4525

Combining a Novel Retinoic Acid Receptorγ Agonist with Checkpoint Blockade Represses Lung Cancer Growth by Altering the Tumor Immune Microenvironment

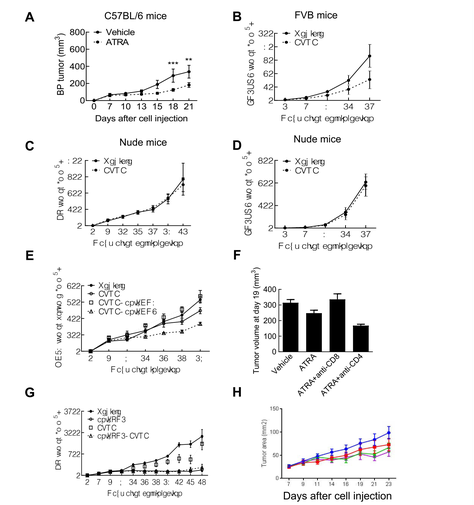
Cheng-Hsin Wei[1], Lu Huang[5], Blair Kreh[1], Xiuxia Liu[1], Liliya Tyutyunyk-Massey[1], Masanori Kawakami[1], Zibo Chen[1], Mi Shi[1], Vidyasagar Vuligonda[7], Martin Sanders[7], Serguei Kozlov[2], King Chan[3], Thorkell Andresson[3], Mary Carrington[4], Amir Horowitz[8], Patrick Hwu[5,6], Weiyi Peng[5], Ethan Dmitrovsky[1], and Xi Liu[1]

[1]Molecular Pharmacology Program, [2]Center for Advanced Preclinical Research, [3]Protein Characterization Laboratory, [4]Basic Science Program, Frederick National Laboratory for Cancer Research; [5]Department of Immunology and [6]Division of Cancer Medicine the University of Texas MD Anderson Cancer Center; [7]Io Therapeutics, Inc.; [8]Icahn School of Medicine at Mount Sinai

Abstract

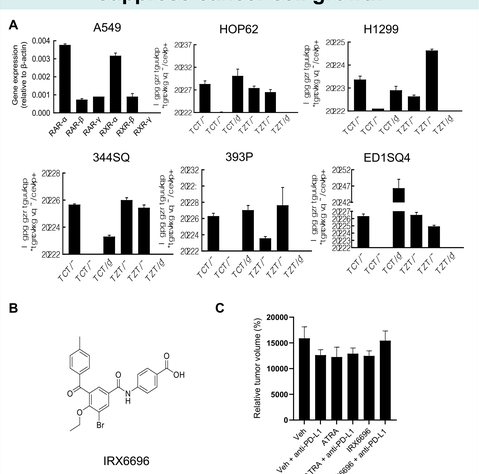
All-*trans*-retinoic acid (ATRA), a pan-agonist for retinoic acid receptors (RARs), regulates diverse cellular functions including growth, differentiation and immune function. We report here that ATRA-treatment represses tumor growth in syngeneic, immunocompetent but not in immunodeficient mice. Tumor immune microenvironment was implicated since depletion of cytotoxic T lymphocytes antagonized these effects in syngeneic mice. Combining ATRA with immune checkpoint blockade did not inhibit lung cancer growth in mice. We sought to augment retinoid anti-tumor effects without affecting its pro-tumorigenicity. We previously reported that CD38 mediated resistance to checkpoint blockade in murine 344SQ lung cancer cells via RARα transcriptional activation of CD38 expression. Yet, combining the RARα antagonist (IRX6696) with anti-PD-L1 did not augment anti-tumorigenicity in transplanted 344SQ cells in syngeneic mice. Prior work implicated RARγ in regulating T cell response. Combining the novel RARγ agonist (IRX4647) with anti-PD-L1 treatment statistically significantly repressed 344SQ lung cancer cell growth in syngeneic mice. This cancer cell line is relatively resistant to checkpoint blockade. Immunofluorescent analysis of these treated tumors revealed that combined IRX4647 and anti-PD-L1 treatments reduced CD38 expression in the tumor stroma relative to IRX4647 or anti-PD-L1 treatment alone. Statistically-significantly elevated helper (CD4+) T cells were detected in treated tumors along with increased IL-5 and IL-13 expression observed in plasma and tumors. These cytokines can activate helper T cells, altering lung cancer growth. These microenvironment effects were associated with *in vivo* anti-tumorigenicity. IRX4647-treatment did not appreciably alter *in vitro* growth of lung cancer cells although retinoid receptors expression profiles were affected. Pharmacokinetic study of IRX4647 found its plasma half-life was 6 hours. Combining an RARγ agonist with immune checkpoint blockade exerted superior anti-neoplastic efficacy against lung cancer xenografts versus an ATRA-based regimen. Given these findings, we propose exploring activity of this RARγ agonist with an optimal checkpoint inhibitor in a lung cancer clinical trial.

ATRA repressed syngeneic tumor growth

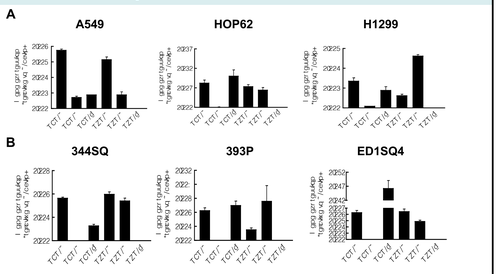


* $P < 0.05$, ** $P < 0.01$

RARα subtype selective ligands did not suppress cancer cell growth

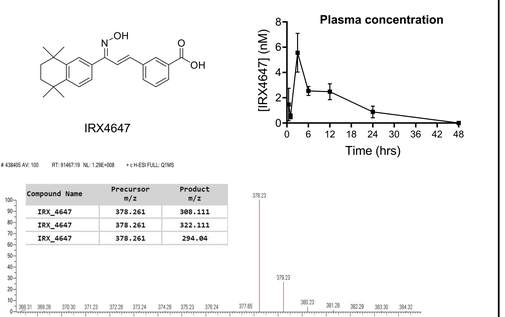


Basal expression of RARs and RXRs

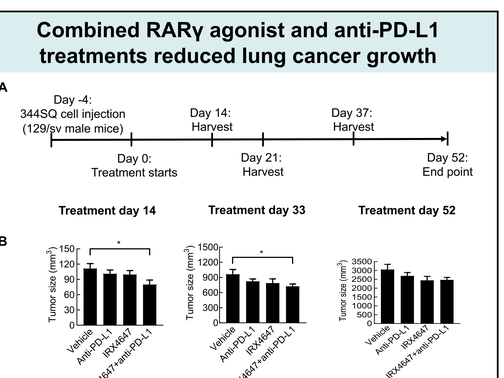


* $P < 0.05$, ** $P < 0.01$

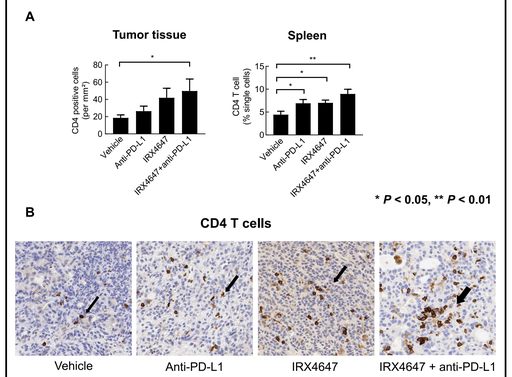
Pharmacokinetics of the RARγ agonist IRX4647



Combined RARγ agonist and anti-PD-L1 treatments reduced lung cancer growth

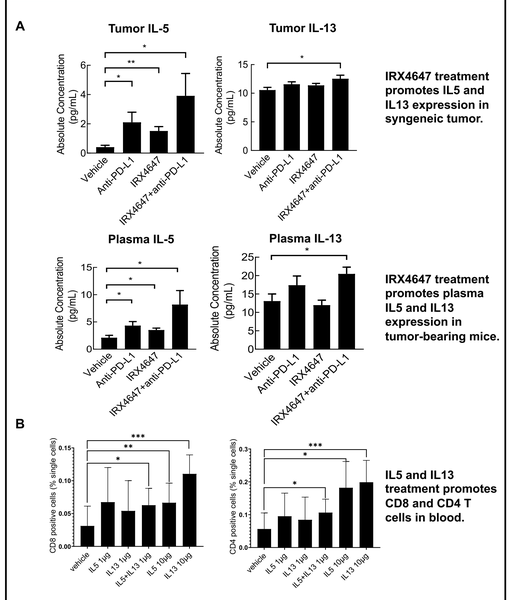


IRX4647 treatment augments CD4 T cells in tissues and tumors



* $P < 0.05$, ** $P < 0.01$

IRX4647 treatment promotes IL5 and IL13 expression in lung cancers



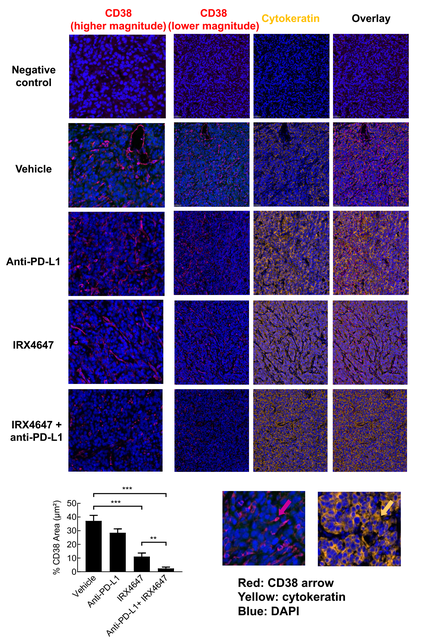
IRX4647 treatment promotes IL5 and IL13 expression in syngeneic tumor.

IRX4647 treatment promotes plasma IL5 and IL13 expression in tumor-bearing mice.

IL5 and IL13 treatment promotes CD8 and CD4 T cells in blood.

* $P < 0.05$, ** $P < 0.01$ *** $P < 0.001$

IRX4647 treatment reduces CD38 expression in the fibrovascular stroma



Red: CD38 arrow
Yellow: cytokeratin
Blue: DAPI

Conclusion

ATRA-treatment represses tumor growth in syngeneic, immunocompetent but not in immunodeficient mice. Combining ATRA with immune checkpoint blockade did not inhibit lung cancer growth in mice. In marked contrast, combined RARγ agonist and anti-PD-L1 treatments reduced lung cancer growth. Elevated helper (CD4+) T cells were detected in treated tumors along with increased IL-5 and IL-13 expression in plasma and tumors. Thus, combining an RARγ agonist with immune checkpoint blockade exerted superior anti-neoplastic efficacy against lung cancers versus an ATRA-based regimen alone. Given these findings, we propose exploring activity of this RARγ agonist with an optimal checkpoint inhibitor in a lung cancer clinical trial.