A Co-clinical Trial in Triple Negative Breast Cancer Patients with Genoproteomic Discovery

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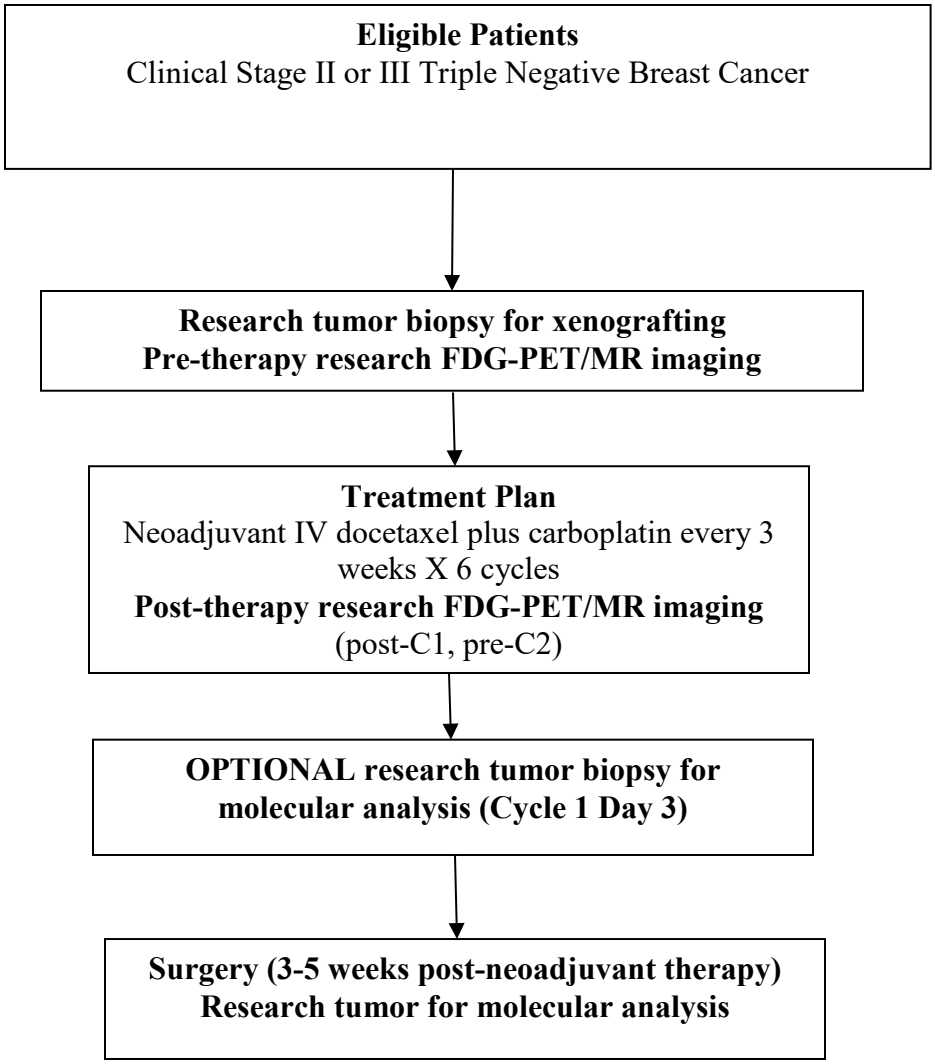

Glossary of Abbreviations

AE	Adverse event
ALT (SGPT)	Alanine transaminase (serum glutamate pyruvic transaminase)
ANC	Absolute neutrophil count
AST (SGOT)	Aspartate transaminase (serum glutamic oxaloacetic transaminase)
B-HCG	Beta human chorionic gonadotropin
BUN	Blood urea nitrogen
CALGB	Cancer and Leukemia Group B
CBC	Complete blood count
CFR	Code of Federal Regulations
CMP	Complete metabolic panel
CNS	Central nervous system
CR	Complete respose
CRF	Case report form
CS	Chemo sensitive
CT	Computed tomography
CTCAE	Common Terminology Criteria for Adverse Events
CTEP	Cancer Therapy Evaluation Program
DFS	Disease-free survival
DNA	deoxyribonucleic acid
DOB	Date of birth
DSM	Data and Safety Monitoring
EBCTCG	Early Breast Cancer Trialists' Cooperative Group
ECOG	Eastern Cooperative Oncology Group
EDTA	ethylenediaminetetraacetic acid
ER	Estrogen receptor
FDA	Food and Drug Administration
FWA	Federal wide assurance
GCP	Good Clinical Practice
G-CSF	Granulocyte colony stimulating factor, filgrastim (Neupogen)
HER2	Human epidermal growth factor receptor 2
HIV	Human Immunodeficiency Virus
HRPO	Human Research Protection Office (IRB)
IND	Investigational New Drug
IRB	Institutional Review Board
IV	Intravenous
LD	Longest diameter
LDH	Lactate dehydrogenase
MRI	Magnetic resonance imaging
NCCN	National Cancer Center Network
NCI	National Cancer Institute

NIH	National Institutes of Health
NSABP	National Surgical Adjuvant Breast and Bowel Project
OHRP	Office of Human Research Protections
OS	Overall survival
pCR	Pathologic complete response
PD	Progressive disease
PDX	Patient-derived xenograft
PET	Positron emission tomography
PI	Principal investigator
PR	Partial response
PR	Progesterone receptor
PS	Performance status
QASMC	Quality Assurance and Safety Monitoring Committee
RECIST	Response Evaluation Criteria in Solid Tumors (Committee)
RNA	Ribonucleic acid
SAE	Serious adverse event
SCC	Siteman Cancer Center
SCID	Severe combined immunodeficiency
SD	Stable disease
TNBC	Triple-negative breast cancer
UPN	Unique patient number
US	Ultrasound
WBC	White blood cell (count)
WHIM	Washington University Human in Mouse
WHO	World Health Organization

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1.0 BACKGROUND AND RATIONALE

1.1 Triple Negative Breast Cancer

Triple negative breast cancer (TNBC) represents approximately 10-20% of breast cancers worldwide and affects approximately 200,000 women annually [1]. It is defined by a lack of expression of the estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2) receptor. The disease is more common in young black women and in women with a deleterious mutation in the BRCA1 gene [2 3].

Established targeted therapies in breast cancer are directed against a nuclear or a surface receptor, such as tamoxifen or trastuzumab, respectively. However, no established targets currently exist for TNBC, so patients receive chemotherapy for systemic control of their disease [4]. Despite neoadjuvant chemotherapy trials showing that TNBC has higher pathological complete response (pCR) rates compared to hormone-receptor-positive subtypes [5], TNBC remains extremely difficult to treat in many cases, and patients have high rates of relapse, particularly due to chemotherapy resistance within the first few years [6]. TNBC patients suffer from a worse initial prognosis among all other breast cancer subtypes, and there is a dire need to develop new treatment strategies for TNBC, particularly for those with disease that is resistant to standard chemotherapy. In addition, it is critical to identify patients who will respond to neoadjuvant docetaxel and carboplatin (NAC) therapy, and to avoid the use of an ineffective treatments in nonresponding patients with an opportunity to devise adaptive treatment strategies.

1.2 Neoadjuvant Chemotherapy in Triple Negative Breast Cancer

Neoadjuvant chemotherapy describes primary systemic therapy that is utilized prior to surgery. It was initially used for patients with locally advanced, inoperable breast cancer in an effort to improve local control and decrease distant metastases when used in combination with surgery [7]. A few years later, the National Surgical Adjuvant Breast and Bowel Project (NSABP) began a clinical trial (B-18) to evaluate the efficacy of neoadjuvant chemotherapy compared to adjuvant therapy in patients with operable breast cancer. Approximately 1500 women were randomized to receive chemotherapy either pre- or post-operatively [8]. Although there were no survival differences or differences in rates of ipsilateral tumor recurrence after lumpectomy, preoperative chemotherapy led to an improvement in rates of breast conservation. Since that time, numerous studies have confirmed the utility of neoadjuvant chemotherapy in patients with locally advanced inoperable disease as well as those with operable disease who desire breast-conserving therapy [9 10]. In addition, the neoadjuvant platform creates an excellent model to assess pathologic tumor response and analyze tissue upon completion of systemic chemotherapy. Ultimately, this allows for the identification of molecular changes induced by treatment, and most importantly, it permits determination of predictors of chemotherapy resistance and chemotherapy sensitivity (CS) to better individualize management.

While several clinical trials involving neoadjuvant traditional chemotherapy, biologics, or different dosing schedules have recently been launched in an attempt to improve the

outcome of patients with TNBC, the initial phase of neoadjuvant studies did not restrict entry criteria to only patients with TNBC. However, a large proportion (41%) of the study population in NSABP protocol B-40 was classified as having TNBC [11]. The goals of this study were to determine if the addition of capecitabine or gemcitabine to docetaxel followed by doxorubicin and cyclophosphamide (AC) would increase pCR rates in patients with palpable and operable HER 2-negative disease and also to determine whether the addition of bevacizumab to docetaxel-based regimens followed by AC will increase pCR rates. In the chemotherapy alone arms, the addition of capecitabine or gemcitabine to docetaxel versus docetaxel alone did not increase the pCR rates (29.7% and 31.8%, respectively, vs. 32.7%; P=0.69). Similarly, the Gepar Trio study reported by Huober et al. showed a pCR rate of 39% in the TNBC patients in a trial that sought to determine the effect of switching neoadjuvant chemotherapy depending on mid-course response on pCR at time of surgery [12]. Patients received 2 cycles of docetaxel, doxorubicin, and cyclophosphamide (TAC), followed by either 4 or 6 more cycles of TAC in responders or 4 cycles of TAC versus capecitabine plus vinorelbine in non-responders. In non-responders, pCR rates were less than 10% in both groups, suggesting a lack of benefit of switching chemotherapy to these non-standard agents or even providing further chemotherapy in the adjuvant setting to that subset of patients.

In contrast to the lack of data that capecitabine, vinorelbine or gemcitabine improve pCR rates, there is increasing data recently indicating a role for platinum agents in the treatment of TNBC. CALGB 40603, recently presented at San Antonio Breast Cancer Symposium 2013, evaluated the impact of the addition of carboplatin and/or bevacizumab to neoadjuvant weekly paclitaxel followed by dose-dense AC on pCR rates in TNBC. This was a 2 X 2 factorial design in which patients on Arm A received weekly paclitaxel at 80 mg/m^2 for 12 weeks followed by dose dense AC for 4 cycles, patients on Arm B received weekly paclitaxel 80 mg/m^2 for 12 weeks plus bevacizumab followed by dose dense AC for 4 cycles, patients on Arm C received weekly paclitaxel 80 mg/m^2 for 12 weeks plus carboplatin followed by dose dense AC for 4 cycles, and patients on Arm D received weekly paclitaxel 80 mg/m^2 for 12 weeks plus carboplatin and bevacizumab followed by dose dense AC for 4 cycles. While there was no clear benefit from the addition of bevacizumab, pCR rates increased from 41% to 54% in patients receiving carboplatin. Similarly, a significant improvement in pCR rate (58.7% vs 37.9%, $p<0.05$) in TNBC was observed with the addition of carboplatin (AUC 1.5 weekly) to 18 weeks of weekly paclitaxel (80mg/m^2) plus non-pegylated liposomal doxorubicin (20mg/m^2) in the GeparSixto trial [13].

In this trial, we therefore aim to develop predictors of response (pCR vs. non-pCR) to neoadjuvant taxane and carboplatin. If sensitive tumors could be identified prospectively, a trial designed to show that the addition of platinum improves survival could be considered. This could lead to a simplified regimen whereby the doxorubicin-based component could be avoided in the treatment of TNBC.

1.3 Patient-derived Xenografts (PDX) in Breast Cancer

Despite progress in our understanding of cancer biology, the translation of research findings into new therapies for cancer is still an enormous barrier. Recent data suggests a 90% rate failure rate for oncology products in clinical development [14]. In part, the development of new therapies in breast cancer is constrained by the scarcity of reliable *in vivo* models of human breast cancer with which to study the biology of tumors and how they spread. Breast cancer cell lines, although informative, do not reliably reflect breast cancer heterogeneity or morphology *in vivo*, and thus poorly predict how drugs will perform in the clinical setting [14-16]. To address this, patient-derived xenografts (PDX) are being explored as surrogates for originating tumors, as reviewed extensively by Landis and colleagues [17].

Interestingly, several groups have found that the take rate of PDX correlate with tumor grade, with the most aggressive grade III and IV TNBCs having higher take rates than ER+ tumors [18-21]. Marangoni and colleagues used estrogen-treated Swiss nude mice to subcutaneously transplant tumor into the subscapular fat pad [18]. A 12.5% rate of engraftment was observed (25 out of 200 samples), with almost all PDX displaying an aggressive phenotype. PDX response rate compared to patient response to treatment was concordant in 5/7 of cases, supporting the utility in these models to predict patient response to therapeutics. Bergmaschi and colleagues used estrogen supplementation and transplantation into the interscapular fat pad of severe combined immunodeficiency (SCID) mice, resulting in a 7% take rate [19]. Of the 2 PDX established, both maintained concordance with the original tumors. Zhang and colleagues established PDX directly from breast cancer patient samples, using epithelium-free mammary fatpad as the transplantation site [20]. Again, most patients yielded triple negative PDX, though lines from other subtypes (ER-PR-HER2+, ER+PR+HER2–, ER+PR-HER2, and ER+PR+HER2+) were also established. PDX were phenotypically consistent with their tumor of origin, and of the 27 lines fully evaluated, 48% developed metastatic lesions to the lungs.

At our institution, we have successfully developed several panels of PDX (Washington University Human in Mouse-WHIM lines) from breast cancer patients under the umbrella of the Longitudinal Cancer Gene Profiling Using Sequential Tissue Biopsies breast cancer protocol (HRPO# 04-0022 / 201102244). Six patients with TNBC have had successful WHIM lines developed from their original tumors. Three of these patients are deceased while the other 3 achieved a pCR and are currently disease free. Table 1 shows characteristics of WHIMs and corresponding patients.

WHIM No.	Race	TP53	PIK3CA	PAM50 subtype	Stage	pathCR	Overall survival in months
2, 5	AA	S166Sins	WT	Basal	3B	No	26.9
6	AA	WT	WT	Basal	4		36
12	Caucasian	R248Q	WT	Claudin low	2B	NA	30.4
17	Caucasian	WT	WT	Basal	2B	Yes	96.4+
30	Caucasian	V216M	WT	Basal	3A	Yes	50.3+
46	Unknown	Unknown	Unknown	Unknown	2B	yes	25.1+

Table 1

Additionally, we have previously shown that whole genome sequencing of a breast cancer primary, brain metastasis, and PDX basal-like breast cancer in one patient demonstrated a PDX model that efficiently captured almost all of the genome-wide somatic mutations from the original tumor and was enriched for mutations present in the metastatic sample. We have also shown that comparative whole-genome sequencing demonstrates that PDX preserves the genomic structural similarities to originating TNBC tumors[22]. A panel of PDX from patients with treatment-resistant breast cancer was established to study the genomic, biological, and pharmacological characteristics of advanced breast cancer. Concordant human and xenografts ER and HER2 status was demonstrated at the mRNA level and confirmed by western blotting.. Array-based mRNA expression data was generated from the originating tumors and matched early and late passage WHIM counterparts. In almost all cases the originating tumor and WHIM lines derived from the same individual clustered adjacently when subjected to unsupervised clustering. In addition, all structural variants (translocations, large deletions, inversions) were preserved upon transplantation, including complex crisscross patterns between 2 chromosomal regions characteristic of chromothripsis (Figure 1). However, there were usually many more supporting reads for any particular structural variant in the WHIM line analysis than in the originating tumor because the xenograft data analysis pipeline removed all background sequence from the mouse genome, thereby "virtually purifying" the human tumor DNA from mouse stromal DNA. The preservation of variant allele frequencies from human to PDX suggests stable interactions between minor and major clones that were not influenced by the xenografting process**.**

This study is based on the hypothesis that PDX are accurate replicas of originating tumors and can recapitulate chemotherapy response. The ability to effectively utilize innovative PDX models may be a unique new starting point for molecular pharmacology in TNBC patients. The development of these models, as outlined here, will allow an exploration of the relationship between PDX phenotype and clinical outcome of patients from whom tumors arose. It will elucidate the mechanisms of response to chemotherapy through analysis of serial biopsies using proteomic technologies, while also correlating molecular changes in PDX to those in originating tumors. The profound impact of this study to clinical breast cancer research is that ultimately, drug discovery using PDX as a living replica may be done more efficiently, especially for those with chemotherapy-resistant TNBC, thus allowing for uniquely individualized management.

1.4 Chemotherapy Agents

Chemotherapy can substantially reduce the risk of breast cancer recurrence and death in early stage breast cancer [23], and there are many chemotherapy regimens with established efficacy and safety data. The value of chemotherapy has been consistently demonstrated from the data of individual randomized trials and affirmed in the Early Breast Cancer Trialists' Collaborative Group's (EBCTCG) 15-year meta-analyses combining data from the individual chemotherapy trials. The meta-analyses have shown that anthracycline-containing therapies, such as sequential doxorubicin and cyclophosphamide (AC) followed by taxanes, and combination regimens such as docetaxel, doxorubicin, and

cyclophosphamide (TAC), reduce the risk of recurrence by 11% and the risk of death by 16% compared with cyclophosphamide, methotrexate, and fluorouracil (CMF) combinations [24].

CALGB 9344 and NSABP B-28 established the role of taxanes as a component of adjuvant therapy of breast cancer. Between May 1994 and April 1999, the CALGB randomized 3121 patients in a 3x2 factorial design to cyclophosphamide combined with one of three doses of doxorubicin given every 3 weeks for 4 cycles followed by paclitaxel on an every-3-week schedule or observation. No differences in efficacy based on doxorubicin dose were observed. However, adding paclitaxel to the AC regimens led to reductions in hazard for recurrence and death of 17% and 18%, respectively [25]. NSABP B-28 randomized 3060 women with resected, node-positive breast cancer to receive 4 cycles of AC followed by 4 cycles of paclitaxel on the every-3-week schedule vs. 4 cycles of AC. The addition of paclitaxel significantly reduced the hazard for disease-free survival (DFS) events by 17% (relative risk 0.83; 95% CI, 0.72– 0.95; p=0.006), but overall survival (OS) was similar for both groups [26].

NSABP B-27 evaluated the potential benefit of administering docetaxel (T) following AC as preoperative therapy for 2411 women with palpable, operable breast cancer. The addition of preoperative or postoperative T after preoperative AC showed a non-significant trend toward improving DFS, primarily by decreasing the incidence of local recurrences, but did not improve OS. Concurrent use of tamoxifen may have limited the impact of adding T [27].

ECOG E1199 employed a 2x2 factorial design to compare paclitaxel to docetaxel following 4 cycles of AC and a weekly schedule vs. an every-3-week schedule of the taxanes in nearly 5,000 women with node-positive or high-risk node-negative breast cancer. Patients were randomly assigned to receive one of the following taxane treatments: docetaxel 35 mg/m^2 once a week; docetaxel 100 mg/m^2 once every 3 weeks; paclitaxel 80 mg/m^2 once a week; or paclitaxel 175 mg/m^2 once every 3 weeks. The primary comparisons showed no differences between the taxanes (paclitaxel vs. docetaxel: HR 1.032; p=0.61) or schedule (q3w vs. q1w: HR 1.062; p=0.33). However, a Cox proportional hazards model, which included the taxane administered, the taxane schedule and their interaction, showed the interaction of docetaxel and the weekly schedule was significant for both DFS (p=0.003) and OS (p=0.01). This complicated the interpretation of the primary endpoints so comparisons were made between the standard every-3-week paclitaxel schedule and each of the other three arms. As compared with the group receiving paclitaxel every 3 weeks, there was better DFS in the group receiving weekly paclitaxel (HR 1.27; p=0.006) and in the group receiving every-3-week docetaxel (HR 1.23; p=0.02). However, while OS was significantly better in the group receiving weekly paclitaxel (HR 1.32; p=0.01), it was not evident in the group receiving docetaxel every 3 weeks (HR 1.13; p=0.25). Serious adverse effects of treatment developed in 71% of patients treated with docetaxel every 3 weeks, and only 28% of patients treated with paclitaxel once a week. The increase in adverse events with docetaxel was primarily due to febrile neutropenia and neutropenia-associated infections which can be substantially diminished with the use of primary prophylactic G-CSF [28] [29]. Most recently, SWOG 0221, compared weekly

paclitaxel to dose-dense (every 2 week) scheduling as adjuvant breast cancer treatment and showed no difference in efficacy endpoints with either method of delivery. Patients had higher rates of grade 3 and 4 neuropathy (17% vs. 10%) with dose-dense delivery and higher rates of hematologic toxicity with weekly scheduling[30].

As sporadic TNBC is clinically similar to BRCA-1 associated breast cancer, there has been significant interest in using platinum compounds in TNBC. BRCA-1 associated breast cancers are sensitive to these agents since they cause double-strand breaks in DNA and these cancers are deficient in homologous recombination mediated DNA repair mechanisms. Several neoadjuvant trials have evaluated platinum agents in TNBC patients. Alba et el. investigated whether the addition of carboplatin to standard chemotherapy in patients with TNBC would lead to an increase in the pCR rates in the neoadjuvant setting.[31] Patients received epirubicin plus cyclophosphamide (EC) followed either by docetaxel or docetaxel plus carboplatin. The addition of the platinum did not improve pCR rates (35% versus 30%) in this study. Silver et al. evaluated the efficacy of neoadjuvant cisplatin in 28 patients with TNBC.[32] All patients received 4 cycles of cisplatin preoperatively, followed by definitive surgery, and then adjuvant chemotherapy and/or radiation as per their treating physicians. The pCR rate was 21% (6 of 28 patients), while 64% (18 of 28) achieved either a clinical complete or partial response. The efficacy of neoadjuvant cisplatin in TNBC versus non-TNBC was also compared in a small retrospective study by Sirohi et al.[33] Complete response rates by clinical exam were higher for those with TNBC (88%) versus the non-TNBC group (51%). Paradoxically survival outcomes were worse for the TNBC group despite higher rates of initial response to chemotherapy. This has also been seen in a study that showed despite a higher rate of chemosensitivity, patients with TNBC had a worse outcome than those with ER positive disease.[34] Multiple other small studies have also evaluated neoadjuvant platinum-based therapy in patients with TNBC with varying results.[35-42] While current data provides insufficient evidence for the routine use of platinums in patients with TNBC, the pCR improvements observed in the neoadjuvant setting have created a conundrum and some physicians are now routinely adding carboplatin. However, until survival data for the addition of carboplatin is available, the use of carboplatin is still considered investigational by other physicians. Since the most significant issue remains the identification of platinum sensitivity, this protocol is therefore designed to have high clinical and scientific impact.

1.5 Study Rationale

While improvements in adjuvant therapy have remarkably improved the outcome of women with resectable breast cancers over the past two decades, biologically defined subsets of patients at a high risk of recurrence and death continue to exist. Improving upon our ability to eradicate micrometastases and prevent recurrences in these high risk individuals, particularly those with TNBC remains a challenge. The relapse rate for patients treated with adjuvant therapy on clinical trials has declined, due, in part, to improvements in treatment. Unfortunately, testing new drugs in the adjuvant setting in breast cancer requires large number of patients as well as long follow up periods. The neoadjuvant platform allows the opportunity to assess clinically meaningful responses in vivo, therefore enabling more rapid identification of effective drugs and consequently changes to

established standards of care. In fact, to facilitate early access of potentially active drugs for patients with high risk disease such as TNBC, the Food and Drug Administration (FDA) has recently outlined a pathway for accelerated drug approval based on pathologic complete response (pCR) from neoadjuvant trials. Additionally, the neoadjuvant platform allows us to obtain serial tissue and blood samples before and after treatment, which may allow identification of subsets of patients less or more likely to benefit from a given treatment.

As TNBC lacks an identified therapeutic target, most individuals receive chemotherapy as part of their systemic management. Unfortunately a large proportion develop recurrences due to chemotherapy resistance, therefore there is a need to develop more effective chemotherapy regimens and also identify predictors of CR and chemotherapy sensitivity (CS) to better individualize management. The use of proteomic and genomic analysis in the design of trials evaluating mechanisms of CR are lacking. By being able to assess pathologic tumor response and molecular changes induced by treatment, the neoadjuvant platform creates an excellent model for such a study. Typical clinical trial experimentation recruiting individuals to adjuvant drug studies, and then assessing survival may not be the most efficient method. Additionally, although cell lines are informative, they do not accurately reflect tumor morphology or heterogeneity in vivo. To address this, PDX are being explored as surrogates for originating tumors.

This research proposal investigates a potential new standard of care for patients with curable TNBC using a combination of docetaxel and carboplatin in the neoadjuvant setting. We propose to treat patients with a similar backbone as the CALGB 40603 regimen by administering six 3-week cycles of docetaxel in combination with 6 cycles of carboplatin to improve pCR with a view to omitting anthracyclines. This strategy will avoid the cardiotoxicity and leukemia risk associated with anthracyclines and will develop evidence for a much more convenient regimen than the Gerpar Sixto protocol of 18 weeks of weekly carboplatin and paclitaxel. It has been postulated that DNA-damaging agents like platinum salts may be particularly effective in TNBC due to its similarities with BRCA-1 associated breast cancer. Studies utilizing taxanes and platinum salts have been performed in unselected breast cancer patients and have shown higher response rates than single agent taxanes and good tolerance with a low incidence of serious side effects. In addition, PDX models will be developed from tumors from individual patients on this trial. This study is therefore based on the hypotheses that neoadjuvant docetaxel and carboplatin will achieve a pCR rate of 54%, similar to the CALGB 40603 carboplatin-containing arms, and that PDX are accurate replicas of originating tumors and can recapitulate chemotherapy response. The promise of this novel strategy is that PDX may be used as drug discovery platform for chemotherapy-resistant patients.

To understand the potential clinical impact of this study, it is valuable to consider a clinical scenario. After diagnosis, a TNBC patient may be treated with neoadjuvant chemotherapy with the ultimate goal of minimizing the risk of disease recurrence. While these treatments are associated with a 20-38% decrease in breast cancer mortality amongst all subtypes [24], patients with TNBC that do not respond to chemotherapy continue to have a substantial risk of recurrent disease within the first 5 years. A novel personalized strategy to assess

drug responsiveness will be to develop a PDX model from a particular patient, either shortly before or during neoadjuvant treatment, treat the PDX model with a view to determining chemotherapy response in the PDX. This then may be able to be a surrogate for whether the TNBC patient will likely respond to the drug being administered. In addition, drug discovery using the PDX platform as a living replica may also be utilized for those with chemotherapy resistant TNBC, thus allowing for individualized management.

1.6 Correlative Studies Background

Development of new cancer therapies is constrained by the scarcity of reliable in vivo models of human breast cancer with which to study the biology of tumors and how they spread, and to use for validation of the efficacy of potential new drugs. This protocol using PDX models may be a unique new starting point for molecular pharmacology in TNBC patients. The development of these models will allow an exploration of the relationship between PDX phenotype and clinical outcome of patients from whom tumors arose. It will also enable investigating the mechanisms of response to chemotherapy through analysis of serial tissue using genomic and proteomic technologies, while also correlating molecular changes in PDX to those in originating tumors.

1.6.1 Multi-parametric Positron Emission Tomography (PET) and Magnetic Resonance (MR) Imaging to Assess Response to Therapy

Advanced quantitative imaging (QI) strategies offer an opportunity to assess and predict response to therapy early in the treatment. ^{18}F-fluorodeoxyglucose (FDG), a glucose analogue, with PET has been extensively used to measure tumor metabolism as an indicator of response to therapy. Similarly, ^{18}F-fluorothymidine (FLT), a cell proliferative marker, with PET imaging is used as a marker of tumor proliferation via the DNA_salvage pathway. Both have shown efficacy in assessing tumor response to therapy. In particular, FDG-PET image metrics have shown utility in stratifying responders from non-responders [43-46] in TNBC. More recently, results from the American college of radiology imaging network (ACRIN) 6688 trial have shown that FLT-PET weakly predicted pCR in the setting of variable NAC and a mixed population of breast cancer patients; with the conclusion that a more uniform patient population/NAC plan is needed to fully assess the utility of FLT-PET [47]. In addition, multi-parametric MR imaging has shown efficacy in assessing response to therapy in breast cancer [48 49]. The primary objective of QI strategies to identify patients who will respond to NAC therapy, in order to avoid the use of ineffective treatments in nonresponding patients with an opportunity to devise adaptive treatment strategies. In this context, one of the objectives of this trial is to initiate a pilot study to assess the utility of advanced QI methods to predict pCR in TNBC.

The addition of a grant (U24CA209837) is to support imaging studies to assess response to therapy. It is critical to identify patients who will respond to NAC therapy, and to avoid the use of an ineffective treatments in nonresponding

patients with an opportunity to devise adaptive treatment strategies. The imaging methodologies have already been included in the protocol, however, they were optional. With the addition of the funding, imaging will be required. All imaging studies will be performed at the Center for Clinical Imaging Research (CCIR). We are requesting to add 30 patients to support the objectives proposed in the grant. Groheux et al. [59] have shown efficacy in differentiating pCR in TNBC with a sample size of 20 using 18FDG PET/MR where change in SUVmax yielded an AUC of 0.88 and with 30% of the sample being pCR. Therefore, conservatively based on this data, a sample of 30 patients (30% being responders) achieves 80% power to detect a difference of 0.3 between the AUC under the null hypothesis of 0.5 and an AUC under the alternative hypothesis of 0.8 using a one-sided z-test at a significance level of 0.05 and assuming a dropout rate of 15%.

1.6.2 Circulating tumor DNA to predict clinical outcome

ctDNA is cell-free tumor-derived fragmented DNA in the bloodstream and has shown promise for disease monitoring and molecular characterization in advanced cancers.[50-56] In advanced TNBC, mutations in ctDNA show high concordance with those seen in primary breast biopsies.[57] In early stage breast cancer, detection of ctDNA can detect minimal residual disease.[58] We suggest that the simplicity of blood sampling will allow for detection and mutation tracking of ctDNA in patients with previously undetectable minimal residual disease at high-risk for clinical recurrence. We will evaluate the potential to detect and track ctDNA in early-stage TNBC and determine if it might predict clinical outcome.

2.0 OBJECTIVES

2.1 Primary Objective

To determine whether neoadjuvant docetaxel and carboplatin will increase the pCR rate in TNBC compared to historical controls.

2.2 Exploratory Objectives

1. To determine the xenografting rate from TNBC patients being treated with neoadjuvant chemotherapy.
2. To compare chemotherapy responses in PDX and TNBC patients being treated with neoadjuvant chemotherapy
3. To investigate genomic and proteomic molecular changes in PDX and corresponding host patients with the intent to identify predictors of drug response and resistance.
4. To assess the utility of multi-parametric FDG-PET/MR imaging in predicting response to therapy after one cycle of therapy.
5. Measure circulating tumor DNA (ctDNA) somatic mutations in serial plasma samples, and determine if ctDNA can predict clinical outcomes.

3.0 PATIENT SELECTION

3.1 Inclusion Criteria

1. Newly diagnosed AJCC7 clinical stage II or III breast cancer with complete surgical excision of the breast cancer after neoadjuvant chemotherapy as the treatment goal.

2. Patients with PR+ tumors are allowed.

3. HER2 negative by FISH or IHC staining 0 or 1+.

4. ER less than Allred score of 3 or less than 1% positive staining cells in the invasive component of the tumor

5. Tumor size at least 2cm in one dimension by clinical or radiographic exam (WHO criteria). Patients with palpable lymph nodes may be enrolled regardless of tumor size.

6. At least 18 years of age.

7. ECOG performance status ≤ 2

8. Normal bone marrow and organ function as defined below:
   a. Leukocytes $\geq$ 3,000/mcL
   b. Absolute neutrophil count $\geq$ 1,500/mcl
   c. Platelets $\geq$ 100,000/mcl
   d. Serum bilirubin within (or under) normal limits (OR total bilirubin $\leq$ 3.0 x IULN with direct bilirubin within normal range in patients with well documented Gilbert Syndrome)
   e. AST(SGOT)/ALT(SGPT) within (or under) normal limits
   f. Creatinine clearance $\geq$ 30 mL/min/1.73 m^2

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

10. Ability to understand and willingness to sign an IRB approved written informed consent document (or that of legally authorized representative, if applicable).

11. Able to tolerate PET/MRI with intravenous contrast administration and must complete the applicable MRI screening evaluation form.

3.2 Exclusion Criteria

1. Prior systemic therapy for the indexed breast cancer.

2. A history of other malignancy ≤ 5 years previous with the exception of basal cell or squamous cell carcinoma of the skin which were treated with local resection only or carcinoma *in situ* of the cervix.

3. Patients with bilateral or inflammatory breast cancer.

4. Currently receiving any other investigational agents.

5. A history of allergic reactions attributed to compounds of similar chemical or biologic composition to docetaxel or carboplatin.

6. 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.

7. Pregnant and/or breastfeeding. Patient must have a negative serum pregnancy test within 7 days of study entry if premenopausal.

8. Known HIV-positivity.

9. Sentinel lymph node biopsy.

10. Renal insufficiency (glomerular filtration rate (GFR) < 30 mL/min/1.73 m^2) measured within the past 60 days which precludes safe administration of the contrast agent

11. On dialysis

12. Prior allergic reaction to gadolinium-based MR contrast agents

3.3 Inclusion of Women and Minorities

Because breast cancer occurs predominantly in women, men will not be eligible for this trial. Women and members of all races and ethnic groups are eligible for this trial.

4.0 REGISTRATION PROCEDURES

Patients must not start any protocol intervention prior to registration through the Siteman Cancer Center.

The following steps must be taken before registering patients to this study:

1. Confirmation of patient eligibility by Washington University
2. Registration of patient in the Siteman Cancer Center OnCore database
3. Assignment of unique patient number (UPN)

4.1 Confirmation of Patient Eligibility

Confirm patient eligibility by collecting the information listed below at least one business day prior to registering patient:

1. The registering MD's name
2. Patient's race, sex, and DOB
3. Three letters (or two letters and a dash) for the patient's initials
4. Copy of signed consent form
5. Completed eligibility checklist, signed and dated by a member of the study team
6. Copy of appropriate source documentation confirming patient eligibility

4.2 Patient Registration in the Siteman Cancer Center Oncore Database

All patients must be registered through the Siteman Cancer Center OnCore database.

4.3 Assignment of UPN

Each patient will be identified with a unique patient number (UPN) for this study. All data will be recorded with this identification number on the appropriate CRFs.

5.0 TREATMENT PLAN

5.1 Premedication Administration

Patients receiving docetaxel should be premedicated with dexamethasone 8 mg (or its equivalent) PO twice a day X 3 days starting the day before docetaxel (alternatively, dexamethasone 10 mg IV 30 to 60 minutes before dosing), diphenhydramine 50 mg (or its equivalent) IV 30 to 60 minutes before dosing, and cimetidine 300 mg or ranitidine 50 mg (or equivalent) IV 30 to 60 minutes or before dosing.

5.2 Agent Administration

Docetaxel will be administered intravenously at a dose of 75mg/m^2 over 60 minutes on Day 1 of each 21-day cycle. Carboplatin AUC 6 will be administered intravenously over 30 minutes on Day 1 of each 21-day cycle immediately following docetaxel infusion. For carboplatin dosing, creatinine clearance (CrCl) will be calculated according to the Cockcroft-Gault equation (Appendix B). CrCl should be capped at 125 mL/min. A total of 6 cycles will be given.

Surgery will take place 3 to 5 weeks after the conclusion of the neoadjuvant regimen. Further adjuvant chemotherapy may be given at the discretion of the treating physician, but we recommend that patients who have a pCR in the breast and lymph nodes do not receive further adjuvant chemotherapy.

5.3 Adjuvant Chemotherapy

Patients who develop grade 3 neuropathy, other intolerable side effects, progressive disease, or who do not achieve a pCR with the docetaxel/carboplatin combination will have the option of receiving anthracycline-based chemotherapy (such as AC or FEC) OR capecitabine post-operatively at the discretion of the treating physician. These patients will be counted as non-pCR.

5.4 General Concomitant Medication and Supportive Care Guidelines

Physicians will perform standard monitoring for toxicity to chemotherapy administration. Patients will be treated for toxicities at the discretion of the physician. Growth factor support with either filgrastim or pegfilgrastim is at the discretion of the investigator.

5.5 Women of Childbearing Potential

Women of childbearing potential (defined as women with regular menses, women with amenorrhea, women with irregular cycles, women using a contraceptive method that precludes withdrawal bleeding, and women who have had a tubal ligation) are required to have a negative serum pregnancy test within 7 days prior to the first dose of docetaxel.

Female patients are required to use one form of acceptable contraception during participation in the study and for 6 months following the last dose of chemotherapy.

If a patient is suspected to be pregnant, chemotherapy should be immediately discontinued. In addition a positive urine test must be confirmed by a serum pregnancy test. If it is confirmed that the patient is not pregnant, the patient may resume dosing.

If a female patient becomes pregnant during therapy or within 6 months after the last dose of chemotherapy, the investigator must be notified in order to facilitate outcome follow-up.

5.6 Duration of Therapy

If at any time the constraints of this protocol are considered to be detrimental to the patient's health and/or the patient no longer wishes to continue protocol therapy, the protocol therapy should be discontinued and the reason(s) for discontinuation documented in the case report forms.

In the absence of treatment delays due to adverse events, treatment may continue for 6 cycles or until one of the following criteria applies:

- Documented and confirmed disease progression
- Death
- Adverse event(s) that, in the judgment of the investigator, may cause severe or permanent harm or which rule out continuation of study drug
- General or specific changes in the patient's condition render the patient unacceptable for further treatment in the judgment of the investigator
- Suspected pregnancy
- Serious non-compliance with the study protocol
- Lost to follow-up
- Patient withdraws consent
- Investigator removes the patient from study
- The Siteman Cancer Center decides to close the study

Patients who prematurely discontinue treatment for any reason will be followed as indicated in the study calendar.

5.7 Duration of Follow-up

Patients will be followed for survival on a yearly basis for 5 years. Patients removed from study for unacceptable adverse events will be followed until resolution or stabilization of the adverse event.

6.0 DOSE DELAYS/DOSE MODIFICATIONS

For patients who are unable to tolerate the protocol-specified dosing schedule, dose adjustments at the discretion of the treating physician are permitted in order to keep the patient on protocol. If administration of chemotherapy must be interrupted because of unacceptable toxicity, drug dosing will be interrupted or modified according to discretion of the treating physician. If carboplatin is to be delayed, administration of docetaxel shall be omitted as well. Toxicity will be assessed using the NIH-NCI Common Terminology Criteria for Adverse Events, version 4.0.

7.0 SIMULTANEOUS PET/MR IMAGING AND ANALYSIS

Patients will be studied with FDG-PET/MR centered at the level of the breasts prior to initiation of Cycle 1 of NAC therapy (preferably on Day 1 of Cycle 1—however, up to 2 weeks prior to the start of therapy will be allowed due to scheduling difficulties) and prior to initiation of Cycle 2 (preferably on Day 1 of Cycle 2—however, imaging performed Days 15 to 21 will be allowed due to scheduling difficulties). The second FDG-PET/MR will be matched in regard to the fasting duration, FDG dose, and PET/MR imaging parameters. All imaging studies will be performed using the Siemens Biograph mMR PET/MRI scanner, located on the 10th floor West Pavilion of Barnes-Jewish Hospital at the Center for Clinical Imaging Research (CCIR). This state-of-the-art scanner allows for true simultaneous image acquisition of PET and MRI data. All subjects will complete an MRI screening evaluation form and have intravenous (IV) access established prior to

entering MRI zone 3 or the scanning room. In the event unforeseen technical difficulties will prevent a patient from being scanned at baseline without causing a delay in starting NAC therapy (for example scanner is not operating within normal limits or FDG is unavailable) a subject can remain on the study and proceed with NAC therapy as planned (without baseline imaging) in order to address the Primary Objective and other Exploratory objectives. A subject who does not receive imaging at baseline will not be asked to return for follow-up early response imaging. Similarly, if technical difficulties arise at the early response imaging time point (prior to cycle 2 of therapy), cycle 2 of therapy should proceed as scheduled and patient may be scheduled to return for imaging up to 10 days after cycle 2 was administered.

7.1 Multi-Modality PET/MR Imaging

This is an exploratory objective to optimize imaging parameters for simultaneous PET/MR imaging and using the breast coil to facilitate MR imaging. Subjects will be positioned prone in the PET/MRI scanner with arms resting above the head or resting by their sides (bed position 1 or BPos1). The multi-parametric PET/MR protocol listed below will be employed for both baseline (pre-treatment) and the early-treatment response time points following Cycle 1 of NAC. All patients will fast for at least 4 hours prior to FDG-PET/MR imaging. Fasting serum glucose levels prior to FDG injection must be less than 200 mg/dL and IV should be in the arm opposite the known breast lesion whenever possible. Approximately 10 mCi of FDG will be administered IV while patient rests comfortably in an uptake room. The patient will be positioned prone in the scanner at BPos1. Following a simple DIXON scan for attenuation correction, 35 min of dynamic PET and simultaneous MRI imaging will be acquired beginning approximately 35 minutes post FDG injection. Following BPos 1, a 2 min image acquisition of the liver will be employed in bed position 2 (BPos 2) to develop PERCIST (PET Response Criteria in Solid Tumors) measures. In parallel to PET imaging the following MR sequences will be implemented which are subject to change depending on study need:

- T2-weighted
- T1-weighted
- T1/T2 mapping
- Diffusion Tensor Imaging (DTI) (multi-shell)
- Research dynamic contrast-enhanced (DCE) protocol—high temporal low spatial resolution
- Localizer
- Dixon

Contrast injection should consist of IV administration of 0.1 mmol/kg of Dotarem, Multihance or other gadolinium-based MRI contrast agent

7.2 Image Analysis

7.2.1 Image Co-registration

To facilitate quantitative image analysis, MR images of a given patient (pre- and early posttherapy) will be co-registered by employing a rigid body algorithm using commercially available software, thus facilitating the translation and applicability to other imaging centers. Anatomical images will be inspected visually to further manually align images, if needed, using the outline of the breast. Since PET and MR images are intrinsically co-registered (acquired simultaneously), the MR-to-MR image co-registration transformation parameters will be applied to PET images. Subsequently, each tumor will be identified by a landmark and annotated.

7.2.2 Tumor Identification

Target tumor lesions will be identified on PET/MR study by expert radiologists with one radiologist who is experienced in Breast MRI (Dr. Steven Poplack) and one observer with expertise in nuclear medicine (Dr. Farrokh Dehdashti).

7.2.3 Quantitative Analysis

In addition to measures of PERCIST measures of tumor response to therapy, we will quantify the pharmacokinetics of FDG in tumors using established methodologies. As another layer, we will apply local masks based on multi-parametric MR images in performing image quantification; for example, by limiting the analysis to regions of decreased apparent diffusion coefficient (ADC), and/or high perfusion, to investigate the interplay between tumor metabolism and perfusion in a constrained microenvironment established by multi-parametric MR. Through this analysis, we will identify unique and independent image features to explore as predictors of response to therapy.

7.3 Toxicities Related to FDG-PET/MR Imaging

Likely:

- Mild discomfort from the placement of the IV in the patient's arm.
- Radiation exposure: The amount of radiation exposure the patient will receive from one FDG-PET/MR imaging session is equivalent to a uniform whole-body exposure of approximately 0.6 rem (total exposure of 1.2 rem for 2 scans).

Less Likely:

- Discomfort from lying still on the PET imaging table.
- There is a slight risk of bruising at sites of vein puncture.
- Claustrophobia from lying inside the PET/MRI scanner

Rare:

- There is a remote risk of infection and an even smaller risk of blood clot at the site of the IV placement.

- There is a rare possibility of an allergic-type or other adverse reaction to radioactively labeled drugs. While none have been reported to date with the radioactive material FDG, such a reaction could be serious and may result in death.
- There is a theoretical risk of nephrogenic systemic fibrosis (NSF), which has been linked to gadolinium contrast agents administered in patients with severe renal dysfunction. This risk is mitigated by the use of renal function screening.

Recent information shows that repeated gadolinium use may cause collection in the brain. The importance of this information and how it impacts patient health are not known.

8.0 REGULATORY AND REPORTING REQUIREMENTS

The entities providing oversight of safety and compliance with the protocol require reporting as outline below.

The Washington University Human Research Protection Office (HRPO) requires that all events meeting the definition of unanticipated problem or serious noncompliance be reported as outlined in Section 8.2.

8.1 Definitions

8.1.1 Adverse Events (AEs)

Definition: any unfavorable medical occurrence in a human subject including any abnormal sign, symptom, or disease.

Grading: the descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for all toxicity reporting. A copy of the CTCAE version 4.0 can be downloaded from the CTEP website.

Attribution (relatedness), Expectedness, and Seriousness: the definitions for the terms listed that should be used are those provided by the Department of Health and Human Services' Office for Human Research Protections (OHRP). A copy of this guidance can be found on OHRP's website:
http://www.hhs.gov/ohrp/policy/advevntguid.html

8.1.2 Serious Adverse Event (SAE)

Definition: any adverse drug experience occurring at any dose that results in any of the following outcomes:

- Death
- A life-threatening adverse drug experience

- Inpatient hospitalization or prolongation of existing hospitalization
- A persistent or significant disability/incapacity (i.e., a substantial disruption of a person's ability to conduct normal life functions)
- A congenital anomaly/birth defect
- Any other experience which, based upon appropriate medical judgment, may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed above

8.1.3 Unexpected Adverse Experience

Definition: any adverse drug experience, the specificity or severity of which is not consistent with the current investigator brochure (or risk information, if an IB is not required or available).

8.1.4 Life-Threatening Adverse Experience

Definition: any adverse drug experience that places the subject (in the view of the investigator) at immediate risk of death from the reaction as it occurred, i.e., it does not include a reaction that, had it occurred in a more severe form, might have caused death.

8.1.5 Unanticipated Problems

Definition:

- unexpected (in terms of nature, severity, or frequency) given (a) the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document; and (b) the characteristics of the subject population being studied;
- related or possibly related to participation in the research ("possibly related" means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and
- suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

8.1.6 Noncompliance

Definition: failure to follow any applicable regulation or institutional policies that govern human subjects research or failure to follow the determinations of the IRB. Noncompliance may occur due to lack of knowledge or due to deliberate choice to ignore regulations, institutional policies, or determinations of the IRB.

8.1.7 Serious Noncompliance

Definition: noncompliance that materially increases risks, that results in substantial harm to subjects or others, or that materially compromises the rights or welfare of participants.

8.1.8 Protocol Exceptions

Definition: A planned deviation from the approved protocol that are under the research team's control. Exceptions apply only to a single participant or a singular situation.

Pre-approval of all protocol exceptions must be obtained prior to the event.

8.2 Reporting to the Human Research Protection Office (HRPO) at Washington University

The PI is required to promptly notify the IRB of the following events:

- Any unanticipated problems involving risks to participants or others which occur at WU, any BJH or SLCH institution, or that impacts participants or the conduct of the study.
- Noncompliance with federal regulations or the requirements or determinations of the IRB.
- Receipt of new information that may impact the willingness of participants to participate or continue participation in the research study.

These events must be reported to the IRB within **10 working days** of the occurrence of the event or notification to the PI of the event. The death of a research participant that qualifies as a reportable event should be reported within **1 working day** of the occurrence of the event or notification to the PI of the event.

8.3 Reporting to the Quality Assurance and Safety Monitoring Committee (QASMC) at Washington University

The PI is required to notify the QASMC of any unanticipated problem occurring at WU or any BJH or SLCH institution that has been reported to and acknowledged by HRPO as reportable. (Unanticipated problems reported to HRPO and withdrawn during the review process need not be reported to QASMC.)

QASMC must be notified within **10 days** of receipt of IRB acknowledgment via email to a QASMC auditor.

8.4 Timeframe for Reporting Required Events

Adverse events will be tracked for 30 days following the last day of study treatment.

9.0 PHARMACEUTICAL INFORMATION

9.1 Docetaxel (Taxotere)

9.1.1 Docetaxel Description

Docetaxel is an antineoplastic agent belonging to the taxoid family. The chemical name for docetaxel is (2R,3S)-N-carboxy-3-phenylisoserine,N-*tert*-butyl ester, 13-ester with 5β-20-epoxy-1,2α,4,7β,13α-hexahydroxytax-11-en-9-one 4-acetate 2-benzoate, trihydrate. Docetaxel is a white to almost-white powder with an empirical formula of $C_{43}H_{53}NO_{14}\cdot 3H_2O$ and a molecular weight of 861.9. It is highly lipophilic and practically insoluble in water.

9.1.2 Clinical Pharmacology

Docetaxel is an antineoplastic agent that acts by disrupting the microtubular network in cells that is essential for mitotic and interphase cellular functions. Docetaxel binds to free tubulin and promotes the assembly of tubulin into stable microtubules while simultaneously inhibiting their disassembly. This leads to the production of microtubule bundles without normal function and to the stabilization of microtubules, which results in the inhibition of mitosis in cells. Docetaxel's binding to microtubules does not alter the number of protofilaments in the bound microtubules, a feature which differs from most spindle poisons currently in clinical use.

9.1.3 Pharmacokinetics and Drug Metabolism

The pharmacokinetics of docetaxel have been evaluated in cancer patients after administration of 20 mg/m2 to 115 mg/m2 in phase 1 studies. The area under the curve (AUC) was dose proportional following doses of 70 mg/m2 to 115 mg/m2 with infusion times of 1 to 2 hours. Docetaxel's pharmacokinetic profile is consistent with a three-compartment pharmacokinetic model, with half-lives for the α, β, and γ phases of 4 min, 36 min, and 11.1 hr, respectively. Mean total body clearance was 21 L/h/m2. *In vitro* drug interaction studies revealed that docetaxel is metabolized by the CYP3A4 isoenzyme, and its metabolism may be modified by the concomitant administration of compounds that induce, inhibit, or are metabolized by cytochrome P450 3 A4.

9.1.4 Supplier(s)

Docetaxel is commercially available.

9.1.5 Dosage Form and Preparation

Docetaxel injection concentrate is a sterile, non-pyrogenic, pale yellow to brownish-yellow solution at 20 mg/mL concentration. Docetaxel injection

concentrate (20 mg/mL) requires NO prior dilution with a diluent and is ready to add to the infusion solution.

Using only a 21 gauge needle, aseptically withdraw the required amount of docetaxel injection concentrate (20 mg docetaxel/mL) with a calibrated syringe and inject via a single injection (one shot) into a 250 mL infusion bag or bottle of either 0.9% sodium chloride solution or 5% dextrose solution to produce a final concentration of 0.3 mg/mL to 0.74 mg/mL.

9.1.6 Storage and Stability

Docetaxel vials should be stored between 2 and 25°C (36 and 77°F). If the vials are stored under refrigeration, allow the appropriate number of vials to stand at room temperature for approximately 5 minutes before use. The final dilution for infusion should be used within 6 hours (including the 1 hour intravenous administration)

9.1.7 Administration

Docetaxel will be administered intravenously at a dose of 75mg/m^2 over 60 minutes on Day 1 of each 21-day cycle. Patients receiving docetaxel should be premedicated with dexamethasone 8 mg (or its equivalent) PO twice a day X 3 days starting the day before docetaxel (alternatively, dexamethasone 10 mg IV 30 to 60 minutes before docetaxel), diphenhydramine 50 mg (or its equivalent) IV 30 to 60 minutes before dosing, and cimetidine 300 mg or ranitidine 50 mg (or equivalent) IV 30 to 60 minutes before dosing.

9.1.8 Special Handling Instructions

Contact of the docetaxel concentrate with plasticized PVC equipment or devices used to prepare solutions for infusion is not recommended. In order to minimize patient exposure to the plasticizer DEHP (di-2-ethylhexyl phthalate), which may be leached from PVC infusion bags or sets, the final docetaxel dilution for infusion should be stored in bottles (glass, polypropylene) or plastic bags (polypropylene, polyolefin) and administered through polyethylene-lined administration sets.

9.1.9 Expected Adverse Events

The most common adverse reactions across all docetaxel indications are infections, neutropenia, anemia, febrile neutropenia, hypersensitivity, thrombocytopenia, neuropathy, dysgeusia, dyspnea, constipation, anorexia, nail disorders, fluid retention, asthenia, pain, nausea, diarrhea, vomiting, mucositis, alopecia, skin reactions, and myalgia.

9.2 Carboplatin

9.2.1 Carboplatin Description

Carboplatin is supplied as a sterile, pyrogen-free, 10 mg/mL aqueous solution of carboplatin. Carboplatin is a platinum coordination compound. The chemical name for carboplatin is platinum, diamine [1,1-cyclobutane-dicarboxylato(2-)-0,0']-,(SP-4-2). It has a molecular formula of $C_6H_{12}N_2O_4Pt$ and a molecular weight of 371.25. It is soluble in water at a rate of approximately 14 mg/mL, and the pH of a 1% solution is 5 to 7. It is virtually insoluble in ethanol, acetone, and dimethylacetamide.

9.2.2 Supplier(s)

Carboplatin is commercially available.

9.2.3 Dosage Form and Preparation

Carboplatin aqueous solution is a premixed aqueous solution of 10 mg/mL carboplatin. It can be further diluted to concentrations as low as 0.5 mg/mL with 5% Dextrose in Water (D_5W) or 0.9% Sodium Chloride Injection, USP.

It is supplied as follows:
50 mg/5 mL aqueous solution in multidose vials
150 mg/15 mL aqueous solution in multidose vials
450 mg/45 mL aqueous solution in multidose vials
600 mg/60 mL aqueous solution in multidose vials

Note: aluminum reacts with carboplatin causing precipitate formation and loss of potency; therefore, needles or intravenous sets containing aluminum parts that may come in contact with the drug must not be used for the preparation or administration of carboplatin.

9.2.4 Storage and Stability

Unopened vials of carboplatin solution are stable for the life indicated on the package when stored at controlled room temperature and protected from light. The multidose vials of carboplatin aqueous solution are reported to maintain microbial and chemical stability at room temperature for up to 14 days following multiple needle entries.

9.2.5 Administration

Carboplatin will be administered over 30 minutes. Dosing will be AUC6 on Day 1 of each 21-day cycle for 6 cycles. Premedication with antiemetics should happen on days when carboplatin dosing occurs and should follow the instructions in Section 5.1.

Hypersensitivity to carboplatin has been reported in 2% of patients. These allergic reactions have been similar in nature and severity to those reported with other platinum-containing compounds, i.e., rash, urticaria, erythema, pruritus, and rarely bronchospasm and hypotension. Anaphylactic reactions have been reported as part of postmarketing surveillance. These reactions have been successfully managed with standard epinephrine, corticosteroid, and antihistamine therapy.

9.2.6 Expected Adverse Events

Myelosuppression, nausea and vomiting (moderately emetogenic), peripheral neuropathy (occurring in < 10% of patients, mild in severity), hepatotoxicity (mild, reversible elevations in liver function tests), and allergic reactions.

10.0 CORRELATIVE STUDIES

10.1 Tumor Biopsy

Tumor biopsies and banked tissues collected on patients enrolled to the WashU protocol and the Baylor protocol will be processed and evaluated in the same manner. Research biopsies will be performed at baseline, Cycle 1 Day 3 (optional), definitive surgery, and at time of relapse. Guidelines are below.

10.1.1 Baseline Tumor Biopsies

Those tumor specimens for human in mouse modeling will be placed in cold DMEM High Glucose media, chilled on wet ice and immediately sent to Dr. Li's laboratory (address below) for further preparation. The procedure of tissue collection will be handled in a sterile fashion because the tissues will be engrafted into highly immunodeficient NOD/SCID mice.

1) First Core—in 10% formalin for clinical or CLIA diagnostics if indicated
2) Second Core—fresh to lab for engrafting
3) Third Core—immediately frozen in OCT blocks at bedside
4) Fourth Core—fresh to lab for engrafting
5) Fifth Core—immediately frozen in OCT blocks at bedside
6) Sixth Core—in 10% formalin to tumor bank

Optional core biopsies of the lymph nodes may be performed during baseline tumor biopsy for patients with nodal involvement. Guidelines are below:

1) First Core—fresh to lab for engrafting
2) Second Core—immediately frozen in OCT blocks at bedside
3) Third Core—in 10% formalin to tumor bank

Attention: Shun Li, M.D.

Current lab address:
Couch Biomedical Building 3rd floor
4515 McKinley Ave.
St. Louis, MO 63110
Tel: 314-747-9311(Lab)
314-362-3244 (Office)
314-596-8476 (Cell)
Pager: 314-424-5911 or 314-508-7804
E-mail: SQLI@dom.wustl.edu

10.1.2 Cycle 1 Day 3, Definitive Breast Surgery, and Time of Relapse Biopsies

Collection of tissue is optional on Cycle 1 Day 3 and time of relapse and mandatory during definitive breast surgery. All samples should be marked with the UPN, initials, and date of the sample using an indelible marker.

Frozen tissue suitable for gene expression and proteomic analysis
The biopsies obtained should be snap frozen in liquid nitrogen and placed on dry ice. The samples should then be immediately sent to Dr. Watson's laboratory (address below). Three frozen core biopsies should be taken and separately processed in OCT blocks or for the routine tissue blocks to be accessed by the principal investigator for analysis.

10%-buffered formalin fixed tissue
One of the samples obtained should be sent at ambient temperature in 10% buffered formalin to Dr. Watson's laboratory (address below).

Mark A. Watson, M.D., Ph.D.
Siteman Cancer Center Tissue Procurement Facility
425 S. Euclid Ave., Rm 5120
St. Louis, MO 63110
Phone: 314-454-7615
Fax: 314-454-5525
Email: tbank@pathology.wustl.edu

Fresh core
2 fresh cores for engraftment). See instructions in Section 10.1.1.

10.2 Research Blood

Blood (50 mL total) will be drawn for research purposes at baseline, Cycle 1 Day 3, at time of surgery, and at time of relapse.

Ten mL of blood should be taken in a red/tiger top tube, allowed to clot for 30 minutes and then immediately centrifuged at 1200G for 10 minutes at 4°C. The serum should then be stored as 1 mL aliquots at -70°C and sent to TPC for analysis.

An additional 30 mL of blood should be taken in:

1) a 10 mL EDTA tube (pink top) for plasma and DNA collection
2) a 10 mL EDTA tube containing protease inhibitors (BD – P100 tube)
3) a 10 mL lithium heparin (green top) tube for heparinized plasma

Ten mL of blood should be taken in Streck Cell-Free DNA BCT for plasma circulating DNA at baseline, Cycle 1 Day 3, at time of surgery, at time of relapse, and every six months after completion of chemotherapy for 5 years.

Mix these tubes several times to ensure adequate anticoagulation and place on ice. Deliver tubes to laboratory within 30 minutes of draw and spin as follows: pink top tubes, green top tubes, and the Streck tubes at 1000G for 10 min at 4°C and P100 tube at 2500G for 20 minutes in a swing bucket or 45 degree fixed angle rotor. The plasma is aspirated off in 1 mL aliquots and transferred to cryovials to be frozen and stored in LN2 vapor or at -70°C. White blood cell pellets are created with the retaining white blood cells. Germ-line DNA will be processed from the white blood cell pellets. These tubes are sent to TPC lab for analysis.

All samples should be marked with the UPN, initials, and date of the sample using an indelible marker.

11.0 STUDY CALENDAR

Baseline evaluations must be done no more than 4 weeks prior to start of treatment.

	Screening / Baseline	C1D1	C1D3[13]	Cycles 2-6, Day 1	Surgery[4]	EOT[9]	F/U[6]	Relapse
Informed consent	X							
Medical history	X							
Physical exam, incl. ECOG PS, tumor measurement (2D)	X	X		X		X		
Vital signs, weight, height[8]	X	X		X		X		
Hematology[1]	X			X		X		
CMP	X	X		X		X		
Pregnancy test[2]	X							
Docetaxel		X		X				
Carboplatin[3]		X		X				
Research blood	X		X		X[12,14]		X	X
Research tissue	X		X[5]		X[14]			X[15]
Adverse events[7]	X	X		X[7]				
FDG-PET/MRI		X[10]		X[11]				

1. WBC plus differential, hemoglobin, and platelets
2. In women of childbearing potential only.
3. Carboplatin is to be given immediately following docetaxel
4. To be performed between 3 and 5 weeks after completing neoadjuvant therapy
5. Optional.
6. Follow annually for progression and survival for 5 years, ctDNA blood drawn every six months.
7. Collect AEs for 30 days following last day of chemotherapy.
8. Height required at screening/baseline only.
9. First visit after surgery.
10. Baseline FDG-PET/MRI can be done prior to Cycle 1, preferably on Day 1 prior to initiation of therapy.
11. Post-therapy FDG-PET/MRI should be after completion of Cycle 1, preferably on Cycle 2 Day 1
12. If blood sample was not collected the day of surgery, the sample can be collected at the patient's next office visit.
13. -1 day / + 2 days window
14. If patients discontinue treatment due to AEs before completing all 6 cycles, and go directly to surgery, blood and tissue samples will be obtained at the time of surgery.
15. Optional research biopsy

12.0 DATA SUBMISSION SCHEDULE

Case report forms with appropriate source documentation will be completed according to the schedule listed in this section.

Case Report Form	Submission Schedule
Original Consent Form	Prior to registration
On-Study Form	Prior to starting treatment
Treatment Form Tumor Measurement Form Treatment Summary Form (ClinPortal)	Every cycle
Toxicity Form	Continuous
Surgery Form	Surgery
Correlatives Form	Baseline Cycle 1 Day 3 Surgery
Imaging Form	Baseline Between Cycles 1 and 2
Follow-Up Form	Annually for 5 years

13.0 MEASUREMENT OF EFFECT

13.1 Clinical Responses

Physical examination: Within 14 days of pre-registration and at the end of each neoadjuvant treatment cycle cycles (that is, at the end of cycles 1-6) the longest axis and the perpendicular axis of the measurable lesion should be measured and recorded in metric notation by tape, ruler or caliper technique on the case report forms. Measurements for clinical responses will be taken from the physical exam that is documented in the treating physician's notes in the electronic medical records system. If the physician's note indicates no palpable mass, we will use the dimensions of 0x0 cm.

WHO criteria will be used to assess clinical response

Complete Response (CR) is defined as the disappearance of all known disease based on a comparison between the pre-treatment measurements and the measurements taken at the completion of neo-adjuvant therapy. In addition there is no appearance of new lesions.

Partial Response (PR) is defined as a 50% or greater decrease in the product of the bi-dimensional measurements of the lesion (total tumor size) between the pre-treatment measurements and the measurements taken at the completion of neo-adjuvant therapy. In addition there can be no appearance of new lesions or progression of any lesion.

No Change (NC) a 50% decrease in total tumor size cannot be established nor has a 25% increase in the size of the lesion been demonstrated.

Progressive Disease (PD): A 25% or greater increase in the total tumor size of the lesion from its pretreatment measurements or the appearance of new lesions.

13.2 Treatment Resistance

A patient is said to have resistant disease if progressive disease is documented any time during neoadjuvant endocrine therapy.

13.3 Surgery

A pathologic complete response (pCR) is defined as no histology evidence of invasive tumor cells in the surgical breast specimen and sentinel or axillary lymph nodes.

All eligible women who have been treatment with combination therapy are included in the analysis of pCR. A patient is considered to not to have a pCR if any of the following are true:

1. There is histologic evidence of invasive tumor cells in the surgical breast specimen or the axillary lymph nodes.

2. The patient has discontinued neo-adjuvant treatment early due to refusal, toxicity, or radiographic or clinical evidence of progression and then goes straight to surgery where there is histologic evidence of invasive tumor cells in the surgical breast specimen and the axillary lymph nodes.

3. The patient has discontinued neo-adjuvant treatment early due to refusal, toxicity or radiographic or clinical evidence of progression and then receives alternative treatment.

4. The patient refuses surgery or is unable to undergo surgery due to a co-morbid condition.

13.4 Diagnosis of Breast Cancer Recurrence and Other Cancer Events

13.4.1 Local Recurrence

Local recurrence is defined as histologic evidence of ductal carcinoma in situ or invasive breast cancer in the ipsilateral breast or chest wall.

13.4.2 Regional Recurrence

Regional recurrence is defined as the cytologic or histologic evidence of disease in the ipsilateral internal mammary, ipsilateral supraclavicular, ipsilateral

infraclavicular and/or ipsilateral axillary nodes or soft tissue of the ipsilateral axilla.

13.4.3 Distant Recurrence

Distant recurrence is defined as the cytologic, histologic, and/or radiographic evidence of disease in the skin, subcutaneous tissue, lymph nodes (other than local or regional metastasis), lung, bone narrow, central nervous system or histologic and/or radiographic evidence of skeletal or liver metastasis.

13.4.4 Second Primary Breast Cancer

Second primary breast cancer is defined histologic evidence of ductal carcinoma in situ or invasive breast cancer in the contralateral breast or chest wall.

13.4.5 Second Primary Cancer (Non-breast)

Any non-breast second primary cancer other than squamous or basal cell carcinoma of the skin, melanoma in situ, or carcinoma in situ of the cervix is to be reported and should be confirmed histologically whenever possible.

13.4.6 Death

Underlying cause of death is to be reported.

14.0 DATA AND SAFETY MONITORING

In compliance with the Washington University Institutional Data and Safety Monitoring Plan, the Principal Investigator will provide a Data and Safety Monitoring (DSM) report to the Washington University Quality Assurance and Safety Monitoring Committee (QASMC) semi-annually beginning six months after accrual has opened (if at least five patients have been enrolled) or one year after accrual has opened (if fewer than five patients have been enrolled at the six-month mark).

The Principal Investigator will review all patient data at least every six months, and provide a semi-annual report to the QASMC. This report will include:

- HRPO protocol number, protocol title, Principal Investigator name, data coordinator name, regulatory coordinator name, and statistician
- Date of initial HRPO approval, date of most recent consent HRPO approval/revision, date of HRPO expiration, date of most recent QA audit, study status, and phase of study
- History of study including summary of substantive amendments; summary of accrual suspensions including start/stop dates and reason; and summary of protocol exceptions, error, or breach of confidentiality including start/stop dates and reason
- Study-wide target accrual and study-wide actual accrual
- Protocol activation date
- Objectives of protocol with supporting data and list the number of participants who have met each objective

- Measures of efficacy
- Measures of efficacy – provide a summary of tissue samples collected by site, tissue expected to obtain, % of tissue received, and % of tissue missing
- Early stopping rules with supporting data and list the number of participants who have met the early stopping rules
- Summary of toxicities
- Abstract submissions/publications
- Summary of any recent literature that may affect the safety or ethics of the study

The study principal investigator and Research Patient Coordinator will monitor for serious toxicities on an ongoing basis. Once the principal investigator or Research Patient Coordinator becomes aware of an adverse event, the AE will be reported to the HRPO and QASMC according to institutional guidelines.

15.0 STATISTICAL CONSIDERATIONS

15.1 Study Design

This is a single arm open label phase II study in women with clinical stage 2 or 3 triple negative breast cancer to assess the anti-tumor activity (in terms of pathologic complete response rate) of neoadjuvant docetaxel in combination with carboplatin.

15.2 Primary Endpoint

The original primary endpoint of the trial is pCR rate which will be calculated as the percentage of patients who achieve pCR among all pCR evaluable patients.

A patient is considered to not to have a pCR if any of the following are true:

1. There is histologic evidence of invasive tumor cells in the surgical breast specimen or the axillary lymph nodes.
2. The patient has discontinued neoadjuvant treatment early due to refusal, toxicity, or radiographic evidence of progression and then goes straight to surgery where there is histologic evidence of invasive tumor cells in the surgical breast specimen and the axillary lymph nodes
3. The patient has discontinued neoadjuvant treatment early due to refusal, toxicity or radiographic evidence of progression and then receives alternative treatment.
4. The patient discontinues study treatment, refuses surgery, or is unable to undergo surgery due to a co-morbid condition.

Thus, any patient who does not receive alternative treatment prior to surgery and has no histologic evidence of invasive tumor cells in the surgical breast specimen and the axillary lymph nodes is considered to have a pCR.

The primary endpoint of pCR rate will be calculated with 95% CI and will be tested against the reference pCR rate of 35% with standard chemotherapy.

15.3 Sample Size and Trial Duration

The expectation for a pathological complete response (no invasive disease in the breast or nodes) after neoadjuvant chemotherapy for TNBC is expected to exceed 35%.

Adverse events, the pace of accrual, other scientific discoveries or changes in standard of care will be taken into account in any decision to terminate this trial earlier than designed.

The single size phase II trial is still undergoing. Based on the 26 patients who have completed surgery to date, the pCR rate is currently estimated to be ~40%, lower than what was expected (54%) at planning the trial. We propose to increase the sample size to 100 patients to be enrolled in WashU, with 30 of the 100 patients to support the Multi-parametric Positron Emission Tomography (PET) and Magnetic Resonance (MR) Imaging study (see Section 15.3.1). If currently the pCR of the trial achieves 40%, the enrollment of 100 eligible patients provides 82.1% power to test the pCR rate of 40% against the null pCR rate of 28% with standard chemotherapy, based on one-sided Binomial exact test at a target 0.05 alpha level. If 36 or more patients achieve a pCR, we conclude that the investigational regimen yields better efficacy than standard chemotherapy. If the pCR rate is higher than the current estimate of 40%, the sample size of 100 provides 90.33% power to test the pCR rate of 50% against the null pCR rate of 35% with standard chemotherapy, based on one-sided Binomial exact test at a target 0.05 alpha level (actual alpha=0.039). If 44 or more patients achieve a pCR, we conclude that the investigational regimen yields better efficacy than standard chemotherapy.

We anticipate that 4 patients per month with clinical stage 2 or 3 TNBC will be screened for trial eligibility. Approximately 75% of the patients will be eligible and enroll. Thus, we anticipate screening approximately 134 patients to obtain 100 eligible patients as calculated above.

The trial has enrolled 35 eligible patients since its opening. The period encompassing enrollment, study treatment, and surgery of the remaining 35 patients will be approximately 23 months [That is, 18 months to enroll 35 eligible patients and then 5 months to await surgical results of last patient enrolled].

The current engraftment rate of the trial is conservatively estimated to be 25~30% based on the 35 patients on the study. Therefore, we expect to establish a minimum of 25 PDX models.

15.4 Amendment to increase sample size for the Multi-parametric Positron Emission Tomography (PET) and Magnetic Resonance (MR) Imaging study

We are requesting to add 30 patients to support the Multi-parametric Positron Emission Tomography (PET) and Magnetic Resonance (MR) Imaging to Assess Response to

Therapy objectives Groheux et al. have shown efficacy in differentiating pCR in TNBC with a sample size of 20 using 18FDG PET/MR where change in SUVmax yielded an AUC of 0.88 and with 30% of the sample being pCR. Therefore, conservatively based on this data, a sample of 30 patients (30% being responders) achieves 80% power to detect a difference of 0.3 between the AUC under the null hypothesis of 0.5 and an AUC under the alternative hypothesis of 0.8 using a one-sided z-test at a significance level of 0.05 and assuming a dropout rate of 15%.

15.5 The Baylor samples

Baylor College of Medicine (BCM) has a parallel protocol the same as this protocol. We expect to have approximately 19 patients who have been enrolled to the BCM protocol and complied with the WashU patient inclusion and exclusion criteria. We will pool patients and data from this protocol and the BCM protocol for analyses. The total sample size will be approximately 119 (100 from the WashU protocol and approximately 19 from the BCM protocol). The power with 119 evaluable patients attains 95.07% to test the pCR rate of 50% against the null pCR rate of 35% with standard chemotherapy, based on one-sided Binomial exact test with a target 0.05 alpha level (actual alpha level = 0.046). If the total number of pCRs is ≥51, we conclude that the investigational regimen yields better efficacy than standard chemotherapy.

15.6 Secondary Clinical Outcomes

Clinical response rate: The clinical response rate will be estimated by the number of patients whose disease meets the WHO criteria of complete or partial response prior to surgery divided by the total number of eligible patients who began combination neo-adjuvant treatment. A ninety percent confidence interval for the true clinical response rate will be calculated using the Duffy-Santer approach.

Adverse Events: All eligible patients that have intitiated treatment will be considered evaluable for assessing adverse event rate(s). The maximum grade for each type of adverse event will be recorded for each patient using the NCI-CTCAE v4.0 coding scheme, and frequency tables will be reviewed to determine patterns. Additionally, the relationship of the adverse event(s) to the study treatment will be taken into consideration.
Grade for each type of adverse event will be recorded for each patient using the NCI-CTCAE v4.0 coding scheme, and frequency tables will be reviewed to determine patterns. Additionally, the relationship of the adverse event(s) to the study treatment will be taken into consideration.

15.7 Explorative Studies

To determine the xenografting rate from TNBC patients being treated with neoadjuvant chemotherapy. The xenografting rate is expected ~50%. The sample size of 70 calculated to achieve the primary objective will provide 85.4% power to detect an observed xenografting rate of 50% against an unacceptably low xenografting rate of 30% based on one-sided binomial test at the 5% significance level.

To compare chemotherapy responses in PDX and TNBC patients being treated with neoadjuvant chemotherapy. The chemotherapy response in PDX and corresponding TNBC will be tabulated and their association will be examined by Fisher's exact test. The response rate in TNBC patients and PDX will be calculated with 95% CI.

To investigate genomic and proteomic molecular changes in PDX and corresponding host patients with the intent to identify predictors of drug response and resistance. Genomic profiling (including gene expression profiling and aCGH) of tumor tissue harvested at day 3 and at completion of combination treatment will be performed to explore the relationship between genoproteomic factors and endpoints including response, etc as well as to explore the longitudinal changing patterns in profiling. Pearson or Spearman correlation coefficient between the paired profiling of PDXs and their associated hosts will be calculated for similarity/difference. Joint unsupervised analysis of gene expression and aCGH will be carried for PDXs and TNBC separately by use of generalized singular value decomposition through which a few meta-genes will be obtained summarizing thousands of individual genes and meanwhile, individual genes sharing similar variation patterns in both data can be identified. The resulting meta-genes will then be correlated between PDXs and TNBCs for similarity/difference summarizing across multiple genes of both gene expression and copy number. Genomic profiling will be associated with endpoints at each time point. Differential genes/copy numbers expression (DE) analysis between responding and non-responding subjects will be performed within PDXs and TNBCs respectively using significance analysis of microarray (SAM), using individual genes or meta-genes derived from joint analysis. The DE gene lists will be compared using Venn diagram for overlapping and unique genes. The genomic profiling will also be used as predictors for an endpoint by using penalized logistic regression (for clinical response) or random forest regression (for continuous endpoints) to identify genes with important predictive effect on binary and continuous endpoints separately. Random forest regression has the advantage of making no underlying assumption on variable distributions, considering interaction effect and providing variable importance measure. To internally validate the gene signatures, leave one out cross validation procedure will be adopted for penalized logistic regression modeling. Breast cancer subtypes, risk of relapse score and risk group derived from genomic profiling will be summarized by descriptive statistics (frequency for categorical variables and mean, standard deviation for continuous variables) at each time point and the changes between time points will be summarized by correlation coefficients and contingency table analyses.

To investigate the utility of combined FDG-PET/MR, we propose to enlist up to 30 patients to optimize imaging parameters for simultaneous FDG-PET/MR imaging and to optimize use of the breast coil to facilitate MR imaging. Image analysis will be carried out as described earlier.

To measure the circulating tumor DNA (ctDNA), malignant breast samples will be subjected to whole exome (WES) to determine driver mutations. We will analyze whether patient-specific mutations in the primary breast tumor are detectable in ctDNA. These patient-specific mutations will be prioritized according to tumor variant allele fractions

from breast tissue, and evaluate if levels and the presence of ctDNA mutations can predict clinical outcomes in pCR and non-pCR patients.

15.8 Pooled analyses with incorporation of the patients enrolled to the BCM protocol

BCM has a parallel protocol on which 19 patients have been enrolled. To unify the eligibility criteria between the two protocols, we will exclude male patients or patients with inflammatory breast cancer, if any, who were enrolled to the BCM protocol. We will conduct a pooled analyses using the data collected on the patients (expected approximately total N=120) enrolled to both the WashU protocol (N=100) and the BCM protocol (N≈19). Using data from more patients will potentially improve statistical power. The patient demographic characteristics and tumor characteristics will be summarized overall and by protocol (WashU vs. BCM) using descriptive statistics (typically, mean and standard deviation for continuous characteristics and count and percentage for categorical characteristics) and difference in characteristics will be compared by two-sample t-test (or Wilcoxon rank sum test in presence of severe violation of normality assumption) for continuous characteristics and Fisher's exact test for categorical characteristics. All the above proposed analyses for primary, secondary and exploratory endpoints will be performed overall pooling all the data from the two protocols and separately by protocol.

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APPENDIX A: ECOG Performance Status Scale

Grade	Description
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.
1	Symptoms, but ambulatory. Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (e.g., light housework, office work).
2	In bed <50% of the time. Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.
5	Dead.

APPENDIX B: Cockcroft-Gault

GFR = (140 – age) * (wt in kg) * (0.85 if female) / (72 * Cr)

Calvert AUC: Total dose (mg) = (target AUC) x (GFR + 25)