Women's Triple-Negative First-Line Study: A Phase II Trial of Liposomal Doxorubicin, Bevacizumab and Everolimus (DAE) in Patients with Localized Triple-Negative Breast Cancer (TNBC) with Tumors Predicted Insensitive to Standard Neoadjuvant Chemotherapy (NACT)

Principal Investigator: Clinton Yam, MD
Co-principal Investigator: Rashmi Murthy, MD
Collaborating Statistician: Roland Bassett Jr.
Clinical Collaborators: Debu Tripathy, MD; Jennifer Litton, MD; Nuhad Ibrahim, MD; Banu Arun, MD; Bora Lim, MD; Naoto Ueno, MD, PhD; Wei Yang, MD
Laboratory Collaborators: Naoto Ueno, MD, PhD; Helen Piwnica-Worms, PhD; W. Fraser Symmans, MD

Department:	**Breast Medical Oncology**
Phone:	**713-792-2817**
Fax:	**713-794-4385**
Research Nurse:	**TBA**

Protocol Type:	**Standard Protocol**
Protocol Phase:	**Phase II**
Version Status:	**11**

Table of Contents

Study Schema: 4

List of abbreviations 5

Glossary of terms 6

1. Introduction 8

Introduction to investigational treatments and other study treatments 11

Overview of Everolimus 11

mTOR pathway and cancer 12

Non-clinical experience 12

2. Study Objectives..... 16

3. Objectives 18

4. Selection of Patients 19

Screening for hepatitis B and C 22

Registration Procedures 23

5. Treatment Plan 23

Everolimus Dosing in Special Populations 25

Drug Information including Adverse Drug Reactions (ADRs) 26

Myelosuppression 26

Cardiac Toxicity 26

Infusion reactions 27

Palmar-plantar erythrodysesthesia (PPE) / Hand-Foot syndrome (HFS) 27

Nausea/Vomiting/Diarrhea 27

Thromboembolism 28

Gastrointestinal (GI) Perforations and Wound Healing Complications 28

Description of selected adverse drug reactions 32

Therapy Administration and Dose Reductions 37

Management of specific toxicities related to everolimus administration: 37

Management of skin toxicity 40

Management of hepatitis reactivation / flare…….44

6. Concomitant medications.....47

7. Reporting of Adverse Events 50

8. Scheduled evaluations.....53

9. Measurement of Effect 56

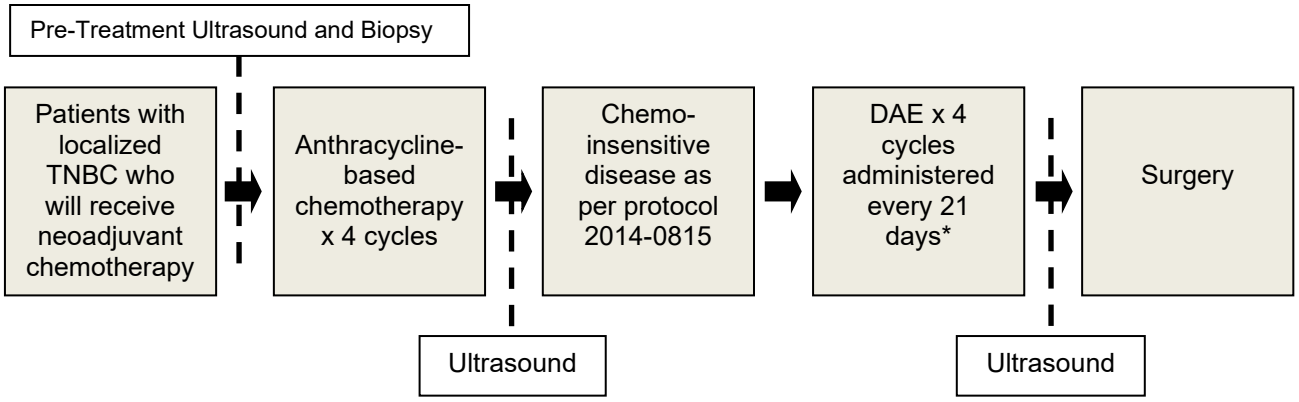
Correlative Studies.....57

Statistical Considerations 57

10. Protocol amendments, or changes in study conduct 58

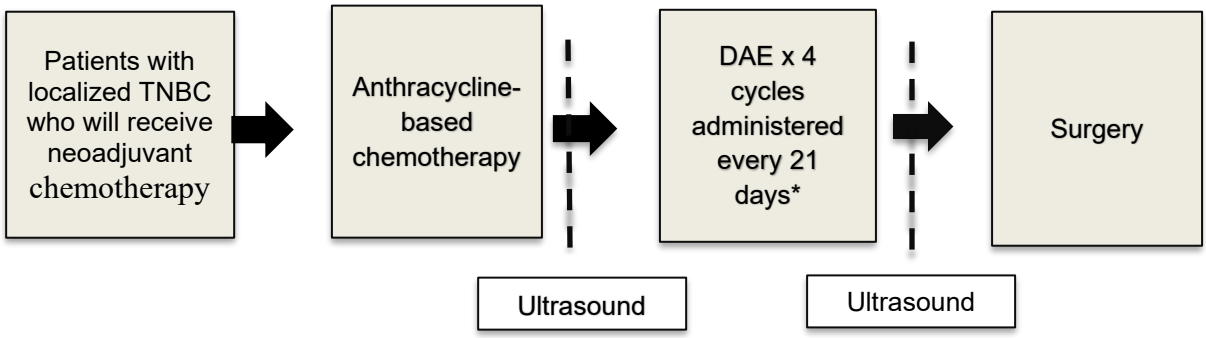
REFERENCES: 59

Study Schema:



*Bevacizumab will be omitted with the fourth cycle

Alternate Study Schema for Patients not Participating on ARTEMIS (2014-0185):



***Bevacizumab will be omitted with the fourth cycle**

List of abbreviations

4E-BP1	4E-binding protein
AE	Adverse event
ALT	Alanine aminotransferase/glutamic pyruvic transaminase/GPT
ANC	Absolute neutrophil count
AST	Aspartate aminotransferase/glutamic oxaloacetic transaminase/GOT
AUC	Area under the curve
BAL	Bronchoalveolar lavage
BCG	Bacillus Calmette-Guérin
CDS	Core data sheet
CoA	Coenzyme A
CPK	Creatine phosphokinase
CRF	Case Report Form
CT	Computer tomography
CTCAE	Common terminology criteria for adverse events
CYP3A4	Cytochrome P450 3A4
DDI	Drug-drug interaction
DLCO	Diffusing capacity of the Lung for Carbon Monoxide
DNA	Deoxyribonucleic acid
DS&E	Drug Safety and Epidemiology
EOT	End of treatment
EU	European Union
FDA	Food and drug administration
GERD	Gastroesophageal reflux disease
HbA1c	Hemoglobin A1c
HBcAb	Hepatitis B core antibody
HbsAb	Hepatitis B surface antibody
HbsAg	Hepatitis B surface antigen
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HMG	3-hydroxy-3-methyl-glutaryl
IB	Investigator brochure
INR	International Normalized Ratio
IRB	Institutional Review Board
IUD	Intrauterine device
IUS	Intrauterine system
IV	Intravenous
$\log_{10}$	Decadic logarithm (common logarithm)
MRI	Magnetic resonance imaging
mTOR	Mammalian target of rapamycin
NCI	National Cancer Institute
PCR	Polymerase chain reaction
PgP	P-glycoprotein
PFT	Pulmonary function tests
PI3K	Phosphoinositide 3-kinase
PNET	Pancreatic neuroendocrine tumor
RCC	Renal cell carcinoma
RMP	Risk management plan
RNA	Ribonucleic acid
SAE	Serious Adverse Event
SEGA	Subependymal giant cell astrocytoma
TS	Tuberous sclerosis
ULN	Upper limit of normal
US	United States
VEGF	Vascular endothelial growth factor

WOCBP Women of child-bearing potential

Glossary of terms

Assessment	A procedure used to generate data required by the study
Baseline	For efficacy evaluations, the baseline assessment will be the last available assessment before or on the date of randomization. For safety evaluations (i.e. laboratory assessments and vital signs), the baseline assessment will be the last available assessment before or on the start date of study treatment. The value obtained at baseline assessments, referred to as "baseline value" will be used as reference for the patient.
Control drug	A study treatment used as a comparator to reduce assessment bias, preserve blinding of investigational drug, assess internal study validity, and/or evaluate comparative effects of the investigational drug
Dose level	The dose of drug given to the patient (total daily or weekly etc.)
Enrollment	Point/time of patient entry into the study; the point at which informed consent must be obtained (i.e. prior to starting any of the procedures described in the protocol)
Investigational drug	The study treatment whose properties are being tested in the study; this definition is consistent with US CFR 21 Section 312.3 and is synonymous with "investigational new drug."
Investigational treatment	Drug whose properties are being tested in the study as well as their associated placebo and active treatment controls (when applicable). This also includes approved drugs used outside of their indication/approved dosage, or that are tested in a fixed combination. Investigational treatment generally does not include other study treatments administered as concomitant background therapy required or allowed by the protocol when used in within approved indication/dosage
Medication number	A unique identifier on the label of each study treatment package which is linked to one of the treatment groups of a study
Other study treatment	Any drug administered to the patient as part of the required study procedures that was not included in the investigational treatment
Patient	A unique identifying number assigned to each patient/subject/healthy volunteer who enrolls in the study
Period	A subdivision of the study timeline; divides stages into smaller functional segments such as screening, baseline, titration, washout, etc.
Premature patient withdrawal	Point/time when the patient exits from the study prior to the planned completion of all study treatment administration and/or assessments; at this time all study treatment administration is discontinued and no further assessments are planned, unless the patient will be followed for progression and/or survival
Stop study participation	Point/time at which the patient came in for a final evaluation visit or when study treatment was discontinued whichever is later

Study treatment	Includes any drug or combination of drugs in any study arm administered to the patient (subject) as part of the required study procedures, including placebo and active drug run-ins. In specific examples, it is important to judge investigational treatment component relationship relative to a study treatment combination; study treatment in this case refers to the investigational and non-investigational treatments in combination.
Study treatment discontinuation	Point/time when patient permanently stops taking study treatment for any reason; may or may not also be the point/time of premature patient withdrawal
Supportive treatment	Refers to any treatment required by the exposure to a study treatment, e.g. premedication of vitamin supplementation and corticosteroid for pemetrexed disodium.
Variable	Identifier used in the data analysis; derived directly or indirectly from data collected using specified assessments at specified time points

1.0 Introduction

Patients with localized TNBC are preferably treated with systemic chemotherapy in the neoadjuvant setting (NACT) as this allows for close monitoring of response in the intact primary tumor and often results in 'downstaging' of tumors, which increases the feasibility of breast conserving surgery. The response to NACT at the time of surgical resection can be determined by measuring the amount of residual cancer remaining in the breast and draining lymph nodes during routine pathologic evaluation and is such a powerful indicator of prognosis that the Food and Drug Administration (FDA) has recognized significant improvement in complete response to neoadjuvant therapy as a pathway to drug approval. Investigators at MDACC have developed and validated a scoring system known as the Residual Cancer Burden (RCB) to quantify the extent of residual disease remaining after NACT and surgical resection.[2] Approximately 50% of patients with localized TNBC treated with standard taxane/anthracycline-based NACT will have either pathologic compete response (pCR/RCB-0) or minimal residual disease (RCB-I) at the time of surgical resection and those patients have identical and exceptionally good long-term prognosis (less than 10% risk of developing distant metastatic disease within 5 years). Unfortunately, those with more extensive residual disease (RCB-II or RCB-III) have a much worse prognosis, with 50%-80% of patients developing distant metastatic disease within 3 years of initial diagnosis.[3] Additionally, clinical trials of NACT in breast cancer have demonstrated that patients without response to their first chemotherapeutic regimen have very low chance (5%) of achieving pCR after their second neoadjuvant chemotherapy regimen.[4] However, this has not been the case with targeted regimens such as trastuzumab in HER2+ tumors, suggesting that intrinsic resistance to chemotherapy can be overcome with appropriate targeted therapy.[5] Though several targeted agents have been tested for treatment in TNBC, so far none have been successful. The underlying causes of this failure are commonly attributed to the molecular heterogeneity of tumors classified within the 'catch all' category of TNBC as well as the dilution effects of chemotherapy sensitive disease which requires clinical trials to enroll larger number of patients to show benefit of combined targeted therapy/chemotherapy regimens over standard chemotherapy. Additionally, clonal selection of resistant cells with escape mechanisms also likely develops. Recent advances in gene expression profiling have identified subgroups of triple-negative breast cancer (TNBC) with distinct molecular features that, if appropriately selected, may be more responsive to targeted therapy with existing FDA-approved drugs, leading to rapid improvement of outcomes in this high-risk breast cancer population[1, 6-8].

Prediction of chemosensitivity and molecular aberrations associated with TNBC subtypes: Our collaborator, Dr. Symmans, and his team have developed gene expression array-based predictive signatures of chemotherapy response using fresh primary tumor tissue obtained from a routine biopsy of breast cancer at the time of diagnosis or surgery. This approach has been retrospectively validated and tested for feasibility using a prospective registry study (MDACC clinical trial 2011-0007).[9] The proposed predictor was tested on an independent cohort of breast cancers irrespective of receptor status (N=198, 99% with clinical Stage II-III) who received neoadjuvant (N=180) or adjuvant (N=18) taxane-anthracycline chemotherapy. Predictions were accurate if ER+/HER2- (30% predicted sensitive, DRFS 97%, CI 91-100; ARR 11%, CI 0.1-21) or ER-/HER2- (26% predicted sensitive, DRFS 83%, CI 68-100; ARR 26%, CI 4-48) (Fig 1).[9] Predicted treatment sensitivity was significantly associated with lower relapse risk (HR 0.19, CI 0.07-0.55) in a multivariate model including ER status, tumor and nodal stage, grade, age, and type of taxane treatment (paclitaxel or docetaxel).

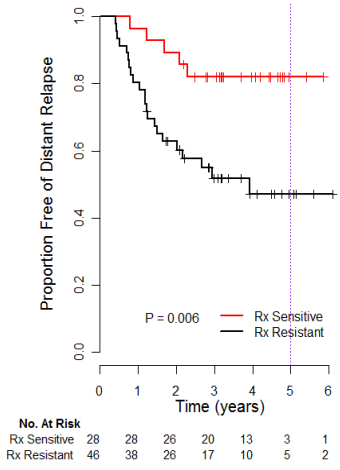


Fig 1: Distant RFS by genomic prediction of chemosensitivity in ER-/HER2- breast cancer

As important as predicting chemosensitivity may be, outcomes for will not be improved unless targeted therapy is developed for

patients with chemoresistant TNBC. Recent advances in gene expression profiling have identified subsets of TNBC (Fig. 2) with distinct molecular features that, if appropriately selected, may be more responsive to targeted therapy with existing FDA approved drugs.[1, 7, 10, 11] Basal-like TNBCs (BL1 and BL2) are heavily enriched in cell cycle and cell division pathways. Gene expression profiling has revealed that these tumors have high expression of proliferation genes such as aurora kinases, *MYC*, *NRAS* and *PLK1* as well as elevated DNA damage response (BL1 subtype). These tumors may have higher rates of pCR to standard chemotherapy compared to other TNBC subtypes. The BL2 subset displays gene ontologies involving growth factor signaling such as EGFR and IGF1R pathways. Cell lines classified as basal-like had high rates of sensitivity to cisplatin and relative resistance to PI3K directed therapy in both cell culture and xenograft models. Though most triple-negative breast cancers are classified as basal-like (BL1 and BL2) by microarray analysis, approximately 30% of triple negative tumors are molecularly distinct from basal-like breast cancers as they display mesenchymal features, including enrichment in epithelial-to-mesenchymal transition (EMT) and stem-cell like characteristics. Subgroups with these features have been identified by independent investigators and have been termed mesenchymal (M), mesenchymal-stem cell like (MSL) and claudin-low. Mesenchymal-like TNBCs carry a high rate of molecular aberrations that activate the PI3K/Akt/mTOR axis suggesting that this subgroup may be responsive to therapeutic regimens targeting this pathway.[1, 6, 7] Approximately 10-15% of TNBC are associated with expression of androgen receptor (AR+ or LAR) and approximately 20-25% are immune modulatory (IM), expressing genes associated with immune activation. Approximately 10-20% of patients with TNBC are carriers of germline BRCA1/2 mutations. When treated with NACT, pCR rates similar to slightly higher than non-carriers.[12] BRCA1 and BRCA2 are required for homologous recombination repair of DNA strand breaks leading to a defect in DNA repair in cancers harboring such mutations. As such, these tumors may be more sensitive to chemotherapy inducing DNA breaks or poly ADP-ribose polymerase (PARP) inhibitors.

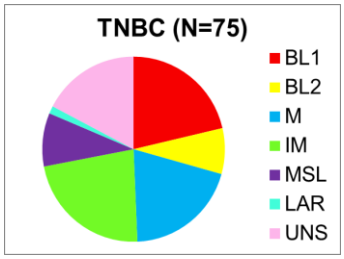


Figure 2: Classification of 75 TNBC samples at MDACC using signatures published by Pietenpol et al.[1]

Notably, investigators at MDACC also determined that rates of pCR/RCB-I to standard NACT were not statistically different amongst the TNBC subtypes, though the lowest rates of pCR were seen in the BL-2 subtype and the highest rates of RCB-III disease was within the mesenchymal subtypes, further validating the need for both predictors of chemotherapy sensitivity as well as molecular characterization of predicted chemotherapy-insensitive disease to determine appropriate therapy for patients with localized TNBC.[13]

Given the disparity of treatment outcomes from NACT for TNBC, a molecular triaging protocol (MDACC 2014-0185) has been developed and IRB approved as a diagnostic platform to identify patients whose tumors are likely or unlikely to achieve pCR or RCB-I, in order to direct predicted responders toward standard chemotherapy and to direct predicted chemotherapy insensitive patients toward potentially more effective experimental therapies within clinical trials (Figure 3). Previously untreated patients with localized TNBC who are candidates for standard NACT (anthracycline → taxane based therapy) will be

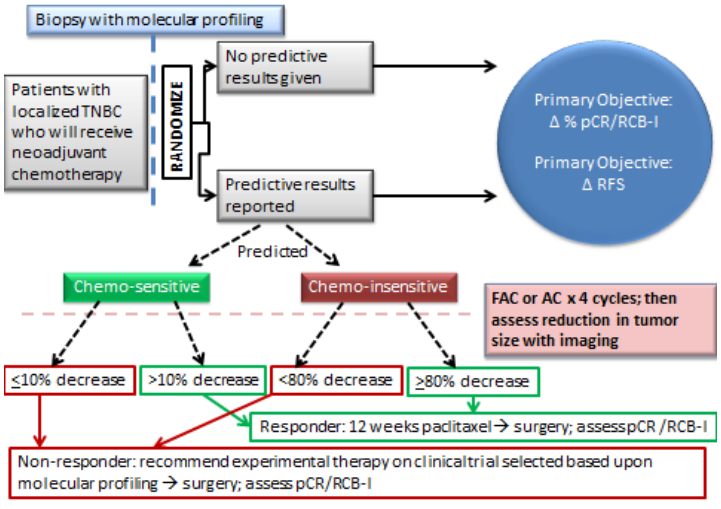


Fig. 3 Clinical Trial Schema for 2014-0185

enrolled into 2014-0185, a non-therapeutic, randomized trial where they will undergo biopsy of the primary tumor for molecular characterization within the MDACC CLIA certified diagnostic lab, including predicted sensitivity to chemotherapy using gene signatures developed at MDACC. Patients will be randomized 2:1 for themselves and their care team to either know or not know the results of the molecular characterization, which will be available within 4-6 weeks after biopsy. All patients will be treated the same except for the assignment of the randomization (Figure 3). The primary endpoint of this triage study is to determine whether molecular profiling improves the overall outcome when used over and above standard indices to identify patients for participation on clinical trials after the mid-point of NACT for those patients with predicted and/or apparent resistance to the first phase of NACT for TNBC.

All patients will then initiate a planned 4 cycles of standard neoadjuvant anthracycline-based chemotherapy based upon physician's choice (AC, dose-dense AC, FAC, EC or FEC are all allowed) with assessment of response by diagnostic imaging after 4 cycles of therapy (or at the time of progression in patients who develop clinical evidence of disease progression on chemotherapy). In our retrospective analysis of molecularly characterized patients with available diagnostic imaging, approximately 10-15% of patients will have obvious, extreme discordance between volumetric measurement of tumor response by ultrasound after 4 cycles of NACT and response prediction by gene signature with the diagnostic imaging best predicting outcome in these extreme cases of response or lack thereof (parameters outlined in Fig. 3). As such, patients with discordant imaging/molecular predictor results will be advised of these results and counseled to proceed based upon imaging. After response assessment, all patients will be offered the option of continuing forward with standard taxane (paclitaxel or docetaxel) or participation in clinical trials (Figure 4). Molecular characterization of the tumor (if known) can also be used along with any other relevant factors by the treating physician to select for clinical trials within the BMO neoadjuvant clinical trials portfolio (Figure 4).

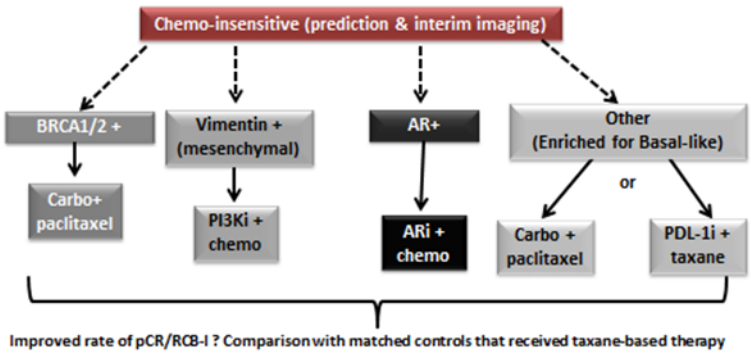


Figure 4: BMO Neoadjuvant Portfolio for Treatment of Chemotherapy Insensitive TNBC

Additionally, because of the 2:1 randomization in 2014-0185 (Figure 3), a subgroup of patients molecularly profiled will not know the results for decision making (control arm). Like the experimental arm, patients on the control arm will be allowed to choose between study participation or to receive standard chemotherapy with paclitaxel based regimen for 12 additional weeks based on standard clinical indices. Essentially, those who do not know the results of the molecular profiling are offered the same options as those who do, they simply will not have the molecular analysis to guide their decision making. Since all patients enrolled in 2014-0185 have biopsies for molecular characterization and diagnostic imaging, for research purposes all patients can be characterized as chemosensitive or chemoresistant and further subtyped into the TNBC molecular subtypes mentioned above. As such, those patients characterized with chemoresistant, subtype specific disease who receive standard NACT with taxane-based regimens can be compared with matched patients treated on clinical trials with phenotypically-matched targeted agents, such as DAE. Though this comparison will not be a randomized comparison, it will provide evidence as to whether patients treated on a single arm phase II trial of targeted therapy derive additional clinical benefit and the comparison can be used to provide more robust data for confirmatory phase III clinical trial design. Likewise, patients whose tumors do not have mesenchymal features can be enrolled on this protocol and receive DAE, which will allow a

control group of non-mesenchymal TNBCs to serve as a control to determine the predictive value of potential biomarkers to define the mesenchymal population.

1.1 Introduction to investigational treatments and other study treatments

Everolimus is a novel derivative of rapamycin. It has been in clinical development since 1996 as an immunosuppressant in solid organ transplantation. Everolimus is approved in Europe and other global markets (trade name: Certican®) for cardiac and renal transplantation, and in the United States (trade name: Zortress®) for the prevention of organ rejection of kidney transplantation.

Afinitor® and was approved for adults with advanced renal cell carcinoma (RCC) after failure of treatment with sunitinib or sorafinib in 2009. In 2010, Afinitor® received United States (US) approval for patients with subependymal giant cell astrocytoma (SEGA) associated with tuberous sclerosis complex (TSC). Everolimus is also available as Votubia® in the European Union (EU) for patients with SEGA associated with TSC who require therapeutic intervention but are not candidates for curative surgical resection. Afinitor® was approved for "progressive pancreatic neuroendocrine tumor (PNET) in patients with unresectable, locally advanced, or metastatic disease" in 2011 in various countries, including the US and Europe. In 2012 Afinitor® received approval for the treatment of postmenopausal women with advanced hormone receptor-positive, HER2- negative breast cancer (advanced HR+ BC) in combination with exemestane, after failure of treatment with letrozole or anastrozole. Furthermore in 2012, Afinitor® received approval for the treatment of patients with TSC who have renal angiomyolipoma not requiring immediate surgery.

Approximately 35,982 cancer patients have been treated with everolimus as of 31-Mar-2014:

- 19,668 patients in Novartis-sponsored clinical trials
- 2,394 patients in the individual patient supply program
- More than 13,930 patients in investigator-sponsored studies.
- In addition, healthy volunteer subjects and non-oncology hepatically impaired subjects have participated in the clinical pharmacology studies as described in Section 7.2.

The following is a brief summary of the main characteristics of Everolimus. More complete information can be obtained from the Everolimus Investigator's Brochure (IB).

1.2 Overview of Everolimus

Everolimus is a derivative of rapamycin which acts as a signal transduction inhibitor (Table 1 and Figure 5). Everolimus selectively inhibits mTOR (mammalian target of rapamycin), specifically targeting the mTOR-raptor signal transduction complex. mTOR is a key serine-threonine kinase in the PI3K/AKT signaling cascade, which is known to be dysregulated in a wide spectrum of human cancers (Boulay and Lane 2007).

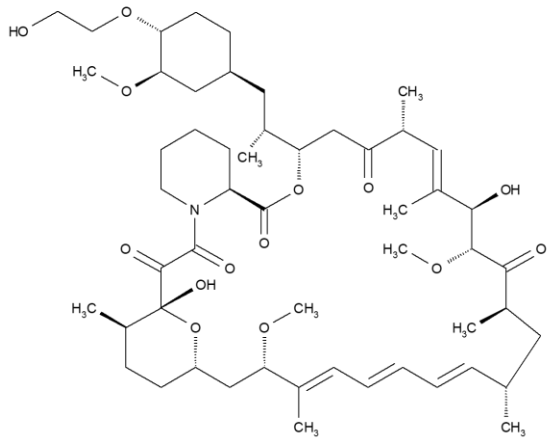
Everolimus is being investigated as an anticancer agent based on its potential to act

- directly on the tumor cells by inhibiting tumor cell growth and proliferation;
- indirectly by inhibiting angiogenesis leading to reduced tumor vascularity (via potent inhibition of tumor cell VEGF (vascular endothelial growth factor) production and VEGF-induced proliferation of endothelial cells).

Table 1: Everolimus - Drug substance

Chemical name	(1R,9S,12S,15R,16E,18R,19R,21R,23S,24E,26E,28E,30S,32S,35R)-1,18-dihydroxy-12-{(1R)-2-[(1S,3R,4R)-4-(2-hydroxyethoxy)-3-methoxycyclohexyl]-1-methylethyl}-19,30-dimethoxy-15,17,21,23,29,35-hexamethyl-11,36-dioxa-4-aza-tricyclo[30.3.1.$0^{4,9}$]hexatriaconta-16,24,26,28-tetraene-2,3,10,14,20-pentaone
International non-proprietary name	Everolimus

1.2.1 Figure 5: Chemical structure of Everolimus



1.2.2 mTOR pathway and cancer

At the cellular and molecular level, Everolimus acts as a signal transduction inhibitor. It selectively inhibits mTOR (mammalian target of rapamycin), a key protein kinase which regulates cell growth, proliferation and survival. The mTOR kinase is mainly activated via the phosphatidylinositol 3-kinase (PI3-Kinase) pathway through AKT/PKB and the tuberous sclerosis complex (TSC1/2). Mutations in these components or in PTEN, a negative regulator of PI3-kinase, may result in their dysregulation. Abnormal functioning of various components of the signaling pathways contributes to the pathophysiology of numerous human cancers. Various preclinical models have confirmed the role of this pathway in tumor development.[14]

The main known functions of mTOR include the following [15]:

- mTOR functions as a sensor of mitogens, growth factors and energy and nutrient levels;
- Facilitating cell-cycle progression from G1-S phase in appropriate growth conditions;
- The PI3K/mTOR pathway itself is frequently dysregulated in many human cancers, and oncogenic transformation may sensitize tumor cells to mTOR inhibitors;
- PI3-kinase mutations have been reported in the primary tumor in 10-20% of human colorectal cancers [16, 17];
- The loss of PTEN protein, either through gene deletion or functional silencing (promoter hypermethylation), is reported in approximately 60% of primary human colorectal cancers [18];
- The mTOR pathway is involved in the production of pro-angiogenic factors (i.e., VEGF) and inhibition of endothelial cell growth and proliferation;
- Through inactivating eukaryotic initiation factor 4E binding proteins and activating the 40S ribosomal S6 kinases (i.e., p70S6K1), mTOR regulates protein translation, including the HIF-1 proteins. Inhibition of mTOR is expected to lead to decreased expression of HIF-1.

1.2.3 Non-clinical experience

Everolimus inhibits the proliferation of a range of human tumor cell lines *in vitro* including lines originating from lung, breast, prostate, colon, melanoma and glioblastoma. IC50s range from sub/low nM to µM. Everolimus also inhibits the proliferation of human umbilical vein endothelial cells (HUVECS) *in vitro*, with particular potency against VEGF-induced proliferation suggesting that Everolimus may also act as an anti-angiogenic agent. The anti-angiogenic activity of Everolimus was confirmed *in vivo*. Everolimus selectively inhibited VEGF-dependent angiogenic

response at well tolerated doses. Mice with primary and metastatic tumors treated with Everolimus showed a significant reduction in blood vessel density when compared to controls.
The potential of Everolimus as an anti-cancer agent was shown in rodent models. Everolimus is orally bioavailable, residing longer in tumor tissue than in plasma in a subcutaneous mouse xenograft model, and demonstrating high tumor penetration in a rat pancreatic tumor model. The pharmacokinetic profile of Everolimus indicates sufficient tumor penetration, above that needed to inhibit the proliferation of endothelial cells and tumor cell lines deemed sensitive to Everolimus *in vitro*.

Everolimus administered orally daily was a potent inhibitor of tumor growth, at well tolerated doses, in 11 different mouse xenograft models (including pancreatic, colon, epidermoid, lung and melanoma) and two syngeneic models (rat pancreatic, mouse orthotopic melanoma). These models included tumor lines considered sensitive and "relatively resistant" *in vitro*. In general, Everolimus was better tolerated in mouse xenograft models than standard cytotoxic agents (i.e., doxorubicin and 5-fluorouracil), while possessing similar anti-tumor activity. Additionally, activity in a VEGF-impregnated subcutaneous implant model of angiogenesis and reduced vascularity (vessel density) of Everolimus-treated tumors (murine melanoma) provided evidence of *in vivo* effects of angiogenesis.

It is not clear which molecular determinants predict responsiveness of tumor cells to Everolimus. Molecular analysis has revealed that relative sensitivity to Everolimus *in vitro* correlates with the degree of phosphorylation (activation) of the AKT/PKB protein kinase and the S6 ribosomal protein; in some cases (i.e., glioblastoma) there is also a correlation with PTEN status.
In vivo studies investigating the anti-tumor activity of Everolimus in experimental animal tumor models showed that Everolimus monotherapy typically reduced tumor cell growth rates rather than produced regressions. These effects occurred within the dose range of 2.5 mg to 10 mg/kg, orally once a day.

In preclinical models, the administration of Everolimus is associated with reduction of protein phosphorylation in target proteins downstream of mTOR, notably phosphorylated S6 (p-S6) and p-4E-BP1, and occasionally with an increase in phosphorylated AKT, a protein upstream of mTOR signaling pathway.

All significant adverse events observed in toxicology studies with Everolimus in mice, rats, monkeys and mini-pigs were consistent with its anticipated pharmacological action as an anti-proliferative and immunosuppressant and at least in part reversible after a 2 or 4-week recovery period with the exception of the changes in male reproductive organs, most notably testes.
In vitro genotoxicity studies covering relevant genotoxicity end-points showed no evidence of clastogenic or mutagenic activity.

In male fertility studies in rats, testicular morphology was affected at 0.5 mg/kg and above, and sperm motility, sperm head count and plasma testosterone levels were diminished at 5 mg/kg which corresponded to 0.7 times the estimated clinical exposure at 10 mg/day, and caused a decrease in male fertility. There was evidence of reversibility. Female fertility was not affected, but everolimus caused an increase of pre-implantation loss in female rats at doses > 0.1 mg/kg, suggesting it could also potentially impact fertility in females. Everolimus crossed the placenta and was toxic to the conceptus. In rats, everolimus caused embryo/fetotoxicity at systemic exposure below the planned therapeutic level comprising mortality and reduced fetal weight. The incidence of skeletal variations and malformations at 0.3 and 0.9 mg/kg (e.g. sternal cleft) was increased. In rabbits, embryo toxicity was evident by an increase in late resorptions. Effects of everolimus on the pre- and postnatal development of rats were limited to slightly affected body weight and survival in the F1-generation at ≥0.1 mg/kg, and did not indicate a specific toxic potential.

The potential reproductive risk for humans is unknown. However, due to the observed malformations in rats, everolimus should be considered potentially teratogenic. Everolimus should

not be given to pregnant women unless the potential benefit outweighs the potential risk for the fetus. Women of childbearing potential should be advised to use highly effective contraception methods while they are receiving everolimus and up to 8 weeks after treatment has been stopped. It is not known whether everolimus is excreted in human milk. In animal studies, everolimus and/or its metabolites were readily transferred into the milk of lactating rats. Therefore women who are taking everolimus should not breastfeed.

Further details can be found in the Everolimus Investigator's Brochure.

1.3 Rationale for DAE in claudin-low/mesenchymal/mesenchymal stem cell-like TNBC

Though most TNBCs are classified as basal-like by gene expression array analysis, approximately 30% of triple-negative tumors are molecularly distinct from basal-like breast cancers as they display mesenchymal features, including enrichment in epithelial-to-mesenchymal transition (EMT) and stem-cell like characteristics.[1, 11, 19] TNBCs and TNBC cell lines with mesenchymal features have been identified by multiple independent investigators and have been labeled 'claudin-low', 'mesenchymal' and 'mesenchymal stem cell-like'.[1, 11] Emerging data suggest that, in contrast to basal subtypes of TNBC, these mesenchymal TNBCs carry a high rate of molecular aberrations that activate the PI3K/Akt/mTOR axis suggesting that this subgroup may be responsive to therapeutic regimens targeting this pathway.[1, 6] Cell lines characterized as mesenchymal/mesenchymal stem-like were noted to have a high rate of molecular aberrations that could result in activation of the PI3K/Akt/mTOR pathway and demonstrated response to PI3K-pathway inhibitors in both cell culture assays and xenograft models.[1]

Many of these cell lines have also been classified as claudin-low. These features were not shared by the basal-like cell lines (BL1 and BL2), which were noted to have high proliferative rates, absence of mutations in PIK3CA, enhanced response to cisplatin and diminished sensitivity to the dual mTOR/PI3kinase inhibitor NVP-BEZ235.[1]

Though evaluation using H&E staining/light microscopy cannot detect distinct morphological features that would distinguish most mesenchymal-like TNBCs from non-mesenchymal TNBCs, approximately 10-30% of tumors molecularly characterized as 'claudin-low' can be morphologically identified as metaplastic breast cancers using these conventional techniques.[6, 11] Metaplastic breast cancers are commonly triple negative, considered refractory to standard therapy, and harbor a high rate of molecular aberrations that lead to activation of the PI3K-pathway.[6] Although genomic profiling, using unsupervised hierarchical clustering of patient samples, initially grouped metaplastic tumors within the spectrum of basal-like invasive ductal carcinomas (IDCs) some transcripts were found to be differentially expressed between metaplastic cancers and basal-like IDCs.[20] For example, DNA repair pathways such as BRCA1, PTEN and TOP2A were significantly down regulated in the metaplastic tumors compared to the basal-like IDCs.[20] Further genomic analyses by Hennessy et al. revealed that metaplastic tumors with mesenchymal features, such as sarcomatoid metaplasia, more closely resembled the claudin-low subtype of breast cancers rather

than basal-like IDCs.[6] Like the mesenchymal/mesenchymal stem-like cell lines, metaplastic tumors with mesenchymal features have been associated with a high rate of mutations in *PIK3CA* (47%) and occasional mutations in *PTEN* (5%), which was noted to be much higher than the 8% (all *PIK3CA*) seen with non-metaplastic, TNBC.[6] Reverse-phase protein array (RPPA) confirmed elevations in the phosphorylation of phosphatidylinositol 3-kinase/Akt pathway proteins in metaplastic tumors (also noted to be higher than non-metaplastic TNBC tumors) further supporting the genomic profiling analyses and suggesting that agents which target the PI3K/mTOR pathway may be a viable option for the treatment of metaplastic breast cancer.[6] Metaplastic breast cancers have also been found to express high levels of vascular endothelial growth factor (VEGF) and hypoxia-induced factor (HIF-1α), a transcription factor important for vasculogenesis and that signals through the mTOR pathway.[21, 22] Therefore, it is likely that

metaplastic tumors act as a morphologically identified 'surrogate of response' to evaluate the activity of targeted therapy regimens in mesenchymal-like TNBC.

Based upon the molecular features of metaplastic breast cancer, 52 patients with metastatic, metaplastic breast cancer have been treated using a regimen using a combination of pegylated liposomal doxorubicin, bevacizumab and the mTOR inhibitors, temsirolimus (DAT) or everolimus (DAE). Most patients were treated on the Phase I trial that established the maximum tolerated dose (MTD) for this regimen.[23] The objective response rate (ORR) was 21% [complete response (CR)=4 (8%); partial response (PR)=7 (13%)] and 10 (19%) pts had stable disease (SD)≥6 months for a clinical benefit rate (CBR) of 40%. One patient had a CR that remains durable for greater than 5 years on maintenance everolimus. Tissue was available for testing in 43 pts and 32 (74%) had a PI3K-pathway activating aberration. PI3K-pathway activation was associated with a significant improvement in ORR (31 vs 0%; P=0.043). It is notable that 12 patients with metastatic non-metaplastic TNBC were also treated with DAT, and only 1 patient had a response (CR/PR=1; SD≥6 months=0).[24]

In this cohort, 13 patients were treated with DAE, with 9 patients being treated with liposomal doxorubicin 30mg/m2 IV Q3W, bevacizumab 15mg/kg IV Q3W and everolimus 7.5mg PO every day for 21 days. In these 9 patients, grade 3-4 toxicities included neutropenia (N=2; 22%), anemia (N=2; 22%), fatigue (N=2; 22%), thrombocytopenia (N=1; 11%), mucositis (N=1; 11%) and transaminitis (N=1; 11%). Grade 2 pulmonary toxicity was seen in one patient who was treated with DAE. Despite previous anthracycline exposure in approximately 85% of patients, no patients treated with DAE developed grade 3-4 heart failure. Although some patients required dose reduction and treatment interruptions due to toxicity, no patients discontinued treatment due to toxicity.

1.4 Rationale for Everolimus

Mesenchymal and mesenchymal stem cell-like tumors are hypothesized to develop from immature breast precursor cells, thus retaining cancer stem cell (CSC)-like features. Metaplastic and claudin-low cancers also closely resemble cancer stem cells (CSCs) and, given the role of the Akt pathway in stem cell maintenance, may be one reason metaplastic tumors are sensitive to mTOR inhibition. In preclinical studies, activation of PI3K/mTOR has been associated with CSC tumorigenicity, survival and capacity for self-renewal.[25, 26] Activation of this pathway is known to play an important role in breast cancer resistance to chemotherapy, HER2 targeted therapy and endocrine therapy as demonstrated in pre-clinical models [27-29] and confirmed in clinical trials [30-32]. The PI3K/mTOR pathway is involved in regulation of the signal transduction and activator of transcription (STAT3) pathway, which has been implicated in CSC survival.[26] Additionally, survival signaling by Notch1, a transmembrane protein involved in embryonic development and highly expressed in CSCs, is mediated through mTOR.[33] Inhibition of mTOR with rapamycin decreased breast CSC fractions in cell culture and impaired breast CSC tumorigenicity in mouse models.[26] Further, in pre-clinical studies, xenograft models of mesenchymal cells lines carrying PIK3CA mutations displayed partial or complete response with the mTOR/PI3kinase inhibitor NVP-BEZ235.[1]

1.5 Rationale for Bevacizumab

CSCs show high adaptability to inhospitable microenvironments or 'tumor niches'. CSCs are able to survive in hypoxic conditions, presumably through the use of anaerobic glycolysis for generation of ATP, a process that involves activation of the PI3K pathway.[34-38] Additionally, hypoxia has been implicated in CSC self-renewal, with increasing oxygen levels resulting in proliferation, maturation and exhaustion of the CSC pool[39]. Under hypoxic conditions, activated Akt promotes accumulation of hypoxia-induced factor (HIF-1), a transcription factor important for vasculogenesis, which is upregulated in stem cell promoting cell culture of breast cancer cell lines (Woodward, data not shown).[40, 41] CSCs also produce much higher levels of vascular endothelial growth factor (VEGF) than non-CSC tumor cells and treatment with bevacizumab

inhibited growth in tumors derived from CSC-positive cells but had no effect upon tumors derived from non-CSC cells in xenograft models[42]. More importantly, early evidence is emerging that suggests that CSCs may be dependent upon growth factors secreted by tumor vasculature.[43] Though most of the data concerning the CSC/vascular niche arises from brain tumors, data exist supporting the effects of the extracellular matrix in maintaining a breast CSCs phenotype, as well as, hypoxic conditions supporting self-renewal[44]. Interestingly, new data have emerged suggesting that treatment with bevacizumab induces intratumoral hypoxia which increases the population of CSCs in breast cancer xenografts.[45] This increase in CSC population was associated with increased phosphorylation of Akt suggesting that treatment with bevacizumab may actually prime tumors with CSC like characteristics for response to temsirolimus. Additionally, under hypoxic conditions, activated Akt also promotes accumulation of hypoxia-induced factor (HIF-1) a transcription factor important for vasculogenesis, which is up-regulated in stem cell promoting cell culture of breast cancer cell lines. As such, the combination of bevacizumab with temsirolimus could impact vasculogenesis by affecting both HIF-1 and VEGF production leading to increased tumor hypoxia.

1.6 Rationale for Liposomal Doxorubicin

The success of doxorubicin in the treatment of sarcoma suggests that other tumors with mesenchymal features may also be responsive to anthracyclines.[46] As confirmation, doxorubicin has shown response in sarcomatoid renal cell carcinoma.[47] Activation of the PI3K/mTOR pathway is known to play an important role in breast cancer resistance to chemotherapy, and it is possible that mTOR inhibition may be able to overcome resistance to anthracyclines. In a study of metastatic metaplastic TNBC patients conducted at MDACC, 23 patients were treated with temsirolimus containing regimens. In patients treated with DAT or DT, the response rate was about 30%.[48] However, in the patients treated with paclitaxel or carboplatin in combination with temsirolimus, the response rate was 0%. Additionally, one patient was treated with temsirolimus alone, and no response was seen. This data suggests synergistic activity of mTOR inhibition with anthracycline therapy but not paclitaxel or carboplatin therapy in this subset of patients. In another study conducted at MDACC, patients with early stage TNBC treated with everolimus in combination with paclitaxel followed by anthracycline containing therapy in the neoadjuvant setting had no increase in pCR rates compared to patients treated with paclitaxel alone followed by anthracycline containing therapy.[49] As further proof of principle, doxorubicin but not paclitaxel was able to inhibit growth in PTEN knockout mammary cell lines, suggesting that anthracycline therapy may be more effective in cell lines exhibiting PI3K pathway activation.[50]

2.0 Study Rationale / Purpose

Based upon the above mentioned data, it is hypothesized that chemo-insensitive TNBCs with mesenchymal features will have higher rates of RCB-0 to RCB-1 with neoadjuvant DAE than historically seen with paclitaxel alone.

2.1 Feasibility

At MDACC, approximately 600 newly diagnosed TNBC patients are seen per year, and the majority will be eligible for screening for the triaging protocol. Of those, approximately 50% will be predicted chemo-insensitive based on molecular analysis performed on the pre-treatment biopsy and confirmed chemo-insensitive based on diagnostic imaging performed after four cycles of anthracycline-based therapy. As such, we estimate that there will be approximately 300 patients within the molecular triaging protocol who will be eligible for experimental therapies yearly. Of these, approximately 30% (90 patients yearly) are expected to have mesenchymal TNBCs. It is estimated that most of these patients will have evidence of some vimentin staining (~80%), which will identify these patients for enrollment in this trial. Additionally, some patients who will not have the results of their molecular profiling will also be recruited to this trial. Our study design requires 37 patients to determine if there is sufficient efficacy data to move to a phase III registration trial. Assuming a 50% accrual rate, we anticipate completing accrual within 15 months with all patients undergoing surgery with assessment for pathologic complete response within 18 months.

2.2 Rationale for Correlative Studies

Unfortunately, neither of the gene expression profiles used to identify claudin-low or mesenchymal/ mesenchymal stem cell-like TNBCs have been CLIA certified or are currently available as an assay that can be performed in formalin-fixed, paraffin-embedded (FFPE) tissue samples; however, staining for vimentin using immunohistochemistry (IHC) is a CLIA certified test that is routinely performed using FFPE tissue.

Vimentin staining has been routinely used to identify cells of mesenchymal origin and has been used to characterize breast tumors or cell lines undergoing EMT. This assay is commercially available, available in or CLIA laboratory and vimentin is commonly expressed in non-tumor stroma, which can be used as a positive control when measuring for expression within cancer cells.

Vimentin expression within breast cancer cells has been associated with a more aggressive triple-negative phenotype in retrospective analyses.[53, 54] Claudin-low tumors are commonly vimentin-positive.[11] Though the Pietenpol group did not use vimentin staining to characterize the mesenchymal and mesenchymal stem-like cell lines, most of the lines identified by these signatures have been previously characterized as vimentin-positive (Table 2) and cell lines which have been characterized as mesenchymal or mesenchymal-stem cell-like have demonstrated high levels of vimentin expression compared to basal-like cell lines (Figure 6, Ueno unpublished data). Interestingly, metaplastic tumors are almost always vimentin-positive (Table 3).[55]

Table 2: Comparison of Vimentin Staining in Cell Lines Identified as Mesenchymal/Mesenchymal Stem-Like vs. Basal-like Subtypes[51, 52]

Cell Line	Molecular Classification	Vimentin
CAL120	Mesenchymal-like	unknown
CAL51	Mesenchymal-like	unknown
MDAMB-157*	Mesenchymal-like	unknown
MDAMB-231*	Mesenchymal-like	positive
MDAMB-436*	Mesenchymal-like	positive
SUM159*	Mesenchymal-like	positive
HS578T*	Mesenchymal-like	positive
BT549*	Mesenchymal-like	positive
MDAMB-468	Basal-like 1	negative
HCC2157, HCC1599, HCC1937, HCC1143, HCC3153, HCC38	Basal-like 1	unknown
SUM149PT	Basal-like 2	negative
HCC70	Basal-like 2	weakly positive (<10%)
HCC1806	Basal-like 2	weakly positive (<10%)
CAL851, HDQ-P1	Basal-like 2	unknown

*indicates cell lines characterized as claudin-low

Table 3: Vimentin staining in human breast cancer TMAs (Gilcrease, unpublished data)

Vimentin staining	Metaplastic TNBC (n=52)	Non-metaplastic TNBC (n=67)
Any amount of tumor cell staining	52 (100%)	36 (54%)

Reproducibility and Potential Clinical Relevance of Vimentin Staining:

To address concerns of reproducibility, tissue Microarrays (TMAs) of non-metaplastic TNBCs (n=64 tumors) were stained x 3 sets of slides and scored for the presence or absence of vimentin in the epithelial carcinoma cells by two MDACC pathologists. Inter-pathologist reliability was assessed using standard metrics as defined in Table 4. The first 4 of these metrics are considered

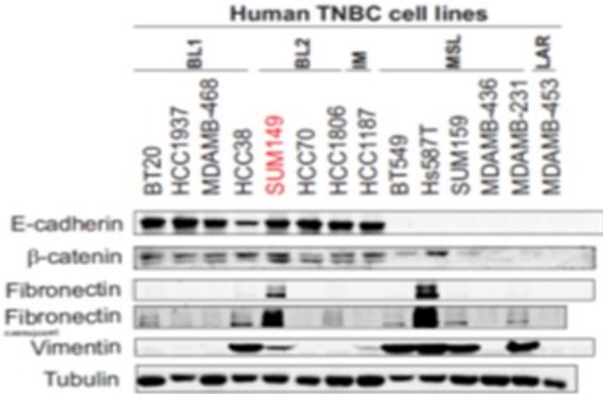


Figure 6: Vimentin Expression in TNBC cell lines.

measures of association and the kappa statistic is a measure of agreement. A generalized linear intercept only model was also fitted to the response data which contained a random subject effect assuming the subject effect had an exchangeable variance-covariance structure (which is the same as a compound symmetry). In this model, there were two observations (pathologist one and pathologist 2) for each unique slide (considered the subject for the model). This linear model was used to estimate the working correlation between paired responses and also to estimate the intra-class correlation. All analyses suggested the inter-pathologist reliability to be above 90%.

To determine the intra-pathologist reliability as a surrogate for staining reproducibility, a generalized linear intercept only model was fitted to the response data which contained a random subject effect assuming the subject effect had an exchangeable variance-covariance structure.

A model for each pathologist was fitted separately in which each subject was each specimen and thus there were three observations for each specimen (TN33, TN34, & TN35). As such, each specimen represented a cluster or class. The linear model was used to estimate the working correlation within cluster and also to estimate the intra-class correlation. An aggregate model was also fitted where each cluster was specimen within pathologists. The estimates are presented in Table 5. All analyses suggested a strong correlation further supporting the reproducibility of this assay

Table 4: Inter-pathologist Reliability

Metric	Value
Kendall's tau	0.9338
Stuart's tau	0.9095
Pearson correlation	0.9338
Spearman correlation	0.9338
Kappa	0.9338
Working Correlation	0.9363
Intra-Class Correlation	0.9341

Finally, as proof of principle, vimentin staining was performed on archived tissue samples from a subset of patients (n=4) with available tissue who carried a diagnosis of metastatic non-metaplastic TNBC and were treated with DAT. Eight additional patients had no archived tissue but were evaluated for response to therapy. Of the 12 unselected non-metaplastic TNBC, all but 1 patient exhibited PD as best response for a RR of 8%; however, of the 4 who had archived tissue available, only the tumor from the patient who developed a partial response had any evidence of vimentin staining.

Table 5: Intra-pathologist reliability

Model	Working Correlation	Intra-Class Correlation
Pathologist 1	0.9338	0.9348
Pathologist 2	0.9558	0.9565
Both	0.9448	0.9452

3. Objectives

3.1 Primary Objective:

Determine the rate of pCR/RCB-0 or RCB-I in patients with anthracycline-based chemotherapy insensitive, localized TNBC who receive 4 cycles of DAE following anthracycline-based chemotherapy in the neoadjuvant setting.

3.2 Secondary Objectives:

1. Determine response rate after 4 cycles of DAE using radiographic imaging.

2. Determine toxicity associated 4 cycles of DAE in the neoadjuvant setting.

3. Pathologic response rates to 4 cycles of DAE in mesenchymal tumors vs. non-mesenchymal tumors.

4. Compare pathologic response rates in mesenchymal tumors to 4 cycles of DAE vs. 12 weeks of weekly paclitaxel (using data collected from standard of care treatment).

3.3 Exploratory Objectives:

1. Determine the correlation between vimentin expression by IHC and the presence of mesenchymal gene signatures at the time of initial tumor biopsy prior to NACT (using gene expression data obtained from protocol 2014-0185); if enrolled to this protocol prior to DAE.

2. Determine the correlation between mutations in *PIK3CA*, *PTEN* or *NF2* or *PTEN* loss by IHC and the presence of mesenchymal gene signatures at the time of initial tumor biopsy prior to NACT (using gene expression data obtained from protocol 2014-0185); if enrolled to this protocol prior to DAE.

3. Determine rates of pCR in patients with mesenchymal tumors identified by gene signatures and compare to pCR rates in non-mesenchymal tumors.

4. Correlate pathologic response with degree of vimentin expression as measured by IHC.

5. Determine rates of pCR in patients whose tumors contain mutations in *PIK3CA*, *PTEN* or *NF2* or *PTEN* loss by IHC and compare to pCR rates in patients whose tumors lacks mutations in these genes.

4.0 Selection of Patients

Patients must have baseline evaluations performed prior to the first dose of study drug and must meet all inclusion and exclusion criteria. Results of all baseline evaluations, which assure that all inclusion and exclusion criteria have been satisfied, must be reviewed by the Principal Investigator or his/her designee prior to enrollment of that patient. In addition, the patient must be thoroughly informed about all aspects of the study, including the study visit schedule and required evaluations and all regulatory requirements for informed consent. The written informed consent must be obtained from the patient prior to enrollment. The following criteria apply to all patients enrolled onto the study unless otherwise specified**.**

4.1 Inclusion Criteria

1. Age ≥ 18 years.
2. ECOG performance status of 0 or 1.
3. Confirmed invasive triple-negative breast cancer defined as ER<10%; PR<10% by immunohistochemistry (IHC) and HER2 0-1+ (by IHC) or 2+ (FISH < 2, gene copy number < 4).
4. Primary tumor sample collected before NACT started and
5. Undergone molecular testing for integral biomarkers including immunohistochemical staining. The tumor must have evidence of mesenchymal differentiation defined as metaplastic breast cancer, or vimentin ≥50% by IHC.

6. Received at least one dose of an anthracycline-based NACT. Patients are eligible if therapy was discontinued due to disease progression or therapy intolerance.

7. At least 1.0 cm of measurable residual disease after neoadjuvant anthracycline-based chemotherapy.

8. Baseline MUGA or echocardiogram showing LVEF ≥ 50% within 6 weeks prior to initiation of NACT.

9. Adequate bone marrow function as shown by:

   ANC ≥1.5 x 10^9/L,
   Platelets ≥100 x 10^9/L,
   Hb >9 g/dL;

10. Adequate liver function as shown by:

    Total serum bilirubin ≤2.0 mg/dL,
    ALT and AST ≤2.5x ULN (≤5x ULN in patients with liver metastases),
    INR ≤2;

11. Adequate renal function as shown by:

    Serum creatinine ≤1.5x ULN;

12. Fasting serum cholesterol ≤300 mg/dL OR ≤7.75 mmol/L, AND fasting triglycerides ≤2.5x ULN.

    NOTE: In case one or both of these thresholds are exceeded, the patient can only be included after initiation of appropriate lipid lowering medication;

13. Signed informed consent obtained prior to any screening procedures.

4.2 Exclusion Criteria

1. Pregnant or lactating women.

2. Presence of metastatic disease.

3. Prior therapy with bevacizumab, liposomal doxorubicin, or everolimus.

4. Prior radiation therapy of the primary breast carcinoma or axillary lymph nodes.

5. Patients who have a history of another primary malignancy, with the exceptions of: non-melanoma skin cancer, and carcinoma in situ of the cervix, uterus, or breast from which the patient has been disease free for ≤3 years;

6. Prior cumulative dose of doxorubicin of greater than 360 mg/m^2 or epirubicin of greater than 640 mg/m^2.

7. Any serious medical illness, other than treated by this study, which would limit survival to less than 1 month or psychiatric illness which would limit informed consent.

8. Patients with history of serious cardiac events defined as:

   New York Heart Association class 3 or 4 heart failure,
   History of myocardial infarction,
   Unstable angina, or CVA within 6 months of protocol registration.
   History of PR prolongation or AV block.

9. Known intolerance or hypersensitivity to rapamycin analogs (e.g. sirolimus, temsirolimus).

10. Known impairment of gastrointestinal (GI) function or GI disease that may significantly alter the absorption of oral Everolimus;

11. Uncontrolled diabetes mellitus as defined by HbA1c >8% despite adequate therapy. Patients with a known history of impaired fasting glucose or diabetes mellitus (DM) may be included, however blood glucose and antidiabetic treatment must be monitored closely throughout the trial and adjusted as necessary;

12. Patients who have any severe and/or uncontrolled medical conditions such as:

    a. serious uncontrolled cardiac arrhythmia, or any other clinically significant cardiac disease
    b. active (acute or chronic) or uncontrolled severe infection, liver disease such as cirrhosis, decompensated liver disease, and active and chronic hepatitis (i.e. quantifiable HBV-DNA and/or positive HBsAg, quantifiable HCV-RNA),
    c. known severely impaired lung function (spirometry and DLCO 50% or less of normal and O_2 saturation 88% or less at rest on room air),
    d. active, bleeding diathesis;
    e. Moderate or severe hepatic impairment (Child-Pugh B or C)

13. Chronic treatment with corticosteroids or other immunosuppressive agents. Topical or inhaled corticosteroids are allowed;

14. Known history of HIV seropositivity;

15. Patients who have received live attenuated vaccines within 1 week of start of Everolimus and during the study. Patient should also avoid close contact with others who have received live attenuated vaccines. Examples of live attenuated vaccines include intranasal influenza, measles, mumps, rubella, oral polio, BCG, yellow fever, varicella and TY21a typhoid vaccines;

16. Patients with a history of non-compliance to medical regimens or who are considered potentially unreliable or will not be able to complete the entire study;

17. Patients who are currently part of or have participated in any clinical investigation with an investigational drug within 1 month prior to dosing;

18. Women of child-bearing potential (WOCBP), defined as all women physiologically capable of becoming pregnant, must use highly effective methods of contraception during the study and 8 weeks after. Highly effective contraception methods include combination of any two of the following:

    a. Placement of an intrauterine device (IUD) or intrauterine system (IUS);
    b. Barrier methods of contraception: condom or occlusive cap (diaphragm or cervical/vault caps) with spermicidal foam/gel/film/cream/ vaginal suppository;
    c. Total abstinence or;
    d. Male/female sterilization.

Note: Women are considered post-menopausal and not of child-bearing potential if they have had 12 months of natural (spontaneous) amenorrhea with an appropriate clinical profile (e.g. age appropriate, history of vasomotor symptoms) or have had surgical bilateral oophorectomy (with or without hysterectomy) or tubal ligation at least six weeks prior to treatment. In the case of oophorectomy alone, only when the reproductive status of the

woman has been confirmed by follow up hormone level assessment is she considered not of child-bearing potential.

19. Male patients whose sexual partner(s) are WOCBP who are not willing to use adequate contraception, during the study and for 8 weeks after the end of treatment.

4.3 Screening for hepatitis B and C

4.3.1 Hepatitis B

Prior to start of Everolimus, the following three categories of patients should be tested for hepatitis B viral load and serologic markers, that is, HBV-DNA, HBsAg, HBs Ab, and HBc Ab: All patients who currently live in (or have lived in) Asia, Africa, Central and South America, Eastern Europe, Spain, Portugal and Greece.

[http://wwwnc.cdc.gov/travel/yellowbook/2012/chapter-3-infectious-diseases-related-to-travel/hepatitis-b.htm]

Patients with any of the following risk factors:

- known or suspected past hepatitis B infection,
- blood transfusion(s) prior to 1990,
- current or prior IV drug users,
- current or prior dialysis,
- household contact with hepatitis B infected patient(s),
- current or prior high-risk sexual activity,
- body piercing or tattoos,
- mother known to have hepatitis B
- history suggestive of hepatitis B infection, e.g., dark urine, jaundice, right upper quadrant pain.
- Additional patients at the discretion of the investigator

The management guidelines, in Section 5.5: Subsection: Therapy Administration and Dose Reductions are provided according to the results of the baseline assessment of viral load and serological markers for hepatitis B.

4.3.2 Hepatitis C

Patients with any of the following risk factors for hepatitis C should be tested using quantitative RNA-PCR:

- known or suspected past hepatitis C infection (including patients with past interferon 'curative' treatment),
- blood transfusions prior to 1990,
- current or prior IV drug users,
- current or prior dialysis,
- household contact of hepatitis C infected patient(s),
- current or prior high-risk sexual activity,
- body-piercing or tattoos.

- At the discretion of the investigator, additional patients may also be tested for hepatitis C.

The management guidelines, in Section 5.5: Subsection: Therapy Administration and Dose Reductions are provided according to the results of the baseline assessment of hepatitis C viral load.

4.4 Registration Procedures

- Patients must not start protocol treatment prior to registration.
- The following information will be requested:
  - Protocol Number
  - Investigator Identification
    - Investigator's name
  - Patient Identification
    - Patient's initials
    - Patient demographics
      - Sex
      - Birth date (mm/yyyy)
      - Race
      - Ethnicity

Eligibility Verification

Patients must meet all of the eligibility requirements listed in section 3. An eligibility checklist will be appended to the protocol.

Additional Requirements

All patients must be provided with a signed and dated, written informed consent form.

4.4.1 Instructions for Patients who Do Not Start Assigned Protocol Treatment

If a patient does not receive any assigned protocol treatment, baseline and follow-up data will still be collected and must be submitted. Document the reason for not starting protocol treatment on one of the baseline forms. Also report the date and type of the first non-protocol treatment that the patient receives.

5 Treatment Plan

5.1 Treatment

Patients will initiate therapy with DAE at the doses and schedules listed in Table 6. Each cycle will be defined as 21 days of therapy (+/- 7days). Patients will undergo a planned 4 cycles of therapy prior to surgical resection. To reduce the rate of surgical complications, patients will not receive bevacizumab during cycle 4 of therapy. Patients who are unable to receive 4 cycles of therapy due to reasons other than toxicity requiring discontinuation of study drug per protocol, or progression will be documented, and will be replaced for the efficacy analysis. Patients who develop disease progression during therapy, or discontinuation of study drug per protocol, due to unmanageable toxicity will be included in the efficacy analysis as treatment failures or non-

pCR/RCB-I. Patients who are unable to receive 4 cycles of therapy due to other reasons will have toxicity and efficacy recorded, however will be replaced for the efficacy analysis.

Table 6: Administration description

Agent	Pre-medications	Dose (IV)	Route/Administration
Bevacizumab [Day 1] 10 mg/kg	Zofran 8 mg in 50 ml NS IVPB 30 min prior to infusion	*100cc NS	Initial infusion over 90 minutes. May be shortened to 60 minutes if initial infusion well tolerated. 3rd and subsequent infusions may be shortened to 30 minutes if 60 minute infusion well tolerated.
Liposomal doxorubicin [Day 1] 30mg/m^2	Solumedrol 20mg PO or IV; Diphenhydramine 50 mg PO 30 min prior to infusion	*250cc D_5W	1st (2) doses infused over 3 hours. Infusion duration may be shortened to 1 hour if 1st two infusions well tolerated.
Everolimus [Days 1-21] 7.5 mg	N/A	N/A	Oral - once daily.

* Diluent / volume; dose prescribed per assigned dose level.

All patients will undergo surgical resection of their primary tumor unless they are deemed not fit for surgery after 4 cycles of therapy. Patients may undergo either lymph node sampling or complete axillary dissection as considered standard of care by their surgeon. In order to decrease wound complications from bevacizumab, patients will be recommended to wait 6 weeks after the last dose of bevacizumab before undergoing surgical resection.

5.2 Schedule of Dosing:

5.2.0 All dosages prescribed and dispensed to the patient and all dose changes during the study must be recorded.

5.2.1 Medication labels will comply with US legal requirements and be printed in English. They will supply no information about the patient. The storage conditions for Everolimus will be described on the medication label.

5.2.2 Everolimus is supplied by Novartis. Everolimus is formulated as tablets for oral administration of 2.5mg, 5mg, and 10mg strength. Tablets are blister-packed under aluminum foil, which should be opened only at the time of administration as drug is both hygroscopic and light-sensitive. Refer to label for expiration date and storage conditions.

5.2.3 The extent of absorption of everolimus through topical exposure is not known. Therefore, caregivers are advised to avoid contact with suspensions of Afinitor Tablets. Wash hands thoroughly before and after preparation of either suspension.

5.2.4 The investigator should promote compliance by instructing the patient to take the study drug exactly as prescribed and by stating that compliance is necessary for the patient's safety and the validity of the study. The patient should be instructed to contact the investigator if he/she is unable for any reason to take the study drug as prescribed.

5.2.5 Everolimus should be administered orally once daily at the same time every day, either consistently with or consistently without food.

5.2.6 The tablets should be swallowed whole with a glass of water and should not be chewed or crushed. For patients unable to swallow tablets, the tablet(s) should be dispersed completely in a glass of water (containing approximately 30 mL) by gently stirring until the tablet(s) is fully disintegrated (approximately 7 minutes), immediately prior to drinking. The glass should be rinsed with the same volume of water and the rinse completely swallowed to ensure the entire dose is administered.

5.2.7 If vomiting occurs, no attempt should be made to replace the vomited dose. Patients should be instructed that if they miss a dose on one day, they must not take any extra dose the next day, but instead to immediately contact the study center as soon as possible to ask for advice.

5.3 Everolimus Dosing in Special Populations

- Geriatrics (≥ 65 years): No dosage adjustment is required.
- Renal impairment: No dosage adjustment is required.
- Ethnicity: Pharmacokinetic characteristics are similar for Caucasian and Japanese subjects.

Pharmacokinetic studies in Black transplant patients have shown an average 20% higher clearance; however doses above 7.5 mg daily will not be administered as individual PK levels will not be evaluated on this trial.

5.4 Drug Information including Adverse Drug Reactions (ADRs)

5.4.1 Liposomal doxorubicin (Doxil®)

Liposomal doxorubicin is pegylated liposomal doxorubicin hydrochloride (HCL). Doxorubicin is an anthracycline topoisomerase inhibitor that acts during the S1 phase of the cell cycle by intercalating irreversibly between DNA base pairs to form a complex that inhibits the progression of the enzyme topoisomerase II. Topoisomerase II functions to unwind the DNA double helix in preparation for transcription. Doxorubicin stabilizes the topoisomerase II complex after it has broken the DNA chain for replication, preventing the DNA double helix from being resealed and thereby stopping the process of replication. This leads to inhibition of mitotic activity and nucleic acid synthesis, induction of mutagenesis and tumor cell death[56]. Liposomal doxorubicin is doxorubicin HCL encapsulated in STEALTH® liposomes. Liposomes are microscopic vesicles composed of a phospholipid bilayer that are capable of encapsulating active drugs. The STEALTH® liposomes of liposomal doxorubicin are formulated with surface-bound methoxypolyethylene glycol (MPEG), a process referred to as pegylation, to protect liposomes from detection by the mononuclear phagocyte system (MPS) and to increase blood circulation time[57]. Liposomal doxorubicin has a half-life of approximately 55 hours in humans. They are stable in blood, and at least 90% of the drug remains liposome encapsulated during circulation. It is believed that because of their small size (~100nm) and persistence in the circulation, the pegylated liposomal doxorubicin liposomes are able to penetrate the altered and often compromised vasculature of tumors. After distribution to the tissue compartment, the encapsulated doxorubicin HCL becomes available[57]. Metabolism of liposomal doxorubicin is extensively metabolized in the liver and eliminated primarily as glucuronide or hydroxylated conjugates[56].

The most common adverse reactions observed with liposomal doxorubicin are asthenia, fatigue, fever, nausea, stomatitis, vomiting, diarrhea, constipation, anorexia, hand-foot syndrome, rash and neutropenia, thrombocytopenia, and anemia[57]. The most clinically important adverse effects are discussed below:

Myelosuppression

Myelosuppression is the dose-limiting toxicity of liposomal doxorubicin. Signs of myelosuppression include pancytopenia, leukopenia, neutropenia, anemia, and thrombocytopenia. In ovarian cancer patients, leukopenia occurred in 42% of patients, neutropenia (ANC < 1000 cells/mm^3) in 19—35%, anemia in 40.2%, and thrombocytopenia in 24%. Severe neutropenia (ANC < 500 cells/mm^3) occurred in 8% and severe thrombocytopenia (< 25,000 cells/mm^3) occurred in 1%. Severe infection (1—5%) and sepsis have been seen in patients with doxorubicin-induced myelosuppression[56].

Cardiac Toxicity

Special attention must be given to the risk of myocardial damage from cumulative doses of doxorubicin HCL. In particular, left ventricular failure may occur in patients who receive a cumulative dosage of doxorubicin exceeding the current recommended limit of 550mg/m2. Lower doses can cause heart failure in patients who have received radiotherapy to the mediastinal area

or concomitant therapy with other potentially cardiotoxic agents such as cyclophosphamide. Prior use of other anthracyclines or anthracenediones should be included in calculations of total cumulative dosage. Cardiac function in patients suspected to have myocardial injury related to liposomal doxorubicin should be considered for multi-gated radionuclide scans or echocardiography[56].

Infusion reactions

An infusion-associated reaction has been identified with liposomal doxorubicin. This reaction is due to the liposomes themselves or one of the surface components Patients who experience this reaction generally do so with the first liposomal doxorubicin dose and not with subsequent dosing. Liposomal doxorubicin should be administered at an initial rate of 1 mg/min to decrease the incidence of this reaction; if no symptoms occur, the infusion rate may be increased to complete the infusion within 1 hour. Symptoms include fever (9.1—21.3%), flushing, dyspnea, facial swelling, headache (5%), chills 1—5%), back pain (1—5%), tightness in the chest and throat, rash (unspecified) (22%), pruritus (1—5%), chest pain (unspecified) (1—5%), hypotension, syncope, sinus tachycardia, apnea, bronchospasm/wheezing, dyspnea(1—5%), cyanosis, and/or asthma. Serious and sometimes life-threatening or fatal anaphylactic shock/anaphylactoid reactions have been reported (1—5%)[56].

Palmar-plantar erythrodysesthesia (PPE) / Hand-Foot syndrome (HFS)

Hand and foot syndrome developed in 50.6% of patients treated for ovarian cancer dosed at 50 mg/m^2; 23.8% of these patients developed a severe reaction (i.e., Grade 3—4). The increased incidence in ovarian cancer patients is due to the higher doses used; liposomal doxorubicin® given at doses of > 20 mg/m^2 or at shorter intervals than recommended are associated with an increased incidence. Among 318 patients with multiple myeloma who received liposomal doxorubicin at 30 mg/m^2 every 3 weeks plus bortezomib, 19% had PPE; in contrast, < 1% of 318 patients who received bortezomib monotherapy had the event. This syndrome usually occurs after the second or third cycle and is associated with the cumulative liposomal doxorubicin dose[56].

Nausea/Vomiting/Diarrhea

Nausea/vomiting occurs in 18-46% and 8-33% of patients treated with liposomal doxorubicin, respectively, and increases with increasing dose. Liposomal doxorubicin is not as emetogenic as conventional doxorubicin but premedication with antiemetics is still necessary. Stomatitis and mucositis occur more frequently with liposomal doxorubicin due to the longer half-life than conventional doxorubicin. In patients treated with higher doses, the incidence of stomatitis is increased (37%) and 7% of patients develop severe reactions. Diarrhea has been reported in 20.9% and constipation in 30.1% of patients treated with liposomal doxorubicin[56].

5.4.2 Bevacizumab (Avastin®)

Bevacizumab is a recombinant humanized monoclonal antibody, specifically an IgG1 antibody that contains human framework regions and murine complementarity-determining regions. Bevacizumab binds to human vascular endothelial growth factor (VEGF) and prevents interaction of VEGF with its receptors (Flt-1, KDR) on the surface of endothelial cells. In vitro models of angiogenesis have shown that interaction of VEGF with its receptors may lead to endothelial cell proliferation and new blood vessel formation. Evidence from animal models has suggested that administration of an anti-VEGF monoclonal antibody (e.g., bevacizumab) may inhibit angiogenesis and thus may reduce microvascular growth of tumors and inhibit metastatic disease progression. Bevacizumab is metabolized and eliminated via the reticuloendothelial system[58]. The estimated half-life of the drug is approximately 20 days. Clearance of the drug is dependent upon gender and tumor burden, with males and patients with a higher tumor burden clearing

bevacizumab faster. In clinical trials, there has been no evidence of lesser efficacy in males or patients with a higher tumor burden as compared to females or patients with a lower tumor burden[59]. Adverse reaction data for bevacizumab are reported from clinical trial results. Because clinical trials are conducted under widely varying conditions, adverse reaction rates observed in the clinical trial of one drug cannot be directly compared to rates in the clinical trials of other drugs and may not reflect the rates observed in clinical practice[59].

Thromboembolism

Bevacizumab therapy is associated with an increased risk of arterial and venous thromboembolism in metastatic colon cancer and non-small cell lung cancer (NSCLC) patients. Additionally, in metastatic colon cancer patients, there is an increased risk of developing a second subsequent thromboembolic event in patients receiving bevacizumab plus chemotherapy versus chemotherapy alone. Among 53 patients who got bevacizumab and chemotherapy for metastatic colon cancer and received warfarin for a venous thromboembolic event, 11 had an additional thromboembolic event. In contrast, 1 of the 30 patients who got chemotherapy alone and received warfarin for a venous thromboembolic event had an additional thromboembolic event. A pooled analysis of randomized controlled clinical trials demonstrated an arterial thromboembolism rate of 4.4% in 963 patients treated with bevacizumab in combination with chemotherapy. Of these events, fatal outcomes occurred in 7 of 963 (0.7%) patients. Arterial thrombotic events have included cerebral infarction (stroke), transient ischemic attack (TIA), myocardial infarction, angina, and a variety of other arterial thromboembolic events. The incidences of both cerebrovascular arterial events and cardiovascular arterial events were increased in patients receiving bevacizumab (1.9% and 2.1%, respectively) as compared with chemotherapy alone (0.5% and 1%, respectively). In addition, there was a correlation between age (>= 65 years vs. < 65 years) and the increase in risk of thromboembolic events (8.5% vs. 2.1%, respectively). In the clinical trial of patients with NSCLC, the incidence of venous thrombosis/embolism was 5% among patients who got bevacizumab and chemotherapy and 3% among chemotherapy alone recipients. An increased risk for a venous thromboembolic event was not found by a post-hoc analysis of data from 5 trials. Other thrombotic events reported during bevacizumab therapy have included axillary/subclavian vein occlusion, superior mesenteric thrombosis, phlebitis (thrombophlebitis), catheter thrombosis, and venous sclerosis. Bevacizumab should be permanently discontinued in patients who experience a severe arterial thromboembolic event[59].

Gastrointestinal (GI) Perforations and Wound Healing Complications

GI perforation complicated by intra-abdominal abscesses or fistula formation occurred more frequently in patients receiving bevacizumab as compared to controls during clinical trials (range 4-11%). In colorectal clinical trials, including single-agent bevacizumab and bevacizumab in combination with other chemotherapy agents, the incidence of GI perforation, fistula formation, and/or intra-abdominal abscess was 2.4%; in non-small cell cancer clinical trials the incidence was 0.9%. GI perforation, fistula (gastrointestinal fistula, enterocutaneous fistula, esophageal fistula, duodenal fistula, rectal fistula), and/or intra-abdominal abscess have also been reported during post-market reports and trials for colorectal cancer and other types of cancer. Of the reported events, 30% were fatal. GI perforation has occurred at various time points during bevacizumab therapy; most events occur within the first 50 days. Of 29 patients with limited-stage small cell lung cancer (SCLC) who were enrolled in a clinical trial, 2 developed a confirmed tracheoesophageal fistula. Information on the background rate of tracheoesophageal fistula is limited, and the presence of other risk factors for tracheoesophageal fistula in the patients is unknown. However, the estimated background rate of tracheoesophageal fistula in patients with limited-stage SCLC is estimated to be < 1%[59]. Two distinct patterns of bleeding have occurred in patients receiving bevacizumab. Minor or moderate bleeding occurs more frequently in patients treated with bevacizumab vs. those treated with chemotherapy alone. These events were generally mild and resolved without medical intervention. NCI-CTC Grade 3—5 hemorrhagic events occurred in 4.7% of non-small cell lung cancer (NSCLC) patients and 5.2% of metastatic colon cancer patients receiving bevacizumab. In a randomized study in patients with NSCLC receiving chemotherapy with or without bevacizumab, 31% (4 of 13) bevacizumab-treated

patients with squamous cell histology and 4% (2 of 53) bevacizumab-treated patients with non-squamous cell histology experienced life-threatening or fatal pulmonary hemorrhage as compared to none of the 32 patients receiving chemotherapy alone. In another study the incidence of pulmonary hemorrhage requiring medical intervention was 2.4% (10 of 427) in patients receiving bevacizumab in combination with carboplatin and paclitaxel versus 0.5% (2 of 441) in patients receiving chemotherapy alone. Seven deaths due to pulmonary hemorrhage have been reported. Of the patients experiencing events of life-threatening pulmonary hemorrhage, many had cavitation and/or necrosis of the tumor, either pre-existing or developing during bevacizumab therapy. The risk of CNS bleeding in patients with CNS metastases receiving bevacizumab has not been evaluated; patients with CNS metastases were excluded from bevacizumab studies after one such patient experienced CNS hemorrhage after receiving bevacizumab in a phase I trial[59]. A study of neoadjuvant bevacizumab in breast cancer patients did show in increase in early and delayed post-operative complications.[60] Serious adverse events in patients treated with bevacizumab included bleeding, thrombotic events and GI perforation.

Hypertension

The incidence of severe hypertension (NCI CTC Grade 3—4) across all bevacizumab clinical trials ranges from 8—18%. In patients with non-small cell lung cancer treated with bevacizumab, the incidence of hypertension was 8% vs. 0.7% in patients receiving chemotherapy alone. Development or worsening of hypertension can require hospitalization or require discontinuation of bevacizumab in 1.7% of patients. Severe hypertension complicated by subarachnoid hemorrhage or hypertensive encephalopathy has been reported and, in some cases, has been fatal. In post-market reports, acute increases in blood pressure have been associated with initial or subsequent infusions of bevacizumab.[59]

Proteinuria

Proteinuria (defined as urine dipstick reading >= 1+) occurs more frequently and is more severe in patients receiving bevacizumab as compared to those receiving chemotherapy alone; the incidence of severe proteinuria (> 3.5 g/24 hours) ranged up to 3% in bevacizumab-treated patients across clinical trials. In patients with non-small cell lung cancer treated with bevacizumab plus carboplatin/paclitaxel, the incidence of proteinuria was 3%. The safety of continued bevacizumab therapy or temporary suspension of the drug in patients with moderate to severe proteinuria has not been evaluated; such patients should be monitored regularly until improvement and/or resolution is observed. In most clinical studies, bevacizumab was interrupted for proteinuria exceeding 2 g per 24 hours and resumed when proteinuria declined below this level[59].

Congestive Heart Failure

Congestive heart failure (CHF), defined as NCI-CTC Grade 2-4 left ventricular dysfunction, was reported in 25 of 1459 (1.7%) patients receiving bevacizumab in manufacturer-sponsored trials. In a controlled trial in patients with breast cancer (non-FDA approved use), CHF occurred in 4% (13 of 299) of patients who received prior anthracyclines and/or left chest wall radiation therapy. The safety of continuation or resumption of bevacizumab therapy in patients with cardiac dysfunction has not been studied[59].

Infusion Reactions

In clinical studies, infusion reactions with the first bevacizumab dose were uncommon (less than 3%). Severe infusion reactions occurred in 0.2% of patients receiving bevacizumab. Infusion reactions include hypertension, hypertensive crises associated with neurologic manifestations, wheezing, oxygen desaturation, grade 3 hypersensitivity, chest pain, headaches, rigors, and diaphoresis[59].

5.4.3 Everolimus (Afinitor®)

The observed antitumor and immunosuppressive properties of rapamycin analogs are due to their ability to disrupt the mTOR-dependent signaling pathway. mTOR, a member of the phosphatidylinositide 3'-kinase (PI3K)-related family, is located predominantly in the nuclear fraction of both neoplastic and normal cells. mTOR activation triggers resting cells to increase the translation of a subset of mRNAs whose proteins are required for cell cycle progression from G_1 to S phase. mTOR regulates essential signal transduction pathways and is involved in the coupling of growth stimuli with cell cycle progression. Experimental data indicate that mTOR acts downstream of the PI3K/Akt-pathway and is phosphorylated in response to mitogenic signals. Early studies reported that mTOR was dedicated to initiating mRNA translation in response to favorable nutrient environments. In fact, cells treated with rapamycin undergo changes that are strikingly similar to those observed during conditions of starvation. These include mTOR inactivation, down regulation of translation, G_1 arrest, accumulation of glycogen stores and altered transcription patterns. More recent studies have demonstrated that mTOR is involved in regulating many aspects of cell growth, including organization of the actin cytoskeleton, membrane traffic, protein degradation, protein kinase C (PKC) signaling, ribosome biogenesis, and transcription[61, 62].

Everolimus reacts with the ubiquitous intracellular FK506-binding protein 12 (FKBP12), forming a everolimus/FKBP12 complex that is a potent inhibitor of the highly conserved kinase mTOR. Inhibition of mTOR leads to suppression of several downstream signaling effectors, including the ribosomal subunit $p70^{S6k}$ and the eukaryotic initiation factor 4 binding protein 1 (4E-BP1). These two proteins play key roles in ribosomal biogenesis and cap-dependent translation, respectively. The extent of phosphorylation of these two downstream proteins ($p70^{S6}$ kinase and 4E-BP1) may therefore serve as indicators of everolimus biologic activity in vivo[63]. Inhibition of the synthesis of ribosomal proteins and elongation factors, required to accelerate the process of cell division, are thought to contribute to the anti-proliferative effects of rapamycin analogs. While everolimus inhibits the translation of only a subset of mRNAs, inhibition of mTOR can lead to a substantial decrease (~15%) in overall protein synthesis[63]. The most common ADRs (incidence ≥1/10 and suspected to be related to treatment by the investigator) from the pooled safety data were (in decreasing order): stomatitis, rash, fatigue, diarrhea, infections, nausea, decreased appetite, anemia, dysgeusia, pneumonitis, hyperglycemia, weight decreased, pruritus, asthenia peripheral edema, hypercholesterolemia, epistaxis, and headache.[64].

The most common grade 3/4 ADRs (incidence ≥1/100 to <1/10 and suspected to be related to treatment by the investigator) were stomatitis, anemia, hyperglycemia, fatigue, infections, pneumonitis, diarrhea, asthenia, thrombocytopenia, neutropenia, dyspnea, lymphopenia, proteinuria, hemorrhage, hypophosphatemia, rash, hypertension, aspartate aminotransferase (AST) increased, alanine aminotransferase (ALT) increased, and pneumonia.

The below table presents the frequency category of ADRs reported in the pooled safety analysis.

ADRs are listed according to MedDRA system organ class. Within each system organ class, the adverse reactions are ranked by frequency, with the most frequent reactions first. In addition, the corresponding frequency category using the following convention (CIOMS III) is also provided for each adverse reaction: very common (≥1/10); common (≥1/100 to <1/10); uncommon (≥1/1,000 to <1/100); rare (≥1/10,000 to <1/1,000); very rare (<1/10,000)

Infections and infestations
Very common - Infections

Blood and lymphatic system disorders
Very common - Anemia,
Common - Thrombocytopenia, neutropenia, leukopenia, lymphopenia
Uncommon - Pancytopenia
Rare - Pure red cell aplasia

Immune system disorders
Uncommon - Hypersensitivity

Metabolism and nutrition disorders
Very common - Decreased appetite, hyperglycemia, hypercholesterolemia
Common - Hypertriglyceridemia, hypophosphatemia, diabetes mellitus, Hyperlipidemia, hypokalemia, dehydration

Psychiatric disorders
Common - Insomnia

Nervous system disorders
Very common - Dysgeusia, headache
Uncommon - Ageusia

Cardiac disorders
Uncommon - Congestive cardiac failure

Vascular disorders
Common - Hemorrhage, hypertension,
Uncommon - Deep vein thrombosis

Respiratory, thoracic and mediastinal disorders
Very common - Pneumonitis, epistaxis,
Common - cough, dyspnea
Uncommon - Hemoptysis, pulmonary embolism
Rare - , acute respiratory distress syndrome

Gastrointestinal disorders
Very common - Stomatitis, diarrhea, nausea
Common - Vomiting, dry mouth, abdominal pain, oral pain, dyspepsia, and dysphagia

Skin and subcutaneous tissue disorders
Very common - Rash, pruritus
Common - Dry skin, nail disorder, acne, erythema, hand-foot syndrome
Rare - Angioedema

Musculoskeletal and connective tissue disorders
Common - Arthralgia

Renal and urinary disorders
Common - Proteinuria, Renal failure
Uncommon - Increased daytime urination, acute renal failure

Reproductive system and breast disorders
Common - Menstruation irregular
Uncommon - amenorrhea

General disorders and administration site conditions
Very common - Fatigue, asthenia, peripheral edema,
Common - Pyrexia, mucosal inflammation
Uncommon - Non-cardiac chest pain,
Rare - impaired wound healing

Investigations
Very common - Weight decreased

Common - aspartate aminotransferase increased, Alanine aminotransferase increased, blood creatinine increased.
a Includes all reactions within the 'infections and infestations' system organ class including common: pneumonia and uncommon: herpes zoster, sepsis and isolated cases of opportunistic infections (e.g. aspergillosis, candidiasis and hepatitis B)
b Includes different bleeding events not listed individually
c Includes common: pneumonitis: interstitial lung disease, lung infiltration, and rare - alveolitis, pulmonary alveolar hemorrhage, and pulmonary toxicity
d Includes very common: stomatitis; common: aphthous stomatitis, mouth and tongue ulceration; uncommon: glossitis, glossodynia
e reported as palmar-plantar erythrodysesthesia syndrome
f frequency is based upon number of women age 10 to 55 years of age in the safety pool

Clinically relevant laboratory abnormalities

In the pooled double-blind phase III safety database, the following new or worsening clinically relevant laboratory abnormalities were reported with an incidence of ≥1/10 (very common, listed in decreasing frequency).

- Hematology: hemoglobin decreased, lymphocytes decreased, white blood cells decreased, platelets decreased, and neutrophils decreased (or collectively as pancytopenia);
- Clinical chemistry: glucose (fasting) increased, cholesterol increased, triglycerides increased, AST increased, phosphate decreased, ALT increased, creatinine increased, and potassium decreased.

Most of the observed abnormalities (≥ 1/100) were mild (grade 1) or moderate (grade 2). Grade 3/4 hematology and chemistry abnormalities include:

- Hematology: lymphocytes decreased, hemoglobin decreased, (very common); neutrophils decreased, platelet count decreased, white blood cells decreased (all common).
- Clinical chemistry: glucose (fasting) increased, phosphate decreased, potassium decreased, AST increased, ALT increased, creatinine increased cholesterol (total) increased, triglycerides increased (all common).

Description of selected adverse drug reactions

In clinical trials and post-marketing spontaneous reports, everolimus has been associated with serious cases of the following:

- Hepatitis B reactivation, including fatal outcome. Reactivation of infections is an expected event during periods of immunosuppression.
- Renal failure events (including fatal ones) and proteinuria. Monitoring of renal function is recommended.
- Amenorrhea (including secondary amenorrhea).
- Pneumocystis jiroveci pneumonia (PJP) some with a fatal outcome.
- Angioedema has been reported with and without concomitant use of everolimus and ACE inhibitors

The table below lists all fatal adverse events reported by investigators across all RAD001 studies as having a suspected causal relationship to everolimus and occurring on or before the cut-off date of 31-Mar-2014.

MedDRA system organ class	Event
Blood and lymphatic system disorders	Thrombocytopenia
Cardiac disorders	Acute cardiopulmonary event, cardiogenic shock, cardio-respiratory arrest, myocardial infarction
Gastrointestinal disorders	Diarrhea, duodenal ulcer, GI hemorrhage, intestinal perforation, esophageal perforation, internal hernia
General disorders/administration site conditions	Cold sweat, concomitant disease progression, general physical health deterioration, multi-organ failure, sudden death
Infections and infestations	Candidal sepsis, EBV pneumonia, neutropenic sepsis, pneumocystis jiroveci pneumonia, reactivation of Hepatitis B, septic shock, septicemia with massive Epstein-Barr viremia
Injury, poisoning and procedural complications	Bone fissure
Metabolism and nutrition disorders	Dehydration, ketoacidosis, **hyperglycemia**
Neoplasms benign, malignant and unspecified (including cysts and polyps)	Squamous cell carcinoma of skin
Nervous system disorders	Cerebral hemorrhage, embolic stroke, restlessness
Renal and urinary disorders	Renal tubular necrosis
Respiratory thoracic and mediastinal disorders	Acute cardiopulmonary event, acute respiratory distress syndrome, cardio-respiratory arrest, dyspnea, chronic obstructive pulmonary disease, interstitial pneumonia, pulmonary embolism, pulmonary edema, pneumonitis
Vascular Disorders	Cerebral hemorrhage, disseminated intravascular coagulation, embolic stroke, hemorrhage, hypovolemic shock, myocardial infarction, pulmonary hypertension

The table below lists all serious, unexpected, life-threatening adverse events considered related to study drug, and reported on at least one occasion up to the cut-off date of 31-Mar-2014. It should be stressed that many of these SAE reports have been reported only on a single occasion making an accurate assessment of causality difficult, if not impossible. Due to the imprecision of causality, the causality assessments should not be assumed that all of these events are indeed the result of therapy with everolimus. Moreover, the assessment of causality is particularly difficult in critically ill patients where confounding factors are present relating mainly to complications of the underlying disease and to the use of prior therapy and/or concomitant medications. In addition to the SAE reports presented below, disease progression has on occasions been reported somewhat paradoxically as an SAE with a suspected causal relationship to everolimus and reported on at least one occasion.

Individual events are presented for each MedDRA system organ class.

MedDRA system organ class	Event
Blood and lymphatic system disorders	Acute leukemia, anemia, bone marrow failure, disseminated intravascular coagulation, febrile neutropenia, hemorrhagic diathesis, leukemoid reaction,

	leukocytosis, leukopenia, lymphadenopathy, lymphedema, lymphopenia, neutropenia, pancytopenia, platelet disorder, retroperitoneal lymphadenopathy, thrombocytopenia, white blood cell disorder, hemolytic uremic syndrome
Cardiac disorders	atrial fibrillation, atrioventricular block, **cardiorespiratory arrest**, cardiopulmonary arrest, congestive cardiomyopathy, cyanosis, diastolic dysfunction, dilatation left atrial and left ventricular, hypertrophy, left ventricular hypertrophy, **left ventricular dysfunction**, myocardial infarction, myocardial ischemia, hypertensive heart disease, palpitations, pericardial effusion, right ventricular failure, sinus tachycardia, stress cardiopathy, supraventricular tachycardia, tachycardia, ventricular dysfunction Valve incompetence: mitral/pulmonary/tricuspid
Ear and labyrinth disorders	Vertigo, **sudden hearing loss, deafness unilateral**
Endocrine disorders	Hyperthyroidism , hyperglycemic non-ketotic syndrome, hypercalcemia, hypothyroidism
Eye disorders	Conjunctivitis, eyelid edema, scleral discoloration, ocular discomfort, ocular surface disease, ophthalmoplegia cranial nerve paralysis, papilloedema, photophobia, retinal artery thrombosis, retinal detachment, retinal hemorrhage, steroid Induced cataract, **senile cataract, visual acuity reduced**.
Gastrointestinal disorders	Abdominal abscess, abdominal adhesions, abdominal: discomfort/distension/pain/tenderness, anorectal discomfort, anal fissure, anal fistula, anastomotic leak, ascites, colitis, constipation, diarrhea, diverticulum, duodenal ulcer, dyspepsia, dysphagia, esophageal perforation, feces discolored, gastric perforation, gastric ulcer hemorrhage, gastric ulceration, gastrointestinal angiodysplasia, gastrointestinal ischemia, gastrointestinal oedema, **gastroenteritis**, hemorrhoids, hematemesis, hematochezia, hiccups, ileus, intestinal perforation, melena, mesenteric vein thrombosis, mouth ulceration, nausea, pancreatitis, proctalgia, proctitis, recall phenomenon radiation enteritis, rectal discharge/hemorrhage, small intestinal obstruction, stomatitis, swollen tongue, vomiting, oral pain, enterovesical fistula, radiation esophagitis, necrotizing esophagitis, salivary gland calculus, small bowel thickness, thickened bowel Wall Gastritis: erosive/ hemorrhagic Lip: edema/swelling/ulceration Gastrointestinal: disorder/hemorrhage/sounds Mucosal: hemorrhage/inflammation
General disorders and administration site conditions	Application site erythema, asthenia, bloody discharge, calcinosis, chest discomfort/pain, chills, concomitant disease progression, condition aggravated, crepitations, death, discomfort, disease progression, drug ineffective, and drug withdrawal syndrome (asthenia and flushing) edema peripheral, face edema, facial pain, fatigue, feeling of body temperature change, general physical health deterioration, generalized edema, **goiter**, granuloma, hypertrophy, hypothermia, impaired healing, inflammation, influenza like illness, irritability, local swelling, malaise, mass, multi-organ failure, necrosis, non-cardiac chest pain, pain, performance status decreased, pitting edema, pyrexia, sudden death, swelling, tenderness, thirst
Hepatobiliary disorders	Cholecystitis, cholelithiasis, cholestasis, cytolytic hepatitis, hepatic cirrhosis**, hepatic failure**, hepatic function abnormal, hepatic necrosis, **hepatorenal failure**, hepatitis B and C reactivation, hepatomegaly, hyperbilirubinemia, jaundice, portal vein thrombosis
Immune system disorders	Acute disseminated encephalomyelitis, autoimmune disorder, cytokine release syndrome, Guillian Barre syndrome, hypersensitivity, immunosuppression, Stevens- Johnson syndrome
Infections and infestations	Bronchitis, bronchopneumonia, bronchopulmonary aspergillosis. candida sepsis, cellulitis, citrobacter sepsis, clostridium bacteremia, endocarditis, escherichia bacteremia, folliculitis, gastroenteritis, hepatitis fulminant. infected lymphocele, klebsiella sepsis, liver abscess, meningitis, herpes, nasopharyngitis, neutropenic sepsis, perirectal/anal abscess, pharyngitis, pneumococcal sepsis, pseudomonal bacteremia, purulent discharge, pyelonephritis, respiratory moniliasis, sepsis syndrome, septic shock, sinusitis, staphylococcal bacteremia, staphylococcal sepsis, streptococcal bacteremia, tonsillitis, tuberculosis, urosepsis

	Infection(s): alpha hemolytic streptococcal/atypical mycobacteriallbacteriallclostridiallenterobacter/enterococcal/epstein-barr virus/ escherichia/escherichia urinary tract/gastrointestinal/haemophilus/herpesvirus/ general/ localized/ morganella/ proteus/ skin/streptococcal/staphylococcal/urinary tract/ wound staphylococcal
Injury, poisoning and Procedural complications	Abdominal adhesion, anastomotic leak, bone fissure, collapse of lung, contusion, eschar, fall, head injury, lung injury, medical device complication, medication error, post procedural swelling, recall phenomenon radiation enteritis, surgical procedure repeated, vena cava injury, wound dehiscence/secretion
Investigations	Aspiration pleural cavity, antinuclear antibody positive, bleeding time prolonged, breath sounds abnormal, cardiac function test abnormal, chest X-ray abnormal, clostridium difficile toxin test positive, computerized tomogram abnormal, ejection fraction decreased, endoscopy upper gastrointestinal tract, general physical condition abnormal, granulocyte count decreased, hematocrit/hemoglobin decreased, hypophosphatemia, liver function test abnormal, neutrophil count abnormal/decreased, occult blood positive, oxygen saturation abnormal/decreased, peak expiratory flow rate decreased, protein urine present, QRS axis abnormal, urine output decreased, X-ray abnormal; Increased: alanine aminotransferase, ammonia, aspartate aminotransferase, aspartate aminotransferase, body temperature, C-reactive protein, eosinophil count, gamma-glutamyltransferase, international normalized ratio, lipase, red blood cell count, sedimentation rate, transaminases, troponin T, troponin Platelet count: abnormal/decreased/increased White blood cell(s): count abnormal/decreased/in urine Lymphocyte count: decreased/increased Blood: amylase increased/albumin decreased/alkaline phosphatase increased/bilirubin increased/creatine phosphokinase increased/creatinine increased/magnesium decreased/glucose increased/lactate dehydrogenase increased/potassium decreased/pressure diastolic decreased/pressure orthostatic abnormal/pressure systolic decreased/triglycerides increased/urea increased/urine present Electrocardiogram: T wave abnormal/T wave amplitude decreased/poor R-wave progression Weight: decreased/ increased
Metabolism and nutrition disorders	Acidosis, anorexia, appetite disorder, cachexia**, Cushing's syndrome**, decreased appetite, dehydration, diabetes mellitus, diabetic ketoacidosis, electrolyte imbalance, failure to thrive, fluid overload/retention, food intolerance, glucose tolerance impaired, gout, hypothyroidism, malnutrition, metabolic acidosis, oral intake reduced, polydipsia, type 2 diabetes mellitus Hyper: cholesterolemia/glycemia/kalemia/lipidemia/calcemia uricemia/ triglyceridemia, glycemic non-ketotic syndrome Hypo: calcemia/glycemia/kalemia/magnesemia/natremia/ proteinemia/ hypophosphatemia
Musculoskeletal and connective tissue disorders	Arthritis, bone fissure, fistula, gout mobility decreased, joint effusion, rhabdomyolysis Pain: back/flank/musculoskeletal/musculoskeletal chest/extremity/neck Muscle: spasms/weakness; myalgia, arthralgia Osteoarthritis; Osteonecrosis, Osteoradionecrosis
Neoplasms benign, malignant and unspecified (incl. cysts and polyps)	Acute leukemia, adenocarcinoma of pancreas, Burkitt's lymphoma, carcinoid syndrome, colon neoplasm, lung neoplasm, lymphoproliferative disorder, malignant neoplasm progression, **malignant melanoma**, malignant pleural effusion, **pancreatic carcinoma**, squamous cell carcinoma of skin, uterine leiomyoma Metastases: central nervous system/lung/lymph nodes/neoplasm; Tumor: hemorrhage/necrosis
Nervous system disorders	Acute disseminated encephalomyelitis, asterixis, ataxia, cerebrovascular accident, cognitive disorder, complex partial seizures, complex regional pain syndrome, convulsion, depressed level of consciousness, dizziness, dyslalia, encephalitis, encephalopathy, facial palsy, headache, hemiparesis, hemiplegia, hyperglycemic non-ketotic syndrome, hypersomnia, hypoglycemic coma, lethargy, loss of consciousness, ophthalmoplegia_cranial nerve paralysis, neuralgia, neuropathy

	peripheral, presyncope, sinus headache, somnolence, speech disorder, tremor, vertigo Cerebral: hemorrhage/ infarction/ischemia; transient ischemic attack
Psychiatric disorders	Agitated depression, anxiety, confusional state, delirium, delusional disorder, disorientation, drug withdrawal syndrome, libido decreased insomnia, mental disorder/status changes, mood altered, persecutory type, **personality disorder**, neurosis, staring
Renal and urinary disorders	Acute renal failure, anuria, bladder tamponade, dysuria, hematuria, hemolytic uremic syndrome, hydronephrosis, leukocyturia, nephrolithiasis, oliguria, pollakiuria, polyuria, postrenal failure, renal failure/acute/impairment, urinary bladder hemorrhage, urinary incontinence, renal atrophy, renal colic, renal tubular necrosis
Reproductive/breast disorders	Azoospermia, **endometrial hyperplasia**, libido decreased, **menorrhagia**, pelvic pain, ovarian cyst, scrotal edema
Respiratory, thoracic and mediastinal disorders	Alveolitis, alveolitis allergic, asthma, atelectasis, bronchomalacia, bronchospasm, cough, cryptogenic organizing pneumonia, diffuse alveolar damage, dysphonia, dyspnea/dyspnea exertional, emphysema, epistaxis, hemoptysis, hydrothorax, hypercapnia, hypoxia, laryngeal edema, interstitial lung disease, laryngeal inflammation, pneumothorax, rales, sinus congestion, stridor, tachypnea, wheezing Acute: respiratory distress syndrome/respiratory failure Lung: consolidation/disorder/infiltration, obstructive airways disorder, orthopnea, pharyngolaryngeal pain, pleural disorder/effusion/pleurisy/pain; pneumonia aspiration, pneumonitis, pneumothorax, productive cough, rhinorrhoea Pulmonary: congestion/alveolar/hemorrhage/artery dilatation/cavitation/embolism/fibrosis/haemorrhage/hypertension/edema/toxicity Respiratory: arrest/disorder/distress/failure/gas exchange disorder
Skin and subcutaneous tissue disorders	Acute febrile neutrophilic dermatosis, Angioedema, blister, dermatitis, dermatitis acneiform, erythema, hyperhidrosis, leukocytoclastic vasculitis, night sweats, petechiae, photosensitivity reaction, pruritus, Stevens-Johnson syndrome Rash: erythematous/generalized/ maculo-papular/pruritic/skin disorder/exfoliation/lesion/reaction/ulcer/palmar plantar erythrodysesthesia/hand foot syndrome
Surgical and medical procedures	Abscess drainage, biliary drainage, bladder irrigation, catheter removal, chest tube insertion, cholecystectomy, debridement, endotracheal intubation, fistula repair, gastric ulcer surgery, gastrointestinal tube insertion, ileojejunal bypass, incisional drainage, laparotomy, mechanical ventilation, pleurodesis, sinus operation, surgery, thoracic cavity drainage, tracheostomy Pericardial: drainage/excision/operation Wound: closure/drainage/treatment
Vascular disorders	Capillary leak syndrome, circulatory collapse, embolism, flushing, gastrointestinal angiodysplasia, gastrointestinal ischemia, hemorrhoids, hematoma, hemodynamic instability, hemorrhage, hypertension, hypertensive angiopathy, hypertensive crisis, hypotension, lymphangiopathy, lymphedema, pallor, shock; transient ischemic attack, retinal hemorrhage, Thrombosis: deep vein/jugular vein

*New events added to IB Editions 12 and 13 are noted in bold

5.5 Therapy Administration and Dose Reductions

NOTE: All toxicities should be graded according to the NCI Common Toxicity Criteria (version 4.0)

All patients will initiate therapy on dose level 1 (Table 7). In a currently accruing phase I study, a dosing schedule of everolimus 7.5mg PO on days 1-21, bevacizumab 15mg/kg IV day 1 and liposomal doxorubicin 30mg/m^2 IV on day 1, every 21 days was well tolerated with no dose limiting toxicity. Although dose escalation within the phase I trial has demonstrated that higher doses of everolimus are feasible, there was a higher incidence of grade 3 mucositis, a known toxicity of both everolimus and liposomal doxorubicin. Since metaplastic patients who developed response were treated with 7.5mg everolimus, this has been the dose chosen to proceed forward in this clinical trial.

Patients experiencing > grade 2 non-hematologic drug toxicity that is unresponsive to symptomatic support may undergo dose reduction of the responsible drug(s) as indicated in Table 7. Other drugs within the regimen can be continued at full dose per the treating physician's discretion. If all three drugs are reduced to dose level -2, the patient will be withdrawn from study. Therapy with everolimus should be held in patients with absolute neutrophil count < 1000/mm^3, hemoglobin < 8/mm^3, platelet count <50,000/mm^3 and/or ≥ grade 3 mucositis and/or hand foot syndrome. Therapy may be resumed if neutrophil count improves to > 1000/mm^3, hemoglobin improves to > 8/mm^3, platelet count improves to ≥50,000/mm^3 and/or mucositis and/or hand foot syndrome improve to grade 1. Neutrophils and platelets must have recovered to grade 2 prior to receiving subsequent cycles of therapy. Treatment may be held for up to 2 weeks for hematologic recovery, but therapy should be discontinued if >2 weeks is required for hematologic recovery. Elevation in blood pressure during therapy should be treated according to guidelines for bevacizumab induced hypertension. Urine dipstick protein values of 2 or higher should be confirmed by 24-hour collection of urine. For proteinuria > 3.5g/24 hours, bevacizumab should be discontinued. For proteinuria 1-3.5g/24 hours, bevacizumab should be held until proteinuria improves to < 2g/24 hours. If improvement to < 2g/24 hours is not seen in 4 weeks, bevacizumab should be discontinued. For proteinuria < 1g/24 hours, bevacizumab can be continued. Therapy will be administered as indicated in Table 7.

Table 7: Drug Dosing and Recommended Reductions for Toxicity*

	Everolimus	Bevacizumab	Liposomal Doxorubicin
Dose level 1	7.5mg PO on days 1-21	10 mg/kg IV on day 1	30 mg/m^2 IV on day 1
Dose level -1	5 mg PO on days 1-21	7.5 mg/kg IV on day 1	25 mg/m^2 IV on day 1
Dose level -2	2.5 mg PO on days 1-21	5 mg/kg IV on day 1	20 mg/m^2 IV on day 1
Dose level -3	Discontinue	Discontinue	Discontinue

*Decisions to reduce doses are made per individual drug based upon known toxicity profiles of each drug. As few as 1 and as many as 3 drugs may be reduced per investigator's discretion upon evaluation of an individual patient's symptoms, severity and likelihood of drug causation.

5.5.1 Management of specific toxicities related to everolimus administration:

Overall, safety data available from completed, controlled and uncontrolled studies indicate that everolimus is generally well tolerated at weekly or daily dose schedules. The safety profile is characterized by manageable adverse events (AEs). These AEs are generally reversible and non-cumulative.

Adverse events most frequently observed with everolimus are stomatitis, rash, diarrhea, fatigue, infections, asthenia, nausea, peripheral edema, decreased appetite, headache, dysgeusia, epistaxis, mucosal inflammation, pneumonitis, weight decreased, vomiting, pruritus, cough, dyspnea, dry skin, nail disorder, and pyrexia. Overall, the most frequently observed laboratory abnormalities include decreased hematology parameters including hemoglobin, lymphocytes,

platelets, and neutrophils (or collectively as pancytopenia).; increased clinical chemistry parameters including cholesterol, triglycerides, glucose, aspartate transaminases, creatinine, alanine transaminases, and bilirubin; and decreased clinical chemistry parameters including phosphate and potassium.. The majority of these AEs have been of mild to moderate severity (NCI CTC grade 1-2).

Table 8 - Dosing guidelines for Everolimus-related non-hematologic toxicities

Toxicity	Action
Non-Infectious Pneumonitis	Please refer to section below, including Table 12
Reactivation of HBV or HCV flare	Please refer to sections below, including Tables 14 and 15
AST or ALT elevation Grade 1 (> ULN - 3.0 x ULN) Grade 2 (> 3.0 - 5.0 x ULN)	Maintain current dose level
AST or ALT elevation Grade 3 (> 5.0 - 20.0 ULN)*	Interrupt Everolimus administration until resolution to ≤ grade 1 (or ≤ grade 2 if baseline values were within the range of grade 2). If resolution occurs ≤ 7 days, Everolimus should be re-started at the dose level prior to interruption. If resolution takes > 7 days, or if event recurs within 28 days, hold Everolimus until recovery to ≤ grade 1 or baseline grade / value and reintroduce Everolimus at one dose level lower, if available.
AST or ALT elevation Grade 4 (> 20 x ULN)*	Interrupt Everolimus administration until resolution to ≤ grade 1 (or ≤ grade 2 if baseline values were within the range of grade 2). If resolution occurs ≤ 7 days, Everolimus should be re-started at one dose level lower. If resolution takes > 7 days, discontinue Everolimus.
Recurrence of grade 4 after dose reduction or toxicity requiring Everolimus interruption for > 28 days	Discontinue Everolimus.
Intolerable grade 2 mucositis, or grade 3 AE, except hyperglycemia or hypertriglyceridemia or hypercholesterolemia (see below, including tables 10 and 11)	Interrupt Everolimus administration until resolution to ≤ grade 1 or baseline grade / value. If resolution occurs within ≤ 7 days, Everolimus should be re-started at the dose level prior to interruption. If resolution takes > 7 days, or if event recurs within 28 days, hold Everolimus until recovery to ≤ grade 1 or baseline grade / value and reintroduce Everolimus at one dose level lower, if available. Patients will be withdrawn from the study if they fail to recover to ≤ grade 1 or baseline grade / value within 28 days.
Any other grade 4	Hold Everolimus until recovery to grade ≤ 1 or baseline value Reintroduce Everolimus at one dose level lower, if available.
Grade 3 or 4 clinical liver failure (asterixis or encephalopathy/coma)	Discontinue Everolimus
Recurrence of intolerable grade 2 mucositis or grade 3 event after dose reduction	Reduce dose to the next lower dose level, if available. The lowest possible dose level of Everolimus is 2.5 mg daily. Below this level, Everolimus must be discontinued. If toxicity recurs at Grade 3, consider discontinuation
Recurrence of grade 4 after dose reduction	Discontinue Everolimus

Toxicity	Action
Any non-hematologic toxicity requiring Everolimus interruption for > 28 days	Discontinue Everolimus
* Should HCV flare be confirmed, the guidelines for flare must take precedence	

Table 9- Dosing guidelines for Everolimus-related hematologic toxicities

Toxicity	Action
Grade 2 thrombocytopenia (platelets <75, ≥ 50x109/L)	No action
Grade 3 thrombocytopenia (platelets <50, ≥ 25 x109/L)	Interrupt Everolimus until resolution to grade ≤1 If resolution occurs ≤ 7 days, reintroduce Everolimus at the dose level prior to interruption. If resolution occurs > 7 days, or event occurs within 28 days, reintroduce Everolimus at one dose level lower, if available.
Grade 4 thrombocytopenia (platelets < 25 x109/L)	Interrupt Everolimus until recovery to grade ≤ 1. Then reintroduce Everolimus at one dose level lower, if available.
Grade 3 neutropenia or anemia (neutrophil <1, ≥0.5 x109/L)	Interrupt Everolimus until resolution to grade ≤1 or baseline value If AE resolution occurs ≤ 7 days, reintroduce Everolimus at the same dose level. If AE resolution occurs > 7 days, or event occurs within 28 days, reintroduce Everolimus at one dose level lower, if available.
Grade 4 neutropenia or anemia	Interrupt Everolimus until recovery to grade ≤ 1 or baseline value. Reintroduce Everolimus at one dose level lower, if available.*
Febrile neutropenia	Interrupt Everolimus until resolution to grade ≤ 1 (or baseline value) and no fever. Reintroduce Everolimus at one dose level lower, if available.*
Recurrence of grade 3 toxicity after dose reduction	Reduce dose to the next lower dose level, if available. The lowest possible dose level of Everolimus is 5 mg every other day (2.5 mg daily). Below this level, Everolimus must be discontinued.
***Recurrence of grade 4 toxicity (including febrile neutropenia) after dose reduction**	**Discontinue Everolimus**
***Any hematologic toxicity requiring Everolimus interruption for > 28 days**	**Discontinue Everolimus**

Management of infections

Everolimus has immunosuppressive properties and may predispose patients to bacterial, fungal, viral or protozoal infections, including infections with opportunistic pathogens. Localized and systemic infections, including pneumonia, other bacterial infections, invasive fungal infections, such as aspergillosis or candidiasis and viral infections including reactivation of hepatitis B virus, have been described in patients taking Everolimus Some of these infections have been severe (e.g. leading to sepsis, respiratory or hepatic failure) and occasionally have had a fatal outcome.

Physicians and patients should be aware of the increased risk of infection with Everolimus Treat pre-existing infections prior to starting treatment with Everolimus. While taking Everolimus, be

vigilant for symptoms and signs of infection; if a diagnosis of infection is made, institute appropriate treatment promptly and consider interruption or discontinuation of Everolimus.

If a diagnosis of invasive systemic fungal infection is made, discontinue Everolimus and treat with appropriate antifungal therapy.

Cases of pneumocystis jirovecii pneumonia (PJP), some with a fatal outcome, have been reported in patients who received everolimus. PJP may be associated with concomitant use of corticosteroids or other immunosuppressive agents. Prophylaxis for PJP should be considered when concomitant use of corticosteroids or other immunosuppressive agents are required.

Management of skin toxicity

For patients with grade 1 toxicity, no specific supportive care is usually needed or indicated. Rash must be reported as an AE. Patients with grade 2 or higher toxicity may be treated with the following suggested supportive measures at the discretion of the investigator: oral minocycline, topical tetracycline, topical clindamycin, topical silver sulfadiazine, diphenhydramine, oral prednisolone (short course), topical corticosteroids, or pimecrolimus.

Management of Hypersensitivity reactions

Hypersensitivity reactions manifested by symptoms including, but not limited to, anaphylaxis, dyspnea, flushing, chest pain or angioedema (e.g. swelling of the airways or tongue, with or without respiratory impairment) have been observed with everolimus.

Angioedema with concomitant use of angiotensin-converting enzyme (ACE) inhibitors

Patients taking concomitant ACE inhibitor therapy may be at increased risk for angioedema (e.g. swelling of the airways or tongue, with or without respiratory impairment).

Renal Failure Events

Cases of renal failure (including acute renal failure), some with fatal outcome, occurred in patients treated with everolimus. Renal function of patients should be monitored particularly where patients have additional risk factors that may further impair renal function.

Elevations of serum creatinine, usually mild, and proteinuria have been reported in patients taking everolimus. Monitoring of renal function, including measurement of blood urea nitrogen (BUN), urinary protein, or serum creatinine, is recommended prior to the start of everolimus therapy and periodically thereafter.

Management of stomatitis, oral mucositis, mouth ulcers

Patients with a clinical history of stomatitis/mucositis/mouth ulcers and those with gastrointestinal morbidity associated with mouth/dental infections, irritation of esophageal mucosa e.g. gastroesophageal reflux disease (GERD) and pre-existing stomatitis/mucositis must be monitored even more closely. Patients should be instructed to report the first onset of buccal mucosa irritation/reddening to their study physician immediately.

Stomatitis/oral mucositis/mouth ulcers due to Everolimus should be treated using local supportive care. Please note that investigators in earlier trials have described the oral toxicities associated with Everolimus as mouth ulcers, rather than mucositis or stomatitis. If your examination reveals mouth ulcers rather than a more general inflammation of the mouth, please classify the adverse event as such. The suggested paradigm for treatment of stomatitis/oral mucositis/mouth ulcers is as follows:

1. For mild toxicity (grade 1), no dose adjustment required. Manage with non-alcoholic mouth wash or salt water (0.9%) mouth wash several times a day until resolution.
2. For more severe toxicity (grade 2 in which case patients have pain but are able to maintain adequate oral alimentation, or grade 3 in which case patients cannot maintain adequate oral alimentation), the suggested treatments are topical analgesic mouth treatments (i.e., local anesthetics such as, benzocaine, butyl aminobenzoate, tetracaine hydrochloride, menthol, or

phenol) with or without topical corticosteroids, such as triamcinolone oral paste 0.1% (Kenalog in Orabase®).

3. Agents containing alcohol, hydrogen peroxide, iodine, and thyme derivatives may tend to worsen mouth ulcers. These agents should be avoided.
4. Antifungal agents should be avoided unless a fungal infection is diagnosed. In particular, systemic imidazole antifungal agents (ketoconazole, fluconazole, itraconazole, etc.) should be avoided in all patients due to their strong inhibition of Everolimus metabolism, therefore leading to higher Everolimus exposures. Therefore, topical antifungal agents are preferred if an infection is diagnosed.

Table 10: Management of stomatitis / oral mucositis / mouth ulcers

Adverse Drug Reaction	Severity	Afinitor Dose Adjustment and Management Recommendations
Stomatitis	Grade 1 (Minimal symptoms, normal diet)	No dose adjustment required. Manage with non-alcoholic or salt water (0.9%) mouth wash several times a day.
	Grade 2 (Symptomatic but can eat and swallow modified diet)	Temporary dose interruption until recovery to grade ≤1. Re-initiate Afinitor at the same dose. If stomatitis recurs at grade 2, interrupt dose until recovery to grade ≤1. Re-initiate Afinitor at a lower dose. Manage with topical analgesic mouth treatments (e.g. benzocaine, butyl aminobenzoate, tetracaine hydrochloride, menthol or phenol) with or without topical corticosteroids (i.e. triamcinolone oral paste)*.
	Grade 3 (Symptomatic and unable to adequately eat or hydrate orally)	Temporary dose interruption until recovery to grade ≤1. Re-initiate Afinitor at lower dose. Manage with topical analgesic mouth treatments (i.e. benzocaine, butyl aminobenzoate, tetracaine hydrochloride, menthol or phenol) with or without topical corticosteroids (i.e. triamcinolone oral paste)*
	Grade 4 (Symptoms associated with life-threatening consequences)	Discontinue Afinitor and treat with appropriate medical therapy.

* using agents containing alcohol, hydrogen peroxide, iodine, and thyme derivatives in management of stomatitis as they may worsen mouth ulcers.

Management of Diarrhea

Appearance of grade 1-2 diarrhea attributed to study drug toxicity may be treated with supportive care such as loperamide, initiated at the earliest onset (for example 4 mg orally followed by 2 mg orally every 2 hours until resolution of diarrhea).

Management of Hyperlipidemia and Hyperglycemia

Treatment of hyperlipidemia should take into account the pre-treatment status and dietary habits of the patient. Grade 2 or higher hypercholesterolemia (>300 mg/dL or 7.75 mmol/L) or grade 2 hypertriglyceridemia or higher (>2.5x upper normal limit) should be treated with a 3-hydroxy-3-

methyl-glutaryl (HMG)-CoA reductase inhibitor (e.g. atorvastatin, pravastatin, fluvastatin) or appropriate triglyceride-lowering medication, in addition to diet.

Note: Concomitant therapy with fibrates and an HMG-CoA reductase inhibitor is associated with an increased risk of a rare but serious skeletal muscle toxicity manifested by rhabdomyolysis, markedly elevated creatine phosphokinase (CPK) levels and myoglobinuria, acute renal failure and sometimes death. The risk versus benefit of using this therapy should be determined for individual patients based on their risk of cardiovascular complications of hyperlipidemia.

Dyslipidemia (including hypercholesterolemia and hypertriglyceridemia) has been reported in patients taking everolimus. Monitoring of blood cholesterol and triglycerides prior to the start of everolimus therapy and periodically thereafter as well as management with appropriate medical therapy is recommended.

Hyperglycemia has been reported in patients taking everolimus. Monitoring of fasting serum glucose is recommended prior to the start of Everolimus and periodically thereafter. More frequent monitoring is recommended when everolimus is co-administered with other drugs that may induce hyperglycemia. Optimal glycemic control should be achieved before starting a patient on Everolimus.

Table 11: Management of hyperlipidemia and hyperglycemia

Adverse Drug Reaction	Severity	Afinitor Dose Adjustment and Management Recommendations
Metabolic events (e.g. hyperglycemia, dyslipidemia)	Grade 1	No dose adjustment required. Initiate appropriate medical therapy and monitor.
	Grade 2	No dose adjustment required. Manage with appropriate medical therapy and monitor.
	Grade 3	Temporary dose interruption. Re-initiate Afinitor at lower dose. Manage with appropriate medical therapy and monitor.
	Grade 4	Discontinue Afinitor and treat with appropriate medical therapy.

Management of non-infectious pneumonitis

Non-infectious pneumonitis is a class effect of rapamycin derivatives. Cases of non-infectious pneumonitis (including interstitial lung disease) have also been described in patients taking Everolimus. Some of these have been severe and on rare occasions, a fatal outcome was observed.

A diagnosis of non-infectious pneumonitis should be considered in patients presenting with non-specific respiratory signs and symptoms such as hypoxia, pleural effusion, cough or dyspnea, and in whom infectious, neoplastic and other non-medicinal causes have been excluded by means of appropriate investigations. Opportunistic infections such as PJP should be ruled out in the differential diagnosis of non-infectious pneumonitis. Patients should be advised to report promptly any new or worsening respiratory symptoms.

Patients who develop radiological changes suggestive of non-infectious pneumonitis and have few or no symptoms may continue Afinitor therapy without dose alteration.

If symptoms are moderate (grade 2), consideration should be given to interruption of therapy until symptoms improve. The use of corticosteroids may be indicated. Afinitor may be reintroduced at a daily dose approximately 50% lower than the dose previously administered.

For cases of grade 3 non-infectious pneumonitis, interrupt Afinitor until resolution to less than or equal to grade 1. Afinitor may be re-initiated at a daily dose approximately 50% lower than the dose previously administered depending on the individual clinical circumstances. If toxicity recurs at grade 3, consider discontinuation of Afinitor. For cases of grade 4 non-infectious pneumonitis, Afinitor therapy should be discontinued. Corticosteroids may be indicated until clinical symptoms resolve.

For patients who require use of corticosteroids for treatment of non-infectious pneumonitis, prophylaxis for pneumocystis jirovecii pneumonia (PJP) may be considered. The two compounds studied most extensively for prophylaxis against PJP have been trimethoprim-sulfamethoxazole, given orally, and pentamidine, given as an aerosol.

If non-infectious pneumonitis develops, the guidelines in Table 12 should be followed. Consultation with a pulmonologist is recommended for any case of pneumonitis that develops during the study.

Table 12: Management of non-infectious pneumonitis

Worst grade pneumonitis	Suggested investigations	Management of pneumonitis	Everolimus dose adjustment
Grade 1 (Asymptomatic, radiographic findings only)	CT scans with lung windows.	No specific therapy is required	No dose adjustment required. Initiate appropriate monitoring.
Grade 2 (Symptomatic, not interfering with Activities of Daily Living)	CT scan with lung windows. Consider pulmonary function testing includes: spirometry, DLCO, and room air O_2 saturation at rest. Consider a bronchoscopy with biopsy and/or BAL. Monitoring at each visit until return to ≤ grade 1. Return to initial monitoring frequency if no recurrence.	Symptomatic only. Consider corticosteroids and/or other supportive therapy if symptoms are troublesome.	Rule out infection and consider interruption of Everolimus until symptoms improve to Grade ≤ 1. Re-initiate Everolimus at one dose level lower. Discontinue Everolimus if failure to recover within ≤ 28 days.
Grade 3 (Symptomatic, Interfering with Activities of Daily Living. O2 indicated	CT scan with lung windows and pulmonary function testing includes: spirometry, DLCO, and room air O2 saturation at rest. Monitoring at each visit until return to ≤ grade 1. Return to initial monitoring frequency if no recurrence. Bronchoscopy with biopsy and/or BAL is recommended.	Consider corticosteroids if infective origin is ruled out. Taper as medically indicated.	Rule out infection and interrupt Everolimus until symptoms improve to Grade ≤ 1. Consider re-initiating Everolimus at one dose level lower (approximately 50% lower than the dose previously administered depending on individual clinical circumstances) Discontinue Everolimus if failure to recover within ≤ 28 days. If toxicity recurs at Grade 3, consider discontinuation
Grade 4 (Life-threatening, ventilator support indicated)	CT scan with lung windows and required pulmonary function testing, if possible, includes: spirometry, DLCO, and room air O2 saturation at rest. Monitoring at each visit until return to ≤ grade 1. Return to initial monitoring frequency if no recurrence. Bronchoscopy with biopsy and/or BAL is recommended if possible.	Consider corticosteroids if infective origin is ruled out. Taper as medically indicated.	Rule out infection and discontinue Everolimus.

5.5.2 Management of hepatitis reactivation / flare

Reactivation of Hepatitis B (HBV) has been observed in patients with cancer receiving chemotherapy.[65] Sporadic cases of Hepatitis B reactivation have also been seen in this setting with everolimus. Use of antivirals during anti-cancer therapy has been shown to reduce the risk of Hepatitis B virus reactivation and associated morbidity and mortality.[66] A detailed assessment of Hepatitis B/C medical history and risk factors must be done for all patients at screening, with testing performed prior to the first dose of everolimus.

Monitoring and prophylactic treatment for hepatitis B reactivation

Table 13 provides detail of monitoring and prophylactic therapy according to the screening results of viral load and serologic markers testing.

Table 13: Action to be taken based on screening hepatitis B results

Test	**Result**	**Result**	**Result**	**Result**	**Result**
HBV-DNA	+	+ or -	-	-	-
HBsAg	+ or -	+	-	-	-
HBsAb	+ or -	+ or -	+ and no prior HBV vaccination	+ or -	- or + with prior HBV vaccination
HBcAb	+ or -	+ or -	+ or -	+	-
Recommendation	Prophylaxis treatment should be started 1-2 weeks prior to first dose of Everolimus Monitor HBV-DNA approximately every 4-8 weeks		No prophylaxis Monitor HBV-DNA approximately every 3-4 weeks		No specific action

Antiviral prophylaxis therapy should continue for at least 4 weeks after last dose of Everolimus. For HBV reactivation definition and management guidelines, see Table 14.

Table 14: Guidelines for the management of hepatitis B reactivation

HBV reactivation (with or without clinical signs and symptoms)*	
For patients with baseline results: Positive HBV-DNA **OR** positive HBsAg ---------- **reactivation is defined as:** [Increase of 1 log in HBV-DNA relative to baseline HBV-DNA value OR new appearance of measurable HBV-DNA]	**Treat**: Start a second antiviral medication AND Interrupt Everolimus administration until resolution: • ≤ baseline HBV-DNA levels **If resolution occurs within ≤ 28 days**, Everolimus should be re-started at one dose lower, if available. (see Table 7 for dose levels available) If the patient is already receiving the lowest dose of Everolimus according to the protocol, the patient should restart at the same dose after resolution. Both antiviral therapies should continue at least 4 weeks after last dose of Everolimus. **If resolution occurs > 28 days** Patients should discontinue Everolimus but continue both antiviral therapies at least 4 weeks after last dose of Everolimus.
For patients with baseline results: Negative HBV-DNA **and** HBsAg **AND** **[**Positive HBsAg (with no prior history of vaccination against HBV), **OR** positive HBcAb**]** ---------- **Reactivation is defined as:** New appearance of measurable HBV-DNA	**Treat :** Start first antiviral medication AND **Interrupt** Everolimus administration until resolution: • ≤ undetectable (negative) HBV-DNA levels **If resolution occurs within ≤ 28 days**, Everolimus should be re-started at one dose lower, if available (see Table 7 for dose levels available). If the patient is already receiving the lowest dose of Everolimus according to the protocol, the patient should restart at the same dose after resolution. Antiviral therapy should continue at least 4 weeks after last dose of Everolimus. **If resolution occurs > 28 days** Patients should discontinue Everolimus but continue antiviral therapy at least 4 weeks after last dose of Everolimus.
* All reactivations of HBV are to be recorded as grade 3 (e.g. CTCAE Version 3.0 - Investigations/Other: Viral Reactivation), unless considered life threatening by the investigator, in which case they should be recorded as grade 4. Date of viral reactivation is the date on which the rise or reappearance of HBV-DNA was recorded.	

Monitoring for hepatitis C flare

The following two categories of patients should be monitored every 4–8 weeks for HCV flare:

- Patients with detectable HCV RNA-PCR test at screening.
- Patients known to have a history of HCV infection, despite a negative viral load test at screening (including those that were treated and are considered ‘cured’)

For definitions of HCV flare and actions to be taken in the event of a flare, please refer to Table 15.

Table 15 Guidelines for the management of hepatitis C flare

Baseline results	HCV flare definition*	HCV flare management
Detectable HCV-RNA	> 2 $\log_{10}$ IU/mL increase in HCV-RNA **AND** ALT elevation > 5 x ULN or 3 x baseline level, whichever is higher.	Discontinue Everolimus
Knowledge of past hepatitis C infection with no detectable HCV-RNA	New appearance of detectable HCV-RNA **AND** ALT elevation > 5 x ULN or 3 x baseline level, whichever is higher.	Discontinue Everolimus

* All flares of HCV are to be recorded as grade 3 (e.g. CTCAE Version 3.0 - Investigations - Other: Viral Flare), unless considered life threatening by the investigator; in which case they should be recorded as grade 4. Date of viral flare is the date on which both the clinical criteria described above were met. (e.g., for a patient whose HCV-RNA increased by 2 logs on 01 JAN 2011 and whose ALT reached > 5 x ULN on 22 JAN 2011, the date of viral flare is 22 JAN 2011).

6. Concomitant medications

Patients must be instructed not to take any medications (over-the-counter or other products) during the protocol treatment period without prior consultation with the investigator. The investigator should instruct the patient to notify the study site about any new medications he/she takes after the start of study drug. All medications (other than study drug) and significant non-drug therapies (including physical therapy and blood transfusions) taken within 28 days of starting study treatment through the 30-day safety follow up visit should be reported in the patient's EMR.

6.1 Permitted concomitant therapy

All supportive measures consistent with optimal patient care should be given throughout the study. Exceptions include the use of erythropoietin to treat therapy induced anemia and use of myeloid growth factor support to treat neutropenia while taking everolimus. If everolimus is held for neutropenia, myeloid growth factors in the form of either GCSF or pegylated GCSF can be administered beginning on either day 2 or 3 of each cycle. The use of growth factor support should follow ASCO guidelines.

6.2 Cytochrome P450 and P-glycoprotein inhibitors/inducers/substrates

Co-administration with strong inhibitors of CYP3A4 or PgP should be avoided; and may cause increased everolimus concentrations. For a current table of Substrates, Inhibitors and Inducers please access the following website:

http://www.fda.gov/Drugs/DevelopmentApprovalProcess/DevelopmentResources/DrugInteractionsLabeling/ucm093664.htm

Everolimus is metabolized by CYP3A4 in the liver and to some extent in the intestinal wall.

Therefore, the following are recommended:

- Co-administration with strong inhibitors of CYP3A4 (e.g., ketoconazole, itraconazole, ritonavir) or P-glycoprotein (PgP) inhibitor should be avoided.
- Co-administration with moderate CYP3A4 inhibitors (e.g., erythromycin, fluconazole) or PgP inhibitors should be used with caution. If a patient requires co-administration of moderate CYP3A4 inhibitors or PgP inhibitors, reduce the dose of everolimus by approximately 50%. Additional dose reductions to every other day may be required to manage toxicities. If the inhibitor is discontinued, the Everolimus dose should be returned to the dose used prior to initiation of the moderate CYP3A4/PgP inhibitor after a washout period of 2 to 3 days.

- **TSC with SEGA:** Everolimus trough concentrations should be assessed approximately 2 weeks after the addition of a moderate CYP3A4/PgP inhibitor. If the inhibitor is discontinued the Afinitor dose should be returned to the dose used prior to initiation of the inhibitor and the everolimus trough concentration should be re-assessed approximately 2 weeks later.
- Grapefruit, Seville oranges, and starfruit affect P450 and PgP activity. Concomitant use should be avoided.
- If patients require co-administration of a strong CYP3A4 inducer, consider doubling the daily dose of Afinitor (based on pharmacokinetic data), using increments of 5 mg or less. This dose of Afinitor is predicted to adjust the AUC to the range observed without inducers. However, there are no clinical data with this dose adjustment in patients receiving strong CYP3A4 inducers. If the strong inducer is discontinued, consider a washout period of at least 3 to 5 days (reasonable time for significant enzyme de-induction), before the Afinitor dose is returned to the dose used prior to initiation of the strong CYP3A4 inducer.
- **TSC with SEGA** Patients receiving concomitant strong CYP3A4 inducers (e.g., the enzyme inducing antiepileptic drugs carbamazepine, phenobarbital, and phenytoin) may require an increased Afinitor dose to attain trough concentrations of 3 to 15 ng/mL. Double the daily dose of Afinitor and assess tolerability. Assess the everolimus trough level two weeks after doubling the dose. Further adjust the dose if necessary to maintain the trough within the 3 to 15 ng/mL range. If the strong inducer is discontinued, the Afinitor dose should be returned to the dose used prior to initiation of the strong CYP3A4 inducer and the everolimus trough concentrations should be assessed approximately 2 weeks later.
- This dose adjustment of Everolimus is intended to achieve similar AUC to the range observed without inducers. However, there are no clinical data with this dose adjustment in patients receiving strong CYP3A4 inducers. If the strong inducer is discontinued the Everolimus dose should be returned to the dose used prior to initiation of the strong CYP3A4/PgP inducer.

Please refer to Table 16 listing relevant inducers and inhibitors of CYP3A and Table 17 for a list of relevant substrates, inducers, and inhibitors of PgP.

Table 16 Clinically relevant drug interactions: inducers, and inhibitors of isoenzyme CYP3A

Inducers
Strong inducers: avasimibe, carbamazepine, mitotane, phenobarbital, phenytoin, rifabutin, rifampin (rifampicin), St. John's wort (hypericum perforatum)
Moderate inducers: bosentan, efavirenz, etravirine, genistein, modafinil, nafcillin, ritonavir, [talviraline], thioridazine, tipranavir
Weak inducers: amprenavir, aprepitant, armodafinil (R-modafinil), bexarotene, clobazam, danshen, dexamethasone, Echinacea, garlic (allium sativum), gingko (ginkgo biloba), glycyrrhizin, methylprednisolone, nevirapine, oxcarbazepine, pioglitazone, prednisone, [pleconaril], primidone, raltegravir, rufinamide, sorafenib, telaprevir, terbinafine, topiramate, [troglitazone] , vinblastine

Inhibitors
Strong inhibitors: boceprevir, clarithromycin, cobicistat, conivaptan, elvitegravir, indinavir, itraconazole, ketoconazole, lopinavir, mibefradil, nefazodone, nelfinavir, posaconazole [67], ritonavir, saquinavir, telaprevir, telithromycin, tipranavir, troleandamycin, voriconazole **Moderate inhibitors:** Amprenavir, aprepitant, atazanavir, casopitant, cimetidine, ciprofloxacin, cyclosporine, darunavir, diltiazem, dronedarone, erythromycin, fluconazole, fosamprenavir, grapefruit juice (citrus parasidi fruit juice), imatinib, schisandra sphenanthera, tofisopam, verapamil

Table 17: Clinically relevant drug interactions: substrates, inducers, inhibitors of PgP and PgP/CYP3A dual inhibitors

Substrates
colchicine, digoxin, fexofenadine, indinavir, paclitaxel, talinolol, topotecan, vincristine, everolimus
Inducers
rifampin, St John's wort
PgP Inhibitors and PgP/CYP3A Dual Inhibitors
amiodarone, azithromycin, captopril, carvedilol, clarithromycin, conivaptan, diltiazem, dronedarone, elacridar, erythromycin, felodipine, fexofenadine, fluvoxamine, ginkgo (ginkgo biloba), indinavir, itraconazole, lopinavir, mibefradil, milk thistle (silybum marianum), nelfinavir, nifedipine, nitrendipine, paroxetine, quercetin, quinidine, ranolazine, rifampin, ritonavir, saquinavir, Schisandra chinensis, St John's wort (hypericum perforatum), talinolol, Telaprevir, telmisartan, ticagrelor, tipranavir, tolvaptan, valspodar, verapamil
Reference: Internal Clinical Pharmacology Drug-drug interaction (DDI) memo, updated Oct. 2, 2011,29-Oct-2012 which summarizes DDI data from three sources including the FDA's "Guidance for Industry, Drug Interaction Studies", the University of Washington's Drug Interaction Database, and Indiana University School of Medicine's Drug Interaction Table.

6.3 Everolimus and drugs influencing CYP3A4 enzyme

Everolimus is a substrate of CYP3A4, and a substrate and moderate inhibitor of the multidrug efflux pump, PgP (PgP, MDR1, and ABCB1). Therefore, extent of absorption and subsequent elimination of systemically absorbed everolimus may be influenced by products that are substrates, inhibitors, or inducers of CYP3A4 and/or PgP. Concurrent treatment with strong CYP3A4-inhibitors should be avoided. Refer to Table 16 for a comprehensive list of inducers and inhibitors of CYP3A4 and Table 17 for a list of relevant substrates, inducers and inhibitors of PgP. Inhibitors of PgP may decrease the efflux of everolimus from brain or tumor and therefore increase everolimus concentrations in these tissues. In vitro studies showed that everolimus is a competitive inhibitor of CYP3A4 and of CYP2D6, potentially increasing the concentrations of products eliminated by these enzymes. Thus, caution should be exercised when co-administering everolimus with CYP3A4 and CYP2D6 substrates with a narrow therapeutic index. Clinical studies have been conducted in healthy subjects to assess pharmacokinetic drug interactions

between everolimus and potential CYP3A modifiers (ketoconazole, verapamil, erythromycin, rifampin, midazolam, and HMGCoA reductase inhibitors (statins).

6.4 Vaccinations

Immunosuppressants may affect the response to vaccination and vaccination during treatment with Everolimus may therefore be less effective. The use of live vaccines should be avoided during treatment with Everolimus. For pediatric patients with SEGA that do not require immediate treatment, complete the recommended childhood series of live virus vaccinations prior to the start of therapy according to local treatment guidelines. Examples of live vaccines are: intranasal influenza, measles, mumps, rubella, oral polio, BCG, yellow fever, varicella, and TY21a typhoid vaccines.

6.5 Prohibited concomitant therapy

The concomitant use of therapeutic doses of anticoagulation is prohibited during treatment with bevacizumab. Patients requiring therapeutic doses of anticoagulation should discontinue bevacizumab, but may be allowed to continue liposomal doxorubicin and everolimus. The use of prophylactic anticoagulation is allowed, but indication must be carefully considered due to the risk of bleeding associated with bevacizumab administration.

Growth factors should not be used for the treatment of anemia or thrombocytopenia as such use has been associated with decreased survival and increased thrombotic events in patients with breast cancer.

7. Reporting of Adverse Events

Definitions and reporting

7.1 Adverse Events

Adverse events that begin or worsen after informed consent should be recorded in the Adverse Events CRF. Conditions that were already present at the time of informed consent should be recorded in the Medical History page of the patient's CRF. Adverse event monitoring should be continued for at least 30 days (or 5 half-lives, whichever is longer) following the last dose of study treatment. Adverse events (including lab abnormalities that constitute AEs) should be described using a diagnosis whenever possible, rather than individual underlying signs and symptoms. When a clear diagnosis cannot be identified, each sign or symptom should be reported as a separate Adverse Event.

The occurrence of adverse events should be sought by non-directive questioning of the patient at each visit during the study. Adverse events also may be detected when they are volunteered by the patient during or between visits or through physical examination, laboratory test, or other assessments. As far as possible, each adverse event should be evaluated to determine:

1. The severity grade (CTCAE Grade 1-4)
2. Its duration (Start and end dates or if continuing at the Safety Follow-up Visit)
3. Its relationship to the study treatment (Reasonable possibility that AE is related: No, Yes)
4. Action taken with respect to study or investigational treatment (none, dose adjusted, temporarily interrupted, permanently discontinued, hospitalized, unknown, not applicable)
5. Whether medication or therapy was given (no concomitant medication/non-drug therapy, concomitant medication/non-drug therapy)
6. Outcome (not recovered/not resolved, recovered/resolved, recovering/resolving, recovered/resolved with sequela, fatal, unknown)
7. Whether it is serious, where a serious adverse event (SAE) is defined as in the Serious Adverse Events subsection.

All adverse events should be treated appropriately. Such treatment may include changes in study drug treatment including possible interruption or discontinuation, starting or stopping concomitant treatments, changes in the frequency or nature of assessments, hospitalization, or any other medically required intervention. Once an adverse event is detected, it should be followed until its resolution, and assessment should be made at each visit (or more frequently, if necessary) of any changes in severity, the suspected relationship to the study drug, the interventions required to treat it, and the outcome.

Information about common side effects already known about the investigational drug can be found in the [Investigators' Brochure] and below. This information should be included in the patient informed consent and should be discussed with the patient during the study as needed.
Adverse event monitoring should be continued for at least 30 days following the last dose of study treatment

Laboratory test abnormalities

Laboratory abnormalities that constitute an Adverse event in their own right (are considered clinically significant, induce clinical signs or symptoms, require concomitant therapy or require changes in study treatment), should be recorded on the Adverse Events CRF. Whenever possible, a diagnosis, rather than a symptom should be provided (e.g. anemia instead of low hemoglobin). Laboratory abnormalities that meet the criteria for Adverse Events should be followed until they have returned to normal or an adequate explanation of the abnormality is found. When an abnormal laboratory or test result corresponds to a sign/symptom of an already reported adverse event, it is not necessary to separately record the lab/test result as an additional event.

Laboratory abnormalities, that do not meet the definition of an adverse event, should not be reported as adverse events. A Grade 3 or 4 event (severe) as per CTCAE does not automatically indicate a SAE unless it meets the definition of serious as defined below and/or as per investigator's discretion. A dose hold or medication for the lab abnormality may be required by the protocol and is still, by definition, an adverse event.

7.2 Serious Adverse Events

A serious adverse event is an undesirable sign, symptom or medical condition which:

- is fatal or life-threatening
- results in persistent or significant disability/incapacity
- constitutes a congenital anomaly/birth defect
- requires inpatient hospitalization or prolongation of existing hospitalization, unless hospitalization is for:
- routine treatment or monitoring of the studied indication, not associated with any deterioration in condition (specify what this includes)
- elective or pre-planned treatment for a pre-existing condition that is unrelated to the indication under study and has not worsened since the start of study drug
- treatment on an emergency outpatient basis for an event not fulfilling any of the definitions of a SAE given above and not resulting in hospital admission
- social reasons and respite care in the absence of any deterioration in the patient's general condition
- is medically significant, i.e., defined as an event that jeopardizes the patient or may require medical or surgical intervention to prevent one of the outcomes listed above

Reporting

The principal investigator has the obligation to report all serious adverse events to the FDA and IRB. If the SAE is deemed definitely, probably or possibly related to everolimus, the investigator is obligated to notify Novartis Pharmaceuticals Drug Safety and Epidemiology Department (DS&E) .

All events reported to the FDA by the investigator are to be filed utilizing the Form FDA 3500A (MedWatch Form).

To ensure patient safety, every SAE, regardless of suspected causality, occurring

- after the patient has provided informed consent and until at least 30 days after the patient has stopped study treatment/participation
- after protocol-specified procedures begin (e.g., placebo run-in, washout period, double-blind treatment, etc.) and 30 days after the patient has stopped study treatment
- after the start of any period in which the study protocol interferes with the standard medical treatment given to a patient (e.g., treatment withdrawal during washout period, change in

treatment to a fixed dose of concomitant medication) and until 30 days after the patient has stopped study treatment

must be reported to Novartis within 24 hours of learning of its occurrence **(fax: 877-778-9739)**. This includes serious, related, labeled (expected) and serious, related, unlabeled (unexpected) adverse experiences. All deaths during treatment or within 30 days following completion of active protocol therapy must be reported within 5 working days.

Any SAEs experienced after this 30 days period should only be reported to Novartis if the investigator suspects a causal relationship to the study drug. Recurrent episodes, complications, or progression of the initial SAE must be reported as follow-up to the original episode within 24 hours of the investigator receiving the follow-up information. A SAE occurring at a different time interval or otherwise considered completely unrelated to a previously reported one should be reported separately as a new event. The end date of the first event must be provided.

The original copy of the SAE Report and the fax confirmation sheet must be kept within the Trial Master File at the study site.

Follow-up information is sent to the same fax number as the original SAE Report Form was sent, using a new fax cover sheet, stating that this is a follow-up to the previously reported SAE, and giving the date of the original report. Each re-occurrence, complication, or progression of the original event should be reported as a follow-up to that event regardless of when it occurs. The follow-up information should describe whether the event has resolved or continues, if and how it was treated, whether the blind was broken or not (if applicable), and whether the patient continued or withdrew from study participation.

If the SAE is not previously documented in the Everolimus Investigator Brochure or Package Insert (new occurrence) and is thought to be related to the Novartis study drug, a DS&E associate may urgently require further information from the investigator for Health Authority reporting. Novartis may need to issue an Investigator Notification (IN), to inform all investigators involved in any study with the same drug that this SAE has been reported. Suspected Unexpected Serious Adverse Reactions (SUSARs) will be collected and reported to the competent authorities and relevant ethics committees in accordance with Directive 2001/20/EC or as per national regulatory requirements in participating countries.

For Comparator Drugs/Secondary Suspects (Concomitant Medications), all serious adverse experiences will be forwarded to the comparator drug company by the investigator.

Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse drug experience when, based upon appropriate 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. All events occurring during the conduct of a protocol and meeting the definition of a SAE must be reported to the IRB in accordance with the timeframes and procedures outlined in “University of Texas M. D. Anderson Cancer Center Institutional Review Board Policy on Reporting Serious Adverse Events”. Unless stated otherwise in the protocol, all SAEs, expected or unexpected, must be reported to ORERM, regardless of attribution (within 5 working days of knowledge of the event). All life-threatening or fatal events, expected or unexpected, and regardless of attribution to the study drug, must have a written report submitted within 24 hours (next working day) of knowledge of the event to the Safety Project Manager in ORERM. The MDACC “Internal SAE Report Form for Prompt Reporting” will be used for reporting to ORERM. Serious adverse events will be captured from the time the patient signs consent until 30 days after the last dose of drug. Serious adverse events must be followed until clinical recovery is complete and laboratory tests have returned to baseline, progression of the event has stabilized, or there has been acceptable resolution of the event. Serious adverse events will be forwarded to FDA by the Safety Project Manager ORERM according to 21 CFR 312.32.

7.3 Pregnancy

Preclinical data regarding reproductive toxicity is described in the most recent Investigator Brochure. The potential reproductive risk for humans is unknown. Women of childbearing potential should be advised to use highly effective contraception methods while they are receiving everolimus and up to 8 weeks after treatment has been stopped.

To ensure patient safety, each pregnancy occurring while the patient is on study treatment must be reported to Novartis within 24 hours of learning of its occurrence. The pregnancy should be followed up to determine outcome, including spontaneous or voluntary termination, details of the birth, and the presence or absence of any birth defects, congenital abnormalities, or maternal and/or newborn complications. The newborn will be followed for at least 12 months.

Pregnancy should be recorded on a Clinical Trial Pregnancy Form and reported by the investigator to the oncology Novartis Drug Safety and Epidemiology Department (DS&E). Pregnancy follow-up should be recorded on the same form and should include an assessment of the possible relationship to the study treatment and any pregnancy outcome. Any SAE experienced during pregnancy must be reported on the SAE Report Form.

7.4. Duration of Therapy

Patients will receive protocol therapy unless:

- Patient experiences unacceptable drug toxicity.
- Patient withdraws consent.
- Patient has progression of disease as defined in section 9.0.2
- The patient completes the required 4 cycles of therapy.

Duration of Follow-up

For this protocol, all patients, including those who discontinue protocol therapy early, will be followed until 1 month after surgical resection of the tumor. Date of last follow up clinic visit should be within 30 days (+/- 10 days) of surgical resection. Investigators also collect data on recurrence 2 years after surgical resection of the tumor.

Protection of human subjects

All current FDA, NCI, state federal and institutional regulations concerning informed consent will be followed.

In order to protect confidential patient information, all study participants will be assigned a study ID number. Specimens will be assigned a separate specimen ID number. All study information will be stored in locked file cabinets and in password-protected computer files. Only authorized study personnel will have access to these files.

8. Scheduled evaluations

8.0 Study Calendar:

Study Week		**Cycle 1** (+/- 7 days)			**Cycle 2** (+/- 7 days)			**Cycle 3** (+/- 7 days)			**Cycle 4** (+/-7 days)				
Study Day	**-28 - 0**	1	8	15	1	8	15	1	8	15	1	8	15	Surgical Resection	30 Day[4] Follow-Up
Informed Consent	X														
Demographics	X														
Medical History	X														
General Physical	X	X[1]			X			X			X				X
Vital Signs, Weight	X	X[1]			X			X			X				X
Performance Status	X	X[1]			X			X			X				X
Baseline Symptoms / Toxicities	X	X[1]			X			X			X				X
CBC	X	X[1]			X			X			X				
Chemistries Chem 12 and LFTs	X	X[1]			X			X			X				
Cholesterol / Triglycerides	X	X[1]						X							
Urinalysis	X	X[1]			X			X			X				
Viral Screening[3]	X														
Pregnancy Test (Urine)	X														
Diagnostic Imaging[2]	X												X		
Cardiac Scan (MUGA or 2D Echo)	X														

1. If screening test is performed >10 days before start of treatment, repeat on Day 1.
2. Imaging within 4 weeks prior to start of treatment, after 4 cycles of treatment, +/- 7 days, (or at the time of clinically suspected disease progression). Imaging will consist of mammogram, ultrasound and/or MRI, as clinically indicated.
3. Viral Screening – for patients with known Hep. B/C, or are in potential risk category, or at the physician's discretion.
4. Follow-Up post-surgical resection may be within +/- 10 days.

8.1 Pretreatment Evaluation

- History and physical examination.
- Laboratory studies: CBC with differential, sodium, potassium, chloride, bicarbonate, BUN, creatinine, glucose, calcium, magnesium, albumin, alkaline phosphatase, total bilirubin, SGOT [AST], SGPT [ALT], Cholesterol, Triglycerides.
- Urinalysis includes analysis (protein, glucose, ketones, blood, and specific gravity).
- Urine pregnancy test (WOCBP).

- Viral Screening: HBV-DNA, HBsAb, HBsAg, HBcAb, HCV-Ab, HCV-RNA for patients with known Hepatitis B or C, or patients who fall into the potential risk category, or at the physician's discretion.
- Radiologic evaluation of measurable disease within 4 weeks prior to start of treatment.
- Patient must sign IRB-approved informed consent prior to any study-specific procedures unless such procedures are part of the standard of care.

8.2 Evaluation during Study

- Physical examination (including vital signs, weight, performance status): on day 1 of (or up to 3 days prior to) each treatment cycle.
- CBC with differential, sodium, potassium, chloride, bicarbonate, BUN, creatinine, glucose, calcium, magnesium, albumin, alkaline phosphatase, total bilirubin, ALT, AST, urine pregnancy test within 48 hours prior to starting each cycle of therapy.
- Blood pressure and urinalysis for evidence of proteinuria within 48 hours of starting each cycle of therapy.
- Patients will undergo repeat imaging, +/- 7 days, (mammogram, ultrasound and/or MRI, as clinically indicated) of the involved breast and axillary nodal basin as standard of care prior to surgical resection (after 4 cycles of therapy) or at the time of clinically suspected disease progression.

8.3 Pregnancy and assessments of fertility

There are no adequate data from the use of everolimus in pregnant women. Studies in animals have shown reproductive toxicity effects including embryo-toxicity and feto-toxicity. The potential risk for humans is unknown. Everolimus should not be given to pregnant women unless the potential benefit outweighs the potential risk to the fetus. If a pregnancy occurs while on study treatment, the newborn will be followed for at least 12 months.

It is not known whether everolimus is excreted in breast milk. However, in animal studies everolimus and/or its metabolites readily passed into the milk of lactating rats. Women taking everolimus should therefore not breast-feed.

Women of child-bearing potential, defined as all women physiologically capable of becoming pregnant, must use highly effective contraception during the study and for 8 weeks after stopping treatment. Highly effective contraception is defined as either:

- Total abstinence: When this is in line with the preferred and usual lifestyle of the subject. [Periodic abstinence (e.g., calendar, ovulation, symptothermal, post-ovulation methods) and withdrawal are not acceptable methods of contraception]
- Sterilization: have had surgical bilateral oophorectomy (with or without hysterectomy) or tubal ligation at least six weeks before starting study treatment. In case of oophorectomy alone, only when the reproductive status of the woman has been confirmed by follow up hormone level assessment
- Male partner sterilization (with the appropriate post-vasectomy documentation of the absence of sperm in the ejaculate). [For female subjects on the study, the vasectomised male partner should be the sole partner for that subject].
- Use of a combination of any two of the following (a+b, or a+c, or b+c):
    a. Use of oral, injected, implanted or other hormonal methods of contraception

b. Placement of an intrauterine device (IUD) or intrauterine system (IUS)

c. Barrier methods of contraception: Condom or Occlusive cap (diaphragm or cervical/vault caps) with spermicidal foam/gel/film/cream/vaginal suppository

- In case of use of oral contraception, women should have been stable on the oral agent before taking study treatment.

8.4 Male Contraception

- Sexually active males must use a condom during intercourse while taking the drug and for 8 weeks after stopping treatment and should not father a child in this period.
- A condom is required to be used also by vasectomised men in order to prevent delivery of the drug via seminal fluid.
- Female partners of male patients must also be advised to use one of the following contraception methods: Use of (1) oral, injected, implanted or other hormonal methods of contraception, or (2) intrauterine device (IUD) or intrauterine system (IUS), or (3) prior male/female sterilization.

8.5 Fertility

The potential for everolimus to cause infertility in male and female patients is unknown. However, menstrual irregularities, secondary amenorrhea and associated luteinizing hormone (LH)/follicle stimulating hormone (FSH) imbalance has been observed. Based on non-clinical findings, male and female fertility may be compromised by treatment with everolimus.

9. Measurement of Effect

9.0.1 Pathologic response

The RCB is a continuous variable derived from the primary tumor dimensions, cellularity of the tumor bed, and axillary nodal burden. RCB can be divided into four classes (RCB-0 to RCB-III) and will be collected as part of the study.

RCB-0 (pCR), Minimal RCB (RCB-I), Moderate RCB (RCB-II), and Extensive RCB (RCB-III)

The following parameters are required from pathologic examination in order to calculate Residual Cancer Burden (RCB) after neoadjuvant treatment:

- The largest two dimensions (mms) of the residual tumor bed in the breast (largest tumor bed if multicentric disease)
- Submission of the entire largest cross-sectional area of the residual tumor bed for histologic mapping, with specific identification of those slides in the pathology report (e.g. "the largest cross-sectional area of primary tumor bed was submitted in cassettes A5 - A9")
- If the residual tumor is large (i.e. largest diameter > 5 cm), then at least 5 representative cassettes from the largest cross-sectional area are sufficient, but should be identified in the original pathology report (e.g. "representative sections from the largest cross-sectional area of primary tumor bed were submitted in cassettes A5 - A9")
- Histologic assessment of the percentage of the tumor bed area that contains carcinoma (all carcinoma, i.e. invasive and in situ), select one of the following:
    - 0%, 1%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%

- To assess cellularity it is helpful to scan across the sections of tumor bed and then estimate the average cellularity from the different microscopic fields.

- When estimating percentage cancer cellularity in any microscopic field, compare the involved area with obvious standards, e.g. more or less than half, one quarter, one fifth, one tenth, one twentieth, etc.

- Expect there to be variable cellularity within the cross section of any tumor bed, but estimate the overall cellularity from the average of the estimates in different microscopic fields of the tumor bed.

    - e.g. if cellularity in different fields of the tumor bed were estimated as 20%, 10%, 20%, 0%, 20%, 30%, then an average estimate of overall cellularity would be 20%.

- Histologic estimate of the percentage of the carcinoma in the tumor bed that is in situ, select one of the following:

    - 0%, 1%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%

- The number of positive (metastatic) lymph nodes

- The largest diameter (mm) of the largest nodal metastasis

9.0.2 Radiographic Imaging

Radiographic criteria of response will be based on the on regional ultrasound examination (decrease in size of the primary tumor and/or fatty replacement in regional lymph nodes)

- A decrease in size of the product of the two largest dimensions should =/> 50% will be considered a partial response.
- Complete disappearance of the primary tumor by physical exam and or ultrasound and normalization of the lymph nodes by ultrasound will be considered a complete clinical response.
- Progression of disease will be defined as 30% increase in the size of the primary tumor and/or lymph nodes by ultrasound.

9.2 Correlative Studies

All molecular correlative studies will involve molecular profiling data generated through biopsy of the primary breast tumor and profiled within the existing BMO molecular triaging protocol that is currently IRB approved (MDACC 2014-0185), for patients who participated on that trial. Potential biomarkers of response will be correlated with pathologic response to DAE using appropriate statistical analyses for the biomarker of interest (see Section 9).

9.3Statistical Considerations

9.3.1 Primary/Secondary Objectives:

This is a non-randomized open label phase II study. Counting pCR (RCB-0) or RCB-I as response, a two-stage Gehan-type design will be employed with 14 patients in the first stage. If at least one patient responds, 23 more patients will be added for a total of 37 patients. This design has a 49% chance of terminating after the first stage if the true response rate is 0.05, 23% chance if the true rate is 0.10, 10% if the true rate is 0.15 and 4% if the true rate is 0.20. If accrual

continues to the second stage and a total of 37 patients are enrolled, the 95% confidence interval for a 0.20 response rate will extend from 0.10 to 0.35. For patients who complete 4 cycles of DAE, the proportion of patients with pCR (RCB-0) or RCB-I as the response rate along with a Wilson (score) 95% confidence interval (CI) will be estimated.

Radiographic response rate (partial response + complete clinical response) will be estimated, and reported with a 95% CI.

Toxicity will be measured according to CTCAE v4.0. Adverse events and serious adverse events will be summarized in order of prevalence, with the highest grade experienced by each patient reported for each adverse event.

PFS will be estimated using the Kaplan-Meier method from the date of enrollment onto this study until the date of progression or death without evidence of progression. Patients alive and disease-free at the latest clinical evaluation will be censored for PFS at the date of that evaluation. PFS will be reported with a 95% CI.

9.3.2 Correlative Studies:

Potential biomarkers of response will be correlated with pathologic response to DAE (this protocol) versus paclitaxel, using appropriate statistical analyses for the biomarker of interest.

It is anticipated that mesenchymal gene signatures currently under development may predict response to therapy and response will be correlated in tumors categorized as mesenchymal using these signatures.

Similarly, it is anticipated that vimentin staining will also be correlated with response. Protein expression will be graded 0, 1+, 2+ or 3+ by IHC by a single collaborating pathologist. Degree of expression will then be correlated with presence of mesenchymal signatures as well as response to therapy.

Presence of mutations in PIK3CA, PTEN and NF2 will also be correlated with response. Further, multivariate logistic regression models will be fitted to the data that include three explanatory variables; namely dichotomous variables for treatment (DAE vs paclitaxel), gene mutation, and the interaction. The interaction will be examined to investigate if the relationship between gene mutation status and pathologic response (RCB-0 or 1) is different for the two treatments. We are interested in fitting three such models; specifically, one for mutations PIK3CA, PTEN and NF2

10. Protocol amendments, or changes in study conduct

Any change or addition (excluding administrative) to this protocol requires a written protocol amendment that must be reviewed by Novartis and the investigator before implementation. Amendments significantly affecting the safety of subjects, the scope of the investigation or the scientific quality of the study require additional approval by the IRB at each study center. A copy of the written approval of the IRB must be provided to Novartis. Examples of amendments requiring such approval are:

1. increases in drug dose or duration of exposure of subjects,
2. significant changes in the study design (e.g. addition or deletion of a control group),
3. increases in the number of invasive procedures,
4. additions or deletions of a test procedure required for monitoring of safety.

These requirements for approval should in no way prevent any immediate action from being taken by the investigator or by Novartis in the interests of preserving the safety of all patients included in the trial. If an immediate change to the protocol is felt to be necessary by the investigator and is implemented for safety reasons Novartis must be notified and the IRB at the center must be informed immediately. Amendments affecting only administrative aspects of the study do not require formal protocol amendments or IRB approval but the IRB must be kept informed of such administrative changes. Examples of administrative changes not requiring formal protocol amendments and IRB approval include:

1. changes in the staff used to monitor trials
2. minor changes in the packaging or labeling of study drug.

REFERENCES:

1. Lehmann BD, Bauer JA, Chen X et al. Identification of human triple-negative breast cancer subtypes and preclinical models for selection of targeted therapies. J Clin Invest 2011; 121: 2750-2767.
2. Symmans WF, Peintinger F, Hatzis C et al. Measurement of residual breast cancer burden to predict survival after neoadjuvant chemotherapy. J Clin Oncol 2007; 25: 4414-4422.
3. Liedtke C, Mazouni C, Hess KR et al. Response to neoadjuvant therapy and long-term survival in patients with triple-negative breast cancer. J Clin Oncol 2008; 26: 1275-1281.
4. von Minckwitz G, Kummel S, Vogel P et al. Neoadjuvant vinorelbine-capecitabine versus docetaxel-doxorubicin-cyclophosphamide in early nonresponsive breast cancer: phase III randomized GeparTrio trial. J Natl Cancer Inst 2008; 100: 542-551.
5. Houssami N, Macaskill P, von Minckwitz G et al. Meta-analysis of the association of breast cancer subtype and pathologic complete response to neoadjuvant chemotherapy. Eur J Cancer 2012; 48: 3342-3354.
6. Hennessy BT, Gonzalez-Angulo AM, Stemke-Hale K et al. Characterization of a naturally occurring breast cancer subset enriched in epithelial-to-mesenchymal transition and stem cell characteristics. Cancer Res 2009; 69: 4116-4124.
7. Lehmann BD, Pietenpol JA. Identification and use of biomarkers in treatment strategies for triple-negative breast cancer subtypes. J Pathol 2014; 232: 142-150.
8. Prat A, Adamo B, Cheang MC et al. Molecular characterization of basal-like and non-basal-like triple-negative breast cancer. Oncologist 2013; 18: 123-133.
9. Hatzis C, Pusztai L, Valero V et al. A genomic predictor of response and survival following taxane-anthracycline chemotherapy for invasive breast cancer. JAMA 2011; 305: 1873-1881.
10. Perou CM, Sorlie T, Eisen MB et al. Molecular portraits of human breast tumours. Nature 2000; 406: 747-752.
11. Prat A, Parker JS, Karginova O et al. Phenotypic and molecular characterization of the claudin-low intrinsic subtype of breast cancer. Breast Cancer Res 2010; 12: R68.
12. Arun B, Bayraktar S, Liu DD et al. Response to neoadjuvant systemic therapy for breast cancer in BRCA mutation carriers and noncarriers: a single-institution experience. J Clin Oncol 2011; 29: 3739-3746.
13. Masuda H, Baggerly KA, Wang Y et al. Differential response to neoadjuvant chemotherapy among 7 triple-negative breast cancer molecular subtypes. Clin Cancer Res 2013; 19: 5533-5540.
14. Cohen HT, McGovern FJ. Renal-cell carcinoma. N Engl J Med 2005; 353: 2477-2490.
15. Bjornsti MA, Houghton PJ. The TOR pathway: a target for cancer therapy. Nat Rev Cancer 2004; 4: 335-348.
16. Frattini M, Signoroni S, Pilotti S et al. Phosphatase protein homologue to tensin expression and phosphatidylinositol-3 phosphate kinase mutations in colorectal cancer. Cancer Res 2005; 65: 11227.
17. Velho S, Oliveira C, Ferreira A et al. The prevalence of PIK3CA mutations in gastric and colon cancer. Eur J Cancer 2005; 41: 1649-1654.
18. Goel A, Arnold CN, Niedzwiecki D et al. Frequent inactivation of PTEN by promoter hypermethylation in microsatellite instability-high sporadic colorectal cancers. Cancer Res 2004; 64: 3014-3021.

19. Herschkowitz JI, Simin K, Weigman VJ et al. Identification of conserved gene expression features between murine mammary carcinoma models and human breast tumors. Genome Biol 2007; 8: R76.
20. Weigelt B, Kreike B, Reis-Filho JS. Metaplastic breast carcinomas are basal-like breast cancers: a genomic profiling analysis. Breast Cancer Res Treat 2009; 117: 273-280.
21. Bellino R, Arisio R, D'Addato F et al. Metaplastic breast carcinoma: pathology and clinical outcome. Anticancer Res 2003; 23: 669-673.
22. Kochhar R, Howard EM, Umbreit JN, Lau SK. Metaplastic breast carcinoma with squamous differentiation: molecular and clinical analysis of six cases. Breast J 2005; 11: 367-369.
23. Moroney JW, Schlumbrecht MP, Helgason T et al. A phase I trial of liposomal doxorubicin, bevacizumab, and temsirolimus in patients with advanced gynecologic and breast malignancies. Clin Cancer Res 2011; 17: 6840-6846.
24. Moulder S, Moroney J, Helgason T et al. Responses to liposomal Doxorubicin, bevacizumab, and temsirolimus in metaplastic carcinoma of the breast: biologic rationale and implications for stem-cell research in breast cancer. J Clin Oncol 2011; 29: e572-575.
25. Miyamoto K, Araki KY, Naka K et al. Foxo3a is essential for maintenance of the hematopoietic stem cell pool. Cell Stem Cell 2007; 1: 101-112.
26. Zhou J, Wulfkuhle J, Zhang H et al. Activation of the PTEN/mTOR/STAT3 pathway in breast cancer stem-like cells is required for viability and maintenance. Proc Natl Acad Sci U S A 2007; 104: 16158-16163.
27. McCubrey JA, Steelman LS, Abrams SL et al. Roles of the RAF/MEK/ERK and PI3K/PTEN/AKT pathways in malignant transformation and drug resistance. Adv Enzyme Regul 2006; 46: 249-279.
28. Lee JT, Steelman LS, Chappell WH, McCubrey JA. Akt inactivates ERK causing decreased response to chemotherapeutic drugs in advanced CaP cells. Cell Cycle 2008; 7: 631-636.
29. Clark AS, West K, Streicher S, Dennis PA. Constitutive and inducible Akt activity promotes resistance to chemotherapy, trastuzumab, or tamoxifen in breast cancer cells. Mol Cancer Ther 2002; 1: 707-717.
30. Shoman N, Klassen S, McFadden A et al. Reduced PTEN expression predicts relapse in patients with breast carcinoma treated by tamoxifen. Mod Pathol 2005; 18: 250-259.
31. Nagata Y, Lan KH, Zhou X et al. PTEN activation contributes to tumor inhibition by trastuzumab, and loss of PTEN predicts trastuzumab resistance in patients. Cancer Cell 2004; 6: 117-127.
32. Perez-Tenorio G, Stal O. Activation of AKT/PKB in breast cancer predicts a worse outcome among endocrine treated patients. Br J Cancer 2002; 86: 540-545.
33. Mungamuri SK, Yang X, Thor AD, Somasundaram K. Survival signaling by Notch1: mammalian target of rapamycin (mTOR)-dependent inhibition of p53. Cancer Res 2006; 66: 4715-4724.
34. Gatenby RA, Gillies RJ. Why do cancers have high aerobic glycolysis? Nat Rev Cancer 2004; 4: 891-899.
35. Bjerkvig R, Johansson M, Miletic H, Niclou SP. Cancer stem cells and angiogenesis. Semin Cancer Biol 2009.
36. Parmar K, Mauch P, Vergilio JA et al. Distribution of hematopoietic stem cells in the bone marrow according to regional hypoxia. Proc Natl Acad Sci U S A 2007; 104: 5431-5436.

37. Das B, Tsuchida R, Malkin D et al. Hypoxia enhances tumor stemness by increasing the invasive and tumorigenic side population fraction. Stem Cells 2008; 26: 1818-1830.
38. Elstrom RL, Bauer DE, Buzzai M et al. Akt stimulates aerobic glycolysis in cancer cells. Cancer Res 2004; 64: 3892-3899.
39. Iwasaki H, Suda T. Cancer stem cells and their niche. Cancer Sci 2009; 100: 1166-1172.
40. Moeller BJ, Dreher MR, Rabbani ZN et al. Pleiotropic effects of HIF-1 blockade on tumor radiosensitivity. Cancer Cell 2005; 8: 99-110.
41. Jain RK, Finn AV, Kolodgie FD et al. Antiangiogenic therapy for normalization of atherosclerotic plaque vasculature: a potential strategy for plaque stabilization. Nat Clin Pract Cardiovasc Med 2007; 4: 491-502.
42. Bao S, Wu Q, Sathornsumetee S et al. Stem cell-like glioma cells promote tumor angiogenesis through vascular endothelial growth factor. Cancer Res 2006; 66: 7843-7848.
43. Calabrese C, Poppleton H, Kocak M et al. A perivascular niche for brain tumor stem cells. Cancer Cell 2007; 11: 69-82.
44. Kai K, Arima Y, Kamiya T, Saya H. Breast cancer stem cells. Breast Cancer 2009.
45. Conley SJ, Gheordunescu E, Kakarala P et al. Antiangiogenic agents increase breast cancer stem cells via the generation of tumor hypoxia. Proc Natl Acad Sci U S A 2012; 109: 2784-2789.
46. Benjamin RS WP, Bachur NR. Adriamycin: a new effective agent in the therapy of disseminated sarcomas. Med Pediatr Oncol 1975; 1: 63.
47. Haas NB LX, Manola J, Pins M, Liu G, McDermott D, Nanus D, Heath E, Wilding G, Dutcher J. A phase II trial of doxorubicin and gemcitabine in renal cell carcinoma with sarcomatoid features: ECOG 8802. Med Oncol 2012; 29: 761-767.
48. Moulder S HT, Janku F, Wheler J, Moroney J, Booser D, Albarracin C, et al. Inhibition of the phosphoinositide 3-kinase pathway for the treatment of patients with metastatic metaplastic breast cancer. Ann Oncol 2015; 26: 1346-1352.
49. Gonzalez-Angulo AM AA, Liu S, Green MC, Murray JL, Chen H, Palla SL, et al. Open-label randomized clinical trial of standard neoadjuvant chemotherapy with paclitaxel followed by FEC versus the combination of paclitaxel and everolimus followed by FEC in women with triple receptor-negative breast cancer. Ann Oncol 2014; 25: 1122-1127.
50. Vitolo MI WM, Szmacinski M, Tahir K, Waldman T, Park BH, et al. Deletion of PTEN promotes tumorigenic signaling, resistance to anoikis, and altered response to chemotherapeutic agents in Human Mammary Epithelial Cells. Cancer Res 2009; 69: 8275-8283.
51. Blick T, Widodo E, Hugo H et al. Epithelial mesenchymal transition traits in human breast cancer cell lines. Clin Exp Metastasis 2008; 25: 629-642.
52. Keller PJ, Lin AF, Arendt LM et al. Mapping the cellular and molecular heterogeneity of normal and malignant breast tissues and cultured cell lines. Breast Cancer Res 2010; 12: R87.
53. Sethi S, Sarkar FH, Ahmed Q et al. Molecular markers of epithelial-to-mesenchymal transition are associated with tumor aggressiveness in breast carcinoma. Transl Oncol 2011; 4: 222-226.
54. Jeong H, Ryu YJ, An J et al. Epithelial-mesenchymal transition in breast cancer correlates with high histological grade and triple-negative phenotype. Histopathology 2012; 60: E87-95.

55. Al Sayed AD, El Weshi AN, Tulbah AM et al. Metaplastic carcinoma of the breast clinical presentation, treatment results and prognostic factors. Acta Oncol 2006; 45: 188-195.
56. Pharmacology C. Clinical Pharmacology: Doxil. In. 2008.
57. FDA. FDA label information: Doxil. In. 2007.
58. FDA. FDA label: Bevacizumab. In. 2008.
59. Pharmacology C. Clinical Pharmacology: Bevacizumab. In. 2008.
60. Sikov WM BD, Perou CM, Singh B, Cirrincione CT, Tolaney SM, Kuzma CS, Pluard TJ, et al. Impact of the addition of carboplatin and/or bevacizumab to neoadjuvant once-per-week paclitaxel followed by dose-dense doxorubicin and cyclophosphamide on pathologic complete response rates in stage II to III triple-negative breast cancer: CALGB 40603 (Alliance). J Clin Oncol 2015; 33: 13-21.
61. FDA. FDA label: Temsirolimus. In. 2007.
62. FDA. FDA Label: Everolimus. In 2012.
63. Meric-Bernstam F, Mills GB. Mammalian target of rapamycin. Semin Oncol 2004; 31: 10-17; discussion 33.
64. Pharmacology C. Clinical Pharmacology: Temsirolimus. In. 2008.
65. Yeo W, Lam KC, Zee B et al. Hepatitis B reactivation in patients with hepatocellular carcinoma undergoing systemic chemotherapy. Ann Oncol 2004; 15: 1661-1666.
66. Loomba R, Rowley A, Wesley R et al. Systematic review: the effect of preventive lamivudine on hepatitis B reactivation during chemotherapy. Ann Intern Med 2008; 148: 519-528.
67. Krishna G, Moton A, Ma L et al. Effects of oral posaconazole on the pharmacokinetic properties of oral and intravenous midazolam: a phase I, randomized, open-label, crossover study in healthy volunteers. Clin Ther 2009; 31: 286-298.
68. Barbareschi M, Buttitta F, Felicioni L et al. Different prognostic roles of mutations in the helical and kinase domains of the PIK3CA gene in breast carcinomas. Clin Cancer Res 2007; 13: 6064-6069.
69. Perez-Tenorio G, Alkhori L, Olsson B et al. PIK3CA mutations and PTEN loss correlate with similar prognostic factors and are not mutually exclusive in breast cancer. Clin Cancer Res 2007; 13: 3577-3584.