RAR Gamma Agonist Compounds Promote Effector Memory Tumor Infiltrating T-cells and are Effective in Multiple Cancer Models

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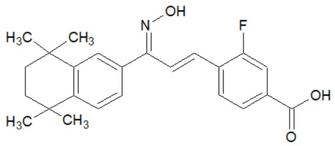
Introduction

Hypothesis: RAR gamma agonism has been shown to play an essential role in CD8 T-cell-mediated immunity to infectious pathogens (Dzhagalov I, *et al.*)[1]. However, before we initiated our drug discovery program for novel RAR gamma agonists, we found no previous reports on effects of RAR gamma agonists on promotion of anti-tumor immunity *in vivo*. Consequently, we undertook a drug discovery and screening program to evaluate whether RAR gamma agonists could play a critical role in T-cell-mediated immunity in various cancers.

Methods and Materials

We synthesized and screened RAR gamma agonist compounds by measuring their effects on increase of gamma interferon production (a marker of memory T-cell activation)) *in vitro,* by CMV immune human PBMCs treated with CMV. We found that as a class, RAR gamma agonists increased gamma interferon production in this assay. We embarked on *in vivo* evaluation of three selective RAR gamma agonist compounds (IRX4647, IRX5010, and tazarotenic acid [a clinically approved dermatologic therapy which is a combined RAR beta/gamma selective agonist]).

We studied these compounds *in vitro* and *in vivo* in syngeneic murine models of non-small cell lung (Lewis Lung Cancer, LLC-1), triple negative breast (**EMT-6**), colorectal (MC38), and prostate cancer (MyC-CaP), to evaluate their effects in models of four prevalent and difficult to treat types of human cancers. We also evaluated one compound (IRX5010) in a murine beta-2 microglobulin deficient mouse model given human PBMCs to establish a humanized immune system, xenografted with the human Her2+ breast cancer JIMT-1. Tumor growth was assessed by serial measurement of tumor size. Flow cytometry was performed to quantitate tumor infiltrating lymphocytes. Serial weights and post-mortem gross pathology examination of major organs were performed to assess toxicity.



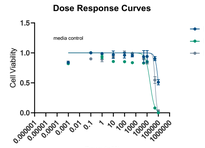
Chemical Structure of IRX5010
Second Generation
RAR Gamma Agonist

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

Effects of RARγ agonist compounds on in vitro EMT-6 and Lewis Lung Cancer (LLC) Proliferation

None of the tested RARγ agonist compounds demonstrated significant inhibitory activity in vitro on EMT-6 or LLC-1 proliferation at lower than 20-100 millimolar.

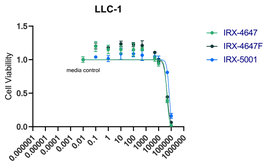
Triple Negative Breast Cancer EMT-6



	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

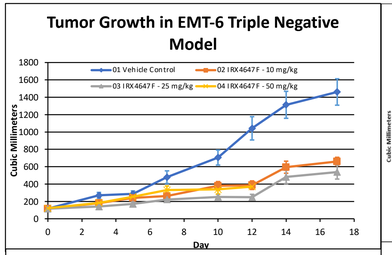
Z-Factor
0.940000
0.910000

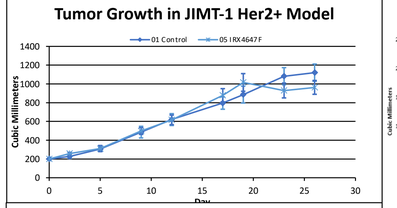
Lewis Lung Cancer

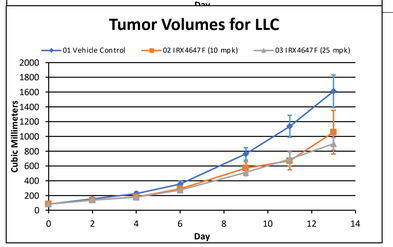


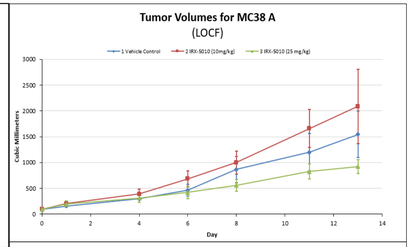
	IC50	R squared	LogIC50	Z factor
IRX-4647	45040	0.8923	4.654	0.49
IRX-4647F	46884	0.8315	4.671	0.49
IRX-5001	69181	0.9426	4.840	0.87

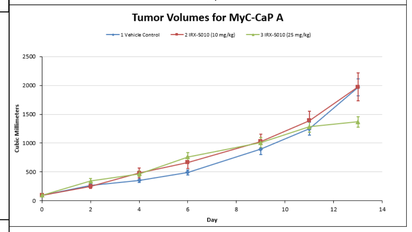
Results











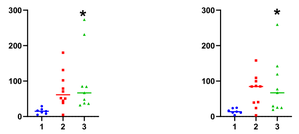
Flow Cytometric Quantitation of Tumor Infiltrating T-cells

IRX5010 demonstrated substantial tumor growth inhibition at 10 and 25 mg/kd/day in the EMT-6 and LLC models. IRX5010 inhibited growth in MC38, and Myc-CAP A models at 25 mg/kg/day. It inhibited growth moderately in the JIMT-1 human Her2+ breast cancer xenograft model (but substantially promoted tumor infiltrating human effector memory phenotype T-cells in this model. Example data of tumor infiltrating effector memory phenotype T-cells in the EMT-6 and JIMT-1 models are shown above right. Similar results for flow cytometric quantitation of effector memory phenotype infiltrating T-cells were observed in the LLC, MC38, and Myc-Cap A models (data not shown).

Flow Cytometry of T-cells in Harvested Breast Cancer Tissues (cells/mg)

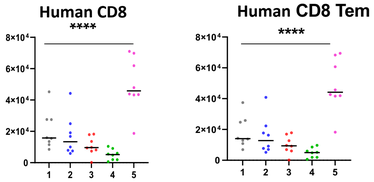
EMT-6 Triple Negative Syngeneic Model

Treatments
Vehicle
10 mg/kg
25 mg/kg



JIMT-1 Her2+ Xenograft Model

Treatments
Vehicle
IRX4204 (RXR agonist)
Anti-PD1
IRX4204 + Anti-PD1
IRX5010



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

Conclusions

Three RAR gamma selective agonist compounds demonstrated inhibition of tumor growth in syngeneic models of murine non-small cell lung, triple negative breast, colorectal, and prostate cancers; and in a murine humanized immune system xenograft model of a human Her2+ breast cancer. The compounds had little to no effect on tumor cell proliferation *in vitro* supporting that their inhibitory effects on *in vivo* tumor growth were not due to direct inhibitory effects on cancer cell proliferation. Inhibition of tumor growth was associated with increased numbers of effector memory phenotype tumor infiltrating T-lymphocytes in the syngeneic and xenograft models. Dose related weight loss, and lymph node and splenic hyperplasia were noted as toxicities. Our second generation RAR gamma agonist IRX5010 was the best tolerated of the three compounds. These data demonstrate that RAR gamma agonism is a potentially new mechanism for immunotherapy of multiple types of the most prevalent, inadequately treated cancers. These studies expand and confirm studies recently published by our collaborators at the Frederick National Laboratory for Cancer Research with ourselves, which demonstrated effects of our first generation RAR gamma agonist IRX4647 on the tumor microenvironment in a syngeneic murine model of non-small cell lung cancer, with increased tumor infiltrating lymphocytes, and combination treatment effects on tumor growth in conjunction with anti-PD-L1 monoclonal antibody (Wei, CH, *et al.*)[2].

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COI Disclosures

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

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.