
     - IHC 0 or 1+
     - IHC 2+ and FISH negative
     - IHC 2+ and FISH equivocal and no indication for HER2 targeted therapy based on the treating investigators discretion (i.e., HER2: CEP17 ratio < 2.0 or HER2 total copy number <6)
2. Planned neoadjuvant treatment with anthracycline and taxane containing chemotherapy
3. ≥ 18 years old at the time of informed consent
4. ECOG Performance Status 0-1
5. Ability to provide written informed consent and HIPAA authorization
6. Women of childbearing potential definition must have a negative pregnancy test within 14 days of registration. All women (regardless of sexual orientation, having undergone a tubal ligation, or remaining celibate by choice) are considered to have childbearing potential unless they meet one of the following criteria:
   - Prior hysterectomy or bilateral oophorectomy;
   - Has not had menses at any time in the preceding 24 consecutive months
7. Adequate organ function for anthracycline and taxane based therapy

- LVEF > LLN based on cardiac ECHO or MUGA
- Hgb $\geq$ 8.5
- ANC > 1,000
- Platelets $\geq$ 100,000
- Creatinine $\leq$ 1.5
- T. bili $\leq$ 1.3
- AST $\leq$ 2.5 x ULN

4.2 Exclusion Criteria

1. Use of prescription PPIs within 12 months prior to study entry
   - Dexlansoprazole (Dexilant)
   - Pantoprazole (Protonix)
   - Rabeprazole (Aciphex)
   - Esomeprazole (Nexium)
   - Lansoprazole (Prevacid)
   - Omeprazole (Prilosec, Zegerid)
2. Use of OTC PPIs within 6 months prior to study entry
   - Esomeprazole (Nexium)
   - Lansoprazole (Prevacid)
   - Omeprazole (Prilosec, Zegerid)
3. Use of Orlistat or any other known FASN inhibitor within 6 months prior to study entry
4. Nursing mothers are excluded.
5. Known hypersensitivity to any component of the formulation or substituted benzimidazoles.
6. Prior osteoporotic fracture

5. STUDY DESIGN

Single arm Phase II

6. PATIENT REGISTRATION

All patients will be registered with the Indiana University Cancer Center Clinical TrialsOffice, Registration must occur prior to the initiation of therapy.

For participating sites to register a patient, the following documents should be completed by the study team and sent to the Coordinating Center Multicenter Network Administrator, (MNA), or designee.

- Signed patient consent form
- Completed and signed eligibility checklist
- Copies of source documentation verifying eligibility

The Coordinating Center will then review the documents to confirm eligibility. To complete the registration process, the MNA, or designee, will:

- Assign a patient study number
- E-mail the patient study number to the participating site
- The participating site will register the patient into OnCore

Regulatory files will be maintained by the Clinical Trials Office. Applicable regulatory documents must be completed and on file prior to registration of any patients. Potential patients will be identified in the Oncology outpatient clinics or by referrals from outside physicians. Patients who appear to be eligible for this trial will undergo the Informed Consent Process and be screened for eligibility utilizing the Eligibility Criteria. The original signed IRB approved Informed Consent Document and completed eligibility checklist will be forwarded to the Clinical Trials Office designee for eligibility verification and registration in the OnCore® database. Notification will be sent to the principal investigator, treating physician and research nurse when registration is complete to confirm registration and inform them of patient ID number.

Study data will be collected and stored in OnCore®, developed by Forte Research Systems, Inc. (www.forteresearch.com). OnCore® Enterprise Research is a comprehensive, web-based, Clinical Trial Management System (CTMS) which utilizes an Oracle database. It has been licensed by Indiana University (IU) to support the operations and data capture of clinical research trials.

The system has been installed and configured within a HIPAA-aligned, Information Technology (IT), operations center supported by IU's IT organization, University Information Technology Services (UITS).

OnCore® provides users secure access with unique IDs/passwords and restricts access by assigned roles, from any location, to record, manage, and report on data associated with the operation and conduct of clinical trials. The system is comprised of three specific applications—Clinical Research Management (CRM), Biospecimen Management (BSM), and Unified Registries Management (URM). Indiana University leverages OnCore® to support clinical research operations specifically as it relates to the following functions/processes: electronic Scientific Review Committee (SRC), regulatory management, protocol and subject life cycle management, coverage analysis, study financials management, subject registration and visit management, subject safety monitoring, protocol deviation monitoring, study auditing and monitoring, electronic data management, correlative study sample management, specimen banking and management, registries management, effort tracking, and reporting.

7. TREATMENT PLAN

7.1 Overview

Patients should begin therapy within 7 working days of study entry. Patients will be treated with omeprazole 80 mg orally BID beginning 4-7 days prior to chemotherapy and continuing until surgery. After the brief period of omeprazole monotherapy, patients will

begin standard neoadjuvant chemotherapy with doxorubicin (60 mg/m^2) and cyclophosphamide (600 mg/m^2) for 4 cycles followed by paclitaxel (80 mg/m^2) weekly x 12.

Doxorubicin and cyclophosphamide (AC) may be administered on a classical every 3 week or dose dense every 2 week (with growth factor support) schedule at the treating physician's discretion. Routine incorporation of carboplatin is not recommended, however use of carboplatin (AUC 6 on week 1, 4, 7, and 10) with paclitaxel is allowed at the treating investigator's discretion. Chemotherapy will be adjusted based on toxicity according to standard treatment guidelines. Additional details regarding neoadjuvant chemotherapy can be found in Sections 7.3 and 7.4. Information contained in these sections is intended to be used as a guideline for administering standard of care neoadjuvant chemotherapy, and modifications to these dosing guidelines can be made based upon institutional standard practice or physician discretion. These modifications will not be recorded as deviations for this protocol.

Patients with overt disease progression during AC should move immediately to paclitaxel therapy. Patients with disease progression during paclitaxel should proceed immediately to surgery. Additional details regarding disease progression can be found in Section 7.5.

Doses should be based upon actual body weight. If subject's weight changes by ≥ 10% during the course of the study, the body surface area and drug dose should be recalculated.

7.2 Omeprazole

Omeprazole 80 mg orally BID beginning 4-7 days prior to chemotherapy and continuing throughout chemotherapy until surgery. Omeprazole should be discontinued the night prior to surgery.

NOTE: Omeprazole inhibits CYP2C19. Patients taking drugs known to be substrates of CYP2C19 (Appendix IV) may enroll but the treating investigator should be aware of the potential drug interaction. When possible, changing to an alternative agent that is not metabolized by CYP2C19 is advised.

7.3 Doxorubicin and Cyclophosphamide (AC)†

Agent	Time	Dose	Route	Day
Fosaprepitant	30 minutes prior to AC	150 mg	IV infusion in 145 mL NS over 20-30 minutes	1
Doxorubicin	NA	60 mg/m^2	IV push through running IV of NS	1
Cyclophosphamide	NA	600 mg/m^2	IV infusion in 150 mL NS over 20-30 minutes	1
Pegfilgrastim* OR	NA	6 mg (regardless of BSA)	SQ	2
Filgrastim*	NA	5 μg/kg**		2-11

* Pegfilgrastim or filgrastim are recommended after each AC treatment (cycle 1-4) for patients receiving dose dense AC. Otherwise, hematologic growth factors are not recommended but may be used in accordance with the ASCO guidelines.
** Rounded to the nearer of 300 or 480 μg.
† Information contained in this table is intended to be used as a guideline for administering standard of care neoadjuvant AC chemotherapy, and modifications to this table can be made based upon institutional guidelines or physician discretion. These modifications will not be recorded as deviations for this protocol.

AC may be administered every 21 days (classical schedule) **OR** every 14 days with growth factor support (dose dense schedule). Choice of AC schedule is at the treating investigator's discretion.

7.4 Paclitaxel (T)†

Agent	Time	Dose	Route	Day
Dexamethasone*	30-60 minutes prior to paclitaxel	12 mg	IV or po	Every 7 days
Diphenhydramine*		50 mg	IV or po	
Cimetadine**		300 mg	IV	
Paclitaxel	NA	80 mg/m^2	IV infusion in 250 mL NS or D_5W over 1 hour	Every 7 days
Carboplatin***	NA	AUC 6	IV infusion	Every 21 days (weeks 1, 4,7, and 10 with paclitaxel)

* The doses of diphenhydramine and dexamethasone may be adjusted after the first dose, based on tolerability.
** Ranitidine (50 mg IV) or famotidine (20 mg IV) may be substituted for cimetidine; however, famotidine is preferred.
*** Incorporation of carboplatin is not recommended, however the use of carboplatin with paclitaxel is allowed at the treating investigator's discretion. Magnesium wasting may be more common in patients receiving concurrent carboplatin. More frequent monitoring of magnesium should be considered in those patients.
† Information contained in this table is intended to be used as a guideline for administering standard of care neoadjuvant paclitaxel chemotherapy, and modifications to this table can be made based upon institutional guidelines or physician discretion. These modifications will not be recorded as deviations for this protocol.

7.5 Management of Disease Progression

Frank disease progression during neoadjuvant chemotherapy is unusual. In the event of disease progression, the treatment plan should be modified as follows:

- Locally progressive disease at any point during AC - defer remaining cycles of AC and begin treatment with paclitaxel (+ carboplatin if desired) according to the original treatment plan.
- Locally progressive disease at any point during paclitaxel - defer remaining cycles of paclitaxel, perform required completion of chemotherapy evaluation and refer for definitive surgery.
- Systemic disease progression - any patient who develops evidence of metastatic disease will be removed from study.

7.6 Breast Surgery and Management of the Axilla

At the completion of neoadjuvant chemotherapy, patients will undergo definitive surgical resection. Decisions regarding the type of surgery (breast conserving surgery, unilateral or bilateral mastectomy), management of the axillary lymph nodes, and incorporation of reconstruction will be according to the patient's preference and treating surgeon's recommendation. Similarly, the use of post-surgery radiation therapy and additional adjuvant systemic therapy are at the treating investigator's discretion and are not specified by this protocol.

When appropriate and desired, patients may enroll in other studies addressing important local therapy and post-neoadjuvant systemic therapy questions.

8. TOXICITIES TO BE MONITORED/DOSAGE MODIFICATIONS

All toxicities should be graded according to the Common Terminology Criteria for Adverse Events (version 4.0).

If a patient develops multiple toxicities, delay treatment or modify dose based on the greatest indicated dose reduction or delay. Dose re-escalations are not allowed.

8.1 Omeprazole

No dose modification of omeprazole is planned during this study. If patients are unable to take omeprazole for more than 14 consecutive days, they should come off the study.

Omeprazole should be discontinued for any of the following:

- Acute interstitial nephritis
- Osteoporosis-related fractures of the hip, wrist, or spine
- Vitamin B-12 deficiency
- *Clostridium difficile* associated diarrhea
- Grade 3 hypomagnesemia
- Any other grade 3 or greater toxicity thought to be related to omeprazole

8.2 AC†

Event	Dose Modification
Neutropenia (on Day 1 of any cycle)	
> 1000/mm^3	No change
≤ 1000/mm^3	Hold until ANC >1000, resume based on timing of recovery: ≤ 1 week – no change or add growth factor support (if not already used) >1 but ≤ 3 weeks - reduce dose 20% for subsequent cycles or add growth factor support (if not already used) > 3 weeks – stop AC, proceed to paclitaxel
Neutropenic Fever (at any point)	
ANC≤ 1000, fever ≥ 38.5	Interrupt until resolved (ANC >1000, fever <38.5), resume according to number of episodes: 1st = no change or add growth factor support (if not already used) 2nd = 20% dose reduction or add growth factor support (if not already used) 3rd = stop AC, proceed with paclitaxel
Thrombocytopenia (on Day 1 of any cycle)	
≥100,000/mm^3	No change
75-99,999/mm^3	Hold until ≥ 100,000, resume based on timing of recovery: ≤ 1 week – no change` >1 but ≤ 3 weeks - reduce dose 20% for subsequent cycles > 3 weeks – stop AC, proceed to paclitaxel
<75,000	Hold until ≥100,000, Resume with 20% dose reduction for subsequent cycles. If > 3 weeks delay is required, stop AC and proceed with paclitaxel.
Anemia	
All grades	No change – erythropoietin or darbepoietin therapy may be initiated at the investigator's discretion. Transfusion support is allowed
Hepatic (on Day 1 of any cycle)	
Grade 0 or 1	No change
≥ Grade 2	Interrupt until ≤ Grade 1, then resume previous dose. If > 3 weeks delay is required, stop AC and proceed with paclitaxel.
Nausea/Vomiting (at any point)	
Grade 0 - 2	No change
≥ Grade 3	Hold until resolved to ≤ Grade 1, reduce dose 20% in subsequent cycles.
Mucositis (at any point)	
Grade 0 - 2	No change
≥ Grade 3	Hold until resolved to ≤ Grade 1, reduce dose 20% in subsequent cycles.

Event	Dose Modification
Cardiac (at any point)	
Grade 0 - 2	No change
≥ Grade 3	Discontinue AC if: ○ a patient has symptoms of CHF and a diagnosis of CHF is confirmed ○ a patient has a myocardial infarction ○ >15% absolute decline in LVEF from baseline, or >10% decline in LVEF from baseline to below LLN Patients who discontinue AC due to cardiac toxicity may receive paclitaxel at the investigator's discretion
Other clinically significant toxicity excluding fatigue, alopecia and leukopenia	
Grade 0 or 1	No change
Grade 2	Hold until resolved to ≤ Grade 1, resume at previous dose. Increase supportive care measures if possible.
≥ Grade 3	Hold until resolved to ≤ Grade 1, resume with 20% dose reduction for subsequent cycles. If Grade 3 or greater toxicity recurs, stop AC and proceed with paclitaxel.

† Information contained in this table is intended to be used as a guideline for AC dose modifications, and variation from this table can be made based upon institutional guidelines or physician discretion. These modifications will not be recorded as deviations for this protocol.

8.3 Paclitaxel (+/- Carboplatin)†

AC dose modification or delay will not impact paclitaxel therapy.

If toxicity requires carboplatin to be held, paclitaxel should be held as well to maintain the same treatment schedule. However patients who discontinue carboplatin may complete the planned paclitaxel.

Infusions that are 'held' will be 'made up' when recovery allows treatment to resume. However the total time for paclitaxel (+carboplatin) therapy may not exceed 16 weeks.

Event	Paclitaxel	Carboplatin
Neutropenia (On day of planned treatment)		
> 1000/mm^3	No change	No change
≤ 1000/mm^3	Hold until ANC >1000, resume based on timing of recovery: < 1 week – no change >1 but < 3 weeks - reduce dose 20% for subsequent cycles > 3 weeks – stop paclitaxel	Hold until ANC >1000, resume based on timing of recovery: < 1 week – no change >1 but < 3 weeks - reduce dose 1 AUC for subsequent cycles > 3 weeks – stop carboplatin
Neutropenic Fever (at any point)		

Event	Paclitaxel	Carboplatin
ANC ≤ 1000, fever ≥ 38.5	Interrupt until resolved (ANC >1000, fever <38.5), resume according to number of episodes: 1st = no change 2nd = 20% dose reduction 3rd = stop paclitaxel	Interrupt until resolved (ANC >1000, fever <38.5), resume according to number of episodes: 1st = no change 2nd = 1 AUC dose reduction 3rd = stop carboplatin
Thrombocytopenia (on day of planned treatment)		
≥ 100,000/mm^3	No change	No change
75-99,999/mm^3	Hold until ≥ 100,000, resume based on timing of recovery: < 1 week – no change >1 but < 3 weeks - reduce dose 20% for subsequent cycles >3 weeks – stop paclitaxel	Hold until ≥ 100,000, resume based on timing of recovery: < 1 week – no change >1 but < 3 weeks - reduce 1 AUC for subsequent cycles >3 weeks – stop carboplatin
<75,000	Hold until ≥ 100,000, Resume with 20% dose reduction for subsequent cycles. If > 3 weeks delay is required, stop paclitaxel	Hold until ≥ 100,000, Resume with 1 AUC dose reduction for subsequent cycles. If > 3 weeks delay is required, stop carboplatin
Anemia		
All grades	No change – erythropoietin or darbepoietin therapy may be initiated at the investigator's discretion. Transfusion support is allowed.	
Hepatic		
Grade 0 or 1	No change	No change
≥ Grade 2	Interrupt until ≤ Grade 1, then resume previous dose. If > 3 weeks delay is required, stop paclitaxel	No change
Renal		
Grade 0-1	No change	No change
≥ Grade 2	No change	Discontinue carboplatin
Nausea/Vomiting (any point)		
Grade 0 - 2	No change	No Change
≥ Grade 3	Hold until resolved to ≤ Grade 1, reduce dose 20% in subsequent cycles.	Hold until resolved to ≤ Grade 1, reduce dose 1 AUC in subsequent cycles.
Mucositis (at any point)		
Grade 0 - 2	No change	No change

Event	Paclitaxel	Carboplatin
≥ Grade 3	Hold until resolved to ≤ Grade 1, reduce dose 20% in subsequent cycles.	Hold until resolved to ≤ Grade 1, reduce dose 1 AUC in subsequent cycles.
Neurotoxicity (at any point)		
Grade 0 - 1	No change	No change
Grade 2	If interval Grade 2 toxicity has resolved to ≤ Grade 1 on the day of treatment, proceed with treatment at the previous dose. If Grade 2 toxicity is present on the day of treatment, reduce dose 20% for all subsequent cycles.	If interval Grade 2 toxicity has resolved to ≤ Grade 1 on the day of treatment, proceed with treatment at the previous dose. If Grade 2 toxicity is present on the day of treatment, reduce dose 1 AUC for all subsequent cycles.
Grade 3	Hold until resolved to ≤ Grade 1, reduce dose 20% in all subsequent cycles. If > 3 weeks delay is required, stop carboplatin	Hold until resolved to ≤ Grade 1, reduce dose 1 AUC in all subsequent cycles. If > 3 weeks delay is required, stop carboplatin
Grade 4	Discontinue paclitaxel	Discontinue carboplatin
Anaphylaxis/Hypersensitivity		
Mild (e.g., mild flushing, rash, pruritis)	No treatment needed. Supervise at bedside and complete paclitaxel infusion.	No treatment needed. Supervise at bedside and complete carboplatin infusion.
Moderate (e.g., moderate flushing, rash, mild dyspnea, chest discomfort)	Stop paclitaxel. Administer diphenhydramine 25 mg and dexamethasone 10 mg IV. After recovery, resume infusion at half the previous rate for 15 minutes. If no further symptoms occur, complete the infusion at the full dose rate. If symptoms recur, stop paclitaxel.	Stop carboplatin. Administer diphenhydramine 25 mg and dexamethasone 10 mg IV. After recovery, resume infusion at half the previous rate for 15 minutes. If no further symptoms occur, complete the infusion at the full dose rate. If symptoms recur, stop carboplatin.
Severe (e.g., hypotension requiring pressors, angioedema, respiratory distress requiring bronchodilators)	Stop paclitaxel. Administer diphenhydramine 25 mg and dexamethasone 10 mg IV. Add epinephrine or bronchodilators as needed. Do not restart paclitaxel.	Stop carboplatin. Administer diphenhydramine 25 mg and dexamethasone 10 mg IV. Add epinephrine or bronchodilators as needed. Do not restart carboplatin.
Other clinically significant toxicity excluding fatigue, alopecia and leukopenia		
Grade 0 or 1	No change	No change
Grade 2	Hold until resolved to ≤ Grade 1, resume at previous dose. Increase supportive care measures if possible.	Hold until resolved to ≤ Grade 1, resume at previous dose. Increase supportive care measures if possible.

Event	Paclitaxel	Carboplatin
≥ Grade 3	Hold until resolved to ≤ Grade 1, resume with 20% dose reduction for subsequent cycles. If Grade 3 or greater toxicity recurs, stop paclitaxel.	Hold until resolved to ≤ Grade 1, resume with 1AUC dose reduction for subsequent cycles. If Grade 3 or greater toxicity recurs, stop carboplatin.

† Information contained in this table is intended to be used as a guideline for AC dose modifications, and variation from this table can be made based upon institutional guidelines or physician discretion. These modifications will not be recorded as deviations for this protocol.

8.4 Supportive Care

- It is anticipated that nausea and vomiting may be a significant side effect of the treatment regimen. Therefore, the following combination regimen is recommended: Dexamethasone, 10 mg IV or PO, plus a 5-HT3 receptor antagonist prior to AC chemotherapy. Prochlorperazine or another antiemetic at the physician's discretion may be used before paclitaxel treatment.
- All supportive measures consistent with optimal patient care will be given throughout the study.
- Pegfilgrastim or filgrastim are recommended after each AC treatment (cycle 1-4) for patients receiving dose dense AC. Otherwise, hematologic growth factors are not required but may be used in accordance with the ASCO guidelines.

8.5 Duration of Follow-up

The primary endpoint of this trial is pCR, therefore study treatment will end at surgery. Patients who developed omeprazole related toxicities will be followed until the toxicity has resolved, returned to baseline, or have been determined to be irreversible. Data regarding therapies administered after surgery, disease recurrence, and overall survival will not be collected.

9. STUDY PARAMETERS/CALENDAR

		Omeprazole monotherapy		AC			
	Baseline[A]	**Day 1 of omeprazole treatment**	**After 4-7 days of omeprazole treatment**	**C1 D1**	**C2 D1**	**C3 D1**	**C4 D1**
REQUIRED ASSESSMENTS					**+/-7 days**	**+/-7 days**	**+/-7 days**
Informed consent	X						
Medical history	X						
Physical exam/ECOG PS	X			X	X	X	X
Urine pregnancy[B]	X						
CBC	X			X	X	X	X
CMP	X			X		X	
Serum B-12	X						
Serum magnesium	X						
LVEF[C]	X						
AE assessment	X		Continuous				
DISEASE ASSESSMENT							
Breast Imaging[D]	X[E]						
TREATMENT							
Omeprazole		X	X	Continuous			
AC[F]				X	X	X	X
Paclitaxel							
Carboplatin							
CORRELATIVE STUDIES							
Tumor biopsy[G]	X		X				
Cell-free DNA	X		X[J]				
Serum lipid profile[H,K]	X		X[J]				
Serum NEFA[H]	X		X[J]				
Omeprazole Level		X[I]			X		X

Calendar continued on page 22

Footnotes:
[A]Baseline assessment should be completed within 21 days of study registration.
[B]Pregnancy testing should be completed with 14 days of study registration in all patients with childbearing potential.
[C]LVEF may be assessed by either ECHO or MUGA.
[D]Breast imaging should include mammogram and ultrasound. Breast MRI is not required and will not be repeated solely for the purposes of this study.
[E]Breast imaging should be completed within 28 days of study registration.
[F]AC may be administered every 3 weeks or every 2 weeks with growth factor support. Cycle 1 should begin within 4-7 days after starting omeprazole monotherapy.
[G]A core biopsy will be obtained after study registration but prior to starting omeprazole treatment (baseline), and after 4-7 days of omeprazole treatment but prior to initiation of AC. IU subjects are required to have a fresh biopsy at baseline. Non-IU subjects may donate archived tissue in lieu of a fresh biopsy at baseline if adequate tissue is available. See Section 13.1 for additional details.
[H]Fasting for 12-14 hours prior to blood collection is required.
[I]Plasma for measurement of omeprazole will be obtained prior to dosing and 2 hours after the first dose of omeprazole only. All other time points will collect a single plasma sample without regard to time from last dose.
[J]These labs can be done on C1D1 of AC instead of the after 4-7 days of omeprazole visit if needed.

[K]This test should be run by the hospital as it is a routine laboratory test. Separate tubes for serum lipid will not be included in the correlative blood collection kits.

	Paclitaxel (+Carboplatin if desired)												
	W1	**W2**	**W3**	**W4**	**W5**	**W6**	**W7**	**W8**	**W9**	**W10**	**W11**	**W12**	**Pre-Surgery**
REQUIRED ASSESSMENTS	**+/-7 days**	**+/-7 days**	**+/-7 days**	**+/-7 days**	**+/-7 days**	**+/-7 days**	**+/-7 days**	**+/-7 days**	**+/-7 days**	**+/-7 days**	**+/-7 days**	**+/-7 days**	
Informed consent													
Medical history													
Physical exam/ECOG PS													
Urine pregnancy													
CBC	X	X	X	X	X	X	X	X	X	X	X	X	
CMP	X			X			X			X			
Serum B-12													X
Serum magnesium	X						X						X
LVEF													
AE assessment	Continuous												
DISEASE ASSESSMENT													
Breast Imaging[A]	X												X
TREATMENT													
Omeprazole	Continuous												
AC													
Paclitaxel[B]	X	X	X	X	X	X	X	X	X	X	X	X	
Carboplatin[C]	(X)			(X)			(X)			(X)			
CORRELATIVE STUDIES													
Tumor biopsy													(X)[D]
Cell-free DNA													X
Serum lipid profile[E,G]													X
Serum NEFA[E]	X												X
Omeprazole Level[F]	X						X						

[A]Breast imaging should include mammogram and ultrasound. Breast MRI is not required and will not be repeated solely for this study.
[B]Infusions that are 'held' will be 'made up' when recovery allows treatment to resume. However the total time for paclitaxel (+carboplatin) therapy may not exceed 16 weeks.
[C]Carboplatin is not recommended but is allowed at the treating investigator's discretion.
[D]Only in patients with clinically apparent residual abnormality. May be obtained intraoperatively.
[E]Fasting for 12-14 hours prior to blood collection is required.
[F]A single plasma sample for measurement of omeprazole will be obtained at each time point without regard to time from last dose.
[G]This test should be run by the hospital, as it is a routine laboratory test. Separate tubes for serum lipid will not be included in the correlative blood collection kits.

10. CRITERIA FOR EVALUATION/REMOVAL FROM STUDY

10.1 Definitions Associated with Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1

10.1.1 Measurable disease - At baseline, tumor lesions/lymph nodes will be categorized measurable or non-measurable as follows:

10.1.2 Measurable

Tumor lesions –will be measured using any of the following methods of measurement:

- Chest X-ray: Lesions on chest x-ray are acceptable as measurable lesions when they are clearly defined and surrounded by aerated lung. However, CT scans are preferable.

- Clinical Lesions: For superficial non-nodal lesions, physical examination is acceptable, but imaging is preferable, if both can be done. Clinical lesions will only be considered measurable when they are superficial (e.g., skin nodules and palpable lymph nodes) and ≥10 mm (≥1 cm) diameter as assessed using calipers (e.g., skin nodules). In the case of skin lesions, documentation by color photography, including a ruler to estimate the size of the lesion, is recommended.

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

Malignant lymph nodes:

To be considered pathologically enlarged and measurable, a lymph node must be ≥ 15 mm in *short* axis when assessed by CT scan (CT scan slice thickness recommended to be no greater than 5 mm). At baseline and in follow-up, only the *short* axis will be measured and followed.

10.1.3 Non-measurable

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

10.1.4 Baseline documentation of "Target" and "Non-Target" lesions

When more than one measurable lesion is present at baseline, all lesions up to a maximum of five lesions total (and a maximum of two lesions per organ) representative

of all involved organs should be identified as target lesions and will be recorded and measured at baseline. This means if a patient has only one or two organ sites involved, a maximum of two and four lesions, respectively, will be recorded.

Target lesions should be selected on the basis of their size (lesions with the longest diameter), be representative of all involved organs and should be those that lend themselves to reproducible repeated measurements. It may be the case that, on occasion, the largest lesion does not lend itself to reproducible measurement in which circumstance the next largest lesion which can be measured reproducibly should be selected.

10.1.4.1 Lymph nodes

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

10.4.1.2 Sum of Diameters

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

All other lesions (or sites of disease) including pathological lymph nodes should be identified as non-target lesions and should also be recorded at baseline. Measurements are not required and these lesions should be followed as 'present', 'absent', or in rare cases 'unequivocal progression'. In addition, it is possible to record multiple non-target lesions involving the same organ as a single item on the case report form (e.g., 'multiple enlarged pelvic lymph nodes' or 'multiple liver metastases').

10.2 Evaluation of Target Lesions

Response	**Evaluation of Target Lesions**
Complete Response (CR)	Disappearance of all target lesions. Any pathological lymph nodes (whether target or non-target) must have reduction in short axis to < 10 mm)
Partial Response (PR)	At least a 30% decrease in the sum of diameters of target lesions, taking as reference the baseline sum of diameters

Stable Disease (SD)	Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum diameters while on study.
Progressive Disease (PD)	At least a 20% increase in the sum of diameters of target lesions, taking as reference the *smallest sum on study* (this includes the baseline sum if that is the smallest on study). In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5 mm. NOTE: the appearance of one or more new lesions is also considered progression.

10.2.1 Special notes on assessment of target lesions

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

10.2.1.2 Target lesions that become 'too small to measure':
All lesions (nodal and non-nodal) recorded at baseline should have their actual measurements recorded at each subsequent evaluation, even when very small (e.g., 2 mm). However, sometimes lesions or lymph nodes become so faint on scan that the radiologist may report them as 'too small to measure'.
When this occurs it is important that a value be recorded on the case report form. If it is the opinion of the radiologist that the lesion has likely disappeared, the measurement should be recorded as 0 mm. If the lesion is believed to be present and is faintly seen but too small to measure, a default value of 5 mm should be assigned (derived from 5 mm slice thickness). Note: It is less likely that this rule will be used for lymph nodes since they usually have a definable size when normal and are frequently surrounded by fat such as in the retroperitoneum; however, if a lymph node is believed to be present and is faintly seen but too small to measure, a default value of 5mm should be assigned in this circumstance as well. However, if the radiologist is able to provide an actual measurement, it should be recorded even if it is below 5 mm.

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

10.3 Evaluation of Non-Target Lesions

Response	**Evaluation of Non-Target Lesions**
Complete Response (CR)	Disappearance of all non-target lesions and normalization of tumor marker level. All lymph nodes must be non-pathological in size (< 10 mm short axis)
Non-CR/ Non-PD	Persistence of one or more non-target lesion(s) and/or maintenance of tumor marker level above the normal limits
Progressive Disease (PD)	Appearance of one or more new lesions and/or unequivocal progression of existing non-target lesions

10.3.1 Special notes on assessment of progression of non-target disease

10.3.1.1 When the patient also has measurable disease

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

10.3.1.2 When the patient has only non-measurable disease

This circumstance arises in some phase III trials when it is not a criterion of study entry to have measurable disease. The same general concepts apply here as noted above, however, in this instance there is no measurable disease assessment to factor into the interpretation of an increase in non-measurable disease burden. Because worsening in non-target disease cannot be easily quantified (by definition: if all lesions are truly non-measurable) a useful test that can be applied when assessing patients for unequivocal progression is to consider if the increase in overall disease burden based on the change in non-measurable disease is comparable in magnitude to the increase that would be required to declare PD for measurable disease: i.e., an increase in tumor burden representing an additional 73% increase in 'volume' (which is equivalent to a 20% increase diameter in a measurable lesion). Examples include an increase in a pleural effusion from 'trace' to 'large', an increase in lymphangitic disease from localised to widespread, or may be described in protocols as 'sufficient to require a change in therapy'. If 'unequivocal progression' is seen, the patient should be considered to have had overall PD at that point. While it would be ideal to have objective criteria to apply to non-measurable disease, the very nature of that disease makes it impossible to do so, therefore the increase must be substantial.

10.4 New Lesions

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

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

If a new lesion is equivocal, for example because of its small size, continued therapy and follow-up evaluation will clarify if it represents truly new disease. If repeat scans confirm

there is definitely a new lesion, then progression should be declared using the date of the initial scan.

10.5 Evaluation of best overall response

The best overall response is the best response recorded from the start of the treatment until disease progression/recurrence (taking as reference for PD the smallest measurements recorded since the treatment started). The patient's best overall response assignment will depend on the findings of both target and non-target disease and will also take into consideration the appearance of new lesions.

Target Lesions	Non-Target Lesions	New Lesion?	Best Overall Response
CR	CR	No	CR
CR	Non CR/ Non PD	No	PR
CR	Not evaluated	No	PR
PR	Non-PD or not all evaluated	No	PR
SD	Non-PD or not all evaluated	No	SD
Not all evaluated	Non-PD	No	NE
PD	Any	Yes or No	PD
Any	PD	Yes or No	PD
Any	Any	Yes	PD

Patients with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be classified as having "symptomatic deterioration". Every effort should be made to document the objective progression even after discontinuation of treatment.

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

10.6 Criteria for Removal from Study

Patients will be removed from study under any of the following conditions:

- When removal from study is thought to be in the best interest of the patient by the patient or the patient's treating physician.
- Unacceptable toxicity in the judgement of the patient or patient's treating physician.
- Pregnancy or failure to practice appropriate contraception.
- Patient's request.
- Evidence of systemic metastatic disease.
- Inability to take omeprazole for 14 continuous days.

11. DRUG INFORMATION

11.1 Drug Name

Omeprazole

11.2 Other Names

PRILOSEC

11.3 Classification

Proton pump inhibitor

11.4 Mode of Action

Omeprazole belongs to a class of antisecretory compounds, the substituted benzimidazoles, that suppress gastric acid secretion by specific inhibition of the H+/K+ ATPase enzyme system at the secretory surface of the gastric parietal cell. Because this enzyme system is regarded as the acid (proton) pump within the gastric mucosa, omeprazole has been characterized as a gastric acid-pump inhibitor, in that it blocks the final step of acid production. This effect is dose-related and leads to inhibition of both basal and stimulated acid secretion irrespective of the stimulus. Animal studies indicate that after rapid disappearance from plasma, omeprazole can be found within the gastric mucosa for a day or more.

11.5 Storage and Stability

Store omeprazole (PRILOSEC) Delayed-Release **Capsules** in a tight container protected from light and moisture. Store between 15°C and 30°C (59°F and 86°F).

11.6 Metabolism

Omeprazole is extensively metabolized by the cytochrome P450 (CYP) enzyme system.

11.7 Dosage Forms and Strengths

Delayed-Release Capsules: 10 mg, 20 mg and 40 mg
Delayed-Release Oral Suspension: 2.5 mg or 10 mg

11.8 Administration

Patients will be instructed to take two 40 mg capsules (80 mg) twice daily. Capsules should not be chewed or crushed. Missed doses should not be made up.

Patients will begin taking the omeprazole 4-7 days before chemotherapy, and will continue taking the medication throughout chemotherapy until surgery (up to 8 months).

11.9 Availability

This study will be conducted in compliance with 21 CFR 312.7 (promotion and charging for investigational drugs). Omeprazole will be obtained, stored, and dispensed by the IUSCC Investigational Drug Service (IDS). Patients will be instructed to bring their bottles with unused omeprazole back to the clinic. After pill counts to document

compliance and drug exposure, remaining omeprazole will be returned to IDS for proper disposal.

11.10 Side Effects

The most common adverse reactions reported in adults (incidence ≥ 2%) are headache, abdominal pain, nausea, diarrhea, vomiting, and flatulence.

The following adverse reactions have been identified during post- approval use of PRILOSEC Delayed-Release Capsules. Because these reactions are voluntarily reported from a population of uncertain size, it is not always possible to reliably estimate their actual frequency or establish a causal relationship to drug exposure.

- Body As a Whole: Hypersensitivity reactions including anaphylaxis, anaphylactic shock, angioedema, bronchospasm, interstitial nephritis, uticaria, (see also Skin below); fever; pain; fatigue; malaise
- Cardiovascular: Chest pain or angina, tachycardia, bradycardia, palpitations, elevated blood pressure, peripheral edema
- Endocrine: Gynecomastia
- Gastrointestinal: Pancreatitis (some fatal), anorexia, irritable colon, fecal discoloration, esophageal candidiasis, mucosal atrophy of the tongue, stomatitis, abdominal swelling, dry mouth, microscopic colitis. During treatment with omeprazole, gastric fundic gland polyps have been noted rarely. These polyps are benign and appear to be reversible when treatment is discontinued.
- Gastroduodenal carcinoids have been reported in patients with ZE syndrome on long-term treatment with PRILOSEC. This finding is believed to be a manifestation of the underlying condition, which is known to be associated with such tumors.
- Hepatic: Liver disease including hepatic failure (some fatal), liver necrosis (some fatal), hepatic encephalopathy hepatocellular disease, cholestatic disease, mixed hepatitis, jaundice, and elevations of liver function tests [ALT, AST, GGT, alkaline phosphatase, and bilirubin] Infections and Infestations: Clostridium difficile associated diarrhea
- Metabolism and Nutritional disorders: Hypoglycemia, hypomagnesemia, hyponatremia, weight gain
- Musculoskeletal: Muscle weakness, myalgia, muscle cramps, joint pain, leg pain, bone fracture
- Nervous System/Psychiatric: Psychiatric and sleep disturbances including depression, agitation, aggression, hallucinations, confusion, insomnia, nervousness, apathy, somnolence, anxiety, and dream abnormalities; tremors, paresthesia; vertigo
- Respiratory: Epistaxis, pharyngeal pain
- Skin: Severe generalized skin reactions including toxic epidermal necrolysis (some fatal), Stevens-Johnson syndrome, and erythema multiforme; photosensitivity; urticaria; rash; skin inflammation; pruritus; petechiae; purpura; alopecia; dry skin; hyperhidrosis
- Special Senses: Tinnitus, taste perversion
- Ocular: Optic atrophy, anterior ischemic optic neuropathy, optic neuritis, dry eye syndrome, ocular irritation, blurred vision, double vision

- Urogenital: Interstitial nephritis, hematuria, proteinuria, elevated serum creatinine, microscopic pyuria, urinary tract infection, glycosuria, urinary frequency, testicular pain
- Hematologic: Agranulocytosis (some fatal), hemolytic anemia, pancytopenia, neutropenia, anemia, thrombocytopenia, leukopenia, leukocytosis

11.11 Nursing Implications

Omeprazole concentrations have been measured in breast milk of a woman following oral administration of 20 mg. The peak concentration of omeprazole in breast milk was less than 7% of the peak serum concentration. This concentration would correspond to 0.004 mg of omeprazole in 200 mL of milk. Because omeprazole is excreted in human milk, because of the potential for serious adverse reactions in nursing infants from omeprazole, and because of the potential for tumorigenicity shown for omeprazole in rat carcinogenicity studies, patients who are nursing are not eligible for this trial.

Omeprazole inhibits CYP2C19. Patients taking drugs known to be substrates of CYP2C19 (Appendix IV) may enroll but the treating investigator and nursing staff should be aware of the potential drug interaction.

12. STATISTICAL METHODS

12.1 General Considerations

Statistical analysis of this study will be the responsibility of the Biostatistics and Data Management Core at the Indiana University Melvin and Bren Simon Cancer Center (IUSCC). Parameter estimates and relevant summary statistics will be reported where appropriate. For continuous variables, summary statistics will include number of subjects, mean, median, standard deviation, minimum and maximum. Categorical endpoints will be summarized using number of subjects, frequency, and percentages. Missing data will not be imputed. Data analysis will be performed in SAS Version 9.4. Additional exploratory analyses of the data will be conducted as deemed appropriate. Changes from this analysis plan will not require an amendment to the protocol unless it changes a significant feature of the protocol.

The statistical analysis methods are outline below.

12.2 Study Design

This is a single arm Phase II study of omeprazole + standard neoadjuvant chemotherapy as an obligate first step toward a definitive trial.

12.3 Analysis Datasets

The enrolled population comprises all patients who meet the eligibility criteria and are registered onto the study.

12.4 Sample Size

The key clinical endpoint is pCR and the primary subgroup of interest is in patients who have the target at baseline. Based on previously reported trials with standard anthracycline/taxane therapy, we estimate a pCR rate of ~40%. Assuming that 70% of

patients with newly diagnosed TNBC have FASN expression, a single stage phase II trial to detect a pCR rate of 60% with power = 80% and alpha=0.10 requires 30 FASN patients (or 42 patients total). This sample size also provides approximately 80% power to identify a change in FASN expression and/or activity of 0.5 SDs using a paired t-test and significance level of 0.05. Patients who are inevaluable for the primary endpoint will be considered non-responders and will not be replaced.

12.5 Patient Characteristics

Demographics, medical history, and physical examination characteristics will be summarized using descriptive statistics.

12.6 Disposition

Frequencies and percentages of all patients enrolled, discontinuing the study, and completing the study will be presented. The reasons for discontinuation will also be summarized.

12.7 Analysis of Primary Objectives

For estimating pCR rate, a 95% exact confidence interval will be calculated.

12.8 Analysis of Secondary Objectives

For number of patients expressing FASN in tumor, a proportion and 95% confidence interval will be reported.

For estimating pCR rate in all patients, 95% exact confidence intervals will be calculated.

For safety, NCI CTC Version 4.0 will be used to summarize adverse events in the assessment of safety for incorporating high dose omeprazole with standard neoadjuvant chemotherapy. Summaries of treatment related adverse events in the population will be tabulated. All adverse events (AEs) will be presented in incidence tables coded by CTC term. An adverse event will be considered treatment related if it occurred on or after date of first dose of omeprazole, and was possibly, probably, or definitely related to treatment. All adverse events will be recorded until off study date. All deaths recoded in this study will be listed and summarized, and the cause documented.

For FASN expressors, mean, standard deviation, median, minimum, and maximum FASN expression and activity in tumor will be calculated. To see how high dose omeprazole impacts biologic activity, changes in FASN expression and activity, FASN downstream target gene expression (e.g., PARP1 and SP1), and lipid levels from baseline to after 4-7 days on omeprazole treatment and at time of surgery will be assessed by linear mixed models. In addition, linear mixed models will be used to assess the association of FASN expression with BMI.

12.9 Analysis of Tertiary Objectives

Descriptive statistics (mean, standard deviation, median, minimum, maximum, 95% confidence intervals) will be use to summarize omeprazole exposure.

Peak omeprazole concentration will be compared to peak based on pre-clinical testing in a purely qualitative manner.

Pharmacokinetics-Pharmacodynamic (PK-PD) modeling of omeprazole drug exposure, FASN target activity (represented by lipid profiling), and clinical outcome will be conducted. A system of PK-PD models will be developed to quantify and predict the clinical outcome, i.e., pCR, based on drug exposure and target levels. The omeprazole pharmacokinetic model will be constructed using the sparse sampling nonlinear mixed effect model, which account for the last dosing time information. The omeprazole-FASN/lipid PK-PD will be constructed using the nonlinear mixed effect with time-dependent covariates model (30). Finally, the correlations between PK (omeprazole) and/or PD (lipid/FASN) and pCR will be evaluated through Cox proportional hazard model with covariates measured in error. The PK and PK-PD modeling will be conducted in NONMEM, while the Cox model will be conducted in SAS.

Change in lipid levels from baseline to after 4-7 days on omeprazole treatment and at time of surgery will also be assessed separately by linear mixed models.

12.10 Interim Analysis

No interim analyses are planned for this study.

12.11 Subgroup Analysis

Additional subgroup analysis may be performed as deemed appropriated.

13. SPECIAL INSTRUCTIONS

13.1 Tumor Biopsy Requirements

Core biopsy samples will be obtained at baseline, after 4-7 days of omeprazole treatment, and in residual disease at surgery. At each time point, 3 core samples should be obtained. Please refer to the Study Procedure Manual for detailed processing instructions for each core.

IU Site: Subjects are required to have a fresh biopsy at baseline to obtain the 3 core samples below.

Non-IU Sites: Archived tissue from a previously performed diagnostic biopsy may be used to obtain the 3 cores listed below at baseline. If adequate tissue from an archived sample is unavailable, subjects will be required to undergo a biopsy at baseline.

- **Core 1 – To be tested for FASN and downstream target gene expression**
- **Core 2 – To be tested for FASN activity**
- **Core 3 – To be tested for NEFA analyses**

13.1.1 FASN expression

We will evaluate FASN expression using immunohistochemistry (IHC) in core biopsy samples obtained at baseline, after 4-7 days of omeprazole treatment, and in residual disease at surgery. Evaluation of the post-treatment samples will determine the potential

impact of omeprazole on FASN expression and upregulation during chemotherapy. Based on our preliminary data, we expect omeprazole to have the greatest potential benefit in those patients with baseline FASN expression. However if FASN is upregulated in response to chemotherapy, omeprazole may prevent or delay emergence of resistance in those without FASN expression at diagnosis. Testing will be coordinated by Dr. JT Zhang using an IHC method as described (2).

13.1.2 Expression of FASN downstream target genes

We will evaluate expression of FASN downstream target genes including PARP1 and SP1 in core biopsy samples obtained at baseline, after 4-7 days of omeprazole treatment, and in residual disease at surgery as described above for IHC of FASN. Analysis of downstream targets will provide verification that inhibition of FASN with omeprazole has functional significance. Downstream target genes will be evaluated in the same sample as used to assess FASN expression. Testing will be coordinated by Dr. JT Zhang using an IHC method as described (2).

13.1.3 FASN activity

We will evaluate FASN activity in core biopsy samples obtained at baseline, after 4-7 days of omeprazole treatment, and in residual disease at surgery. FASN activity will be determined using coupled enzymatic NDAPH oxidation assay as we previously described (3, 4). Based on our preliminary data we expect omeprazole will inhibit FASN activity and we will see a difference in FASN activity between pre- and post-treatment samples. Testing will be coordinated by Dr. JT Zhang using an IHC method as described (2).

13.1.4 Tumor NEFA

Inhibition of FASN in also predicted to decrease the level of non-esterified fatty acids in the tumor samples. NEFA will be quantified using the coupled enzyme NEFA Assay Kit from BIOO Scientific Corp in the laboratory of Dr. JT Zhang. NEFA quantification will be performed on the same sample used for assessment of FASN activity. Given the need for fresh/immediate tumor processing, this will be obtained only in patients enrolled at IU.

13.1.5 Tumor Autophagy Markers

Cell death is predicted as a response to omeprazole. We predict that FASN and autophagy could be a downstream result of FASN, leading to cell death. FASN is also predicted to trigger autophagic mediated aptosis or autophagic cell death by itself. Testing will be completed on tissue between pre- and post- treatment and Testing will be coordinated by Dr. Lacey McNally using an IHC method as described (2). This will be conducted after IU has completed their processing.

13.2 Serum Lipid Profile

As FASN is important in lipid metabolism, inhibition of FASN by high dose PPIs may alter circulating lipids. A fasting serum sample for assessment of lipid profile including total cholesterol, triglycerides, high-density lipoprotein, and low-density lipoprotein, will be obtained at baseline, after 4-7 days on omeprazole treatment and prior to surgery. Samples will be analyzed in the IU Health University Hospital clinical laboratory or the clinical laboratory of participating institutions.

13.3 Serum NEFA

A fasting serum sample for assessment of NEFA will be obtained at baseline, after 4-7 days of omeprazole, at the mid-point of chemotherapy (i.e., week 1 of paclitaxel), and prior to surgery. Samples will be analyzed in the laboratory of JT Zhang using the coupled enzyme NEFA Assay Kit from BIOO Scientific Corp. Detailed information regarding collection and processing can be found in the laboratory manual for this study.

13.4 Omeprazole Exposure

Plasma for measurement of omeprazole exposure will be obtained serially during therapy. Samples will be obtained prior to the first dose and 2 hours after the first dose. Other samples will be obtained during planned clinic visits without regard to time from last dose at Cycles 2 and 4 of AC and Week 1 and 7 of paclitaxel. The time last dose prior to sampling was taken will be recorded to facilitate population PK modeling to estimate peak concentration and time above the therapeutic threshold. Samples will be analyzed in the CPAC laboratory under the direction of Dr. David Jones. Detailed information regarding collection and processing can be found in the laboratory manual for this study.

13.5 Plasma for Cell-Free DNA

Plasma will be obtained to facilitate development of assays to measure cancer-derived cell-free DNA in the laboratory of Dr. Milan Radovich. Plasma samples will be obtained at baseline, after 4-7 days of omeprazole treatment, and at surgery. As these samples will be used in the future for assay development, no formal statistical analysis is planned and samples will solely be banked at this time. Detailed information regarding collection and processing can be found in the laboratory manual for this study.

14. PATIENT CONSENT AND PEER JUDGMENT

The protocol and informed consent form for this study must be approved in writing by the appropriate Institutional Review Board (IRB) prior to any patient being registered on this study.

Changes to the protocol, as well as a change of principal investigator, must also be approved by the Board. Records of the Institutional Review Board review and approval of all documents pertaining to this study must be kept on file by the investigator (housed in the Clinical Trials Office) and are subject to inspection at any time during the study. Periodic status reports must be submitted to the Institutional Review Board at least yearly, as well as notification of completion of the study and a final report within 3 months of study completion or termination.

The study will be conducted in compliance with ICH guidelines and with all applicable federal (including 21 CFR parts 56 & 50), state or local laws.

15. DATA AND SAFETY MONITORING

15.1 Data Safety Monitoring Committee

The Data Safety Monitoring Committee (DSMC) of the Indiana University Simon Cancer Center (IUSCC) is responsible for patient safety and privacy protection, compliance with required reporting, and study integrity for all trials conducted at IUSCC. Members are subject matter experts from multiple disciplines including medical oncology, pediatrics, biostatistics, behavioral oncology, radiation oncology, urology, surgery, gynecologic oncology, data and project management and research administration who are appointed by the DSMC Chair. The DSMC will provide independent oversight of the clinical trial so that study integrity is assured. However, the DSMC is not serving as a Data and Safety Monitoring Board (DSMB) for this study. The DSMC will meet per the currently approved DSMP, led by the DSMC Chair and Coordinator, and will review all adverse events, monitoring and auditing reports, unanticipated problems and study non-compliance events that require expedited reporting. Meeting minutes will be maintained in the IUSCC Clinical Trials Office (CTO). Specifically the DSMC has the following responsibilities:

- Assessment of the adequacy of trial-specific Data Monitoring and Safety Plan (DSMP) of establish risk based monitoring determination of trial specific DSMB.
- Review safety data for investigator initiated trials including all adverse events, unanticipated problems and study non-compliance events requiring expedited reporting.
- Conduct routine study monitoring and auditing in compliance with the IUSCC data quality control review process.

15.2 Data Safety Monitoring Plan

This trial will comply with the current requirements of the Data and Safety Monitoring Plan (DSMP) of the IUSCC. The CTO of the IUSCC will be the Coordinating Center for this multicenter phase *II* trial.

In accordance with the DSMP of the IUSCC, Investigators will conduct continuous review of data and patient safety. **Monthly** review meetings for moderate risk trials are required and will include the principal investigator, clinical research specialist and/or research nurse (other members per principal investigator's discretion). Monthly meeting summaries should include the number of patients, significant toxicities as described in the protocol, dose adjustments, responses observed, eligibility of patients enrolled at each site, serious adverse events (SAEs) or unanticipated problems (UPs) (both IUSCC and those reported from other institutions), dose adjustments, and protocol deviations. Meeting minutes will be submitted and reviewed by the DSMC at dsmc@iupui.edu.

In addition, **conference calls** with investigators and staff at participating sites will be scheduled **at least monthly** (and more often as needed) to discuss study progress. If there are no patients on treatment or in follow-up, email communication will be used in lieu of a teleconference.

16.2.1 Study Auditing and Monitoring

All trials are subject to auditing and/or monitoring per the currently approved DSMC Charter.

16.2.2 Reporting Guidelines
The DSMC has streamlined the reporting process by utilizing reports from OnCore®. This has allowed direct view of reports within the Clinical Trials Management System (CTMS); thus discontinuing paper reports. SAE reports are entered into OnCore® monthly and reviewed by the DSMC chair and/or coordinator monthly. Findings will be reported to the full DSMC at the time of study review.

16.2.3 Reporting Death
Death will be reported per local IRB reporting guidelines (Section 5.8 of the Unanticipated Problems and Noncompliance SOP).

16.2.4 Study Accrual Oversight
Accrual data will be entered into the IU Simon Cancer Center OnCore® system. The Protocol Progress Committee (PPC) reviews study accrual twice per year while the PPC coordinator reviews accrual quarterly.

16.2.5 Continuing Review
All Continuing Reviews (CR) will be reviewed annually or as dictated by the Institutional Review Board. Participating sites will submit a copy of the CR with attachments to the IUSCC MNA, or designee.

15.2.6 Protocol Deviations
Investigators are required to submit protocol deviations to the DSMC via the OnCore® database

15.2.7 Early Study Closure
At any time during the conduct of the trial, if it is the opinion of the investigators that the risks (or benefits) to the patient warrant early closure of the study, this recommendation should be made in writing to the Data Safety Monitoring Committee. Alternatively, the DSMC may initiate suspension or early closure of the study based on its review of the investigator reports.

15.3 Data Safety Monitoring Board

This study will have a Data and Safety Monitoring Board (DSMB) that will review and monitor study progress, toxicity, safety and other data from this trial. The board is chaired by an independent medical oncologist or another qualified individual external to this trial. Questions about participant safety or protocol performance will be addressed with the Principal Investigator, statistician and study team members. Should any major concerns arise, the DSMB will offer recommendations regarding whether or not to suspend the trial.

The DSMB will meet annually to review accrual, toxicity, response and other information reported to the IRB. Information to be provided to the DSMB may include: participant accrual, treatment regimen information, adverse events and serious adverse events reported by category, summary of any deaths on study, audit and/or monitoring results.

The DSMB will provide a written recommendation to the PI and team after all information is reviewed. The report will indicate one or more of the following decisions:

- Continuation of the trial without change
- Continuation of protocol/project with modifications as outlined by the Board
- Immediate suspension of trial for safety reasons with recommended plan of follow up to minimize subject harm
- Study placed on clinical hold
- Termination of trial

The IUSCC will submit the DSMB report(s) to the participating sites as well. The DSMB report(s) should be provided to the local IRB at the time of continuing review

15.4 Data Acquisition

Case Report Forms and Data Submission: This study will utilize electronic Case Report Form completion in the OnCore® database. A calendar of events and required forms are available in OnCore® at https://cancer.iu.edu/oncore. The OnCore® database is a comprehensive database used by the IUSCC CTO and supported by the Indiana University Cancer Center. Access to data through OnCore® is restricted by user accounts and assigned roles. Once logged into the OnCore® system with a user ID and password, OnCore® defines roles for each user which limits access to appropriate data.

All source documents are to remain in the patient's clinic file. All documents should be kept according to applicable federal guidelines. Clinical trial data in OnCore® are periodically monitored by the IU Simon Cancer Center per the DSMC Charter.

16. REPORTING ADVERSE EVENTS

Adverse events (AEs) will be recorded from the time of first study drug administration and for at least 30 days after treatment discontinuation, regardless of whether or not the event(s) are considered related to trial medications. All AEs considered related to trial medication will be followed until resolution, return to baseline, or deemed clinically insignificant, even if this occurs post-trial.

16.1 Definitions of Adverse Events

16.1.1 Adverse Event (AE)

Adverse event (AE): An adverse event is an unplanned, unwanted event which occurs to a study participant and which is possibly, but not necessarily, related to the use of protocol therapy. While some events may not initially appear to be associated with the use of the study treatment, a relationship may not emerge until sufficient numbers of reports accumulate from participating sites. An AE may also include a newly occurring event or a previous condition that has increased in severity or frequency since the

administration of the investigational product. Assessment of the occurrence of an AE will be based on changes in the subject's physical examination, laboratory results and/or signs and symptoms. AEs will be monitored until they are resolved or are clearly determined to be due to a subject's stable or chronic condition or intercurrent illness. Medical care will be provided, as defined in the informed consent document, for any AE related to participation in this clinical trial

16.1.2 Suspected Adverse Reaction (SAR)

Suspected adverse reaction is any adverse event for which there is a reasonable possibility that the drug caused the adverse event. For the purposes of IND safety reporting, 'reasonable possibility' means there is evidence to suggest a causal relationship between the drug and the adverse event. A suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction, which means any adverse event caused by a drug. Suspected adverse reactions are the subset of all adverse events for which there is a reasonable possibility that the drug caused the event.

Examples of types of evidence that would suggest a causal relationship between the drug and the adverse event:

- A single occurrence of an event that is uncommon and known to be strongly associated with drug exposure (e.g., angioedema, hepatic injury, Stevens-Johnson Syndrome).
- One or more occurrences of an event that is not commonly associated with drug exposure, but is otherwise uncommon in the population exposed to the drug (e.g., tendon rupture).
- An aggregate analysis of specific events observed in a clinical trial (such as known consequences of the underlying disease or condition under investigation or other events that commonly occur in the study population independent of drug therapy) that indicates those events occur more frequently in the drug treatment group than in a concurrent or historical control group.

16.1.3 Unanticipated Problem (UP)

Unanticipated Problem (UP): any incident, experience, or outcome that meets all of the following criteria:

1) unexpected (in terms of nature, severity, or frequency) given (a) the research procedures are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document; and (b) the characteristics of the subject population being study;

2) related or possibly related to participation in the research; and

3) suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) related to the research than was previously known or recognized.

Only a small subset of adverse events occurring in human subjects participating in research will meet these three criteria for an unanticipated problem. Furthermore, there are other types of incidents, experiences, and outcomes that occur during the

conduct of human subjects research that represent unanticipated problems but are not considered adverse events. For example, some unanticipated problems involve social or economic harm instead of the physical or psychological harm associated with adverse events. In other cases, unanticipated problems place subjects or others at increased risk of harm, but no harm occurs.

16.1.4 Adverse Reaction (AR)

An adverse reaction is any adverse event caused by a drug. Adverse reactions are a subset of all suspected adverse reactions where there is reason to conclude that the drug caused the event.

16.1.4 Serious Adverse Event (SAE)

An adverse event or suspected adverse reaction is considered "serious" if it results in any of the following outcomes:

- Results in death
- Is life-threatening. Life-threatening is defined as an adverse event or suspected adverse reaction that places the subject at immediate risk of death. It does not include an adverse event or suspected adverse reaction that, had it occurred in a more severe form, might have caused death.
- Requires inpatient hospitalization ≥ 24 hours or prolongation of existing hospitalization

  NOTE: Hospitalizations that are not considered SAEs are:
  - Hospitalization planned prior to first administration of study drug
  - Hospitalization for less than 24 hours
  - Hospitalization for elective treatment of a pre-existing condition unrelated to the study medication

  Results in persistent or significant disability/incapacity
- Is a congenital anomaly or birth defect
- Is an important medical event (defined as a medical event(s) that may not be immediately life-threatening or result in death or hospitalization but, based upon appropriate medical and scientific judgment, may jeopardize the patient or may require intervention (e.g., medical, surgical) to prevent one of the other serious outcomes listed in the definition above). Examples of such events include, but are not limited to, intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions not resulting in hospitalization; or the development of drug dependency or drug abuse.
- Pregnancy
  - Pregnancy of a patient or of the female partner of a male patient during the study or within 30 days after the last dose of study drug should be reported via an SAE report. Should pregnancy occur in a female participant during the treatment period, study drug should be discontinued. Should a pregnancy occur in a female companion of a male participant during the treatment period, the male participant can continue treatment. Any such pregnancy is to be followed until final outcome.

16.1.5 Unexpected Adverse Event

An adverse event, of which the specificity or severity is not listed in the study protocol, product inserts, investigator brochure or informed consent document.

16.1.6 Determining Attribution to the Investigational Agent(s)

Attribution: An assessment of the relationship between the AE and the medical intervention. CTCAE does not define an AE as necessarily *"caused by a therapeutic intervention"*. After naming and grading the event, the clinical investigator must assign an attribution to the AE using the following attribution categories:

Relationship	**Attribution**	**Description**
Unrelated to investigational agent/intervention	Unrelated	The AE is clearly **NO**T related
	Unlikely	The AE is **doubtfully related**
Related to investigational agent/intervention	Possible	The AE **may be related**
	Probable	The AE **is likely related**
	Definite	The AE **is clearly related**

16.1.7 Classification and Grading

Classification and grading of adverse event will utilize the National Cancer Institute Common Terminology Criteria for Adverse events (CTCAE) version 4.0 to determine the severity of the reaction for adverse event reporting. A copy of the current CTCAE version 4 is available at http://ctep.cancer.gov/reporting/.

16.2 Reporting of Adverse Events:

16.2.1 Participating Site Reporting Responsibilities:

Any serious adverse event or unanticipated problem occurring within 30 days of the last study treatment or procedure must be reported to the IU Simon Cancer Center **within 1 business day** of notification or discovery of the incident, using the SAE reporting Form found in the study procedures manual. SAEs that occur greater than 30 days from last dose of study treatment or procedure must be reported to the IU Simon Cancer Center if the event is possibly, probably, or definitely related to the study treatment or procedure. This form must be accompanied by a cover letter which: identifies the event, is signed by the local principal investigator or treating physician, includes the applicable study number and title, and contains the following:

- Site assessment of the event attribution to investigational product or study procedure
- Site assessment of event expectedness (expected vs. unexpected)
- Assessment of whether or not the research places subjects at a greater risk of harm than was previously known or recognized
- Assessment of the event's effect on the risk to benefit ratio
- Statement as to whether the informed consent statement should reflect changes in the potential risks involved

- Statement as to whether the event has been reported previously, and if so, whether the frequency is considered unusually high

Send to: IUSCC Clinical Trials Office
ATTN: Multicenter coordinator/ Protocol # IUSCC-0555
Fax: (317) 274-8022
E-mail: IUSCCSAE@iu.edu

The MNA, or designee, will distribute the reports to all participating sites per section 10.5.2 below. Copies of all serious adverse event reports or unanticipated problems reports will be kept on file in the IU Simon Cancer Center Clinical Trials Office.

16.2.3 Reporting to the IRB:

Each participating site will report adverse events and unanticipated problems to their IRB per local guidelines. Any event that requires expedited reporting to the local IRB will also be submitted to the IU Simon Cancer Center

16.2.1.4 Coordinating Center Reporting Responsibilities

The Coordinating Center will maintain documentation of all adverse event reports for each participating site in the case report forms in OnCore®. AE and SAE reports will be reviewed at weekly meetings. If an adverse event or SAE requires modification of the informed consent, protocol or other study documents, participating sites will be informed by way of an amendment and during monthly teleconferences, or sooner as deemed necessary by the Principal Investigator

16.2.1.5 Reporting to the IUSCC Data Safety Monitoring Committee:

Regardless of study sponsorship, the study team must enter all initial and follow-up SAE, expedited, and noncompliance reports into OnCore® for review by the DSMC chair and/or coordinator. Expedited reports may include IRB Prompt Report Forms, AdEERS reports, MedWatch, and additional SAE forms as required by the sponsor. When follow-up information is received, a follow-up report should also be created in OnCore®. This DSMC reporting requirement is **in addition to any other** regulatory bodies to be notified (i.e., IRB, pharmaceutical company, etc.). The DSMC chair and/or coordinator will review all SAE, expedited, and noncompliance reports monthly.

- Statement as to whether this adverse event has been reported previously, and if so, whether the frequency is considered unusually high

17. MULTICENTER GUIDELINES

17.1 Study Documents

Each participating site must submit regulatory documents (informed consents, 1572s, Financial Disclosures, IRB approval documents, Continuing Reviews, Amendments, patient brochures or recruitment material etc.) to the Coordinating Center. The Coordinating Center will provide each site with a comprehensive list of the required documents prior to study start-up, throughout the duration of the study and upon study close-out. It is the responsibility of the participating site to maintain copies of all documentation sent to the Coordinating Center.

17.2 Study Initiation

Before activating the clinical trial at each participating site, the IUSCC CTO MNA, or designee, will ensure that:

- Full **Institutional Review Board (IRB) approval** has been obtained.
- Research staff at the participating site has been trained in data entry into *OnCore*®
- A **start-up meeting** with each institution has taken place via telephone conference. The start-up meeting will cover protocol details (including eligibility criteria, treatment plan, etc.), responsibilities of the participating investigators, and reporting procedures.
- A financial **conflict of interest statement** from each investigator has been obtained.

17.3 Patient Enrollment

After eligibility is confirmed by the participating site staff, a completed eligibility checklist, supporting source documentation, and signed consent will be sent to IUSCC for verification. The MNA, or designee, will assign the patient a study number and return the enrollment information to the site. The site staff will then register the patient in OnCore®. *Additional details of this process can be found in the Study Procedure Manual.*

17.5 Data Monitoring

All multicenter investigator initiated trials conducted at the IUSCC are subject to data monitoring by the MNA, or designee. External sites will be notified of upcoming monitoring visits and will be expected to provide the MNA, or designee, with de-identified source documents for remote monitoring of patients. Queries will be issued in OnCore® and a detailed monitoring report will be provided to the participating site. The IUSCC will also forward any monitoring and/or auditing reports to the DSMC.

When a patient enrolled on this trial, or the trial itself, is selected for local monitoring or auditing, the participating site will forward the results to the MNA, or designee. In addition, if a participating site patient is selected for local auditing by the IUSCC DSMC, the site will be responsible for sending IUSCC de-identified source documents.

17.6 Record Retention

All documentation of adverse events, records of study drug receipt, dispensation, destruction, and all IRB correspondence will be stored in accordance with all applicable federal guidelines.

Following closure of the study, each participating site will maintain a copy of all site study records in a safe and secure location. The Coordinating Center will inform the investigator at each site at such time that the records may be destroyed.

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19. APPENDICES

19.1 Appendix I: NCI Common Toxicity Criteria Version 4.0

Due to the size of the latest version of the Common Toxicity Criteria, copies of this appendix are not included with this protocol document.

An electronic copy is available on the CTEP web site, http://ctep.cancer.gov/reporting/ctc.html

19.2 Appendix II: Performance Status Scales/Scores

ECOG or Zubrod		Karnofsky		Lansky	
Score	Activity	Score	Activity	Score	Activity
0	Fully active, able to carry on all pre-disease performance without restriction.	100	Normal, no complaints, no evidence of disease.	100	Fully active, normal.
		90	Able to carry on normal activity; minor signs or symptoms of disease.	90	Minor restrictions in physically strenuous activity.
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light housework, office work.	80	Normal activity with effort; some signs or symptoms of disease.	80	Active, but tires more quickly.
		70	Cares for self, unable to carry on normal activity or do active work.	70	Both greater restriction of and less time spent in play activity.
2	Ambulatory and capable of all selfcare but unable to carry out any work activities. Up and about more than 50% of waking hours.	60	Requires occasional assistance, but is able to care for most of his/her needs.	60	Up and around, but minimal active play; keeps busy with quieter activities.
		50	Requires considerable assistance and frequent medical care.	50	Gets dressed, but lies around much of the day; no active play; able to participate in all quiet play and activities.
3	Capable of only limited selfcare, confined to bed or chair more than 50% of waking hours.	40	Disabled, requires special care and assistance.	40	Mostly in bed; participates in quiet activities.
		30	Severely disabled, hospitalization indicated. Death not imminent.	30	In bed; needs assistance even for quiet play.
4	Completely disabled. Cannot carry on any selfcare. Totally confined to bed or chair.	20	Very sick, hospitalization indicated. Death not imminent.	20	Often sleeping; play entirely limited to very passive activities.
		10	Moribund, fatal processes progressing rapidly.	10	No play; does not get out of bed.

19.3 Appendix III: DSMC Check Sheet

Meeting Date:			
Team/Program: (include meeting sign in sheet)			
Protocol & Status (open/closed to accrual)			
PI:			
CRS:			
	Y	N	N/A
Weekly and Monthly meetings should include discussion on data, patient safety, dose levels, accrual numbers, significan toxicities, dose adjustments and responses observed, deviation summaries and SAE reports. (per IUSCC DSMP)			
Has patient safety data been discussed/reviewed? Have all SAE's and deviations been reported in Oncore for all IUSCC programs.			
Has all data been entered into Oncore per the *"Data Requirements for Cancer Center Reporting"* SOP?			
Deviation Log reviewed, discussed and signed by <u>ALL</u> team members including PI			
Has accrual been entered into Oncore for all IUSCC programs per the *"Data Requirements for Cancer Center Reporting"* SOP			
***Notes**			

19.4 Appendix IV: Potential Drug Interactions

Omeprazole inhibits CYP2C19. Patients taking drugs known to be substrates of CYP2C19 may enroll but the treating investigator should be aware of the potential drug interaction. When possible, changing to an alternative agent that is not metabolized by CYP2C19 is advised.

CYP2C19 substrates include:
cyclophosphamide
citalopram
amitriptyline
clopidogrel
voriconazole
diazepam
phenytoin
S-mephenytoin
imipramine
thalidomide
phenobarbitone
carisoprodol
chloramphenicol
clomipramine
hexobarbital
indomethacin
labetalol
R-mephobarbital
moclobemide
nelfinavir
nilutamide
primidone
progesterone
proguanil
teniposide

Specific guidance is available for the following potential interactions
Atazanavir and nelfinavir: omeprazole reduces plasma levels of atazanavir and nelfinavir.
Saquinavir: omeprazole increases plasma levels of saquinavir. Monitor for toxicity and consider dose reduction of saquinavir.
May interfere with drugs for which gastric pH affects bioavailability (e.g., ketoconazole, iron salts, erlotinib, ampicillin, esters, digoxin and mycophenolate mofetil). Patients treated with omeprazole and digoxin may need to be monitored for increases in digoxin toxicity.
Clopidogrel: omeprazole decreases exposure to the active metabolite of clopidogrel.
Cilostazol: omeprazole increases systemic exposure of cilostazol and one of its active metabolites. Consider dose reduction of cilostazol.
Drugs metabolized by cytochrome P450 (e.g., diazepam, warfarin, phenytoin, cyclosporine, disulfiram, benzodiazepines): omeprazole can prolong their elimination. Monitor and determine need for dose adjustments. Patients treated with proton pump inhibitors and warfarin may need to be monitored for increases in INR and prothrombin time.
Combined inhibitor of CYP2C19 and 3A4 (e.g., voriconazole) may raise omeprazole levels.
Tacrolimus: omeprazole may increase serum levels of tacrolimus.
Methotrexate: omeprazole may increase serum levels of methotrexate.