Local Protocol #: OSU-18317

ClinicalTrials.gov Identifier: NCT04205903

FDA IND#: 143553

TITLE: A PHASE Ib STUDY OF THE SAFETY AND PHARMACOLOGY OF NILOTINIB TO PREVENT PACLITAXEL-INDUCED PERIPHERAL NEUROPATHY IN PATIENTS WITH BREAST CANCER

Principal Investigators:

Nicole Williams, MD
Division of Medical Oncology
1800 Cannon Drive
1310K Lincoln Tower
Columbus, OH 43210
Tel: (614) 293-0066
Fax: (614) 293-7264
Email: nicole.williams@osumc.edu

Shuiying Hu, PhD
Pharmaceutics & Pharmacology
406 Biomedical Research Tower
460 W. 12th Ave.
Columbus, OH 43210
Tel: (614) 685-8028
Email: hu.1333@osu.edu

Other Agent(s): Nilotinib, Paclitaxel

Protocol Type:

Original / Version #1.0 / Version Date: 11/1/2/2018
Version #2: 2/19/2019
Version #3: 03/01/2019
Version #4: 10/22/2019
Version #5: 12/31/2019
Version #6: 04/24/2020
Version #7: 11/03/2020
Version #8: 01/29/2021
Version #9: 08/20/2021
Version #10: 07/28/2023
Version #11: 10/17/2024

Funding: National Cancer Institute 1R01CA238946-01 to Lustberg and Hu 04/01/2019

Table of Contents

1.0 TRIAL SUMMARY & SCHEMA

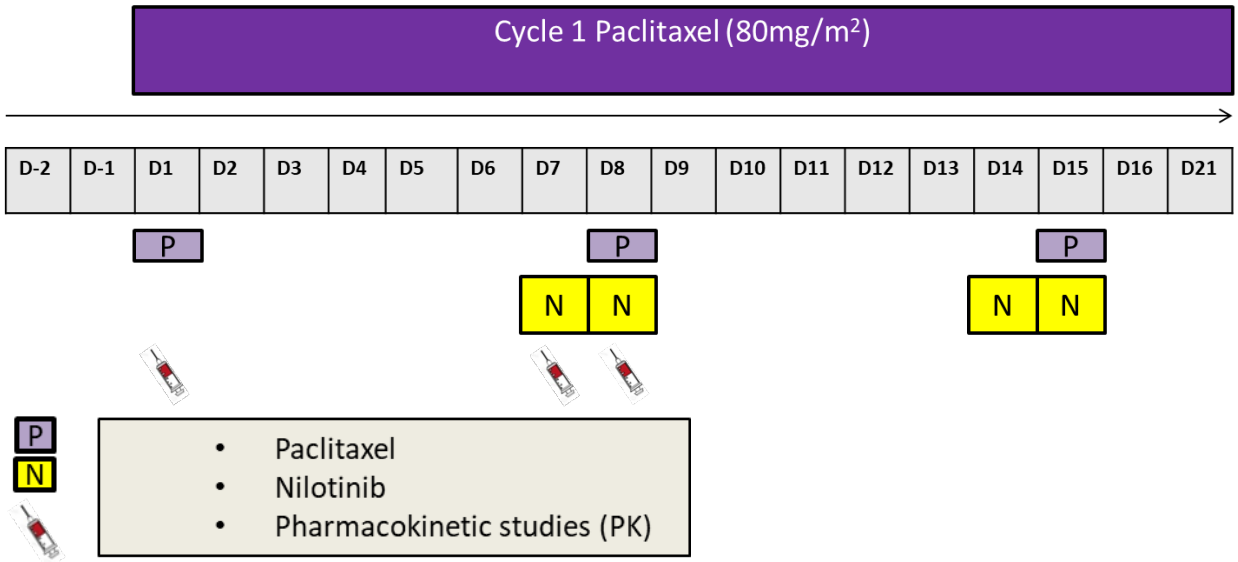
Abbreviated Title	PHASE Ib STUDY OF SAFETY AND PHARMACOLOGY OF NILOTINIB TO PREVENT PACLITAXEL-INDUCED PERIPHERAL NEUROPATHY
Trial Phase	Ib
Clinical Indication	Stages I-III Breast Cancer
Trial Type	Single arm Phase 1b
Type of control	None
Route of administration	Oral nilotinib IV paclitaxel
Trial Blinding	None
Treatment Groups	All study subjects will receive nilotinib.
Number of trial subjects	Approximately up to 20
Estimated enrollment period	2 years
Estimated duration of trial	Patients will be followed for 6 months after last paclitaxel dose
Duration of Participation	Approximately 6-9 months including observation. Active treatment maximum 12 weeks

1.1 Trial Design

This is a phase Ib trial in men and women with stage I-III breast cancer who are initiating chemotherapy with paclitaxel. Intermittent doses of nilotinib will be administered (Day-1 to Day 1; each dose administered with paclitaxel except cycle 1 day 1). Nilotinib will be administered orally **once a day** 24 hours before each paclitaxel infusion (excluding cycle 1 day 1) and then repeated approximately 30 minutes prior to paclitaxel infusion to evaluate DLTs, MTD, or MAD.

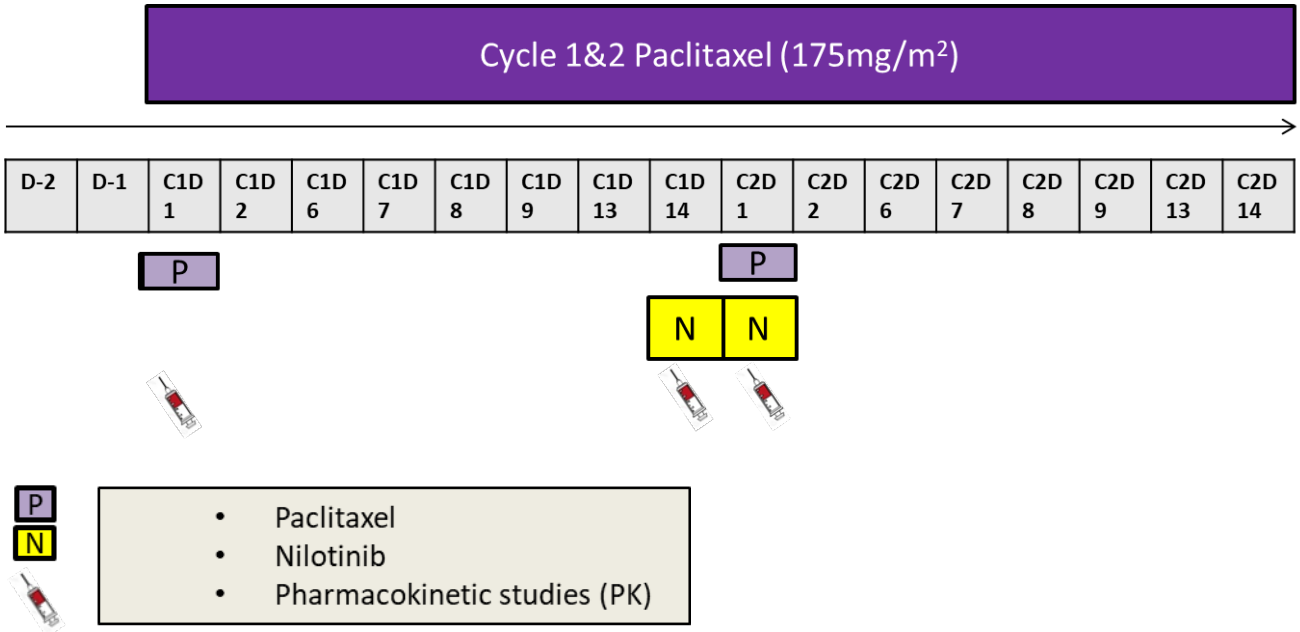
1.2 Study Schema Phase 1b

There are two paclitaxel dosing schedules administered in the clinic based on investigator discretion – either weekly paclitaxel or dose-dense paclitaxel given every two weeks. The study schemas below describe the interventions for the two dosing schedules.



Schedule A:

- weekly dose of 80mg/m^2, paclitaxel will be given weekly on days 1, 8, 15, of every 21 days.
- Nilotinib will be given orally on cycle 1 Days 7, 8, 14, 15 **once a day** 24 hours prior to the paclitaxel infusion and again 30 minutes prior to the paclitaxel infusion on days 8, 15.
- During the cycle 1, PK will be obtained at baseline, during, and up to 6 hours after paclitaxel or nilotinib administration on the days shown above.
- Patients will continue paclitaxel without nilotinib after cycle 1 as part of standard of care at the discretion of the treating investigator.



Schedule B:

- For dose dense schedule of 175mg/m2, paclitaxel will be given once every two weeks on

day 1.

- Nilotinib will be given orally on cycle 1 Days 14, once a day 24 hours prior to the cycle 2 paclitaxel infusion and again 30 minutes prior to the paclitaxel infusion on day 1.
- During the cycle 1 & 2, PK will be obtained at baseline, during, and up to 6 hours after paclitaxel or nilotinib administration on the days shown above.
- Patients will continue paclitaxel without nilotinib after cycle 2 as part of standard of care at the discretion of the treating investigator.

2.0 TRIAL OBJECTIVES AND ENDPOINTS

2.1. Primary Objectives

(1) To determine the recommended phase 2 dose (RP2D) of nilotinib in combination with paclitaxel for early stage breast cancer, defined as the lowest intermittent dose of nilotinib that can temporarily inhibit the function of OATP1B1 without affecting the plasma pharmacokinetics of paclitaxel.

(2) To determine the toxicity profile (based on CTCAE v. 5.0) of nilotinib in combination with paclitaxel in early stage breast cancer patients.

2.2 Secondary Objectives

(1) To determine the effect of paclitaxel on PK of nilotinib in the study population. To determine the effect of nilotinib on PK of paclitaxel in the study population.

2.3 Exploratory Objectives:

(1) To evaluate quality of life and chemotherapy induced neuropathy symptoms using the CIPN20 questionnaire

(2) To determine the disease-free survival (DFS), event free survival (EFS) and overall survival (OS) of nilotinib in combination with paclitaxel.

3.0 BACKGROUND

3.1 Chemotherapy-Induced Peripheral Neuropathy (CIPN)

Breast cancer detection and treatment has improved significantly in recent years, with breast cancer patients currently comprising the largest groups of cancer survivors. However, more than 30% of patients receiving chemotherapy experience chemotherapy-induced peripheral neuropathy (CIPN), which occurs as a result of several commonly used classes of chemotherapy drugs including taxanes, vinca alkaloids and platinum compounds. CIPN is predominantly a sensory neuropathy with symptoms of paresthesia and pain, but also includes a component of motor neuropathy. In many populations including the elderly and diabetics, as well as cancer patients, CIPN is also associated with not only pain, but also with falls and difficulty in walking and performing activities of daily living (ADLs).[1-3] Although symptoms may improve over time in many patients, symptoms can persist for years, and for some patients, full reversibility of symptoms may never occur. In addition to peripheral nervous system impairments, chemotherapy treatment is also associated with central nervous system impairments, including detrimental changes to blood flow in frontal lobes of the brain, which are important for motivation and neurocognition, as well as in brain areas responsible for processing pain. Combined, these nervous

system changes induced by chemotherapy and CIPN hinder the ability for patients to perform various ADLs.

3.2 Complications associated with CIPN

Among the taxanes, the drug paclitaxel is associated with an acute pain syndrome, known as paclitaxel-associated pain syndrome (P-APS). This syndrome occurs within 7 days of drug administration, and recent studies suggest that patients with P-APS are also at increased risk of CIPN.[4] As a consequence of this toxicity, dose modification and early completion of treatment may result and affect patients' progress. Preventative and therapeutic interventions have been attempted but have had limited success or have produced additional toxicity. To date, no preventative strategy for CIPN has been confirmed by randomized placebo controlled trials. A recently reported study with duloxetine as treatment for CIPN was promising.[5] Improved understanding of the pathophysiology of CIPN would help in the rational design of studies for the prevention and treatment of this very common cytotoxicity.

3.3 New Strategies for Prevention of CIPN: Targeting Paclitaxel Toxicity

To date, more than 40 randomized controlled clinical trials have been conducted to evaluate efficacy of prevention or treatment of peripheral neuropathy, but none have provided convincing evidence for a clinically-beneficial agent.[6] These studies have been limited by the failure to develop interventions based on a firm mechanistic rationale. The translational exploration of many of the proposed intervention strategies has been hampered by findings that (*i*) paclitaxel has multiple intracellular targets, hence blocking a single injurious event will only have partial protective effects; and (*ii*) the protective approach may diminish the antitumor effects of paclitaxel given the potential overlap in cell death signaling pathways between normal cells and tumor cells. Therefore, an optimal approach is to simultaneously protect the peripheral nerves against toxicity from paclitaxel without affecting its therapeutic effects in tumors. The development of such an approach would rely on the identification of the critical differences between normal and malignant cells that drive toxic responses to paclitaxel. We recently found that paclitaxel accumulates in cells through a process mediated by the murine organic anion transporting polypeptide OATP1B2 (OATP1B1 and OATP1B3 in humans; **Fig 1**).[7] We also found that paclitaxel induces acute and chronic forms of neurotoxicity in mice in an OATP1B2-dependent manner, and that these phenotypes can be completely reversed by pre-treatment with the tyrosine kinase inhibitor (TKI) nilotinib (**Fig 2**).[7]

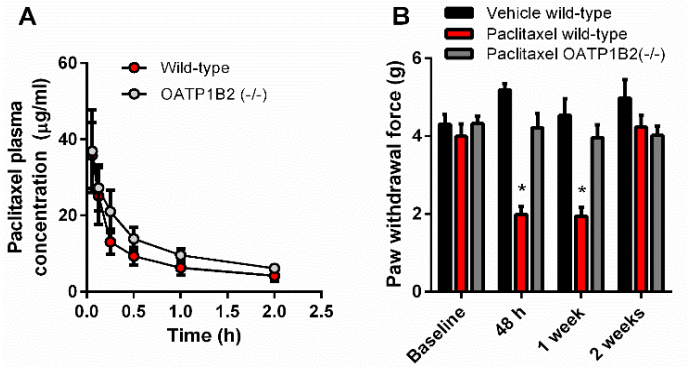


Figure 1. Dependence of acute paclitaxel-induced peripheral neuropathy on OATP1B2. (**A**) Plasma levels of paclitaxel in wild-type and OATP1B2(-/-) mice (n=4); (**B**) Mechanical allodynia as determined by a Von Frey Hairs test (n=8). Data present mean ± SE after one (**A**) or 2 (**B**) doses of paclitaxel (10 mg/kg, i.v.; time 0 and 72 h). The star (*) denotes significant differences from baseline and OATP1B2(-/-) mice (P<0.01).

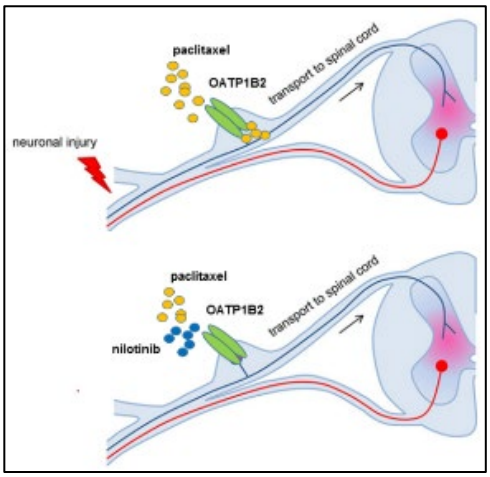


Figure 2. Proposed model of paclitaxel-induced injury to the peripheral nervous system. Paclitaxel enters the nervous system through OATP1B2, ultimately leading to peripheral neuropathy (top). These effects can be blocked by the OATP1B2 inhibitor nilotinib (bottom).

Compared to other TKIs, nilotinib has features that suggest it might be an excellent modulator of paclitaxel toxicity, including good oral absorption, a relatively slow clearance, and a long half-life.[8] In our studies, we aim to interrogate the response of the peripheral nervous system to the nilotinib-paclitaxel combination following acute or intermittent exposure to the TKI. Therefore, we anticipate that nilotinib will not be intrinsically toxic in such combination therapy regimens We have detailed, below, additional data that supports that the proposed once a day doses of nilotinib are lower than currently approved labeling doses for this drug, are safe, and do not increase paclitaxel toxicity (Please see section 3.6). In order to assess the degree of OATP1B1 inhibition by nilotinib, analysis of glycochenodeoxycholate sulfate (GCDGA-S) and chenodeoxycholate-24-glucuronide (CDCA-24G) levels will be performed in plasma samples as validated surrogate endogenous substrates of OATP1B1. Previous clinical studies have indicated that the area under the curve (AUC) of GCDCA-S can be increased >20-fold following treatment with OATP1B1 inhibitors, whereas CDCA-24G is only detectable after the administration of OATP1B1 inhibitors.[9] Based on our preclinical studies, we expect that the administration of nilotinib will result in dose-dependent changes in levels of GCDAGA-S and CDCA-24G caused by inhibition of OATP1B1. Since the initial approval of the protocol, significant progress has been made in the identification of other endogenous biomarkers to assess the OATP1B transporter function. With rich clinical datasets, emerging evidence suggests that coproporphyrin-I (CP-I) and coproporphyrin-III (CPIII), compounds formed in the body as byproducts of heme synthesis, have higher selectivity and sensitivity and can be used to better assess in vivo OATP1B1 activity.[10-12] Furthermore, we recently identified α-tocopherol as a DRG-specific OATP1B-type transporter biomarker that can be measured in the circulation[13], analysis of additional biomarker CP-I, CPIII as well as α-tocopherol could provide more accurate assessment of OATP1B1 transporter activity with nilotinib, which could serve as a companion diagnostic to guide dose selection of pharmacological inhibitors in the future development of combinatorial regimens with chemotherapy drugs.

Although combining paclitaxel with nilotinib could possibly reduce the incidence and severity of neuropathy, it is important to establish that the anticancer efficacy of paclitaxel is not compromised by nilotinib. We already confirmed that the OATP1B1 and OATP1B3 genes are expressed at low levels in MBC samples and that the cellular uptake and cytotoxicity of paclitaxel are not reduced by nilotinib in MBC cell lines.[7] In addition, the nilotinib-paclitaxel combination was recently identified as a synergistic drug pair *in vivo*, resulting in tumor regressions in a MBC xenograft model, with no tumor regrowth observed for more than 80 days following the end of therapy.[14] Although *in vivo* confirmation is required in patient-derived xenograft models that more faithfully represent the characteristics of primary human MBC,[15] these initial observations indicate that combining paclitaxel with nilotinib has the potential to simultaneously reduce toxicities and increase anticancer effects.

3.4. Measuring CIPN

Patient reported outcomes (PRO) are the gold standard for evaluation of CIPN symptoms.

Multiple validated instruments have been evaluated in large scale studies. For the purposes of this study, CIPN-20 will be used.[16]

3.4.1 CIPN 20

CIPN 20 is a validated instrument for longitudinal evaluation of neuropathy symptoms induced by chemotherapy. This 20-item self-report questionnaire includes three subscales evaluating sensory, motor and autonomic symptoms. It is easy to use and is completed by patients independently. This instrument was designed and validated to evaluate patients' symptoms of CIPN during chemotherapy treatment with a number of chemotherapeutic agents, including taxanes.[17]

3.4.2 Other Assessments

Each participant will also have a diary to report daily changes in symptoms. Changes in the chemotherapy dosing, dose delays, discontinuation of therapy, and the amount of analgesic use during the study period will also be recorded by research coordinator. All toxicities will be assessed by CTCAE V5.

3.5 Rationale and Feasibility

There are multiple gaps in our understanding of CIPN despite its commonness and pervasive negative impact on drug adherence and quality of life in many cancer survivors. Safe and affordable strategies that may help prevent the onset or ameliorate joint symptoms are therefore needed. The overall objectives of this application are to evaluate a promising targeted therapy intervention for prevention of taxane induced CIPN in breast cancer patients by first confirming the MTD of the intermittent dosing of nilotinib with paclitaxel. The MTD will be evaluated in a future randomized phase II study to evaluate preliminary efficacy for prevention of CIPN.

3.6 Preliminary safety and tolerability of nilotinib with paclitaxel

Based on personal communication and a submitted confidential abstract, submitted International Conference on Molecular Targets and Cancer Therapeutics the preliminary data from the ongoing study at NCI (Clinicaltrials.gov ID NCT02379416) patients with solid tumors (n=30) treated for up to 41 cycles with weekly paclitaxel and twice daily nilotinib. The MTD was 80 mg/m2 paclitaxel and nilotinib 300 mg bid, the combination has been well tolerated. **There have been no paclitaxel dose delays due to myelosuppression and no QTC prolongation.** No participants have terminated study protocol due to toxicity. Preliminary response data are promising, especially considering the PRs observed in pts who had previously been unresponsive to paclitaxel therapy. Of the 24 pts assessable for response to date, there are 4 confirmed partial responses (PRs; 17%, 1 pt with endometrial cancer, 1 pt with anal cancer and 2 pts with granulosa cell of the ovary) and 12 pts with a best response of stable disease (SD; 50%), including 6 pts with SD for ≥8 cycles. Mean time on study to date is 8.7 months (range: 2 – 41 months). Three of 4 responding pts had undergone prior paclitaxel-based therapy and experienced a best response of only SD (1 pt) or progressive disease (2 pts) to carboplatin/paclitaxel or paclitaxel monotherapy, respectively. Preliminary results from NCT02379416 are described below in Table 1.

Table 1. Preliminary Data from NCT02379416

Condition	Number of Patients
Paclitaxel Dose Delays due to Myelosuppression	0
Paclitaxel Dose Delays due to QTC Elongation	0
Termination from Study due to Toxicity	0
Partial Responses to Paclitaxel after Prior Unresponsiveness	4 (of the 24 with available data)
Maintained Stable Disease	12 (of the 24 with available data) *6 for 8 or more months

Data collected from 30 patients with solid tumors treated with up to 41 cycles of weekly paclitaxel and twice daily with nilotinib. MTD = 80mg/m2 paclitaxel and 300 mg nilotinib bid. Mean time on study is 8.7 months (2-41).

Given the intermittent dosing of nilotinib, we expect toxicity to be very low. Additionally, all our preclinical modeling suggests no pharmacokinetic interaction between paclitaxel and nilotinib. Additionally, in phase 1b, interaction between the two drugs will be closely monitored.

4.0 METHODOLOGY

4.1 Entry Criteria

4.1.1 Inclusion Criteria

1. Men or Women with a known diagnosis of breast cancer stages I-III
2. Be eligible for weekly or dose dense single agent paclitaxel therapy based on physician assessment
3. Be ≥18 years of age.
4. Have an ECOG performance status ≤ 2
   - Patients with ECOG scores of 3 or greater typically do not receive chemotherapeutic intervention
5. Demonstrate adequate organ and marrow function as defined in Table 2.

Table 2. Adequate Organ and Marrow laboratory values

	Laboratory values
Leukocytes	≥ 2,000/μL
Absolute neutrophil count	≥ 1,500/μL
Platelets	≥ 100,000/μL
Total bilirubin	≤ UNL
AST (SGOT)/ALT (SGPT)	≤ 2.5 × Institutional upper limit of normal*

Creatinine	Within normal institutional limits **OR**
	≥50mL/min for patients with creatinine levels above institutional normal.
QTc	<450 milliseconds

6. If a female subject is with child bearing potential, she must have a negative pregnancy test at screening.
7. Female subjects of child-bearing potential and men must agree to use adequate contraception prior to study entry, for the duration of study participation and for 3 months after completion of study treatment administration. Adequate contraception includes methods such as oral contraceptives, double barrier method (condom plus spermicide or diaphragm), or abstaining from sexual intercourse.
8. Be willing and able to understand and sign the written informed consent document.
9. Demonstrate adequate electrolyte values as defined below. Hypokalemia and/or hypomagnesemia must be corrected prior to initiating nilotinib:
   - Calcium 8.6-10.5mg/dL
   - Magnesium 1.6-2.6mg/dL

4.1.2 Exclusion Criteria

1. Known distant metastatic disease
2. Is HER2+ and is receiving paclitaxel in conjunction with trastuzumab +/- pertuzumab
3. Has experienced > grade 1 neuropathy during previous therapies for early stage breast cancer.
4. Has experienced prior treatment-related toxicities that have not recovered to grade 1 or less (except for alopecia).
5. Has a history of grade 3-4 immediate hypersensitivity reaction to paclitaxel.
6. Has a history of clinically significant allergic reactions attributed to compounds of similar chemical or biologic composition to nilotinib or paclitaxel.
7. Has uncontrolled intercurrent illness including, but not limited to, ongoing or active infection, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situations that would limit compliance with study requirements.
8. Is currently pregnant or breast feeding as there is an unknown but potential risk for adverse events in nursing infants secondary to treatment of the mother with nilotinib and paclitaxel.
9. Has any other medical or psychiatric condition that in the opinion of the investigator would make the study therapy unsafe for the patient.
10. Has gastrointestinal (GI) disorders or impairment of GI function that is likely to significantly alter the absorption of nilotinib
11. Has a marked baseline abnormal heart rhythm such as prolongation of QT/QTc interval (e.g., repeated demonstration of a QTc of > 450msec)
12. Has a history of additional risk factors for TdP (e.g., heart failure, hypokalemia,

hypomagnesemia, family history of Long QT Syndrome)

13. Uses potent CYP3A4 inhibitors (grapefruit juice, cyclosporine, ketoconazole, ritonavir) and if treatment cannot be either safely discontinued or switched to a different medication prior to starting nilotinib.
14. Has a known diagnosis of HIV and is currently taking combination antiretroviral therapy known or suspected to affect paclitaxel PK.
15. Is concurrently using potent OATP1B1 inhibitors, including antibiotics (rifampicin, rifamycin SV, systemic fusidic acid, clarithromycin, erythromycin, roxithromycin, telithromycin), antiretrovirals (indinavir, saquinavir, ritonavir), cyclosporine, and gemfibrozil.

Please note: Anthracycline regimens are often used sequentially with paclitaxel based regimens in breast cancer. However, whether anthracyclines are used prior or after paclitaxel treatment has not been shown to significantly impact outcomes. Prior exposure to anthracyclines or subsequent anthracyclines should not impact our study. Therefore, any patient who meets the above eligibility criteria and is receiving single dose paclitaxel over the duration of the present study is still eligible, regardless of prior anthracycline regimens.

4.2. Study Location and Sample

This study will be open for accrual at the Stefanie Spielman James Comprehensive Breast Center, part of The Ohio State University Comprehensive Cancer Center. Patient eligibility will be determined according to the eligibility criteria listed. The study will enroll up to approximately up to 20 patients in this phase Ib trial.

4.3 Registration and Stratification

To register a patient, the following documents must be completed by the study coordinator:
(1) Patient Consent Form - signed
(2) Patient HIPAA Authorization Form - signed
(3) Consent Documentation Note
(4) Eligibility Checklist - completed & signed
(5) Source documents verifying every inclusion & exclusion criteria. Note: Every inclusion and exclusion criteria must be documented in the patient's medical record (emails or other notes outside the medical record will not be considered source documentation)
(6) Results from baseline tests required per the Study Calendar.
(7) Registration Form - completed
Upon receipt of all required registration documents and upon verification the patient meets all eligibility criteria, the study coordinator will:

- Assign the patient a study sequence ID.
- Register the patient on the study.

Following registration, patients should begin protocol treatment within 14 business days. Issues that would cause treatment delays should be discussed with the Principal Investigator as soon as possible. If a patient does not receive protocol therapy following registration, the patient's registration on the study may be canceled, after discussion with the Principal Investigator and study coordinator.

All the patients enrolled in this study will receive nilotinib.

4.4 Study Treatment

In Schedule A (weekly paclitaxel), the starting dose of nilotinib will be 100mg p.o. once per day on 2 occasions 24hrs before and 30 min before paclitaxel on C1D7, C1D8, C1D14, C1D15. Paclitaxel will be given at 80 mg/m^2 throughout all dose levels (unless dose reduction is needed for other clinically standard criteria). In Schedule B (dose dense paclitaxel), nilotnib will be given as 100mg p.o. once per day on two occasions 24hrs before and 30min before paclitaxel on C1D14 and C2D1. Duration of paclitaxel for both schedules is for 4 cycles of doses unless there is a clinical indication to terminate therapy earlier per treating physician. Paclitaxel will be administered in the standard regimen and followed the package insert and institutional guidelines for its administration and management of hypersensitive reactions (both acutely and for subsequent doses) will be followed (Section 1.2 study schema).

4.5 Clinical Assessments

Participants will be evaluated for toxicity at baseline and on D1 of each cycle as part of clinic visit. In addition, clinical research coordinator will evaluate patients as part of a research encounter prior to each infusion. Toxicity evaluation of neuropathy will be recorded based on CTCAE criteria v. 5 for sensory and motor neuropathy in addition to CIPN 20.

4.6 Evaluation of Neuropathy Symptoms

4.6.1 Patient Reported Outcomes

Patient reported outcomes (PRO) are the gold standard for evaluation of CIPN symptoms. Multiple validated instruments have been evaluated in large scale studies. For the purposes of this study, CIPN-20 will be used.[16] Each participant will also have a diary to report daily changes in symptoms. Changes in the chemotherapy dosing, dose delays, discontinuation of therapy, and the amount of analgesic use during the study period will also be recorded by research coordinator.

4.7 Duration of Therapy

In Schedule A (weekly paclitaxel), nilotinib will be given only on C1D7, C1D8, C1D14, C1D15. Patients will remain on study until C4D21 of paclitaxel (unless they come off due to progression of disease) for the purpose of monitoring for the occurrence of neuropathy as defined by NCI CTCAE V5 and data on quality of life using CIPN20 questionnaire. In Schedule B (dose dense paclitaxel), nilotnib will be given on C1D14 and C2D1 only. Patient will remain on study until Cycle 4 Day 1. Further continuation of paclitaxel will be at the discretion of the treating investigator. In the absence of treatment delays due to adverse event(s), patient will stay on study until one of the following criteria applies:

- Therapy completion,
- Disease progression,
- Intercurrent illness that prevents further administration of treatment,
- Unacceptable adverse event(s),
- Patient decides to withdraw from the study, or
- General or specific changes in the patient's condition render the patient unacceptable for further treatment in the judgment of the investigator

4.8 Duration of Follow up

Patients will be followed 3 months and 6 months after completion of paclitaxel. Patients removed from study for unacceptable adverse event(s) will be followed until resolution or stabilization of the adverse event.

4.9 Criteria for Removal from Study

The following criteria will be used for removal of patients from the study: (1) patient withdraws their participation; (2) patient has a disease progression or dies (whichever occurs first); (3) the study is terminated by the Principal Investigator; (4) patient develops neuropathy or other condition that prohibits continued treatment with paclitaxel; (5) pregnancy or failure to practice appropriate contraception. The reason for study removal and the date the patient is removed must be documented in the Case Report Form.

4.10 Adherence

Participant adherence to the study intervention will be encouraged and monitored in several ways. Subjects will be encouraged to maintain a daily diary in which to check off the doses of study drug when taken. At study visits, participants will return their pill containers to study personnel, who will count and record leftover pills.

5.0 PHARMACEUTICAL INFORMATION

5.1 Paclitaxel

Taxanes (including paclitaxel, docetaxel and nab-paclitaxel) are among the most active cytotoxic agents for the treatment of breast cancer.[18-20] Paclitaxel was originally isolated from the bark of the Pacific yew tree (taxus brevifolia) and subsequently found to have anti-tumor properties in the early 1970s.[21, 22] Its mechanism of action relates to binding and stabilization of microtubules causing inhibition of their depolymerization, leading to mitotic arrest.[23] Paclitaxel is hydrophobic and has poor solubility in water. Therefore, this agent is solubilized in 50% polyoxyethylated castor oil (Cremophor EL) and 50% ethanol. Cremaphor EL vehicle is associated with hypersensitivity reactions which require premedication with corticosteroids and histamine receptor blockers to minimize its incidence and severity.[24] The majority of hypersensitivity reactions occur during the first 1-2 administrations of paclitaxel, which has led to studies demonstrating that these premedications can be safely withdrawn in patients for whom hypersensitivity to paclitaxel formulation does not develop after the first 2 treatments.[25]

Paclitaxel administered intravenously at a dose of 175 mg/m^2 for 3 hours every third week has emerged as an active and safe initial and salvage therapy for early stage breast cancer with associated response rates around 30-40% and survival of about 19 months in patients with untreated early stage breast cancer.[18, 26] Since the activity of paclitaxel is directly related to the cell cycle, shortening the interval between treatments might improve efficacy.[27] In addition, paclitaxel administered in a more continuous manner exhibits pro-apoptotic and antiangiogenic properties, increasing its antineoplastic effects.[28] Indeed, subsequent randomized studies confirmed that weekly paclitaxel administration at 80mg/m2 was superior to every-3-week schedules in treatment of early stage and operable breast cancer.[29-31] Weekly paclitaxel almost

doubled the time to progression and increased the response rate from 29% to 42% without affecting quality of life in patients with stages I-III breast cancer.[29] Additionally, dose dense paclitaxel at 175 mg/m2 has also been shown to be an effective regimen in breast cancer.[32]

5.1.1 Adverse Events of Paclitaxel

Please see section 7.3 for detailed toxicities reported on paclitaxel.

5.1.2 Dose and Schedule of Paclitaxel

This study will utilize the weekly paclitaxel schedule and the dose dense paclitaxel schedule, as these regimens have been extensively found to have superior efficacy to 3 week schedule paclitaxel.[29-31] We are therefore proposing the use of either the standard weekly 80 mg/m2 paclitaxel dose schedule or the 175 mg/m^2 dose dense paclitaxel every two weeks dose schedule. The information on the safety profile of the combination and effect of nilotinib pharmacokinetics of paclitaxel will be collected in this trial.

5.2 Nilotinib

Nilotinib (Tasigna^R) will be purchased in doses specified. Nilotinib is indicated for the treatment of adult and pediatric patients greater than or equal to 1 year of age with newly diagnosed Philadelphia chromosome positive chronic myeloid leukemia (Ph+ CML) in chronic phase. For CML, Nilotinib is taken twice daily at approximately 12-hour intervals and must be taken on an empty stomach. No food should be consumed for at least 2 hours before the dose is taken and for at least 1 hour after the dose is taken. Please note this is not the dosing recommended for this proposal and we are proposing once daily intermittent dosing.

5.2.1 *Adverse Events of Nilotinib*

Please see section 7.3 for detailed toxicities reported on nilotinib. Please note that these toxicities are for continuous dosing of drug but in this study only intermittent dosing will be used. Therefore, anticipated toxicity is expected to be much less.

5.2.2 *Dose and Schedule of Nilotinib*

The recommended dose of nilotinib in CML is 300 mg orally twice daily. However, in this study nilotinib will be administered p.o. once per day on 2 occasions 24hrs before and 30 min before paclitaxel on unless DLT encountered. A total of up to 4 nilotinib dose levels will be considered; 50, 100, 200, and 300mg in the phase Ib portion of the trial (**Tables 3a and 3b**). The maximum sample size will be 20 with cohort size of 2. The first cohort of patients will be enrolled at dose level 1 (nilotinib 100 mg). After each cohort is treated and evaluated for toxicity and efficacy as defined in the context of this trial, these data will inform our dynamic, adaptive model to determine dose escalation decisions based on changes to estimated probabilities for toxicity and for efficacy given the data observed. Then next cohort of patients will be treated on the dose level determined from the Bayesian adaptive model.

Nilotinib must be taken on an empty stomach and no food should be consumed for at least 2 hours before the dose is taken. Food should be avoided for at least 1 hour after the dose is taken. All participants in the study will be advised of this.

5.3 Paclitaxel and nilotinib interactions

Based on the NCI data Clinicaltrials.gov ID NCT02379416, the risk of myelosuppression and dose delays with our regimen of intermittent nilotinib dosing is expected to be unlikely. Based on our recent preclinical experience of nilotinib given in combination with paclitaxel, we anticipate that nilotinib will not affect the disposition profile of paclitaxel and vice versa. The expectation that nilotinib is unlikely to influence the pharmacokinetics of paclitaxel would be consistent with previously reported studies confirming that other OATP1B1-inhibiting TKIs identified from our screens, such as axitinib, and sorafenib, do not alter the systemic exposure to paclitaxel. The above noted NCI study confirmed our preclinical anticipation, there were no observed adverse PK interactions between nilotinib and paclitaxel. Although the drug-drug interaction in a clinical setting is highly unlikely, we will perform real-time PK analyses for both paclitaxel, nilotinib and in combination for parent and metabolites.

6.0 POTENTIAL TOXICITY, DOSE MODIFICATIONS, AND MANAGEMENT

Toxicity will be monitored during study visits and telephone calls using the National Cancer Institute Common Terminology Criteria for Adverse Events version 5.0 (CTCAE) of the National Cancer Institute will be used: (https://ctep.cancer.gov/protocoldevelopment/electronic_applications/docs/CTCAE_v5_Quick_Reference_5x7.pdf). Any Grade 1 or greater toxicities will be reported as adverse events.

Patients with any **Grade ≥3 adverse reactions or** with an absolute QTc≥ 500msec,or increase of baseline QTc of > 60msec during the first cycle will count toward DLT determination. If a patient has a DLT, nilotinib will be stopped and paclitaxel will be continued per treating physician. See Section 6.1 for the definition of DLTs.

Table 3a. Phase 1b dose modifications for nilotinib and paclitaxel during cycle 1 – Schedule A (weekly paclitaxel)

Dose Level	***Nilotinib (Refer to study schema)***	***Weekly Paclitaxel (mg/m^2 IV on days 1, 8, 15)***
Level -1	50 mg (one 50 mg capsule once daily 24 hours before and day of paclitaxel treatment)	80
Level 1 **(Starting dose level)**	100 mg (two 50 mg capsules once daily 24 hours before and day of paclitaxel treatment)	80

Level 2	200mg (four 50mg capsules once daily 24 hours before and day of Paclitaxel treatment)	80
Level 3	300mg (six 50mg capsules once daily 24 hours before and day of paclitaxel treatment)	80

Table 3b. Phase 1b dose modifications for nilotinib and paclitaxel during cycle 1 – Schedule B (dose dense paclitaxel)

Dose Level	***Nilotinib (Refer to study schema)***	***Dose dense Paclitaxel (mg/m^2 IV)***
Level -1	50 mg (one 50 mg capsule once daily 24 hours before and day of paclitaxel treatment	175
Level 1 **(Starting dose level)**	100 mg (two 50 mg capsules once daily 24 hours before and day of paclitaxel treatment	175
Level 2	200mg (four 50mg capsules once daily 24 hours before and day of Paclitaxel treatment)	175
Level 3	300mg (six 50mg capsules once daily 24 hours before and day of paclitaxel treatment)	175

We do not anticipate significantly increased toxicities given intermittent dosing of drug and administration of drug nilotinib only during cycles 1 and 2, if using the dose dense schedule, and excellent tolerability and lack of myelosuppression with continuous dosing noted in Clinicaltrials.gov ID NCT02379416.

Dose levels for phase Ib will follow **Tables 3a and 3b.** Movement across these levels will be per statistical models as outlined in section 11.2

Nilotinib is started at dose level 1. Dose escalations and de-escalations will be based on the observed dose limiting toxicities and efficacy (OATP1B1 inhibition) of the patients who have been treated as described in section 11.2.

Dose modification after cycle 1 (and cycle 2 for the dose dense schedule) for paclitaxel will be left up to treating physician discretion since patients are no longer on nilotinib.

Note: In order to receive weekly paclitaxel during cycle 1 the following criteria must be met:

- ANC ≥ 1000/μL
- Platelets ≥ 75,000/μL
- No grade 3 toxicity

If paclitaxel is held for any indication, nilotinib will not be administered. If nilotinib is held for any indication, paclitaxel can be given based on treating physician discretion but the patient will need to come off study. In phase 1b, if any dose holds of nilotinib or paclitaxel for any reason other than dose-limiting toxicity, happens within the first cycle, will need to discuss with PI whether participant needs to be replaced.

In order to initiate nilotinib, the following criteria must be met:

- Calcium ≥8.6mg/dL
- Magnesium ≥1.6.dL

If these criteria are not met, treatment must be delayed until toxicity is improved to grade 2 or better. Hypokalemia and/or hypomagnesemia must be corrected prior to initiating nilotinib. Filgrastim may not be used during cycle 1 or cycle 2 for weekly paclitaxel. For the dose dense schedule, standard of care pegfilgastrim will be used to maintain adequate blood counts per routine care practice.

For any grade ≥ 2 QTC prolongation or any grade ≥ 2 cardiac event, nilotinib should be held as per the United States Prescribing Information for nilotinib, and cardiology should be consulted prior to restarting the patient on nilotinib.

6.1 Assessment of Dose Limiting Toxicities

The DLT observation period for this trial will be the first cycle for schedule A and the second cycle during schedule B; thus, toxicities observed during the specified DLT period of treatment will be used to direct decisions related to dose escalation and ultimately to determine the RP2D. Patients who do not complete the first cycle for schedule A and cycle 1 and 2 for schedule B of combination treatment for reasons other than toxicity or other treatment-related reasons will not be considered evaluable for DLT and will be replaced with another patient at that dose level.

Grading of toxicities will be assessed by the use of Common Terminology Criteria for Adverse Events (CTCAE) version 5.0. DLTs will consist of any adverse events that are at least possibly attributed to nilotinib in the first cycle of phase Ib schedule A and in cycle 2 for schedule B and are further defined as follows:
We acknowledge that the toxicities due to nilotinib and paclitaxel can be similar and any possible attribution to nilotinib will count toward DLT determination if it fits definitions below. Further, if the adverse event is not a known toxicity specific to paclitaxel and is felt to be at least possibly related to treatment, we will conservatively attribute it as possibly related to nilotinib. All adverse events of the specified grades will count as DLTs except those that are clearly and incontrovertibly due to disease progression or extraneous causes.

Hematologic DLTs

- Grade 4 neutropenia
- Grade ≥ 3 neutropenia, complicated by a fever
- Grade 4 thrombocytopenia
- Grade 3 thrombocytopenia, complicated by bleeding

Non-hematologic DLTs

- Any CTCAE version 5.0 Grade ≥ 3 non-hematologic toxicity, unless the event is clearly unrelated to treatment -- EXCEPT the following:
  - Grade ≥ 3 nausea, vomiting, or diarrhea that resolves to Grade ≤2 within 48 hours, with or without medical intervention or prophylaxis.
  - Grade ≥ 3 fatigue lasting for 1 week or longer.
  - Transient (< 14 days) increase in LFTs (of ≤ 1 Grade in severity) compared to baseline levels in patients with baseline liver metastases.
  - Grade 3 maculopapular rash for which symptoms are easily managed with supportive care and no evidence of superinfection or limitation of self-care ADLs.
  - Grade ≥ 3 electrolyte abnormality lasting longer than 72 hours, unless the patient has clinical symptoms in which case all grade ≥ 3 abnormalities regardless of duration. Grade ≥ 3 amylase or lipase elevation not associated with symptoms or clinical manifestation of pancreatitis do not need to be counted.

Patients with an absolute QTc≥ 500msec, QTc< 320msec, or increase of baseline QTc of > 60msec.

If, during the DLT observation period, a patient experiences a toxicity that does not clearly fit any of the above DLT criteria but in the opinion of the investigator is highly clinically significant, the toxicity may be considered a DLT after discussion with the

7.0 TREATMENT PLAN

7.1 Treatment Plan and Overview

Eligible patients will be approached by study personnel in the clinics at Stefanie Spielman Comprehensive Breast Center. The person obtaining informed consent will tell the patient that 1) participation is voluntary, 2) participation or non-participation will not affect their usual care and management, and 3) patient confidentiality will be maintained in the event that the results of the study are published. The potential toxicities associated with nilotinib will be explained fully to the patient. Patients will be informed of the need for, blood tests, physical examinations, questionnaires prior to entry into the study and at several specified intervals during study period. Patients will be provided with a consent form to review, and all questions will be answered.

In this **phase Ib** study**,** all participants will receive nilotinib during first cycle (Schedule A with weekly paclitaxel) and during the second cycle (Schedule B with dose dense paclitaxel).

Schedule A (weekly paclitaxel): On cycle 1 days 1, 8, 15 paclitaxel infusion will be given at a dose of 80mg/m^2 as shown in Schema. Duration of paclitaxel is for 12 weekly doses unless there is a clinical indication to terminate therapy per treating physician.

- On cycle 1 day 7 and day 14, nilotinib will be taken once a day 24 hours before paclitaxel infusion. On day 8 and day 15, nilotinib will be taken once a day 30 minutes before paclitaxel infusion.

- Clinic visits and or research visits (see calendar) will take place prior to nilotinib administration, 24 hour before paclitaxel infusion to ensure that patients are cleared for therapy. When no nilotinib is being administered, patients are seen on day of paclitaxel infusion.

Schedule B (dose dense paclitaxel): On cycle 1 day 1 paclitaxel infusion will be given at a dose of 175mg/m^2 every 2 weeks as shown in Schema. Duration of paclitaxel is for 4 doses unless there is a clinical indication to terminate therapy per treating physician.

- On cycle 1 day 14, nilotinib will be taken once a day 24 hours before paclitaxel infusion. On day 1 cycle 2, nilotinib will be taken once a day 30 minutes before paclitaxel infusion.

- Clinic visits and or research visits (see calendar) will take place prior to nilotinib administration, 24 hour before paclitaxel infusion to ensure that patients are cleared for therapy. When no nilotinib is being administered, patients are seen on day of paclitaxel infusion.

Self-report diaries (adverse events, record of study drug doses taken/missed) will be collected, and pill count will be recorded by the research coordinator. Medications and supplements will be reviewed with participants. A history and physical examination will be performed at beginning of each cycle. Study drugs will be dispensed once at the initiation of study protocol with enough pills for a cycle of treatment. Pill compliance will be conducted with the start of

each cycle through pill counts and pill diaries to ensure proper reconciliation and adherence.

If the patient's chemotherapy is held, the patients should also hold study medication.

7.2 Study Calendar

Baseline evaluations are to be conducted within 2 weeks prior to start of protocol therapy. In the event that the patient's condition is deteriorating, laboratory evaluations should be repeated within 48 hours prior to initiation of the next cycle of therapy. All study clinic visits starting with cycle 1, day 1 have +/- 2 day window, unless otherwise noted. Off-treatment follow up will occur at 3 months and at 6 months after completion of paclitaxel therapy. Patients removed from study for unacceptable adverse event(s) will be followed until resolution or stabilization of the adverse event. (see study calendars, below)

Study Calendar Phase Ib – Schedule A (weekly paclitaxel)

	Pre study	**C 1 D 1**	**C1 D7**	**C1 D8**	**C1 D 14**	**C1 D 15**	**C2 D1**	**C2 D8**	**C2 D 15**	**C3-4 D1**	**C3-4 D8**	**C3-4 D 15**	**(3 month follow up after completion of paclitaxel)**	**6 month follow up) after completion of paclitaxel**
Nilotinib			X	X	X	X								
Paclitaxel		X		X		X	X	X	X	X	X	X		
Informed consent	X													
Medical History	X													
Concurrent Meds	X	X	X		X		X			X			X	X
Physical Exam	X	X												
Vital Signs	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Height	X	X												
Weight	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Performance Status	X	X	X	X	X	X	X			X			X	X
CBC w/ diff, plts	X	X	X	X	X	X	X	X	X	X	X	X		
Serum chemistry magnesium	X	X	X	X	X	X	X			X				
ECG (perfomed in triplicate)	X	X	X		X									
Adverse Event evaluation by CRC		X	X	X	X	X	X	X	X	X	X	X	X	X
B-HCG for premenopausal women		X												
PK studies*		X	X	X										
CIPN 20	X	X		X		X	X			X			X	X

***Please see section 8.1 for detailed PK schedule**

Study Calendar Phase Ib – Schedule B (dose dense paclitaxel)

	Pre study	**C1 D1**	**C1D 14**	**C2D1**	**C3D1**	**C4D1**	**(3 month follow up after completion of paclitaxel)**	**6 month follow up) after completion of paclitaxel**
Nilotinib			X	X				
Paclitaxel		X		X	X	X		
Informed consent	X							
Medical History	X							
Concurrent Meds	X	X	X		X		X	X
Physical Exam	X	X						
Vital Signs	X	X	X	X	X	X	X	X
Height	X	X						
Weight	X	X	X	X	X	X	X	X
Performance Status	X	X	X	X	X		X	X
CBC w/ diff, plts	X	X	X	X	X	X		
Serum chemistry magnesium	X	X	X	X	X			
ECG (perfomed in triplicate)	X	X	X					
Adverse Event evaluation by CRC		X	X	X	X	X	X	X
B-HCG for premenopausal women		X						
PK studies*(175mg/m2)		X	X	X				
CIPN 20	X	X		X	X		X	X

***Please see section 8.1 for detailed PK schedule**

7.3 Adverse Events Associated with Nilotinib and Paclitaxel

7.3.1 Adverse Events Reported on Nilotinib Trials

- **Blood and lymphatic system disorders:** myelosuppression, including grade 3-4 thrombocytopenia, neutropenia and anemia, serious hemorrhagic events including intracranial hemorrhage.
- **Cardiac disorders:** QTC prolongation, ischemia, pericardial effusions, hypertension.
- **Eye disorders:** N/A
- **Gastrointestinal disorders:** pancreatitis and elevated serum lipase, upper abdominal pain, nausea, constipation or diarrhea.
- **General disorders and administration site conditions:** fatigue, pyrexia, asthenia, fluid retention.
- **Hepatobiliary disorders:** AST/ALT elevations, hyperbilirubinemia.
- **Investigations:** elevated lipase, hyperglycemia, elevated bilirubin, elevated ast, elevated alt, elevated alkaline phosphatase, elevated lipoprotein cholesterol, elevated total cholesterol, elevated blood triglycerides.
- **Metabolism and nutrition disorders:** hyperglycemia, electrolyte abnormalities, including hypophosphatemia, hypokalemia, hypocalcemia, hyponatremia. Nilotinib capsules contain lactose.
- **Musculoskeletal and connective tissue disorders**: myalgias, arthralgias, muscle spasm.
- **Nervous system disorders:** headache.
- **Psychiatric disorders:** N/A
- **Renal and urinary disorders:** N/A
- **Respiratory, thoracic and mediastinal disorders:** pleural effusions.
- **Skin and subcutaneous tissue disorders:** dry skin, alopecia.
- **Vascular disorders:** peripheral arterial occlusive disease (increased frequency at higher doses.

There are no genotoxicity, carcinogenicity, developmental and reproductive studies conducted with nilotinib. Women of childbearing potential should not become pregnant or breastfeed and men should not father a child during the study. All subjects must use acceptable contraceptive

measures during the treatment of nilotinib and 3 months after the last dose of the investigational drug.

If a patient is suspected to be pregnant, nilotinib should be IMMEDIATELY discontinued and the study physician contacted. A positive urine test must be confirmed by a serum pregnancy test. If it is confirmed that the patients is not pregnant, the patient may resume dosing with nilotinib.

If a female patient becomes pregnant during therapy or within 3 months after the last dose of nilotinib, or if abortion occurs, whether accidental, therapeutic, or spontaneous, it should always be classified as serious. Any congenital anomaly/birth defect in a child conceived during the study or within 12 months after the last dose of nilotinib to a female patient within 12 months after the last dose of nilotinib should be recorded and reported as an SAE.

• Female patients should not breastfeed a baby while on this study.
• Female patients must NEVER donate ova while or after being treated with nilotinib.

All patients are prohibited from donating blood while on study and for 12 months after the last dose of nilotinib.

For any grade ≥ 2 QTC prolongation or any grade ≥ 2 cardiac event, nilotinib should be held as per the United States Prescribing Information for nilotinib, and cardiology should be consulted prior to restarting the patient on nilotinib.

7.3.2 Adverse Events Reported for Paclitaxel

Consult the package insert for the most current and comprehensive list of side effects.

- **Hematologic**: The most common DLT is myelosuppression, primarily leukopenia.

- **Anaphylaxis and severe hypersensitivity reactions**: Patients with a history of severe hypersensitivity reactions to products containing Cremophor® EL (e.g., cyclosporin for injection concentrate and teniposide for injection concentrate) should not be treated with paclitaxel. In order to avoid the occurrence of severe hypersensitivity reactions, all patients treated with paclitaxel should be premedicated with corticosteroids (such as dexamethasone), diphenhydramine and H2 antagonists (such as famotidine or ranitidine). Since most of these reactions occur during the first 2 infusions, discontinuation of these premedications may be a consideration at the discretion of the treating physician. Minor symptoms such as flushing, skin reactions, dyspnea, hypotension, or tachycardia do not require interruption of therapy. However, severe reactions, such as hypotension requiring treatment, dyspnea requiring bronchodilators, angioedema, or generalized urticaria require immediate discontinuation of paclitaxel and aggressive symptomatic therapy. Patients who have developed severe hypersensitivity reactions should not be re-challenged with paclitaxel.

- **Cardiac**: Cardiovascular events observed with paclitaxel include hypotension and bradycardia. Severe conduction abnormalities have been documented in < 1% of patients during Paclitaxel therapy and in some cases requiring pacemaker placement. If patients develop significant conduction abnormalities during paclitaxel infusion, appropriate

therapy should be administered and continuous cardiac monitoring should be performed during subsequent therapy with paclitaxel.

- **Neurologic**: The frequency and severity of neurologic events are dose-dependent. Peripheral neuropathy is rarely severe and may be the cause of paclitaxel discontinuation in 1% of patients. Sensory symptoms usually improve or resolve within several months of completion of treatment. Serious neurologic events such as grand mal seizures, syncope, ataxia and neuroencephalopathy are rare. Although the occurrence of peripheral neuropathy is frequent, the development of severe symptomatology is unusual and requires a dose reduction of 20% or greater for all subsequent courses of paclitaxel.

- **Gastrointestinal**: The most common GI toxicities, which include nausea, vomiting, diarrhea and mucositis, are typically mild or moderate in severity. These respond well to supportive care with anti- emetics such as prochlorperazine, anti-diarrheal agents such as loperamide and topical analgesics for mucositis. Weekly administration of paclitaxel at 80 mg/m2 is rarely associated with nausea/vomiting and antiemetic pre-medication is generally not needed prior to infusion. If patient experience significant nausea/vomiting during or post-infusion, appropriate antiemetic(s) may be added as prophylaxis prior to infusion at the treating physician's discretion.

- **Injection Site Reaction**: Injection site reactions, including reactions secondary to extravasation, were usually mild and consisted of erythema, tenderness, skin discoloration, or swelling at the injection site. These reactions have been observed more frequently with the 24-hour infusion than with the 3-hour infusion. Recurrence of skin reactions at a site of previous extravasation following administration of paclitaxel at a different site, i.e., ―recall‖, has been reported rarely. Rare reports of more severe events such as phlebitis, cellulitis, induration, skin exfoliation, necrosis, and fibrosis have been received as part of the continuing surveillance of paclitaxel safety. In some cases the onset of the injection site reaction either occurred during a prolonged infusion or was delayed by a week to ten days. Given the possibility of extravasation, it is advisable to closely monitor the infusion site for possible infiltration during drug administration. If extravasation is discovered or the patient develops localized infusion reaction the following cold compression protocol is recommended:

    - Immediately after medical treatment is completed, apply ice pack to the affected for 15-20 minutes at least 4 times per day for the first 24-48 hours by any of the following means:
        - Cool wash cloth
        - Instant cool/ice pack
    - The limb should be elevated at all times and exercised at least every 4-6 hours to reduce immobility.

- **Other**: Although 60% of all patients experience arthralgia and myalgia, there is no consistent relationship between the dose or schedule of paclitaxel and the frequency or these events. The symptoms, which usually begin 2 or 3 days after paclitaxel treatment, are generally transient. Almost all patients receiving paclitaxel experience alopecia. Nail changes (changes in pigmentation or discoloration of nail bed; lifting of the nails) are uncommon with frequency of about 2%. Occasionally, edema is seen at a rate of about

5%. It is usually of mild severity and gradually reversible following discontinuation of paclitaxel.

- **Pregnancy**: Paclitaxel can cause fetal harm when administered to a pregnant woman. Administration of paclitaxel during the period of organogenesis to rabbits at doses of 3.0 mg/kg/day (about 0.2 the daily maximum recommended human dose on a mg/m^2 basis) caused embryo and fetotoxicity, as indicated by intrauterine mortality, increased resorptions, and increased pregnancy loss. There are no adequate and well-controlled studies in pregnant women. If paclitaxel is used during pregnancy, or if the patient becomes pregnant while receiving this drug, the patient should be apprised of the potential hazard to the fetus. Women of childbearing potential should be advised to avoid becoming pregnant.

- **Carcinogenesis, Mutagenesis, Impairment of Fertility**: The carcinogenic potential of paclitaxel has not been studied. Paclitaxel has been shown to be clastogenic *in vitro* (chromosome aberrations in human lymphocytes) and *in vivo* (micronucleus test in mice). Paclitaxel was not mutagenic in the Ames test or the CHO/HGPRT gene mutation assay. Administration of paclitaxel prior to and during mating produced impairment of fertility in male and female rats at doses equal to or greater than 1 mg/kg/day (about 0.04 the daily maximum recommended human dose on a mg/m^2 basis). At this dose, paclitaxel caused reduced fertility and reproductive indices, and increased embryo- and fetotoxicity.

7.3.3 Management of QTC prolongation during study

For participants who have a QTC greater than 480 msec while receiving nilotinb, the following measures are required:

1) Withhold nilotinib, and perform an analysis of serum potassium and magnesium, and if below lower limit of normal, correct with supplements to within normal limits. Concomitant medication usage must be reviewed
2) Resume within 14 days at prior dose if QTc returns to less than 450msec and to within 20msec of baseline. Repeat ECG in triplicate with weekly dosing of nilotinib
3) Discontinue nilotinib and end study participation if QTc remains elevated to greater than 480msec after 14 days drug hold

Patients who have a marked baseline prolongation of QT/QTc interval (e.g., repeated demonstration of a QTc of > 450msec) or a history of additional risk factors for TdP (e.g., heart failure, hypokalemia, family history of Long QT Syndrome) will be excluded from study. Patients with an absolute QTc≥ 500msec, QTc< 320msec or increase of baseline QTc of > 60msec will discontinue treatment but will still be followed.

8.0 CORRELATIVE STUDIES

The focus of this trial will be an assessment of safety, tolerability and determination of a recommended future phase II dose of the study regimen. The future phase II component of the trial will ascertain the efficacy of nilotinib vs placebo in preventing paclitaxel-related

neuropathy. Correlatives for the trial are as follows:

The effect of nilotinib on pharmacokinetics (PK) of paclitaxel and the effect of paclitaxel on PK of nilotinib will be evaluated to ensure that these agents do not have clinically significant interactions. We will evaluate real time PK data to ensure that there is no adverse negative effect on paclitaxel PKs. We will assess all patient data for the first 6 patients before proceeding, to ensure that all PKs are within expected level of no interaction between the two drugs.

8.1 Integrated Correlative Studies: PK of Nilotinib and Paclitaxel

The effect of nilotinib on the PK of paclitaxel, as well as the effect of paclitaxel on the PK of nilotinib will be studied.

Based on our recent preclinical experience of nilotinib given in combination with paclitaxel, we anticipate that nilotinib will not affect the disposition profile of paclitaxel and vice versa. The expectation that nilotinib is unlikely to influence the pharmacokinetics of paclitaxel would be consistent with previously reported studies confirming that other OATP1B1-inhibiting TKIs identified from our screens, such as axitinib, and sorafenib, do not alter the systemic exposure to paclitaxel. The noted NCI study confirmed our preclinical anticipation, there were no observed adverse PK interactions between nilotinib and paclitaxel. Although the drug-drug interaction in a clinical setting is highly unlikely, we will perform real-time PK analyses for both paclitaxel, nilotinib and in combination for parent and metabolites.

The PK assessments of nilotinib and paclitaxel are summarized below.

8.1.1 Schedule for collection of specimen(s):

During the Phase Ib trial, PK will be obtained at baseline (within 15 min of pre-dose), during drug administration (within 5 min of the end of infusion), and at 4 defined time-points ranging up to 4.5 hours after drug administration, for up to 6 blood withdrawals for paclitaxel. The PK of nilotinib will be measured at pre- and post-dose for a total of 7 blood samplings. The PK of both paclitaxel and nilotinib will be assessed prior to administration of nilotinib, prior to administration of paclitaxel, during the administration of paclitaxel, and at 3 defined time-points ranging up to 2.5 hours after paclitaxel administration, for up to 7 blood samplings for combination treatment.

For Schedule A: weekly dose of 80mg/m^2 paclitaxel

- The PK of paclitaxel will be measured on Day 1 (paclitaxel given as a single agent) at the following times:

  - Pre-dose (within 15 min)
  - Immediately prior to end of infusion of paclitaxel (within 5 min)
  - 0.5 hour after end of infusion (±5 min)
  - 1.5 hour after end of infusion (±5 min)
  - 2.5 hours after end of infusion (±5 min)
  - 4.5 hours after end of infusion (±5 min)

- The PK of nilotinib will be measured during Day 7. Nilotinib is given as a single agent, 24 hours prior to paclitaxel infusion at following times:

- Pre-dose (within 15 min)
- 0.5 hour after taking the pill (±5 min)
- 1.5 hour after taking the pill (±5 min)
- 2 hours after taking the pill (±5 min)
- 3 hours after taking the pill (±5 min)
- 4 hours after taking the pill (±10 min)
- 6 hour after taking the pill (±10 min)

- The PK of nilotinib and paclitaxel will be measured on Day 8. Nilotinib given 30 min before paclitaxel infusion at the following times:

  - Pre-dose of nilotinib (within 15 min)
  - Prior to starting paclitaxel infusion (within 5 min)
  - Immediately prior to end of infusion of paclitaxel (within 5 min)
  - 0.5 hour after end of paclitaxel infusion (±5 min)
  - 1.5 hours after end of paclitaxel infusion (±5 min)
  - 2.5 hours after end of paclitaxel infusion (±5 min)
  - 4 hours after end of paclitaxel infusion (±10 min)

For Schedule B: Every two weeks dose dense of 175mg/m^2 paclitaxel

- The PK of paclitaxel will be measured on Day 1 (paclitaxel given as a single agent) at the following times:

  - Pre-dose (within 15 min)
  - 1.5 hour after infusion of paclitaxel (within 5 min)
  - Immediately prior to end of infusion of paclitaxel (within 5 min)
  - 0.5 hour after end of infusion (±5 min)
  - 1.5 hour after end of infusion (±5 min)
  - 2.5 hours after end of infusion (±5 min)

- The PK of nilotinib will be measured during Day 14, nilotinib is given as a single agent, 24 hr prior to paclitaxel infusion at following times:

  - Pre-dose (within 15 min)
  - 0.5 hour after taking the pill (±5 min)
  - 1.5 hour after taking the pill (±5 min)
  - 2 hours after taking the pill (±5 min)
  - 3 hours after taking the pill (±5 min)
  - 4 hours after taking the pill (±10 min)
  - 6 hour after taking the pill (±10 min)

- The PK of nilotinib and paclitaxel will be measured on Cycle 2 Day 1, nilotinib is given 30 min before paclitaxel infusion at the following times:

  - Pre-dose of nilotinib (within 15 min)
  - Prior to starting paclitaxel infusion (within 5 min)
  - 1.5 hour after infusion of paclitaxel (within 5 min)

- Immediately prior to end of infusion of paclitaxel (within 5 min)
- 0.5 hour after end of paclitaxel infusion (±5 min)
- 1.5 hours after end of paclitaxel infusion (±5 min)
- 2.5 hours after end of paclitaxel infusion (±5 min)

8.1.2 Procedure for collection of specimen(s):

Samples should be collected via a peripheral blood draw in the contralateral arm to the infusion. In the event when a patient has poor venous access and has an existing and functional central venous catheter, sampling through the central line is acceptable. Patients without venous access will be allowed to have a port placed prior to initiation of study therapy per discretion of the treating physician. Patients without a port will have an option of having a central line inserted during treatment visits, especially on days that require timed blood draws. Each sample will be collected into a 6-mL lithium heparin (green top) vacutainer tube. Immediately after collection, the tube is inverted 8-10 times to ensure proper mixing and place tube on ice until centrifugation. The PK specimen will then be centrifuged at 1,200 xg for 10 min at 4°C within 1 hour of sample collection. The plasma layer will be transferred into three (3) 2-mL cryovials in roughly equal proportions and frozen immediately at (-70) ºC. An accompanied PK requisition form must be completed for each course of PK sampling. A copy of the completed form must be included with the shipment of the samples for analysis.

8.1.3 Procedure for analysis of specimen(s):

We anticipate that OATP1B1 function can be modulated by nilotinib without simultaneously influencing the systemic clearance of paclitaxel. This would be consistent with our prior work indicating that the plasma concentration-time profiles of paclitaxel are inversely related to corresponding drug levels in liver, and that the presence of Kolliphor EL in the clinical paclitaxel formulation is the main determinant of drug disposition properties, and that this excipient masks an effect of OATP1B-type transporters on paclitaxel levels in plasma. This hypothesis will be prospectively tested in all patients enrolled on the Phase 1b study by evaluating the plasma pharmacokinetics of paclitaxel when given either alone or with nilotinib. Plasma samples will be analyzed in real time for paclitaxel and its main liver metabolites by LC/MS/MS in the laboratory of Dr. Shuiying Hu in the College of Pharmacy. In addition, we anticipate that paclitaxel will not alter the absorption and distribution profiles of nilotinib, considering that the principal elimination pathway of nilotinib, CYP3A4-mediated metabolism, is insensitive to inhibition by paclitaxel. To confirm this hypothesis, plasma samples will be also analyzed for nilotinib by UPLC/MS/MS. All pharmacokinetic parameters will be calculated using standard non-compartmental methods.

8.1.4 Procedure for banking of specimen(s):

After samples are analyzed, any unused, leftover samples will be retained to allow for other biomarker analysis in the future as the pre-clinical research progresses. Samples will be stored indefinitely in -70 ºC freezers in the laboratory of Dr. Shuiying Hu, 460 12th Ave, 436D BRT, Columbus, OH, 43210. No genetic testing will take place on leftover samples.

8.2 Integrated Correlative Studies: Biomarkers of OATP1B1

In order to assess the degree of OATP1B1 inhibition by nilotinib, analysis of

glycochenodeoxycholate sulfate (GCDGA-S) and chenodeoxycholate-24-glucuronide (CDCA-24G) levels will be performed as validated surrogate endogenous substrates of OATP1B. Previous clinical studies have indicated that the AUC of GCDCA-S can be increased >20-fold following treatment with OATP1B1 inhibitors, whereas CDCA-24G is only detectable after the administration of OATP1B1 inhibitors. Based on our preclinical studies, we expect that the administration of nilotinib will result in dose-dependent changes in levels of GCDAGA-S and CDCA-24G caused by inhibition of OATP1B1. With recent significant progress in the identification of endogenous OATP1B1 biomarkers, we will measure newly emerged biomarkers with higher selectivity and sensitivity, such as CP-I, CP-III, as well as α-tocopherol which can serves as a bona fide DRG-specific endogenous biomarker of OATP1B-type transporter function. This will be done using the same plasma samples already collected for paclitaxel and nilotinib analyses during the phase Ib, and provide more reliable prediction of nilotinib inhibition of OATP1B transporter function. Within each cohort of patients, plasma samples obtained at baseline and at timed-intervals after the first nilotinib dose will be collected and subjected to LC/MS/MS in a negative multiple reaction monitoring mode to quantify concentrations of the test compounds. Concentrations of total and unbound nilotinib will also be determined in the same samples by validated assays based on LC/MS/MS and micro-equilibrium dialysis, respectively. This information will be utilized to confirm that plasma levels of nilotinib associated with the applied test doses are in the anticipated ranges and are consistent with the inhibition constant of nilotinib for OATP1B1 derived from cell culture studies.

9.0 ADVERSE EVENT REPORTING

Adverse event: Any unfavorable and unintended sign (including abnormal laboratory finding), symptom, or disease temporally associated with the use of a medical treatment or procedure, regardless of whether it is considered related to the medical treatment or procedure; also an "unanticipated problem" of any nature (e.g., psychological or social harm) (designated as unrelated, definitely related, probably related, or possibly related; see below)

Serious adverse event: Any adverse event that is fatal or life threatening, is permanently disabling, requires inpatient hospitalization or prolongs hospitalization, or results in a congenital anomaly or birth defect.

Life-threatening event: Any adverse event in which the subject is at immediate risk of death from the reaction as it occurs; does not include a reaction that, if it were to occur in a more serious form, might cause death

Unexpected event: Any adverse event that is not identified in nature, severity, or frequency in the investigator brochure, study protocol, consent form, or IND application; or the event was more serious than anticipated.

9.1 Attribution of the Adverse Events

Definite: AE is CLEARLY RELATED to the study treatment.
Probable: AE is LIKELY RELATED to the study treatment.
Possible: AE MAY BE RELATED to the study treatment.

Unlikely: AE is DOUBTFULLY RELATED to the study treatment.
Unrelated: AE is CLEARLY NOT related to the study treatment

9.2 Documentation

CTCAE version 5.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 5.0. A copy of the CTCAE version 5.0 can be downloaded from the CTEP web site http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

For expedited reporting purposes only:

- AEs for the agent that are bold and italicized in the CAEPR (i.e., those listed in the SPEER column, Section 7.1.1) should be reported through CTEP-AERS only if the grade is above the grade provided in the SPEER.

All adverse events must be documented in detail within the medical record. The patient will be observed and monitored carefully until the condition resolves, stabilizes, or its cause is identified. All adverse events, including laboratory abnormalities, will be followed up according to good medical practices. Information to be recorded includes the following:

1. Specific type of reaction.
2. Duration of reaction.
3. Severity/grade of reaction according to the NCI Common Terminology Criteria for Adverse Events v5.0 (CTCAE).
4. Suspected cause of the reaction (i.e. possibly or probably related to one of the following: study intervention, progression of disease, concurrent medications, concurrent illness, or other factors).
5. Patient's response to medical interventions.

9.3 Reporting

Adverse events during standard chemotherapy are not attributable to study intervention (gait and balance assessment) and therefore not subject to reporting. However, they will be collected in order to evaluate associations with gait and balance deficits. Since it is difficult to discern which AEs may impact gait and balance testing, all AEs will be collected, not just neuropathy symptoms. Although unlikely, some unrelated symptoms may impact gait and balance and may also be indirectly related to neuropathy symptoms (e.g. cytopenias and fatigue may impact balance and gait).

10.0 DATA REPORTING / REGULATORY REQUIREMENTS

10.1 Ethical and Regulatory Considerations

This trial will be conducted in compliance with the protocol, Good Clinical Practice guidelines, and all applicable regulatory requirements.

10.2 Institutional Review Board

The Principal Investigator will have obtained written approval to conduct the study from The Ohio State University IRB and the Clinical Scientific Review Committee of the James Cancer Hospital and Solove Research Institute. All amendments must be approved by the Institutional Review Board of The Ohio State University prior to implementation.

10.3 Informed Consent

All potential candidates for the study will be given a copy to read of the consent form for the study. The Principal Investigator and/or designee will explain all aspects of the study in lay language and answer all the candidate's questions regarding the study. If the candidate desires to participate in the study, she will be asked to sign the Informed Consent. The study agent will not be released to a subject without a signed Informed Consent. Elements of informed consent include explanations of 1) the purpose of the trial, 2) what the study entails, 3) alternate treatments, 4) expenses and inconveniences to be incurred, 5) discomfort and risks to the subject, 6) whether she will receive payment for participation in the study, 7) contact person to call in the event of an emergency, 8) subject rights as a result of illness or injury from trial participation, 9) subjects right to withdraw from the trial at any time without prejudice, 10) confidentiality of trial participation.

10.4 Patient Confidentiality

The information obtained during the conduct of this study is considered confidential and will not be released without the written permission of the subject, except as necessary for monitoring by the FDA or other regulatory agencies. All laboratory specimens will be labeled with coded identifiers in order to maintain confidentiality. Signed consent forms, data sheets, and laboratory notebooks will be kept in locked cabinets in Dr. Nicole Williams' office and/or research laboratories.

10.5 Publication and Research Findings

Publications of the research findings will present data in a format that will not reveal the identity of the participants.

10.6 Compliance Monitoring

In accordance with IRB guidelines, the study program will be reviewed by the IRB every 12 months or less. Deviations from the protocol must be documented in the medical record and reported immediately to the PI. Deviations that meet the criteria for Immediate Event Reporting (http://orrp.osu.edu/irb/event/index.cfm) such as those that increase risks to subjects and/or compromise scientific integrity will be reported immediately to the IRB.

10.7 Data Safety Monitoring

The data and safety monitoring plan will involve the continuous evaluation of safety, data quality and data timeliness. Investigators will conduct continuous review of data and patient safety at their regular Disease Group meetings (at least monthly) and the discussion will be documented in the minutes. The PI of the trial will review toxicities and responses of the trial where applicable at these disease center meetings and determine if the risk/benefit ratio of the trial changes. Frequency and severity of adverse events will be reviewed by the PI and compared to what is

known about the agent/device from other sources; including published literature, scientific meetings and discussions with the sponsors, to determine if the trial should be terminated before completion. Serious adverse events and responses will also be reviewed by the OSUCCC Data and Safety Monitoring Committee (DSMC). The PI will also submit a progress report biannually that will be reviewed by the committee per the DSMC plan. All reportable Serious Adverse Events (SAE) will also be reported to the IRB of record as per the policies of the IRB.

10.8 Responsibility for Data Submission

Data are to be submitted via OnCore on a real-time basis, but no less than once every 2 weeks.

10.9 Data and Records

Primary source documents will include forms routinely used at the Stefanie Spielman Comprehensive Breast Center, namely the Breast Patient Information Form, clinic and office notes as well as laboratory and radiology reports, including documentation found in the electronic medical record (EPIC).

10.10 Safety and Monitoring

Adverse events will be monitored by self-reporting of signs and symptoms. Patients will maintain a daily diary of time of drug intake and any possible ill effects, with instructions to contact the PI or Research Nurse to discuss and manage any possible side effects.

10.11 Accountability

Study drug, nilotinib, will be provided at no cost to the patient. Pill bottles will be provided to the patient, with the start date and number of pills recorded. The drugs will be provided in sufficient supply for one cycle of taxane chemotherapy. Pill bottles will be collected at each cycle's follow-up visits, and any unused capsules will be documented and counted to ensure proper adherence. The pill bottle will be returned to the patient after pill reconciliation until the end of study protocol. Additionally, there will be no charge for research-related blood work. Imaging is considered as part of standard of care testing.

10.12 Documentation

All adverse events must be documented in detail within the medical record. The patient will be observed and monitored carefully until the condition resolves, stabilizes, or its cause is identified. All adverse events, including laboratory abnormalities, will be followed up according to good medical practices. Information to be recorded includes the following:

1. Specific type of reaction.
2. Duration of reaction.
3. Severity/grade of reaction according to the NCI Common Terminology Criteria for
4. Adverse Events 5.0 (CTCAE).
5. Suspected cause of the reaction (i.e. possibly or probably related to one of the following: study treatment, progression of disease, concurrent medications, concurrent illness, or other factors).
6. Changes made in the administration of the study drugs and other actions taken to alleviate the clinical event.

7. Patient's response to medical interventions.

10.13 Reporting

According to FDA regulations (21 CFR 312.32), IND safety reports shall address "any adverse experience associated with the use of a drug that is both serious and unexpected." The IRB will be notified of any adverse event that occurs on the study regardless of severity.

Federal policy [45 CFR 46.116(b)(5)] also requires that investigators inform subjects of any important new information that might affect their willingness to continue participating in the research. When an adverse event necessitates changes to the consent/assent form(s) and/or protocol, or that notification is given to currently or previously enrolled subjects, an amendment request will be submitted in conjunction with the adverse event report. The IRB will make a determination whether any new findings, new knowledge, or adverse effects should be communicated to subjects.

In accordance with IRB guidelines, serious adverse events will be reported within 10 days of the investigator's or research staff members' learning of the event to The Ohio State University Institutional Review Board. OSU IRB Event Reports should be submitted through BuckIRB at: http:/orrp.osu.edu/irb/buck-IRB/. Events resulting in temporary or permanent interruption of study activities by the investigator or sponsor to avoid potential harm to subjects should be reported within 48 hours whenever possible. In addition, all adverse events regardless of severity will be reported in the same manner within 10 calendar days of the investigator's or research staff members' learning of the event.

All events that may represent unanticipated problems involving risks to subjects or others will be promptly reported (as described above), regardless of whether they occur during or after the study, or involve a subject who has withdrawn from or completed study participation. If changes to the research or consent process are proposed as a result of the event or if additional information will be provided to current and/or past participants, an amendment request will also be submitted for IRB review. In addition, all adverse events regardless of grade will be reported as per the FDA reporting requirements below (21 CFR Part 312) including Grade 1 and Grade 2 events, which will be reported within 10 calendar days whether participants were hospitalized or not.

Related events involving risk but not meeting the prompt reporting requirements will also be reported to the IRB in summary form at the time of continuing review.

Phase I Studies: Expedited Reporting Requirements for Adverse Events that Occur on Studies under an IND/IDE within 30 Days of the Last Administration of the Investigational Agent/Intervention

FDA REPORTING REQUIREMENTS FOR SERIOUS ADVERSE EVENTS (21 CFR Part 312)
NOTE: Investigators **MUST** immediately report to the sponsor (NCI) **ANY** Serious Adverse Events, whether or not they are considered related to the investigational agent(s)/intervention (21 CFR 312.64)
An adverse event is considered serious if it results in **ANY** of the following outcomes:

1) Death
2) A life-threatening adverse event

3) An adverse event that results in inpatient hospitalization or prolongation of existing hospitalization for ≥ 24 hours

4) A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions

) A congenital anomaly/birth defect.

6) Important Medical Events (IME) that may not result in death, be life threatening, or require hospitalization may be considered serious when, based upon medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. (FDA, 21 CFR 312.32; ICH E2A and ICH E6).

ALL SERIOUS adverse events that meet the above criteria MUST be immediately reported to the NCI via electronic submission within the timeframes detailed in the table below.

Hospitalization	**Grade 1 and Grade 2 Timeframe**	**Grade 3-5 Timeframes**
Resulting in Hospitalization ≥ 24 hrs	10 Calendar Days	24 hour 5 calendar days
Not resulting in Hospitalization ≥ 24 hrs	Not required	

NOTE: Protocol specific exceptions to expedited reporting of serious adverse events are found in the Specific Protocol Exceptions to Expedited Reporting (SPEER) portion of the CAEPR.

Expedited AE reporting timelines are defined as:

o "24-Hour; 5 Calendar Days" - The AE must initially be submitted electronically within 24 hours of learning of the AE, followed by a complete expedited report within 5 calendar days of the initial 24-hour report.

o "10 Calendar Days" - A complete expedited report on the AE must be submitted electronically within 10 calendar days of learning of the AE.

Serious adverse events that occur more than 30 days after the last administration of investigational agent/intervention and have an attribution of possible, probable, or definite require reporting as follows:

Expedited 24-hour notification followed by complete report within 5 calendar days for:

• All Grade 3, 4, and Grade 5 AEs

Expedited 10 calendar day reports for:

• Grade 2 AEs resulting in hospitalization or prolongation of hospitalization

Effective Date: May 5, 2011

11.0 STATISTICAL CONSIDERATIONS

11.1 Study endpoints

Definition of Primary Endpoint

- Safety, tolerability and RP2D of nilotinib in combination with paclitaxel.

Definition of Secondary Endpoints

- PK endpoints such as AUC, CL, Vd, and t1/2 for nilotinib and paclitaxel.

- Quality of life using CIPN-20 questionnaire, disease-free survival (DFS), event free survival (EFS) and overall survival (OS) of nilotinib in combination with paclitaxel.

11.2 Study Design and Sample Size Calculation

The primary objective of the Phase 1b study is to obtain data to support the selection of an optimal, safe nilotinib dose that can temporarily inhibit the function of OATP1B1 in patients with breast cancer without affecting the plasma pharmacokinetics of paclitaxel. The proposed study design is derived from an adaptive Bayesian method for dose-finding based on trade-offs between the probabilities of treatment efficacy and toxicity (EffTox). In this design, treatment efficacy is defined as statistically significant inhibition of OATP1B1 activity by nilotinib, without causing changes in the pharmacokinetic profiles of paclitaxel. Tolerability of the combination regimen will be evaluated within the first 4 weeks of treatment (cycles 1 & 2). Doses are selected for successive patient cohorts based on a set of efficacy-toxicity trade-off contours that partition the two-dimensional outcome probability domain. Priors are established by solving for hyper-parameters that optimize the fit of the model to elicited mean outcome probabilities. Published studies have demonstrated that, under a wide range of dose-outcome scenarios, the EffTox method has high probabilities of making correct decisions as compared to classic dose-escalation studies such as the '3+3 Design' in which the assessment is mainly based on establishing the dose levels associated with dose-limiting toxicity (DLT). Moreover, the EffTox method allows treatment of most patients at doses with desirable efficacy-toxicity trade-offs. In the proposed Phase 1b study, the maximum sample size will be 20 with cohort size of 2. A total of 4 nilotinib dose levels will be considered, including 50 mg (dose level -1), 100 mg (dose level 1), 200 mg (dose level 2), and 300 mg (dose level 3), with the goal to find an optimal dose.

During the proposed EffTox Phase 1b study, both efficacy and toxicity will be monitored and these data used to iteratively inform the dynamic, adaptive model to make dose escalation or de-escalation decisions. Efficacy in this setting will be OATP1B1 inhibition as defined by a ≥5-fold increase in the AUC of GCDCA-S from pre- to post-treatment and/or detectable CDCA-24G levels post-treatment; although preliminary data in other populations suggest a potentially much larger effect, a 5-fold increase would be sufficiently large to indicate effective inhibition.

We specified prior distributions and threshold probabilities of efficacy and toxicity for this model; the maximum allowable probability of toxicity will be 0.33, the minimum probability of efficacy will be 0.90, and cut-offs of 0.10 will be used for both stopping probability criteria. The maximum sample size will be 20 with cohort size of 2. The first cohort of patients will be enrolled at dose level 1 (nilotinib 100 mg). After each cohort is treated and evaluated for toxicity and efficacy as defined in the context of this trial, these data will inform our dynamic, adaptive model to determine dose escalation decisions based on changes to estimated probabilities for toxicity and for efficacy given the data observed. Then next cohort of patients will be treated on the dose level determined from the Bayesian adaptive model. This iterative process is repeated until the maximum sample size or pre-defined stopping criteria are reached. For this phase 1b study, our primary objective is to select an optimal, safe nilotinib dose that can temporarily inhibit the function of OATP1B1 in patients with breast cancer without affecting the plasma pharmacokinetics of paclitaxel. We are interested in both toxicity (DLT) and efficacy (OATP1B1 inhibition). The traditional 3+3 design can determine the MTD only accounting for the associated

DLTs at each dose level, but could not determine the optimal dose level using both toxicity and efficacy. Therefore, an adaptive Bayesian method for dose-finding based on trade-offs between the probabilities of treatment efficacy and toxicity (EffTox) will be used.

After all evaluable patients have been treated, the recommended dose for the phase II will be the highest dose level that satisfies those two EffTox dose acceptability rules (dose has acceptable toxicity if Prob[prob (Toxicity) < 0.33|data] ≥ 0.1; dose has acceptable efficacy if Prob[proc(Efficacy) <.90|data)≥0.1). A feature of this study design is that we are not bound to start our dosing at the lowest dose but rather an expected effective dose; thus, the first cohort will be treated at dose level 1 (100 mg). Assessment of OATP1B1 inhibition and paclitaxel plasma levels will be performed within one week of sample collection to avoid delays in enrollment of patients on subsequent dose levels. These models will identify admissible sets of doses, where a modification will be that no untested doses will be skipped during dose escalation. The prior hyper-parameters will be computed based on the assumed prior means Prob(Toxicity | dose) = {0.02, 0.04, 0.06, 0.08}, and Prob(Efficacy | dose) = {0.2, 0.4, 0.6, 0.8}, for each of the 4 nilotinib dose levels, respectively, with the overall prior effective sample size (ESS) of 1. Simulations are carried out using EffTox version 5.0.0, with 2000 replications per scenario, and the operating characteristics of this design are summarized in **Table 4** If it is highly likely (ie, probabilities >0.90) that more than one dose is associated with acceptable rates of efficacy and toxicity, the next cohort of patients will be treated at the dose associated with the stronger degree of OATP1B1 inhibition. The trial will be conducted using EffTox Software (version 5.0.0) developed by MD Anderson. Adverse events will be classified and attributed using NCI-CTCAE v5.0 and will be summarized within and across dose levels.

Table 4: Operating characteristics of the EffTox study design planned, where P_T is the probability of toxicity and P_E is the probability of efficacy

Scenario	Model characteristics	Nilotinib dose level				
		-1 (50 mg)	**1** (100 mg)	**2** (200 mg)	**3** (300 mg)	None
1	True P_T, P_E	0.02, 0.20	0.04, 0.40	0.06, 0.80	0.08, 1.00	--
	Utility (trade-off)	-3.02	-2.04	-0.10	0.73	--
	% selected	0	0	7	93	0
	# patients treated	0.0	2.0	3.3	14.6	--
2	True P_T, P_E	0.05, 0.10	0.10, 0.50	0.15, 0.85	0.33, 0.95	--
	Utility (trade-off)	-3.55	-1.64	-0.07	-0.22	--
	% selected	0	0	45	48	7
	# patients treated	0.0	2.1	8.1	9.0	--
3	True P_T, P_E	0.01, 0.50	0.10, 0.70	0.20, 0.90	0.50, 1.00	--
	Utility (trade-off)	-1.51	-0.66	0.00	-0.67	--
	% selected	0	4	83	8	6
	# patients treated	0.0	2.9	12.8	3.7	--
4	True P_T, P_E	0.01, 0.70	0.20, 0.80	0.30, 1.00	0.60, 1.00	--
	Utility (trade-off)	-0.51	-0.43	0.00	1.00	--
	% selected	0	30	62	0	8
	# patients treated	0.1	6.2	11.7	1.1	--

11.3 Statistical Data Analysis

Adverse events (AEs) will be classified and attributed using NCI CTCAE v5.0 and will be summarized within and across dose levels using descriptive statistics. The overall number and percentage of patients experiencing AEs and toxicities will be summarized and reported as across all event types, non-hematologic AEs, hematologic AEs, and for each type. Specific focus will be in summarizing any neuropathy-related AEs by dose level and how this corresponds to our measures of OATP1B1 inhibition. All patients who have received at least one dose of the therapeutic agents will be evaluable for toxicity and tolerability.

Overall response rate (partial response + complete response) will be summarized and 95% exact binomial confidence interval will be provided. Progression Free Survival (PFS) will be defined as the time from initiation of therapy to the time of RECIST progression or death. Overall Survival (OS) will be defined as time from initiation of therapy to death. Event free survival (EFS) will be defined as time from initiation to breast cancer disease progression. Survival will initially be modeled using Kaplan-Meier methods, resulting in median survival times with 95% CI. Survival will be further modeled using Cox proportional hazards models resulting in HRs with 95% CI, allowing for adjustment for critical covariates such as age, gender, race/ethnicity.

We will explore PK endpoints such as AUC, CL, Vd, and t1/2 computed using non-compartmental and compartmental methods. Our 6 hour PK sampling schedule is sufficient to provide complete PK profile to characterize for the rest of parameters, based on well-established PK profile for both paclitaxel and nilotinib. We will use graphical analyses as well as repeated measure models (linear or nonlinear mixed models, generalized estimating equations [GEE]) to assess the PK and PD markers described above in relation to clinical treatment outcomes, recognizing some inherent limitations due to sample size.

12.0 REFERENCES

1. Tinetti ME. Clinical practice. Preventing falls in elderly persons. N Engl J Med. 2003;348(1):42-9. Epub 2003/01/03. doi: 10.1056/NEJMcp020719. PubMed PMID: 12510042.
2. Balducci S, Iacobellis G, Parisi L, Di Biase N, Calandriello E, Leonetti F, Fallucca F. Exercise training can modify the natural history of diabetic peripheral neuropathy. J Diabetes Complications. 2006;20(4):216-23. Epub 2006/06/27. doi: 10.1016/j.jdiacomp.2005.07.005. PubMed PMID: 16798472.
3. Bakitas MA. Background noise: the experience of chemotherapy-induced peripheral neuropathy. Nurs Res. 2007;56(5):323-31. Epub 2007/09/12. doi: 10.1097/01.NNR.0000289503.22414.79. PubMed PMID: 17846553.
4. Loprinzi CL, Reeves BN, Dakhil SR, Sloan JA, Wolf SL, Burger KN, Kamal A, Le-Lindqwister NA, Soori GS, Jaslowski AJ, Novotny PJ, Lachance DH. Natural history of paclitaxel-associated acute pain syndrome: prospective cohort study NCCTG N08C1. J Clin Oncol. 2011;29(11):1472-8. Epub 2011/03/09. doi: 10.1200/JCO.2010.33.0308. PubMed PMID: 21383290; PMCID: PMC3082985.
5. Smith EM, Pang H, Cirrincione C, Fleishman S, Paskett ED, Ahles T, Bressler LR, Fadul CE, Knox C, Le-Lindqwister N, Gilman PB, Shapiro CL, Alliance for Clinical Trials in O. Effect of duloxetine on pain, function, and quality of life among patients with chemotherapy-induced painful peripheral neuropathy: a randomized clinical trial. JAMA. 2013;309(13):1359-

67. Epub 2013/04/04. doi: 10.1001/jama.2013.2813. PubMed PMID: 23549581; PMCID: PMC3912515.
6. Hershman DL, Lacchetti C, Dworkin RH, Lavoie Smith EM, Bleeker J, Cavaletti G, Chauhan C, Gavin P, Lavino A, Lustberg MB, Paice J, Schneider B, Smith ML, Smith T, Terstriep S, Wagner-Johnston N, Bak K, Loprinzi CL, American Society of Clinical O. Prevention and management of chemotherapy-induced peripheral neuropathy in survivors of adult cancers: American Society of Clinical Oncology clinical practice guideline. J Clin Oncol. 2014;32(18):1941-67. doi: 10.1200/JCO.2013.54.0914. PubMed PMID: 24733808.
7. Leblanc AF, Sprowl JA, Alberti P, Chiorazzi A, Arnold WD, Gibson AA, Hong KW, Pioso MS, Chen M, Huang KM, Chodisetty V, Costa O, Florea T, De Bruijn P, Mathijssen RH, Reinbolt RE, Lustberg MB, Sucheston-Campbell LE, Cavaletti G, Sparreboom A, Hu S. OATP1B2-deficiency protects against paclitaxel-induced neurotoxicity. J Clin Invest. 2018;128(2):[Epub ahead of print].
8. Xia B, Heimbach T, He H, Lin TH. Nilotinib preclinical pharmacokinetics and practical application toward clinical projections of oral absorption and systemic availability. Biopharmaceutics & drug disposition. 2012;33(9):536-49. Epub 2012/10/26. doi: 10.1002/bdd.1821. PubMed PMID: 23097199.
9. Takehara I, Terashima H, Nakayama T, Yoshikado T, Yoshida M, Furihata K, Watanabe N, Maeda K, Ando O, Sugiyama Y, Kusuhara H. Investigation of Glycochenodeoxycholate Sulfate and Chenodeoxycholate Glucuronide as Surrogate Endogenous Probes for Drug Interaction Studies of OATP1B1 and OATP1B3 in Healthy Japanese Volunteers. Pharm Res. 2017;34(8):1601-14. Epub 2017/05/28. doi: 10.1007/s11095-017-2184-5. PubMed PMID: 28550384.
10. Galetin A, Brouwer KLR, Tweedie D, Yoshida K, Sjostedt N, Aleksunes L, Chu X, Evers R, Hafey MJ, Lai Y, Matsson P, Riselli A, Shen H, Sparreboom A, Varma MVS, Yang J, Yang X, Yee SW, Zamek-Gliszczynski MJ, Zhang L, Giacomini KM. Membrane transporters in drug development and as determinants of precision medicine. Nat Rev Drug Discov. 2024;23(4):255-80. Epub 20240124. doi: 10.1038/s41573-023-00877-1. PubMed PMID: 38267543; PMCID: PMC11464068.
11. Kikuchi R, Chothe PP, Chu X, Huth F, Ishida K, Ishiguro N, Jiang R, Shen H, Stahl SH, Varma MVS, Willemin ME, Morse BL. Utilization of OATP1B Biomarker Coproporphyrin-I to Guide Drug-Drug Interaction Risk Assessment: Evaluation by the Pharmaceutical Industry. Clin Pharmacol Ther. 2023;114(6):1170-83. Epub 20231011. doi: 10.1002/cpt.3062. PubMed PMID: 37750401.
12. Yoshida K, Jaochico A, Mao J, Sangaraju D. Glycochenodeoxycholate and glycodeoxycholate 3-O-glucuronides, but not hexadecanedioate and tetradecanedioate, detected weak inhibition of OATP1B caused by GDC-0810 in humans. Br J Clin Pharmacol. 2023;89(6):1903-7. Epub 20230214. doi: 10.1111/bcp.15679. PubMed PMID: 36735594.
13. Li Y, Drabison T, Nepal M, Ho RH, Leblanc AF, Gibson AA, Jin Y, Yang W, Huang KM, Uddin ME, Chen M, DiGiacomo DF, Chen X, Razzaq S, Tonniges JR, McTigue DM, Mims AS, Lustberg MB, Wang Y, Hummon AB, Evans WE, Baker SD, Cavaletti G, Sparreboom A, Hu S. Targeting a xenobiotic transporter to ameliorate vincristine-induced sensory neuropathy. JCI Insight. 2023;8(14). Epub 20230724. doi: 10.1172/jci.insight.164646. PubMed PMID: 37347545; PMCID: PMC10443802.
14. Holbeck SL, Camalier R, Crowell JA, Govindharajulu JP, Hollingshead MG, Anderson LW, Polley EC, Rubinstein L, Srivastava AK, Wilsker DF, Collins JM, Doroshow JH. The National Cancer Institute ALMANAC: A Comprehensive Screening Resource for the Detection of Anticancer Drug Pairs with Enhanced Therapeutic Activity. Cancer research. 2017. doi:

10.1158/0008-5472.CAN-17-0489. PubMed PMID: 28446463.
15. Hidalgo M, Amant F, Biankin AV, Budinska E, Byrne AT, Caldas C, Clarke RB, de Jong S, Jonkers J, Maelandsmo GM, Roman-Roman S, Seoane J, Trusolino L, Villanueva A. Patient-derived xenograft models: an emerging platform for translational cancer research. Cancer discovery. 2014;4(9):998-1013. doi: 10.1158/2159-8290.CD-14-0001. PubMed PMID: 25185190; PMCID: 4167608.
16. Wolf SL, Barton DL, Qin R, Wos EJ, Sloan JA, Liu H, Aaronson NK, Satele DV, Mattar BI, Green NB, Loprinzi CL. The relationship between numbness, tingling, and shooting/burning pain in patients with chemotherapy-induced peripheral neuropathy (CIPN) as measured by the EORTC QLQ-CIPN20 instrument, N06CA. Support Care Cancer. 2012;20(3):625-32. Epub 2011/04/12. doi: 10.1007/s00520-011-1141-9. PubMed PMID: 21479990; PMCID: PMC3329939.
17. Postma TJ, Aaronson NK, Heimans JJ, Muller MJ, Hildebrand JG, Delattre JY, Hoang-Xuan K, Lanteri-Minet M, Grant R, Huddart R, Moynihan C, Maher J, Lucey R. The development of an EORTC quality of life questionnaire to assess chemotherapy-induced peripheral neuropathy: the QLQ-CIPN20. Eur J Cancer. 2005;41(8):1135-9. Epub 2005/05/25. doi: 10.1016/j.ejca.2005.02.012. PubMed PMID: 15911236.
18. Seidman AD, Tiersten A, Hudis C, Gollub M, Barrett S, Yao TJ, Lepore J, Gilewski T, Currie V, Crown J, et al. Phase II trial of paclitaxel by 3-hour infusion as initial and salvage chemotherapy for metastatic breast cancer. J Clin Oncol. 1995;13(10):2575-81. Epub 1995/10/01. doi: 10.1200/JCO.1995.13.10.2575. PubMed PMID: 7595709.
19. Hudis CA, Seidman AD, Crown JP, Balmaceda C, Freilich R, Gilewski TA, Hakes TB, Currie V, Lebwohl DE, Baselga J, Raptis G, Gollub M, Robles M, Bruno R, Norton L. Phase II and pharmacologic study of docetaxel as initial chemotherapy for metastatic breast cancer. J Clin Oncol. 1996;14(1):58-65. Epub 1996/01/01. doi: 10.1200/JCO.1996.14.1.58. PubMed PMID: 8558221.
20. Gradishar WJ, Tjulandin S, Davidson N, Shaw H, Desai N, Bhar P, Hawkins M, O'Shaughnessy J. Phase III trial of nanoparticle albumin-bound paclitaxel compared with polyethylated castor oil-based paclitaxel in women with breast cancer. J Clin Oncol. 2005;23(31):7794-803. Epub 2005/09/21. doi: 10.1200/JCO.2005.04.937. PubMed PMID: 16172456.
21. Wani MC, Taylor HL, Wall ME, Coggon P, McPhail AT. Plant antitumor agents. VI. The isolation and structure of taxol, a novel antileukemic and antitumor agent from Taxus brevifolia. J Am Chem Soc. 1971;93(9):2325-7. Epub 1971/05/05. PubMed PMID: 5553076.
22. Horwitz SB. Personal recollections on the early development of taxol. J Nat Prod. 2004;67(2):136-8. Epub 2004/02/28. doi: 10.1021/np0304464. PubMed PMID: 14987047.
23. Schiff PB, Fant J, Horwitz SB. Promotion of microtubule assembly in vitro by taxol. Nature. 1979;277(5698):665-7. Epub 1979/02/22. PubMed PMID: 423966.
24. Rowinsky EK, Donehower RC. Paclitaxel (taxol). N Engl J Med. 1995;332(15):1004-14. Epub 1995/04/13. doi: 10.1056/NEJM199504133321507. PubMed PMID: 7885406.
25. Berger MJ, Vargo C, Vincent M, Shaver K, Phillips G, Layman R, Macrae E, Mrozek E, Ramaswamy B, Wesolowski R, Shapiro CL, Lustberg MB. Stopping paclitaxel premedication after two doses in patients not experiencing a previous infusion hypersensitivity reaction. Support Care Cancer. 2015;23(7):2019-24. Epub 2014/12/19. doi: 10.1007/s00520-014-2556-x. PubMed PMID: 25519756; PMCID: PMC4804339.
26. Sledge GW, Neuberg D, Bernardo P, Ingle JN, Martino S, Rowinsky EK, Wood WC. Phase III trial of doxorubicin, paclitaxel, and the combination of doxorubicin and paclitaxel as front-line chemotherapy for metastatic breast cancer: an intergroup trial (E1193). J Clin Oncol.

2003;21(4):588-92. Epub 2003/02/15. doi: 10.1200/JCO.2003.08.013. PubMed PMID: 12586793.
27. Norton L. Theoretical concepts and the emerging role of taxanes in adjuvant therapy. Oncologist. 2001;6 Suppl 3:30-5. Epub 2001/05/11. PubMed PMID: 11346683.
28. Symmans WF, Volm MD, Shapiro RL, Perkins AB, Kim AY, Demaria S, Yee HT, McMullen H, Oratz R, Klein P, Formenti SC, Muggia F. Paclitaxel-induced apoptosis and mitotic arrest assessed by serial fine-needle aspiration: implications for early prediction of breast cancer response to neoadjuvant treatment. Clin Cancer Res. 2000;6(12):4610-7. Epub 2001/01/13. PubMed PMID: 11156210.
29. Seidman AD, Berry D, Cirrincione C, Harris L, Muss H, Marcom PK, Gipson G, Burstein H, Lake D, Shapiro CL, Ungaro P, Norton L, Winer E, Hudis C. Randomized phase III trial of weekly compared with every-3-weeks paclitaxel for metastatic breast cancer, with trastuzumab for all HER-2 overexpressors and random assignment to trastuzumab or not in HER-2 nonoverexpressors: final results of Cancer and Leukemia Group B protocol 9840. J Clin Oncol. 2008;26(10):1642-9. Epub 2008/04/01. doi: 10.1200/JCO.2007.11.6699. PubMed PMID: 18375893.
30. Green MC, Buzdar AU, Smith T, Ibrahim NK, Valero V, Rosales MF, Cristofanilli M, Booser DJ, Pusztai L, Rivera E, Theriault RL, Carter C, Frye D, Hunt KK, Symmans WF, Strom EA, Sahin AA, Sikov W, Hortobagyi GN. Weekly paclitaxel improves pathologic complete remission in operable breast cancer when compared with paclitaxel once every 3 weeks. J Clin Oncol. 2005;23(25):5983-92. Epub 2005/08/10. doi: 10.1200/JCO.2005.06.232. PubMed PMID: 16087943.
31. Sparano JA, Wang M, Martino S, Jones V, Perez EA, Saphner T, Wolff AC, Sledge GW, Jr., Wood WC, Davidson NE. Weekly paclitaxel in the adjuvant treatment of breast cancer. N Engl J Med. 2008;358(16):1663-71. Epub 2008/04/19. doi: 10.1056/NEJMoa0707056. PubMed PMID: 18420499; PMCID: PMC2743943.
32. Budd GT, Barlow WE, Moore HC, Hobday TJ, Stewart JA, Isaacs C, Salim M, Cho JK, Rinn KJ, Albain KS, Chew HK, Burton GV, Moore TD, Srkalovic G, McGregor BA, Flaherty LE, Livingston RB, Lew DL, Gralow JR, Hortobagyi GN. SWOG S0221: a phase III trial comparing chemotherapy schedules in high-risk early-stage breast cancer. J Clin Oncol. 2015;33(1):58-64. doi: 10.1200/JCO.2014.56.3296. PubMed PMID: 25422488; PMCID: PMC4268253.