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Onyinye Offor

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**Exploring a therapeutic role for IGF1R inhibitors in triple-negative breast cancer**

A Thesis Submitted to the  
Yale University School of Medicine  
in Partial Fulfillment of the Requirements for the  
Degree of Doctor of Medicine

by  
Onyinye Offor  
2010

## EXPLORING A THERAPEUTIC ROLE FOR IGF1R INHIBITORS IN TRIPLE-NEGATIVE BREAST CANCER

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### SUMMARY

There is substantial preclinical and clinical data suggesting that triple-negative breast cancer (TNBC), a breast cancer subtype that lacks HER-2, estrogen- and progesterone-receptor expression is associated with obesity, insulin resistance and metabolic derangements involving the insulin-like growth factor (IGF) pathway. We hypothesized that IGF-1 receptor (IGF1R) targeted therapy will be active in TNBC and will enhance the activity of chemotherapeutic agents used for breast cancer. We aimed (1) to determine if AG1024, an experimental tyrosine kinase inhibitor of IGF-1R, or Figitumumab, a human anti-IGF1R antibody, has a cytotoxic effect on TNBC cell lines as a single agent and (2) to determine if combining AG1024 or Figitumumab with conventional chemotherapeutic agents, doxorubicin or paclitaxel, in TNBC cell lines would enhance their cytotoxic effects. To evaluate the effect of these agents, cytotoxicity assays were conducted using four TNBC cell lines (MDA-MB-231, MDA-MB-468, SUM149 and BT20) and a non-TNBC cell line, MCF7, for comparison.

Our results showed that AG1024 caused a dose-dependent decrease in cell viability in TNBC cell lines and that TNBC cell lines were more sensitive to AG1024 than non-TNBC cell lines. Also, the cytotoxic effects of AG1024 were enhanced in all TNBC cell lines by the addition of paclitaxel and in three out of four TNBC cell lines upon adding doxorubicin. Figitumumab monotherapy failed to have cytotoxic effects on TNBC cell lines but the anti-IGF1R antibody cytotoxic effects were enhanced by addition of doxorubicin in two TNBC cell lines and by addition of paclitaxel in one TNBC cell line. This study suggests that therapies targeting the IGF1R may have clinical application in the treatment of TNBC and tyrosine kinase inhibitors, such as AG1024, may be better suited for treating TNBC than monoclonal antibodies.

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## **1. INTRODUCTION**

Microarray profiling studies have led to the identification of distinct breast cancer subtypes<sup>1</sup>. Breast malignancies are designated “triple-negative” when immunohistochemical and fluorescence *in situ* hybridization testing reveals a lack of HER2/neu expression, estrogen- and progesterone-receptor expression<sup>1</sup>. This subtype of breast cancer frequently resembles the outer or basal layer of the breast duct rather than the inner layer or lumen of the breast duct. However, the triple-negative subtype is not synonymous with basal-like breast cancer. Basal-like malignancies express one or more the basal cytokeratins (CK 5/6, CK14, CK17 and CK903), laminin and fatty acid binding protein<sup>1</sup>. They also tend to be estrogen- and progesterone-receptor negative. 55-85% of basal-like malignancies lack estrogen- and progesterone-receptor expression. Depending on the study, between 65-90% of triple-negative tumors are basal-like. Clearly, there are basal-like malignancies that are not triple-negative and vice-versa.

Triple-negative breast cancer (TNBC) accounts for 10-17% of all breast cancer cases and is more prevalent in pre-menopausal African-American and Hispanic women, compared to Caucasian and post-menopausal African-American women<sup>2</sup>. Moreover, it carries a worse prognosis than its counterparts. Up to 50% of patients will relapse and die of their disease even when it is detected during its early stages<sup>3, 4</sup>. Furthermore, they cannot be treated with therapies such as tamoxifen or trastuzumab (Herceptin®) that have significantly improved outcomes in estrogen receptor-positive or HER-2 positive breast

cancer patients, respectively. Therefore, it is imperative to identify novel therapies for this patient population.

Although the exact pathogenesis of TNBC is not clear, TNBC is more common in women with an elevated waist-hip ratio<sup>5</sup> and increased body mass index (BMI)<sup>6</sup>. Obesity, defined as a body mass index (BMI)  $\geq 30\text{kg/m}^2$ , is an established risk factor for breast cancer in post-menopausal women and prognostic factor in both pre- and post-menopausal women<sup>7, 8</sup>. Obesity causes changes in steroid metabolism that directly or indirectly contribute to breast carcinogenesis; one of these consequences is the up-regulation of insulin and insulin-like growth factors (IGFs)<sup>9, 10</sup>. This suggests that abnormalities in metabolism may be implicated in the development of TNBC.

Insulin receptors (IRs) were first detected on mammary carcinoma samples and shown to bind insulin in 1977<sup>11</sup>. Insulin receptors and insulin-like growth factor receptors (IGFRs) are both tetrameric tyrosine kinases found on the cell surface. These tetramers are comprised of two half-receptors consisting of an extracellular  $\alpha$ -chain that mediates ligand binding and an intracellular  $\beta$ -chain that contains the tyrosine kinase domain. The gene encoding the insulin receptor can be differentially spliced to yield the fetal splice variant, IRA, or the classic adult isoform, IRB<sup>12-14</sup>. IRA is thought to be involved in growth and proliferation whereas IRB is associated with carbohydrate metabolism<sup>15</sup>.

*In vitro* studies have shown that insulin, at physiologic concentrations, stimulates DNA synthesis and thus cell proliferation in breast cancer cells<sup>16, 17</sup>. In patients

with hyperinsulinemia, prospective studies have shown that women with insulin levels in the highest quartile have twice the risk of recurrence and triple the mortality risk from all breast cancer subtypes, compared to women with normal insulin levels<sup>18</sup>. Type II diabetics with breast cancer are known to have more rapid disease progression and approximately 40% increase in 5-year mortality due to their breast cancer<sup>19</sup>. Of note, 1,1-dimethylbiguanide hydrochloride (Metformin®), an anti-diabetic drug that stabilizes glucose flux and reduces insulin resistance via activation of the AMP-kinase dependent pathway, lowers insulin levels by 22% in early stage breast cancer patients<sup>20</sup> and improves survival in diabetic cancer patients<sup>21</sup>. Also, higher pathologic complete response rates are observed in diabetics who take Metformin® compared to those who do not (24% versus 8%)<sup>22</sup>. Finally, Metformin® inhibits cellular proliferation and induces apoptosis in TNBC *in vitro* and *in vivo*<sup>23</sup>. These effects have not been demonstrated in non-TNBC cell lines.

Given the common ancestry and similarities in the signaling pathways downstream of the insulin and insulin-like growth factor 1 receptor (IGF1R), the associations between elements of the IGF axis and carcinoma have also become of interest to the scientific community. The insulin-like growth factor (IGF) ligands I and II play important roles in the regulation of cellular proliferation, differentiation and survival. They are primarily synthesized in the liver and both bind to IGF1R to exert their effects via the RAS-MAPK and PI3K pathways. IGF2R, on the other hand, does not appear to transmit proliferation and survival

signals; rather, it seems to sequester the ligands and thereby act as a negative regulator of the pathway. Of note, IRs and IGF1R can form hybrid receptors; malignant neoplasms have been shown to preferentially express IRA-IGF1R hybrid receptors<sup>24, 25</sup>. Several landmark studies have also shown that IGF1R is implicated in mitogenesis, malignant transformation, invasion and resistance to some anticancer therapies particularly those that target the ER and the epidermal growth factor receptor (EGFR) members, EGFR<sup>26</sup> and HER2<sup>27</sup>.

By 1984, *in vitro* studies had shown IGF I and II have mitogenic activity in breast carcinoma cell lines<sup>28</sup>. In 1987, IGF1R was detected in primary human breast carcinoma specimens<sup>29</sup>. As early as 1989, blockade of the IGF1R using  $\alpha$ -IR-3, a monoclonal antibody, was proven to inhibit growth *in vitro* and thwart the mitogenic effect of IGF-I in breast cancer cell lines. Of note,  $\alpha$ -IR-3 inhibited the growth of TNBC in nude mice but failed to act similarly in estrogen-receptor positive breast cancer cells<sup>30</sup>.

Several studies have also attempted to correlate expression of IGF components with prognosis in breast cancer patients. Initially, high IGF1R expression was believed to be a favorable prognostic factor<sup>31</sup>. In a series of 184 breast cancer specimens, the average IGF1R content was nearly ten times higher in breast carcinomas than in normal breast tissue and IGF1R levels were significantly higher in the low-risk group (ER+/PR+) than in high-risk individuals (ER-/PR-). However, a recent study by Law *et al*<sup>32</sup> suggests that detection of phosphorylated IGF1R (p-IGF1R), not total IGF1R, better predicts survival and is

associated with poor prognosis. They evaluated 438 cases of invasive breast cancer using p-IGF1R antibody on tumor tissue microarrays. Their data suggest that p-IGF1R rather than total IGF1R was indicative of survival and that p-IGF1R was detected in all subtypes of breast cancer represented in their cohort (triple-negative 41.9%, luminal 48.1%, HER2 64.3%). An IGF-I molecular signature associated with poor disease outcome and negative prognostic factors has also been postulated. Through *in vitro* stimulation of breast cancer cells with IGF-I, Creighton *et al* defined an expression pattern of over 800 genes that were either up- or down-regulated. Analysis of gene expression in clinical breast tumors revealed that ER-negative tumors displayed high expression of genes induced by IGF-I and low expression of genes repressed by IGF-I<sup>33</sup>.

Despite evidence suggesting a role for IGFs and IGF1R in breast carcinoma, it was not until the beginning of the 21<sup>st</sup> century that the pharmaceutical industry began to manufacture therapies targeted to IGF1R<sup>34</sup>. There are two main classes of anti-IGF1R therapies: receptor-specific antibodies and receptor kinase inhibitors. IGF1R-specific antibodies include CP-751871 (Figitumumab: Pfizer), AMG479 (Amgen), h10h5 (Genentech), AVE1642 (Sanofi-Aventis), A12 (Imclone), MK0646 (Merck) and R1507 (Roche) and BMS-536924 (Bristol Myers Squibb). AG538 and AG1024 (3-bromo-5-*t*-butyl-4-hydroxy-benzylidenemalonitrile) are two of the IGF1R tyrophostins, receptor tyrosine kinase inhibitors, which have been developed. AG1024 is only used experimentally and has not been tested in humans.

AG1024 binds to the active site of both IGF1R and IR to induce conformational changes that prevent substrates such as IRS-1 and ATP from binding to the receptors. It has a lower affinity for IR and a lower IC50 for IGF1R (7 $\mu$ M for IGF1R compared to 57 $\mu$ M for IR<sup>35</sup>). Early studies of AG1024 in hormone receptor-positive and TNBC cell lines have shown that as a single agent, it is effective at reducing proliferation and inducing apoptosis<sup>36-38</sup>. Using hormone receptor-positive MCF7 cells, Chakraborty *et al* showed that combining  $\alpha$ -IR3, a murine anti-IGF1R monoclonal antibody or AG1024, an IGF1R tyrosine kinase inhibitor, with anti-estrogen reagents leads to greater growth inhibition than using these reagents alone<sup>39</sup>. Other studies have corroborated these findings<sup>40</sup>.

Initial studies of Figitumumab in breast cancer were conducted using MCF7, an estrogen- and progesterone-receptor positive breast carcinoma cell line<sup>41</sup>. Figitumumab binds to the extracellular domain of IGF1R homodimers and heterodimers; it does not cross-react with IR. Furthermore, consecutive immunoblots showed that culturing MCF7 cells with 1 $\mu$ g/mL of Figitumumab caused a time-dependent decrease in IGF1R with maximal effect attained between 3 and 4 hours of exposure. Confocal microscopy also revealed that the same concentration of Figitumumab induced internalization of IGF1R receptors within 15 minutes of exposure. In tumor xenograft models using MCF7 cells, Figitumumab alone inhibited tumor growth and when combined with tamoxifen, an anti-estrogen therapy for estrogen-receptor positive patients, inhibited tumor growth more than Figitumumab or tamoxifen alone<sup>41</sup>. A similar effect was

produced when Figitumumab and doxorubicin (Adria) were combined in 3T3/IGF1R-transfected tumors<sup>41</sup>.

In recent clinical trials involving patients with multiple myeloma, lung cancer or other solid tumors, several agents have induced responses when acting as single-agents and the IGF1R antibodies generally have a favorable toxicity profile<sup>42, 43</sup>. These antibodies cause increases in the serum concentration of human growth hormone and IGF-I but there is no evidence to suggest that increases in IGF-I can overcome the suppression caused by IGF1R inhibitors<sup>42</sup>. To date, clinical trials of IGF1R targeted therapies in breast cancer include a Phase I trial of neoadjuvant Figitumumab in early stage, operable breast cancer (ClinicalTrials.gov identifier: NCT006352) and a Phase I trial of Figitumumab and Pegvisomant, a growth hormone antagonist, in advanced solid tumors (ClinicalTrials.gov identifier: NCT00976508).

Although there is evidence that antagonizing IGF1R in TNBC may be of clinical benefit, most of the studies involving the newly developed IGF1R antibodies have focused on hormone receptor-positive breast cancer models and there are few preliminary studies of IGF1R antibodies in TNBC or TNBC cell lines. A recent study by Zha *et al*<sup>40</sup> examined the effect of h10H5, an anti-human IGF1R monoclonal antibody, in several hormone receptor-positive and TNBC cell lines. The cell lines that showed sensitivity to h10H5 were primarily ER-positive with intermediate to high levels of IGF1R expression. MCF7, a hormone receptor-positive breast carcinoma cell line, was shown to respond to h10H5

whereas MDA-MB-231 and MDA-MB-468, two TNBC negative cell lines failed to show sensitivity to this agent. Nonetheless, the proliferation of three ER-negative cell lines (HCC1143, HDQP1 and SW527) with low to intermediate IGF1R expression was inhibited by h10H5.

Our study proposes to evaluate the activity of IGF1R receptor antibodies and the tyrophostin, AG1024, in TNBC cell lines to explore the therapeutic potential of these agents in this breast cancer subtype. In this study, we will investigate TNBC cell lines that were not represented in previously published studies. Finally, we will explore the potential for IGF1R targeted therapies to enhance the cytotoxicity of conventional chemotherapeutic agents that are the mainstay of treatment for TNBC patients. In this study, we combine doxorubicin, an anthracycline that interferes with DNA synthesis, and paclitaxel, a taxane that stabilizes microtubules, with Figitumumab and AG1024 to determine if greater cellular inhibition is achieved using these conventional chemotherapeutic agents with IGF1R antagonists.

## **2. PURPOSE AND HYPOTHESIS**

There is substantial preclinical and clinical data suggesting that TNBC is associated with obesity, insulin resistance and metabolic derangements (involving IGF and IGF1R). We hypothesize that IGF1R targeted therapy will be active in TNBC [cell lines](#) and will enhance the activity of chemotherapeutic agents used for breast cancer.

## **3. SPECIFIC AIMS**

Specific Aim #1: To confirm that AG1024, an experimental tyrosine kinase inhibitor of IGF-1R, has an anti-proliferative effect on TNBC cell lines as a single agent.

Specific Aim #2: To determine if Figitumumab, a human anti-IGF1R antibody targeting the extracellular domain of the receptor, has an anti-proliferative effect on TNBC cell lines as a single agent.

Specific Aim #3: To determine if AG1024 has an additive cytotoxic effect when combined with conventional chemotherapeutic agents, doxorubicin or paclitaxel, in TNBC cell lines.

Specific Aim #4: To determine if Figitumumab has an additive cytotoxic effect when combined with conventional chemotherapeutic agents, doxorubicin or paclitaxel, in TNBC cell lines.

Specific Aim #5: To compare the effects of AG1024 and Figitumumab on TNBC cell lines with their effects on an ER- and PR-positive breast cancer cell line, MCF7 (alone or in combination with chemotherapeutic agents).

Specific Aim #6: To compare the relative levels of IGF1R expression in the TNBC and hormone-receptor positive cell lines utilized in this study and determine if total IGF1R expression correlates with response to IGF1R-targeted agents.

## **4. MATERIALS AND METHODS**

### Cell Culture

TNBC cell lines (MDA-MB-231, MDA-MB-468, BT-20) were maintained in RPMI 1640 supplemented with 10% fetal bovine serum (FBS), L-glutamine, sodium pyruvate and gentamicin. SUM-149 was maintained in F-12 media containing 5 $\mu$ g/mL insulin, 1 $\mu$ g/mL hydrocortisone, 10mM or 1% HEPES, 5% FBS and gentamicin. The MCF-7 cell line was maintained in Dulbecco's Modified Eagles Medium (DMEM) supplemented with 10% fetal bovine serum, L-glutamine, sodium pyruvate, 7 $\mu$ g/mL insulin and gentamicin. All reagents were purchased from Invitrogen except HEPES and hydrocortisone which were purchased from Sigma. All cell lines were cultured in a 37°C humidified atmosphere containing 95% air and 5% CO<sub>2</sub>.

### Reagents

AG1024, an IGF1R tyrosine kinase inhibitor (Calbiochem: San Diego, CA), was dissolved in 164 $\mu$ L of DMSO to prepare a 20mM stock solution. This solution was stored at -20°C. CP-751871 (Figitumumab) was given as part of collaboration with Dr. Michael DiGiovanna (Yale Cancer Center) and was received as a stock solution of 6.1mg/mL stored at 4°C. Paclitaxel (Taxol: Bristol Myers Squibb, Princeton, NJ) and doxorubicin (Adria: Adria Laboratories Incorporated, Columbus, OH) were stored at concentrations of 7mM and 2mg/mL respectively. Paclitaxel was stored at room temperature and doxorubicin

was stored at 4°C. All of the stock solutions were diluted in OptiMEM (Invitrogen) prior to their addition to the cells.

### Western Blotting

All cell lines were trypsinized, centrifuged and washed in PBS with EDTA-free protease inhibitor cocktail (Roche: Indianapolis, IN). Cells were centrifuged, PBS was aspirated and the cell pellet was stored at -80°C. After thawing the frozen pellet, the cells were resuspended in 100-120µL of CelLytic M lysis reagent (Sigma-Aldrich, St Louis, MO) containing protease inhibitor cocktail (Roche). This mixture was sonicated on ice for 10 seconds then incubated for 15 minutes at 4°C followed by centrifugation at maximum speed for 15 minutes at 4°C. A standard curve was calibrated using BSA (2µg/µL). Nine microliters of distilled water and 200µL of BioRad protein assay dye were added to 1µL of each sample to calculate each sample protein concentration. 10µL of lysis buffer and Laemmli sample buffer was added to 30µg of protein from each cell line. These mixtures were boiled for 5 minutes then cooled on ice for 5 minutes and proteins were separated on Bio-Rad Ready 4-20% Tris-HCl gels. Proteins were separated on an SDS-PAGE 4-20% Tris-HCl gel (Bio-Rad: Hercules, CA). Proteins were then transferred onto a nitrocellulose membrane (Bio-Rad) in a transfer buffer containing 20% methanol. Western blot analysis was performed with anti-IGF1R antibody (Cell Signaling Technology: Danvers, MA) and appropriate secondary antibodies. Images were taken on a ChemiDoc XL (Bio-Rad).

### Cell Proliferation Assays

To determine the optimal concentration of cells per well for 5-day drug assays, a proliferation assay was performed for each cell line. All cell lines were harvested, resuspended in OptiMEM and plated in triplicate in 96-well plates at four or more different concentrations of cells per well. Cells were allowed to adhere for 24 hours then 10 $\mu$ L of Cell Proliferation Reagent, WST-1 (Roche Diagnostics: Mannheim, Germany), was added to each well. Plates were incubated for 2 hours when optical density at 450nm was determined using a microplate reader (EL-800 Universal microplate reader: Bio-Tek instruments, Winooski, VT). The WST-1 colorimetric assay was performed daily and a proliferation curve was produced using the readings from Days 0-5. The optimal cell concentration at Day 0 was defined as the concentration at which cells remain in the log phase of growth on Day 5. One thousand cells/well was estimated as the optimal concentration for MCF7 and BT-20 cells. Fifteen hundred cells/well was estimated to be the optimal concentration for MDA-MB-231 cell line. Three thousand cells/well was estimated to be the optimal concentration for MDA-MB-468 and SUM149 cells.

### Cytotoxicity Assays

A. All cells were harvested, resuspended in OptiMEM and plated in triplicate in 96-well plates at their optimal concentration. After a 24-hour incubation period at

37°C, drugs were added to the cells. Cells were not confluent at the time drug was added. Five concentrations of each drug were tested in triplicate. Paclitaxel was used at 0.2nM, 2nM, 20nM, 200nM and 2000nM. AG1024 and doxorubicin were tested at 0.01μM, 0.1μM, 1μM, 10μM and 100μM. Figitumumab was tested at 0.1μM, 1μM, 10μM, 100μM and 1000μM. The WST-1 colorimetric assay was performed on Day 5. The optical densities of the controls (the cells not exposed to the drugs) were averaged to obtain the mean. This number represented 100% survival of cells. The percent of surviving cells in each well containing drug was calculated using the following equation: (optical density in drug-treated well / average optical density of the controls) x 100. For each drug concentration, the average of the three calculated percentages represented the percent of surviving cells for that concentration. The half maximal inhibitory concentration was calculated using GraphPad Prism® (GraphPad Software Incorporated: La Jolla, CA).

B. All cell lines were harvested, resuspended in OptiMEM and plated at the optimal concentration of cells per well. After a 24-hour incubation period, drugs were added as needed in each well. On Day 5, a WST-1 colorimetric assay was performed to determine the cytotoxicity of the drug(s) on the cells. Calculations were performed as previously described under IC50 determination assays. For each concentration, the mean  $\pm$  two standard deviations are reported. P-values were calculated using an unpaired t-test.

## **5. RESULTS**

### **Expression of IGF1R in MCF7 (hormone receptor-positive) and triple-negative breast cancer cell lines.**

Western blots were performed to compare the relative total IGF1R expression levels in MCF7, MDA-MB-231, MDA-MB-468, BT-20 and SUM149 cell lines (Figure 1). As expected, BT-20 cell lines exhibited the highest levels of total IGF1R<sup>44</sup>. An intermediate level of expression was noted in MCF7 cells while MDA-MB-468 and SUM-149 cells expressed the lowest levels of IGF1R. MDA-MB-231 total IGF1R expression was not analyzed due to loss of the cell lysate, however, other studies have shown that MDA-MB-231 has a similar expression to MDA-MB-468<sup>40</sup>.

### **AG1024 inhibits the proliferation of hormone receptor-positive and TNBC cells in a dose-dependent fashion.**

Dose-response curves were generated to demonstrate the effect of AG1024, an IGF1R tyrosine kinase inhibitor, on each cell line. AG1024 significantly inhibited the proliferation of the MDA-MB-231 (Fig. 2B), MDA-MB-468 (Fig. 2C) and SUM149 (Fig. 2E) cell lines in a dose-dependent manner (<30% cell viability when incubated with 40 $\mu$ M AG1024). AG1024 also caused intermediate growth inhibition in BT20 (Fig. 2D) cells (<40% cell viability when incubated with 40 $\mu$ M AG1024) and was least effective in MCF7 (Fig. 2A) cells (<60% cell viability when incubated with 40 $\mu$ M AG1024.)

**Figitumumab, when used as a single agent, has no anti-proliferative activity in hormone receptor-positive and triple-negative breast cancer cell lines.**

Dose-response curves were generated to demonstrate the effect of Figitumumab, an IGF1R targeted antibody, on each cell line (Figure 3). At concentrations ranging from 0 $\mu$ M to 0.585 $\mu$ M, Figitumumab had no discernible inhibitory effect on the proliferation of MCF7, MDA-MB-231, MDA-MB-468, BT20 and SUM149 cells.

**AG1024 enhances the anti-proliferative activity of doxorubicin in MDA-MB-231, MDA-MB-468 and SUM149 triple-negative breast cancer cell lines.**

AG1024 was combined with doxorubicin to determine if it had an additive effect on the anti-proliferative action of paclitaxel. Bar graphs depicting the effect of AG1024 alone and in combination with doxorubicin were created for each cell line. Drug combinations were interpreted to cause additive or enhanced cytotoxicity if the combination caused a statistically significant decrease in cell viability compared to either drug alone.

The proliferation of MCF7 cells was not inhibited by any of the tested doxorubicin concentrations. When 1 $\mu$ M AG1024 was used alone, it was associated with 90% cell viability ( $\pm$ 6%, 95% CI), however when either 0.01 $\mu$ M or 0.05 $\mu$ M doxorubicin was added, the combination led to an 11% decrease in cell viability. When 1 $\mu$ M AG1024 was combined with 0.1 $\mu$ M doxorubicin there was no evidence of additive

cytotoxicity. Hence, the observed additive cytotoxicity at lower doses is modest and not consistent at all dose ranges (Figure 4A).

In contrast, there was a dramatic decrease in cell viability of MDA-MB-231 cells when increasing concentrations of doxorubicin were added to 1 $\mu$ M AG1024 (Figure 5A). Doxorubicin alone at 0.01 $\mu$ M resulted in 84% cell viability ( $\pm$ 2%, 95% CI) and 1 $\mu$ M AG1024 alone led to 67% cell viability ( $\pm$ 8%, 95% CI). However, adding 0.01 $\mu$ M doxorubicin to 1 $\mu$ M AG1024 led to 45% cell viability ( $\pm$ 8%,  $p=0.002$ ) whereas adding 0.05 $\mu$ M doxorubicin produced 20% cell viability ( $\pm$ 2%,  $p=0.001$  for 1 $\mu$ M AG1024 alone versus combined with 0.05 $\mu$ M doxorubicin). Combining 0.05 $\mu$ M doxorubicin with 1 $\mu$ M AG1024 produced the same level of inhibition as that achieved by 0.4 $\mu$ M doxorubicin used as monotherapy.

Growth of MDA-MB-468 cells was inhibited by doxorubicin in a dose-dependent manner. A decrease in cell viability was appreciated when 0.05 $\mu$ M doxorubicin was added to 1 $\mu$ M AG1024 (Figure 6A). Incubating MDA-MB-468 cells with only 0.05 $\mu$ M doxorubicin led to approximately 48% cell viability. Incubation of cells with 1 $\mu$ M AG1024 alone caused 48% cell viability ( $\pm$ 5%) compared to 23% ( $\pm$ 1%) when 0.05 $\mu$ M doxorubicin was added. It is possible that there is an enhanced cytotoxic effect when 1 $\mu$ M AG1024 is combined with 0.01 $\mu$ M doxorubicin but this effect may have been obscured by the variation and wide standard deviation among the replicates of the 0.01 $\mu$ M doxorubicin assay. Additive cytotoxic effects were also observed to a lesser degree when comparing 5 $\mu$ M AG1024 alone (40% $\pm$ 1%) to 5 $\mu$ M AG1024 plus 0.01 $\mu$ M doxorubicin

(34%±4%, p=0.006) and 5µM AG1024 plus 0.05µM doxorubicin (29%±4%, p=0.001).

In BT20 cells, doxorubicin monotherapy caused growth inhibition only at the highest concentration tested - 0.4µM led to 75% cell viability ±15%. Enhancement of AG1024's effect was observed when 1µM AG1024 was combined with 0.1µM doxorubicin (Figure 7A). This combination caused 80% cell viability (±2%) compared to 93% cell viability (±5%) when 1µM of AG1024 was used alone or 94% cell viability (±6%,) when 0.1µM of doxorubicin was used alone. However, a higher concentration of doxorubicin (0.2µM) failed to produce additive cytotoxicity with 1µM AG1024 and inhibited cell viability to a lesser extent than 0.1µM doxorubicin plus 1µM AG1024. These findings suggest that the optimal dose-range for AG1024 and doxorubicin is likely to be achievable *in vivo*.

In SUM149 cells, 80% cell viability (±3%) was seen when 1µM AG1024 was used alone and 59% cell viability (±7%) was noted when 0.2µM doxorubicin alone was used. Cell viability decreased to 45% (±1%) when 0.2µM doxorubicin was combined with 1µM AG1024. Addition of doxorubicin to 5µM and 10µM AG1024 failed to produce greater inhibition of cell proliferation than 5µM or 10µM AG1024 alone (Figure 8A).

**AG1024 enhances the anti-proliferative activity of paclitaxel in MDA-MB-231, MDA-MB-468, BT-20 and SUM149 triple-negative breast cancer cell lines.**

AG1024 was combined with paclitaxel to determine if it had an additive effect on paclitaxel's anti-proliferative action. Bar graphs depicting the effect of AG1024 alone and in combination with paclitaxel were created for each cell line. The proliferation of MCF7 cells was not inhibited by any of the tested paclitaxel concentrations and there was no increase in cytotoxicity when paclitaxel and AG1024 were combined (Figure 4B).

Paclitaxel inhibited the growth of all triple negative cell lines in a dose-dependent manner and enhanced cytotoxicity with combinations of paclitaxel and AG1024 was observed in all of the triple-negative cell lines (Figures 5B-8B). In MDA-MB-231 cells, incubation with 1 $\mu$ M AG1024 alone led to 67% cell viability ( $\pm$ 8%). Treatment with 1.136nM and 2.27nM paclitaxel alone resulted in 93% cell viability ( $\pm$ 16%) and 54% cell viability ( $\pm$ 9%), respectively. Compared to 1 $\mu$ M AG1024 alone, adding 1.136nM and 2.27nM paclitaxel resulted in an 18% decrease ( $\pm$ 11%,  $p=0.008$ ) and 36% decrease in MDA-MB-231 cell viability ( $\pm$ 7%,  $p=0.0003$ ), respectively. Combining 5 $\mu$ M AG1024 with 1.136nM paclitaxel failed to produce greater growth inhibition than 5 $\mu$ M AG1024 alone in MDA-MB-231 cells. However, 49% cell viability ( $\pm$ 4%) was seen after treatment with 5 $\mu$ M

AG1024 alone compared to 28% cell viability ( $\pm 8\%$ ,  $p=0.001$ ) after incubation with  $5\mu\text{M}$  AG1024 and  $2.27\text{nM}$  paclitaxel.

In MDA-MB-468 cells, treatment with  $1.136\text{nM}$  paclitaxel failed to inhibit cell proliferation while using  $2.27\text{nM}$  paclitaxel alone caused 69% cell viability ( $\pm 10\%$ .) Incubation with  $1\mu\text{M}$  AG1024 alone led to 48% cell viability ( $\pm 5\%$ ) and adding  $2.27\text{nM}$  paclitaxel to  $1\mu\text{M}$  AG1024 caused a decrease to 30% cell viability ( $\pm 2\%$ ). Also, adding  $1.136\text{nM}$  paclitaxel to  $5\mu\text{M}$  AG1024 led to a 7% decrease in cell viability ( $40\% \pm 1\%$  using  $5\mu\text{M}$  AG1024 alone compared to  $33\% \pm 1\%$  when combined with  $1.136\text{nM}$  paclitaxel) and adding  $2.27\text{nM}$  led to an 11% decrease in cell viability ( $\pm 3\%$ ).

Paclitaxel also inhibited the growth of BT20 cells with increasing doses and additive cytotoxicity was observed between the lowest dose of paclitaxel and the two highest concentrations of AG1024 (Figure 7B). Treating BT20 cells with  $1.136\text{nM}$  paclitaxel alone resulted in 79% cell viability ( $\pm 3\%$ ). Treating these cells with  $5\mu\text{M}$  AG1024 alone caused 57% cell viability ( $\pm 1\%$ ) whereas adding  $1.136\text{nM}$  paclitaxel led to 51% cell viability ( $\pm 4\%$ ,  $p=0.03$ ). Similarly,  $10\mu\text{M}$  AG1024 alone resulted in 57% cell viability ( $\pm 3\%$ ) but with addition of  $1.136\text{nM}$  paclitaxel, the cell viability fell to 51% ( $\pm 2\%$ ,  $p=0.005$ ).

In SUM149 cells, enhanced cytotoxicity was evident with multiple combinations of paclitaxel and AG1024 (Figure 8B). Treatment of SUM149 cells with  $1\mu\text{M}$  AG1024 alone led to 80% cell viability ( $\pm 3\%$ ) and incubation with  $1.136\text{nM}$  paclitaxel alone resulted in 69% cell viability ( $\pm 11\%$ ); adding  $1.136\text{nM}$

paclitaxel to 1 $\mu$ M AG1024 further inhibited cell viability to 53% ( $p=0.04$  for 1.136nM paclitaxel alone versus combined with 1 $\mu$ M AG1024). Treatment of SUM149 cells with 5 $\mu$ M AG1024 alone produced 49% cell viability ( $\pm 4\%$ ) and adding 1.136nM paclitaxel led to 36% cell viability ( $\pm 1\%$ ,  $p=0.004$  for 5 $\mu$ M AG1024 alone versus combined with 1.136nM paclitaxel). Combinations of 10 $\mu$ M AG1024 and paclitaxel also provided evidence of increased cytotoxicity when the two compounds were combined. 10 $\mu$ M AG1024 alone caused 34% cell viability ( $\pm 2\%$ ) whereas adding 1.136nM paclitaxel led to 28% cell viability. Using concentrations of paclitaxel greater than 1.136nM in combination with any dose of AG1024 led to approximately the same level of cellular growth inhibition.

**Figitumumab enhances the anti-proliferative activity of doxorubicin in MCF7, MDA-MB-468 and SUM149 breast cancer cell lines.**

Figitumumab was combined with doxorubicin to determine if it had an additive effect on the anti-proliferative action of doxorubicin. Bar graphs depicting the effect of Figitumumab, a targeted IGF1R antibody, alone and in combination with doxorubicin were created for each cell line (Figures 4C-8C).

Neither doxorubicin nor Figitumumab significantly inhibited the proliferation of MCF7 cells as single agents at any of the tested concentrations (Figure 4C). However, increased cytotoxicity was appreciated when 0.175 $\mu$ M Figitumumab was combined with 0.05 $\mu$ M doxorubicin. Adding 0.05 $\mu$ M

doxorubicin to 0.175 $\mu$ M Figitumumab caused cell viability to decrease by 11% ( $\pm$ 6%,  $p=0.005$ ).

The proliferation of MDA-MB-231 and MDA-MB-468 cells was inhibited by doxorubicin alone (0.05 $\mu$ M-0.4 $\mu$ M); however, Figitumumab alone did not inhibit proliferation of these cell lines (Figures 5C and 6C respectively). Increased cytotoxicity was noted when 0.175 $\mu$ M or 0.585 $\mu$ M of Figitumumab was combined with 0.05 $\mu$ M of doxorubicin in MDA-MB-468 cells (47% $\pm$ 4% and 43% $\pm$ 3% cell viability, respectively compared to 81% $\pm$ 26 cell viability when 0.05  $\mu$ M of doxorubicin was used alone.)

Doxorubicin and Figitumumab combinations also increased cytotoxicity in SUM149 cells. Treatment with 0.01 $\mu$ M doxorubicin alone did not inhibit cellular proliferation. Treatment with 0.1 $\mu$ M and 0.2 $\mu$ M doxorubicin alone caused approximately 100% cell viability, 85% cell viability ( $\pm$ 7%) and 59% ( $\pm$ 7%) respectively. When treated with 0.0585 $\mu$ M Figitumumab alone, SUM149 cells did not experience inhibition of cell proliferation but adding 0.01 $\mu$ M, 0.1 $\mu$ M and 0.2 $\mu$ M doxorubicin led to 88% ( $\pm$ 11%), 54% ( $\pm$ 4%) and 35% ( $\pm$ 4%) cell viability, respectively (Figure 8C). Adding doxorubicin to 0.175 $\mu$ M Figitumumab and 0.585 $\mu$ M Figitumumab led to cell viability levels similar to those seen with 0.0585 $\mu$ M Figitumumab. An enhanced cytotoxic effect was not appreciated in MDA-MB-231 or BT-20 cells with any combinations of Figitumumab and doxorubicin.

**Figitumumab enhances the anti-proliferative activity of paclitaxel in the MCF7 and MDA-MB-231 breast cancer cell lines.**

Figitumumab was combined with paclitaxel to determine if it had an additive effect on paclitaxel's anti-proliferative action. Bar graphs depicting the effect of Figitumumab alone and in combination with paclitaxel were created for each cell line.

As single agents, paclitaxel and Figitumumab did not inhibit the proliferation of MCF7 cells. However, increased cytotoxicity was detected at all concentrations of Figitumumab when either 2.27nM or 9.09nM paclitaxel was added (Figure 4D). The addition of 2.27nM or 9.09nM paclitaxel to 0.0585 $\mu$ M Figitumumab both caused a 30% decrease in cell viability ( $\pm$ 9%). When the same concentrations of paclitaxel were used in combination with 0.175 $\mu$ M Figitumumab, a 23-26% decrease in cell viability was noted. Combining 0.585 $\mu$ M Figitumumab with 2.27nM or 9.09nM paclitaxel led to approximately 13-19% decrease in cell viability.

Paclitaxel alone significantly inhibited the proliferation of MDA-MB-231 and MDA-MB-468 cells (2.27nM-18.18nM, Figures 5D and 6D) as well as BT20 and SUM149 cells (1.136nM-18.18nM, Figures 7D and 8D) whereas Figitumumab alone did not inhibit proliferation of these cells. However, the

lowest and intermediate concentrations of Figitumumab (0.0585 $\mu$ M and 0.175 $\mu$ M) had an increased cytotoxic effect when combined with 2.27nM paclitaxel in MDA-MB-231 cells (52% $\pm$ 6% and 56% $\pm$ 9%, respectively compared to 69% $\pm$ 1 with 2.27nM paclitaxel alone.) This effect was not discerned in MDA-MB-468, BT20 or SUM149 cells.

## **6. DISCUSSION**

The primary aim of this study was to explore whether antagonizing the IGF1R in triple-negative breast cancer models is a useful therapeutic strategy. The association between triple-negative breast cancer, obesity and metabolic derangements involving the IGF1R pathway make IGF1R a logical target. To examine the activity of IGF1R antagonists in triple-negative breast cancer cell lines, we performed cytotoxicity assays using AG1024, an IGF1R tyrosine kinase inhibitor, and Figitumumab, an IGF1R specific antibody, as single agents. As triple-negative patients are often treated with doxorubicin or paclitaxel, two chemotherapeutic agents, cytotoxicity assays testing combinations of these reagents with doxorubicin or paclitaxel were also performed. Although AG1024 has been studied in triple-negative breast cell lines, there are no studies documenting the effect of Figitumumab in these breast cancer cell lines. Furthermore, no prior publications have explored if enhanced cytotoxicity is achieved when IGF1R inhibitors are combined with doxorubicin or paclitaxel in TNBC cell lines.

Our results indicate that AG1024 effectively inhibits the proliferation of both hormone receptor-positive and TNBC cell lines. However, in our study, AG1024 produced higher levels of inhibition in TNBC cell lines, compared to the ER- and PR-positive cell line, MCF7. After treatment with 40 $\mu$ M AG1024, MCF7

cells exhibited 52% cell viability whereas treatment of MDA-MB-231 cells, MDA-MB-468, BT20 and SUM149 cells resulted in 15%, 26%, 34% and 18% cell viability, respectively. Cell lines with the highest level of IGF1R expression

(MCF7 and BT20) were least sensitive to AG1024 whereas the cell lines with relatively low levels of IGF1R expression were the most sensitive to AG1024 (MDA-MB-231, MDA-MB-468 and SUM149.)

Evidence of additive cytotoxic effects due to AG1024 and doxorubicin combinations was seen in MDA-MB-231, MDA-MB-468 and SUM149 triple-negative cell lines. Additive cytotoxicity was evident when the lowest concentration of AG1024 ( $1\mu\text{M}$ ) was combined with the lowest concentration of doxorubicin ( $0.01\mu\text{M}$ ) in MDA-MB-231 cell lines. However, this concentration of doxorubicin did not increase cytotoxicity when added to  $1\mu\text{M}$  AG1024 in MDA-MB-468 and SUM149 cell lines. A higher concentration of doxorubicin,  $0.05\mu\text{M}$  in MDA-MB-468 cell lines and  $0.2\mu\text{M}$  in SUM149, was needed to produce additive cytotoxic effects. Also, MDA-MB-468 cells were the only cell line to exhibit this effect when  $5\mu\text{M}$  AG1024 was combined with doxorubicin. The dose of doxorubicin at which additive cytotoxic effects are elicited appears to depend on the cell line's sensitivity to doxorubicin. The cell lines that were more sensitive to doxorubicin (MDA-MB-231 and MDA-MB-468) exhibited increased cytotoxicity with AG1024 at a lower concentration of doxorubicin.

Of note, the cell lines with the highest IGF1R expression levels (MCF7 and BT20) did not exhibit enhanced cytotoxicity with combinations of AG1024

and doxorubicin. In this study, the proliferation of MCF7 cells was not inhibited after treatment with concentrations of doxorubicin ranging from 0.01 $\mu$ M to 0.4 $\mu$ M.

Our findings are consistent with other studies that have shown an IC<sub>50</sub> as high as 5 $\mu$ M doxorubicin for MCF7 cells<sup>45</sup>.

Combinations of AG1024 and paclitaxel only produced increased cytotoxicity in the TNBC cell lines. The proliferation of all the TNBC cell lines was inhibited by paclitaxel alone. The cell lines that were most sensitive to AG1024 (MDA-MB-231, MDA-MB-468 and SUM149) exhibited increased cytotoxicity due to AG1024 and paclitaxel at the lowest concentration of AG1024 (1 $\mu$ M) whereas the cell lines that were less sensitive to AG1024 (BT20) demonstrated this effect with a higher dose of AG1024 (5 $\mu$ M) but not with the lowest dose of AG1024 (1 $\mu$ M). Treatment with 1.136nM paclitaxel alone failed to inhibit MDA-MB-468 proliferation while cell viability fell to 93% in MDA-MB-231 cells and approximately 80% in BT20 and SUM149 cells. Of note, the lowest concentration of paclitaxel, 1.136nM, increased cytotoxicity in all cell lines regardless of their sensitivity to paclitaxel. In this study, concentrations of paclitaxel ranging from 1.136nM to 18.18nM failed to inhibit the cellular proliferation of MCF7 cells. These findings are corroborated by studies that have demonstrated an IC<sub>50</sub> as high as 200nM for MCF7 cells<sup>45</sup>.

Figitumumab, when used as a sole agent, did not inhibit cell proliferation regardless of hormone receptor status. However, there was evidence of

increased cytotoxicity due to combinations of Figitumumab and doxorubicin or paclitaxel. At the lowest concentration of Figitumumab that we tested (0.0585 $\mu$ M), Figitumumab enhanced the cytotoxic effects of both low and high

concentrations of doxorubicin in SUM149 cells. In MCF7 cells, this effect between Figitumumab and doxorubicin was evident at an intermediate dose of Figitumumab (0.175 $\mu$ M) and an intermediate dose of doxorubicin (0.05 $\mu$ M). A similar response was noted in MDA-MB-468 cells which demonstrated increased cytotoxicity when intermediate to high doses of Figitumumab (0.175 $\mu$ M and 0.585 $\mu$ M) were combined with an intermediate dose of doxorubicin (0.05 $\mu$ M.)

Of note, even though MDA-MB-468 cells were the most sensitive to doxorubicin when used as a single agent, an intermediate dose of doxorubicin was needed to elicit increased cytotoxicity with Figitumumab. In contrast, SUM149 cells were slightly less sensitive to doxorubicin than MDA-MB-468 cells yet they demonstrated this response at low and high doses of doxorubicin. Therefore, doxorubicin sensitivity did not predict the concentration at which doxorubicin and Figitumumab combinations would enhance cytotoxicity in our study.

Sensitivity to doxorubicin alone also did not predict whether doxorubicin and Figitumumab would enhance cytotoxicity. For instance, MDA-MB-231 cells were sensitive to doxorubicin when used as a single agent. Doxorubicin at 0.01 $\mu$ M failed to inhibit the proliferation of MDA-MB-231 cells but 0.05 $\mu$ M

doxorubicin caused the cell viability to drop sharply to 39%. However, there was no discernible increase in cytotoxicity due to Figitumumab and doxorubicin

combinations in MDA-MB-231 cells whereas MCF7 cells that failed to respond to doxorubicin as a single agent, exhibited this effect.

In MCF7 and MDA-MB-231 cells, Figitumumab enhanced the anti-proliferative activity of paclitaxel. In MCF7 cells, this effect was noted at all concentrations of Figitumumab and intermediate concentrations of paclitaxel. In MDA-MB-231 cells, increased cytotoxicity was noted when low and intermediate concentrations of Figitumumab were combined with a low concentration of paclitaxel. Of note, sensitivity to paclitaxel did not predict that increased cytotoxicity would occur with Figitumumab and paclitaxel combinations since MDA-MB-468, BT20 and SUM149 cells were sensitive to paclitaxel alone but failed to experience enhanced cytotoxic effects with Figitumumab and paclitaxel combinations.

This study demonstrates that antagonizing IGF1R is effective in inhibiting the proliferation of several triple-negative breast cancer cell lines. AG1024, a receptor tyrosine kinase inhibitor, appeared to be more effective as a single agent than Figitumumab, an IGF1R specific antibody. Moreover, our data show that combining AG1024 with paclitaxel enhanced the anti-proliferative activity of AG1024 in all the TNBC cell lines. This effect was not as striking in the hormone

receptor-positive cell line, MCF7. Furthermore, combinations of AG1024 and doxorubicin caused additive cytotoxicity in three out of four TNBC cell lines.

The toxicity of doxorubicin and paclitaxel are well-documented. Significant adverse reactions associated with doxorubicin include acute or delayed

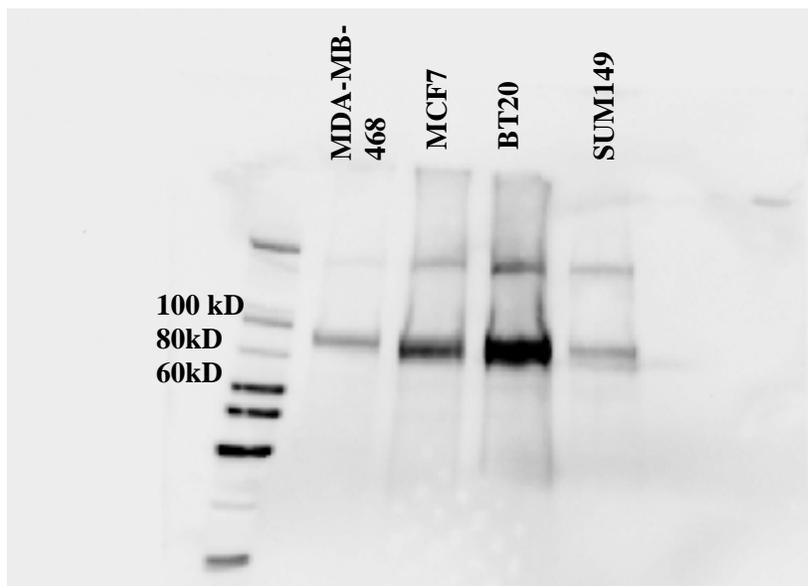
cardiotoxicity, colon necrosis, gastrointestinal ulceration and infertility. Paclitaxel's side effects include peripheral neuropathy (seen in up to 70% of patients), nausea, vomiting, mucositis and increases in creatinine and liver enzymes. Decreasing the amount of doxorubicin or paclitaxel used in chemotherapy regimens is a desirable goal since it could reduce the risk of adverse reactions and improve the quality of life of patients.

Although Figitumumab did not inhibit proliferation as a single agent, addition of doxorubicin caused additive cytotoxicity in MCF7 cells and one TNBC cell lines. Combining Figitumumab and paclitaxel improved Figitumumab's ability to antagonize cell proliferation in hormone receptor-positive MCF7 cells and two TNBC cell lines.

Our investigation is a preliminary study of IGF1R antagonists alone and in combination with chemotherapy in TNBC cell lines. Limitations of this study include the small number of cell lines employed. Testing other TNBC and hormone receptor-positive breast cancer cell lines, besides those used in this study, is required to further elucidate the effect of AG1024 and Figitumumab on these subtypes of breast cancer. In addition, each cytotoxicity assay will need to be repeated several times in order to ensure that the results we obtained

are reproducible. Furthermore, analysis of IGF1R expression after treatment with AG1024 and Figitumumab (alone and in combination with chemotherapy) would also provide useful data about the effect of targeted IGF1R therapies. Overall, our findings suggest that there is a role for IGF1R antagonism in the treatment of TNBC and that kinase inhibitors, like AG1024, may be more effective agents than anti-IGF1R monoclonal antibodies.

## 7. FIGURE REFERENCES AND LEGENDS



**Figure 1: Total IGF1R protein expression in breast carcinoma cell lines.** 30 $\mu$ g of protein from each cell line was loaded on BioRad Ready 4-20% Tris-HCl gel. Western blots were performed by Sofya Rodov (SR). IGF1R, a 95 kiloDalton protein, was detected in each of the cell lines. BT-20 cell lines exhibited the highest levels of total IGF1R<sup>44</sup>. An intermediate level of expression was noted in MCF7 cells while MDA-MB-468 and SUM-149 cells expressed the lowest levels of IGF1R. MDA-MB-231 total IGF1R expression was not analyzed due to loss of the cell lysate, however, other studies have shown that MDA-MB-231 has a similar expression to MDA-MB-468<sup>40</sup>

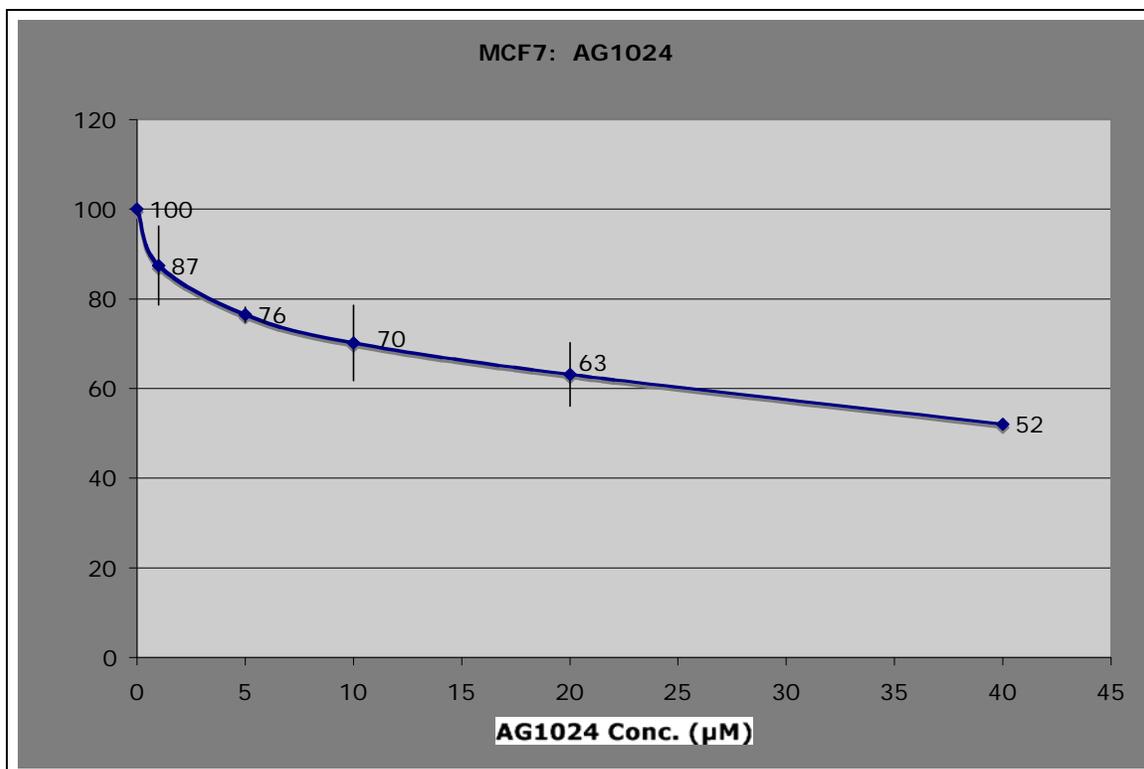


Figure 2A

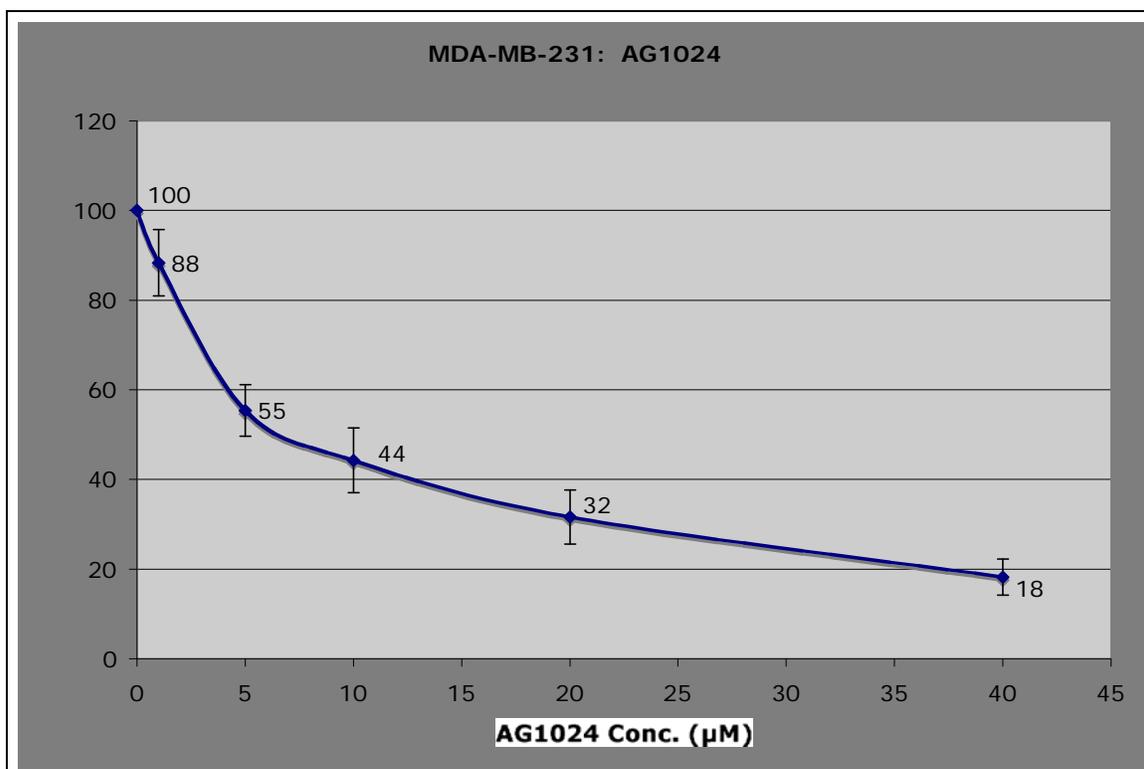


Figure 2B

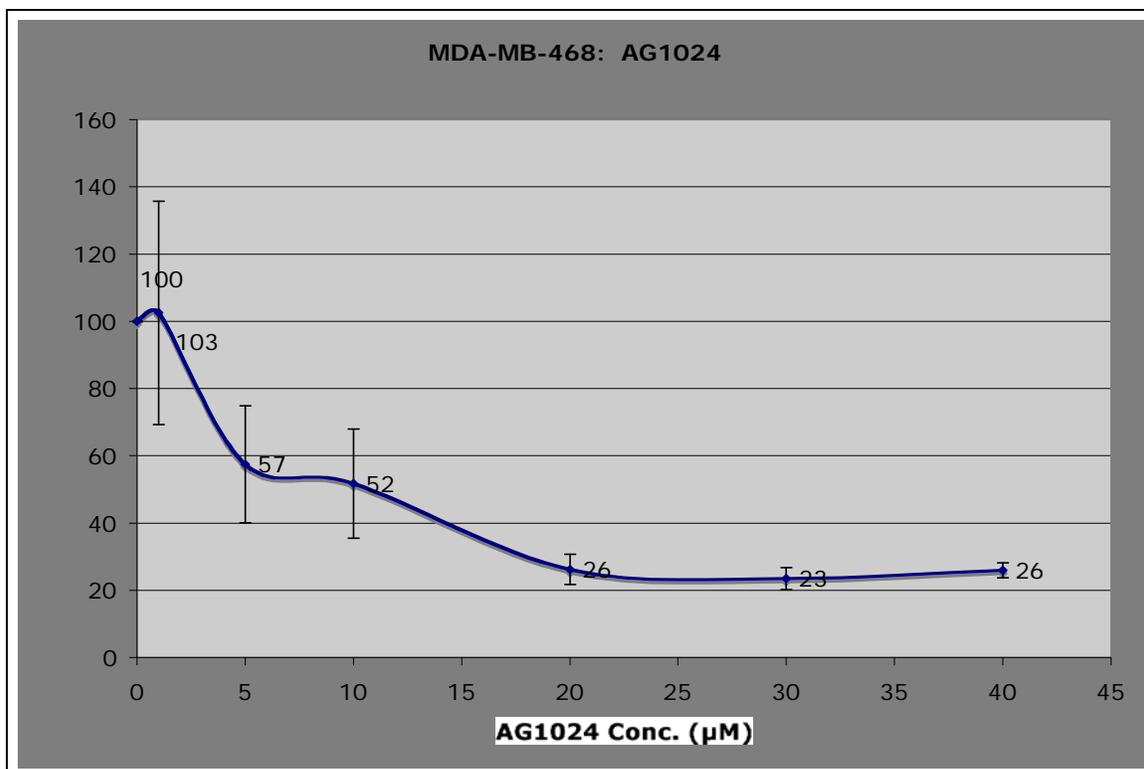


Figure 2C

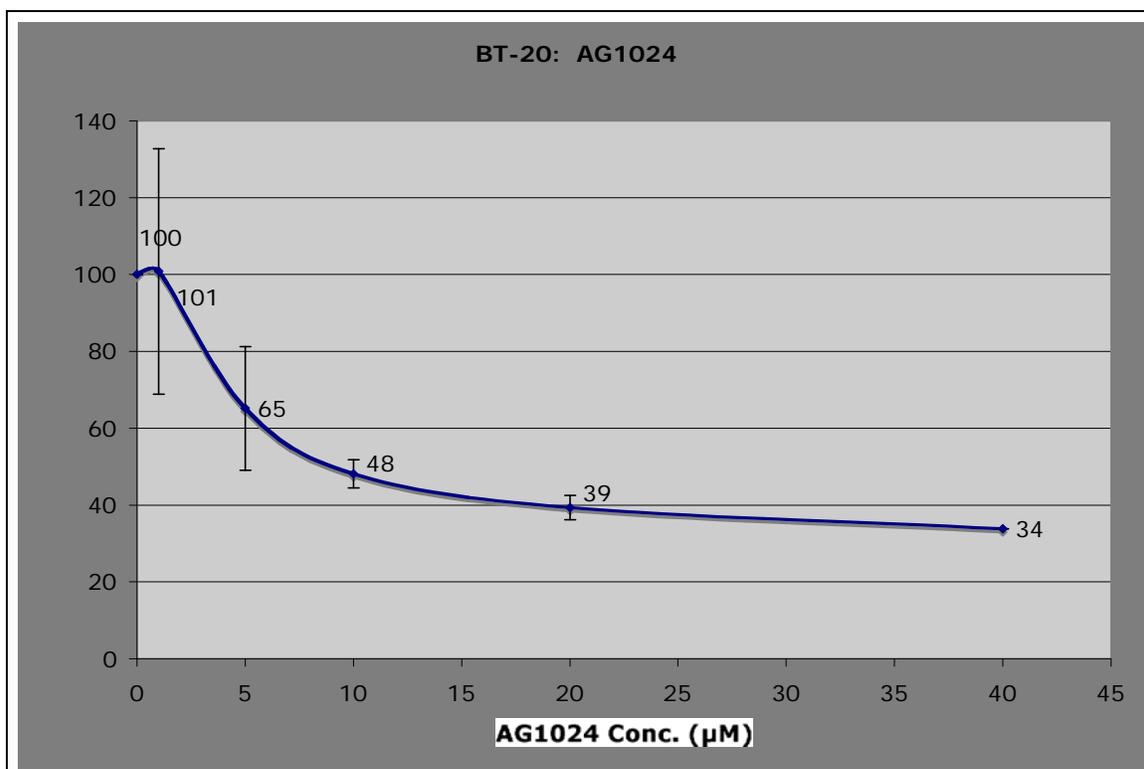


Figure 2D

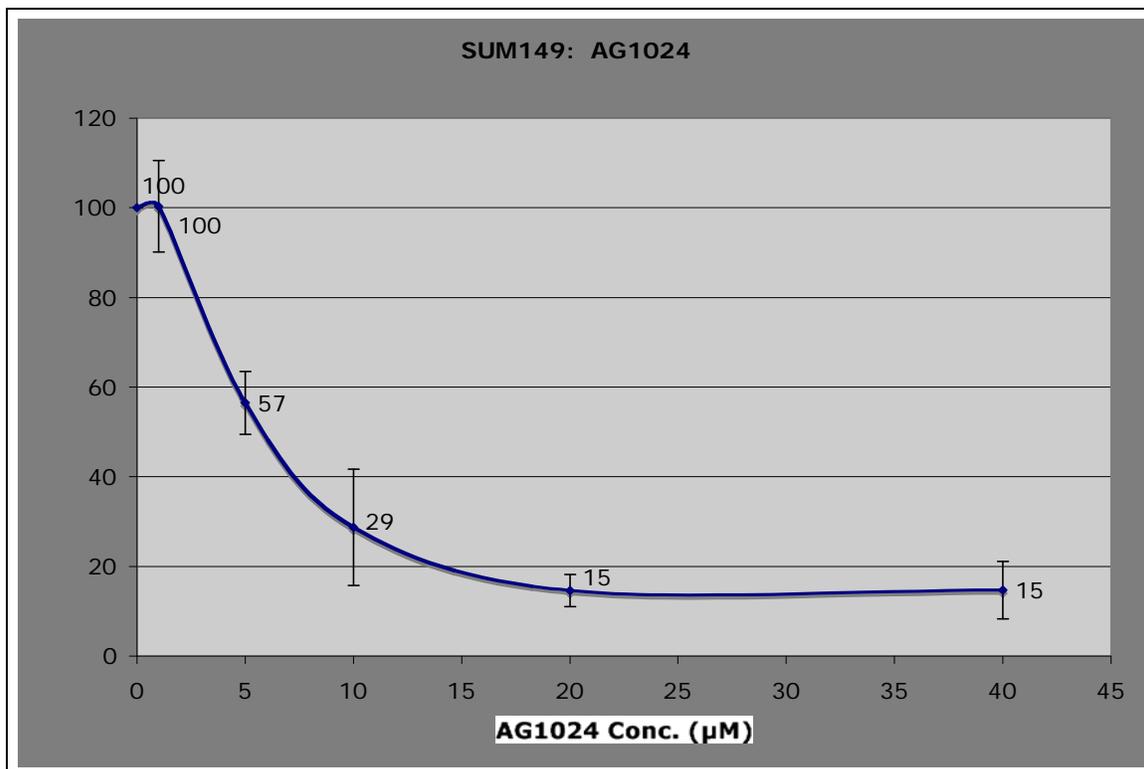


Figure 2E

Figure 2A-E: Effect of AG1024, a tyrosine kinase inhibitor of IGF1R, on MCF7 (2A), a hormone receptor positive breast cancer cell line and triple-negative breast cancer cell lines, MDA-MB-231(2B), MDA-MB-468(2C), BT20 (2D) and SUM149 (2E). Cells were seeded at their optimal concentration in 96 well plates then allowed to adhere overnight. AG1024 was added the following day (Day 0). After 5 days of incubation, the WST-1 assay was performed. Each point represents the percent of surviving cells compared to control (cells not exposed to AG1024). The mean of three wells  $\pm 2SD$  (95% CI) is shown. AG1024 decreased the viability of all cell lines in a dose-dependent fashion. The MCF7 cell line was the least sensitive to AG1024. Assays in Figures 2A-2C were executed by Onyi Offor (OO). Assays in Figures 2D-2E were executed jointly by OO and SR.

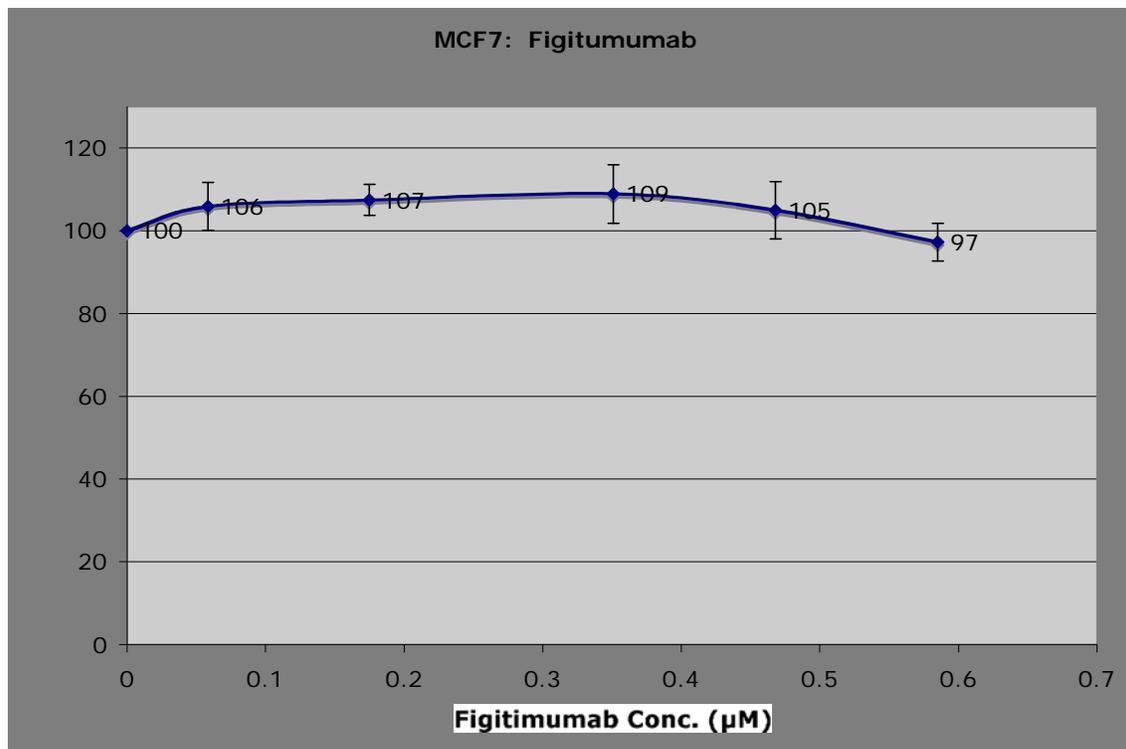


Figure 3A

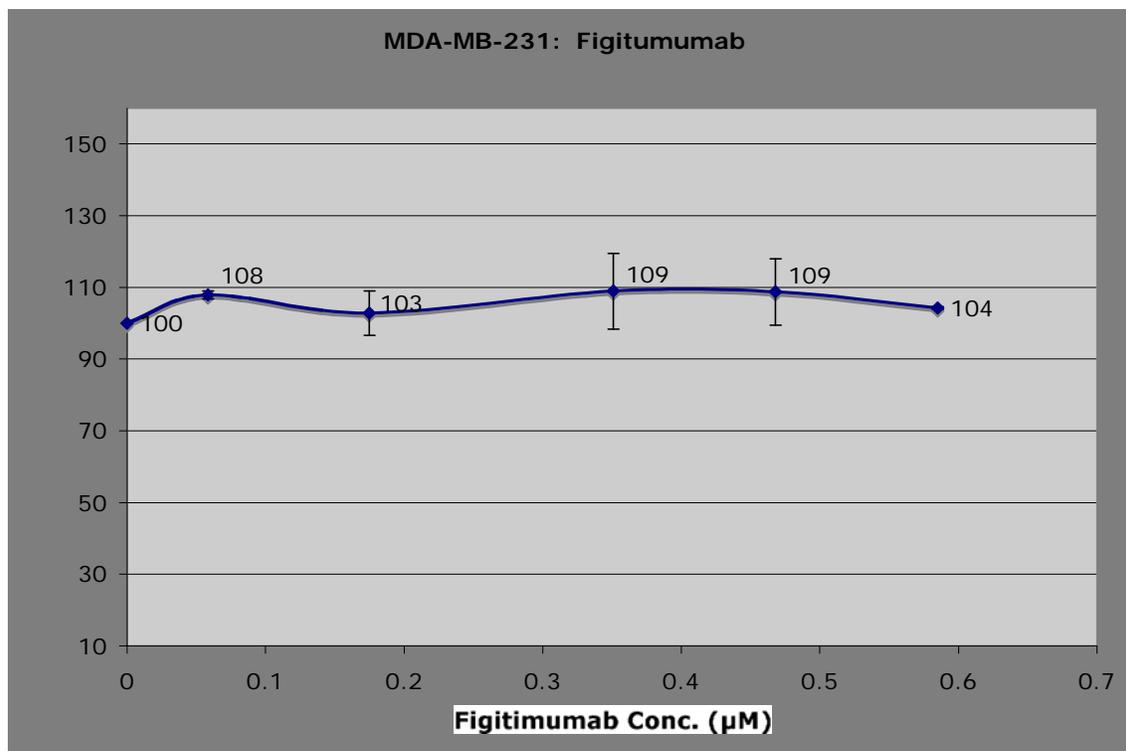


Figure 3B

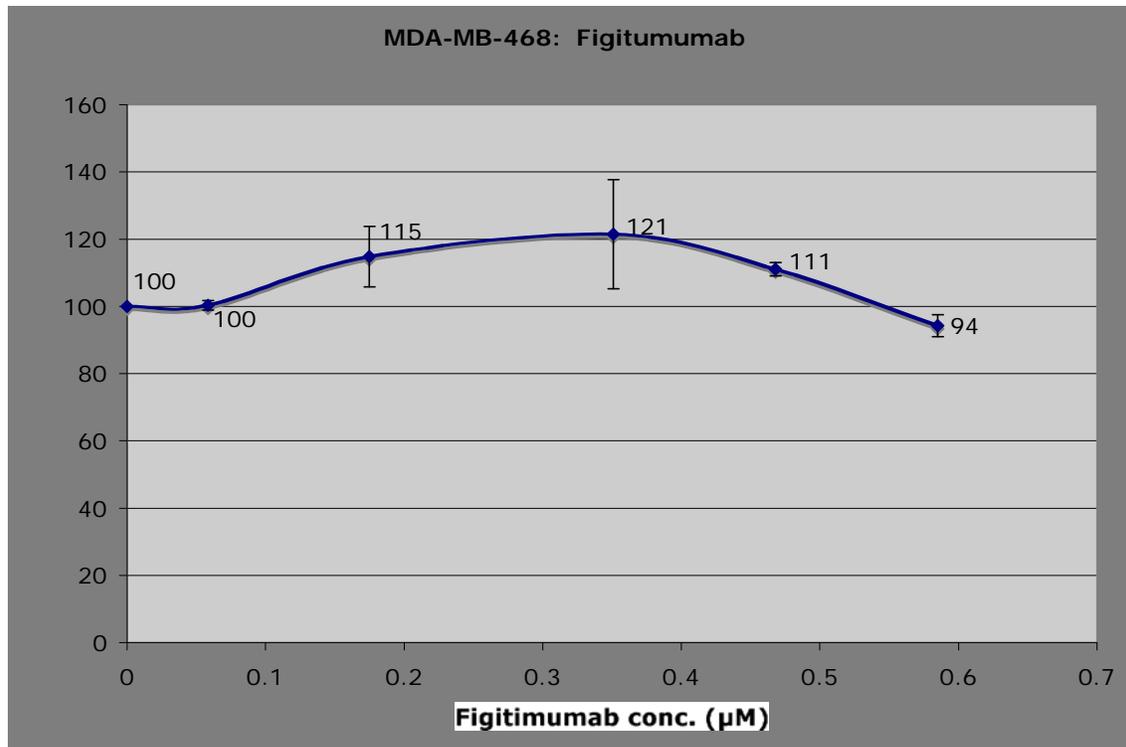


Figure 3C

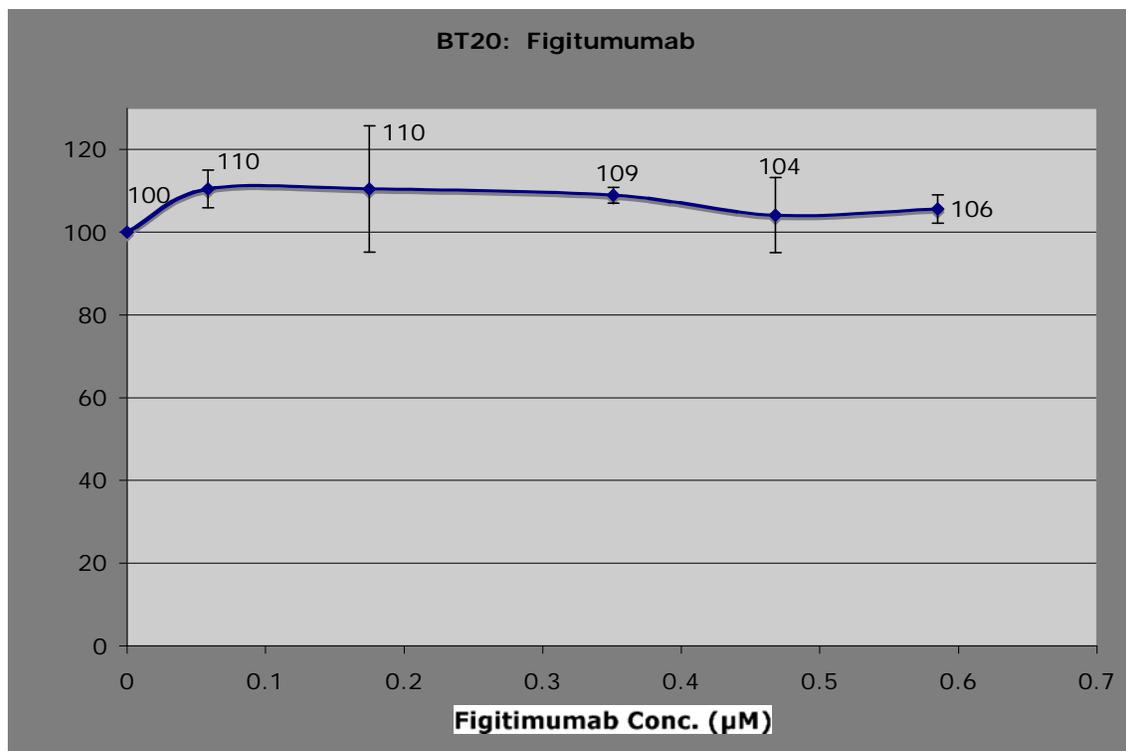


Figure 3D

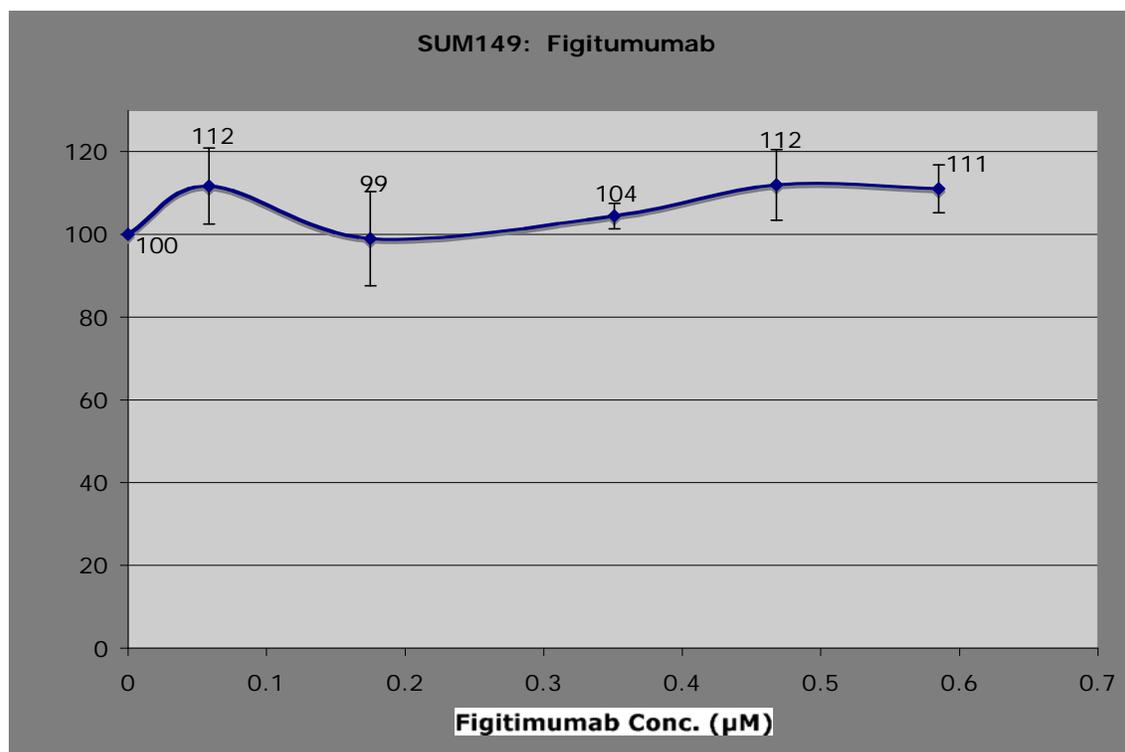


Figure 3E

Figure 3A-E: Effect of Figitumumab, an IGF1R targeted antibody, on MCF7 (3A), a hormone receptor positive breast cancer cell line and triple-negative breast cancer cell lines, MDA-MB-231 (3B), MDA-MB-468(3C), BT20 (3D) and SUM149 (3E). Cells were seeded at their optimal concentration in 96 well plates then allowed to adhere overnight. Figitumumab was added the following day (Day 0). After 5 days of incubation, the WST-1 assay was performed. Each point represents the percent of surviving cells compared to control (cells not exposed to Figitumumab.) The mean of three wells  $\pm 2SD$  (95% CI) is shown. Figitumumab monotherapy failed to have a cytotoxic effect in all of the cell lines. Assay in Figure 3A, 3D and 3E was executed by SR. Assays in Figures 3B and 3C were executed by OO.

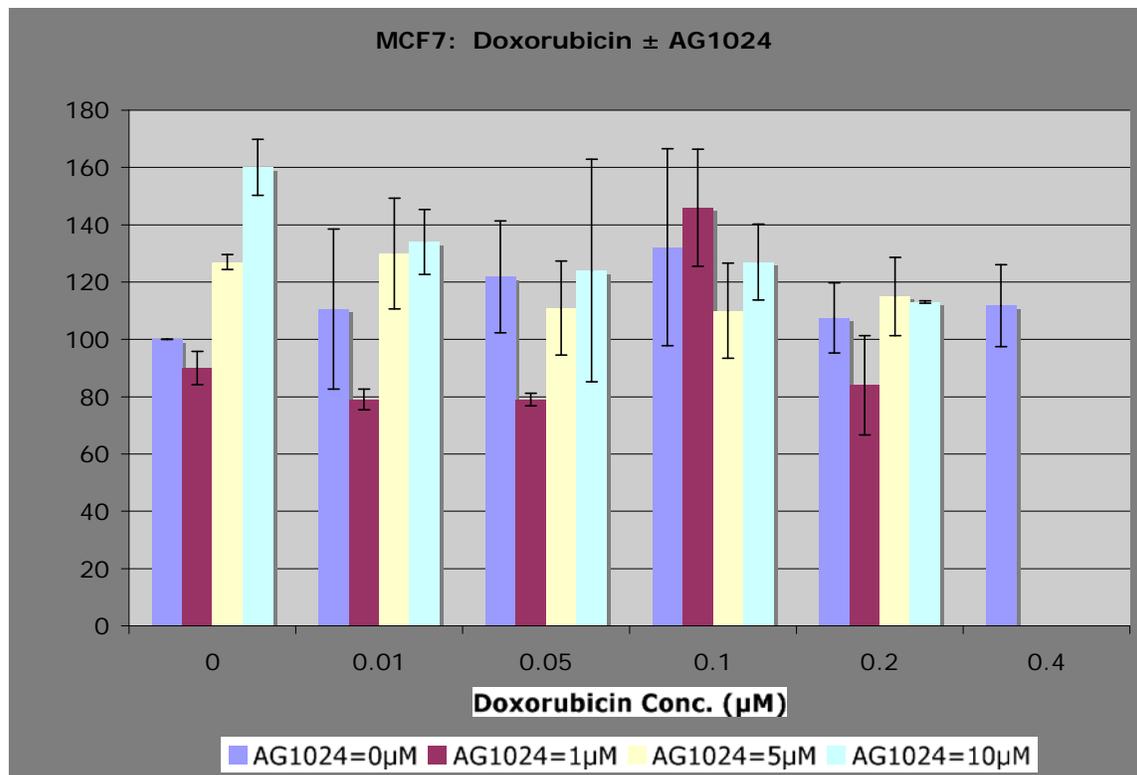


Figure 4A

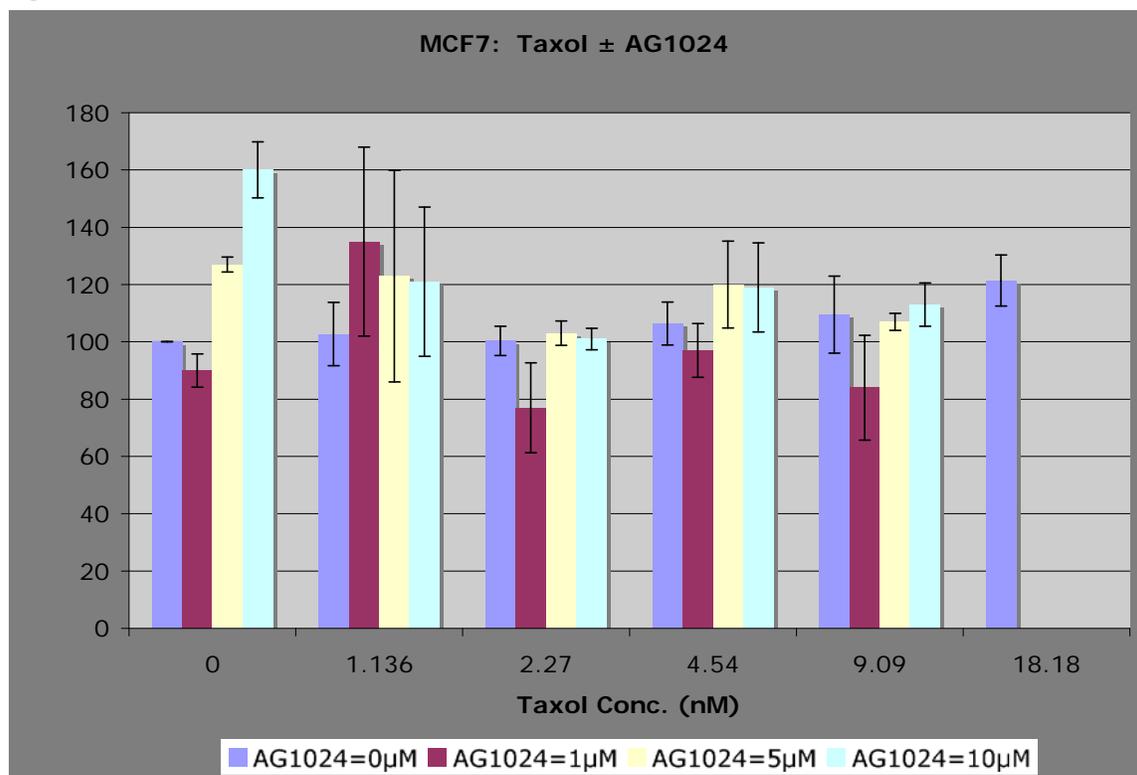


Figure 4B

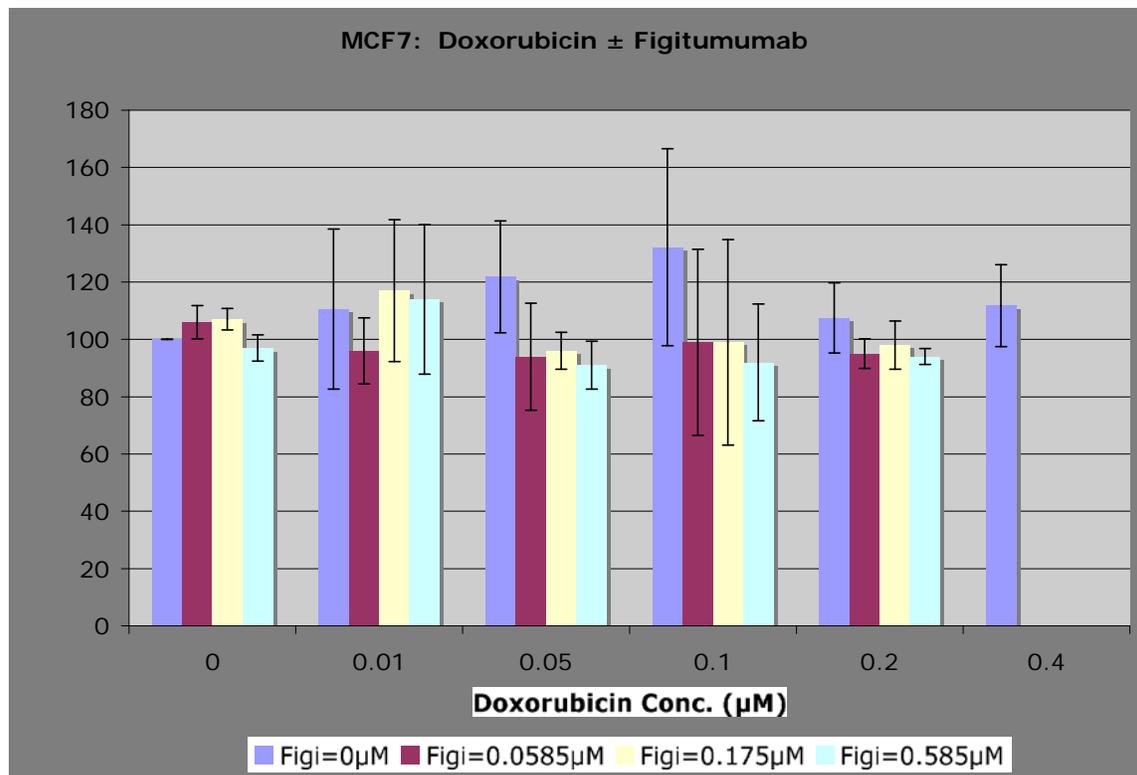


Figure 4C

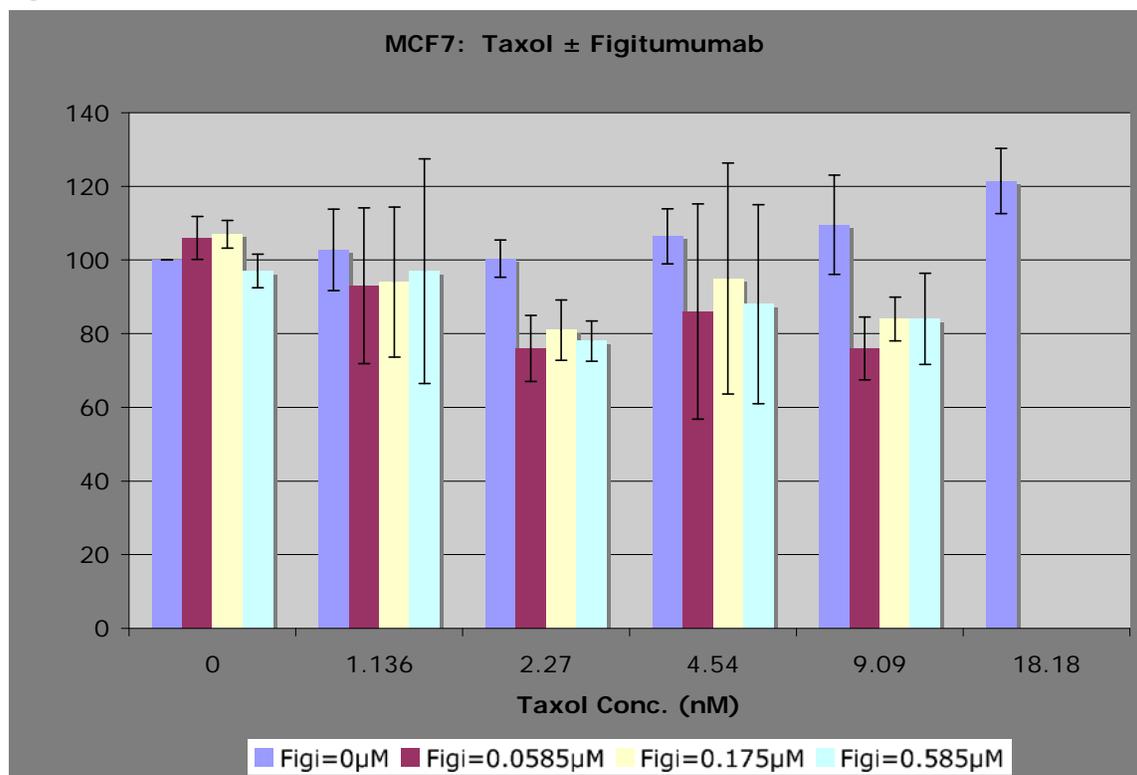


Figure 4D

Figure 4: Effect of AG1024, an IGF1R tyrosine kinase inhibitor, and Figitumumab, an IGF1R targeted antibody, alone and in combination with conventional chemotherapeutic agents, doxorubicin and paclitaxel, in MCF7 cells. Cells were plated at 1000 cells/well and allowed to adhere overnight. Drugs were applied the next day (Day 0). WST-1 assay was performed on Day 5. Each bar represents the average of three wells  $\pm$  2SD (95% CI). Doxorubicin or paclitaxel concentrations increase along the x-axis. Each color represents a different concentration of AG1024 or Figitumumab tested (blue bars = 0 $\mu$ M AG1024 in Figs. 4A and 4B or 0 $\mu$ M Figitumumab in Figs. 4C and 4D, maroon bars = 0.01 $\mu$ M doxorubicin in Figures 4A and 4B or 0.0585 $\mu$ M Figitumumab in Figures 4C and 4D and so forth). Increased cytotoxicity was seen with combinations of Figitumumab and AG1024 (Fig. 4C) and Figitumumab and paclitaxel (Fig. 4D.)

Fig. 4C: Adding 0.05 $\mu$ M doxorubicin to 0.175 $\mu$ M Figitumumab caused cell viability to decrease by 11% ( $\pm$ 6%,  $p=0.005$ ) compared to using 0.175 $\mu$ M Figitumumab alone.

Fig. 4D: Increased cytotoxicity was detected at all concentrations of Figitumumab when either 2.27nM or 9.09nM paclitaxel was added (Figure 4D). The addition of 2.27nM or 9.09nM paclitaxel to 0.0585 $\mu$ M Figitumumab both caused a 30% decrease in cell viability ( $\pm$ 9%). When the same concentrations of paclitaxel were used in combination with 0.175 $\mu$ M Figitumumab, a 23-26% decrease in cell viability was noted. Combining 0.585 $\mu$ M Figitumumab with 2.27nM or 9.09nM paclitaxel led to approximately 13-19% decrease in cell viability. Assays in Figures 4A and 4D were executed by OO and SR respectively. Assays in Figures 4B and 4C were executed jointly by OO and SR.

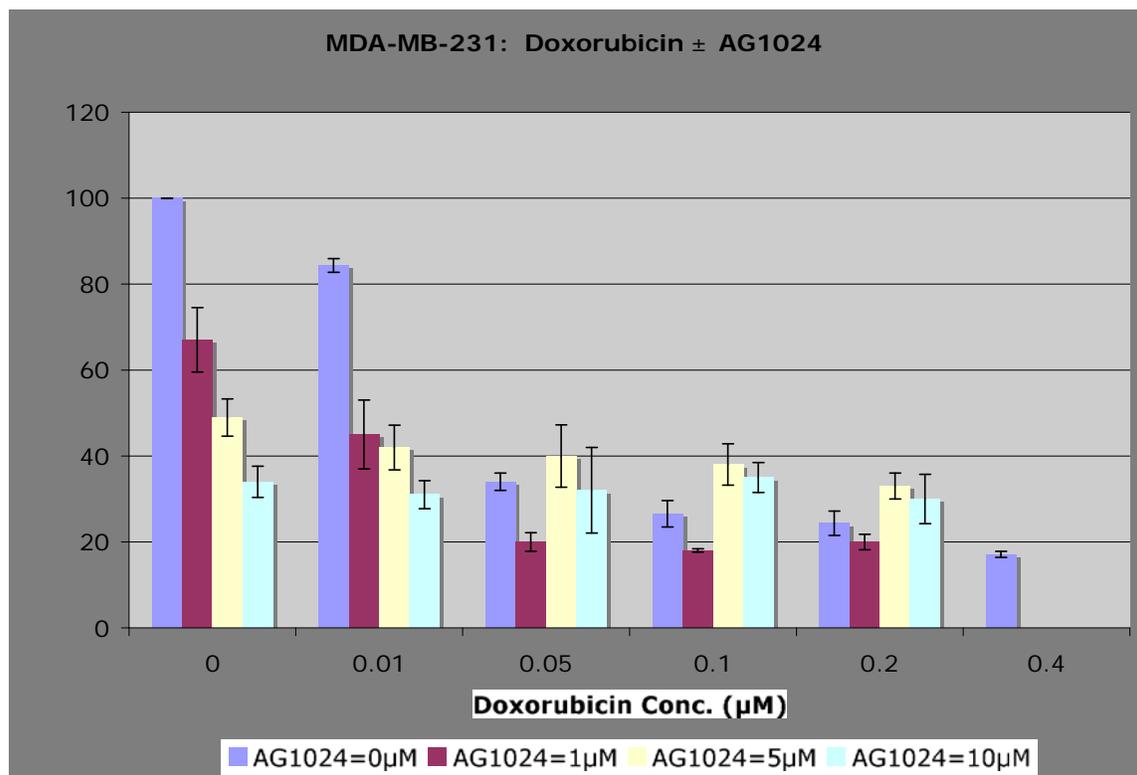


Figure 5A

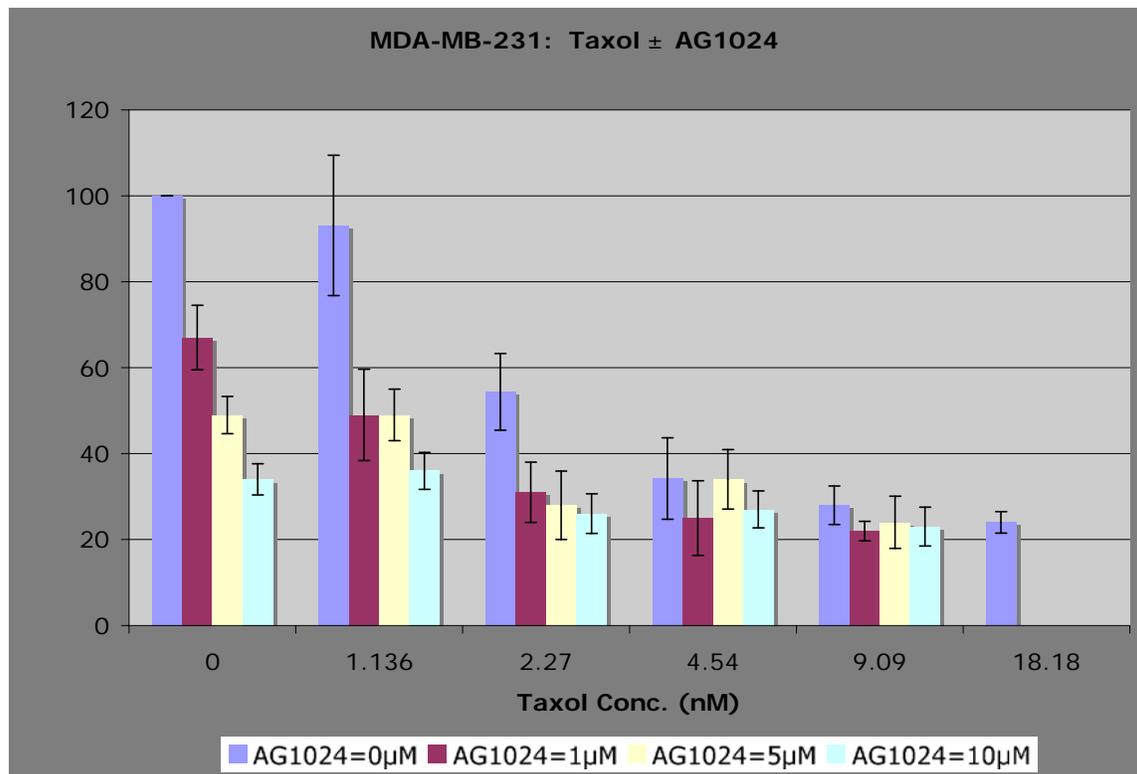


Figure 5B

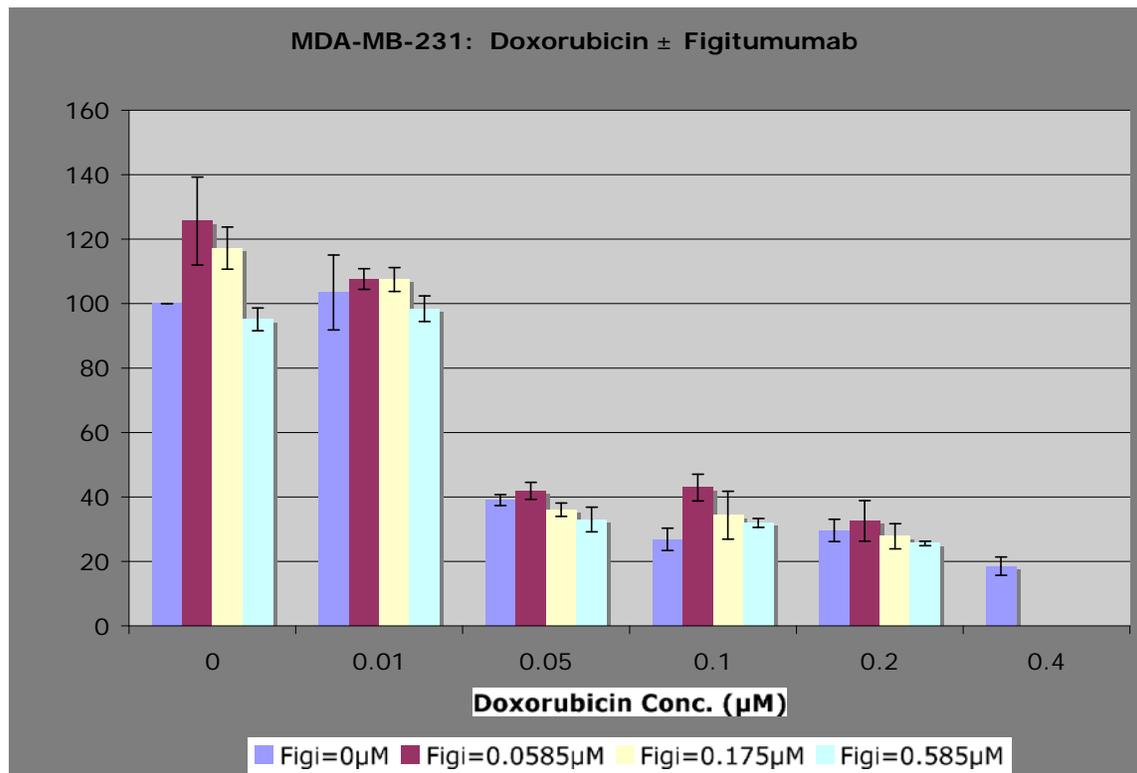
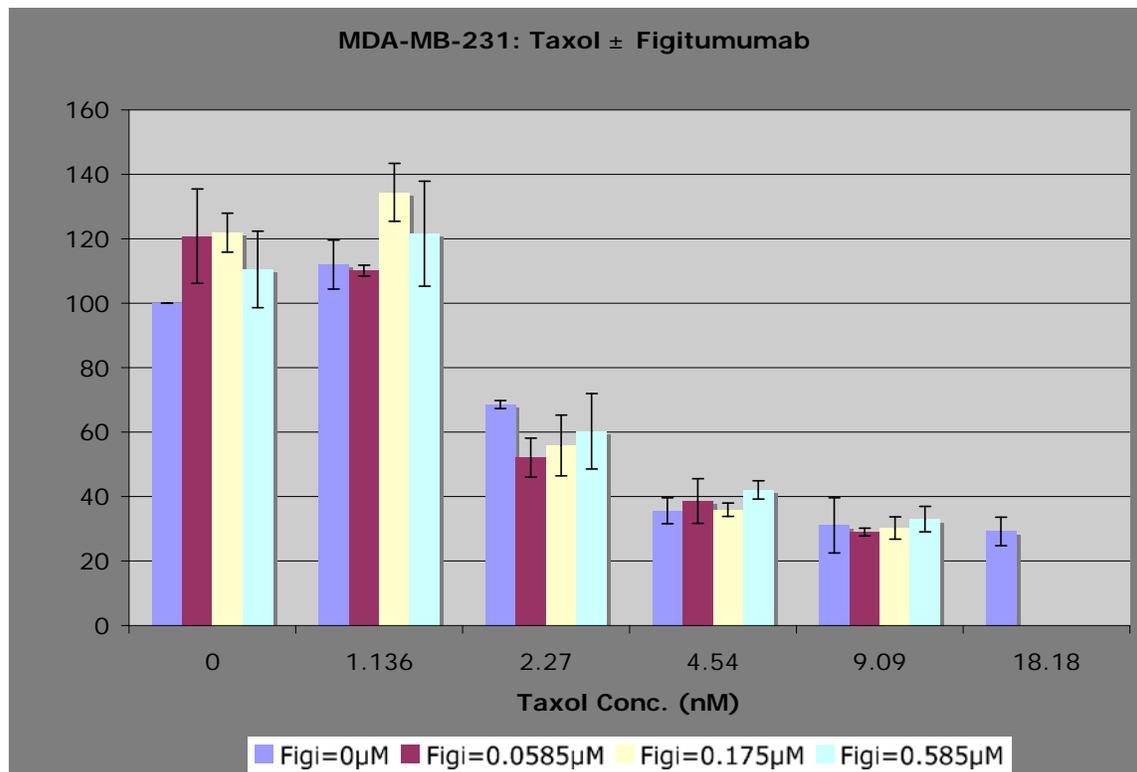


Figure 5C



## Figure 5D

Figure 5: Effect of AG1024, an IGF1R tyrosine kinase inhibitor, and Figitumumab, an IGF1R targeted antibody, alone and in combination with conventional chemotherapeutic agents, doxorubicin and paclitaxel, in MDA-MB-231 cells. Cells were plated at 1500 cells/well and allowed to adhere overnight. Drugs were applied the next day (Day 0). WST-1 assay was performed on Day 5. Each bar represents the average of three wells  $\pm$  2SD (95% CI). Each color represents a different concentration of AG1024 or Figitumumab tested (blue bars = 0 $\mu$ M AG1024 in Figs. 5A and 5B or 0 $\mu$ M Figitumumab in Figs. 5C and 5D, maroon bars = 0.01 $\mu$ M doxorubicin in Figures 5A and 5B or 0.0585 $\mu$ M Figitumumab in Figures 5C and 5D and so forth). Figitumumab monotherapy failed to cause a decrease in cell viability. Combining AG1024 and doxorubicin (Figure 5A), AG1024 and paclitaxel (Fig. 5B) as well as Figitumumab and paclitaxel (Fig. 5D) showed an increased cytotoxic effect compared to these agents alone.

Fig. 5A: Doxorubicin alone at 0.01 $\mu$ M resulted in 84% cell viability ( $\pm$ 2%, 95% CI) and 1 $\mu$ M AG1024 alone led to 67% cell viability ( $\pm$ 8%, 95% CI). However, adding 0.01 $\mu$ M doxorubicin to 1 $\mu$ M AG1024 led to 45% cell viability ( $\pm$ 8%,  $p=0.002$ ) whereas adding 0.05 $\mu$ M doxorubicin produced 20% cell viability ( $\pm$ 2%,  $p=0.001$  for 1 $\mu$ M AG1024 alone versus combined with 0.05 $\mu$ M doxorubicin).

Fig. 5B: Compared to 1 $\mu$ M AG1024 alone, adding 1.136nM and 2.27nM paclitaxel resulted in an 18% decrease ( $\pm$ 11%,  $p=0.008$ ) and 36% decrease in MDA-MB-231 cell viability ( $\pm$ 7%,  $p=0.0003$ ), respectively. Combining 5 $\mu$ M AG1024 with 1.136nM paclitaxel failed to produce greater growth inhibition than 5 $\mu$ M AG1024 alone in MDA-MB-231 cells. However, 49% cell viability ( $\pm$ 4%) was seen after treatment with 5 $\mu$ M AG1024 alone compared to 28% cell viability ( $\pm$ 8%,  $p=0.001$ ) after incubation with 5 $\mu$ M AG1024 and 2.27nM paclitaxel.

Fig. 5D: The addition of 2.27nM or 9.09nM paclitaxel to 0.0585 $\mu$ M Figitumumab both caused a 30% decrease in cell viability ( $\pm$ 9%). When the same concentrations of paclitaxel were used in combination with 0.175 $\mu$ M Figitumumab, a 23-26% decrease in cell viability was noted. Combining 0.585 $\mu$ M Figitumumab with 2.27nM or 9.09nM paclitaxel led to approximately 13-19% decrease in cell viability. Assays in Figures 5C and 5D were executed by OO and SR respectively. Assays in Figures 5A and 5B were executed jointly with SR and OO

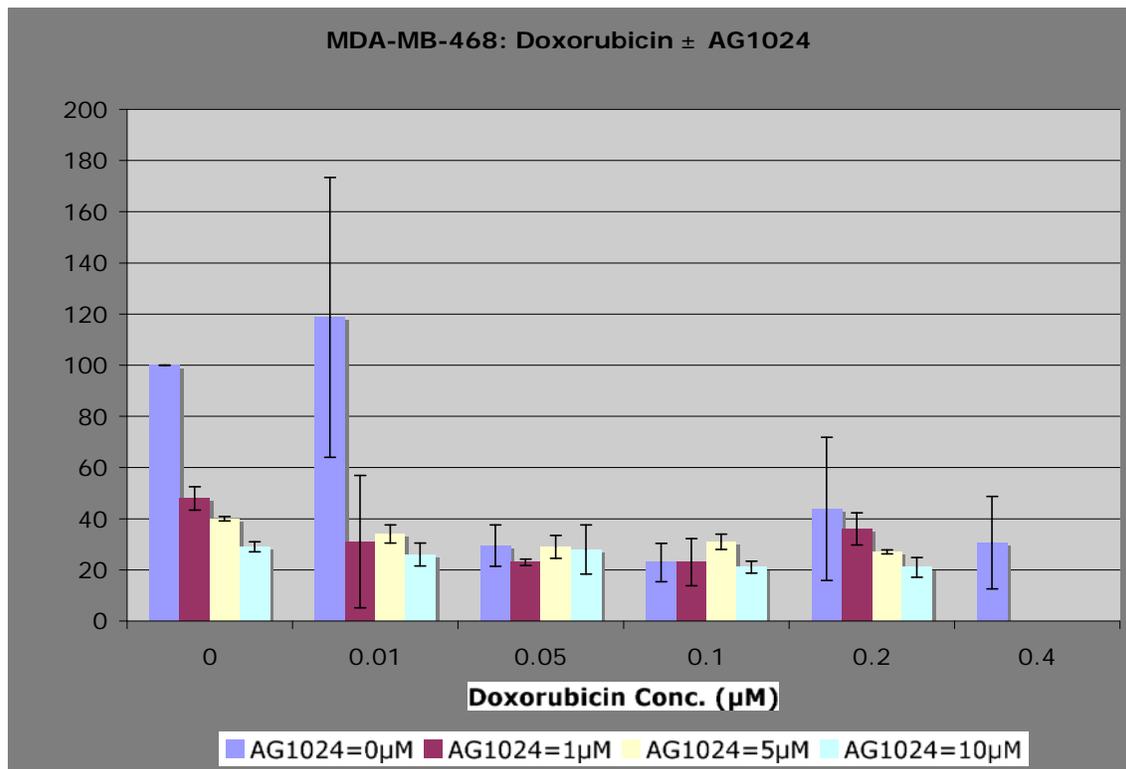


Figure 6A

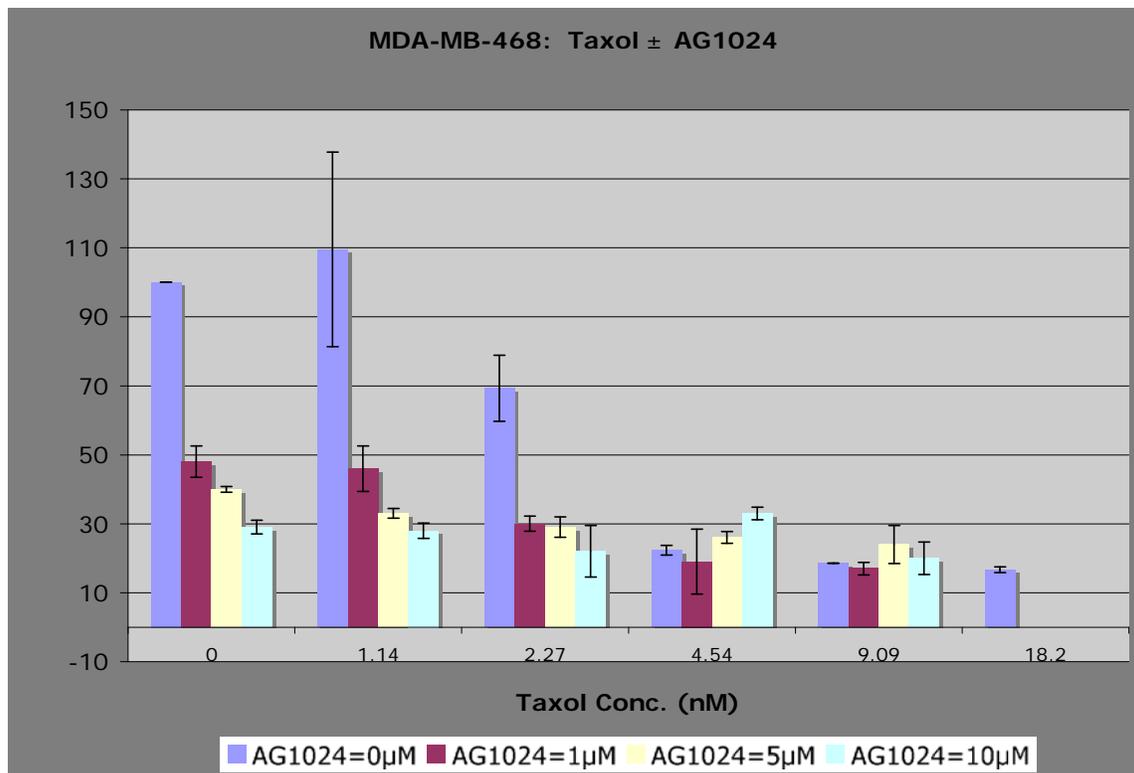


Figure 6B

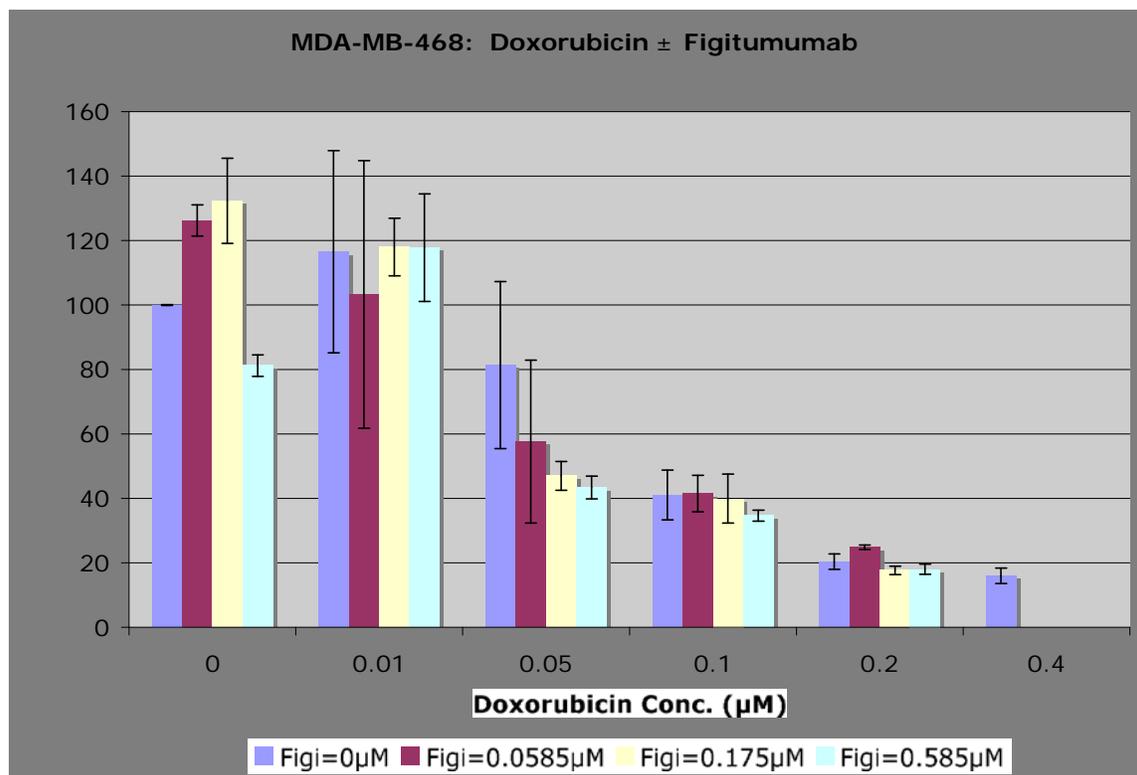


Figure 6C

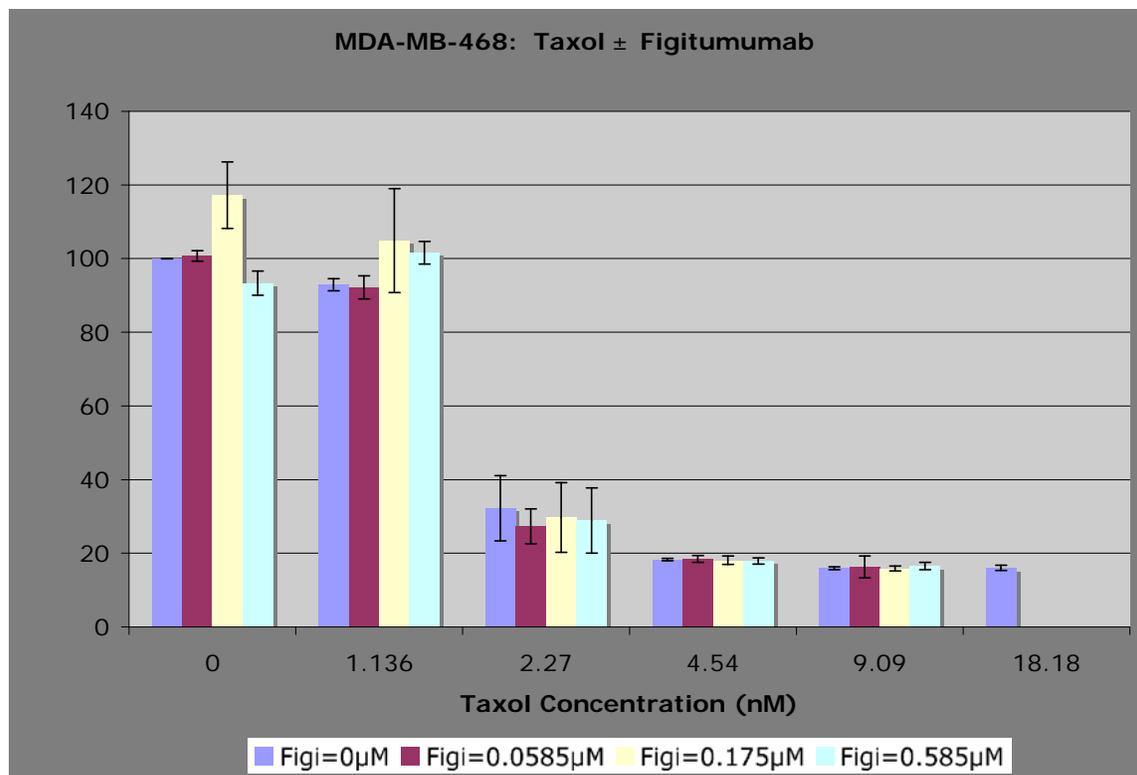


Figure 6D

Figure 6: Effect of AG1024, an IGF1R tyrosine kinase inhibitor, and Figitumumab, an IGF1R targeted antibody, alone and in combination with conventional chemotherapeutic agents, doxorubicin and paclitaxel, in MDA-MB-468 cells. Cells were plated at 3000 cells/well and allowed to adhere overnight. Drugs were applied the next day (Day 0). WST-1 assay was performed on Day 5. Each bar represents the average of three wells  $\pm$  2SD (95% CI). Each color represents a different concentration of AG1024 or Figitumumab tested (blue bars = 0 $\mu$ M AG1024 in Figs. 6A and 6B or 0 $\mu$ M Figitumumab in Figs. 6C and 6D, maroon bars = 0.01 $\mu$ M doxorubicin in Figures 6A and 6B or 0.0585 $\mu$ M Figitumumab in Figures 6C and 6D and so forth). Combining AG1024 and doxorubicin (Figure 6A) and Figitumumab and doxorubicin (Fig. 6C) showed an increased cytotoxic effect compared to these agents alone.

Fig. 6A: Treating MDA-MB-468 cells with only 0.05 $\mu$ M doxorubicin or only 1 $\mu$ M AG1024 led to approximately 48% cell viability compared to 23% ( $\pm$ 1%) when 1 $\mu$ M AG1024 and 0.05 $\mu$ M doxorubicin were combined (Fig. 6A). Additive cytotoxic effects were also observed to a lesser degree when comparing 5 $\mu$ M AG1024 alone (40% $\pm$ 1%) to 5 $\mu$ M AG1024 plus 0.01 $\mu$ M doxorubicin (34% $\pm$ 4%,  $p=0.006$ ) and 5 $\mu$ M AG1024 plus 0.05 $\mu$ M doxorubicin (29% $\pm$ 4%,  $p=0.001$ ).

Fig. 6C: Increased cytotoxicity was noted when 0.175 $\mu$ M or 0.585 $\mu$ M of Figitumumab was combined with 0.05 $\mu$ M of doxorubicin in MDA-MB-468 cells (47% $\pm$ 4% and 43% $\pm$ 3% cell viability, respectively compared to 81% $\pm$ 26 cell viability when 0.05  $\mu$ M of doxorubicin was used alone.) Assays in Figures 6A, 6C and 6D were executed by OO. Assay in Figure 6B was executed jointly by OO and SR.

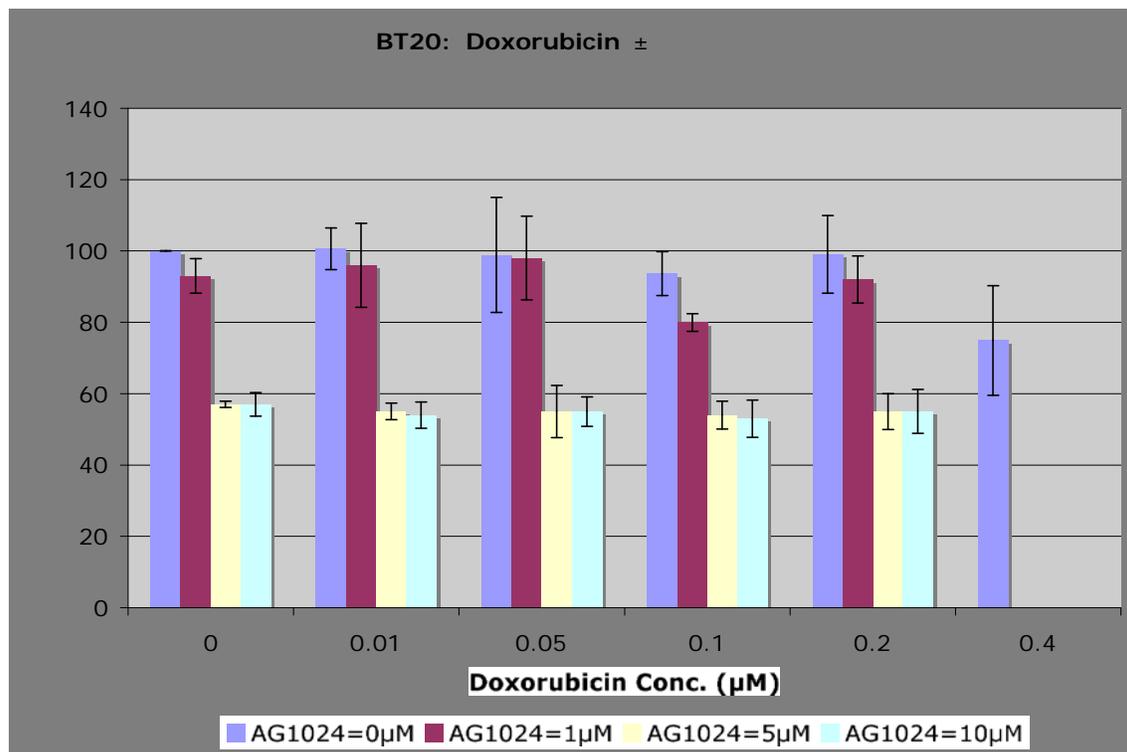


Figure7A

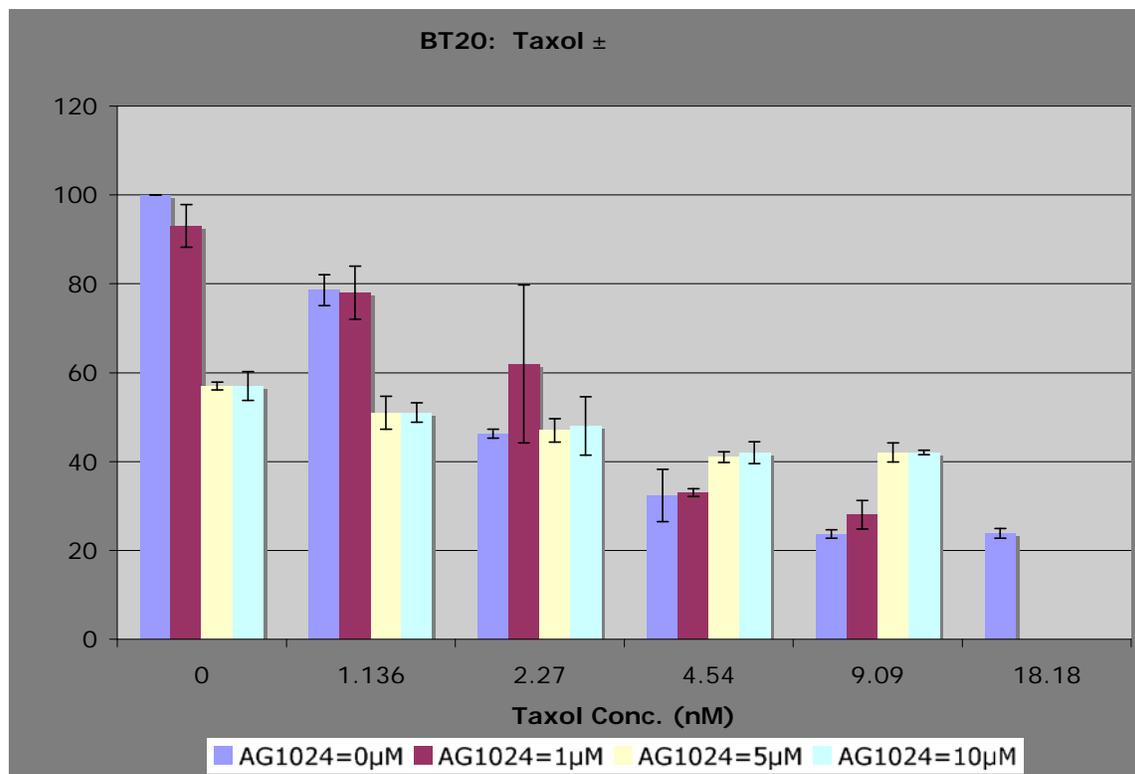


Figure 7B

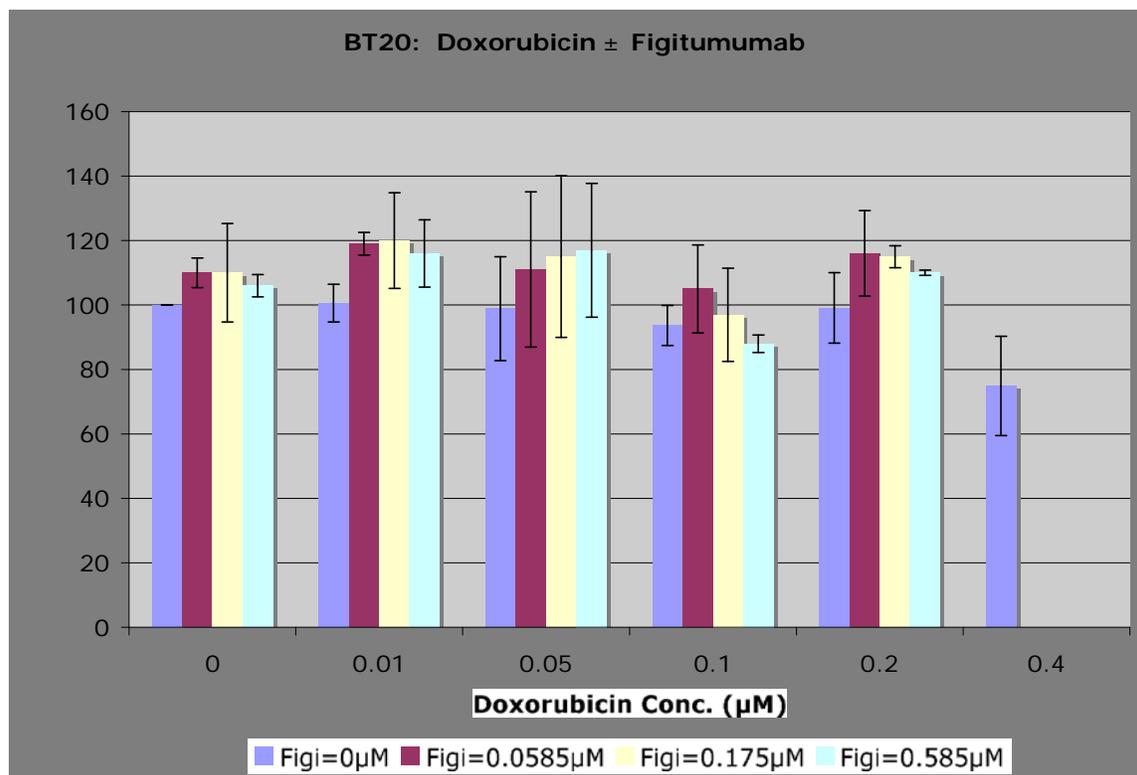


Figure 7C

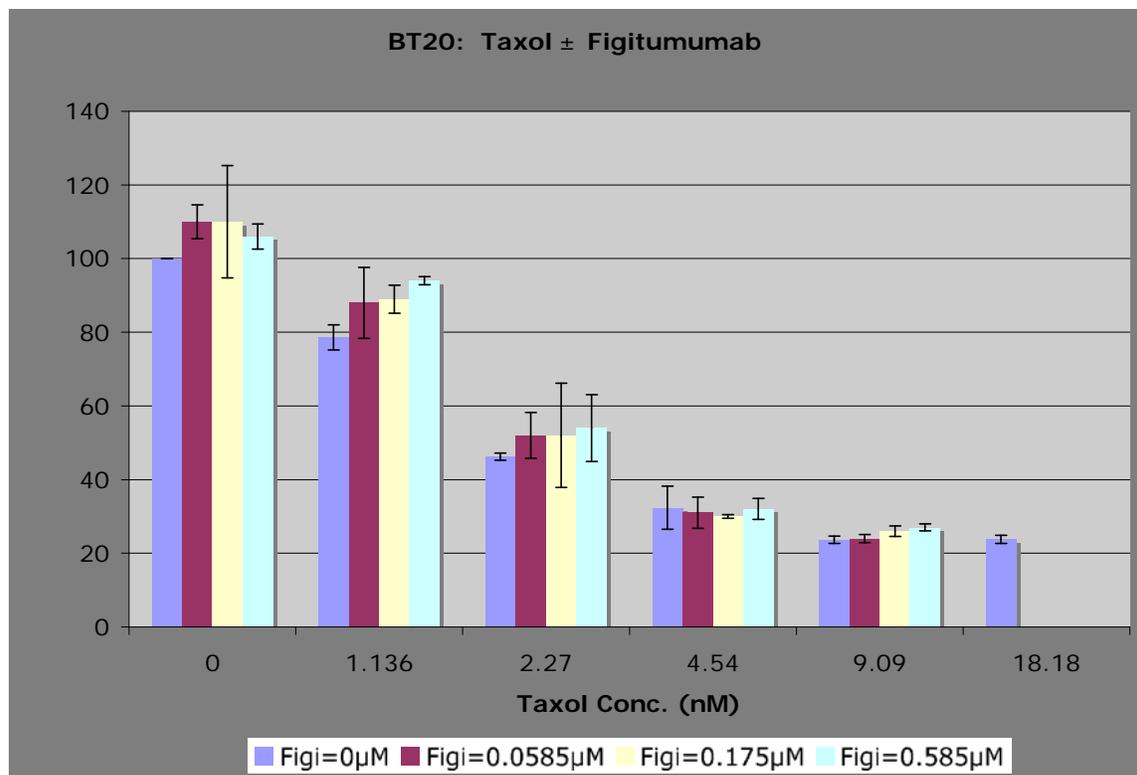


Figure 7D

Figure 7: Effect of AG1024, an IGF1R tyrosine kinase inhibitor, and Figitumumab, an IGF1R targeted antibody, alone and in combination with conventional chemotherapeutic agents, doxorubicin and paclitaxel, in BT20 cells. Cells were plated at 1000 cells/well and allowed to adhere overnight. Drugs were applied the next day (Day 0). WST-1 assay was performed on Day 5. Each bar represents the average of three wells  $\pm$  2SD (95% CI). Color-coding of the bars is similar to that described in Figs. 4-6. Combining AG1024 and paclitaxel (Fig. 7B) showed an increased cytotoxic effect compared to these agents alone. Treating BT20 cells with 1.136nM paclitaxel alone resulted in 79% cell viability ( $\pm$ 3%). Treating these cells with 5 $\mu$ M AG1024 alone caused 57% cell viability ( $\pm$ 1%) whereas adding 1.136nM paclitaxel led to 51% cell viability ( $\pm$ 4%,  $p=0.03$ ). Similarly, 10 $\mu$ M AG1024 alone resulted in 57% cell viability ( $\pm$ 3%) but with addition of 1.136nM paclitaxel, the cell viability fell to 51% ( $\pm$ 2%,  $p=0.005$ ). Assays in Figures 7A and 7D were executed by OO and SR respectively. Assays in Figures 7B and 7C were executed jointly by OO and SR.

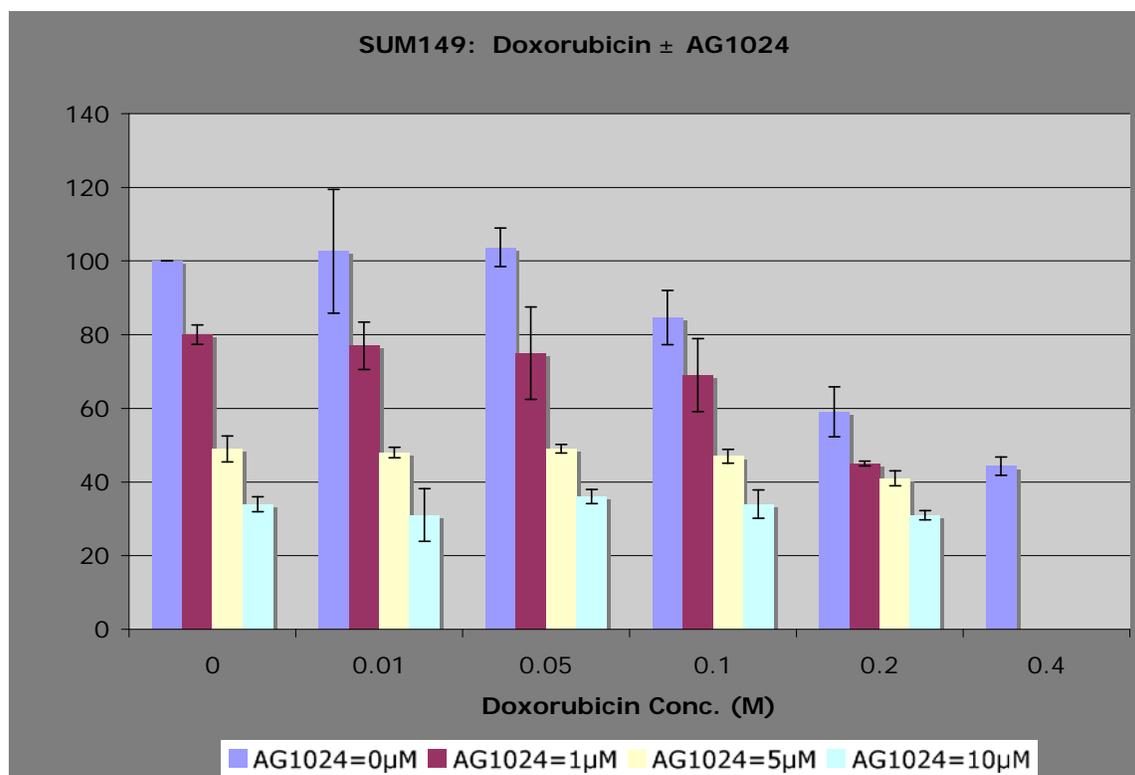


Figure 8A

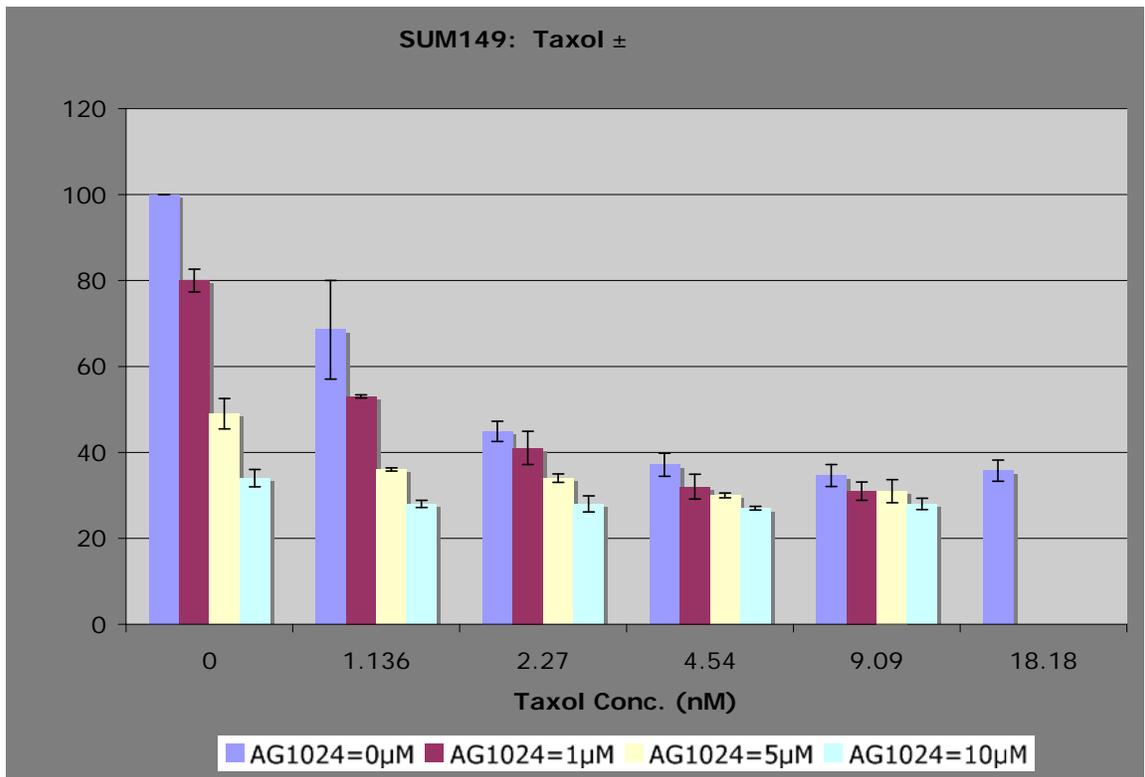


Figure 8B

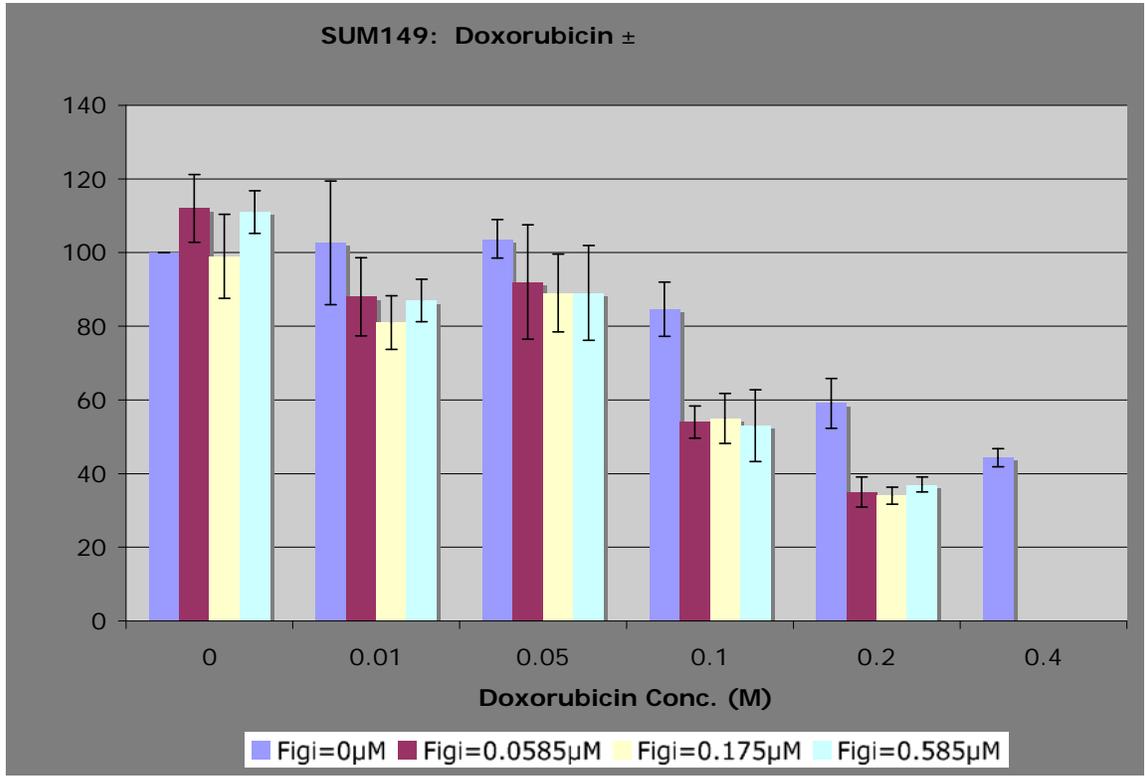


Figure 8C

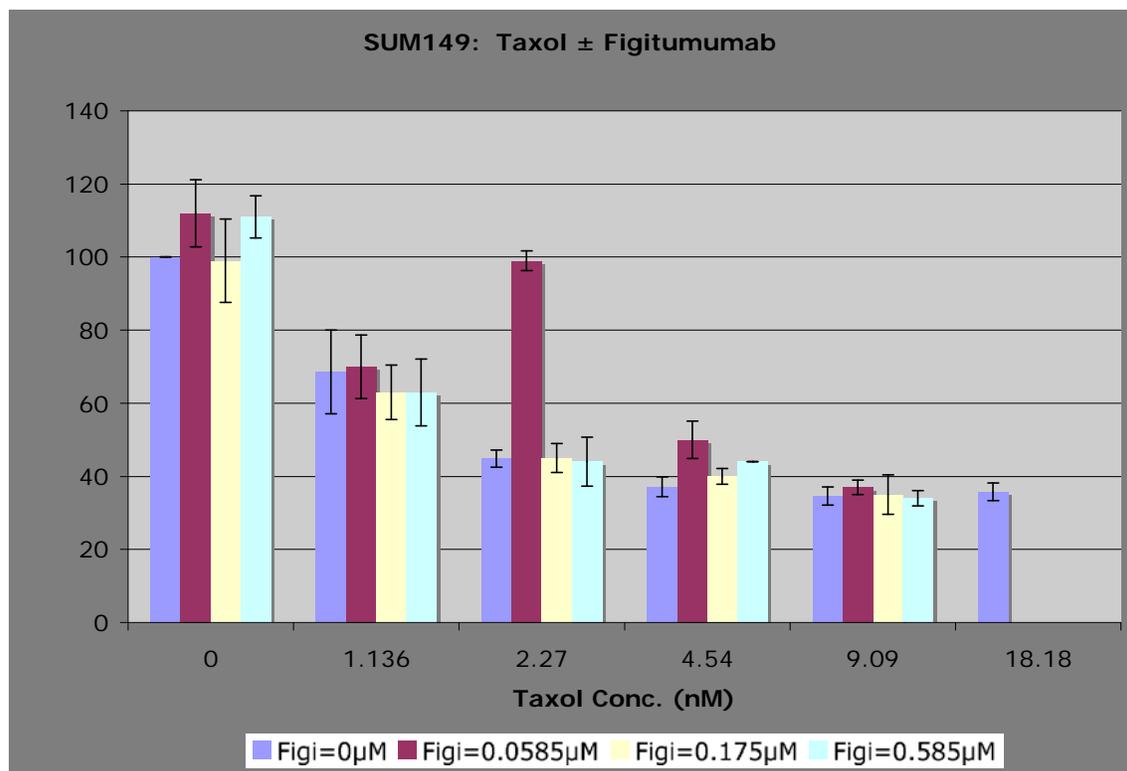


Figure 8D

Figure 8: Effect of AG1024, an IGF1R tyrosine kinase inhibitor, and Figitumumab, an IGF1R targeted antibody, alone and in combination with conventional chemotherapeutic agents, doxorubicin and paclitaxel, in SUM149 cells. Cells were plated at 3000 cells/well and allowed to adhere overnight. Drugs were applied the next day (Day 0). WST-1 assay was performed on Day 5. Each bar represents the average of three wells  $\pm$  2SD (95% CI). Color-coding of the bars is similar to that described in Figs. 4-6. Combining AG1024 and doxorubicin (Figure 8A), AG1024 and paclitaxel (Fig. 8B) and Figitumumab and doxorubicin (Fig. 8C) showed an increased cytotoxic effect compared to these agents alone.

Fig. 8A: Cell viability decreased to 45% ( $\pm$ 1%) when 0.2 $\mu$ M doxorubicin was combined with 1 $\mu$ M AG1024 compared to 80% cell viability ( $\pm$ 3%) when 1 $\mu$ M AG1024 was used alone or 59% cell viability ( $\pm$ 7%) 0.2 $\mu$ M doxorubicin monotherapy was used.

Fig. 8B: Combining 1.136nM paclitaxel with any concentration of AG1024 led to enhanced cytotoxic effects compared to either reagent alone. Treatment of SUM149 cells with 1 $\mu$ M AG1024 alone led to 80% cell viability ( $\pm$ 3%) and incubation with 1.136nM

paclitaxel alone resulted in 69% cell viability ( $\pm 11\%$ ); adding 1.136nM paclitaxel to 1 $\mu$ M AG1024 further inhibited cell viability to 53% ( $p=0.04$  for 1.136nM paclitaxel alone versus combined with 1 $\mu$ M AG1024). Treatment of SUM149 cells with 5 $\mu$ M AG1024 alone produced 49% cell viability ( $\pm 4\%$ ) and adding 1.136nM paclitaxel led to 36% cell viability ( $\pm 1\%$ ,  $p=0.004$  for 5 $\mu$ M AG1024 alone versus combined with 1.136nM paclitaxel). Treatment using 10 $\mu$ M AG1024 alone caused 34% cell viability ( $\pm 2\%$ ) whereas adding 1.136nM paclitaxel led to 28% cell viability. Treatment with 0.01 $\mu$ M or 0.05 $\mu$ M doxorubicin alone did not inhibit cellular proliferation but treatment with 0.1 $\mu$ M and 0.2 $\mu$ M doxorubicin alone caused approximately 85% cell viability ( $\pm 7\%$ ) and 59% ( $\pm 7\%$ ) respectively.

Fig. 8C: When treated with 0.0585 $\mu$ M Figitumumab alone, SUM149 cells did not experience inhibition of cell proliferation but adding 0.01 $\mu$ M, 0.1 $\mu$ M and 0.2 $\mu$ M doxorubicin led to 88% ( $\pm 11\%$ ), 54% ( $\pm 4\%$ ) and 35% ( $\pm 4\%$ ) cell viability, respectively (Figure 8C). Assays in Figures 8A and 8D were executed by OO and SR respectively. Assays in Figures 8B and 8C were executed jointly by OO and SR. Doxorubicin and Figitumumab combinations also increased cytotoxicity in SUM149 cells.

## 8. REFERENCES

1. Perou CM, Sorlie T, Eisen MB, et al. Molecular portraits of human breast tumours. Nature 2000;406:747-52.
2. Kang SP, Martel M, Harris LN. Triple negative breast cancer: current understanding of biology and treatment options. Curr Opin Obstet Gynecol 2008;20:40-6.
3. Eley JW, Hill HA, Chen VW, et al. Racial differences in survival from breast cancer. Results of the National Cancer Institute Black/White Cancer Survival Study. JAMA 1994;272:947-54.
4. Clegg LX, Li FP, Hankey BF, Chu K, Edwards BK. Cancer survival among US whites and minorities: a SEER (Surveillance, Epidemiology, and End Results) Program population-based study. Arch Intern Med 2002;162:1985-93.
5. Millikan RC, Newman B, Tse CK, et al. Epidemiology of basal-like breast cancer. Breast Cancer Res Treat 2008;109:123-39.
6. Yang XR, Sherman ME, Rimm DL, et al. Differences in risk factors for breast cancer molecular subtypes in a population-based study. Cancer Epidemiol Biomarkers Prev 2007;16:439-43.
7. Cleary MP, Maihle NJ. The role of body mass index in the relative risk of developing premenopausal versus postmenopausal breast cancer. Proc Soc Exp Biol Med 1997;216:28-43.
8. Huang Z, Hankinson SE, Colditz GA, et al. Dual effects of weight and weight gain on breast cancer risk. JAMA 1997;278:1407-11.
9. Gupta K, Krishnaswamy G, Karnad A, Peiris AN. Insulin: a novel factor in carcinogenesis. Am J Med Sci 2002;323:140-5.
10. Pollak M. Insulin and insulin-like growth factor signalling in neoplasia. Nat Rev Cancer 2008;8:915-28.
11. Holdaway IM, Friesen HG. Hormone binding by human mammary carcinoma. Cancer Res 1977;37:1946-52.
12. Ebina Y, Edery M, Ellis L, et al. Expression of a functional human insulin receptor from a cloned cDNA in Chinese hamster ovary cells. Proc Natl Acad Sci U S A 1985;82:8014-8.
13. Ebina Y, Ellis L, Jarnagin K, et al. The human insulin receptor cDNA: the structural basis for hormone-activated transmembrane signalling. Cell 1985;40:747-58.
14. Ullrich A, Bell JR, Chen EY, et al. Human insulin receptor and its relationship to the tyrosine kinase family of oncogenes. Nature 1985;313:756-61.
15. Frasca F, Pandini G, Scalia P, et al. Insulin receptor isoform A, a newly recognized, high-affinity insulin-like growth factor II receptor in fetal and cancer cells. Mol Cell Biol 1999;19:3278-88.
16. Heuson JC, Coune A, Heimann R. Cell proliferation induced by insulin in organ culture of rat mammary carcinoma. Exp Cell Res 1967;45:351-60.
17. Osborne CK, Bolan G, Monaco ME, Lippman ME. Hormone responsive human breast cancer in long-term tissue culture: effect of insulin. Proc Natl Acad Sci U S A 1976;73:4536-40.

18. Goodwin PJ, Ennis M, Pritchard KI, et al. Fasting insulin and outcome in early-stage breast cancer: results of a prospective cohort study. J Clin Oncol 2002;20:42-51.
19. Lipscombe LL, Goodwin PJ, Zinman B, McLaughlin JR, Hux JE. The impact of diabetes on survival following breast cancer. Breast Cancer Res Treat 2008;109:389-95.
20. Goodwin PJ, Pritchard KI, Ennis M, Clemons M, Graham M, Fantus IG. Insulin-lowering effects of metformin in women with early breast cancer. Clin Breast Cancer 2008;8:501-5.
21. Goodwin PJ. Insulin in the adjuvant breast cancer setting: a novel therapeutic target for lifestyle and pharmacologic interventions? J Clin Oncol 2008;26:833-4.
22. Jiralerspong S, Palla SL, Giordano SH, et al. Metformin and pathologic complete responses to neoadjuvant chemotherapy in diabetic patients with breast cancer. J Clin Oncol 2009;27:3297-302.
23. Liu B, Fan Z, Edgerton SM, et al. Metformin induces unique biological and molecular responses in triple negative breast cancer cells. Cell Cycle 2009;8:2031-40.
24. Pandini G, Vigneri R, Costantino A, et al. Insulin and insulin-like growth factor-I (IGF-I) receptor overexpression in breast cancers leads to insulin/IGF-I hybrid receptor overexpression: evidence for a second mechanism of IGF-I signaling. Clin Cancer Res 1999;5:1935-44.
25. Pandini G, Frasca F, Mineo R, Sciacca L, Vigneri R, Belfiore A. Insulin/insulin-like growth factor I hybrid receptors have different biological characteristics depending on the insulin receptor isoform involved. J Biol Chem 2002;277:39684-95.
26. Chakravarti A, Loeffler JS, Dyson NJ. Insulin-like growth factor receptor I mediates resistance to anti-epidermal growth factor receptor therapy in primary human glioblastoma cells through continued activation of phosphoinositide 3-kinase signaling. Cancer Res 2002;62:200-7.
27. Lu Y, Zi X, Zhao Y, Mascarenhas D, Pollak M. Insulin-like growth factor-I receptor signaling and resistance to trastuzumab (Herceptin). J Natl Cancer Inst 2001;93:1852-7.
28. Myal Y, Shiu RP, Bhaumick B, Bala M. Receptor binding and growth-promoting activity of insulin-like growth factors in human breast cancer cells (T-47D) in culture. Cancer Res 1984;44:5486-90.
29. Pollak MN, Perdue JF, Margolese RG, Baer K, Richard M. Presence of somatomedin receptors on primary human breast and colon carcinomas. Cancer Lett 1987;38:223-30.
30. Arteaga CL, Kitten LJ, Coronado EB, et al. Blockade of the type I somatomedin receptor inhibits growth of human breast cancer cells in athymic mice. J Clin Invest 1989;84:1418-23.
31. Papa V, Gliozzo B, Clark GM, et al. Insulin-like growth factor-I receptors are overexpressed and predict a low risk in human breast cancer. Cancer Res 1993;53:3736-40.

32. Law JH, Habibi G, Hu K, et al. Phosphorylated insulin-like growth factor-I/insulin receptor is present in all breast cancer subtypes and is related to poor survival. Cancer Res 2008;68:10238-46.
33. Creighton CJ, Casa A, Lazard Z, et al. Insulin-like growth factor-I activates gene transcription programs strongly associated with poor breast cancer prognosis. J Clin Oncol 2008;26:4078-85.
34. Gualberto A, Pollak M. Clinical development of inhibitors of the insulin-like growth factor receptor in oncology. Curr Drug Targets 2009;10:923-36.
35. Parrizas M, Gazit A, Levitzki A, Wertheimer E, LeRoith D. Specific inhibition of insulin-like growth factor-1 and insulin receptor tyrosine kinase activity and biological function by tyrphostins. Endocrinology 1997;138:1427-33.
36. Wen B, Deutsch E, Marangoni E, et al. Tyrphostin AG 1024 modulates radiosensitivity in human breast cancer cells. Br J Cancer 2001;85:2017-21.
37. Camirand A, Zakikhani M, Young F, Pollak M. Inhibition of insulin-like growth factor-1 receptor signaling enhances growth-inhibitory and proapoptotic effects of gefitinib (Iressa) in human breast cancer cells. Breast Cancer Res 2005;7:R570-9.
38. Chong YM, Colston K, Jiang WG, Sharma AK, Mokbel K. The relationship between the insulin-like growth factor-1 system and the oestrogen metabolising enzymes in breast cancer tissue and its adjacent non-cancerous tissue. Breast Cancer Res Treat 2006;99:275-88.
39. Chakraborty AK, Welsh A, Digiovanna MP. Co-targeting the insulin-like growth factor I receptor enhances growth-inhibitory and pro-apoptotic effects of anti-estrogens in human breast cancer cell lines. Breast Cancer Res Treat 2009.
40. Zha J, O'Brien C, Savage H, et al. Molecular predictors of response to a humanized anti-insulin-like growth factor-I receptor monoclonal antibody in breast and colorectal cancer. Mol Cancer Ther 2009;8:2110-21.
41. Cohen BD, Baker DA, Soderstrom C, et al. Combination therapy enhances the inhibition of tumor growth with the fully human anti-type 1 insulin-like growth factor receptor monoclonal antibody CP-751,871. Clin Cancer Res 2005;11:2063-73.
42. Haluska P, Shaw HM, Batzel GN, et al. Phase I dose escalation study of the anti insulin-like growth factor-I receptor monoclonal antibody CP-751,871 in patients with refractory solid tumors. Clin Cancer Res 2007;13:5834-40.
43. Tolcher AW, Mita M, Meropol NJ, et al. Phase I pharmacokinetic and biologic correlative study of mapatumumab, a fully human monoclonal antibody with agonist activity to tumor necrosis factor-related apoptosis-inducing ligand receptor-1. J Clin Oncol 2007;25:1390-5.
44. Peyrat JP, Bonneterre J, Dusanter-Fourt I, Leroy-Martin B, Djiane J, Demaille A. Characterization of insulin-like growth factor 1 receptors (IGF1-R) in human breast cancer cell lines. Bull Cancer 1989;76:311-9.
45. Tassone P, Tagliaferri P, Perricelli A, et al. BRCA1 expression modulates chemosensitivity of BRCA1-defective HCC1937 human breast cancer cells. Br J Cancer 2003;88:1285-91.