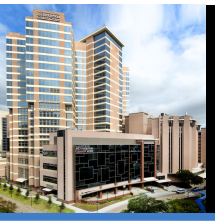

The RXR Agonist, IRX4204, Increases the Anti-tumor Activity of HER2-targeted Therapies in HER2-amplified Breast Cancer

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Background & Significance

An estimated 40,000 women in the United States are living with HER2-overexpressed metastatic breast cancer, for which there is currently no cure. Despite the development of highly successful targeted therapies for HER2-amplified primary tumors, the challenge persists that current anti-HER2 therapies cause unwanted cardiotoxicity, that primary and acquired resistance to anti-HER2 therapy is common, and that most anti-HER2 targeted therapies still fail to achieve a cure in the metastatic setting. There is a critical need for safe and effective therapies that can overcome anti-HER2 drug resistance to eliminate breast cancer metastases.

Highly specific agonists of the nuclear retinoid X receptor (RXR) have been shown to modulate the immune system and inhibit the growth of HER2 amplified breast cancer (1). More importantly, these agonists are well tolerated in humans (2) and less toxic than current breast cancer therapies. Here, we present data demonstrating the activity of IRX4204, a highly specific agonist of RXR, to inhibit the growth of HER2-amplified breast cancer cell lines alone and in combination with current anti-HER2 therapies.

Hypothesis

We hypothesize that treatment with IRX4204 in combination with existing targeted therapies can eradicate HER2-overexpressing breast cancer, including those with developed resistance to anti-HER2 therapy.

Methods

Cell growth assays (single agent): Cells were seeded in duplicate 24-well plates and treated with IRX4204 [1uM] or DMSO. Cells were collected in quadruplicate, stained with Trypan Blue dye and total live cell number was calculated on days 1, 3, 5, and 7.

Cell growth assays (drug combinations): Cells were seeded in 96-well plates and treated with anti-HER2 and chemotherapy drugs (1nM to 1uM) in combination with IRX4204 (10 to 1000nM) for 6 days. Nuclei were stained with DAPI, imaged using ImageXpress Pico (Molecular Devices) and counted using CellReporterXpress Analysis Software.

Drug synergy analysis: Total number of nuclei from combination treatment and single drug treatment was compared to DMSO control treated cells to calculate the fraction affected (Fa) of each drug treatment. CompuSyn software (ComboSyn, Inc) was used to calculate combination index.

Apoptosis assay: Cells were seeded in 6-well plates and treated with DMSO, IRX4204 [1uM-96 hours] or staurosporine [2uM-4 hours] and labeled with AnnexinV-FITC and propidium iodide (PI). Positive cells were counted by flow cytometry.

Senescence assay: Cells were seeded in 12 well plates and treated with DMSO, etoposide [10uM], doxorubicin [100nM] or IRX4204 [1uM] for 24-48 hours before maintained in normal growth media for 7 days. Senescence β-Galactosidase (β-gal) Staining Kit (#9860, Cell Signaling) was used to stain cells for β-gal accumulation.

Results

Effect of IRX4204 on panel of breast cancer cell lines

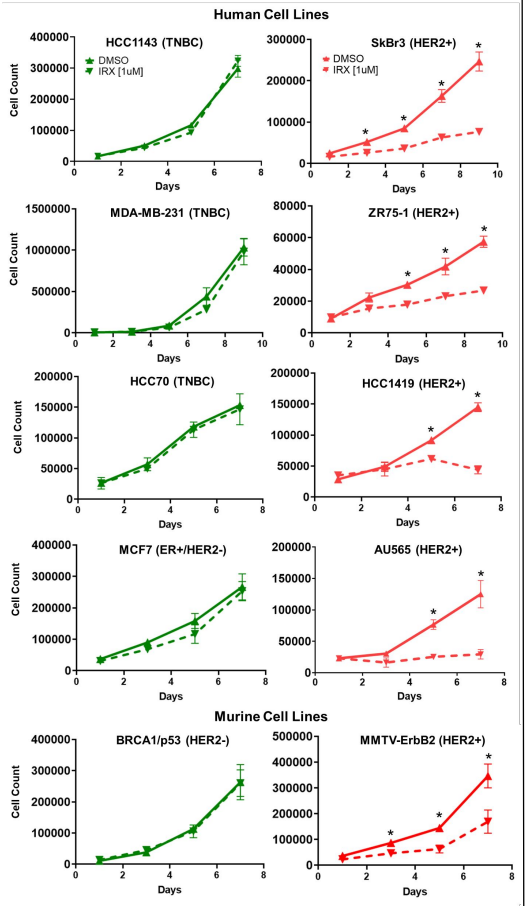

Figure 1. IRX4204 inhibits HER2-overexpressing breast cancer cell lines *in vitro*. Top: Four human HER2- breast cancer cell lines and four human HER2+ cell lines treated with IRX4204 [1uM] or DMSO (control) for 7-9 days. Bottom: Murine BRCA1/p53 (HER2-) and MMTV-ErbB2 (HER2+) treated with IRX4204 [1uM] or DMSO (control) for 7 days. Statistical significance was determined using the Bonferroni-Dunn method of multiple t-tests. Each day was analyzed individually, without assuming a consistent SD (*p<0.01).

Effect of IRX4204 in combination with current breast cancer therapies

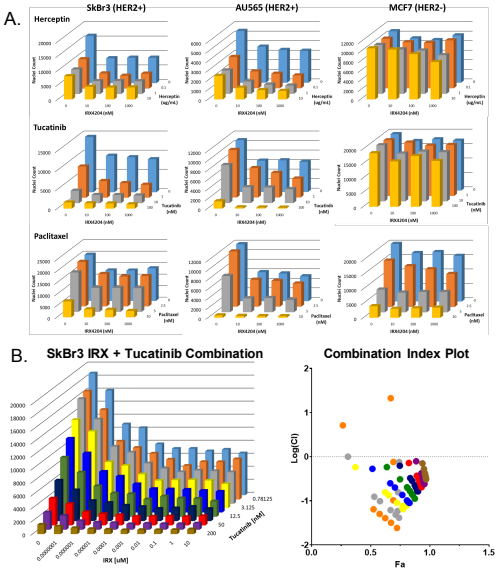

Figure 2. IRX4204 synergizes with anti-HER2 therapy *in vitro*. A) SkBr3, AU565 and MCF7 human breast cancer cells were treated *in vitro* with IRX4204 and Herceptin, tucatinib or paclitaxel for 6 days. Nuclei were stained and counted. The average nuclei count of 6 replicates for each combination is displayed. Percent CV among replicates is less than 7%. B) SkBr3 were treated *in vitro* with 100 dose combinations of IRX4204 and tucatinib for 6 days. Nuclei were stained and counted. The average nuclei count of 6 replicates for each combination is displayed. Percent CV among replicates is less than 7%. The combination index (CI) for each dose combination was plotted against fraction affected (Fa). All data points below 0 show a synergistic drug interaction.

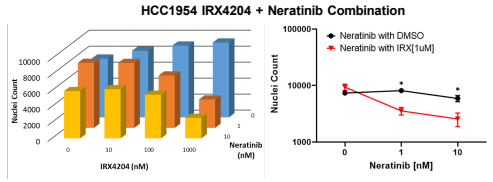

Figure 3. Treatment with IRX4204 overcomes anti-HER2 resistance *in vitro*. Anti-HER2 resistant HCC1954 human breast cancer cells were treated *in vitro* with IRX4204 and neratinib for 6 days. Only the combination of neratinib with IRX4204 resulted in growth inhibition of the drug resistant cell line. Each data point represents six replicates. Each dose was analyzed individually, using the Bonferroni-Dunn method of multiple t-tests (*p<0.01).

Mechanism by which IRX4204 inhibits breast cancer cell line growth

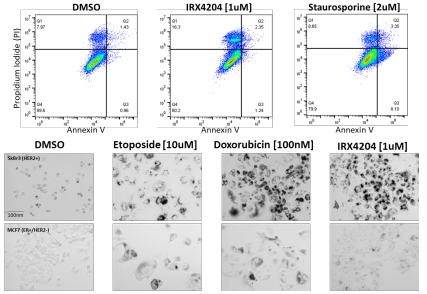

Figure 4. Apoptosis and senescence of breast cancer cells treated with IRX4204. A) AnnexinV-PI staining of SkBr3 cells treated with DMSO, IRX4204 [1uM] or apoptosis inducing staurosporine. Treatment with IRX4204 shows an increase in PI (necrosis) but not AnnexinV (apoptosis). B) SkBr3 and MCF7 human breast cancer cells were treated *in vitro* with DMSO (control), etoposide [10uM], doxorubicin [100nM] or IRX4204 [1uM] for 24-48 hours and stained for β-gal. As expected, an accumulation of β-gal was observed in the etoposide and doxorubicin treated cells over DMSO. Increased β-gal was also observed in the IRX4204 treated cells; more notably in the HER2-amplified SkBr3 cell line which also showed a change in morphology.

Conclusions

- IRX4204 inhibits the growth of HER2-amplified breast cancer as a single agent *in vitro*
- IRX4204 synergizes with anti-HER2 therapy to inhibit the growth of HER2 amplified breast cancer *in vitro*
- IRX4204, in combination with anti-HER2 therapy, overcomes anti-HER2 resistance *in vitro*
- IRX4204 induces necrosis and senescence in HER2-amplified breast cancer cell lines

Future Directions

- Replicate IRX4204 and anti-HER2 drug synergy *in vivo*
- Define the mechanism by which IRX4204 inhibits HER2-amplified breast cancer
- Examine the efficacy of IRX4204 in the setting of HER2-amplified breast cancer brain metastases

References

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