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TITLE: A Window of Opportunity Study of Dasatinib in Operable Triple Negative Breast Cancers with Nuclear Epidermal Growth Factor Receptor

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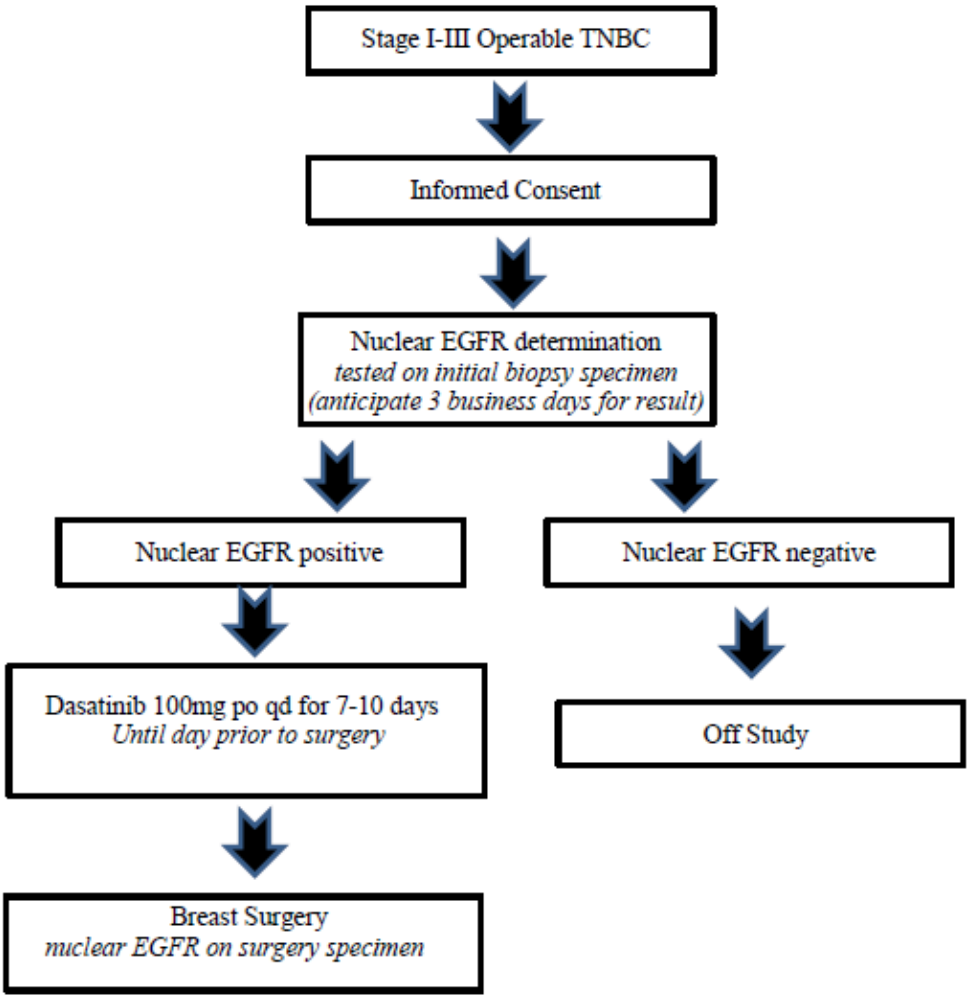
Project Summary/SCHEMA



It is estimated that 95 subjects will need to have EGFR testing on the primary tumor to identify a total of 19 evaluable subjects for the primary endpoint. Dasatinib will be planned for a minimum of 7 days, but up to a maximum of 10 days to allow for flexibility in case of adjustments in surgery schedule.

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1. OBJECTIVES

1.1 Primary Objectives

To determine if dasatinib, an inhibitor of the Src family kinases, can prevent the nuclear translocation of the epidermal growth factor receptor (EGFR) in Stage I-III nuclear EGFR positive, triple negative breast cancers (TNBC).

1.2 Secondary Objectives

1.2.1 To examine the safety and tolerability of dasatinib in patients with operable TNBC

1.2.2 To explore potential intracellular mechanisms which impact dasatinib effect on cellular localization of EGFR in operable TNBC.

1.2.3 To examine the pathologic complete response (pCR) rates to standard neoadjuvant chemotherapy in nEGFR+ TNBC

1.2.4 To examine breast cancer recurrence rates and patterns of metastatic recurrence in nEGFR+ TNBC.

2. BACKGROUND

2.1 Triple negative breast cancer

Breast cancer remains an important global health issue. One of eight U.S. women is diagnosed with breast cancer during her lifetime.[1] The American Cancer Society estimates that 234,190 new cases of invasive breast cancer will be diagnosed in the United States in 2015 with 40,730 deaths. [2 2 2 2] Triple-negative breast cancer (TNBC) constitutes approximately15% of breast cancers and lacks expression of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor-2 (HER2). TNBC is associated with African-American and Hispanic race, younger age at diagnosis, advanced stage, high mitotic index, and BRCA1 mutations.[3-7] Due to the absence of any targeted therapies, cytotoxic chemotherapy remains the mainstay of medical treatment for TNBC, but outcomes are poor compared with other subtypes.[8,9] Median survival for women with advanced TNBC remains a dismal 13 months.[9] Improved understanding of this disease to advance our treatment approaches are urgently required.

For stage I-III TNBC, despite optimal local therapy with breast and axillary surgery with or without radiation, the risk for distant early recurrence remains significant. Thus, systemic combination chemotherapy is part of the standard of care for the majority of these patients. Neoadjuvant (also known as preoperative) chemotherapy is often recommended in this setting based on trials that have shown equivalent long term outcomes.[10] Furthermore, neoadjuvant chemotherapy has been shown to allow for conversion from mastectomy to breast conservation in a portion of patients and potentially reduce need for full axillary dissection.[11] In addition, studies in TNBC have shown that patients who are able to achieve a pathologic complete

response (pCR) are likely to have improved survival over those who have residual disease. Patient's with TNBC who achieve a pCR have similar survival to non-TBNC.[12] Neoadjuvant therapy is now considered a standard of care for many of these patients.

2.2 Epidermal Growth Factor Receptor

2.2.1 Overview of Epidermal Growth Factor Receptor (EGFR) in TNBC

EGFR is an oncogene that promotes tumor growth.[13] Targeting EGFR has been a successful therapeutic strategy for multiple solid tumors.[14-17] Approximately 50% of TNBCs over-express EGFR, which is associated with poor patient prognosis.[18-23] However, clinical trials investigating the anti-EGFR antibody cetuximab in TNBC have largely been unsuccessful when used alone, and only modestly effective in combination with platinum chemotherapy.[24,25] The failure of cetuximab in TNBC patients may be due to the fact that cetuximab only targets the classical EGFR signaling pathway emanating from plasma membrane-bound EGFR. We hypothesize that the failure of cetuximab in TNBC patients may be due to the fact that cetuximab only targets classical EGFR signaling that emanates from plasma membrane-bound EGFR and ***not*** the nEGFR-signaling pathway. This represents a fundamental obstacle in the field of EGFR targeting in TNBC, and suggests that nEGFR expressing patients will be resistant to cetuximab therapy.

2.2.2 Nuclear EGFR

Emerging data suggests a new mode of EGFR function in which EGFR undergoes nuclear translocation in a series of well-defined steps.[26-35] At the plasma membrane the dimerized receptor is phosphorylated at Y1101 by SFKs and this represents a critical, early event for movement of the EGFR to the nucleus.[29] These early events lead to receptor internalization to the early endosome followed by retrograde trafficking to the Golgi via microtubule-dependent movement by interacting with dynein and fusion with the Golgi apparatus through syntaxin 6-mediated membrane fusion[34]. This is followed by COPI-mediated retrograde trafficking from the Golgi to the ER where the nuclear localization sequence (NLS)[36] of the EGFR interacts with importinβ1.[30] Here the EGFR/importinβ1 complex moves to the outer nuclear membrane (ONM) where importinβ1 interacts with nup358 of the nuclear pore complex (NPC) and is shuttled to the inner nuclear membrane (INM). Here, EGFR interacts with the Sec61 translocon for nuclear import.[32] Within the nucleus, two functions have been identified for EGFR. Firstly, EGFR serves as a co-transcription factor regulating genes involved in tumor progression (Cyclin D1, iNOS, B-myb, Myc, BCRP, Aurora Kinase A and COX2).[37-43] Secondly, nEGFR serves as a kinase that phosphorylates proliferating cell nuclear antigen (PCNA) increasing its stability and function in the proliferating cell[44] and activates DNA-PK within the nucleus to enhance DNA repair.[45] These identified nuclear functions have been linked with: 1) inverse correlation with overall survival in breast, ovarian, oropharyngeal, gallbladder and lung cancer[46-51], 2) enhanced tumor growth[52-54] and 3) resistance to therapeutic agents including radiation, gefitinib, cisplatin and cetuximab.[41,52,55,56] Although there are several reported mechanisms of resistance to cetuximab including altered angiogenesis[57-59], increased EGFR activity[60], increased expression of HER family ligands[52,61] increased EGFR degradation[60,62], oncogenic shift[60], epithelial to mesenchymal shift[63], and PTEN degradation,[64] findings from Wheeler et al. suggest that targeting nEGFR can sensitize nEGFR positive TNBC cells to cetuximab therapy.[29,52,60,65-67] Unfortunately, nEGFR

cannot be targeted by cetuximab and since EGFR regulation of genes is kinase-independent, gefitinib and erlotinib, small molecule inhibitors of the EGFR, are of little utility.[68]

2.2.3 Preliminary Data

Nuclear EGFR plays a role in cetuximab resistance

Cetuximab-resistant tumor cell lines were established by exposing the cetuximab sensitive human NSCLC line NCI-H226 (H226) and the HNSCC line UMSCC-1 (SCC1) to increasing doses of cetuximab for a time period of six-months until single cell resistant clones emerged.[60,69] This process resulted in six stable resistant clones for the H226 NSCLC line designated HC1, HC4, HC5, HC6, HC7 and HC8. The sensitive parental line was designated HP. For the SCC1 HNSCC line, six stable resistant clones were generated (SC1, SC2, SC5, SC6, SC7, SC8), and the sensitive parental line was designated SP. All clones displayed a robust cetuximab-resistant phenotype when challenged with increasing concentrations of cetuximab as compared to parental controls. Sequence analysis of the EGFR in selected resistant clones indicated no mutations developed during the selection process in either the resistant or parental cells.[60]

Cetuximab-resistant clones have increased steady-state expression and dependency on the EGFR

After successful establishment of cetuximab-resistant clones in two different model systems, activity of 42 RTKs were screened using an antibody-based array in selected clones.[60] Analysis of selected cetuximab-resistant clones (HC1, HC4 and HC8, SC1 and SC2) indicated that the EGFR steady-state expression was dramatically increased, secondary to decreased ubiquitination[60]) as well as its overall activity as indicated by phosphorylation of several key tyrosines on the EGFR (**Figure 1A**). Further, experiments using siRNAs directed against the EGFR indicated that cetuximab-resistant clones remained dependent on the EGFR for proliferation (**Figure 1B&C**). *Collectively this data suggested that cetuximab-resistant clones upregulated the steady-state expression of the EGFR and maintained dependence on the EGFR.[60]*

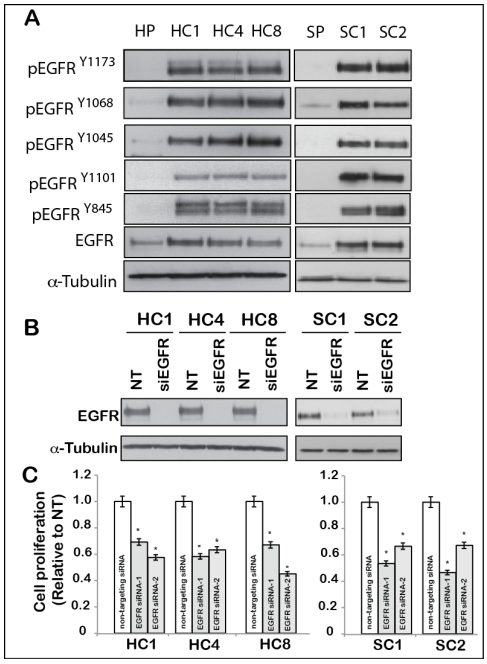

Figure 1: Cetuximab-resistant clones have increased expression and dependency of EGFR. A) Western blot analysis of the EGFR. **B&C)** siRNAs directed against the EGFR decreases proliferation of cetuximab resistant clones. * P<0.005

Cetuximab-resistant clones have increased nEGFR

To investigate why cetuximab-resistant clones maintain dependency on the EGFR, *yet are resistant to cetuximab*, we performed biochemical fractionation and automated quantitative IHC analysis (AQUA, *data not shown*) and found that cetuximab-resistant clones exhibit increased nEGFR expression relative to cetuximab-sensitive parental controls (HP and SP, respectively) (**Figure 2A**). To determine if this increased nEGFR resulted in upregulation of *known* nEGFR gene targets we analyzed expression of Cyclin D1, B-myb, iNOS and Aurora Kinase A by quantitative PCR (qPCR). These results, relative to the cetuximab-sensitive parental controls (HP and SP, respectively), showed robust increased expression (**Figure 2B**). *Collectively these findings indicate that cetuximab-resistant clones, in two independent model systems with multiple clones, have increased nEGFR and upregulation of known nEGFR target genes.*

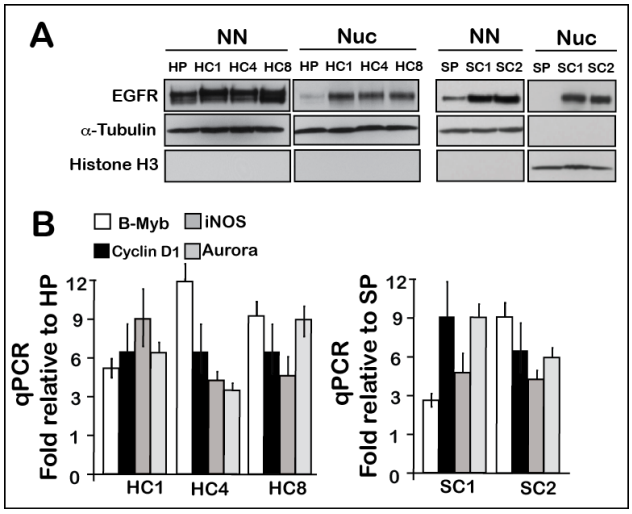

Figure 2: Cetuximab-resistant clones have increased nEGFR. A) Western blot analysis of EGFR from [illegible] fracti[illegible]n nEGF[illegible]

Nuclear EGFR plays a role in resistance to cetuximab therapy

To isolate the effects of nEGFR and its role in resistance, we designed a retrovirus expressing wild-type human EGFR fused to a SV40 nuclear localization sequence tag (NLS) (EGFR-NLS/Myc[52], **Figure 3A**). We infected the *cetuximab-sensitive* parental lines, HP and SP, with a retrovirus containing either the WT-EGFR or EGFR-NLS vectors. From these experiments we obtained one WT-EGFR and two EGFR-NLS-expressing clones (EGFR-NLS1 and EGFR-NLS2) for the LSCC model (**Figure 3B**) and one WT-EGFR and one EGFR-NLS expressing clones (EGFR-NLS1) for the HNSCC model (**Figure 3E**) that were highly localized to the nucleus**.** To determine if increased nEGFR had functionality we measured the expression of four known EGFR target genes, cyclin D1, COX2, iNOS and B-myb: All four genes had greater than six-fold increase in expression (*data not shown*).[52]

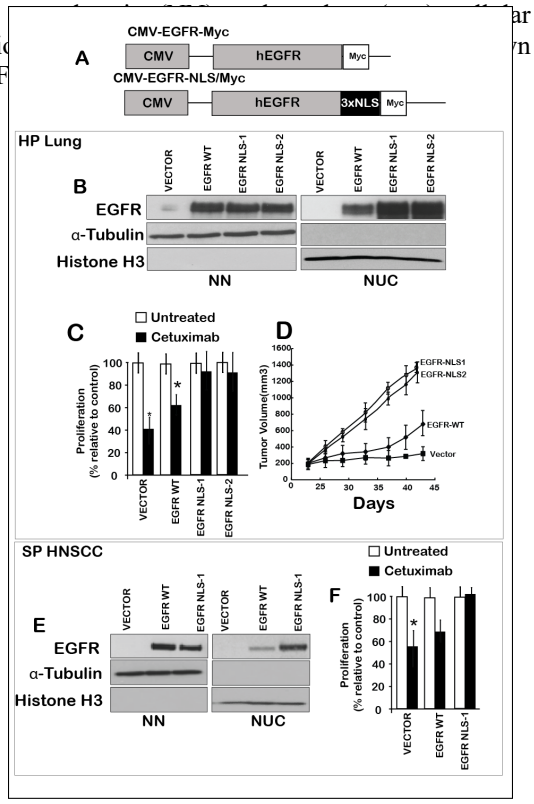

Figure 3: nEGFR leads to resistance to cetuximab. A) Schematics for viral vectors of EGFRWT and EGFR fused to NLS (EGFR-NLS)**. B&E)** Nuclear (Nuc) fraction showing increased expression of EGFR in the nucleus in the EGFR-NLS clones. **C&F)** Proliferation assays showing EGFR-NLS clones were more resistant to cetuximab. **D)** EGFR-NLS clones are resistant to cetuximab *in vivo*. * P<0.005

Next, we used these model systems to test the hypothesis that directing EGFR to the nucleus would result in increased resistance to cetuximab therapy. The results indicated that, relative to the wildtype control, each clone exhibited an approximate 50—75% increase in resistance to cetuximab treatment *in vitro* (**Figure 3C&F**)[52]. These results were expanded for the lung system in xenografts models challenged with cetuximab for the time indicated (Figure 3D).[52] Further, analysis of established NSCLC, HSNCC and TNBC cell lines, exhibited a correlation between nEGFR levels and intrinsic resistance to cetuximab (data not shown).[52,65] *Collectively, these data support a role for nEGFR in cetuximab resistance.*

Cetuximab-resistant clones have increased SFK activity

To further investigate activated pathways in cetuximab-resistant models we performed proteomic analysis measuring activity, by phosphorylation, using intracellular phospho-arrays (*data not shown*). Results of this analysis indicated that all cetuximab-resistant clones, in both models of acquired resistance, had marked increased phosphorylation of SFKs on Y419, a critical tyrosine site in the activation loop of the kinase (*data not shown*).[66,67] Further, SFK kinase assays demonstrated robust activation in resistant clones tested (*data not shown*).[67] *Collectively these results suggest that cetuximab-resistant clones have increased SFK activity relative to cetuximab sensitive parental controls.*

SFKs regulate EGFR translocation to the nucleus by phosphorylation of tyrosine 1101

Our findings in acquired resistance to cetuximab[52,67] indicated that cetuximab-resistant cells have **1)** increased nEGFR[52,66] and **2)** increased SFK activity.[66,67] To understand if SFKs played a direct role in nuclear translocation of the EGFR we analyzed known SFK phosphorylation sites on the EGFR (Y845 and Y1101).[70,71] This analysis showed increased phosphorylation of Y845 and Y1101 in cetuximab-resistant clones **(Figure 1A).** To determine whether phosphorylation of one or both of these tyrosines were involved in EGFR nuclear translocation, the MCF-7 cell line was transfected with wild-type (WT) EGFR or the following EGFR mutants: EGFR-Y845F or EGFR-Y1101F **(Figure 4A).** To induce nuclear translocation of the EGFR we treated the transfected cells with EGF for 45 min before nuclear fractionation. The results of this experiment indicated that EGF induced nuclear translocation of EGFR in both EGFR-WT and EGFR-Y845F mutant cells **(Figure 4B).** However, EGF- induced EGFR nuclear translocation was diminished ~70% in EGFR-Y1101F mutant cells as compared to EGFR-WT expressing cells.[29] *Collectively, these data suggest that the phosphorylation of Y1101 is important for the nuclear translocation of EGFR and suggests that SFK blockade may represent a unique opportunity to block nEGFR translocation and thus function.*

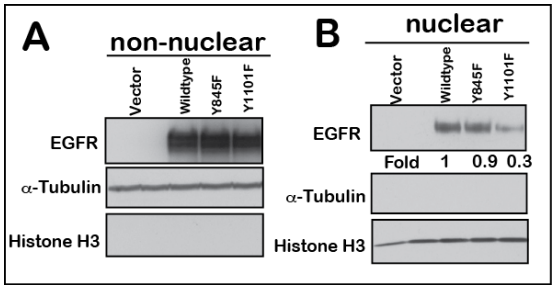

Figure 4: Tyrosine 1101 of the EGFR plays a critical role in EGFR nuclear translocation. A) Western blot analysis of cytosolic lysate showing equal expression of WT, Y845F and Y1101 mutant stable clones**. B)** Western blot analysis of nuclear lysate showing Y1101F is defective in nuclear translocation of the EGFR as compared to WT and Y845F.

TNBC cell lines and human tumors express nuclear localized EGFR

Six established (BT549, MBAMD231, SUM149, SUM159, SUM229 and MDAMB468) TNBC cell lines were evaluated for EGFR and SFK expression **(Figure 5A).** Further, all TNBC cell lines expressing EGFR also expressed variant levels of nEGFR (Figure 5B). Immunogold labeling of the EGFR and subsequent transmission electron microscopy (EM) validated nEGFR expression (**Figure 5C**). Given that nEGFR was expressed in established TNBC cell lines, we probed a human tissue microarray (TMA) containing 74 TNBC patient tumors for EGFR expression and localization. Analysis of tumors stained for EGFR via immunohistochemistry (IHC) indicated that 20% of the tumors expressed nEGFR (**Figure 5D**). *Together, these results indicated that established human TNBC lines and TNBC patients express nEGFR.*

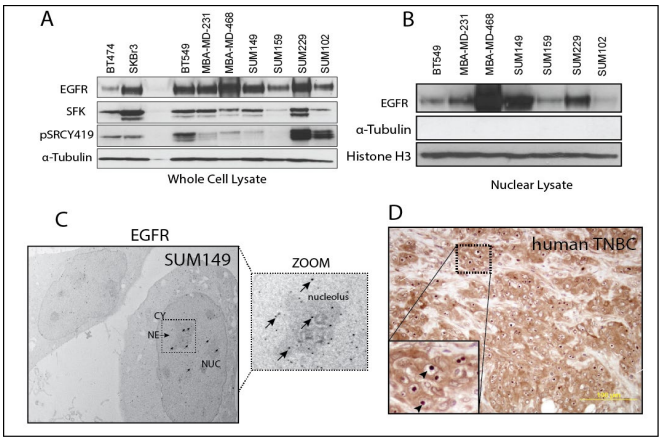

Figure 5: TNBC cell lines and human tumors express nuclear localized EGFR. A) TNBC cells express EGFR and SFKs**. B and C)** TNBC cells express nEGFR as indicated by nuclear fractionation (B) and immunogold labeling and EM (C). **D)** 20% of Human TNBC tumors express nEGFR**.** IHC staining for EGFR was performed on a total of 74 TNBC patient tumor sections.

TNBC cells are resistant to cetuximab therapy, but dependent on EGFR for proliferation

To determine the role of EGFR in TNBC proliferation, genetic ablation studies were performed in six TNBC cell lines using an EGFR directed siRNA pool: SUM159, MDAMB231, SUM229, SUM149, SUM102 and MDAMB468 (depicted) (Figure 6A and B). Loss of EGFR expression led to a 23-50% reduction in cell proliferation as compared to cells treated with vehicle or non-targeting (NT) siRNA (**Figure 6**). Each cell line challenged with increasing doses of cetuximab (from 0.01nM to 100nM) demonstrated only minor reductions in proliferation indicating cetuximab resistance (**Figure 6C**). SUM102 was sensitive to cetuximab therapy (*data not shown*). *These results indicate that TNBC cell lines depend on EGFR for proliferation but are resistant to cetuximab.*

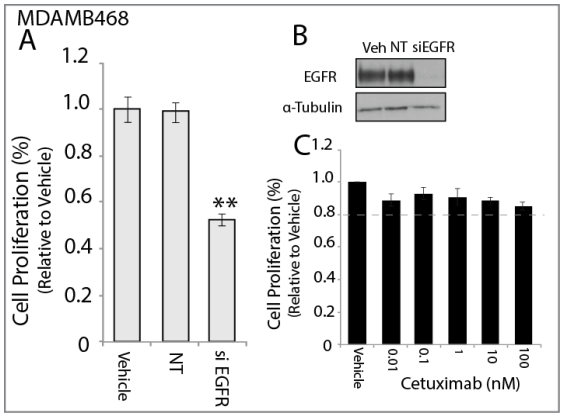

Figure 6: TNBC cell lines are dependent on EGFR for proliferation, but are intrinsically resistant to cetuximab. A and B) Six TNBC cell lines (MDAMB468 depicted) were incubated with siEGFR, non-targeting (NT) siRNA, or vehicle for 72-96 hr prior to performing proliferation assays. **C)** Cells were treated with cetuximab at indicated doses for the same time course. Proliferation is plotted as a percentage of growth relative to vehicle treated cells (n=3). Whole cell lysate was harvested from all cell lines at the same time point to confirm knockdown of EGFR. Data points are represented as mean±s.e.m. **p<0.01.

Nuclear EGFR trafficking is abrogated by SFK inhibition leading to increased EGFR membrane expression

Since Y1101 phosphorylation by SFKs is necessary for EGFR nuclear translocation (**Figure 7A**) we utilized the SFK inhibitor dasatinib to determine if it could abrogate EGFR translocation from the membrane to nucleus. Treatment of TNBC cells SUM149, SUM229 and MDAMB468 with dasatinib led to potent decreases in nEGFR expression (see arrow) and robust increases in non-nuclear EGFR levels (see arrow) at 72 hours post treatment (SUM 148 and MDAMB468 depicted, Figure 7B). Additionally, significant increases in cell surface EGFR levels were detected by flow cytometry 72 hours post treatment. These results were also verified using genetic approaches with SFK mutants and a negative regulator of Src (known as the SLAP protein) to ensure these findings were *not off target* effects of dasatinib. **Increase in membrane EGFR was also seen after dasatinib treated mice with MDAM8468 xenografts** (**Figure 7C**). *These results indicate that SFK blockade decreases nEGFR and increases plasma membrane EGFR levels.*

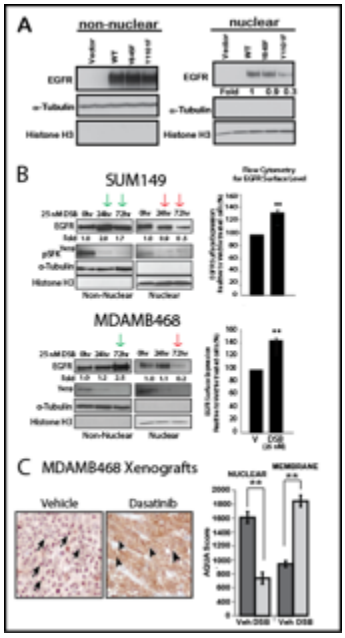

Figure 7: Therapeutic inhibition of SFKs blocks nEGFR translocation in TNBC cell lines A) Western blot analysis of cytoplasmic and nuclear fractions demonstrates that mutation of Y1101 prevents nEGFR translocation. **B)** Dasatinib (25nM) inhibits nEGFR translocation (red arrows) and increases nonnuclear EGFR levels (green arrows). SFK blockade via dasatinib increases EGFR surface levels measured by flow cytometry. C) Tumors harvested from mice treated with dasatinib have decreased nEGFR and increased plasma membrane E

Cetuximab resistant TNBC cell lines are sensitized to Cetuximab resistant TNBC cell lines are sensitive upon inhibition of nEGFR translocation

Since SFK inhibition of nEGFR increased plasma membrane EGFR levels we hypothesized that cells would now be more sensitive to cetuximab. To investigate this possibility, we pre-treated TNBC cells (SUM129, SUM149, MBAMB231, MDAMB468 depicted) with dasatinib or vehicle for 24 hours, the time point where increased surface level EGFR was detected, and subsequently treated cells with increasing doses of cetuximab therapy for an additional 72 hours **(Figure 8).** Analysis of cellular proliferation post treatment indicated that TNBC cell lines that received dasatinib for 24 hours prior to cetuximab treatment demonstrated significant reductions in proliferation over a wide range of cetuximab doses. *Collectively, these data suggest that the blockade of nEGFR translocation via SFK inhibition can increase TNBC cell sensitivity to cetuximab.*

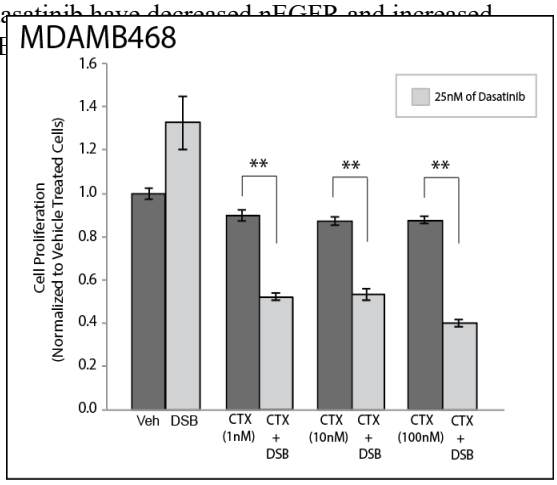

Figure 8. Therapeutic inhibition of SFK activity can sensitize TNBC cells to cetuximab[19]. Six TNBC cell lines (MDAMB468 depicted) were pretreated with vehicle or dasatinib (25 nM) for 24 hr prior to adding cetuximab to the growth medium at the indicated doses for an additional 72 hr. Proliferation assays were performed and plotted as a percentage of growth relative to vehicle treated cells (n=3). Data points are represented as mean±s.e.m. **p<0.01.

2.3 Dasatinib

Dasatinib is an oral tyrosine kinase inhibitor that targets BCR/ABL, Src, c-Kit, ephrin and other receptors.[72] It was first approved in 2006 for chronic phase-chronic myelogenous leukemia (CML) who are intolerant or resistant to prior therapy including imatinib.[73] It was also approved in 2010 for treatment of newly diagnosed patients with Philadelphia chromosome + CML during the chronic phase.[74] Since then, dasatinib approval has now been extended to use in all phases of CML in patient who are intolerant of imatinib and Philadelphia chromosome + acute lymphoblastic leukemia (ALL) with resistance or intolerance to prior therapy.[75]

At nanomolar concentrations, dasatinib is shown to inhibit BCR/ABL, SRC family (Src, Lck, Yes, Fyn), c-KIT, EPHA2, and PDGFRβ. *In vitro*, dasatinib was found to be active in leukemic cell lines overexpressing BCR/ABL that were sensitive and resistant to imatinib. In addition, dasatinib was found to overcome BCR/ABL kinase domain mutations leading to imatinib resistance by activating alternate signaling pathways involving SRC family kinases and multi-drug resistance gene expression.

Pharmacokinetics

Maximum plasma concentrations of dasatinib were observed between 0.5-6hours after oral administration. There is a dose proportional increase in AUC and linear elimination characteristics between 15mg per day to 240mg per day. Overall terminal half-life of 3 to 5 hours. Volume of distribution if 2.5L and is extensively distributed in the extravascular space. Binding of the drug and its active metabolite to human plasma proteins *in vitro* was approximately 96 and 93%, respectively.

Metabolism: Dasatinib is extensively metabolized in humans by the CYP3A4 enzyme (a cytochrome P450 enzyme) leading to its active metabolite. Flavin-containing monooxygenase 3 and uridine diphosphate-glucoronosyltransferase enzymes are also involved in the formation of dasatinib metabolites. The active metabolite of dasatinib, which is equipotent to dasatinib, is about 5% of the AUC, indicating that the active metabolite does not play a significant in the pharmacology of the drug.Dasatinib is a weak inhibitor of CYP3A4 enzyme. It does not inhibit CYP1A2, 2A6, 2C8, 2C9, 2C19, 2D6, or 2E1 at clinically relevant concentrations. It does not induce the CYP enzymes in humans.

Elimination: After a single oral dose of [^{14}C]-labeled dasatinib, 4% was recovered in the urine and 85% recovered in the feces within 10 days. Unchanged dasatinib was found in urine at a rate of 0.1% and 19% in feces.

Effects of age and gender: There are no clinically relevant effects of age and gender on the pharmacokinetics of dasatinib.

Hepatic impairment: Differences in Cmax and AUC are not clinically relevant and dose adjustments are not needed in hepatic impairment. Dasatinib administered in patients with Child Pugh B and Child Pugh C at doses of 50 and 20mg, respectively, and compared to patients with normal hepatic function at 70mg. Patients with Child Pugh B hepatic impairment had decreases in dose-normalized C_{max} and AUC by 47% and 8%, respectively. Patients with Child Pugh C hepatic impairment had decreases in dose-normalized C_{max} and AUB by 43% and 28%, respectively.

Renal impairment: There are no clinical studies with dasatinib in renal impairment. Less than 4% of dasatinib and its metabolites are excreted by the kidney.

Drug interactions

Drugs that may increase dasatinib plasma concentrations include CYP3A4 inhibitors: In a trial evaluating pharmacokinetics of dasatinib, it was found that dasatinib 20mg daily with ketoconazole 200mg twice daily (a potent CYP3A4 inhibitor) showed that C_{max} and AUC were increased by four- and five-fold, respectively. Use of dasatinib in combination with a CYP3A4 inhibitor may increase dasatinib levels and recommended to be avoided. Patients taking dasatinib should be monitored for toxicity and a dose reduction is recommended if CYP3A4 inhibitor cannot be discontinued. Common medications that inhibit P450 system and grapefruit juice may increase plasma concentrations of dasatinib and should be avoided. Use of an alternative medication with no or minimal enzyme inhibition potential should be considered. If dasatinib must be used in conjunction with a CYP3A4 inhibitor, a dose decreased should be considered with recommendation to reduce dose 20mg daily if starting at 100mg daily. Reduced doses of dasatinib are predicted to adjust for area under the curve to the range observed if no CYP3A4 inhibitor was used. However, there is no clinical data to support these dose adjustments in patients.

Drugs that may decrease dasatinib plasma concentrations include CYP3A4 inducers, antacids and proton pump inhibitors: Concominant use of a potent CYP3A4 inducer (rifampin) and dasatinib showed the mean C_{max} and AUC of dasatinib were decreased by 81% and 82%, respectively. If a CYP3A4 inducer must be utilized, consider an alternative drug that has less CYP3A4 activity. If dasatinib must be used with a CYP3A4 inducer, a dose increase is recommended. Common medications that induce P450 system and St. John's Wort may decrease plasma concentrations of dasatinib unpredictably. If patients must use a strong CYP3A4 inducer, pharmacokinetics suggest a dose increase. Patient should be monitored for drug toxicity.

Drugs that may have plasma concentration altered by dasatinib: Mean Cmax and AUC of simvastatin were increased by 37% and 20%, respectively, when administered in combination. CYP3A4 substrates known to have a narrow therapeutic index should be administered in caution while taking dasatinib.

Adverse Events

In the overall population of 2712 dasatinib-treated patients, 88% of patients experienced adverse reaction at some time and 19% had adverse reactions that led to treatment discontinuation.[72-81] In a randomized trial in patients with newly diagnosed chronic phase CML, 16% of patients had to discontinue dasatinib due to adverse reactions at 60 month follow up and 39% of patients discontinued dasatinib for all reasons.[78] The reported frequency of specific adverse events is in tables below. Significant adverse events include:

Myelosuppression: Dasatinib is associated with severe (Grade 3 or 4) thrombocytopenia, neutropenia, and anemia. This is generally reversible and managed by holding dasatinib or dose reducing, depending on severity. This was commonly reported in all patient populations. Severe adverse reaction rate was higher in patients in patients with advanced CML than in chronic phase CML. These occurred earlier and more frequently in patients with advanced CML or Ph+ ALL than in chronic phase CML. In patients on dasatinib for CML, it is recommended for CBC monitoring for every 1-2 weeks for first 2-3 months and, thereafter, as clinically indicated.

Bleeding related events: Dasatinib is associated with thrombocytopenia and platelet dysfunction *in vitro*. Most bleeding events in studies were associated with severe thrombocytopenia. Co-administration of medications that inhibit platelet function or anticoagulation may increase risk of hemorrhage. In all CML or PH+ ALL studies: ≥ Grade 3 CNS hemorrhages, including fatalities, occurred in <1% of patients; ≥ Grade 3 gastrointestinal hemorrhage, including fatalities, occurred in 4% of patients and required treatment interruptions and transfusions;
≥ Grade 3 other hemorrhages occurred in 2% of patients.

Fluid retention: Dasatinib may cause fluid retention. Evaluation of patients who develop symptoms of pleural effusion or other fluid retention, such as new or worsened dyspnea, pleuritic chest pain, or dry cough with chest X-ray or additional diagnostic imaging as clinically indicated. Management includes supportive care measures as diuretics or steroids. Severe pleural effusions are managed by thoracentesis and oxygen. Dasatinib was either dose reduced or held depending on severity of adverse reaction. At 5 years of follow-up with chronic dasatinib use, grade 3 or 4 fluid retention was reported in 5-8% of CML and ALL pts.

Cardiovascular events: Dasatinib has been associated with cardiovascular events. At 5 years follow-up, patients on continuous dasatinib for chronic phase CML reported cardiac ischemic events in 3.9%, cardiac related fluid retention in 8.5%, arrhythmias or palpitations in 7.0% and Transient ischemic attacks in 0.8%.

Pulmonary arterial hypertension: Dasatinib may be associated with an increased risk of developing pulmonary arterial hypertension, occurring at any time after initiation, including after more than 1 year of treatment. Symptoms included dyspnea, fatigue, hypoxia, fluid retention.

These were found to be reversible after discontinuation of dasatinib and recommend to be permanently discontinued

QT prolongation: Based on in vitro studies, dasatinib potentially prolonged cardiac ventricular repolarization. This was more evident in patients with hypokalemia, hypomagnesemia, congenital long QT syndrome, patient on anti-arrhythmic medicine or other medications that also prolong QT, and cumulative high-dose anthracycline therapy. In a study of 2440 patients at all doses of dasatinib, <1% of patients reported QT prolongation with 1% experiences a QT prolongation of >500ms. Maximum changes in QTc from baseline ranged from 7.0 to 13.4ms

Severe dermatological reactions: Cases of severe mucocutaneous dermatologic reaction have been reported, including Stevens-Johnson syndrome and erythema multiforme.

Embryo-fetal toxicity: Limited human data suggests that dasatinib may cause fetal harm with patient is pregnancy. Resulting complication include hydrops fetalis, fetal leukopenia, and fetal thrombocytopenia. Recommend that females of reproductive potential to avoid pregnancy and recommend the use of contraception while taking dasatinib and for 30 days after final dose.

Table 1. Non-laboratory adverse reactions in ≥10% of patients (or those of special interest) in a randomized trial of dasatinib vs imatinib in patients with newly diagnosed chronic phase CML with minimum follow-up of 60 months (N=258).[72] Dasatinib dose of 100mg daily

Adverse reaction	All grades (%)	Grade 3 or 4 (%)
Fluid retention	38	5
Pleural effusion	28	3
Superficial local edema	14	0
Pulmonary hypertension	5	1
Generalized edema	4	0
Pericardial effusion	4	1
CHF/cardiac dysfunction*	2	<1
Pulmonary edema	1	0
Diarrhea	22	1
Musculoskeletal pain	14	0
Rash$	14	0
Headache	14	0
Abdominal pain	11	0
Fatigue	11	<1
Nausea	10	0
Myalgia	7	0
Arthralgia	7	0
Hemorrhage	8	1
GI bleeding	2	1
CNS bleeding	<1	0
Other bleeding^	6	0

Vomiting	5	0
Muscle spasms	5	0

*Cardiac dysfunction includes acute cardiac failure, congestive cardiac failure, cardiomyopathy, diastolic dysfunction, decreased EF, and LV dysfunction
$Rash includes erythema, erythema multiforme, rash, generalized rash, macular rash, popular rash, pustular rash, skin exfoliation, and vasicular rash
^Other bleeding includes conjunctival hemorrhage, ear hemorrhage, ecchymosis, epistaxis, eye hemorrhage, gingival bleeding, hematoma, hematuria, intra-abdominal hematoma, petechiae, scleral hemorrhage, uterine hemorrhage, and vaginal hemorrhage

Table 2. Grade 3/4 laboratory abnormalities in patients with newly diagnosed chronic phase CML (N=258) with minimum 60 months follow-up.[72] Dasatinib dose of 100mg daily

Laboratory Abnormality	Percentage (%) of Patients
Hematologic	
Neutropenia	29
Thrombocytopenia	22
Anemia	13
Non-Hematologic	
Hypophosphatemia	7
Hypokalemia	0
Elevated SGPT (ALT)	4
Elevated SGOT (AST)	<1
Elevated Bilirubin	<1
Elevated Creatinine	1

At 60 months of continuous dasatinib use, there were 26 deaths in dasatinib-treated patients (10.1%).

Table 3. Additional adverse reactions from pooled data from clinical trials[72]

Adverse reaction	≥10%	1%-10%	0.1%-<1%	<0.1%
GI disorders		Mucosal inflammation, dyspepsia, abdominal distension, constipation, gastritis, colitis, oral soft tissue disorder	Ascites, dysphagia, anal fissure, upper GI ulcer, esophagitis, pancreatitis, GERD	Protein losing gastroenteropathy, ileus, acute pancreatitis, anal fistula
General disorders and administration	Peripheral edema, face	Asthenia, chest pain, chills	Malaise, other superficial edema	Gait disturbance

site-conditions	edema			
Skin and subcutaneous tissue disorders		Alopecia, acne, dry skin, hyperhidrosis, urticarial, dermatitis	Pigmentation disorder, skin ulcer, bullous conditions, photosensitivity, nail disorder, neutrophilic dermatosis, panniculitis, palmar-planta erythrodysesthesia, hair disorder	Leukocytoclastic vasculitis, skin fibrosis
Respiratory, thoracic, and mediastinal disorders		Lung infiltration, pneumonitis, cough	Asthma, bronchospasm, dysphonia, pulmonary arterial hypertension	ARDs, pulmonary embolism
Nervous system disorders		Neuropathy, dizziness, dysgeusia, somnolence	Amnesia, tremor, syncope, balance disorder	Convulsion, CVA, TIA, optic neuritis, VII nerve palsy, dementia, ataxia
Blood and lymphatic system disorders			Lymphadenopathy, lymphopenia	pure red cell aplasia
Musculoskeletal and connective tissue disorders		Muscular weakness, musculoskeletal stiffness	Rhabdomyolysis, tendonitis, muscle inflammation, osteonecrosis, arthritis	
Investigations		Weight increase, weight decrease	Blood creatine phosphokinase increased, gamma-glutamyltransferase increased	
Infection and infestations		Pneumonia, URI/inflammation, herpes virus infection, entercolitis infection, sepsis		
Metabolism and nutrition disorders		Appetite disturbances, hyperuricemia	Hypoalbuminemia, tumor lysis syndrome, dehydration,	Diabetes mellitus

			hypercholesterolemia	
Cardiac disorders		Arrhythmia, palpitations	Angina pectoris, cardiomegaly, pericarditism ventricular arrhythmia, T-wave abnormality, troponin increase	Cor pulmonale, myocarditis, acute coronary syndrome, cardiac arrest, PR prolongation, coronary artery disease, pleuropericarditis
Eye disorders		Visual disorder (visual disturbance, blurred vision, reduced visual acuity), dry eye	Conjunctivitis, visual impairment photophobia, increased lacrimation	
Vascular disorders		Flushing, hypertension	Hypotension, thrombophlebitism thrombosis	Livedo reticularis, DVT, embolism
Psychiatric disorders		Insomnia, depression	Anxiety, labile affect, confusion, decreased libido	
Pregnancy, puerperium, and perinatal conditions				Abortion
Reproductive system and breast disorders			Gynecomastia, menstrual disorder	
Injury, poising, and prodecureal complications		Contusion		
Ear and labyrinth disorders		Tinnitus	Vertigo, hearing loss	
Hepatobiliary disorders			Cholestasis, cholecystitis, hepatitis	
Renal and urinary disorders			Urinary frequency, renal failure, proteinuria	Renal impairment
Immune system disorders			Hypersensitivity	
Endocrine disorders			Hypothyroidism	Hyperthyroidism, thyroiditis

In a 2011 phase II study, 44 patients with triple negative breast cancer (locally advanced or metastatic) were treated with single agent dasatinib. Results showed that initial dosing of 100mg BID was poorly tolerated and required dose reduction to 70mg BID, which was better tolerated. While single agent dasatinib had limited activity (objective response rate was 4.7%), dasatinib was tolerated at 70mg BID. While no grade 4 adverse events were reported, there were grade 3 adverse reactions related to fatigue, diarrhea, pleural effusions, and dyspnea, occurring in >5% of patients. Mean duration of drug use was 1.6 months, limited by development of adverse reactions.[82]

2.4 Rationale

Figure 9 demonstrates our working hypothesis for cetuximab resistance in TNBC. The goal of this window clinical trial is to evaluate in women with TNBC that harbors nuclear EGFR whether dasatinib can be used to re-establish EGFR on the plasma membrane.

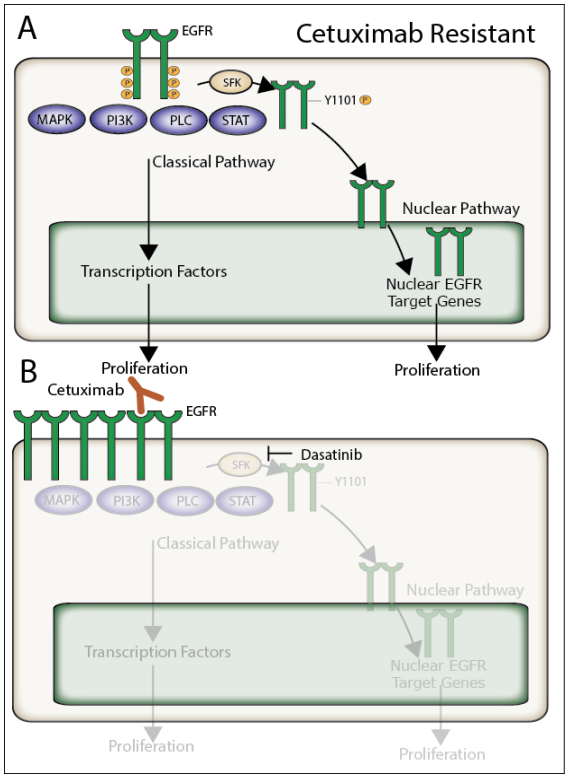

Figure 9: Dual targeting of both nEGFR and plasma membrane-bound EGFR is necessary for the complete inhibition of EGFRs oncogenic functions in TNBC[19]: *A working hypothesis*: SFK phosphorylation of Y1101 of the EGFR is a crucial, early event, in nuclear translocation of the EGFR[18,20]. We have previously shown that TNBC tumors resistant to cetuximab have dependence on the nEGFR pathway (**Figure 9A**)[19]. Blockade of EGFR translocation to the nucleus leads to increased expression on the membrane of the tumor cell where it is available for inhibition by cetuximab (**Figure 9B**).

A window of opportunity design allows this important research question to be asked in a treatment- naïve population of patients as exposure to prior therapies can impact mechanisms of resistance in tumors. If successful, this will provide proof-of-principle for a clinical trial of the combination of dasatinib and cetuxumab in nEGFR positive TNBC. Notably, the combination has already been demonstrated to be safe in phase I studies[83], so rapid translation of this data to the next step clinical trial is feasible.

Since many patients with Stage I-III TNBC are treated upfront with neoadjuvant chemotherapy rather than primary breast surgery, this study will allow for the window of dasatinib to be given prior to either of these modalities. Potential interactions with with neoadjuvant chemotherapy by dasatinib will be avoided with a minimum 2 day washout period between dasatinib use and start of neoadjuvant therapy. This window of time to chemotherapy initiation is often needed in standard practice to obtain pre-chemotherapy testing and insurance approvals. A research biopsy will be obtained in these patients at the end of dasatinib to meet the primary endpoint of this study.

3. PATIENT SELECTION

3.1 Eligibility Criteria for nEGFR testing

3.1.1 Patients must have histologically or cytologically confirmed Stage I- III triple negative breast cancer.

3.1.1.1 ER and PR must be <10% by standard assay methods

3.1.1.2 HER2 must be either 0, 1+ by immunohistochemistry (if 2+, in situ hybridization method used to define HER2) OR have HER2: 17 centromere signal of <2.0 using a standard in situ hybridization method.

3.1.2 No prior therapy for current breast cancer

3.1.3 Meet criteria for neoadjuvant chemotherapy or primary breast surgery, as determined by primary oncologist and surgeon.

3.1.4 Women age ≥18 years.

3.2 Eligibility Criteria for study therapy

3.2.1 nEGFR positive

3.2.2 ECOG performance status ≤1 (see Appendix A).

3.2.3 Patients must have normal organ and marrow function as defined below:

– leukocytes	≥3,000/mcL
– absolute neutrophil count	≥1,500/mcL
– platelets	≥150,000/mcL
– total bilirubin	<1.25x institutional upper limit of normal
– AST(SGOT)/ALT(SGPT)	≤2.5 × institutional upper limit of normal
– creatinine	within normal institutional limits OR
– creatinine clearance	≥60 mL/min/1.73 m^2 for patients with creatinine levels above institutional normal.

3.2.4 Women of child-bearing potential and men 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 and for 30 days after the final dose. Should a woman become pregnant or suspect she is pregnant while she or her partner is participating in this study, she should inform her treating physician immediately.

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

3.3 Exclusion Criteria

3.3.1 Patients who are receiving any other investigational agents.

3.3.2 Patients not able to swallow oral medications or with gastrointestinal conditions that may impact absorption of dasatinib.

3.3.3 History of allergic reactions attributed to compounds of similar chemical or biologic composition to dasatinib.

3.3.4 Patients receiving any medications or substances that are moderate or strong inhibitors or inducers of CYP3A4 are ineligible. Because the lists of these agents are constantly changing, medications should be reviewed by the UW Pharmacy Research Center for any contraindicated medications. As part of the enrollment/informed consent procedures, the patient will be counseled on the risk of interactions with other agents, and what to do if new medications need to be prescribed or if the patient is considering a new over-the-counter medicine or herbal product.

3.3.5 H2 antagonists and proton pump inhibitors are not allowed

3.3.6 Anticoagulants (ie. Coumadin, heparin, anti-Xa inhibitors) and anti-platelet agents (ie. aspirin) are not allowed. NSAIDS and acetaminophen are allowed on study.

3.3.7 Medications known to prolong QTC are not allowed (See Appendix B)

3.3.8 No history of prolonged QTC or cardiomyopathy unless normal QTC and ejection fraction confirmed within 1 month prior to study entry.

3.3.9 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.

3.3.10 Pregnant women are excluded from this study because dasatinib is a pregnancy category D agent with the potential for teratogenic or abortifacient effects. Because there is an unknown but potential risk for adverse events in nursing infants secondary to treatment of the mother with dasatinib, breastfeeding should be discontinued if the mother is treated with dasatinib and not resumed until at least 2 weeks after the final dose.

3.3.11 HIV-positive patients on combination antiretroviral therapy are ineligible because of the potential for pharmacokinetic interactions with dasatinib. In addition, these patients are at increased risk of lethal infections when treated with marrow-suppressive therapy. Appropriate studies will be undertaken in patients receiving combination antiretroviral therapy when indicated.

3.3.12 Contraindication to repeat breast biopsy (neoadjuvant chemotherapy group)

3.4 Inclusion of Women and Minorities

Women and Minorities are not excluded from participation in this trial.

4. REGISTRATION PROCEDURES

4.1 Patient Registration

Eligible patients will be entered on study using the University of Wisconsin Carbone Cancer Center OnCore Database.

To register a patient, the following documents should be completed by the study coordinator or data manager and maintained in the subject study chart:

- Copy of required eligibility tests.
- Signed patient consent form.
- Signed HIPAA authorization form.

The research coordinator at the site will then register the subject into the OnCore database prior to starting study treatment. OnCore will assign the unique subject number.

There will not be access to the OnCore database registration until documented IRB approval is obtained and entered into the OnCore database.

4.2 General Guidelines

Following registration, patients should begin protocol treatment within 30 days. Issues that would cause treatment delays should be discussed with the Principal Investigator. If a patient does not receive protocol therapy following registration, the patient's registration on the study may be canceled.

5. TREATMENT PLAN

5.1 Dasatinib Administration

Treatment will be administered on an outpatient basis. No investigational or commercial agents or therapies other than those described below may be administered with the intent to treat the patient's malignancy.

Regimen Description					
Agent	***Premedications; Precautions***	***Dose***	***Route***	***Schedule***	***Cycle Length****
Dasatinib	*None*	*100mg*	*Oral*	*Once daily*	*7-10 days*

**Dose should be continued up to the day prior to planned surgery or research biopsy (neoadjuvant chemotherapy group)*					

Dasatinib should be taken at the same time daily (+/- 4 hours). It may be taken with or without food, in the morning or evening. If a dose is missed, it should not be made up. Do not crush or cut tablets.

Patients should be informed that the tablet contains lactose, in the event they are lactose intolerant

The patient will be requested to maintain a medication diary of each dose of medication (Appendix C). The medication diary will be returned to clinic staff at the end of the therapy.

5.2 General Concomitant Medication and Supportive Care Guidelines

Because there is a potential for interaction of dasatinib with other concomitantly administered drugs, the case report form must capture the concurrent use of all other drugs, over-the-counter medications, or alternative therapies. The Principal Investigator should be alerted if the patient is taking any agent known to affect or with the potential for drug interactions.

Drug interactions most commonly associated in the absorption and metabolism of dasatinib are ones that affect the CYP3A4, both inducers and inhibitors, antacids, and H2 antagonists along with proton-pump inhibitors.

CYP3A4: Moderate or Strong CYP3A4 inducer or inhibitors are not allowed. CYP3A4 substrates may be administered, but caution and close monitoring should occur if the substrate has a narrow therapeutic index.

Proton pump inhibitors and H2 antagonists are not allowed. If antacid therapy is needed, the antacid dose should be administered at least 2 hours prior to or after the dose of dasatanib.

Anticoagulants and anti-platelet agents are not allowed. NSAIDS and acetaminophen are allowed on study. However, NSAIDS should be discontinued as per standard of care for pre-surgical management.

5.3 Duration of Therapy

Treatment may continue for a maximum of 10 days or until one of the following criteria applies:

- 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.

5.4 Duration of Follow Up

Patients treated with dasatinib will be followed for adverse events for 30 (+/-14) days after breast surgery date (if surgery first) or through completion of cycle 1 of chemotherapy (if neoadjuvant chemotherapy given). Patients removed from study therapy for unacceptable adverse event(s) will be followed until resolution or stabilization of the adverse event. All patients will have long-term follow up until 2 years after primary breast surgery.

5.5 Criteria for Removal from Study

Patients will be removed from study when any of the criteria listed in Section 5.4 applies. The reason for study removal and the date the patient was removed must be documented in the Case Report Form.

6. DOSING DELAYS/DOSE MODIFICATIONS

Due to the limited therapeutic intent of this clinical trial, study drug will be discontinued for any grade 3 or higher adverse event or any intolerable grade 2 event if the event is attributed at least possible or greater to dasatinib. No dose modifications are allowed on study. Once study drug is discontinued for an adverse event it may not be resumed.

Adverse events (AEs) will be graded using the National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE) (version 4.0). Attribution of the AE should be also recorded as:

- Definite – The AE *is clearly related* to the study treatment.
- Probable – The AE *is likely related* to the study treatment.
- Possible – The AE *may be related* to the study treatment.
- Unlikely – The AE *is doubtfully related* to the study treatment.
- Unrelated – The AE *is clearly NOT related* to the study treatment.

Although impact on surgery or chemotherapy is not anticipated, any delays in planned breast surgery or initiation of neoadjuvant chemotherapy by >1 week related to the study drug or any unanticipated complications from surgery reported by the surgery team will also be recorded and reviewed at regular DOWG meetings.

7. PHARMACEUTICAL INFORMATION

7.1 Dasatinib

Availability: Dasatinib is a commercial agent which will be purchased for the investigational use in this study.

Product description: Tablet – White to off-white, biconvex, oval, film-coated tablet with "BMS" debossed on one side and medication dosage on other side. Available preparations in 20mg, 50mg, 70mg, 80mg, **100mg,** and 140 mg.

Solution preparation: Not applicable – oral medication available in dose required in study

Storage requirements: should be stored at 20°C to 25°C (68°F to 77°F); excursions permitted between 15°C and 30°C (59°F and 86°F).

Handling and Disposal: Dasatinib is an antineoplastic product. Dasatinib tablets consist of a core tablet (containing the active drug substance), surrounded by a film coating to prevent exposure of pharmacy and clinical personnel to the active drug substance. However, if tablets are inadvertently crushed or broken, pharmacy and clinical personnel should wear disposable chemotherapy gloves. Personnel who are pregnant should avoid exposure to crushed or broken tablets.

Stability: Include the stability of the original dosage form, reconstituted solution, and final diluted product, as applicable.

Route of administration: Take orally, once a day. If a patient misses a dose, they should take the next dose at its regular schedule. They should not take multiple doses at once. To be taken with or without food, swallowed whole without crushing, and not with grapefruit juice.

Agent Ordering: Ordering and distribution will be handled by the UW Pharmaceutical Research Center

8. BIOMARKER, CORRELATIVE, AND SPECIAL STUDIES

8.1 Nuclear EGFR

nEGFR determination on the diagnostic biopsy will be performed by immunohistochemistry utilizing a protocol developed by the Wheeler lab and interpreted by a pathologist (D. Yang) who has been collaborating with the Wheeler lab in evaluating nEGFR for over 5 years. Briefly, 5 micron tissue sections from the patient's diagnostic biopsy with be de-paraffinized and subjected to citrated buffer antigen retrieval followed by overnight incubation with EGFR polyclonal antibody (sc-03, Santa Cruz Biotechnology Inc., Santa Cruz, CA) at 4C. Peroxidase conjugated secondary antibody is then applied for 1 hour followed by standard DAB staining and hematoxylin counterstaining. Each case will be evaluated for nEGFR expression by counting 300 tumor cells at 400x magnification by light microscopy. Tumor cells with strong nucleolar DAB staining will be counted as positive and cases with ≥20% positive cells will be categorized as nEGFR positive. To ensure preservation of sufficient biopsy tissue for clinical care, no more than three 5um sections will be cut from the tissue block for nEGFR immunohistochemical evaluation.

In patients with nEGFR positive tumors treated with dasatinib who after dasatinib are proceeding with neoadjuvant chemotherapy, a research biopsy of the primary breast tumor will be obtained after 7-10 days of dasatinib. This biopsy will be palpation or ultrasound guided and include 2-4

cores using a standard breast biopsy needle. Samples will be formalin-fixed and paraffin embedded, coded and stored in the TRIP laboratory. The risks of a breast biopsy are overall low and include risk for bleeding, infection and pain. For non-UWCCC sites, please refer to study procedure manual for sample shipping instructions and tissue requirements.

After completion of the window trial, a tissue microarray (TMA) comprised of all tissue samples will be prepared. This will include all nEGFR positive cases who consented to study. Tumor tissue will include the initial breast cancer tumor biopsies (thus, pretreatment or baseline samples), follow up tumor tissue from surgical specimens or biopsies..The TMA will be constructed to evaluate the effect of dasatinib therapy on plasma membrane and nEGFR expression. Sections of the TMA will be fluorescently triple stained with anti-EGFR, anti-E-cadherin, and DAPI per previously established protocol[84] and images will be acquired through the VECTRA imaging system (PerkinElmer, Waltham, MA). Following multispectral de-convolution, co-localization of EGFR with DAPI or E-cadherin will be assessed by fluorescence intensity to quantitatively determine nuclear and plasma membrane EGFR expression, respectively. Again, to ensure preservation of sufficient biopsy and resection tissue for clinical care, no more than 50% of the tissue available in each paraffin block will be utilized for TMA construction. If there is not sufficient tumor material for creation of a TMA, unstained slides will be used.

8.2 Exploratory Studies

If consent is obtained for future studies, additional tumor tissue (blocks or slides) remaining will be stored for future studies. This may include studies evaluating mechanisms of resistance to dasatinib therapy impact on EGFR location or other studies related to cancer.

Please see section 9.0 further information on storage for future research.

9. CONFIDENTIALITY & DATA MANAGEMENT

To protect the confidentiality of subjects in this study the tissue samples will be coded. The key will be saved in computer files that are protected via passwords and access rights. The key will include: subject name, medical record number, procedure dates or pathology accession number (biopsy and surgery), the sample identification codes (one per subject per sample), and the nEGFR status of the biopsy samples. For subjects who are deemed eligible and are registered to the trial their subject ID number will also be listed on the key. The rest of the research data will also be saved in secure computer files or in the OnCore database which is only accessible via username, password, and access rights.

After subjects sign the informed consent form, the biopsy samples will be obtained and coded prior to the samples being provided to the Wheeler lab or the Translational Research Initiatives in Pathology (TRIP) lab. Both of these labs are only accessible to individuals involved with the research completed in those labs. Subjects whose biopsy tissue is nEGFR negative will not be eligible for the dasatinib component of the trial but the remaining slides of tumor tissue collected will be analyzed as a control cohort for the nEGFR testing and any tissue remaining after completion of the nEGFR testing for these subjects will be destroyed.

Subjects whose tissue is nEGFR positive, and who meet all other eligibility criteria, will be enrolled in the dasatinib treatment component of the study. Further samples of their original biopsy tissue, as well as tissue from their definitive surgery (post dasatinib treatment or post neo-adjuvant chemotherapy) will be obtained (for those subjects who go directly to surgery following the dasatinib treatment). For those patients receiving dasatinib prior to neoadjuvant chemotherapy, a research biopsy will be obtained the day after 7-10 days of dasatinib. This tissue will be coded and stored in the TRIP lab. Archived tumor tissue may be obtained from surgical pathology by either the UWCCC research office or the staff in the TRIP lab. The UWCCC research staff or the TRIP lab would then code the tissue. For those subjects who did not consent to future banking any excess tissue obtained from pathology, but not required for study related testing, would be returned to surgical pathology. For non-UWCCC sites, please refer to study procedure manual for sample shipping instructions and tissue requirements.

After study related testing is completed the tissue samples from subjects who consent to future banking would be stored in the Wheeler lab. These samples will be kept until they are exhausted. The link between the study and the samples will be broken one year after completion of the primary analysis. A limited data set would be kept associated with these samples as listed in the table below. Subjects would be permitted to withdraw their samples from the study or the bank up until the time that the link is broken. They would withdraw their samples by informing the study PI.

Table 4. Identifiers and information to be collected

Identifiers and information to be collected for this study:
Name
Medical record number
Date of birth
Race and Ethnicity
Address
Telephone and fax numbers
E-mail address
Pathology accession numbers
Health Insurance Information
Tumor characteristics
Medical history including dates of treatments and procedures
Health information generated from participation in the study
Identifiers and information to be kept for the sample bank for future research:
Tumor characteristics
Age at diagnosis
Race & Ethnicity
Health information generated from participation in the study including information related to treatment and study follow-up

10. STUDY CALENDAR

Patients will have EGFR testing done on initial tumor biopsy/excision done for standard of care. If EGFR testing meets criteria for eligibility for dasatinib treatment, remaining of pre-study screening tests will then be completed and the on-study calendar below will be followed. Pre-study evaluations are to be conducted within 2 weeks prior to start of protocol therapy (day 1). A standard of care visit can be used for a pre-study evaluation if within this time window. In the event that the patient's condition is deteriorating, laboratory evaluations should be repeated within 48 hours prior to initiation of the therapy. For patients with negative nEGFR testing, they will proceed off study to standard of care treatment.

	nEGFR screen	Pre-Study Screen[g]	Days 1-6	One – Three days prior to surgery (Days 7- 10) or can be same day as biopsy (Days 8-11)	Day of Breast Surgery or breast tumor biopsy (if planning neoadjuvant chemotherapy) (Day 8- 11)	Day of breast surgery for neoadjuvant chemotherapy treated nEGFR+ group	Follow-up[d]
Dasatinib (if nEGFR+)			X----------X				
Informed Consent	X						
Demographics	X						
Medical history	X	X					
Concurrent meds		X----------X					
Physical exam		X		X			X
Vital signs		X		X			X
Height		X					
Weight		X					X
Performance status		X		X			X
CBC w/diff, plts		X		X			
Serum chemistry[a]		X		X			
EKG (as clinically indicated)		X		X			
Adverse event evaluation[c]		X----------X					
B-HCG		X[b]					
Radiologic testing (per SOC)[c]		X----------X					
Tumor tissue collection for nEGFR testing and	X				X	X	

inclusion in TMA[f]							

a. Albumin, alkaline phosphatase, total bilirubin, bicarbonate, BUN, calcium, chloride, creatinine, magnesium, potassium, total protein, SGOT [AST], SGPT [ALT], sodium.
b. Serum pregnancy test (women of childbearing potential).
c. Radiologic testing at any time up prior to surgery per SOC
d. Long term clinical follow up for interval treatment and disease status every 4-6 months for 2 years after breast surgery.
e. AE evaluation only required in those who received dasatinib. Required 30 (+/- 14) days after surgery date (if neoadjuvant chemotherapy not given) or through completion of cycle 1 for patients who receive neoadjuvant chemotherapy.
f. After initial nEGFR testing, archived tumor tissue will be collected for all patients for inclusion in the TMA. For those proceeding to surgery, this will include the initial breast biopsy and the surgery specimen. For those receiving neoadjuvant chemotherapy, this will include the initial breast biopsy, a research biopsy the day after dasatinib and prior to neoadjuvant chemotherapy (if nEGFR+ and treated with dasatinib) and from the final breast surgery specimen (if residual tumor).
g. Pre-study evaluations, including history and physical, are to be done within 2 weeks prior to start of protocol therapy (Day 1)
h. In the event that the patient's condition is deteriorating, laboratory evaluations should be repeated within 48 hours prior to initiation of the therapy, per MD discretion.

11. MEASUREMENT OF EFFECT

11.1 Antitumor Effect – Solid Tumors

Although significant tumor response is not anticipated with 7-10 days of dasatinib, for the purposes of this study, patients will be evaluated for pathologic evidence of response at the time of breast surgery. Evidence of response will be described using tumor cell viability.

11.1.1 Definitions

Evaluable for toxicity. All patients will be evaluable for toxicity from the time of their first treatment with dasatinib

Evaluable for primary endpoint: All patients who complete at least 7 days of the dasatinib with a dose of this medication taken within the 48 hours prior to breast surgery and who have adequate breast tissue for nuclear EGFR testing from surgery specimen.

12. DATA SAFETY AND MONITORING PLAN

Oversight And Monitoring Plan

The UWCCC Data and Safety Monitoring Committee (DSMC) is responsible for the regular review and monitoring of all ongoing clinical research in the UWCCC. A summary of DSMC activities are as follows:

- Reviews all clinical trials conducted at the UWCCC for subject safety, protocol compliance, and data integrity.
- Reviews all Serious Adverse Events (SAE) requiring expedited reporting, as defined in the protocol, for all clinical trials conducted at the UWCCC, and studies conducted at external sites for which UWCCC acts as an oversight body.
- Reviews all reports generated through the UWCCC DSMS elements (Internal Audits, Quality Assurance Reviews, Response Reviews, Compliance Reviews, and Protocol Summary Reports) described in Section II of this document.

- Notifies the protocol Principal Investigator of DSMC decisions and, if applicable, any requirements for corrective action related to data or safety issues.
- Notifies the CRC of DSMC decisions and any correspondence from the DSMC to the protocol Principal Investigator.
- Works in conjunction with the UW Health Sciences IRB in the review of relevant safety information as well as protocol deviations, non-compliance, and unanticipated problems reported by the UWCCC research staff.
- Ensures that notification is of SAEs requiring expedited reporting is provided to external sites participating in multi-institutional clinical trials coordinated by the UWCCC.

Monitoring and Reporting Guidelines

UWCCC quality assurance and monitoring activities are determined by study sponsorship and risk level of the protocol as determined by the PRMC. This study falls into the category for:

Intermediate Monitoring

Protocols subject to intermediate monitoring generally include UW Institutional Phase I/II and Phase II Trials. These protocols undergo review of subject safety at regularly scheduled DOWG meetings where the results of each subject's treatment are discussed and the discussion is documented in the DOWG meeting minutes. The discussion includes the number of subjects enrolled, significant toxicities, dose adjustments, and responses observed. Protocol Summary Reports are submitted on a semi-annual basis by the study team for review by the DSMC.

The UWCCC Data and Safety Monitoring Committee has approved the request to provide oversight for the conduction of this study at University of Illinois at Chicago. All instances of SAEs, unanticipated problems and non-compliance will be reported to UWCCC DSMC and UW Health Sciences IRB via notification to Dr. Wisinski and Tammy Koehn. Deviations will be entered into Oncore directly by the UIC study team.

REVIEW AND OVERSIGHT REQUIREMENTS

a) Serious Adverse Event – Reported Within 24 Hours

Serious Adverse Events requiring reporting within 24 hours (as described in the protocol) must also be reported to the Data and Safety Monitoring Committee (DSMC) Chair via an email to saenotify@uwcarbone.wisc.edu within one business day. The OnCore SAE Details Report must be submitted along with other report materials as appropriate (NCI AdEERS form or FDA Medwatch Form #3500 and/or any other documentation available at that time of initial reporting). The DSMC Chair will review the information and determine if immediate action is required. Within 10 working days, all available subsequent SAE documentation must be submitted electronically along with a 24 hour follow-up SAE Details Report and a completed UWCCC SAE Routing Form to saenotify@uwcarbone.wisc.edu. All information is entered and tracked in the UWCCC database.

The Principal Investigator notifies all investigators involved with the study at the UWCCC, the IRB, the sponsor, and the funding agency and provides documentation of these notifications to

the DSMC.

If the SAE occurs on a clinical trial in which the UW PI serves as the sponsor-investigator, the PI reviews the event to determine whether the SAE requires reporting to the FDA and other participating investigators

For a multiple-institutional clinical trial the PI is responsible for ensuring SAEs are reported to the FDA as well as to all participating investigators.

See Section *below* for detailed instructions on SAE reporting.

b) Serious Adverse Event – Reported within 10 Days

Serious Averse Events requiring reporting within 10 days (as described in the protocol) must also be reported to the Data and Safety Monitoring Committee (DSMC) Chair via an email to saenotify@uwcarbone.wisc.edu. The OnCore SAE Details Report must be submitted along with other report materials as appropriate (NCI AdEERS form or FDA Medwatch Form #3500 and/or any other documentation available at that time of initial reporting). The DSMC Chair will review the information and determine if further action is required. All information is entered and tracked in the UWCCC database.

The Principal Investigator notifies all investigators involved with the study at the UWCCC, the IRB, the sponsor, and the funding agency and provides documentation of these notifications to the DSMC.

If the SAE occurs on a clinical trial in which the UW PI serves as the sponsor-investigator, the PI reviews the event to determine whether the SAE requires reporting to the FDA and other participating investigators.

For a multi-institutional clinical trial the PI is responsible for ensuring SAEs are reported to the FDA as well as to all participating investigators.

See Section *below* for detailed instructions on SAE reporting.

c) Sponsor-Investigator Responsibilities for SAE Review

In the event the UWCCC Principal Investigator is acting as the Sponsor-Investigator (i.e., the PI holds the IND), the PI assumes responsibilities of the study sponsor in accordance with FDA 21 CFR 312.32. In this capacity, the UWCCC PI reviews all reports of serious adverse events occurring on the study at the UWCCC and participating external sites and makes a determination of 1) **suspectedness** (i.e., whether there is a reasonable possibility that the drug caused the AE); and 2) **unexpectedness** (the event is not listed in the Investigator's Brochure or is not listed at the specificity or severity that has been observed) in the context of this study. SAE with suspected causality to study drug and deemed unexpected are reported as IND Safety Reports by the UWCCC PI to the FDA, all participating investigators on the study, and the external global sponsor (if applicable) within 15 calendar days. All fatal or life-threatening SAE that are unexpected and have suspected causality to the study drug will be reported by the UWCCC PI to the FDA, all participating investigators on the study, and the external global sponsor (if applicable) within 7 calendar days.

Refer to Section *below* for UWCCC PI instructions for reporting to the FDA.

d) Study Progress Review

Protocol Summary Reports (PSR) are required to be submitted to the DSMC in the timeframe determined by the risk level of the study (quarterly; semi-annually; or annually). The PSR provides a cumulative report of SAEs, as well as instances of non-compliance, protocol deviations, and unanticipated problems, toxicities and responses that have occurred on the protocol in the timeframe specified. PSRs for those protocols scheduled for review are reviewed at each DSMC meeting.

Protocol Summary Reports enable DSMC committee members to assess whether significant benefits or risks are occurring that would warrant study suspension or closure. This information is evaluated by the DSMC in conjunction with other reports of quality assurance activities (e.g., reports from Internal Audits, Quality Assurance Reviews, etc.) occurring since the prior review of the protocol by the DSMC. Additionally, the DSMC requires the study team to submit external DSMB or DSMC reports, external monitoring findings for industry-sponsored studies, and any other pertinent study-related information.

In the event that there is significant risk warranting study suspension or closure, the DSMC will notify the PI of the DSMC findings and ensure the appropriate action is taken for the protocol (e.g., suspension or closure). The DSMC ensures that the PI reports any temporary or permanent suspension of a clinical trial to the sponsor (e.g., NCI Program Director, Industry Sponsor Medical Monitor, Cooperative Group Study Chair, etc.) and other appropriate agencies. DSMC findings and requirements for follow-up action are submitted to the CRC.

I. EXPEDITED REPORTING OF SERIOUS ADVERSE EVENTS

Depending on the nature, severity, and attribution of the serious adverse event an SAE report will be phoned in, submitted in writing, or both according to Table below. All serious adverse events must also be reported to the UWCCC Data and Safety Monitoring Committee Chair. All serious adverse events must also be reported to the UW IRB (if applicable), and any sponsor/funding agency not already included in the list.

Determine the reporting time line for the SAE in question by using the following table. Then refer to sections A and B below if the SAE occurred at the UWCCC.

Expedited Reporting Requirements for Adverse Events that occur on Studies within 30 Days of the Last Administration of the investigational Agent/Intervention

FDA Reporting Requirements for Serious Adverse Events (21 CRF Part 312)
NOTE: Investigators MUST immediately report to the *UWCCC* and any other parties outlined in the protocol 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.
5) 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 UWCCC within the timeframes detailed in the table below:

Hospitalization	Grade 1 Timeframes	Grade 2 Timeframes	Grade 3 Timeframes	Grade 4 & 5 Timeframes
Resulting in hospitalization ≥ 24 hrs	10 Calendar Days			24-Hour; 5 Calendar Days
Not resulting in Hospitalization ≥ 24 hrs	Not required		10 Calendar Days	

Expedited AE reporting timelines are defined as:

- 24-Hour; 5 Calendar Days – The AE must initially be reported within 24 hours of learning of the AE, followed by a complete expedited report within 5 calendar days of the initial 24-hour report.
- 10 Calendar Days – A complete expedited report on the AE must be submitted within 10 calendar days of learning of the AE

[1] 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 4 and Grade 5 AEs

Expedited 10 calendar day reports for:

- Grade 2 adverse events resulting in hospitalization or prolongation of hospitalization
- Grade 3 events

[2] For studies using PET or SPECT IND agents, the AE reporting period is limited to 10 radioactive half-lives, rounded UP to the nearest whole day, after the agent/intervention was last administered. Footnote "1" above applies after this reporting period.

A. SAE Requiring *[24]* Hour Reporting Occurs at UWCCC:

1. Report to the UWCCC:

Reference the **SAE SOP** (Standard Operating Procedure) and the **SAE Reporting Workflow for DOWGs** on the UWCCC website (http://www.uwccc.wisc.edu) for specific instructions on how and what to report to the UWCCC for *[24]* hour initial and follow-up reports. **A follow-up report is required to be submitted within 10 days of the initial *[24]* hour report.**

For this protocol, the following UWCCC entities are required to be notified:

a) saenotify@uwcarbone.wisc.edu
b) Kari B. Wisinski, MD (kbwisinski@medicine.wisc.edu)
c) Tammy Koehn (tmkoehn@medicine.wisc.edu)

d) Any other appropriate parties listed on the SAE Routing Form (for follow-up reports only)

2. Report to the IRB:

Consult the UW-IRB website for reporting guidelines.

If the SAE Requiring 24 Hour Reporting occurs at UIC:
The UW PI is responsible for the submission of the SAE to the UW Health Sciences IRB for any site for which the UW serves as the IRB of record. The outside site should enter the initial information and upload any supporting documentation into Oncore and send an email (within 24 hours) with the subject line of "UW15114 24 Hour SAE Notification" to:

a) Dr. Kari Wisinski (kbwisinski@medicine.wisc.edu)
b) Tammy Koehn (tmkoehn@medicine.wisc.edu)

Upon receipt of the outside SAE report, it will be reviewed by the PI and the Breast DOT will be responsible for reporting it to UW Health Sciences IRB and UWCCC DSMC via reporting outlines for SAEs requiring 24 hour reporting at UWCCC.

A follow up report should be entered into Oncore within 10 days and email notification sent to:

c) Dr. Kari Wisinski (kbwisinski@medicine.wisc.edu)
d) Tammy Koehn (tmkoehn@medicine.wisc.edu)

B. SAE Requiring *[10]* Day Reporting Occurs at UWCCC:

1. Report to the UWCCC:

Reference the **SAE SOP** and the **SAE Reporting Workflow for DOWGs** on the UWCCC website (http://www.uwccc.wisc.edu) for specific instructions on how and what to report to the UWCCC for *[10]* day reports.

For this protocol, the following entities are required to be notified:

a) saenotify@uwcarbone.wisc.edu
b) Any appropriate parties listed on SAE Routing Form

2. Report to the IRB:

Consult the UW-IRB website for reporting guidelines.

If the SAE Requiring 10 day reporting occurs at UIC:
The UW PI is responsible for the submission of the SAE to the UW Health Sciences IRB for any site for which the UW serves as the IRB of record. The outside site should enter the initial information and upload any supporting documentation into Oncore and send an email

with the subject line of "UW15114 10 Day SAE Notification" to:

e) Dr. Kari Wisinski @ kbwisinski@medicine.wisc.edu
f) Tammy Koehn @ tmkoehn@medicine.wisc.edu

Upon receipt of the outside SAE report, it will be reviewed by the PI and the Breast DOT will be responsible for reporting it to UW Health Sciences IRB and UWCCC DSMC via reporting outlines for SAEs requiring 10 day reporting at UWCCC.

A follow up report should be entered into Oncore within 10 days and email notification sent to:

g) Dr. Kari Wisinski (kbwisinski@medicine.wisc.edu)
h) Tammy Koehn (tmkoehn@medicine.wisc.edu)

C. Other Reporting Requirements

Reporting to the FDA

Serious Adverse Events occurring on studies on which a UW PI is acting as sponsor-investigator must be reported to the FDA within the appropriate time frame. Mandatory and voluntary reporting guidelines and instructions are outlined on the FDA website: http://www.fda.gov/Safety/MedWatch/HowToReport/default.htm

Data Management: Study personnel are responsible for entering case report forms directly into the OnCore database in accordance with the UWCCC data management policy. Data must be submitted within 2 weeks of a subject visit and will be monitored monthly by the Breast DOWG Cancer Research office at the UWCCC.

Outside sites (UIC) will complete data entry case report forms and submit them to UWCCC for data entry. Data must be submitted within 2 weeks of a subject visit. Data will be verified via submission of de-identified source documentation from UIC to UWCCC. Remote auditing will occur a minimum of once per year.

13. STATISTICAL CONSIDERATIONS

The primary endpoint is the increase in plasma membrane EGFR expression as measured by VECTRA. An increase of at least 25% from baseline to post-dasatinib treatment will be considered significant. We hypothesize that more than 50% of patients will have such increase. If there are fewer than 20% who reach this increase, then this would not be considered an effective approach. With a type I error set at 5% and power at 80%, 19 patients will be needed to evaluate the primary endpoint. Analysis will be conducted using exact binomial confidence intervals. It is expected that 20% of all consented TNBC cases will have nEGFR, thus the total patient population would be estimated to be approximately 95, with 19 patients treated with dasatinib for the primary endpoint. The secondary endpoint of safety and tolerability of dasatinib in patients with operable TNBC will be based on NCIC Adverse Events Version 4.0 and will be assessed by frequency tables. In addition, the secondary endpoint of pCR will be defined as ypT0 ypNO and assessed by the investigator. The point estimate of the pCR endpoint and its exact 95% confidence intervals (CI) will be calculated. In evaluating pCR, subjects with

missing data will be considered non-responders. Breast cancer recurrence rates and patterns of metastatic recurrence in TNBC by nEGFR status will be described in tabular formats and compared by Chi-Square or Fisher's exact test. Also, potential intracellular mechanisms which impact dasatinib effect will be correlated with cellular localization of EGFR in operable TNBC to explore the effects. Descriptive statistics will be given. Formal statistical tests will be performed based on the outcome types and no multiple comparison adjustment will be made due to the exploratory nature of the analyses.

With an estimated accrual of one nEGFR positive TNBC patient proceeding to primary breast surgery or neoadjuvant chemotherapy every 1-2 months, we anticipate study completion within 36-48 months

REFERENCES

1. Howlader N NA, Krapcho M, Neyman N, Aminou R, Waldron W, Altekruse SF, Kosary CL, Ruhl J, Tatalovich Z, Cho H, Mariotto A, Eisner MP, Lewis DR, Chen HS, Feuer EJ, Cronin KA: SEER Cancer Statistics Review, 1975-2009 (Vintage 2009 Populations), National Cancer Institute. Bethesda, MD, http://seer.cancer.gov/csr/1975_2009_pops09/, based on November 2011 SEER data submission, posted to the SEER web site, April 2012.,
2. American Cancer Society, *Cancer Facts & Figures 2015.*,
3. Hudis CA, Gianni L: Triple-negative breast cancer: an unmet medical need. Oncologist 16 Suppl 1:1-11, 2011
4. Boyle P: Triple-negative breast cancer: epidemiological considerations and recommendations. Ann Oncol 23 Suppl 6:vi7-12, 2012
5. Bauer KR, Brown M, Cress RD, et al: Descriptive analysis of estrogen receptor (ER)-negative, progesterone receptor (PR)-negative, and HER2-negative invasive breast cancer, the so-called triple-negative phenotype: a population-based study from the California cancer Registry. Cancer 109:1721-8, 2007
6. Carey LA, Perou CM, Livasy CA, et al: Race, breast cancer subtypes, and survival in the Carolina Breast Cancer Study. Jama 295:2492-502, 2006
7. Lara-Medina F, Pérez-Sánchez V, Saavedra-Pérez D, et al: Triple-negative breast cancer in Hispanic patients: high prevalence, poor prognosis, and association with menopausal status, body mass index, and parity. Cancer 117:3658-69, 2011
8. Gelmon K, Dent R, Mackey JR, et al: Targeting triple-negative breast cancer: optimising therapeutic outcomes. Ann Oncol 23:2223-34, 2012
9. Kassam F, Enright K, Dent R, et al: Survival outcomes for patients with metastatic triple-negative breast cancer: implications for clinical practice and trial design. Clin Breast Cancer 9:29-33, 2009
10. Wolmark N, Wang J, Mamounas E, et al: Preoperative chemotherapy in patients with operable breast cancer: nine-year results from National Surgical Adjuvant Breast and Bowel Project B-18. Journal of the National Cancer Institute. Monographs:96-102, 2001
11. Boughey JC, Suman VJ, Mittendorf EA, et al: Sentinel lymph node surgery after neoadjuvant chemotherapy in patients with node-positive breast cancer: the ACOSOG Z1071 (Alliance) clinical trial. JAMA 310:1455-61, 2013
12. Cortazar P, Zhang L, Untch M, et al: Pathological complete response and long-term clinical benefit in breast cancer: the CTNeoBC pooled analysis. Lancet 384:164-72, 2014
13. Brand TM, Iida M, Luthar N, et al: Nuclear EGFR as a molecular target in cancer. Radiotherapy and oncology : journal of the European Society for Therapeutic Radiology and Oncology 108:370-7, 2013
14. Cappuzzo F, Ciuleanu T, Stelmakh L, et al: Erlotinib as maintenance treatment in advanced non-small-cell lung cancer: a multicentre, randomised, placebo-controlled phase 3 study. The Lancet. Oncology 11:521-9, 2010
15. Sobrero AF, Maurel J, Fehrenbacher L, et al: EPIC: phase III trial of cetuximab plus irinotecan after fluoropyrimidine and oxaliplatin failure in patients with metastatic colorectal cancer. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 26:2311-9, 2008

16. Van Cutsem E, Kohne CH, Lang I, et al: Cetuximab plus irinotecan, fluorouracil, and leucovorin as first-line treatment for metastatic colorectal cancer: updated analysis of overall survival according to tumor KRAS and BRAF mutation status. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 29:2011-9, 2011

17. Zhou C, Wu YL, Chen G, et al: Erlotinib versus chemotherapy as first-line treatment for patients with advanced EGFR mutation-positive non-small-cell lung cancer (OPTIMAL, CTONG-0802): a multicentre, open-label, randomised, phase 3 study. The Lancet. Oncology 12:735-42, 2011

18. Nielsen TO, Hsu FD, Jensen K, et al: Immunohistochemical and Clinical Characterization of the Basal-Like Subtype of Invasive Breast Carcinoma. Clinical Cancer Research 10:5367-5374, 2004

19. Rakha EA, El-Sayed ME, Green AR, et al: Prognostic markers in triple-negative breast cancer. Cancer 109:25-32, 2007

20. Tsutsui S, Ohno S, Murakami S, et al: Prognostic value of epidermal growth factor receptor (EGFR) and its relationship to the estrogen receptor status in 1029 patients with breast cancer. Breast Cancer Research and Treatment 71:67-75, 2002

21. Zhang M, Zhang X, Zhao S, et al: Prognostic value of survivin and EGFR protein expression in triple-negative breast cancer (TNBC) patients. Target Oncol, 2013

22. Park HS, Jang MH, Kim EJ, et al: High EGFR gene copy number predicts poor outcome in triple-negative breast cancer. Mod Pathol, 2014

23. Zhang J, Wang Y, Yin Q, et al: An associated classification of triple negative breast cancer: the risk of relapse and the response to chemotherapy. Int J Clin Exp Pathol 6:1380-91, 2013

24. Baselga J, Gomez P, Greil R, et al: Randomized phase II study of the anti-epidermal growth factor receptor monoclonal antibody cetuximab with cisplatin versus cisplatin alone in patients with metastatic triple-negative breast cancer. Journal of Clinical Oncology 31:2586-92, 2013

25. Carey LA, Rugo HS, Marcom PK, et al: TBCRC 001: Randomized Phase II Study of Cetuximab in Combination With Carboplatin in Stage IV Triple-Negative Breast Cancer. Journal of Clinical Oncology 30:2615-2623, 2012

26. Wang YN, Yamaguchi H, Hsu JM, et al: Nuclear trafficking of the epidermal growth factor receptor family membrane proteins. Oncogene 29:3997-4006, 2010

27. Wang SC, Hung MC: Nuclear translocation of the epidermal growth factor receptor family membrane tyrosine kinase receptors. Clin Cancer Res 15:6484-9, 2009

28. Lo HW, Hung MC: Nuclear EGFR signalling network in cancers: linking EGFR pathway to cell cycle progression, nitric oxide pathway and patient survival. British journal of cancer 94:184-8, 2006

29. Iida M, Brand TM, Campbell DA, et al: Yes and Lyn play a role in nuclear translocation of the epidermal growth factor receptor. Oncogene 32:759-67, 2013

30. Wang YN, Wang H, Yamaguchi H, et al: COPI-mediated retrograde trafficking from the Golgi to the ER regulates EGFR nuclear transport. Biochemical and biophysical research communications 399:498-504, 2010

31. Lo HW, Ali-Seyed M, Wu Y, et al: Nuclear-cytoplasmic transport of EGFR involves receptor endocytosis, importin beta1 and CRM1. J Cell Biochem 98:1570-83, 2006

32. Wang YN, Yamaguchi H, Huo L, et al: The translocon Sec61beta localized in the inner nuclear membrane transports membrane-embedded EGF receptor to the nucleus. The Journal of biological chemistry 285:38720-9, 2010
33. Liao HJ, Carpenter G: Role of the Sec61 translocon in EGF receptor trafficking to the nucleus and gene expression. Mol Biol Cell 18:1064-72, 2007
34. Du Y, Shen J, Hsu JL, et al: Syntaxin 6-mediated Golgi translocation plays an important role in nuclear functions of EGFR through microtubule-dependent trafficking. Oncogene 33:756-70, 2014
35. Brand TM, Iida M, Li C, et al: The nuclear epidermal growth factor receptor signaling network and its role in cancer. Discov Med 12:419-32, 2011
36. Hsu SC, Hung MC: Characterization of a novel tripartite nuclear localization sequence in the EGFR family. J Biol Chem 282:10432-40, 2007
37. Lin SY, Makino K, Xia W, et al: Nuclear localization of EGF receptor and its potential new role as a transcription factor. Nat Cell Biol 3:802-8, 2001
38. Lo HW, Hsu SC, Ali-Seyed M, et al: Nuclear interaction of EGFR and STAT3 in the activation of the iNOS/NO pathway. Cancer Cell 7:575-89, 2005
39. Hanada N, Lo HW, Day CP, et al: Co-regulation of B-Myb expression by E2F1 and EGF receptor. Mol Carcinog 45:10-7, 2006
40. Jaganathan S, Yue P, Paladino DC, et al: A functional nuclear epidermal growth factor receptor, SRC and stat3 heteromeric complex in pancreatic cancer cells. PloS one 6:e19605, 2011
41. Huang WC, Chen YJ, Li LY, et al: Nuclear Translocation of Epidermal Growth Factor Receptor by Akt-dependent Phosphorylation Enhances Breast Cancer-resistant Protein Expression in Gefitinib-resistant Cells. The Journal of biological chemistry 286:20558-68, 2011
42. Hung LY, Tseng JT, Lee YC, et al: Nuclear epidermal growth factor receptor (EGFR) interacts with signal transducer and activator of transcription 5 (STAT5) in activating Aurora-A gene expression. Nucleic Acids Res 36:4337-51, 2008
43. Lo HW, Cao X, Zhu H, et al: Cyclooxygenase-2 is a novel transcriptional target of the nuclear EGFR-STAT3 and EGFRvIII-STAT3 signaling axes. Molecular cancer research : MCR 8:232-45, 2010
44. Wang SC, Nakajima Y, Yu YL, et al: Tyrosine phosphorylation controls PCNA function through protein stability. Nat Cell Biol 8:1359-68, 2006
45. Dittmann K, Mayer C, Kehlbach R, et al: Radiation-induced caveolin-1 associated EGFR internalization is linked with nuclear EGFR transport and activation of DNA-PK. Mol Cancer 7:69, 2008
46. Traynor AM, Weigel TL, Oettel KR, et al: Nuclear EGFR protein expression predicts poor survival in early stage non-small cell lung cancer. Lung Cancer 81:138-41, 2013
47. Psyrri A, Yu Z, Weinberger PM, et al: Quantitative determination of nuclear and cytoplasmic epidermal growth factor receptor expression in oropharyngeal squamous cell cancer by using automated quantitative analysis. Clin Cancer Res 11:5856-62, 2005
48. Lo HW, Xia W, Wei Y, et al: Novel prognostic value of nuclear epidermal growth factor receptor in breast cancer. Cancer Res 65:338-48, 2005
49. Xia W, Wei Y, Du Y, et al: Nuclear expression of epidermal growth factor receptor is a novel prognostic value in patients with ovarian cancer. Mol Carcinog 48:610-7,

2009

50. Hoshino M, Fukui H, Ono Y, et al: Nuclear expression of phosphorylated EGFR is associated with poor prognosis of patients with esophageal squamous cell carcinoma. Pathobiology 74:15-21, 2007

51. Li CF, Fang FM, Wang JM, et al: EGFR Nuclear Import in Gallbladder Carcinoma: Nuclear Phosphorylated EGFR Upregulates iNOS Expression and Confers Independent Prognostic Impact. Annals of surgical oncology, 2011

52. Li C, Iida M, Dunn EF, et al: Nuclear EGFR contributes to acquired resistance to cetuximab. Oncogene 28:3801-13, 2009

53. Gururaj AE, Gibson L, Panchabhai S, et al: Access to the nucleus and functional association with c-Myc is required for the full oncogenic potential of DeltaEGFR/EGFRvIII. J Biol Chem 288:3428-38, 2013

54. Lo HW, Cao X, Zhu H, et al: Cyclooxygenase-2 is a novel transcriptional target of the nuclear EGFR-STAT3 and EGFRvIII-STAT3 signaling axes. Mol Cancer Res 8:232-45, 2010

55. Hsu SC, Miller SA, Wang Y, et al: Nuclear EGFR is required for cisplatin resistance and DNA repair. American journal of translational research 1:249-58, 2009

56. Dittmann K, Mayer C, Fehrenbacher B, et al: Radiation-induced epidermal growth factor receptor nuclear import is linked to activation of DNA-dependent protein kinase. J Biol Chem 280:31182-9, 2005

57. Viloria-Petit A, Crombet T, Jothy S, et al: Acquired resistance to the antitumor effect of epidermal growth factor receptor-blocking antibodies in vivo: a role for altered tumor angiogenesis. Cancer Res 61:5090-101, 2001

58. Ciardiello F, Bianco R, Caputo R, et al: Antitumor activity of ZD6474, a vascular endothelial growth factor receptor tyrosine kinase inhibitor, in human cancer cells with acquired resistance to antiepidermal growth factor receptor therapy. Clin Cancer Res 10:784-93, 2004

59. Bianco R, Rosa R, Damiano V, et al: Vascular endothelial growth factor receptor-1 contributes to resistance to anti-epidermal growth factor receptor drugs in human cancer cells. Clin Cancer Res 14:5069-80, 2008

60. Wheeler DL, Huang S, Kruser TJ, et al: Mechanisms of acquired resistance to cetuximab: role of HER (ErbB) family members. Oncogene 27:3944-56, 2008

61. Hatakeyama H, Cheng H, Wirth P, et al: Regulation of heparin-binding EGF-like growth factor by miR-212 and acquired cetuximab-resistance in head and neck squamous cell carcinoma. PLoS One 5:e12702, 2010

62. Lu Y, Li X, Liang K, et al: Epidermal growth factor receptor (EGFR) ubiquitination as a mechanism of acquired resistance escaping treatment by the anti-EGFR monoclonal antibody cetuximab. Cancer Res 67:8240-7, 2007

63. Fuchs BC, Fujii T, Dorfman JD, et al: Epithelial-to-mesenchymal transition and integrin-linked kinase mediate sensitivity to epidermal growth factor receptor inhibition in human hepatoma cells. Cancer Res 68:2391-9, 2008

64. Kim SM, Kim JS, Kim JH, et al: Acquired resistance to cetuximab is mediated by increased PTEN instability and leads cross-resistance to gefitinib in HCC827 NSCLC cells. Cancer Lett 296:150-9, 2010

65. Brand TM, Iida M, Dunn EF, et al: Nuclear Epidermal Growth Factor Receptor Is a Functional Molecular Target in Triple-Negative Breast Cancer. Mol Cancer Ther,

2014

66. Li C, Iida M, Dunn EF, et al: Dasatinib blocks cetuximab- and radiation-induced nuclear translocation of the epidermal growth factor receptor in head and neck squamous cell carcinoma. Radiother Oncol 97:330-7, 2010

67. Wheeler DL, Iida M, Kruser TJ, et al: Epidermal growth factor receptor cooperates with Src family kinases in acquired resistance to cetuximab. Cancer Biol Ther 8:696-703, 2009

68. Brand TM, Iida M, Luthar N, et al: Nuclear EGFR as a molecular target in cancer. Radiother Oncol, 2013

69. Benavente S, Huang S, Armstrong EA, et al: Establishment and characterization of a model of acquired resistance to epidermal growth factor receptor targeting agents in human cancer cells. Clin Cancer Res 15:1585-92, 2009

70. Boerner JL, Demory ML, Silva C, et al: Phosphorylation of Y845 on the epidermal growth factor receptor mediates binding to the mitochondrial protein cytochrome c oxidase subunit II. Mol Cell Biol 24:7059-71, 2004

71. Biscardi JS, Maa MC, Tice DA, et al: c-Src-mediated phosphorylation of the epidermal growth factor receptor on Tyr845 and Tyr1101 is associated with modulation of receptor function. J Biol Chem 274:8335-43, 1999

72. Sprycel (dasatinib) [prescribing information]. Princeton, NJ: Bristol-Myers Squibb Company; August 2015.

73. Apperley JF, Cortes JE, Kim DW, et al: Dasatinib in the treatment of chronic myeloid leukemia in accelerated phase after imatinib failure: the START a trial. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 27:3472-9, 2009

74. Kantarjian H, Shah NP, Hochhaus A, et al: Dasatinib versus imatinib in newly diagnosed chronic-phase chronic myeloid leukemia. The New England journal of medicine 362:2260-70, 2010

75. Ottmann O, Dombret H, Martinelli G, et al: Dasatinib induces rapid hematologic and cytogenetic responses in adult patients with Philadelphia chromosome positive acute lymphoblastic leukemia with resistance or intolerance to imatinib: interim results of a phase 2 study. Blood 110:2309-15, 2007

76. Kantarjian HM, Shah NP, Cortes JE, et al: Dasatinib or imatinib in newly diagnosed chronic-phase chronic myeloid leukemia: 2-year follow-up from a randomized phase 3 trial (DASISION). Blood 119:1123-9, 2012

77. Saglio G, Hochhaus A, Goh YT, et al: Dasatinib in imatinib-resistant or imatinib-intolerant chronic myeloid leukemia in blast phase after 2 years of follow-up in a phase 3 study: efficacy and tolerability of 140 milligrams once daily and 70 milligrams twice daily. Cancer 116:3852-61, 2010

78. Shah NP, Guilhot F, Cortes JE, et al: Long-term outcome with dasatinib after imatinib failure in chronic-phase chronic myeloid leukemia: follow-up of a phase 3 study. Blood 123:2317-24, 2014

79. Shah NP, Kantarjian HM, Kim DW, et al: Intermittent target inhibition with dasatinib 100 mg once daily preserves efficacy and improves tolerability in imatinib-resistant and -intolerant chronic-phase chronic myeloid leukemia. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 26:3204-12, 2008

80. Shah NP, Kim DW, Kantarjian H, et al: Potent, transient inhibition of

BCR-ABL with dasatinib 100 mg daily achieves rapid and durable cytogenetic responses and high transformation-free survival rates in chronic phase chronic myeloid leukemia patients with resistance, suboptimal response or intolerance to imatinib. Haematologica 95:232-40, 2010

81. Talpaz M, Shah NP, Kantarjian H, et al: Dasatinib in imatinib-resistant Philadelphia chromosome-positive leukemias. The New England journal of medicine 354:2531-41, 2006

82. Finn RS, Bengala C, Ibrahim N, et al: Dasatinib as a single agent in triple-negative breast cancer: results of an open-label phase 2 study. Clinical cancer research : an official journal of the American Association for Cancer Research 17:6905-13, 2011

83. Argiris A, Feinstein TM, Wang L, et al: Phase I and pharmacokinetic study of dasatinib and cetuximab in patients with advanced solid malignancies. Investigational New Drugs 30:1575-84, 2012

84. Brand TM, Iida M, Dunn EF, et al: Nuclear epidermal growth factor receptor is a functional molecular target in triple-negative breast cancer. Mol Cancer Ther 13:1356-68, 2014

APPENDIX A PERFORMANCE STATUS CRITERIA

ECOG Performance Status Scale		**Karnofsky Performance Scale**	
Grade	Descriptions	Percent	Description
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.	100	Normal, no complaints, no evidence of disease.
		90	Able to carry on normal activity; minor signs or symptoms of disease.
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).	80	Normal activity with effort; some signs or symptoms of disease.
		70	Cares for self, unable to carry on normal activity or to do active 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.	60	Requires occasional assistance, but is able to care for most of his/her needs.
		50	Requires considerable assistance and frequent medical care.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.	40	Disabled, requires special care and assistance.
		30	Severely disabled, hospitalization indicated. Death not imminent.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.	20	Very sick, hospitalization indicated. Death not imminent.
		10	Moribund, fatal processes progressing rapidly.
5	Dead.	0	Dead.

Appendix B: Medications That May Cause QTc Prolongation

Drugs that are generally accepted to have a risk of causing Torsades de Pointes	Drugs that in some reports have been associated with Torsades de Pointes and/or QTc prolongation but at this time lack substantial evidence for causing Torsades de Pointes	Drugs that, in some reports, have been weakly associated with Torsades de Pointes and/or QTc prolongation but that are unlikely to be a risk for Torsades de Pointes when used in usual recommended dosages and in subjects without other risk factors (e.g., concomitant QTc prolonging drugs, bradycardia, electrolyte disturbances, congenital long QTc syndrome, concomitant drugs that inhibit metabolism)
Generic/Brand Name	**Generic/Brand Name**	**Generic/Brand Name**
Amiodarone /Cordarone®	Alfuzosin /Uroxatral®	Amitriptyline /Elavil®
Amiodarone /Pacerone®	Amantadine /Symmetrel®	Ciprofloxacin /Cipro®
Arsenic trioxide /Trisenox®	Atazanavir /Reyataz®	Citalopram /Celexa®
Astemizole /Hismanal®	Azithromycin /Zithromax®	Clomipramine /Anafranil®
Bepridil /Vascor®	Chloral hydrate /Noctec®	Desipramine /Pertofrane®
Chloroquine /Aralen®	Clozapine /Clozaril®	Diphenhydramine /Benadryl®
Chlorpromazine /Thorazine®	Dolasetron /Anzemet®	Diphenhydramine /Nytol®
Cisapride /Propulsid®	Dronedarone /Multaq®	Doxepin /Sinequan®
Clarithromycin /Biaxin®	Felbamate /Felbatrol®	Fluconazole /Diflucan®
Disopyramide /Norpace®	Flecainide /Tambocor®	Fluoxetine /Sarafem®
Dofetilide /Tikosyn®	Foscarnet /Foscavir®	Fluoxetine /Prozac®
Domperidone /Motilium®	Fosphenytoin /Cerebyx®	Galantamine /Reminyl®
Droperidol /Inapsine®	Gatifloxacin /Tequin®	Imipramine /Norfranil®
Erythromycin /Erythrocin®	Gemifloxacin /Factive®	Itraconazole /Sporanox®
Erythromycin /E.E.S.®	Granisetron /Kytril®	Ketoconazole /Nizoral®
Halofantrine /Halfan®	Indapamide /Lozol®	Mexiletine /Mexitil®
Haloperidol /Haldol®	Isradipine /Dynacirc®	Nortriptyline /Pamelor®
Ibutilide /Corvert®	Lapatinib /Tykerb®	Paroxetine /Paxil®
Levomethadyl /Orlaam®	Lapatinib /Tyverb®	Protriptyline /Vivactil®
Mesoridazine /Serentil®	Levofloxacin /Levaquin®	Sertraline /Zoloft®
Methadone /Dolophine®	Lithium /Lithobid®	Solifenacin /VESIcare®
Methadone /Methadose®	Lithium /Eskalith®	Trimethoprim-Sulfa /Sulfa®
Pentamidine /Pentam®	Moexipril/HCTZ /Uniretic®	Trimethoprim-Sulfa /Bactrim®
Pentamidine /NebuPent®	Moxifloxacin /Avelox®	Trimipramine /Surmontil®
Pimozide /Orap®	Nicardipine /Cardene®	
Probucol /Lorelco®	Nilotinib /Tasigna®	
Procainamide /Pronestyl®	Octreotide /Sandostatin®	
Procainamide /Procan®	Ofloxacin /Floxin®	
Quinidine /Cardioquin®	Ondansetron /Zofran®	
Quinidine /Quinaglute®	Oxytocin /Pitocin®	
Sotalol /Betapace®	Paliperidone /Invega®	

Drugs that are generally accepted to have a risk of causing Torsades de Pointes	Drugs that in some reports have been associated with Torsades de Pointes and/or QTc prolongation but at this time lack substantial evidence for causing Torsades de Pointes	Drugs that, in some reports, have been weakly associated with Torsades de Pointes and/or QTc prolongation but that are unlikely to be a risk for Torsades de Pointes when used in usual recommended dosages and in subjects without other risk factors (e.g., concomitant QTc prolonging drugs, bradycardia, electrolyte disturbances, congenital long QTc syndrome, concomitant drugs that inhibit metabolism)
Generic/Brand Name	**Generic/Brand Name**	**Generic/Brand Name**
Sparfloxacin /Zagam®	Perflutren lipid microspheres /Definity®	
Terfenadine /Seldane®	Quetiapine /Seroquel®	
Thioridazine /Mellaril®	Ranolazine /Ranexa®	
	Risperidone /Risperdal®	
	Roxithromycin* /Rulide®	
	Sertindole /Serlect®	
	Sertindole /Serdolect®	
	Sunitinib /Sutent®	
	Tacrolimus /Prograf®	
	Tamoxifen /Nolvadex®	
	Telithromycin /Ketek®	
	Tizanidine /Zanaflex®	
	Vardenafil /Levitra®	
	Venlafaxine /Effexor®	
	Voriconazole /VFend®	
	Ziprasidone /Geodon®	

Appendix C

MEDICATION DIARY for Dasatinib

Patient Initials:___ ___ ___

This is a medication diary on which you are to record the dasatinib you take. Enter the time when the medication is taken, as well as the dose of medication. You will begin taking the dasatinib on Study Day 1 and continuing through Day 7-10, depending on when your breast surgery or biopsy date is scheduled. The last dose of dasatinib should be the day prior to surgery or breast biopsy.

- You should take 1 tablet (100mg) once daily with or without food
- If antacids (ie. tums/Maalox) are needed, the antacid dose should be administered at least 2 hours prior to or after the dose of dasatanib.
- Try to schedule dasatinib around the same time each day (preferably in the morning). Daily dose may be taken 4 hours early or after the scheduled time. If you do not take a medication in the window of scheduled time, do not make it up later or the next day.
- If the medication is not taken, please specify the reason. If you vomit after taking the dose, please indicate on study diary, but do not repeat dose.
- You are to return this diary and your medication bottle to your Research Staff.

Study Day	**Date MM/DAY/YEAR**		**Time Taken**	**Dose (mg)**	**Comments** (Side effects, complaints, other medication)
1*		Dasatinib Dose	Time:_____	_____mg	
2		Dasatinib Dose	Time:_____	_____mg	
3		Dasatinib Dose	Time:_____	_____mg	
4		Dasatinib Dose	Time:_____	_____mg	
5		Dasatinib Dose	Time:_____	_____mg	
6		Dasatinib Dose	Time:_____	_____mg	
7		Dasatinib Dose	Time:_____	_____mg	
8		Dasatinib Dose	Time:_____	_____mg	
9		Dasatinib Dose	Time:_____	_____mg	
10		Dasatinib Dose	Time:_____	_____mg	