The RXR agonist IRX4204 promotes cytotoxicity of HER2+ breast cancer cells by HER2-targeted CAR-T cells

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Introduction

IRX4204 is a third generation RXR agonist compound. It is highly potent and highly RXR nuclear receptor- selective. IRX4204 is far more RXR selective than other RXR agonist compounds, being devoid of RAR, LXR, and PPAR gamma nuclear receptor agonism. This profile makes IRX4204 more safe than earlier generation RXR agonist compounds. It has been safely administered to 100 patients with cancers and other diseases, for up to 20 months of continuous treatment, with few, mild to moderate adverse effects. It is in IND phase II clinical trials for various indications.

With our collaborators, we have previously reported that: (1) IRX4204 has synergistic cytotoxic effects with HER2-targeted antibodies, HER2 targeted-tyrosine kinase inhibitors, and paclitaxel on HER2+ breast cancer cell lines; (2) IRX4204 promotes apoptosis of HER2+ breast cancer cells; and (3) IRX4204 promotes *in vivo* CD8 T-cell infiltration into murine Brca1mutant breast cancer tumors[1,2].

CAR-T cells targeted against various types of hematologic malignancies have been highly clinically effective. However, progress in developing effective CAR-T cells against solid tumors, such as breast cancers, has been slow. Based on the previously reported combination effects of IRX4204 with anti-HER2-targeted agents on HER2+ breast cancers, we hypothesized that IRX4204 may have combination effects with HER2-targeted CAR-T cells on killing of HER2+ breast cancer. We now report that IRX4204 promotes increased infiltration and cytotoxicity of cultured spheroids of the HER2+ human breast cancer cell line SKBR3 by HER2-targeted CAR-T cells.

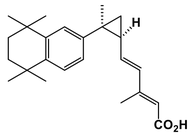
Methods and Materials

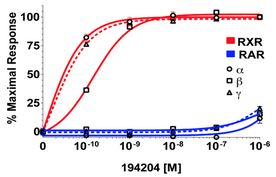
Initial experiments examined effects of IRX4204 on expression of cell surface HER2 and various adhesion molecules on cultured SKBR3 cells by flow cytometry.

To evaluate combination treatment effects with IRX4204 and HER2-targeted CAR-T cells, we obtained HER2-targeted human CAR-T cells from a commercial source (Promab: PM-CAR1024-1M; Her2 scf-v-CD28CD3zeta). SKBR3 cells were stained with cell tracker deep red and cultured till forming spheroids. HER2-targeted CAR-T cells were stained with cell trace violet and added to the SKBR3 cells at effector to target ratios of 0, 2.5, 5, and 10 to 1.

Following 72 hours of co-incubation of SKBR3 cells with CAR-T cells, IRX4204 was added to the cultures at 10, 100, and 1000 nM concentrations, for 24 hours. Sytox Green Ready reagent was added to the cultures to quantitate cell death. Images were analyzed using automated systems to quantitate T-cell infiltration and cytotoxicity.

IRX4204 – A Potent and selective RXR Homodimer Agonist with no RAR Activity at nM Concentrations



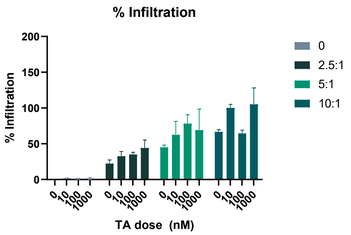


Results

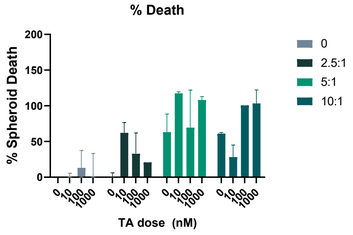
Flow Cytometry SKBR3 Cells

IRX4204	Marker	% positive	MFI
None	HER2	100	53755
	ICAM-1	4	7516
10 nM.	HER2	100	53633
	ICAM-1	6	8919
100 nM	HER2	100	50846
	ICAM-1	6	8799
1000 nM	HER2	100	52805
	ICAM-1.	6	8918

CAR-T Infiltration of Of SKBR3 Spheroids



Anti-HER2 CAR-T Cytotoxicity



- IRX4204 did not affect expression of cell surface Her2 on SKBR3 cells.
- IRX4204 slightly increased expression of the adhesion molecule ICAM-1 on SKBR3 cells,
- IRX4204 increased infiltration of CAR-T cells into SKBR3 spheroids in a dose dependent manner.
- IRX4204 alone had minimal cytotoxic effect on SKBR3 cells under these test conditions.
- IRX4204 synergistically promoted Her-2 targeted CAR-T cytotoxicity of SKBR3 HER2+ breast cancer cells.

Conclusions

Combination of the RXR agonist IRX4204 with HER2-targeted CAR-T cells showed a dose dependent increase in CAR-T infiltration and killing of spheroids of human HER2+ SKBR3 breast cancer cells *in vitro*. These promising results support further development of HER2-targeted CAR-T cells, and combination of such cells with the RXR agonist IRX4204 as a therapeutic option for patients with advanced Her2+ breast cancer. These data add to our recently reported studies of synergistic killing of human multiple myeloma by the combination of IRX4204 plus BCMA CAR-T cells *in vitro* and *in vivo* in a mouse xenograft model of human multiple myeloma by our collaborators in the laboratory of Professor Yubin Kang, M.D., at Duke University School Medicine[3]. In the xenograft myeloma model, IRX4204 inhibited CAR-T exhaustion and promoted CAR-T survival *in vivo,* allowing the CAR-T cells to have increased duration of anti-cancer efficacy. Cumulatively, our present studies demonstrating synergistic effects of IRX4204 in combination with HER2-targeted CAR-T cells for killing HER2+ breast cancer cells *in vitro* by yet to be defined mechanisms of action, and the recently reported results of IRX4204 in combination with BCMA CAR-T cells for killing myeloma, support that IRX4204 may be effective for increasing anti-cancer responses in multiple types of solid tumor and hematologic cancers.

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COI Disclosures

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

Acknowledgement

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References

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2. Moyer *et. al.:* Prevention of Breast Caner Using RXR Agonists, *Cancer Prevention Research,* 2025
3. Wu, *et. al.* , RXR agonist IRX4204 improves BCMA 'CAR-T functionality by suppressing ferroptosis via CHAC1 downregulation and promoting mitophagy., abstract presented at 2025 *American Society of Hematology Annual Meeting*