The RAR gamma nuclear receptor agonist IRX5010 has combination inhibitory effects with an anti-PDL-1 checkpoint inhibitor on the growth of EMT-6 triple negative breast cancer

Martin E. Sanders, M.D., Vidyasagar Vuligonda, Ph.D.
Io Therapeutics, Inc., Spring, TX

San Antonio Breast Cancer Symposium, December 11, 2024

Introduction

RARγ agonism plays an essential role in CD8 T-cell-mediated immunity to infectious pathogens (1). However, before we initiated our drug discovery program for novel RARγ agonists, we found no previous reports on effects of RARγ agonists on promotion of anti-tumor immunity *in vivo*. We undertook a drug discovery program to evaluate whether RARγ agonists could promote T-cell-mediated immunity in cancers. With our collaborators at the Frederick National Laboratory for Cancer Research we recently published 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-PDL-1 monoclonal antibody checkpoint inhibitor. Concurrently with these lung cancer studies, we evaluated a new second generation RARγ compound, IRX5010, which is an analogue of the earlier compound IRX4647. IRX5010 demonstrates greater potency than IRX4647 *in vitro* in RARγ transactivation reporter assays and was better tolerated *in vivo* in mice.

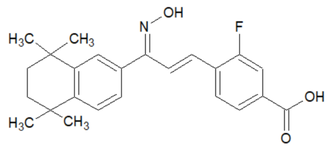
We previously presented (2023 SABCS) that monotherapy with our second generation RAR gamma agonist IRX5010 inhibited tumor growth in the triple negative breast cancer EMT-6 syngeneic mouse model; and in a human Her2+ JIMT-1 breast cancer xenograft mouse model. Growth inhibitory effects of IRX5010 in both models were accompanied by increased tumor infiltrating effector memory T-cells. We recently presented at the 2024 Society for Immunotherapy of Cancer Annual Research Conference positive effects of monotherapy with IRX5010 on inhibition of tumor growth accompanied by development of tumor infiltrating effector memory T-cells in mouse models of colorectal (MC38) and prostate cancers (MyC-CaP). In these models we also observed inhibition of tumor infiltration by myeloid derived suppressor cells, suggesting that combination treatment with RAR**γ** agonists such as IRX5010 with checkpoint inhibitors could have combination inhibitory effects on tumor growth in models of multiple cancers. We now present data on the effects of IRX5010 treatments with anti-PD-1 or anti-PDL-1 monoclonal antibody checkpoint inhibitors on tumor growth, tumor infiltrating T-cells, and myeloid derived suppressor cells in a syngeneic murine EMT-6 triple negative breast cancer model.

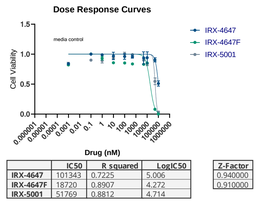
Methods and Materials

Experimental Methods: We synthesized and screened compounds for selective transactivation of RARγ in reporter assays, and promotion of anti-viral memory T-cell responses in assays in CMV immune human PBMCs by measuring gamma interferon release. A compound demonstrating highly selective and highly potent RARγ transactivation, and human memory T-cell activation 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. We have focused our current studies on compound IRX5010, which is one of the best compounds in this analogue series for both potency and tolerability.

EMT-6 cells were injected into Balb/c mice and allowed to grow to 50-150 mm^3. Then treatments were started and maintained until tumors grew to approximately 1500 mm^3. Treatment was administered as daily oral doses of IRX5010 at 10 mg/kg/day, or vehicle. Murine monoclonal anti-PD-1 or anti-PDL-1 antibodies were injected ip every three days at 10 mg/kg. Tumor sizes were assessed by calipers every three days. Toxicity assessment was done by weighing the mice every three days. Flow cytometric quantitation of Tumor Infiltrating T-cells (TIL) and Myeloid Derived Suppressor Cells (MDSC) were performed at study end. Total T cells, and CD4 and CD8 effector memory cells were quantified as number of cells per mg of harvested tumor tissue expressing CD3, and CD4 or CD8, and for effector memory cells, are CD44+ and CD62L-. Granulocytic MDSC (G-MDSC) were quantified as cells per mg of harvested tissue expressing CD11b that are Ly-6C-low and Ly-6G+.

Chemical Structure of IRX5010





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

Z-Factor
0.940000
0.910000

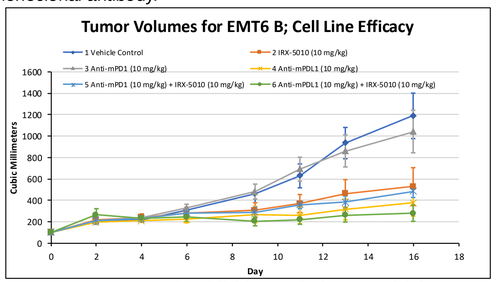
EC50 of IRX5010 in RARγ transactivation assay is less than 0.1 nM EC50 and in RARα transactivation assay is greater than 100 nM

Effects of RARγ agonist compounds on EMT-6 proliferation: IRX5010 (IRX4647F) and other RARγ agonist compounds demonstrated no significant inhibitory activity *in vitro* on EMT-6 proliferation at less than 20 mM

Results

Effects of IRX5010 and Checkpoint inhibitors on Tumor Growth

IRX5010 demonstrated substantial tumor growth inhibition as monotherapy at 10 and 25 mg/kg/day in the EMT-6 model (see figure at right). IRX5010 10 mg/kg/day showed additive inhibitory effect on tumor growth in combination with an anti-PDL-1 monoclonal antibody, and no additive effect with an anti-PD-1 monoclonal antibody.



Mean Tumor Growth Inhibition Relative to Vehicle Control

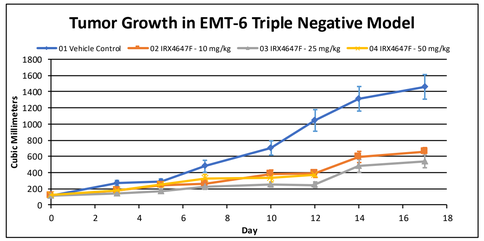
Vehicle	0%	IRX5010	61%
Anti-PD-1	14%	Anti-PDL-1	75%
Anti-PD-1+IRX5010	65%	Anti-PD-L-1+IRX5010	84%

Mean Body Weight Change from Baseline

Vehicle	7.5%	IRX5010	-4.2%
Anti-PD-1	8.5%	Anti-PDL-1	3.8%
Anti-PD-1+IRX5010	-6.8%	Anti-PD-L-1+IRX5010	-4.5%

Effects of IRX5010 and Checkpoint Inhibitors on Tumor infiltrating T-cells and G-Myeloid Derived Suppressor Cells

	Median Cells/mg Harvested Tumor			
	Total T	CD4 Tem	CD8 Tem	G-MDSC
Vehicle	1311	107	36	3349
IRX5010	2702	664	194	2246
Anti-PD-1	1555	443	90	2758
Anti-PDL-1	1707	601	186	1741
Anti- PD-1+IRX5010	2090	661	406	2530
Anti-PD-L-1+IRX5010	3862	1059	659	1568

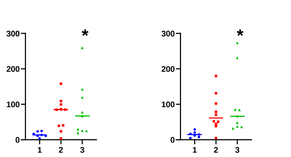


Dose range evaluation of monotherapy with IRX5010 (IRX4647F) (presented at 2023 SABCS)

Tumor Growth Inhibition Relative to Vehicle Control

10 mg/kg/day 70.7%
25 mg/kg/day. 85.7%

Flow Cytometry of Effector Memory T-cells in Harvested Tumors



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

Conclusions

Treatment of the EMT-6 murine model of triple negative breast cancer with the RAR**γ** selective nuclear receptor agonist compound IRX5010 plus a checkpoint inhibitor murine monoclonal anti-PDL-1 resulted in 84% inhibition of tumor growth, a 9% increase of inhibitory treatment effect over anti-PDL-1 alone. Combination treatment with IRX5010 with monoclonal anti-PD-1 had no additive effects on inhibition of EMT-6 tumor growth. IRX5010 and IRX5010+anti-PDL-1 treatment regimens were well tolerated as assessed by change in body weight. Flow cytometry showed that treatment with IRX5010 increased tumor infiltration of total and effector memory CD4 and CD8 phenotype T-cells, and inhibited tumor infiltration by G-MDSC. The effects were additive in combination with anti-PDL-1 but not anti-PD-1. These findings are the first demonstration of additive effects of combination treatment with an RAR**γ** nuclear receptor agonist and an anti-PDL-1 monoclonal antibody checkpoint inhibitor in a triple negative breast cancer mouse model. They support that the observed inhibitory effects of IRX5010 on tumor growth have multiple immune mechanisms of action rather than direct inhibitory effects on tumor proliferation. They also provide a strong rationale for future translation of combination treatment with IRX5010 plus an anti-human-PDL-1 monoclonal antibody checkpoint inhibitor into clinical trials in triple negative breast cancer patients.

Contact

Martin E. Sanders, M.D.
Io Therapeutics, Inc.
msanders@io-therapeutics.com
www.io-therapeutics.com

COI Disclosures

Martin Sanders and Vidyasagar Vuligonda are patent inventors, directors, officers, and equity owners of Io Therapeutics, Inc.

Acknowledgement

All studies were conducted at Champions Oncology, a contract research organization.

References

1. Dzhagalov I, et al., Regulation of CD8+ T lymphocyte effector function and macrophage inflammatory cytokine production by retinoic acid receptor gamma. J Immunol. 2007 Feb 15;178(4):2113-21.
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.
3. Sanders, M.E., Vuligonda, V. RAR**γ** agonist compound IRX5010 inhibits tumor growth, promotes effector memory tumor infiltrating T- cells, and inhibits tumor infiltrating myeloid derived suppressor cells in multiple cancer models. SITC Annual Research Conference, 2024.