# Circulating FGF21 Levels and Efficacy of Exemestane, Everolimus and Metformin in Postmenopausal Women with Hormone Receptor Positive Metastatic Breast Cancer and BMI ≥25

**Chair:** **Vicente Valero, MD, F.A.C.P.**
Professor
Department of Breast Medical Oncology
The University of Texas MD Anderson Cancer Center
1515 Holcombe Boulevard, Unit 1354
Houston, TX 77030
Tel: (713) 792-2817
Fax: (713) 563-0739
vvalero@mdanderson.org

**Co-Chair:** **Sai-Ching J. Yeung, MD, PhD**
Associate Professor
Department of Emergency Medicine
Department of Endocrine Neoplasia and Hormonal Disorders

The University of Texas MD Anderson Cancer Center

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**List of abbreviations**

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|---|---|
| ABC | Advanced or Metastatic Breast Cancer: Advanced Breast Cancer |
| ADR | Adverse Drug Reaction |
| AE | Adverse Event |
| AI | Aromatase Inhibitor |
| AKT / PKB | Protein Kinase B |
| ALT | Alanine Aminotransferase / Glutamic Pyruvic Transaminase / sGPT |
| ALP | Alkaline Phosphatase |
| ANC | Absolute Neutrophil Count |
| aPTT | activated Partial Thromboplastin Time |
| ASCO | American Society of Clinical Oncology |
| AST | aspartate aminotransferase |
| AUC | Area Under the Concentration Time Curve |
| BAP | Bone Alkaline Phosphatase |
| BC | Breast Cancer |
| bFGF | Basic Fibroblast Growth Factor |
| BSA | Body Surface Area |
| BUN | Blood Urea Nitrogen |
| CBC | Complete Blood Count |
| CBR | Clinical Benefit Rate |
| CFR | Code of Federal Regulation |
| CI | Confidence Interval |
| CL/F | Oral Clearance |
| Cmax | Maximum Concentration |
| CMC | Chemistry / Manufacturing / Controls |
| CNS | Central Nervous System |
| CPK | Creatine Phosphokinase |
| CPO | Country Pharma Organization |
| CR | Complete Response |
| CRD | Clinical Research and Development |
| CRF | Case Report/Record Form |
| CRO | Contract Research Organization |
| CT | Computed Tomography |
| CTA | Clinical Trials Application |
| CTC | Common Terminology Criteria |
| CTCAE | Common Terminology Criteria for Adverse Events |
| CTX | C-terminal cross linking telopeptide of type I collagen |
| CV | Coefficient of Variation |
| CYP | Cytochrome P450 |
| CYP3A4 | Cytochrome P450 3A4 isoenzyme |
| DCR | Disease Control Rate |
| DDI | Drug-Drug Interaction |

| | |
|---|---|
| DLCO | Diffusing Capacity for Carbon Monoxide |
| DLT | Dose Limiting Toxicities |
| DNA | Deoxyribonucleic Acid |
| ECG | Electrocardiogram |
| ECOG | Eastern Cooperative Oncology Group |
| (e)CRF | (electronic) Case Report / Record Form |
| EDC | Electronic Data Capture |
| EDTA | ethylenediaminetetraacetic acid |
| EGFR | Epidermal Growth Factor Receptor |
| eIF | eukaryotic translation initiation factor |
| 4E-BP1 | 4E-binding proteins 1 |
| ELISA | Enzyme Linked ImmunoSorbent Assay |
| EMEA | European Medicines Evaluation Agency |
| EORTC | European Organization for Research and Treatment of Cancer |
| EOT | End of Treatment |
| ER | Estrogen Receptor |
| EU | European Union |
| FAS | Full Analysis Set |
| FDA | Food and Drug Administration (USA) |
| FEV1 | Forced Expiratory Volume in one second |
| GCP | Good Clinical Practice |
| GGT | Gamma-Glutamyltransferase |
| GI | Gastrointestinal |
| GM-CSF | Granulocyte Macrophage Colony Stimulating Factor |
| GTP | Guanosine Triphosphate |
| HER2 | Human Epidermal Growth Factor Receptor 2 |
| Hgb | Hemoglobin |
| HIF | Hypoxia-Inducible Factor |
| HIV | Human Immunodeficiency Virus |
| HMG-CoA | 3-Hydroxy-3methyl-glutaryl coenzyme A |
| HPLC | High Performance Liquid Chromatography |
| HR | Hazard Ratio |
| HR | Hormone Receptor |
| HUMECS | Human Umbilical Vein Endothelial Cells |
| i.v. | intravenous(ly) |
| IB | Investigator's Brochure |
| IC50 | Inhibitory Concentration at 50% |
| ICH | International Conference on Harmonization |
| IDMC | Independent Data Monitoring Committee |
| IEC | Independent Ethics Committee |
| IHC | Immunohistochemistry |
| IMS | Integrated Medical Safety |
| IND | Investigational New Drug Application (USA) |

| | |
|---|---|
| INR | International Normalized Ratio IRB Institutional Review Board |
| ITT | Intent-To-Treat |
| LDH | Lactate Dehydrogenase |
| LDL | Low-Density Lipoprotein |
| LH-RH | Luteinizing hormone-releasing hormone |
| MBC | Metastatic Breast Cancer |
| MPD | Molecular Pharmacodynamics MRI Magnetic Resonance Imaging |
| mTOR | mammalian Target of Rapamycin |
| NADPH | Nicotinamide Adenine Dinucleotide Phosphate |
| NCCN | National Comprehensive Cancer Network |
| NCI | National Cancer Institute |
| PR | Progesterone Receptor |
| PI3K | Phosphatidylinositol 3-Kinase |
| PK | Pharmacokinetics |
| PLGF | Placental Growth Factor |
| PR | Partial Response |
| PS | Performance Status |
| PTEN | Phosphatase and Tensin Homolog (deleted on chromosome 10) |
| QoL | Quality of Life |
| RAPTOR | Regulatory Associated Protein of mTOR |
| RBC | Red Blood Cell Count |
| RECIST | Response Evaluation Criteria In Solid Tumors |
| RIA | Radioimmunoassay |
| RICTOR | Rapamycin-Insensitive Companion of mTOR |
| RNA | Ribonucleic acid |
| S6K1 | serine / threonine kinase p70S6 kinaseS6 Kinase 1 |
| SAE | Serious Adverse Events |
| SD | Stable Disease |
| SmPC | Summary of Product Characteristics |
| SNPs | Single Nucleotide Polymorphisms |
| SOP | Standard Operating Procedure |
| t1/2 | Half Life |
| TKI | Tyrosine Kinase Inhibitor |
| tmax | Time to Maximum Concentration |
| TTP | Time to Progression |
| ULN | Upper Limit of Normal |
| WBC | Total White Blood Cell Count |
| WHO | World Health Organization |

# 1.0 OBJECTIVES

## 1.1 Primary Objective

The primary objective of this study is to assess the efficacy of exemestane and everolimus combined with metformin in overweight and obese post-menopausal women with metastatic breast cancer. The primary endpoint is progression-free survival (PFS), defined as the time from the date of registration to the date of the first documented progression or death due to any cause.

**For patients with measurable disease at baseline**, progression will be determined according to RECIST **1.1** [1].

**In the absence of measurable disease at baseline**, patients with bone lesions only, lytic or mixed (lytic + sclerotic), will be allowed to enter the study and the following will be considered disease progression among these patients:

- The appearance of one or more new lytic lesions in bone
- The appearance of one or more new lesions in tissues other than bone
- Unequivocal progression of the size(s) of existing bone lesion(s)

## 1.1 Secondary objectives

1.1.1 To determine the predictive role of serum FGF21 levels in overweight and obese postmenopausal women with metastatic breast cancer treated with exemestane, everolimus and metformin on PFS.

1.1.2 To identify novel predictive markers of response to metformin added to exemestane and everolimus in overweight and obese postmenopausal women with hormone receptor-positive metastatic breast cancer.

1.1.3 To assess the overall response rate (ORR) and the clinical benefit rate (CBR) in this population. ORR is defined as the proportion of patients whose best overall response is either complete response (CR) or partial response (PR) according to RECIST. CBR is defined as the proportion of patients with CR, PR or stable disease (SD) with a duration of 24 weeks or longer.

1.1.4 To determine the tolerability and toxicity of exemestane, everolimus and metformin in this population. Safety will be assessed by the Common Terminology Criteria (CTCAE), version 3.0. Incidence of adverse events, serious adverse events, changes from baseline in vital signs and laboratory results (hematology, blood chemistry) will be reported.

## 2.0 BACKGROUND

### 2.1 Obesity and breast cancer

Breast cancer is the most common cancer in women, and the second leading cause of female cancer death. More than 200,000 women are diagnosed with breast cancer yearly, and 40,000 breast cancer related deaths were expected in 2011 in the US [2, 3]. There is a need to identify and take steps to alter modifiable breast cancer risks. Among the identified risk factors for breast cancer [4], **obesity** in postmenopausal women and use of **estrogen** are two readily modifiable risk factors. Unfortunately, the prevalence of obesity is rapidly increasing and has reached epidemic proportions.

*The Rising Prevalence of Obesity:* The World Health Organization (WHO) recognized obesity as one of the top 10 global health problems. The WHO estimates that around one billion people throughout the world are overweight and that over 300 million of these are obese and if current trends continue, the number of overweight persons will increase to 1.5 billion by 2015 (http://www.who.int). Overweight and obesity are defined by the WHO as a BMI of 25–30 and >30 kg/m$^2$, respectively. Normal percentages of fat in the body mass are 9–18% in males and 14–28% in females, but it may be as high as 60–70% in morbid obesity. As a consequence of the worldwide obesity epidemic, there is an explosion of obesity- and overweight-related illnesses [5]. Given the association between breast cancer and obesity, it is critical to address the impact of obesity on breast cancer based on our understanding of the mechanisms by which obesity promotes cancer.

*The Impact of Obesity on Breast Cancer Carcinogenesis:* Obesity is associated with an increased risk of developing postmenopausal breast cancer [6]. Twenty percent of all postmenopausal breast cancers may be attributable to obesity [7]. Up to 50% of postmenopausal breast cancer deaths in the US may be attributed to obesity [8]; the data from the Cancer Prevention Study II supported that up to 18,000 deaths in US women older than 50 years could be avoided if these women were lean (BMI <25 kg/m$^2$) throughout their adult life [8]. Estrogens, both exogenous and endogenous, are etiologic factors for breast cancer [9, 10]. Estrogens stimulate cell proliferation through nuclear receptor-mediated gene regulation as well as other effects that increased mutation rates and aneuploidy. The biosynthesis of estrogens in adipocytes in postmenopausal women is particularly important in the pathogenesis of estrogen receptor (ER)-positive breast carcinoma [11]. Hyperinsulinemia [12-15] is associated with increased risk of breast cancer, and it remains a significant risk factor independent of adiposity or fat distribution [15]. The effect of obesity on breast cancer risk is mediated by both insulin resistance and estrogen metabolism [16]. Both retrospective and prospective observational studies support a potential role of IGFs in breast carcinogenesis [17]. Obese patients also have elevated serum levels of leptin (an adipokine) contributing to increased risk of developing breast cancer compared with lean patients with the normal leptin levels [18]. Adiponectin (another adipokine) is a risk factor for breast cancer in postmenopausal females [19]. Thus, estrogens, adipokines such as leptin and adiponectin, insulin and IGF-1 have been viewed as important factors in estrogen receptor-positive (ER+) breast cancer carcinogenesis.

*The Impact of Obesity on Breast Cancer Progression:* Obesity is also associated with worse prognosis of breast cancer after disease onset [20-23]. Obesity at the time of diagnosis predicts poor prognosis and adverse outcomes in both pre- and post-menopausal women with breast cancer [20]. **Obesity** is a **preventable risk factor for breast cancer death** [21]. The adverse effect of obesity on breast cancer prognosis is not an artifact of delayed diagnosis in obese patients, but is due to hormonal changes associated with obesity [24]., Obese women had 35% more estrone and 130% more estradiol in the plasma than lean women [25]. Concentrations of free estradiol are 2-3 times higher in obese women than in lean women. These hormonal differences potentially explain the association between obesity and breast cancer prognosis [25]. IGF-1 protected breast cancer cells from apoptosis induced by chemotherapy [26]. Inhibition of IGF-I receptor results in suppression of breast cancer cell adhesion, invasion, and metastasis [27]. Adipokine, estrogen and insulin/IGF-1 signaling pathways are again involved in the impact of obesity on breast cancer progression.

*Molecular Mechanisms Mediating the Impact of Obesity on Breast Cancer:* In breast cancer, at least three signaling pathways have been postulated to explain its association with breast cancer: **insulin/IGF-1**, **adipokines** and **estrogens**. There are **synergistic interactions** among these pathways. The combination of estradiol and IGF-1 results in synergistic proliferation of breast cancer cells [28]. Estrogens stimulate breast cancer proliferation at least in part via paracrine/autocrine growth factors (TGF-$\alpha$ and IGF-1) [29, 30], their receptors and down-stream signaling components [31]. For instance, the 5'-untranslated region of the TGF-$\alpha$ gene contains two imperfect estrogen responsive elements (EREs) [32, 33], and IGF-I receptor is induced by interactions between ER-$\alpha$ and Sp1 in breast cancer cells [34]. Estradiol induces the expression of insulin receptor substrate 1 (IRS-1) and the p85/p110 subunits of phosphoinositide 3' kinase (PI3K) [35], and also enhanced tyrosine phosphorylation of IRS-1 after IGF-1 stimulation, followed by enhanced mitogen activated protein kinase (MAPK) and protein kinase B (Akt) activation. Estradiol also enhances the effect of IGF-1 on expression of cyclin D1 and cyclin E, and phosphorylation of retinoblastoma protein (Rb) [28, 36, 37]. Estradiol stimulated c-Myc expression but had little direct effect on expression of c-Jun, Jun B, Jun D [38], or c-Fos, but it induces expression of TGF-$\alpha$ [29, 33] which induces expression of c-Jun, Jun B, and c-Fos [38]. Similarly, while estradiol has no effect on p21, it induces IGF-1 which increases p21 expression and reduces p27 expression [36].

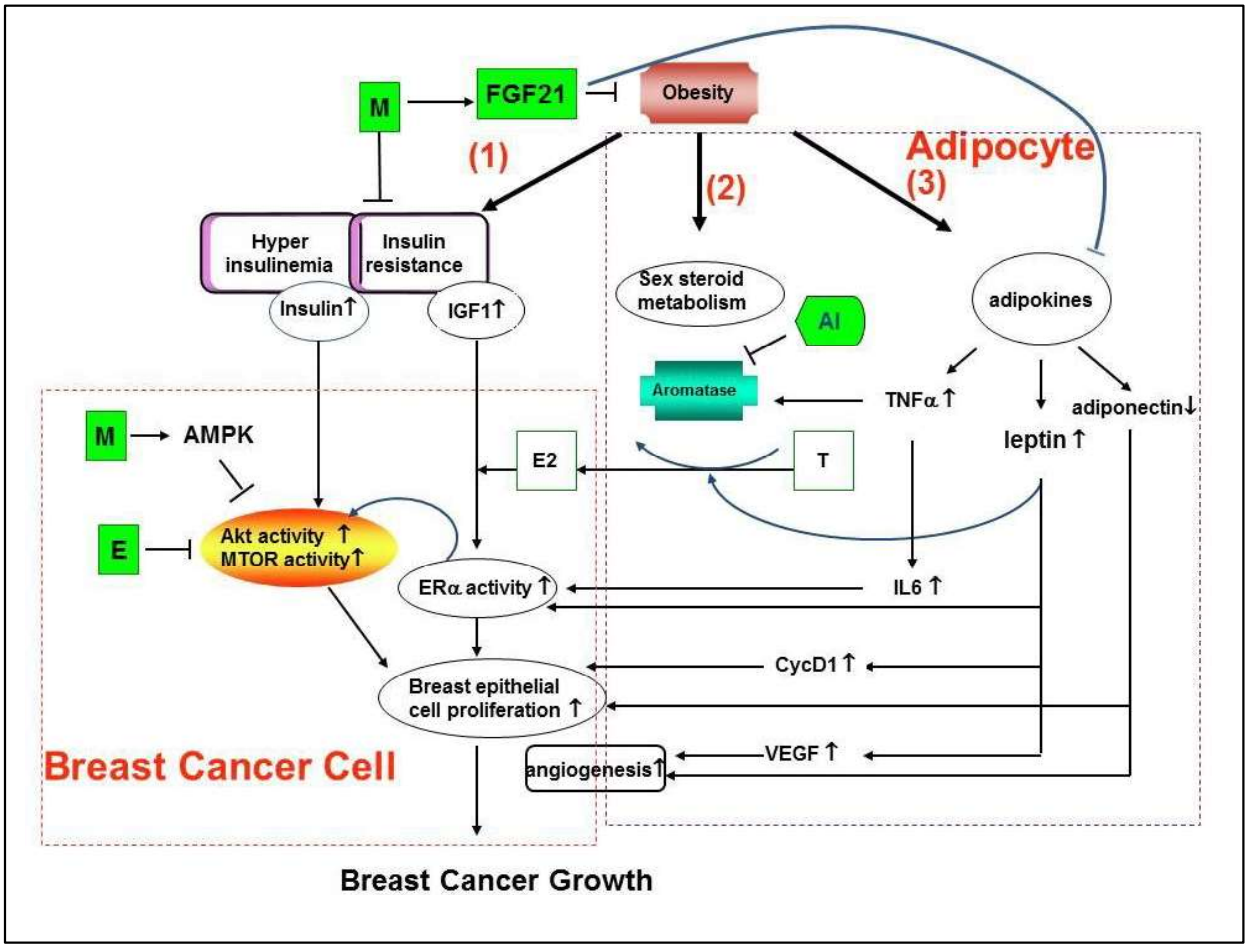


Figure 1. A molecular model for the impact of obesity on breast cancer. Obesity triggers three signaling pathways to promote breast cancer cell growth: (1) High levels of insulin and IGF-I caused by obesity can stimulate breast cancer cell growth. (2) Adipocyte, serving as an endocrine cell, can modify sex steroid metabolism leading to production of estrogen. (3) Adipocyte releases a number of hormones or cytokines, including TNFα, leptin, and adiponectin. As a result, these factors can stimulate or promote breast cancer cell growth or angiogenesis. Obese postmenopausal breast cancer patients have more estrogen and more IGF-1 and insulin than lean women, thus the combination of anti-obesity or insulin-sensitizing drugs with aromatase inhibitors may serve as an important treatment strategy for obese ER+ postmenopausal breast cancer patients. M, metformin; Ev, everolimus; E2, estradiol; FGF21, fibroblast growth factor-21; AI, aromatase inhibitor; AMPK, adenosine 5-monophosphate (AMP)-activated protein kinase; PTEN, phosphatase and tensin homolog; ER , estrogen receptor; mTOR, mammalian target of rapamycin; E2, estradiol; IL6, interleukin 6; Akt, protein kinase B; TNF, Tumor necrosis factor; IGF, insulin like growth factor; VEGF, Vascular endothelial growth factor; CycD1, Cyclin D1.

While estrogens stimulate expression of IGF-1 and components in its signaling pathway, IGF-1 reciprocates. The IGF-1 ligand-receptor system is an important regulator of metabolic enzymes

involved in estrogen production. IGF-I activates transcription of endogenous ER in human breast cancer cells [39]. IGF-1 potentiates the effects of estradiol on ERE sites [40]. Inhibition of estrogen action by IGFBP-1 suggests that IGF-1 signaling may be required for maximal activation of estrogen receptors [39].

Breast cancer cells express adiponectin receptors (AdipoR1 and AdipoR2) but not adiponectin. Expression of AdipoR1 is higher in breast cancer than in adjacent or control tissues [41]. Lean women with high adiponectin levels had a substantial reduction in the risk of breast cancer. Adiponectin inhibits angiogenesis by inducing endothelial cell apoptosis [42]. In contrast, it had no effect on breast cancer cell apoptosis, and it inhibits breast cancer cell proliferation by activation of ERK1/2 but not AMP-activated protein kinase (AMPK) or p38MAPK [41].

The molecular basis for the link between obesity and breast cancer is formed by the complex interaction of at least three signaling pathways: **insulin/IGF-1**, **adipokines** and **estrogens** (Figure 1). There is crosstalk among the pathways leading to amplification of mitogenic signal [43]. Estrogen signaling may be especially important in obese **postmenopausal** patients with **ER+** breast cancer. Therefore, obesity and these signaling pathways are therapeutic targets for obese breast cancer patients.

## 2.2 Treatment options for ER/PR positive advanced breast cancer

Treatment goals for advanced breast cancer (ABC) are palliative in nature, primarily focused at reducing tumor size, slowing progression and metastasis and reducing complications such as fatigue, bone fracture and hypercalcemia.

The presence of estrogen receptor (ER) and/or progesterone receptor (PR) is one of the most important predictive and prognostic markers in human breast cancers. Approximately 70% of all invasive breast cancers are positive for ER and/or PR expressions at the time of diagnosis. Consequently, anti-estrogen therapies that antagonize ER functions (such as tamoxifen) or inhibit estrogen production (e.g. aromatase inhibitors [AIs]) have been extensively developed in oncology [44]. Deprivation of estrogenic signaling with the anti-estrogen tamoxifen has been the main form of hormonal treatment for over 30 years. Tamoxifen is indicated for the treatment across the whole continuum of breast cancer, ranging from risk reduction for women with increased risk of breast cancer, as an adjuvant treatment and also for metastatic disease. Besides tamoxifen, progesterone analogues or progestins have been used for the treatment of breast cancer for almost 50 years. Although the exact mechanism of action of progestins has not been established, their effect on estrogen levels is thought to play a role. Megestrol acetate, a synthetic derivative of progesterone, is the only progestin indicated in the USA for use in postmenopausal ABC patients. Once the standard second-line therapy after tamoxifen, progestins are now mainly used for patients whose cancer does not respond well to other hormonal treatments.

While therapies which interfere with ER functions such as tamoxifen have significantly contributed to mortality reduction in advanced breast cancer patients, at best 50-60% of ER-positive patients respond to anti-estrogen therapy. Consequently, a number of aromatase inhibitors (AIs) that reduce peripheral estrogen synthesis have been developed for the treatment

of ABC. The AIs block the conversion of androgens to estrogens, which is the primary way estrogens are produced in post-menopausal women. Aminoglutethimide, a first generation AI, was the first non-steroidal (reversible-competitive) inhibitor of aromatase evaluated clinically for the treatment of ABC. For many years, Aminoglutethimide was considered suitable second-line therapy after tamixofen, as an alternative to progestins. Prompted by the efficacy of aminoglutethimide, more selective, less toxic, reversible second generation AIs were developed [45]. At present, third generation aromatase inhibitors have been approved for use in postmenopausal women with hormone receptor-positive BC after tamoxifen. The third generation AIs can be broadly classified into two groups: non-steroidal aromatase inhibitors (NSAI), mainly letrozole and anastrozole and steroidal aromatase inactivator, exemestane.

Everolimus and letrozole synergistically inhibit proliferation in BC cells. This combination (L-R) was evaluated in a randomized double blind phase II trial against letrozole + placebo (L-P) as a 4-month neoadjuvant treatment for postmenopausal women with early BC. 270 patients were enrolled in this trial (138 L-R vs. 132 L-P). Response rates on L-R and L-P were 68% vs. 59% (palpation, $p = 0.062$) and 58% vs. 47% (ultrasound, $p= 0.021$) respectively. Pharmacodynamic changes in each treatment arm were observed. Marked downregulation in progesterone receptor and cyclin D1 were seen in response to letrozole. Phospho-S6 levels showed dramatic down-regulation only in response to everolimus. Cell cycle response, as defined by the proportion of patients with $< 2.7$ % $Ki67^{+}$ tumor cells at day 15, was also significantly higher in the everolimus + letrozole arm (57% everolimus treated patients vs. 30% placebo treated patients were cell cycle responder at day 15, $p<0.01$). Baselga and collaborators [46] concluded that everolimus significantly increased the efficacy of letrozole in newly diagnosed ER+ BC, with regard to both clinical and cell cycle response. The increased cell cycle response rate on everolimus was found in all subsets of tumors, including PTEN-positive, PI3KCA wild-type tumors. The most common grade 3/4 adverse events in the L-R arm were hyperglycemia (5%), stomatitis (2%), pneumonitis (2%), and infections (2%). All three cases of grade 3 pneumonitis completely resolved after discontinuation of everolimus. Daily therapy with everolimus plus letrozole (NSAI) showed no PK interaction. This trial demonstrated that mTOR inhibition provides additional efficacy to long term estrogen deprivation and has an acceptable level of tolerability in the neoadjuvant setting. A daily dose of everolimus 10 mg was recommended for further trials [46].

A randomized phase II study comparing two schedules of everolimus (10 mg daily and 70 mg weekly) in patients with recurrent/metastatic breast cancer [47] also demonstrated that in general, adverse effects were those predicted from preclinical and early clinical studies [48] including hyperglycemia and hyperlipidemia (generally in patients with preexisting abnormalities), and were reversible. The most common grade 3 or 4 adverse events were fatigue, non infectious pneumonitis and neutropenia. Pneumonitis occurred more frequently than anticipated, but was reversible in all affected patients and in general, manageable, although some patients required discontinuation. None of the 16 patients recruited to the weekly 70 mg arm responded, with four patients with a stable disease. Among the 33 patients treated in the daily 10 mg arm, response was evaluable in 30 patients; one patient had a complete response, three patients had a partial response and 15 patients had stable disease. The four responding patients were ER+ and the HER2 status was normal in 3 patients and unknown in one. As previously demonstrated in a

pharmacodynamic model [49], supported by a clinical tumor pharmacodynamic study [48], in which the 10mg daily dosage was achieving more profound and sustained suppression of mTOR activity than could be achieved with weekly dosing, Ellard and coworkers [47] suggested that continuous daily dosing of everolimus, but not weekly dosing, has single agent activity in this disease setting. While ER positive and HER-2 normal status appeared to be predictive for response and/or prolonged stable disease, no associations were noted between molecular markers and efficacy.

Everolimus in combination with trastuzumab and paclitaxel or vinorelbine is also being studied in patients with HER2-overexpressing metastatic breast cancer. In a phase I trial of daily and weekly everolimus in combination with weekly vinorelbine and trastuzumab in heavily pretreated patients with HER2-overexpressing MBC with prior resistance to trastuzumab, everolimus was well tolerated at doses of 5 mg daily and 30 mg weekly. The main toxicities were neutropenia and stomatitis. Efficacy data based on the 25 patients on the 5mg daily arm showed 20 patients with clinical benefit (ORR= 20%, 1 CR, 4 PR). The median duration of progression free survival was 32 weeks. The combination also showed encouraging responses in the subgroup of patients who were resistant to both taxanes and trastuzumab. The dose of everolimus selected for further development was 5 mg daily [50]. In another phase I of daily and weekly everolimus in combination with weekly paclitaxel and trastuzumab in patients with HER2 overexpressing MBC with prior resistance to trastuzumab, everolimus was well tolerated at doses of 5 mg and 10 mg daily and 30 mg weekly. The main toxicities were blood cell disorders, stomatitis and metabolic disorders . The combination showed encouraging responses in heavily pretreated patients including patients refractory to both taxanes and trastuzumab. Disease Control Rate (CR+PR+SD) was 85%. The dose of everolimus selected for the phase II part of the trial was 10 mg daily [51]. Morrow and collaborators completed a phase I/II trial of trastuzumab and everolimus in patients with metastatic breast cancer who had progressed after trastuzumab-based chemotherapy. The dose of everolimus was 10 mg orally daily. The regimen was well tolerated, and the clinical benefit rate was 30% [52].

Baselga and collaborators [53] completed a multicenter randomized phase III trial of everolimus in combination with exemestane versus exemestane with placebo in postmenopausal women with advanced breast cancer who were refractory to letrozole or anastrozole. This study showed a significant prolongation in progression-free survival for patients treated with exemestane plus everolimus. However, patients continue to develop progressive disease and novel therapeutic approaches are needed. Furthermore, limited outcome data are available for this combination in overweight and obese women.

## 2.3 Metformin as potential cancer therapy

Metformin has become the most common anti-diabetic medication for Type II diabetes [54]. Metformin reduces levels of circulating glucose, increases insulin sensitivity, and reduces insulin resistance-associated hyperinsulinemia. Experimental data indicate metformin also has anticancer activity in breast cancer models [55, 56]. Epidemiological and retrospective data support the antineoplastic effects of metformin, and provide the rationale to study the role of metformin for breast cancer therapy in a variety of clinical settings [57]. In one study, patients

with breast cancer and diabetes mellitus had a higher pathologic response to neoadjuvant chemotherapy if treated with concomitant metformin [58]. The North American Breast Cancer Intergroup is participating in a large randomized trial (MA.32, led by NCI-Canada) to determine the efficacy and safety of metformin in the adjuvant setting in patients with early-stage breast cancer who have completed local regional therapy and adjuvant chemotherapy.

### 2.4 Fibroblast growth factor 21 (FGF21)

FGF21 is a metabolic hormone devoid of mitogenic activity despite being a member of the FGF family [59]. It is a starvation hormone that regulates lipid metabolism and insulin sensitivity and is currently under clinical development as pharmacologic interventions for type 2 diabetes mellitus and obesity. Preclinical data generated by our PROMISE grant team showed that:

a) FGF21 increased adiponectin secretion while it decreased the majority of other adipokines secreted by mature adipocytes in cell culture.
b) Metformin increased the circulating level of FGF21 in a mouse model of obesity and hormone receptor-positive breast cancer.

## 3.0 STUDY RATIONALE

The molecular basis for the link between obesity and breast cancer is formed by the complex interaction of at least three signaling pathways: estrogens, insulin/IGF-1/PI3K and adipokines. There is crosstalk among the pathways leading to amplification of mitogenic signal. Estrogen signaling may be especially important in overweight and obese postmenopausal patients with ER/PR+ breast cancer. Therefore, overweight, obesity and these signaling pathways are therapeutic targets in this population.

**Our over-arching hypothesis is that the biochemical effects of metformin on insulin/IGF-1, adipokines and the PI3K pathway will combine with the anti-estrogenic effects of exemestane and inhibition of mTOR signaling by everolimus to improve progression-free survival in postmenopausal overweight and obese women with ER/PR+ metastatic breast cancer.** Synergistic crosstalk between estrogen, adipokine and insulin/IGF-1/AKT/mTOR signaling underlies the adverse impact of obesity on postmenopausal ER/PR+ breast cancer. While aromatase inhibitors decrease estrogen, metformin improves insulin resistance and lowers insulin and IGF-1 levels. In addition, metformin therapy has been associated with inhibition of the mTOR signaling pathway through activation of AMPK and may act synergistically together with the mTOR inhibitor everolimus.

## 4.0 ELIGIBILITY CRITERIA

### 4.1 Inclusion criteria

1. Postmenopausal overweight or obese women with a history of biopsy-proven hormone receptor-positive breast cancer and clinical evidence of metastatic disease. Overweight is

defined as body mass index (BMI) of 25 – 29.9 kg/$m^2$ while obese is defined as BMI ≥ 30 kg/$m^2$. Postmenopausal status is defined by one of the following: a) no spontaneous menses for over 1 year, in women ≥55 years; b) no spontaneous menses within the past 1 year in women < 55 years with postmenopausal gonadotrophin levels (LH and FSH levels > 40 IU/L) or postmenopausal estradiol levels (by local laboratory range); or c) bilateral oophorectomy.

2. Prior hormonal therapy for metastatic breast cancer is allowed. Patients who develop progressive metastatic disease on a non-steroidal aromatase inhibitor are eligible. Patients who develop metastatic disease while receiving a non-steroidal aromatase inhibitor in the adjuvant setting are eligible.

3. One prior chemotherapy line for metastatic breast cancer is allowed if there is evidence of progressive disease. Patients treated with chemotherapy to best response and no evidence of progression are not eligible.

4. Prior tamoxifen, LH/RH agonist, anastrozole or letrozole therapy in the adjuvant and/or neoadjuvant settings is allowed. Prior adjuvant and/or neoadjuvant chemotherapy is allowed.

5. Patients must have: [1] at least one lesion that can be accurately measured in at least one dimension ≥ 20 mm with conventional imaging techniques or ≥ 10 mm with spiral CT or MRI; or [2] bone lesions: lytic or mixed (lytic + sclerotic) in the absence of measurable disease; the following will be considered disease progression among these patients: a) the appearance of one or more new lytic lesions in bone; b) the appearance of one or more new lesions outside of bone; c) unequivocal progression of existing bone lesions.

6. Localized radiotherapy, which does not influence the signal of evaluable lesion, is allowed prior to the initiation of study medications.

7. ECOG performance status ≤ 2.

8. Absolute neutrophil count (ANC) >/= 1000/microliter, platelets >/= 75,000/microliter, hemoglobin >/= 8.5 gm/dL; creatinine clearance >60 mg/dL; bilirubin < 1.5 mg/dL (</= 3 × ULN for patients known to have Gilbert Syndrome); ALT <3 x upper limit of normal (or </= 5 if hepatic metastases are present); alkaline phosphatase < 3 x upper limit of normal; calcium </= 11.0 mg/dL.

9. Fasting serum cholesterol ≤ 300 mg/dl or 7.75 mmol/L and fasting triglycerides ≤ 2.5 × ULN. In case one or both of these thresholds are exceeded, the patient can only be included after initiation of statin therapy and when the above mentioned values have been achieved.

10. Bisphosphonate treatment is permitted for the management of bone loss and/or bone metastases.

11. Patients must be competent to give informed consent and to state that they understand the investigational nature of the proposed treatment.

### 4.2 Exclusion criteria

1. HER2-overexpressing breast cancer (IHC 3+ staining or in situ hybridization positive).

2. Diabetes mellitus on active treatment or hemoglobin A1C $\geq$ 6.5% or random plasma glucose > 200 mg/dL in patients without known diabetes.

3. Treatment with metformin in the 30 days prior to enrollment.

4. Known hypersensitivity or intolerance to metformin.

5. Previous treatment with exemestane or mTOR inhibitors.

6. Known hypersensitivity to mTOR inhibitors, e.g. sirolimus (rapamycin).

7. History of acromegaly, Cushing's syndrome, Cushing's disease, Addison's disease (treated or untreated).

8. Patients with unstable angina, uncontrolled ischemic cardiac disease or symptomatic congestive heart failure (e.g. Class III or IV New York Heart Association's Functional Classification).

9. Other investigational drugs within the past 3 weeks or concurrently.

10. Patients with known chronic liver diseases (e.g., chronic active hepatitis, and cirrhosis).

11. Another malignancy within 5 years prior to registration, with the exception of adequately treated in-situ carcinoma of the cervix, uteri, basal or squamous cell carcinoma or non-melanomatous skin cancer.

12. Radiotherapy within four weeks prior to registration except in case of localized radiotherapy for analgesic purpose or for lytic lesions at risk of fracture which can then be completed within two weeks prior to registration. Patients must have recovered from radiotherapy toxicities.

13. History of brain or other central nervous system metastases.

14. Bilateral diffuse lymphangitic carcinomatosis.

15. Presence of life-threatening metastatic visceral disease, defined as extensive hepatic involvement, or any degree of brain or leptomeningeal involvement (past or present), or symptomatic pulmonary lymphangitic spread. Subjects with discrete pulmonary

parenchymal metastases are eligible, provided their respiratory function is not compromised as a result of disease.

16. Patients receiving concomitant immunosuppressive agents or chronic corticosteroids use, at the time of study entry except in cases outlined below: Topical applications (e.g. rash), inhaled sprays (e.g. obstructive airways diseases), eye drops or local injections (e.g. intra-articular) are allowed. Patients on stable low dose of corticosteroids for at least two weeks before enrollment are allowed.

17. Any severe and / or uncontrolled medical conditions such as:

- Unstable angina pectoris, symptomatic congestive heart failure, myocardial infarction ≤6 months prior to enrollment, serious uncontrolled cardiac arrhythmia;
- Uncontrolled diabetes as defined by fasting serum glucose > 1.5 × ULN;
- Acute and chronic, active infectious disorders (except for Hep B and Hep C positive patients) and nonmalignant medical illnesses that are uncontrolled or whose control may be jeopardized by the complications of this study therapy;
- Impairment of gastrointestinal function or who have gastrointestinal disease that may significantly alter the absorption of study drugs (e.g., ulcerative disease, uncontrolled nausea, vomiting, diarrhea, malabsorption syndrome);
- Active skin, mucosa, ocular or GI disorders of Grade > 1;
- Significant symptomatic deterioration of lung function. If clinically indicated, pulmonary function tests including measures of predicted lung volumes, DLco, O2 saturation at rest on room air will be considered to exclude restrictive pulmonary disease, pneumonitis or pulmonary infiltrates.

18. Patients being treated with drugs recognized as being strong inhibitors or inducers of the isoenzyme CYP3A (Rifabutin, Rifampicin, Clarithromycin, Ketoconazole, Itroconazole, Voriconazole, Ritinavir, Telithromycin) within the last 5 days prior to registration (List of clinically relevant drug interaction listed in Table 1).

## 5.0 TREATMENT PLAN

Exemestane is formulated as tablets of 25 mg strength (Aromasin, Pfizer, New York NY). Everolimus is formulated as tablets of 5.0 mg strength is commercially available. Metformin is formulated as extended release tablets of 500 mg strength (Glucophage XR, Bristol-Myers Squibb Company, New York, NY).

All patients will take exemestane orally once daily and everolimus 10 mg orally once daily. The starting dose of metformin will be 500 mg daily for 3 days. If there are no dose-limiting toxicities, the dose of metformin will be increased by 500 mg every 3 days to reach the target dose of 2000 mg/day (1,000 mg orally twice daily). Drugs will be taken immediately after a meal at the same time each day.

Patients will be treated until disease progression, unacceptable toxicity, or until the patient

withdraws consent. For patients who are unable to tolerate the protocol-specified dosing schedule, dose adjustments are permitted (Table 2).

## 6.0 PATIENT EVALUATION

Table 6 lists all of the assessments and indicates with an "X" the visits when they are performed. All data obtained from these assessments must be supported in the patient's source documentation.

**Screening and baseline**

Written informed consent must be obtained before any study specific medical procedures are performed.

Screening assessments to confirm eligibility must be performed prior to the first dose of study drug.

- Laboratory baseline assessments (including hematology, chemistry, coagulation, and urinalysis), physical examination including performance status, height and weight must be performed within 28 days prior to first dose of study treatment. **Note: Day 1 laboratory assessments do not need to be repeated if screening is completed within 7 days.**
- Patients with potassium, sodium, and/or calcium levels that are below the LLN at screening must have their potassium, sodium and/or calcium replenished through supplementation and the levels must be within normal limits prior to the first dose of study drug.

All attempts should be made to complete the visits within 48 hours of the day indicated on the evaluation schedule. One month is considered as 4 weeks (i.e., 28 days).

The following assessments must be obtained within 28 days prior to treatment administration to establish eligibility:

- Physical Exam;
- Relevant medical history;
- Current medical conditions;
- Concomitant medications;
- Vital signs, height and weight;
- ECOG performance status;
- Hematology tests include a complete blood count (CBC). A total white blood cell (WBC) with absolute differentials (including neutrophil count plus bands, lymphocyte, monocyte, eosinophil, basophil counts), hemoglobin (Hgb), and a platelet count. Serum Chemistry must include: BUN or uric acid, creatinine, LDH, total protein, electrolytes

(sodium, potassium and calcium), total bilirubin, , albumin, alkaline phosphatase, AST/sGOT and HbA1C after fasting glucose. Serum fasting lipid profile must include: total cholesterol and triglycerides.

- Tumor assessment as standard diagnostic imaging test(s) to determine diagnosis and extent of cancer. These include CT of the chest and abdomen and a bone scan or skeletal survey. Positive areas on bone scans must be assessed by X-ray, CT scan with bone windows or MRI Skin lesions should be photographed in addition to measuring. Brain scan (CT scan or MRI with i.v. contrast) must be performed if CNS symptoms are present.

### 6.1 Assessments During the Study

The following assessments are to be performed at weeks 4, 8, 12, and every 2 months (± 7 days) of medication. Laboratory assessments may be performed up to 3 days before the actual clinic visit in order to allow for flexibility in scheduling.

- History and physical examination;
- ECOG Performance Status;
- Vital signs;
- Record any non-serious and serious adverse events and assign appropriate toxicity grade (NCI CTCAE version 3);
- Record all concomitant medication(s) added and/or changed;
- Hematology tests include a complete blood count (CBC). A total white blood cell (WBC) with absolute differentials (including neutrophil count plus bands, lymphocyte, monocyte, eosinophil, basophil counts), hemoglobin (Hgb), and a platelet count. Serum Chemistry must include: BUN or uric acid, creatinine, LDH, total protein, electrolytes (sodium, potassium and calcium), total bilirubin, , albumin, alkaline phosphatase, AST/HbA1C after fasting glucose. Serum fasting lipid profile must include: total cholesterol and triglycerides.

Tumor assessment of target metastatic lesions using the same imaging modality as baseline (e.g., CT, MRI) will be performed every 2 months (+/-7 days).

## 7.0 DRUG INFORMATION

### 7. 1 Exemestane

Exemestane is 6-methylenandrosta-1,4-diene-3,17-dione. It is structurally related to androstenedione, acting as a false substrate for aromatase, and is processed to an intermediate that binds irreversibly to the active site of aromatase causing “suicide inhibition”. It inhibits the in vivo aromatization of androstenedione to estrone by an average of 97.9% [60]. It significantly lowers circulating estrogen concentrations in postmenopausal women, but has no detectable effect on adrenal biosynthesis of corticosteroids or aldosterone.

Exemestane has demonstrated efficacy in the treatment of postmenopausal patients with ABC [61]. It is indicated for adjuvant treatment of postmenopausal women with Estrogen receptor positive (ER+) early BC who have received two to three years of tamoxifen and are switched to exemestane for completion of a total of five consecutive years of adjuvant hormonal therapy. It is also indicated for the treatment of ABC in postmenopausal women whose disease has progressed following tamoxifen therapy.

The recommended daily dose of exemestane is 25 mg via oral administration. Exemestane is rapidly absorbed from the gastrointestinal tract. Its bioavailability is limited by first-pass metabolism, but is increased when taken with food. Exemestane is widely distributed, and is extensively bound to plasma proteins. It appears to be more rapidly absorbed in women with breast cancer [time to peak blood levels (tmax) of 1.2 hours] than in healthy women (tmax of 2.9 hours). The terminal half life for exemestane is 18-24 hours. The time needed to reach maximal estradiol suppression is 7 days [62-64]. Exemestane is metabolized by CYP3A4 and aldoketoreductases. It does not inhibit any of the major CYP isoenzymes, including CYP 1A2, 2C9, 2D6, 2E1 and 3A4. Although no formal drug-drug interaction studies have been conducted, significant effects on exemestane clearance by CYP isoenzyme inhibitors appear unlikely [64, 65]. A randomized trial compared exemestane to fulvestrant in patients with ER+ metastatic breast cancer who had been previously treated with a non-steroidal AI (anastrozole or letrozole). The time to progression was the same for both groups (3.7 months) [66].

### 7.2 Everolimus

Everolimus has been in clinical development in solid organ transplantation since 1996 and is approved in more than 60 countries. Everolimus is commercially available for use as prophylaxis for organ rejection in adult patients receiving an allogeneic renal or cardiac transplant and is administered in association with cyclosporine and glucocorticoids. Starting in November 2002, everolimus has been in development to treat cancer patients both as monotherapy and in combination with a number of other anticancer agents.

Everolimus is a derivative of rapamycin that acts as a signal transduction inhibitor. The target of this class of agents is mTOR, a multi-functional signal transduction protein, which obtains signals from many upstream inputs, and propagates the information via regulation of multiple downstream pathways. A serine-threonine kinase, mTOR acts as a nutrient sensor and monitor of the cellular metabolic state, regulating protein synthesis and ultimately cell growth and cell proliferation, angiogenesis and survival. mTOR serves a key role in normal mammalian cell physiology, and is centrally involved in tumor-cell physiology, (for example, facilitating cell-cycle progression from G1-S phase) and consequently inhibition of this target has received considerable attention as an anti-cancer approach [67].

mTOR regulates global mRNA translation [68]. Indeed, downstream from mTOR is the serine / threonine kinase p70S6 kinase (S6K), of which there are two forms (S6K1 and S6K2). S6K phosphorylates key residues on the ribosomal protein S6, permitting its activation and full function as a protein involved in ribosomal biogenesis. The mTOR kinase also modulates phosphorylation of 4E-BP1, releasing its inhibition of eIF-4E and consequently permitting

efficient cap-dependent translation [67]. Specifically, the rapamycin-sensitive signaling pathway of mTOR occurs through the mTOR-RAPTOR complex (mTORC1), while a rapamycin-insensitive pathway occurs when mTOR is complexed with RICTOR (mTORC2) [69]. Rapamycins inhibit the activity of mTOR by directly adhering to FKBP-12 which binds to mTORC1 (i.e. the mTOR-RAPTOR complex), and also indirectly inhibit the mTOR-RICTOR complex (mTORC2) by sequestering free mTOR and thus also preventing its assembly into mTORC2 complexes. Therefore, mTOR is a downstream component in the PI3K/Akt/mTOR pathway which is known to be dysregulated in numerous human cancers.

Molecular epidemiological studies show that in addition to a high frequency of activation in specific cancers, activation of the PI3K/Akt/mTOR pathway is frequently a characteristic of worsening prognosis through increased aggressiveness, progression and resistance to treatment [70]. A variety of preclinical studies have confirmed the role of this pathway in tumor development. Gain of function models have demonstrated that constitutive activation of kinases such as Akt can lead to the inexorable development of cancers resembling those in patients which are characterized by frequent activation of the same kinase. This is complemented by the demonstration of anti-tumor activity of kinase inhibitors acting in vitro and in vivo. Everolimus is capable of inhibiting the growth of a wide spectrum of tumor cell lines and tumors. The mTOR inhibitory activity presumably contributes to the antiproliferative activity of everolimus against tumor cell lines. However, everolimus may also exert an antitumor effect through the inhibition of angiogenesis in vivo. Indeed, both rapamycin and everolimus potently inhibit proliferation of endothelial cells [71] and have antiangiogenic activity in vivo [71, 72].

An important aspect of the anti-tumor effect of everolimus is indeed its potential to act both on tumor cells directly (to inhibit growth) and indirectly (by inhibiting angiogenesis and displaying anti-vascular properties). The observation of in vivo sensitivity of xenografts comprised of tumor cells showing insensitivity to everolimus in vitro is attributed to the drug's potential to act on the vascular component of the supporting peritumoral stroma. The anti-angiogenic property of everolimus has been confirmed through experiments demonstrating the effect of everolimus in countering vascular endothelial growth factor (VEGF)-induced proliferation of human umbilical endothelial cells (HUVECs) in vitro, VEGF-driven angiogenesis in a chamber implant murine model, and neovascularisation in murine orthotopic melanoma and xenograft models [72-74].

***Everolimus pharmacokinetics.*** Everolimus is rapidly absorbed after oral administration, with a median tmax of 1-2 hours post dose. The extent of absorption is estimated at above 11%. The area under the blood concentration-time curve (AUC) is dose-proportional over the dose range tested while maximum blood concentration (Cmax) appears to plateau at dose levels higher than 20 mg. The elimination half-life in cancer patients averaged 30 hours, which is similar to that in healthy subjects. Inter-patient variability is moderate with the coefficient of variation (CV) of approximately 50%. In healthy subjects, high fat meals reduced systemic exposure to Afinitor 10 mg (as measured by AUC0-$\infty$) by 22% and the peak plasma concentration Cmax by 54%. Light fat meals reduced AUC0-$\infty$ by 32% and Cmax by 42%. Food, however, had no apparent effect on the post absorption phase concentration-time profile [Everolimus (RAD001) Investigator Brochure, Clinical Study Report RAD001C2120]. Steady-state trough levels are highly predictive of AUC, with a coefficient of determination of 0.96, as has been reported in renal

transplantation patients [75]. Everolimus is mainly metabolized by the cytochrome P450 isoenzyme 3A4 (CYP3A4) in the liver and to some extent in the intestinal wall. Everolimus is also a substrate of P-glycoprotein (P-gp). Therefore, absorption and subsequent elimination of systematically absorbed everolimus may be influenced by medicinal products that interact with CYP3A4 and/or P-gp. Strong CYP3A inhibitors (such as ketoconazole, itraconazole, ritonavor) and inducers (such as rifampicin, rifabutin) should be avoided. In Study C2108, everolimus, at a daily dose of either 5 mg or 10 mg, was added to the daily letrozole regimen of 2.5 mg in breast cancer patients. Letrozole is mainly mediated by CYP3A4 and CYP2A6 with minor contribution of renal clearance. Due to the low affinity of letrozole to CYP3A4, the potential for pharmacokinetic interaction is remote. Pharmacokinetic profiles of letrozole were investigated before addition of everolimus and after everolimus reached steady state (day 15). Data suggested that, indeed, coadministration of everolimus with 2.5 mg/day letrozole did not influence the pharmacokinetics of letrozole. The exposure of everolimus in the presence of letrozole was similar to the historical data obtained from the amended Study C2102 CP report [Everolimus (RAD001) Investigator Brochure, Clinical Study Report RAD001C2102 and 2108].

### 7.3 Metformin

Metformin hydrochloride (N,N-dimethylimidodicarbonimidic diamide hydrochloride) is a biguanide anti-diabetic agent. Metformin is approved for the treatment of type 2 diabetes mellitus. Metformin acts primarily by decreasing endogenous hepatic glucose production. Clinically, metformin lowers fasting and postprandial hyperglycemia. The decrease in fasting plasma glucose is approximately 25-30%. Metformin demonstrates an anti-hyperglycemic action rather than a hypoglycemic action. Metformin does not cause weight gain and in fact, may cause a modest weight loss. Metformin also can improve the fasting lipid profile by decreasing triglyceride, and is known to improve steatohepatitis.

## 8.0 DOSE MODIFICATIONS

If the patient experiences drug-related grade 2 toxicity, everolimus and metformin will be withheld until the toxicity is resolved to ≤ Grade 1 with the exception of fatigue and grade 2 diarrhea treated with loperamide. If diarrhea does not resolve to grade 1 with loperamide then hold everolimus and metformin until it resolves to grade 1. Everolimus and metformin may then be resumed at the same daily dose.

If the Grade 2 toxicity recurs, Metformin and everolimus will be withheld until the toxicity has resolved to ≤ Grade 1, and the daily dose will be reduced by 50%. If the patient experiences Grade 3/4 toxicity, everolimus and metformin will be withheld until the toxicity has resolved to ≤ Grade 1, and the daily dose of metformin will be reduced by 50%.

Metformin therapy will be temporarily discontinued prior to intravascular administration of iodinated contrast media. Metformin will be withheld for 48 hours after the radiologic study is performed, and then resumed.

Please refer to Tables 3 and 4 for dose modification guidelines for hematologic and non-hematologic toxicities, respectively.

## 9.0 MANAGEMENT OF SPECIFIC TOXICITIES

### 9.1 Management of stomatitis/oral mucositis/mouth ulcers

Mouth ulcers, stomatitis and oral mucositis have been seen in patients treated with everolimus. Stomatitis/oral mucositis/mouth ulcers due to everolimus will 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 examination reveals mouth ulcers rather than a more general inflammation of the mouth, please classify the adverse event as such. Please follow the paradigm below for treatment of stomatitis/oral mucositis/mouth ulcers:

For mild toxicity (grade 1), use conservative measures such as **non-alcoholic mouth wash or salt water (0.9%) mouth wash** several times a day until resolution.

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®).

Agents containing hydrogen peroxide, iodine, and thyme derivatives may tend to worsen mouth ulcers. It is preferable to avoid these agents.

Antifungal agents will be avoided unless a fungal infection is diagnosed. In particular, systemic imidazole antifungal agents (ketoconazole, fluconazole, itraconazole, etc.) will 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. Similarly, antiviral agents such as acyclovir will be avoided unless a viral infection is diagnosed.

### 9.2 Management of hyperlipidemia and hyperglycemia

Treatment of hyperlipidemia will take into account the pre-treatment status and dietary habits. Blood tests to monitor hyperlipidemia will be taken in the fasting state. Hyperlipidemia and hypertriglyceridemia will be treated according to local best clinical practice. Patients will be monitored clinically and through serum biochemistry for the development of rhabdomyolysis and other adverse events as required in the product label/data sheets for HMG-CoA reductase

inhibitors, if this class of medications are used to treat dyslipidemia.

Hyperglycemia has been reported in clinical trials of everolimus. Monitoring of fasting serum glucose is recommended prior to the start of everolimus therapy, periodically (according to the follow up schedule as stated above) thereafter and as warranted by symptoms. Optimal glycemic control should be achieved before starting trial therapy.

### 9.3 Management of Diarrhea

Appearance of diarrhea attributed to everolimus, metformin or their combination may be treated with loperamide. Other medications for diarrhea may be used as needed.

### 9.4 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 will 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. Patients will be advised to report promptly any new or worsening respiratory symptoms.

Diagnosis is generally suspected in individuals receiving mTOR inhibitors who develop these symptoms or in asymptomatic individuals in whom a routine chest CT scan reveals a new ground glass-like alveolar infiltrate.

In an analysis of pneumonitis in breast cancer patients treated with everolimus the observed incidence of noninfectious pneumonitis in 4 studies totaling 238 patients did not exceed 3% [53]. In another study involving 49 patients [47], the incidence was approximately 10-fold higher. Although such a marked difference remains poorly explained, some considerations can be addressed:

- Different monitoring methods across the studies have been applied: more extensive chest assessment by CT scan in NCIC CTG, J2101, and J2102 studies;
- Sponsor management guidelines for noninfectious pneumonitis based on CTCAE grade were developed in 2006, therefore fully implemented only in the J2101 and J2102 studies;
- The regular premedication with corticosteroids before the infusion of weekly chemotherapy could in part explain the low incidence observed in J2101 and J2102 studies.

The frequency of symptomatic pulmonary toxicity (all grades) was approximately 13% in a phase III study of RAD001 in patients with metastatic renal cell carcinoma (CRAD001C2240). Severe (CTC grade 3) pneumonitis occurred in 4% of patients, and an occasional fatality was reported. The lung toxicity was partly or completely reversible in the majority of cases with interventions including drug interruption, discontinuation and the use of corticosteroids.

Consultation with a pulmonologist is recommended for any case of pneumonitis that develops during the study. Table 5 summarizes the management of non-infectious pneumonitis.

## 9.5 Management of infections

Everolimus has immunosuppressive properties and may predispose patients to bacterial, fungal, viral or protozoan 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 respiratory or hepatic failure) and occasionally have had a fatal outcome.

Physicians and patients will 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.

## 9.6 Management of hematologic toxicities

A reduction in blood cell counts is frequent when everolimus therapy is initiated. Without clinical significance and infrequently, anemia and thrombocytopenia have been reported. Patients will be monitored regularly hematologically, especially at the beginning of treatment. Suspected drug-related hemorrhages have been exceptional. Nevertheless, everolimus will be considered as predisposing patients to hemorrhage, potentially fatal, will they develop severe drug-related thrombocytopenia. Patients with ongoing thrombocytopenic or with a known bleeding diathesis will be subject to careful evaluation and more frequent monitoring.

Please refer to the Investigator Brochure for more details.

## 9.7 Management of rash and similar dermatologic adverse events (AE's)

Rash and similar dermatologic AE's were common among patients receiving everolimus. The proportion of patients experiencing any rash related AE was 29.2% and 33.4% in the everolimus treatment groups [76] and the pooled dataset, respectively. Rash and other dermatologic findings

were present in 6.6% of patients in the placebo arm. Although toxicity by grade is not presented, most cases of rash and related events were of low grade.

### 9.8 Management of metabolic events

Elevations of serum creatinine, usually mild, have been reported in clinical trials. Monitoring of renal function, including measurement of blood urea nitrogen (BUN) or serum creatinine, is recommended prior to the start of everolimus therapy and periodically thereafter. Hypercholesterolemia, hypertriglyceridemia, had at least a 2-fold increase in incidence in patients receiving everolimus compared with placebo.

### 9.9 Follow-up for toxicities

Patients whose treatment is interrupted or permanently discontinued due to an adverse event or abnormal laboratory value will be followed at least once a week for 4 weeks, and subsequently at 4-week intervals, until resolution or stabilization of the event. All patients will be followed for onset of any new serious adverse events for 28 days following the last dose of study treatment.

All patients who discontinue study treatment for any reason other than progression will continue to follow the protocol tumor assessments until progression. After progression, patient will be followed for survival every 3 months for up to approximately 3 years after the registration of the last patient.

If, because of toxicity, a patient requires a dose delay of > 4 weeks from the intended day of dose, then the patient will discontinue study treatment. However, the patient will continue to be followed for toxicity and tumor assessments as previously described.

### 9.10 Permitted study drug adjustments

For patients who may require dose adjustments because of concomitant medication, please refer to Section 10.

For patients who are unable to tolerate the protocol-specified dosing schedule, dose adjustments are permitted in order to keep the patient on study drug. The following guidelines will be followed. These changes will be recorded on the Dosage Administration Record eCRF.

If treatment is interrupted due to toxicity, study drug will not be resumed until recovery to Grade ≤ 1. Then it could be reintroduced at the initial dose or a lower dose level depending on the toxicity type and Grade. These changes will be recorded on the Dosage Administration Record eCRF.

If a patient has already decreased 2 dose levels, no further dose reduction is permitted. Patients requiring an additional dose reduction will be required to discontinue study treatment.

Patients who interrupt therapy for more than 4 weeks will be discontinued from the study.

## 10.0 CONCOMITANT THERAPY

Patients will be instructed not to take any additional medications (over-the-counter or other products) during the study without prior consultation with the investigator. All medications taken within 28 days of starting study treatment will be reported on the Concomitant Medication eCRF pages.

The following concomitant treatments are not allowed during the study:

- Chronic concomitant bisphosphonate therapy for the prevention of bone metastases are not permitted during the study. Bisphosphonate therapy for the treatment of osteoporosis is permitted during the study. Bisphosphonate therapy for the management of bone metastases is recommended as standard of care. Please refer to prescribing information for details of administration. If bisphosphonate therapy is initiated after registration, the reason for its use must be clearly documented.

- Investigational or commercial anticancer agents, such as chemotherapy, immunotherapy, targeted therapy, biological response modifiers, or endocrine therapy other than exemestane (including steroids) will not be given to patients.

- Hormone replacement therapy, topical estrogens (including any intra-vaginal preparations), megestrol acetate and selective estrogen-receptor modulators (e.g. raloxifene) are prohibited.

- Prolonged systemic corticosteroid treatment, except for topical applications (e.g. rash), inhaled sprays (e.g. obstructive airways diseases), eye drops or local injections (e.g. intra-articular) will not be given. A short duration (<2 weeks) of systemic corticosteroids is allowed (e.g. chronic obstructive pulmonary disease, anti-emetic).

- Hematopoietic growth factors (e.g. erythropoietins, G-CSF and GM-CSF) are not to be administered prophylactically. Use of these will be reserved to cases of severe neutropenia and anemia as per the labeling of these agents.

- Inhibitors of CYP3A4 and/or PgP: Co-administration with strong inhibitors of CYP3A4 (e.g., ketoconazole, itraconazole, ritonavir) or P-glycoprotein (PgP) will be avoided. Co-administration with moderate CYP3A4 inhibitors (e.g., erythromycin, fluconazole) or PgP inhibitors will be used with caution. If patient requires co-administration of moderate CYP3A4 inhibitors or PgP inhibitors, reduce the dose of everolimus to half the currently used dose. Additional dose reductions to every other day may be required to manage toxicities. If the inhibitor is discontinued the everolimus dose will be returned to the dose used prior to initiation of the moderate CYP3A4/PgP inhibitor. Seville orange, star fruit, grapefruit and their juices affect P450 and PgP activity. Concomitant use will be avoided.

- Inducers of CYP3A4 and/or PgP: Avoid the use of strong CYP3A4 inducers. If patient requires co-administration of strong CYP3A4 inducers (i.e., phenytoin, carbamazepine, rifampin, rifabutin, phenobarbital, St. John's wort), an increase in the dose of everolimus up to twice the currently used daily dose will be considered, using 5mg increments. Enzyme induction usually occurs within 7-10 days, therefore everolimus dose will be increased by one increment 7 days after the start of the inducer therapy. If no safety concerns are seen within the next 7 days, the dose can be increased again one additional increment up to a maximum of twice the daily dose used prior to initiation of the strong CYP3A4 inducer. 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 will be returned to the dose used prior to initiation of the strong CYP3A/PgP inducer.

- Local radiotherapy for analgesic purposes or for lytic lesions at risk of fracture may be carried out if required. Whenever possible, these patients will have a tumor assessment of the lesion(s) before they actually receive the radiotherapy. No dose modification of study treatment is needed during radiotherapy.

- Everolimus may affect the response to vaccinations making it less effective. Live vaccines will be avoided while a patient is treated with everolimus.

- Lipid-lowering drugs may be given in case of hyperlipidemia.

Otherwise, the use of other concomitant medication/therapy deemed necessary for the care of the patient is allowed. The investigator will instruct the patient to notify the study site about any new medications he/she takes after the start of the study drug. All medications (other than study drug) and significant non-drug therapies (including physical therapy and blood transfusions) administered after the patient starts study treatment and for up to 28 days after study drug discontinuation must be listed on the Concomitant medications/Significant Non-drug Therapy After Start of Study Drug eCRF. The first regimen of antineoplastic medication/treatments received after study treatment discontinuation must also be recorded in the eCRF antineoplastic treatment modules.

## 11.0 ASSESSMENT OF EFFICACY

Patients will have either at least one lesion that can be measured as per RECIST [77] OR have bone lesions: lytic or mixed (lytic + sclerotic) in the absence of measurable disease as defined above.

For patients with measurable disease at baseline (as per RECIST), efficacy (overall tumor response and progression) will be evaluated every **8** weeks according to RECIST (see Post-text

supplement 1).

A bone scan or a skeletal survey will be performed at baseline for all patients within 6 weeks before registration. Any abnormalities (i.e. hotspots) identified on the bone scan will be confirmed by X-ray, CT scan with bone windows or MRI.

Bone lesions identified at baseline will follow the same assessment schedule as for measurable lesions. Additional bone scans or skeletal surveys will be performed if clinically indicated. Abnormalities found on subsequent bone scans will also be confirmed by X-ray, CT scan, or MRI.

The same method of assessment and the same technique will be used to characterize each identified and reported lesion at baseline for each study tumor assessment after start of study treatment.

### 11.1 Radiological assessment of tumor

Tumor response will be assessed using RECIST [77]. A CT scan or a MRI of the Chest, Abdomen and Pelvis will be performed at screening (≤ 21 days prior to the first dose of everolimus and ≤ 28 days for patients with positive baseline Hepatitis B and C results) and periodically as indicated in Table 6 (chest CT performed if clinically needed for the surveillance of pneumonitis will be available during the regular follow-up). CT Scan and MRI with contrast media will be used except for patients who are allergic/sensitive to the radiographic contrast media. Ultrasound scans will not be used to measure tumor lesions.

Tumor response will be assessed every 8 weeks (± 1 week) until disease progression and until further anti-cancer therapy is initiated. A partial or a complete response warrants a confirmation no sooner than 4 weeks after its observation.

All patients who discontinue study treatment for any reason (i.e. an adverse event, administrative reasons etc.) other than disease progression and consent withdrawal will continue to have tumor assessments as per the schedule and until disease progression and until further anti-cancer therapy is initiated. Documented disease progression will be determined by a local radiologist and/or the investigator.

All patients will have at least one lesion that can be accurately measured in at least one dimension ≥ 20 mm with conventional imaging techniques or ≥ 10 mm with spiral CT or MRI OR bone lesions such as lytic or mixed (lytic + sclerotic) in the absence of measurable disease as defined above.

Bone scans or skeletal survey will be performed at baseline. Positive areas on bone scans will be assessed by X-ray, CT scan with bone windows or MRI, prior to registration and will continue to be assessed using the same modality (X-ray, CT scan or MRI) every 6 weeks until disease progression as described above. Additional bone scans or skeletal surveys will be performed if clinically indicated. Abnormalities found on subsequent bone scans will also be confirmed by X-

ray, CT scan, or MRI.

If the patient presents with both irradiated and non-irradiated bone lesions (either therapeutic high dose or low dose for pain), only the non-irradiated lesions will be followed for tumor assessments unless progression is documented after the radiation.

### 11.2 Response assessment

For patients with measurable disease at baseline, progression will be determined according to the RECIST [77].

In the absence of measurable disease at baseline, the following will be considered progression among patients with lytic or mixed (lytic + sclerotic) bone lesions:

- The appearance of one or more new lytic lesions in bone
- The appearance of one or more new lesions outside of bone
- Unequivocal progression of existing bone lesions

Note: Pathologic fracture, new compression fracture, or complications of bone metastases will not be considered as evidence of disease progression, unless one of the above-mentioned criteria is fulfilled.

Patients with symptoms of rapidly progressing disease without radiological (or photographical) evidence will not be considered to have progressed for efficacy analyses.

The evaluation of overall lesion response will be performed according to RECIST. For patients with only bone lesions, the RECIST will be extended to include the evaluation of overall lesion response, which will be based solely on non-target lesion responses. Specifically, in absence of new lesions, the overall lesion response at each assessment will be one of the following: complete response, stable disease, unknown, or progressive disease based on non-target lesion responses. Stable disease would include all assessments not qualifying for complete response, progressive disease or unknown. In presence of any new lesion, the overall lesion response will be progressive disease.

## 12.0 TOXICITY MONITORING

Toxicity will be assessed using the NCI Common Toxicity Criteria for Adverse Events v3.0.

### 12.1 Adverse Event Reporting Requirements

Information about all adverse events, whether volunteered by the subject, discovered by investigator questioning, or detected through physical examination, laboratory test or other means, will be collected and recorded and followed as appropriate. An adverse event is any undesirable sign, symptom or medical condition occurring after starting study drug (or therapy)

even if the event is not considered to be related to study drug (or therapy). Study drugs (or therapy) include exemestane, everolimus and metformin.

Medical conditions/diseases present before starting study treatment are only considered adverse events if they worsen after starting study treatment (any procedures specified in the protocol). Adverse events occurring before starting study treatment but after signing the informed consent form are recorded. Abnormal laboratory values or test results constitute adverse events only if they induce clinical signs or symptoms or require therapy, and are recorded.

### 12.1.1 Serious adverse events

Information about all serious adverse events will be collected and recorded on the MDACC "Internal SAE Report Form for Prompt Reporting" if requirements for prompt reporting have been met. All serious internal adverse events that do not fall under the prompt reporting requirements will be reported during continuing review using the Internal SAE Log. To ensure patient safety each serious adverse event occurring during the conduct of the protocol and meeting the definition of a SAE must also be reported to the UT MD Anderson Cancer Center Institutional Review Board (IRB) in accordance with the timeframes and procedures outlined in the Policy on Reporting Serious Adverse Events.

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

1. is fatal or life-threatening,
2. requires prolonged hospitalization,
3. results in persistent or significant disability/incapacity,
4. is medically significant such that medical or surgical intervention to prevent one of the outcomes listed above is required.

Any serious adverse event occurring in a patient after providing informed consent and until 30 days after stopping the trial will be reported. The period after discontinuing study drug may be extended if there is a strong suspicion that the drug has not yet been eliminated. All serious adverse events will also be reported for the 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).

### 12.1.2 Non-serious adverse events

Events not considered to be serious adverse events are hospitalizations for the:

1. routine treatment or monitoring of the studied indication, not associated with any deterioration in condition,
2. treatment, which was elective or pre-planned, for a pre-existing condition that did not worsen,
3. treatment on an emergency, outpatient basis for an event not fulfilling any of the definitions of serious given above and not resulting in hospital admission.

## 12.2 Adverse Drug Reaction Reporting

All adverse experiences occurring after administration of the first dose of study medication and on or before the final visit will be reported on the Adverse Experience form in the patient's CRF.

Adverse experiences will be evaluated at each visit/assessment. Adverse experiences not previously documented in the study will be recorded in the adverse experience record form within the patient's CRF. The nature of each experience, date and time (where appropriate) of onset, outcome, course (i.e. intermittent or constant), maximum intensity, action taken with respect to dosage and relationship to treatment will be established. Details of changes to the dosage schedule or any corrective treatment will be recorded on the appropriate pages of the CRF.

Any serious adverse experiences which occur at any time during the clinical study or within 30 days of receiving the last dose of study medication, whether or not related to the study drug, will be reported immediately to the IRB.

## 12.3 Instructions for rapid notification of serious adverse events

### 12.3.1 Reporting responsibility

Each serious adverse event (but not pregnancies) will be reported by the investigator to IRB within 24 hours of learning of its occurrence, even if it is not felt to be treatment-related. Follow-up information about a previously reported serious adverse event will also be reported to IRB within 24 hours of receiving it. If warranted, an investigator alert may be issued, to inform all investigators involved in any study with the same drug (or therapy) that this serious adverse event has been reported.

### 12.3.2 Reporting procedures

The investigator will complete the Serious Adverse Event Report Form in English, assess the relationship to study treatment and send the completed form by fax within 24 hours to the PI. The PI and co-PI will ensure that the form is accurately and fully completed, and will then fax it to IRB within 2 to 3 calendar days for deaths or life-threatening events and 5 calendar days for other serious adverse events. The original and the duplicate copies of the Serious Adverse Event Form, and the fax confirmation sheet will be kept with the case report forms at the study site.

Follow-up information will describe whether the event has resolved or continues, if and how it was treated, and whether the patient continued or discontinued study participation. The form and fax confirmation sheet will be retained.

## 13.0 DURATION OF THERAPY

In the absence of treatment delays due to adverse event (AE), treatment may continue for the duration of funding (up to 5 years) or until one of the following criteria applies:

1. Disease progression.
2. Intercurrent illness that prevents further administration of treatment.
3. Unacceptable adverse event(s) or a dose-limiting toxicity that does not resolve within 2 weeks of stopping therapy.
4. Patient's decision to withdraw from the study.
5. General or specific changes in the patient's condition rendering the patient unacceptable for further treatment in the judgment of the investigator.
6. Protocol violation (including non-compliance).
7. Eligibility criteria not fulfilled.

### 13.1 Procedures for Discontinuation

Patients who discontinue will always be asked about the reason(s) for their discontinuation and about the presence of any AEs. If possible, they will be seen and assessed by an investigator(s). AEs are to be followed-up, and the patient will return any investigational products.

## 14.0 CORRELATIVE STUDIES

The prevalence of obesity is rapidly increasing and has reached epidemic proportions. Obesity is associated with an increased risk of developing postmenopausal breast cancer, and it is associated with worse prognosis of breast cancer after disease onset [20]. In breast cancer, at least three signaling pathways have been postulated to explain its association with breast cancer: **insulin/IGF-1**, **adipokines** and **estrogens**. There are **synergistic interactions** among these pathways (Figure 1). There is crosstalk among the pathways leading to amplification of mitogenic signal. Estrogen signaling may be especially important in obese **postmenopausal** patients with hormone receptor breast cancer. Therefore, overweight/obesity and these signaling pathways are therapeutic targets in breast cancer patients.

**Experimental approach, validated assays employed and expertise of PI:** The specific aims of this translational medicine proposal are: (a) to determine whether changes in insulin resistance [as assessed by the Homeostasis Model Assessment (HOMA), estimated from a single fasting glucose and insulin], hormones and adipokines (including FGF21) after 4 weeks (+/-3 days) of metformin treatment correlate with PFS; (b) to determine whether metformin-induced decreased signaling through the mTOR pathway, activation of AMPK (a marker of metformin action), and down regulation of ER-alpha expression correlate with a longer PFS; (c) to determine whether stromal FGF21 signaling was changed in tumor and stroma biopsies before and after drug treatment.

Logistically, this will involve collection of blood prior to and after starting treatment for assessment of glucose and glycohemoglobin. For correlative studies, after fasting blood glucose specimens will be collected at baseline and at weeks 4, 8, 16 and 24 (+/- 7 days). Plasma glucose in gray top vacutainer tubes (containing sodium fluoride) will be measured expeditiously at the

clinical laboratory because levels of glucose are affected by storage of blood samples. Glycohemoglobin will also be measured in the clinical laboratory. Serial blood samples (two 10 mL lavender-top tubes) will be collected for plasma at weeks 8, 16 and 24 (+/- 7 days), to coincide with imaging studies. Multiple aliqouts of plasma will be stored at -80 C. A representative diagnostic formalin fixed paraffin embedded tumor block will be requested from all patients in the study (estimated >95% of subjects). In patients with tumors safely accessible for biopsy, informed consent will be obtained for imaging-guided core biopsy of breast cancer at baseline and 8 weeks (+/-3 days) after drug treatment. At each time point, a core biopsy specimen will be fixed and embedded in paraffin and processed for immunohistochemistry and molecular studies. If any tissue is leftover and the patient agrees, it will be stored in a MD Anderson laboratory for future research related to cancer. Before the banked samples can be used for research, the people doing the research must get specific approval from the Institutional Review Board (IRB) of MD Anderson. The banked samples will be given a code number. No identifying information will be directly linked to these samples. Only the principal investigator and the laboratory coordinator will have access to the code numbers and be able to link the samples to the patient. This is to allow medical data related to the samples to be updated as needed. Other researchers using your samples will not be able to link this data to the patient.

***Experimental Approach.*** We will determine whether changes in characteristics such as HOMA, age, body mass index (BMI), tumor markers of mTOR signaling, and circulating levels of estradiol, progesterone, insulin, IGF-1, IGF-2, leptin, adiponectin, FGF21, TNF-alpha and IL-6 are prognostic and predictive for PFS. To address hypothesis 1, a Cox proportional hazards model will be used to identify significant predictors of PFS. To address hypothesis 2, the pre- and post-treatment tumor biopsies will examine whether decrease in signaling through the AKT/mTOR pathway, activation of AMPK (a marker of metformin action), and a down regulation of ER-alpha expression occur after drug treatment. The automated objective measure of immunostaining described below will be statistically correlated with PFS. To address hypothesis 3, the pre- and post-treatment tumor biopsies will examine whether changes in FGF21 signaling was changed in tumor and stroma biopsies before and after drug treatment.

***Laboratory Methods.*** Glucose and glycohemoglobin (hemoglobin A1c) will be assayed in the clinical laboratory, and blood samples will be assayed in Dr. Yeung's laboratory at MDACC for hormones and adipokines. Assay kits from Immuno-Biological Laboratories-America, Inc. Minneapolis, MN will be used according to protocols provided by the manufacturer. The characteristics of each assay kit are as follows:

1. Insulin ELISA -- Catalog Number IB79401; range: 0 / 5 - 500 microIU/mL; sensitivity: 0.15 µIU/mL.
2. C-Peptide ELISA -- Catalog Number IB79101; range: 0 / 0.2 - 16 ng/mL; sensitivity: 0.064 ng/mL.
3. IGF-1 ELISA -- Catalog Number E20; range: 0 / 1 - 50 ng/mL; sensitivity: 0.09 ng/mL.
4. IGF-2 ELISA -- Catalog Number E30; range: 0 / 0.45 - 9 ng/mL; sensitivity: 0.02 ng/mL.
5. IGFBP-3 ELISA -- Catalog Number E03; range: 0 / 2 - 150 ng/mL; sensitivity: 0.6 ng/mL.
6. Estradiol 17-Beta ELISA -- Cat Num IB79103; range: 0 / 25 - 2000 pg/mL; sensitivity: 9.7 pg/mL.

7. Progesterone ELISA -- Cat Num IB79105; range: 0 / 0.3 - 40 ng/mL; sensitivity: 0.045 ng/mL.
8. SHBG ELISA -- Catalog Number IB59106; range: 0 / 3.3 - 295 nmol/L; sensitivity: 0.1 nmol/L.
9. Leptin ELISA -- Catalog Number 27775; range: 0 / 15.63 - 1000 pg/mL; sensitivity: 2.13 pg/mL.
10. Adiponectin ELISA -- Catalog Number E09; range: 0 / 1 - 100 ng/mL; sensitivity: < 0.6 ng/mL.
11. TNF-alpha ELISA -- Catalog Number IB49623; range: 0 / 7.8 - 500 pg/mL; sensitivity: 2.3 pg/mL.
12. IL-6 ELISA -- Catalog Number IB49612; range: 0 / 1.6 - 100 pg/mL; sensitivity: 0.92 pg/mL.
13. FGF21 ELISA – R&D Systems, Catalog Number DF2100; range: 0 / 31.3 - 2000 pg/mL; sensitivity: 31 pg/mL

*Immunohistochemical Assays.* All immunohistochemistry (IHC) assays will be completed by a pathologist specialized in breast cancer. Tissue microarrays will be prepared from the tumor blocks. If blocks are not available, we will request 15 unstained slides. Briefly, IHC staining will be performed on 4-μm slices of formalin fixed paraffin-embedded tissue sections. Estrogen receptor (ER-alpha) and progesterone receptor will be immunostained according to standard procedures in clinical pathology. Primary antibodies against Insulin Receptor (IR) (CT-3, 1:100; Chemicon/Millipore, Billerica, MA), IGF-1R (alpha-subunit, clone 24-31, !:50; Cell Signaling), p-AMPK-alpha (Thr172; Upstate/Millipore), p-AKT (Ser473, 736E11, IHC-specific, 1:50; Cell Signaling), p-mTOR (Ser2448, 49F9, IHC-specific, 1:100; Cell Signaling) and p-p70S6K (Thr389, 1A5, 1:700; Cell Signaling) will be used. The p-AKT and p-p70S6K stainings have been validated by Western blotting and other studies. After deparaffinization, rehydration and heat-induced epitope retrieval, slides will be immunostaining according standard procedures as previously described. Positive control slides and negative control slides will be included within each batch for staining. IHC assays will be scored /evaluated based on the staining intensity (SI) and percentage of positive cells (PP) within the whole tissue section. Protein expression will be objectively and quantitatively measured using a DAKO automatic cellular imaging system (ACIS III) equipped with an automatic microscopy and advanced computerized image analysis. We define the expression index (EI) as the product of Percentage Staining (PP) and Average Brown Intensity (SI) (both quantitative values measured by the ACIS III system).

## 15.0 STATISTICAL CONSIDERATIONS

A phase 3 randomized trial compared everolimus and exemestane versus exemestane and placebo (randomly assigned in a 2:1 ratio) in 724 patients with hormone-receptor–positive metastatic breast cancer who had recurrence or progression while receiving previous therapy with a nonsteroidal aromatase inhibitor in the adjuvant setting. The primary end point of this study (known as the BOLERO-2 trial) was progression-free survival (PFS). At the interim analysis, median PFS was 6.9 months with everolimus plus exemestane and 2.8 months with placebo plus exemestane, according to assessments by local investigators (hazard ratio for progression or death, 0.43; 95% confidence interval [CI], 0.35 to 0.54; P<0.001). [53].

The primary objective of our study is to assess the efficacy of exemestane and everolimus combined with metformin in overweight and obese post-menopausal women with metastatic breast cancer. The primary endpoint is progression-free survival (PFS), defined as the time from the date of registration to the date of the first documented progression or death due to any cause. We will use a median PFS of 7 months as the comparator, based on the local investigators assessment of PFS in the BOLERO-2 trial [53]. Using STPLAN, with 40 patients accrued at a rate of 2 patients per month (i.e., over a 20-month period), 1-sided 5% alpha, and a post-accrual follow-up of 3 months, we would have 80% power to detect a control median PFS of 7 months as statistically significantly lower than an experimental group median PFS of 12 months. We will use Cox proportional hazards regression analysis to assess the effects of the usual prognostic factors for breast cancer (hormone receptor status, age, HER2 status, nuclear grade, and stage) on PFS.

The method of Thall, Simon, and Estey (1995, Stat Med) will be employed to perform interim safety monitoring. We will assume a Beta (1, 5) prior distribution for the DLT rate, which in particular has mean DLT rate of 17%. We will target a 30% DLT rate and we will terminate enrollment into the trial if

Pr{DLT rate > 30%| data} > 0.925

That is, if at any time during the study we determine that there is more than a 92.5% chance that the DLT rate is more than 30% we will stop enrollment into the study. Stopping boundaries corresponding to this probability criterion are to terminate the trial if

[# of patients with DLT/ # of patients evaluated] >=

5/5, 7/9, 8/12, 9/14, 10/17, 11/20, 12/22, 13/25, 14/28, 15/31, 16/33, 17/36, or 18/39

The operating characteristics of this rule in the trial are shown in the following table:

Operating Characteristics for Safety Monitoring Rule

| True DLT Rate | PET | N: | LQ | Med | UQ |
|---|---|---|---|---|---|
| 10% | 0% | | 40 | 40 | 40 |
| 20% | 1% | | 40 | 40 | 40 |
| 30% | 9% | | 40 | 40 | 40 |
| 40% | 44% | | 23 | 40 | 40 |
| 50% | 85% | | 11 | 19 | 30 |

PET = Probability of Early Termination; LQ = lower quartile; Med = median; UQ = upper quartile

We will conduct exploratory biomarker studies to identify the population most likely to benefit. Our preclinical data suggest that metformin increases FGF21 serum levels, and this may enhance the efficacy of exemestane and everolimus by dual blockade of the mTOR pathway. If we assume the serum FGF21 data follow a log-normal distribution, then using the data from Zhang *et al.* [78] and the log scale, we get a normal distribution with mean = 5.677 and a standard deviation of about 0.940. Assuming a 50% clinical benefit rate, 40 patients, a two-sided 5% alpha, 80% power, and a two-sample, equal variance t-test, we can detect an increase to a mean of 6.509 (a 15% increase) or larger as statistically significant. If the clinical benefit rate is only 30%, then the smallest detectable mean increases to 6.585.

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Table 1. Clinically Relevant Drug Interactions: Inducers and Inhibitors of Isoenzyme CYP3A

| |
|---|
| **INDUCERS**<br><br>Barbiturates, carbamazepine, glucocorticoids, modafinil, oxcarbazepine, phenobarbital, phenytoin, pioglitazone, rifabutin, rifampin, St. John's wort, troglitazone, efavirenz, nevirapine, topiramate |
| **INHIBITORS**<br><br>**Strong inhibitors:**<br>clarithromycin, conivaptan, indinavir, itraconazole, ketoconazole, lopinavir, mibefradil, nefazodone,<br>nelfinavir, ritonavir, saquinavir, telithromycin, troleandamycin, voriconazole, Posaconazole<br><br>**Moderate inhibitors:**<br>aprepitant, atazanavir, cimetidine, ciprofloxacin, darunavir, diltiazem, erythromycin, fluconazole,<br>grapefruit juice, imatinib, tofisopam, verapamil. |

Table 2. Everolimus Dose Reductions

| **Dose level** | **Dose and schedule** |
|---|---|
| 0 = starting dose | 10 mg daily (2 × 5 mg daily) |
| -1 dose level | 5 mg daily |

Table 3. Dose Modification Guidelines for Hematologic Toxicities

| Toxicity | Actions |
|---|---|
| Thrombocytopenia<br>Platelet count | **≥ 75000/mm3:** No change<br>**50000/ mm3 to 75000/ mm3**<br>Hold everolimus treatment until recovery to **≥ 75000/mm3**<br>Reintroduce everolimus at the same dose level<br>**< 50000/ mm3**<br>Hold everolimus treatment until recovery to **≥ 75000/mm3**<br>Reintroduce everolimus at the next lower dose level, if available. |
| Absolute Neutrophil count (ANC) | **≥ 1000/ mm3** :<br>No change<br>**500/ mm3 to 1000/ mm3**<br>Hold everolimus treatment until recovery to ≥ 1000/<br>mm3<br>Reintroduce everolimus at the same dose level<br>**< 500/ mm3**<br>Hold until recovery to ≥ 1000/ mm3.<br>Reintroduce everolimus at the next lowest dose level, if available. |
| Febrile neutropenia | Hold further dosing until ANC ≥ 1250/mm3 and no fever. Then resume dosing at the next lower dose level if available. |
| Toxicity requiring interruption for > 4 weeks | Permanently discontinue everolimus treatment |
| Physicians should always manage patients according to their medical judgment based on the particular clinical circumstances. | |

Table 4. Dose Modification Guidelines for Non-Hematologic Toxicities

| Toxicity | Actions |
|---|---|
| Hyperlipidemia and/or hypertriglyceridemia | **Any grade:**<br>Treat according to best clinical practice. No specific dose reductions are needed. |
| Hyperglycemia | **Any grade:**<br>Treat according to best clinical practice. No specific dose reductions are needed. |
| Stomatitis | **Grade 2:**<br>Interrupt everolimus until resolution to ≤ grade 1. Restart at the same dose.<br>**Grade 3:**<br>Interrupt everolimus until recovery to grade ≤1.<br>Reintroduce everolimus at the next lower dose level.<br>Discontinue everolimus if stomatitis doesn't recover to ≤ grade 1 within 4 weeks.<br>**Grade 4:**<br>Discontinue everolimus treatment |
| Pneumonitis | See Table 6-4 |
| Other toxicities | **Grade 2 and 3**<br>Interrupt administration until resolution to ≤ grade 1. Restart at the same dose.<br>**Grade 4**<br>Hold everolimus treatment until recovery to ≤ grade 1.Reintroduce everolimus at the lower dose level, if available. |
| Toxicity requiring interruption for >4 weeks | Permanently discontinue treatment. |
| No specific dose adjustments are recommended for Grade 1 toxicity. However, physicians should always manage patients according to their medical judgment based on the particular clinical circumstances. | |

Table 5. Management of Non-Infectious Pneumonitis

| Worst Grade Pneumonitis | Required Investigations | Management of Pneumonitis | Everolimus Dose Adjustment |
|---|---|---|---|
| Grade 1 Asymptomatic, radiographic findings only | CT scans with lung windows. Repeat at least every 12 weeks until return to within normal limits. | No specific therapy is required | Administer 100% of study treatment dose. |
| Grade 2 Symptomatic, not interfering with ADL | CT scan with lung windows. Consider pulmonary function testing includes: spirometry, DLCO, and room air O2 saturation at rest. Repeat at least every 12 weeks until return to within normal limits. Consider a bronchoscopy with biopsy and / or BAL. | Symptomatic only. Consider corticosteroids if symptoms are troublesome. | Reduce study treatment dose by 1 dose level (see Table 6-5) until recovery to ≤ Grade 1. Study treatment may also be interrupted if symptoms are troublesome. Patients will discontinue study treatment if they fail to recover to ≤ Grade 1 within 3 weeks. |
| Grade 3 Symptomatic, interfering with ADL; O2 indicated | CT scan with lung windows and pulmonary function testing includes: spirometry, DLCO, and room air O2 saturation at rest. Repeat at least every 6 weeks until return to within normal limits. Bronchoscopy with biopsy and / or BAL is recommended. | Consider corticosteroids if infective origin is ruled out. Taper as medically indicated. | Hold treatment until recovery to ≤ Grade 1. May restart study treatment within 3 weeks at a reduced dose (by one level) if evidence of clinical benefit. |
| Grade 4 Lifethreatening; ventilatory support indicated | CT scan with lung windows and required pulmonary function testing, if possible, includes: spirometry, DLCO, and room air O2 saturation at rest. Repeat at least every 6 weeks until return to within normal limits. Bronchoscopy with biopsy and / or BAL is recommended if possible. | Consider corticosteroids if infective origin is ruled out. Taper as medically indicated. | Discontinue treatment. |

| Table 6. Visit Evaluation Schedule | **Screening (days -28 to 1)** | **Day 1 (+/- 7 days)** | **4 wks (+/- 7 days)** | **8 wks (+/- 7 days)** | **12 wks (+/- 7 days)** | **16 wks (+/- 7 days)** | **Every 2 months (+/- 7 days)** | **EOT** |
|---|---|---|---|---|---|---|---|---|
| Demography/informed consent | X | | | | | | | |
| Inclusion/exclusion criteria | X | | | | | | | |
| Relevant medical history/current medical conditions | X | | | | | | | |
| Diagnosis and extent of cancer[1] | X | | | | | | | |
| Prior antineoplastic therapy | X | | | | | | | |
| Vital signs | X | X | X | X | X | X | X | X |
| Height | X | | | | | | | |
| Weight | X | X | X | X | X | X | X | |
| Physical examination | X | X | X | X | X | X | X | X |
| ECOG performance status | X | X | X | X | X | X | X | |
| Hematology[2] | X | X | X | X | X | X | X | X |
| Hemoglobin A1C | | | X | X | X | X | | |
| Coagulation | X | | | | | As clinically indicated | | |
| Biochemistry[3] | X | X | X | X | X | X | X | X |
| Serum Lipid Profile[4] | X | | X | X | X | X | X | X |
| Prior/concomitant medications | X | | | | | Continuous during the study | | |
| Adverse events | | | | | | Continuous during the study | | |
| Tumor evaluation | | | | | | | | |
| CT or MRI of chest, abdomen[5] | X | | | X | | X | X | X[6] |
| CT or MRI of Brain[7] | X | | | | | | | |
| Bone Scan or skeletal Survey[8] | X | | | | | | | |
| Bone X-Ray, CT or MRI13 | X | | | X | | X | X | X |
| Blood samples for plasma and serum soluble biomarkers[9] | X | | | X | | X | | X |
| Request of archival tumor block/slides | X | | | | | | | |
| Blood samples for DNA extraction[10] | X | | | | | | | |
| Optional biopsy of metastatic tumor[11] | X | | | X | | | | |

[1]CT of the chest and abdomen. Skin lesions should be photographed in addition to measuring. Brain scan (CT scan or MRI with i.v. contrast) must be performed

if CNS symptoms are present. Note: If the patient had history of brain or other CNS metastases, she is ineligible. [2]Hematology tests include a complete blood count (CBC). A total white blood cell (WBC) with absolute differentials (including neutrophil count plus bands, lymphocyte, monocyte, eosinophil, basophil counts), hemoglobin (Hgb), and a platelet count. In the event of Grade 2, Grade 3 or Grade 4 hematological toxicities that require study drug dose modifications or interruptions, hematological tests must be repeated **weekly** until recovery to the baseline value or Grade 1. [3]Serum Chemistry must include: BUN or uric acid, creatinine, LDH, total protein, electrolytes (sodium, potassium and calcium), total bilirubin, GGT, albumin, alkaline phosphatase, AST/sGOT fasting glucose. [4]Serum fasting lipid profile must include: total cholesterol and triglycerides. Patients should be in fasting state. [5]CT of chest and abdomen must be repeated at each tumor assessment visit (including if negative at baseline). Scans for complete and partial responses must be repeated at least at 4 weeks but no later than the next scheduled tumor assessment following the first documented response. Skin lesions should be photographed in addition to measuring. [6]Except in case of discontinuation from treatment due to progression, tumor assessment at EOT is not necessary if the previous evaluation was done within 6 weeks of EOT. [7]Brain scan (CT scan or MRI with i.v. contrast) must be performed if CNS symptoms are present. Note: If the patient had history of brain or other CNS metastases, she is ineligible. [8]A bone scan or skeletal survey is to be performed at baseline only. Positive areas on bone scans must be assessed by X-ray, CT scan with bone windows or MRI, and should continue to be assessed using the same modality (X-ray, CT scan with bone windows or MRI) every 8 weeks until disease progression and new anticancer therapy is started. Additional bone scans or skeletal surveys should be performed if clinically indicated. Abnormalities found on subsequent bone scans must also be confirmed by X-ray, CT scan, or MRI. [9]Soluble biomarkers will need a sample of 20 mL blood for plasma and 40 mL blood for serum. Plasma and serum samples will be collected at screening, at 4 weeks (pre-dose), 8 weeks (pre-dose) and EOT. There can be an important circadian rhythm variation of these markers, samples should thus optimally be collected fasting in the morning and specimen collection should be consistent during study visits. [10]One 5 ml blood sample for DNA extraction will be collected at baseline from all patients. This will be used for DNA analysis of SNPs relevant to response to therapy. [11]In patients with tumors safely accessible for biopsy, informed consent will be obtained for imaging-guided core biopsy of breast cancer at baseline and 8 weeks (+/-3 days) after drug treatment. At each time point, a core biopsy specimen will be fixed and embedded in paraffin and processed for immunohistochemistry and molecular studies.