IRX5010 (IRX4647F), a Highly Selective RARγ Nuclear Receptor Agonist Compound Inhibits *In Vivo* Growth of Murine EMT-6 Triple Negative Breast Cancer and Human JIMT-1 Her2+ Breast Cancer By Induction of Tumor-Infiltrating Effector Memory T-cells

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Abstract

RARγ agonism has been shown to play an essential role in CD8 T-cell-mediated immunity to infectious pathogens.[1] However, we found no previous reports on effects of RARγ agonists on *in vivo* tumor growth of triple negative or Her2+ breast cancers. Here we explored whether RARγ could play a critical role in T-cell-mediated immunity in these breast cancers, using a highly potent and highly selective novel RARγ agonist.

None of the RARγ agonist compounds we evaluated demonstrated significant inhibitory effects on *in vitro* growth of EMT-6 triple negative breast cancer cells even at concentrations as high as 20-100 millimolar, indicating that these compounds have insignificant direct inhibitory effects on EMT-6 cancer cell growth *in vitro*. We then examined the effects of one of the compounds *in vivo* in a syngeneic mouse model of EMT-6 triple negative breast cancer. Treatment with one of the most potent of the our RARγ agonist compounds, IRX5010 (IRX4647F), demonstrated dose dependent inhibition of *in vivo* growth of EMT-6 triple negative breast cancer. Inhibition of tumor growth at day 17 relative to vehicle was 59% and 69% respectively in mice treated with IRX5010 (IRX4647F) at 10 or 25 mg/kg/day. A cohort treated with 50 mg/kg/day was sacrificed early on day 12 due to excessive weight loss and deaths of some of the animals. This cohort experienced a 73% inhibition of tumor growth relative to vehicle. Gross necropsies of animals in the 25 and 10 mg/kg/day dose groups did not show observable pathologic findings in any major organ. Flow cytometry was performed on freshly excised tumors on the day of termination of the in-life portion of the study in the 10 and 25 mg/kg/day dose groups. Tumors from mice treated with IRX5010 (IRX4647F) had increased numbers of total CD45+ cells; total CD3+, total CD4+, and total CD8+ T-cells per mg of tumor tissue, relative to vehicle controls. IRX5010 (IRX4647F) treated mice also had increased CD4+CD62L- and CD8+CD62L- T-effector memory T-cells (Tem) per mg of tumor tissue relative to vehicle-treated control mice.

We also conducted an experiment of treatment with IRX5010 (IRX4647F) in mice transplanted with human immune cells (to provide a human immune system) to examine the effects on xenografted human Her2+ JIMT-1 cancer cells. In this model, treatment with IRX5010 (IRX4647F) at 10 mg/kg/day resulted in a 17% inhibition of tumor growth relative to vehicle control over 26 days of treatment. Flow cytometric analysis showed that tumors from the IRX5010 (IRX4647F) treated mice contained increased numbers per mg of tumor tissue of human CD8+ T-cells, most of which were CD8+ CD7- CD45RA+ terminally differentiated effector memory phenotype T-cells (TEFF).

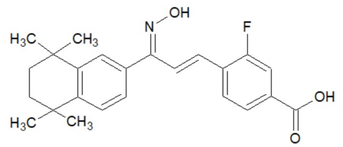
Introduction

We recently reported that our first generation RARγ agonist compound, IRX4647, was effective in murine models of non-small cell lung cancers, even though it had minimal or no direct inhibitory effect on tumor cell growth *in vitro*.[2] *In vivo* in NSCLC models, IRX4647 demonstrated inhibitory effects on tumor growth, and tumor immunity promoting effects in the tumor immune microenvironment. Further, IRX4647 demonstrated combination inhibitory effects on tumor growth *in vivo* when co-administered with an anti-PDL1 checkpoint inhibitor.

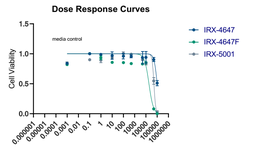
Concurrently with these lung cancer studies, we evaluated a new second generation RARγ compound, IRX5010 (IRX4647F), which is an analogue of the earlier compound IRX4647. IRX5010 demonstrates greater potency than IRX4647 in *in vitro* RARγ transactivation assays.

Methods and Materials

We synthesized and screened compounds for selective transactivation of RARγ in reporter assays. A compound demonstrating highly selective and highly potent RARγ transactivation, IRX5010 (IRX4647F), was selected for further evaluation of potential anti-cancer activities against tumor cells *in vitro*; and *in vivo* in the EMT-6 syngeneic mouse model of triple negative breast cancer; and in a JIMT-1 human Her2+ breast cancer xenograft model in immuno-incompetent mice transplanted with human PBMCs to introduce a human immune system. IRX5010 (IRX4647F) or vehicle was administered orally once daily in both models. We focused our current studies on IRX5010 (IRX4647F) which we view as one of our best compounds in this analogue series.



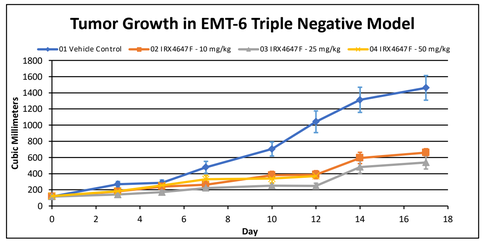
Chemical structure of IRX5010 (IRX4647F)
EC_{50} of IRX5010 (IRX4647F) in RARγ transactivation reporter assay Is less than 0.1 nanomolar. EC_{50} in RARα transactivation reporter assay is greater than 100 nanomolar.

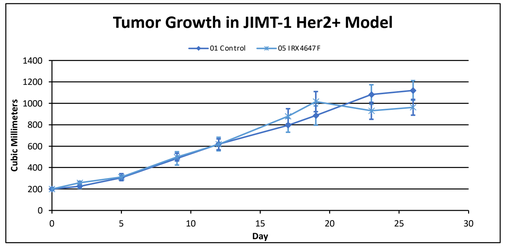


	IC50	R squared	LogIC50	Z-Factor
IRX-4647	101343	0.7225	5.006	0.940000
IRX-4647F	18720	0.8907	4.272	0.910000
IRX-5001	51769	0.8812	4.714	

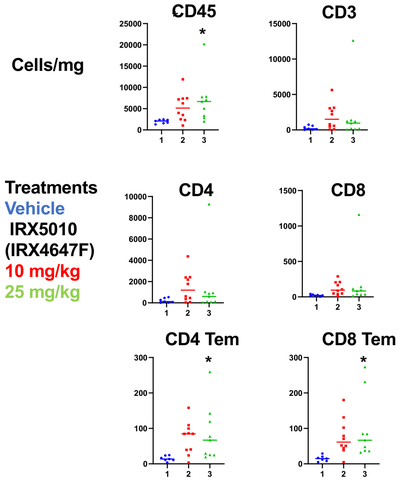
Effects of RARγ agonist compounds on *in vitro* EMT-6 proliferation
None of the tested RARγ agonist compounds demonstrated significant inhibitory activity *in vitro* on EMT-6 proliferation at concentrations lower than 20-100 millimolar.

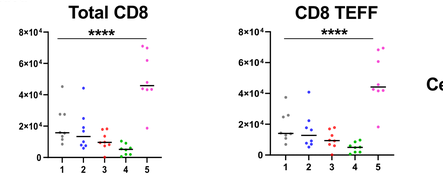
Results





Flow Cytometry of T-cells in Harvested Tumors





* P< 0.05 **** P< 0.0001
one-way ANOVA with Dunnett's test compared to vehicle control group

Conclusions

IRX5010 (IRX4647F), a second generation, highly potent and highly selective RARγ nuclear receptor agonist compound, demonstrated substantial dose dependent treatment effects relative to vehicle control (59-73%) on inhibition of growth of EMT-6 syngeneic murine triple negative breast cancer *in vivo*, despite having essentially no growth inhibitory effect on this cancer *in vitro*. Dose dependent weight loss was observed, but gross necropsies in the two lowest dose groups did not identify specific organ toxicities.

Treatment of xenografted human Her2+ tumor JIMT-1 in immuno-incompetent mice transplanted with human PBMCs to produce humanized immune systems, resulted in a moderate 17% inhibition of Her2+ breast cancer growth relative to vehicle control.

In both models, treatment with IRX5010 (IRX4647F) induced tumor infiltrating T-lymphocytes of effector memory phenotypes, demonstrating immunity promoting effects in the tumor immune microenvironment, be it of murine or human origin. Such effects were not observed with an RXR agonist (IRX4204), or anti-PD1, or their combination.

These data identify RARγ as a target and RARγ agonists as a novel treatment modality for breast cancers, especially for the remaining highly unmet need for new and better treatments for triple negative breast cancer.

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Martin Sanders and Vidyasagar Vuligonda are directors, officers, shareholders, and patent inventors of Io Therapeutics, Inc.
Mary Topalovski collaborated in study designs, and is an employee of Champions Oncology which conducted the presented studies.

References

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2. Wei CH, et al., A novel retinoic acid receptor-γ agonist antagonizes immune checkpoint resistance in lung cancers by altering the tumor immune microenvironment. Sci Rep. 2023 Sep 9;13(1):14907.