Io Therapeutics Phase II Clinical Stage and Preclinical Retinoid Compounds for Treatment of Cancers

Io Therapeutics, Inc.
Martin E. Sanders, M.D.
Chairman and CEO

Io Therapeutics
Cancer Treatments Pipeline

- **IRX4204: Phase II Clinical Stage Selective RXR Agonist for Her2+ Breast Cancer and Other Cancers**
- **IRX5183: Phase II Clinical Stage RARα Agonist for Multiple Myeloma and AML**
- **RARγ Agonists for Triple Negative and Her2+ Breast Cancers, Non-Small Cell Lung Cancer, Colon Cancer, and Prostate Cancer**

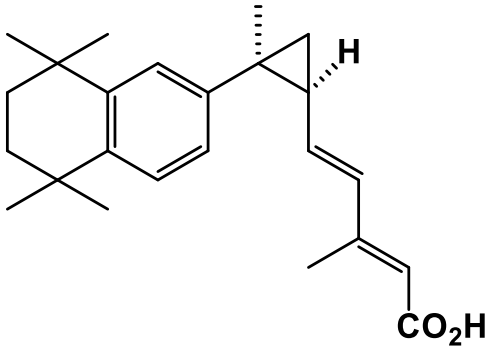
IRX4204

A Phase II Clinical Stage Selective RXR Agonist for Her2+ Breast Cancer and Other Cancers

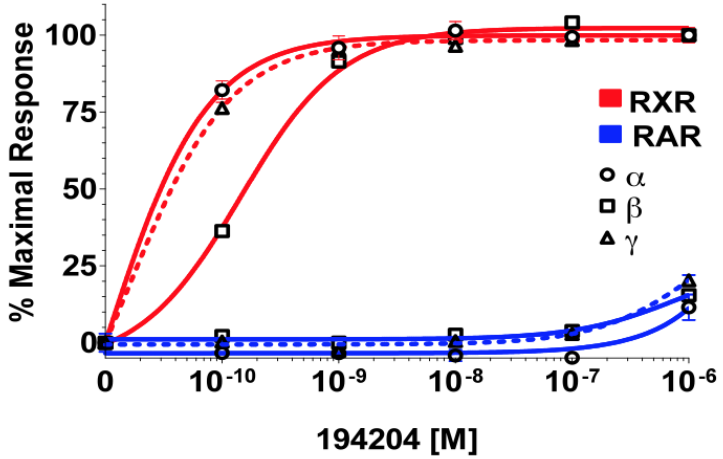
IRX4204 Summary

- IRX4204 is a potent, and highly selective RXR agonist with demonstrated safety and anti-cancer activities in preclinical and phases I and II clinical studies.
- 100 patients have been treated with IRX4204, including 85 with various cancers and 15 with early-stage Parkinson's disease under US INDs.
- IRX4204 has been safe and well tolerated with up to 20 months of treatment in humans.
- Oral PK in humans is consistent with once per day dosing.
- Common side effects include dose related suppression of TSH with resultant hypothyroidism, mild to moderate increased triglycerides, and transient mild to moderate reversible leukopenia.
- Open label phase I and IIA clinical trials in cancers and Parkinson's disease support clinical safety and efficacy, and selection of dose level.
- Preclinical data demonstrate synergy of therapeutic effects of IRX4204 plus anti-Her2 mab trastuzumab; HER2 tyrosine kinase inhibitors; or paclitaxel in HER2+ breast cancer.
- Preclinical data demonstrate therapeutic activity on trastuzumab resistant, and HER2 tyrosine kinase resistant HER2+ breast cancers.
- IRX4204 has been shown to be brain penetrant, with potential use in the treatment of brain metastasis in HER2+ breast cancer.

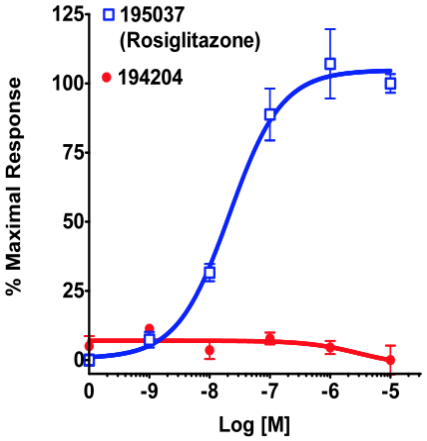
IRX4204: A Phase II Clinical Stage RXR Specific Agonist for the Treatment of Cancers



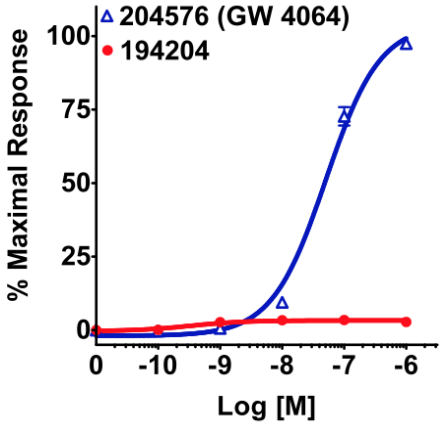
IRX4204 – A Potent RXR Homodimer Agonist with no RAR Activity at nM Concentrations



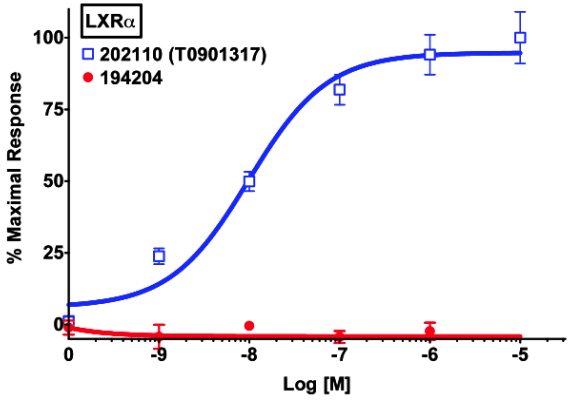
IRX4204 is highly selective for RXR
It does not transactivate PPARγ, FXR, or LXR



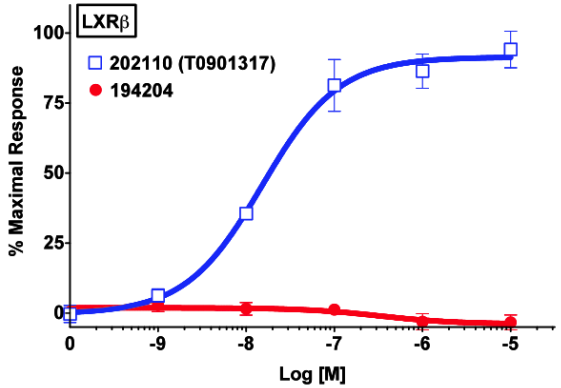
PPARγ/RXR



FXR/RXR



LXR/RXR



LXR/RXR

IRX4204 is More Potent and More Selective than Bexarotene (The Only Currently FDA Approved RXR Agonist)

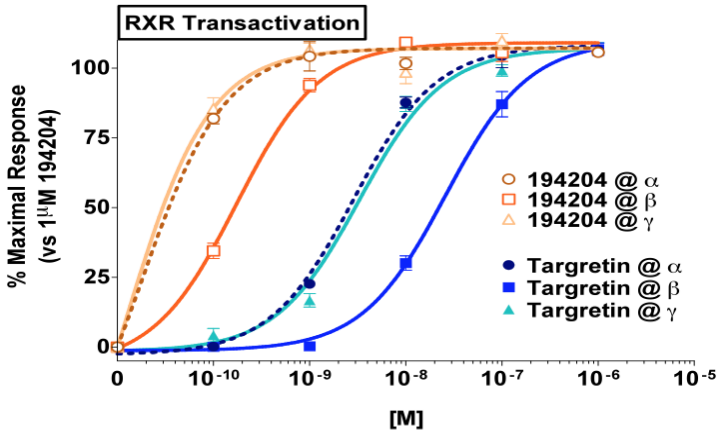
Bexarotene

- Bexarotene is a non-selective, less potent RXR agonist – approved by FDA for T-Cell lymphoma
- Bexarotene transactivates RXRs, RARs, PPARγ, and LXRs
- Bexarotene is more toxic in humans than IRX4204 (rashes, greater lipid elevations)

IRX4204

- IRX4204 is a highly selective RXR agonist, 100x more potent than bexarotene
- IRX4204 is specific for RXRs; not active at RARs, PPARγ, and LXRs
- IRX4204 was well tolerated in 100 humans in Phase I/II clinical trials; less cutaneous rash and less lipid elevations than bexarotene

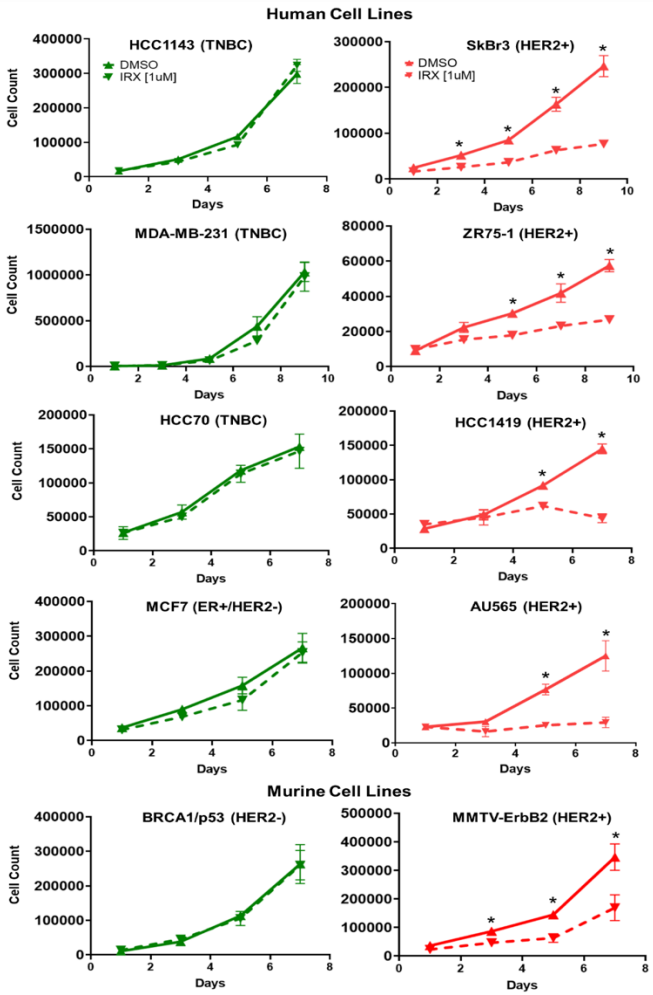
IRX4204 is >100 Fold More Potent Than Targretin (Bexarotene) in Transactivation of RXR Homodimers



IRX4204 Pharmacology in Cancer Models

- Dose dependent inhibition of proliferation of breast (especially HER2+), NSCLC, SCLC, prostate, colon, pancreatic, cervical, lymphoma, leukemia, melanoma, glioblastoma, and multiple types of sarcomas.
- Effective in *in vivo* cell line xenograft models of breast (HER2+), lung, and prostate cancers.
- **Synergistic inhibition of HER2+ breast cancer proliferation** *in vitro* with trastuzumab, neratinib, lapatinib, tucatinib, and paclitaxel.
- Inhibits proliferation *in vitro* of some **trastuzumb or HER2 TKI resistant HER2+ breast cancers**.
- Effective in *in vivo* models of breast (HER2+) and lung cancer prevention.
- Induces apoptosis in HER2+ breast cancer cells.

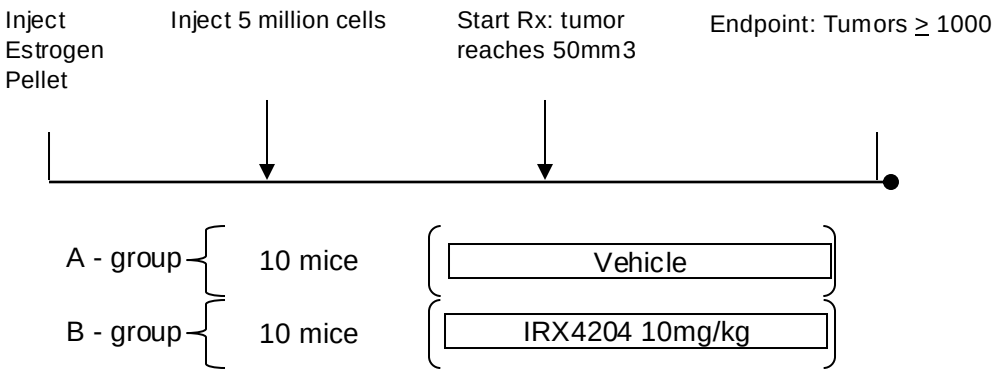
IRX4204 Inhibits Tumor Growth *in Vitro* in Most HER2+ Breast Cancers, but Not in Most HER2- Breast Cancers



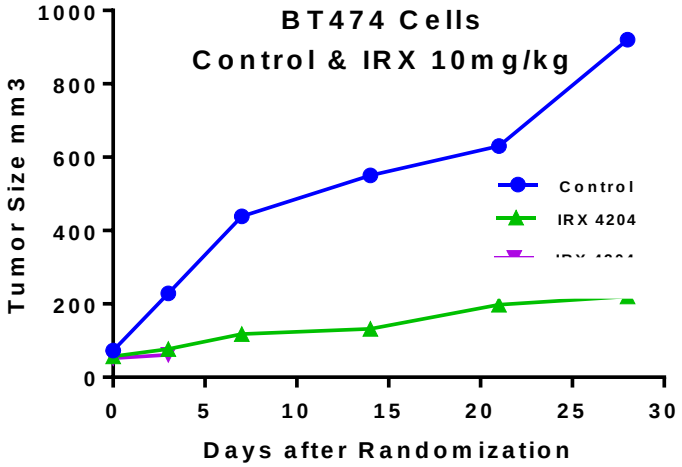
Data from PH Brown, MD, PhD; A Mazumdar, PhD and Cassandra Moyer, PhD; MD Anderson Cancer Center

Effect of IRX4204 Monotherapy Treatment on BT474 (ER+ / HER2+) Tumor Growth in *In Vivo*

Treatment Schema:



Results:

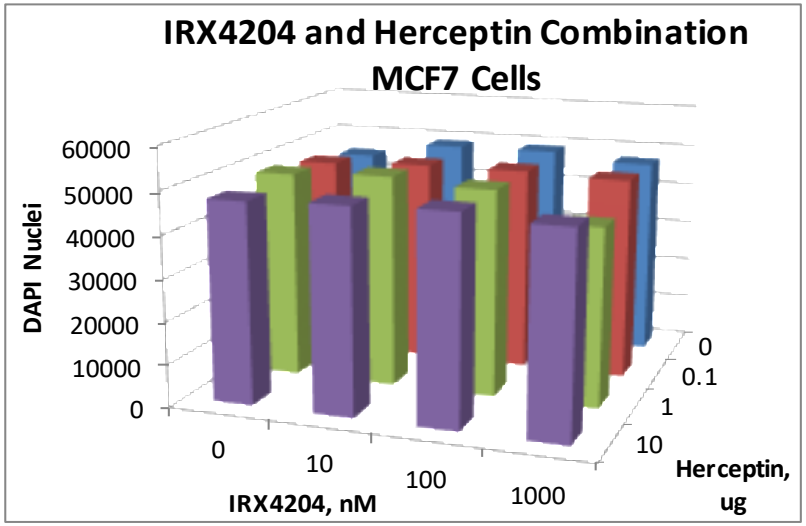


ER-pos
HER2-pos

Data from P Brown, MD, PhD
MD Anderson Cancer Center

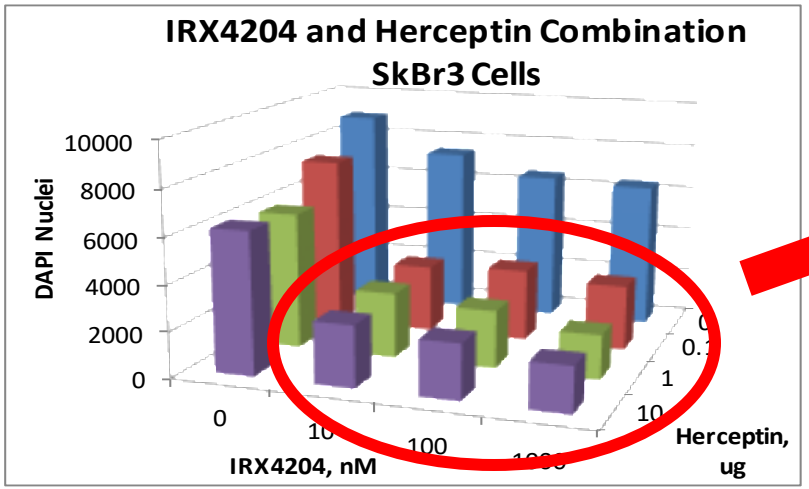
IRX4204 plus Trastuzumab (anti-HER2 Mab) Have Synergistic Inhibitory Effects on Human HER2+ Breast Cancer Growth *in Vitro*

MCF7
ER-pos / HER2-neg



ER-pos
HER2-neg

SkBR3 cells
ER-neg / HER2-pos

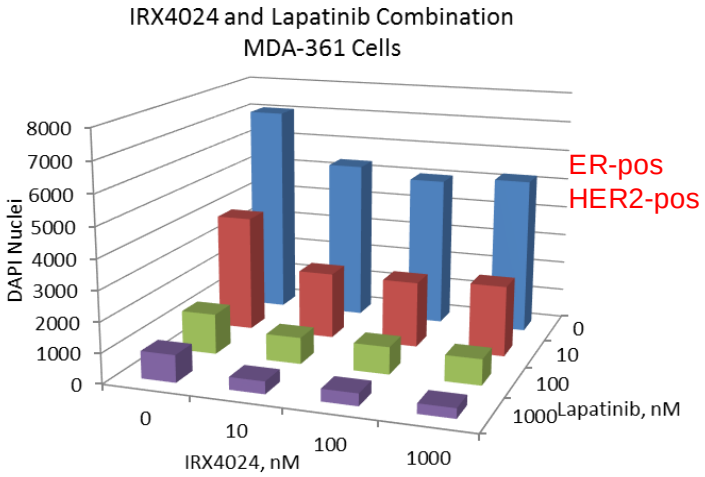


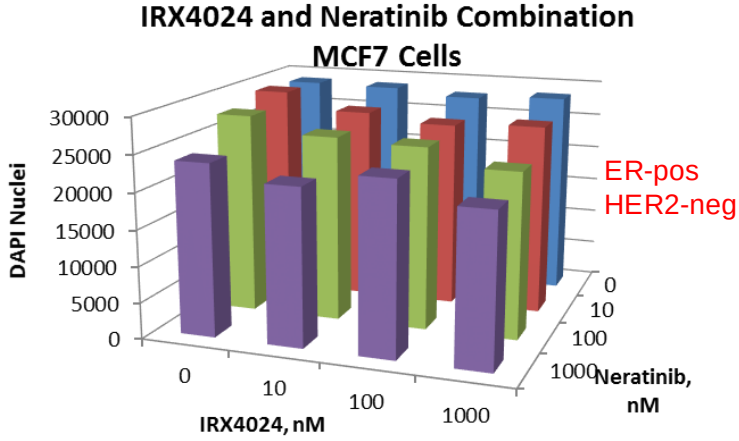
ER-neg
HER2-pos

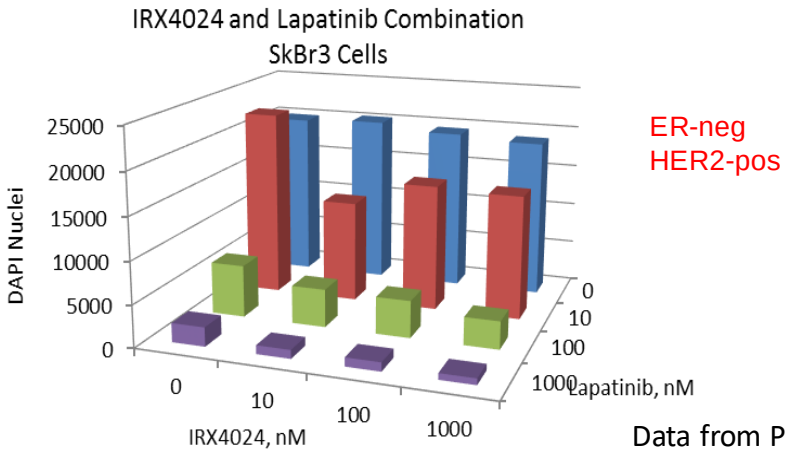
Synergistic Activity !

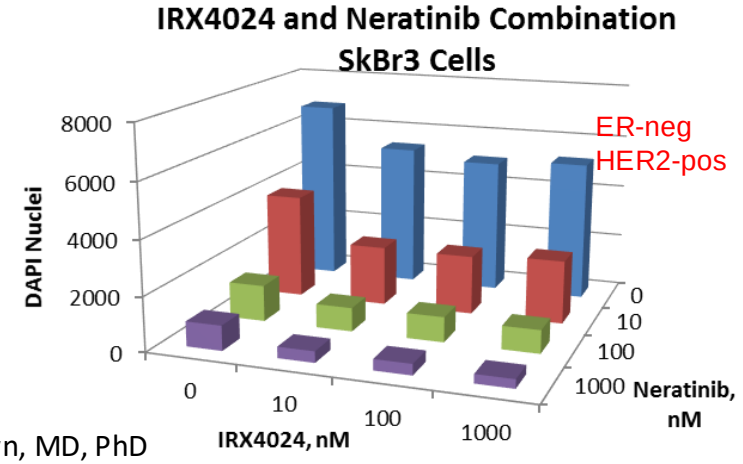
Data from P Brown, MD, PhD
MD Anderson Cancer Center

Combination Therapy for HER+: IRX4204 plus Small Molecule TKI Inhibitors Lapatinib and Neratinib Have Synergistic Effects



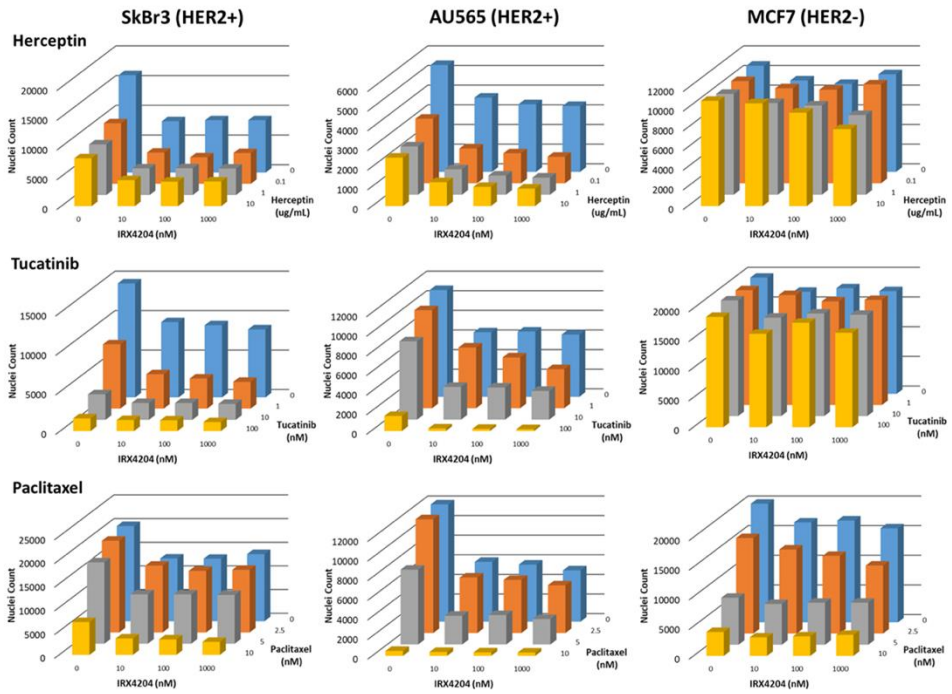


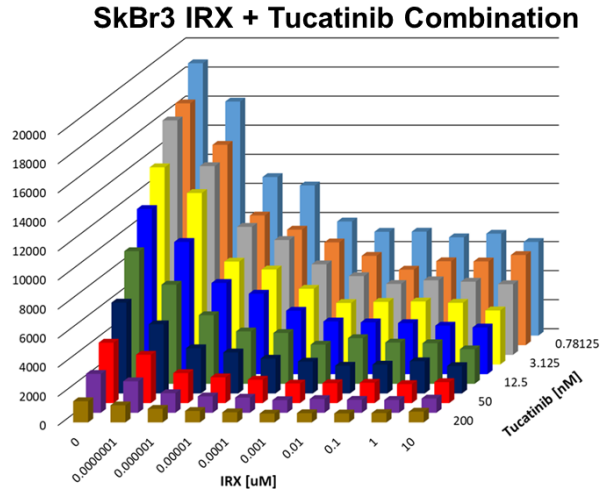


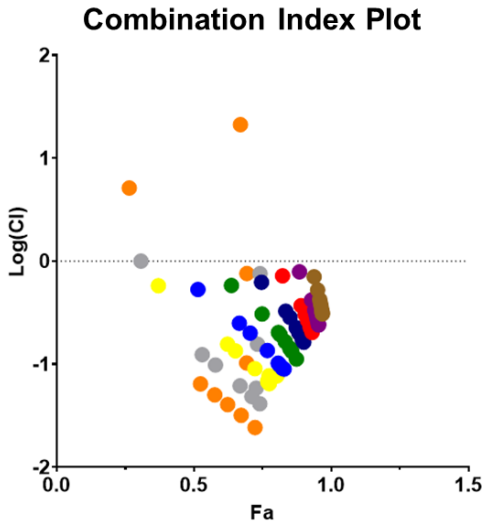


Data from P Brown, MD, PhD
MD Anderson Cancer Center

IRX4204 has Synergistic Inhibitory Effects with Tucatinib on Human HER2+ Breast Cancer Cells *in Vitro*

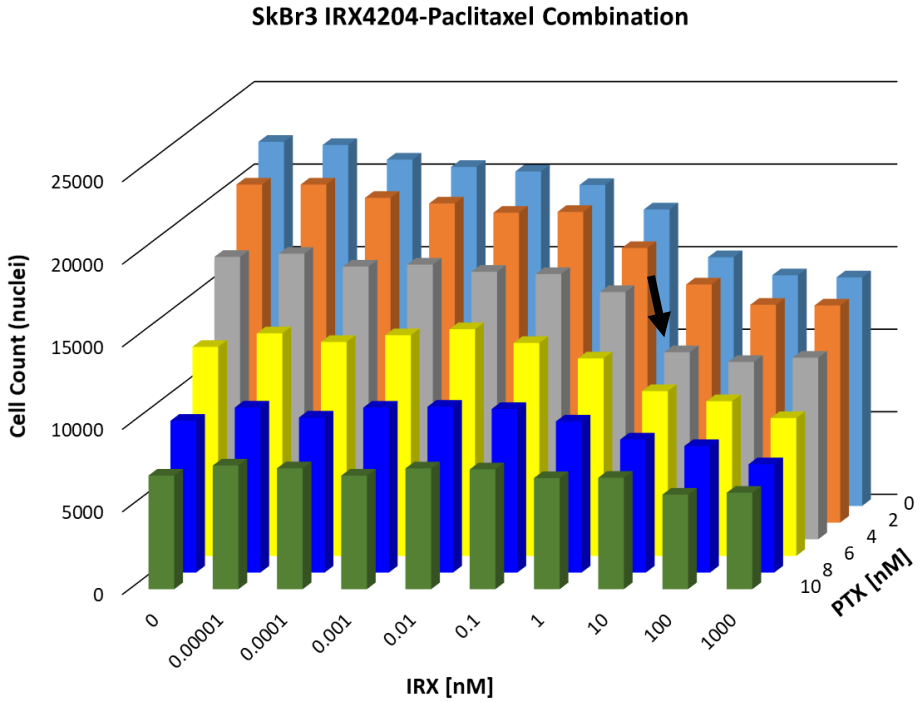


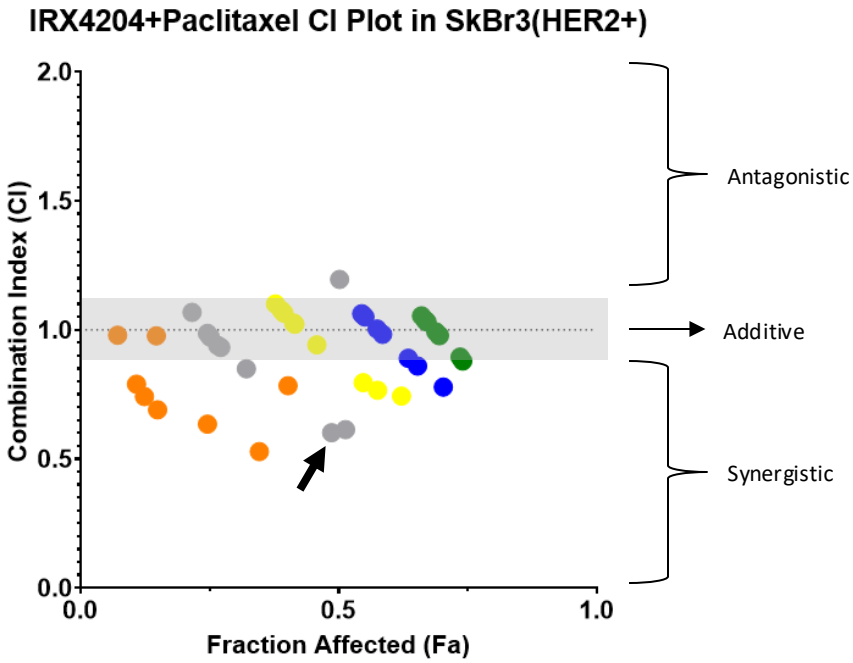




Data from P Brown, MD, PhD; A Mazumdar, PhD; and Cassandra Moyer, PhD; MD Anderson Cancer Center

IRX4204 has Synergistic Inhibitory Effects with Paclitaxel on HER2+ Human SkBr3 Breast Cancer Cells *in Vitro*





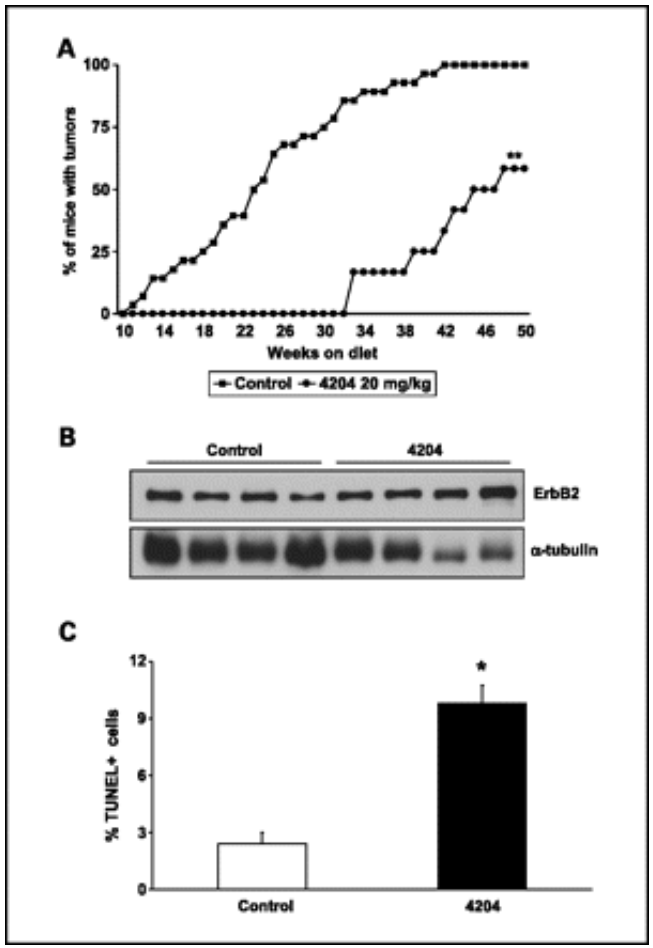
Data from P Brown, MD, PhD; A Mazumdar, and Cassandra Moyer, PhD; MD Anderson Cancer Center

IRX4204 Monotherapy Prevents Development of Her2+ Breast Cancer in MMTV-ErbB2 Mouse Model; and Induces Apoptosis of Mammary Cells

Prevents ER-neg / HER2-pos BC in MMTV-ErbB2 mice (Dose: 20 mg/ kg in feed)

NRX194204 treatment does not affect the ErbB2 transgene expression

NRX194204 treatment induces apoptosis of mammary cells



Liby et al. Clin Cancer Res, 2007
Dartmouth School of Medicine

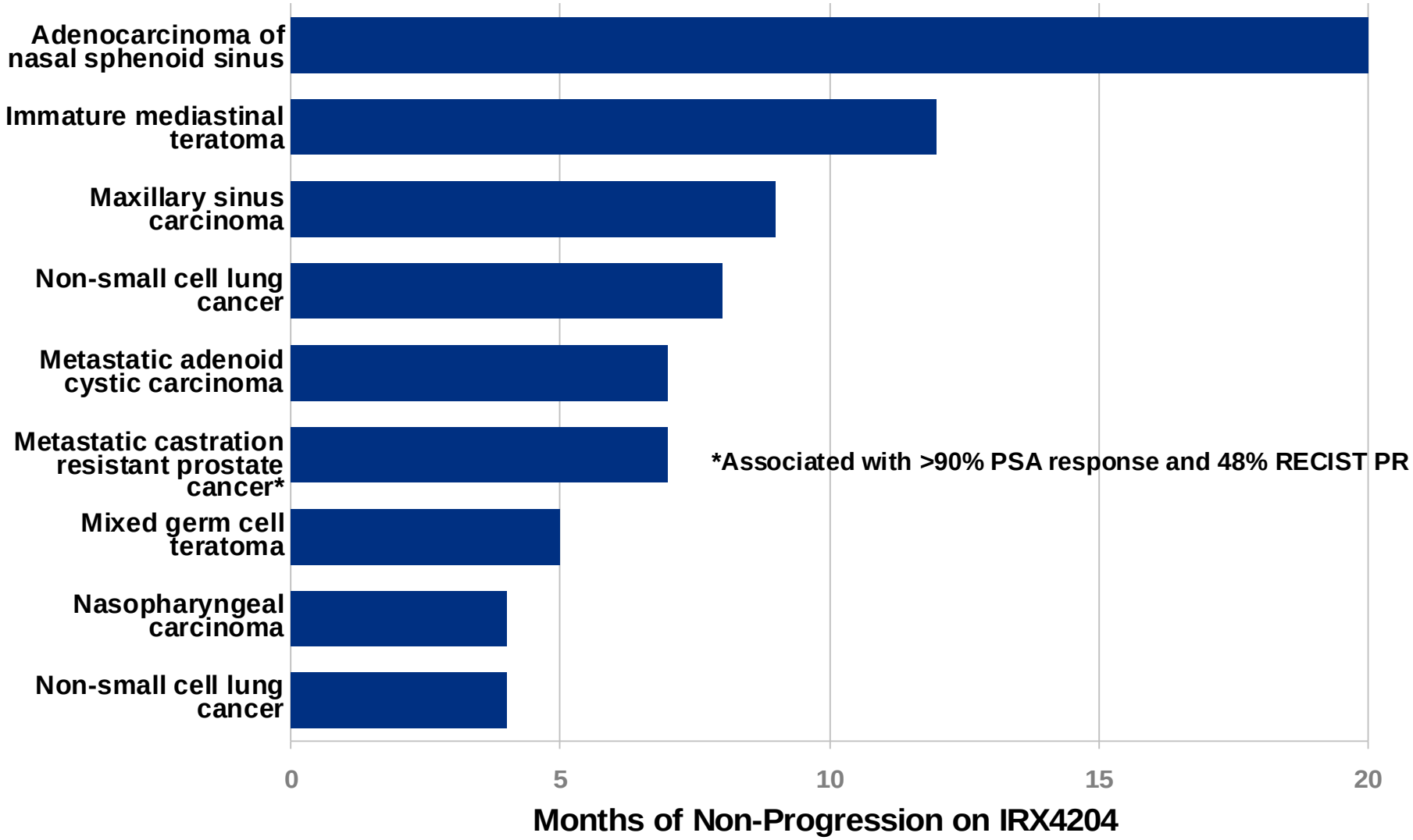
Patients Treated with IRX4204 To Date

Study/Site	# of Patients
Phase I study, various solid tumors	18
Phase II NSCLC, USC	6
Phase II NSCLC, Dartmouth	5
Phase II Mesothelioma, USC	6
Ph II Castration Resistant Prostate, UCLA/consortium	37
Ph II NSCLC + erlotinib, Dartmouth	10
Compassionate use, adenoid cystic carcinoma	3
Phase I Parkinson's Disease	15
Total	**100**

Phase I/II Study of IRX4204 in Refractory Malignancies

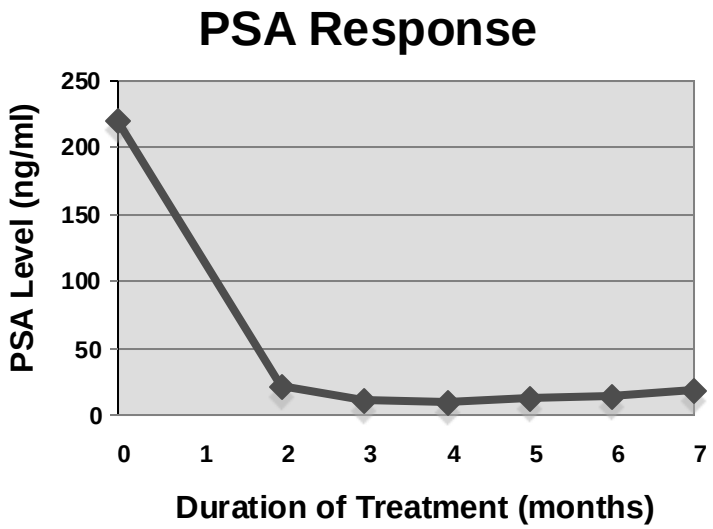
- Multi-center, open-label study of once-daily oral IRX4204 in patients with refractory malignancies
  - Dose groups of 3, 6, 12 and 24 mg/m^2
  - Treatment for a minimum of 4 weeks, with longer treatment possible for patients with disease stabilization
- Enrolled 18 patients with progressive solid tumors
- No serious adverse events considered possibly or probably related to study drug
- Adverse events included triglyceride elevation and hypothyroidism, both types of events treatable

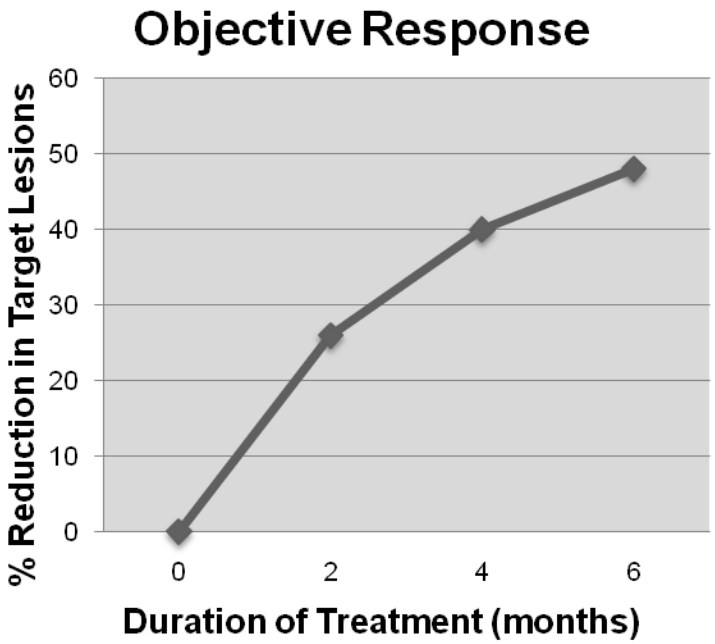
50% of Patients in Phase I Study had Non-Progression for ≥4 Months



PSA and Objective Response in Phase I Prostate Cancer Patient

- 73 year old patient with advanced, metastatic disease, post-castration, taxane-resistant, failed multiple prior therapies.
- Received 7 months of treatment with IRX4204
- Patient had a rapid, dramatic, and durable PSA response.





- Concurrent with biochemical response, patient experienced a ~50% objective response (RECIST)

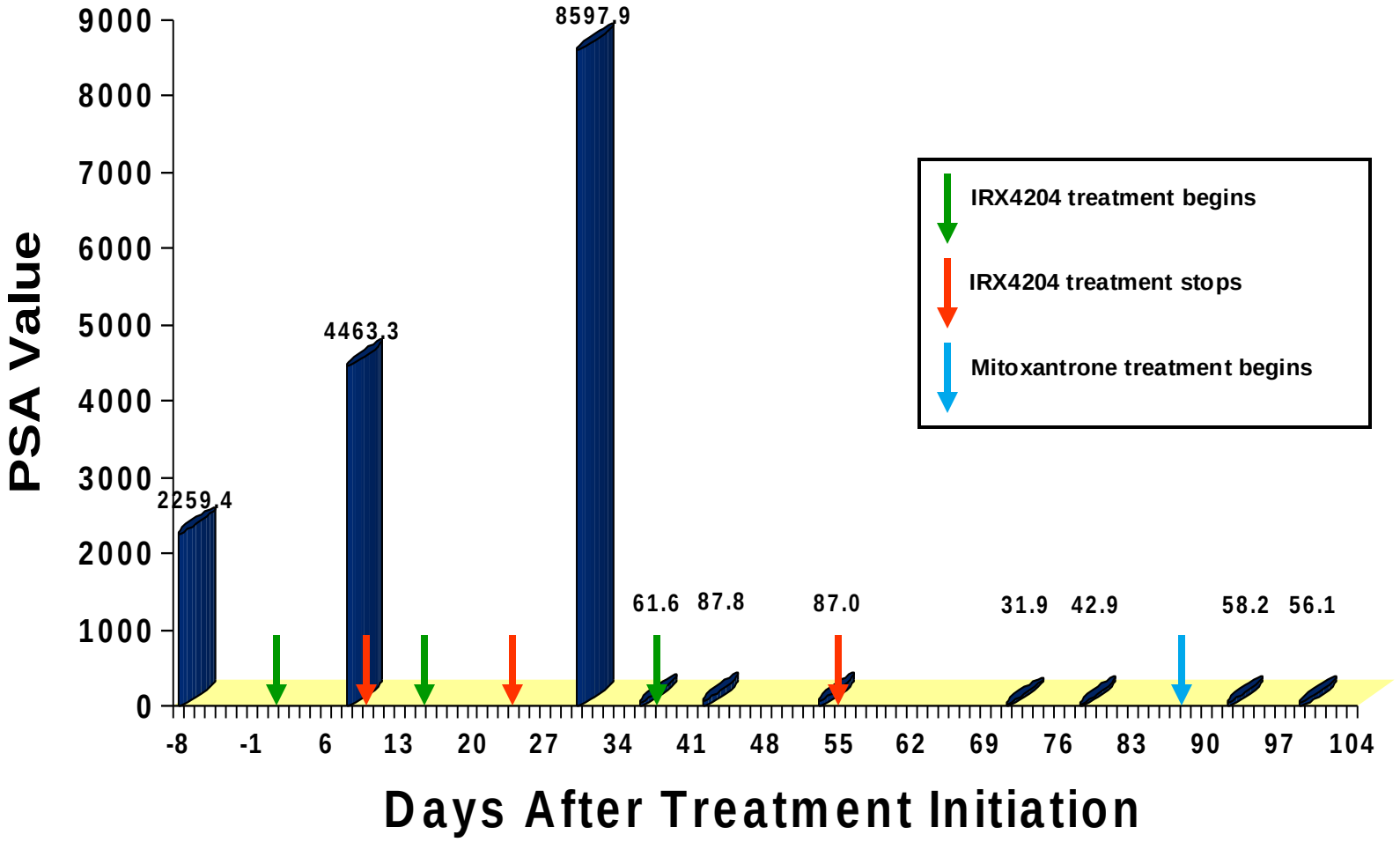
Phase IIA Castration Resistant Prostate Cancer Clinical Trial

- 37 patients with progressive castration resistant prostate cancer were enrolled in this open label uncontrolled clinical trial and treated with IRX4204 at 20 mg/day. Continued hormonal therapy was allowed.
- Primary objective was assessment of safety. Efficacy outcomes were collected.
- IRX4204 was generally safe and well tolerated for up to 540 days. Drug related adverse events included increased lipids and hypothyroidism.
- "Clinical Benefit" was prospectively defined as greater than two cycles (56 days) without observed progression. The study was prospectively defined to be positive if 6 of 37 patients were treated for at least 56 days without progression.
- 19 of the 37 patients were treated for more than 56 days without progression.
- One patient with baseline PSA of 2259 experienced rapid tumor lysis (peak PSA of 8598) associated with serious coagulopathy, followed by sustained PSA values of 88 or less.
- One additional patient experienced a low magnitude 50% decrease in PSA while being treated for 540 days without progression. No other patients experienced a 50% drop in PSA.

IRX4204: Clinical Development Status

- 100 patients have been treated with IRX4204, including 85 with various cancers, and 15 with early Parkinson's disease.
- IRX4204 was safe and well-tolerated for up to 20 months of continuous oral treatment over a range of doses.
- PK in humans is consistent with once per day oral dosing.
- Common side effects include dose related reversible suppression of TSH with resultant treatable hypothyroidism; mild to moderate reversible increased triglycerides; and mild to moderate reversible leukopenia.
- Open label uncontrolled phase I and II clinical trials in cancers and Parkinson's disease support clinical safety, efficacy, and effective doses.
- Synthesis of GMP clinical trial drug supply API is completed. Manufacture of drug and placebo capsules for Phase II trials completed.
- Planning phase II clinical trials in Her2+ breast cancer and NSCLC.

PSA Response in Phase IIA Prostate Cancer Patient



IRX4204: Product Development Status

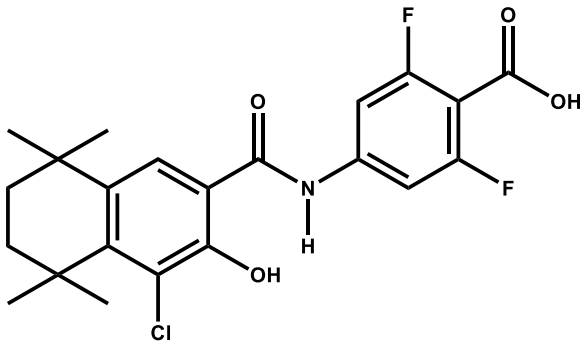
- INDs for IRX4204 for treatment of cancers and Parkinson's disease;
- GLP 6-month rat, 9-month dog toxicology completed; dose related ocular cataracts noted in rats and dogs; milder findings than in bexarotene toxicology (which also showed ocular cataracts)
- PK in rats, dogs and humans consistent with once daily oral dosing
- Brain penetrance demonstrated in rodents and humans
- Panlabs binding assays on panel of diverse CNS receptors negative
- hERG binding assay negative
- GLP gene toxicology panel negative
- GLP *in vivo* safety pharmacology panel (CNS, CV, Resp) negative
- GMP manufacturing completed on multi-kg scale; producing API with chemical and chiral purity 99+% without requiring a terminal chromatographic purification step
- Multi-year compound stability demonstrated
- Formulated in oral capsules for Phase II clinical trials
- Can be formulated for intravenous, inhaled nose to brain, inhaled lung, dermal, and ocular administration

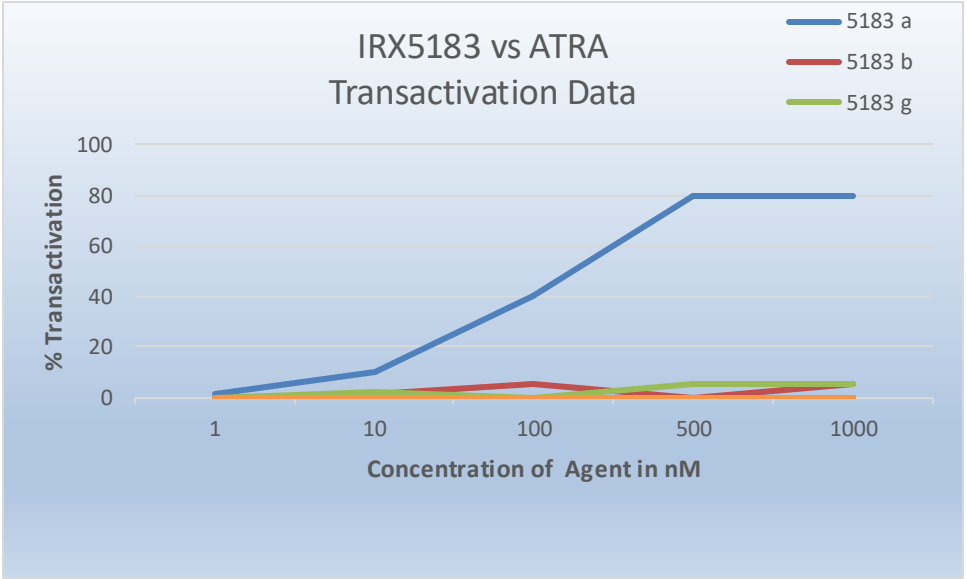
IRX5183

A Phase II Clinical Stage

RARα Selective Agonist for the Treatment of Multiple Myeloma and AML

IRX5183 is a Highly Selective RARα Agonist with no RAR β or γ Activity at µM Concentrations



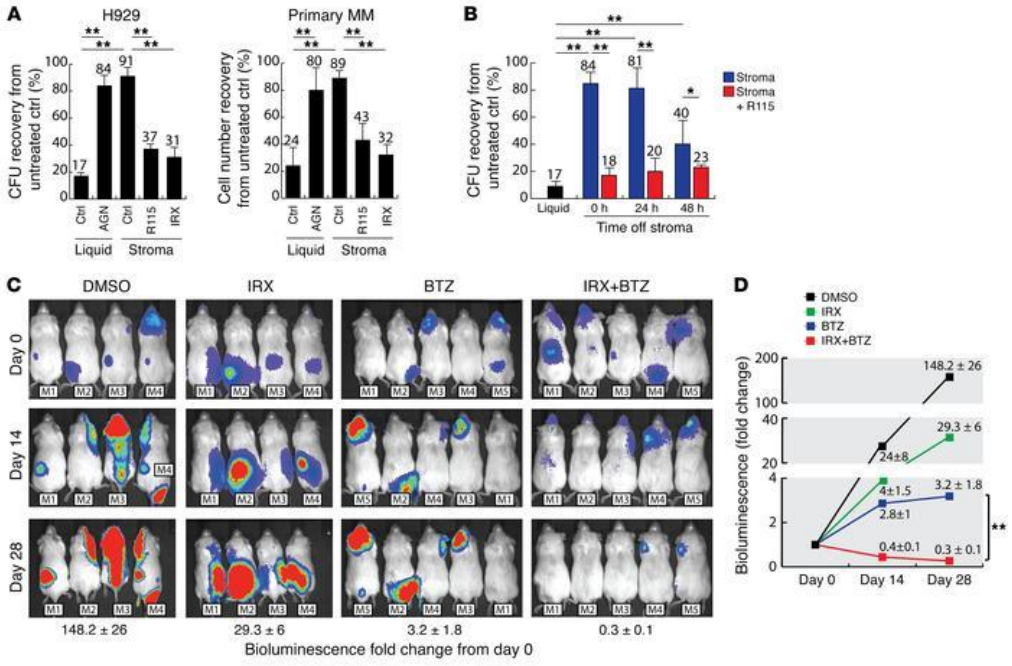


Patients Treated to Date with IRX5183

- Phase I Trial in Patients with solid tumors — 15 Patients
- Phase I Trial in Patients with Lymphoma or Multiple Myeloma with G-CSF for Peripheral Blood Progenitor Cell Mobilization — 8 Patients
- Phase I Monotherapy Trial in Patients with AML — 11 Patients
- Compassionate Treatment of APL — 2 Patients
- **Total = 36 Patients**

- IRX5183 was generally safe and well tolerated for up to 112 days of treatment.
- Drug related adverse events included elevations of triglycerides and possibly low-grade elevation of liver enzymes.
- IRX5183 induces AML leukemic blast maturation *in vivo* in humans.
- **One AML patient treated with IRX5183 monotherapy achieved a morphological complete remission with incomplete hematologic recovery (CRi).**
- Four of eleven AML patients (36%) had stable disease or better while receiving IRX5183.

IRX5183 Increases Bortezomib (BTZ) Sensitivity of the Multiple Myeloma (MM) cell line H929 and Primary CD138 MM Cells in Various Retinoid-low and -high Conditions; and is Effective in a Xenograft Model of Human MM

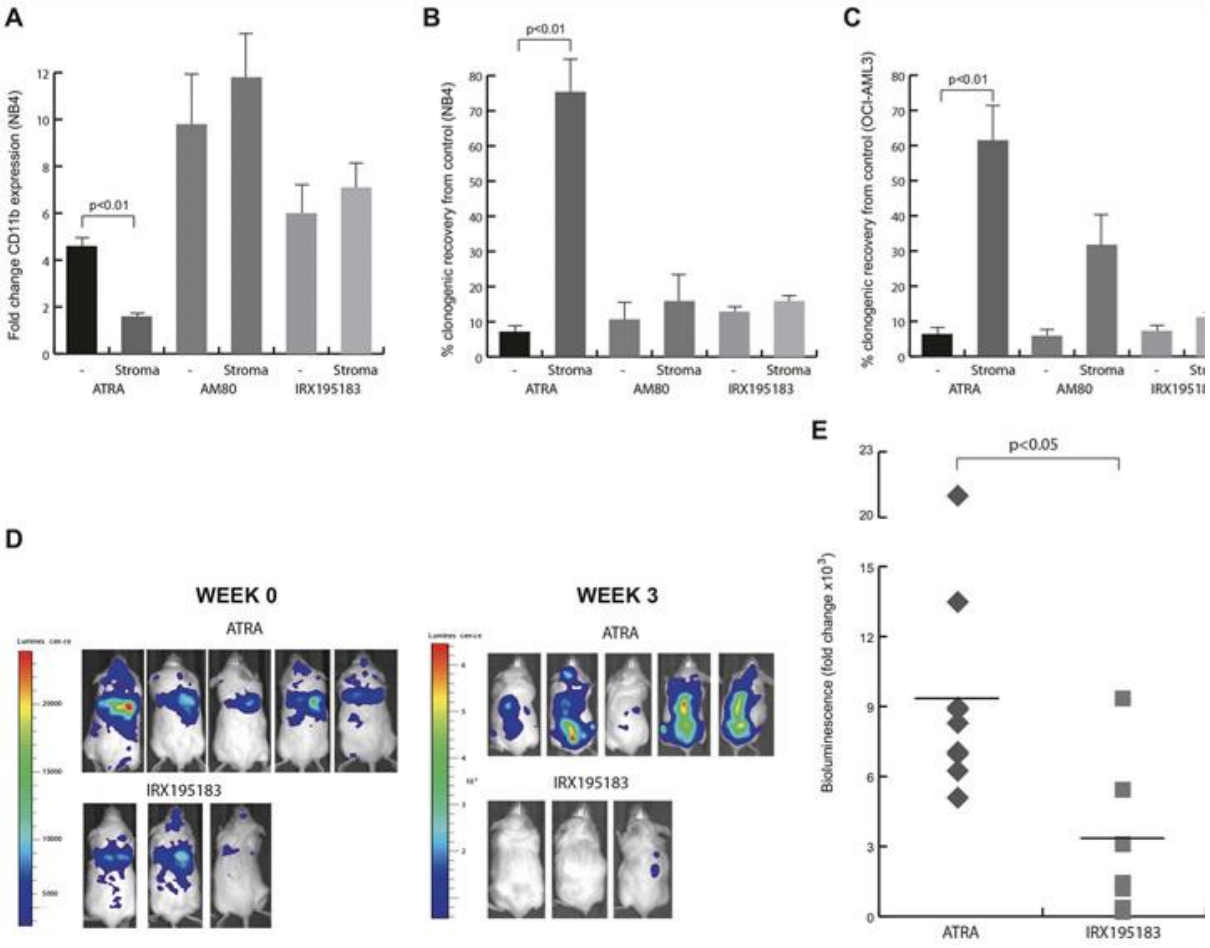


(A) Clonogenic recovery (CFU) of H929 cells or cellular recovery of primary CD138$^+$ MM cells from 3 different patient samples. (B) Clonogenic recovery of H929 cells treated with BTZ (2.5 nM). (C) Bioluminescent images of systemic MM xenografts. Following engraftment of H929 Luc$^+$ cells, mice were treated with IRX (n = 4), BTZ (n = 5), or a combination of both (n = 5) for 4 weeks. (D) Fold change in bioluminescence from day 0.

J Clin Invest 2016; DOI: 10.1172/JCI88152

Data from G Ghiaur Laboratory
Johns Hopkins School of Medicine

Effects of CYP26 Resistant RARα Selective Agonist Compound IRX5183 on Differentiation of AML Cells in Stroma Co-culture and in Xenograft AML Model



A-C: Fold change CD11b expression (A), and clonogenic recovery (B) of NB4 cells as well as clonogenic recovery of OCI-AML3 cells (C), treated with various retinoids in the presence or absence of mesenchymal stroma. **D:** Tumor burden by bioluminescence activity of xenograft mice transplanted with OCI-AML3-luciferase and treated with ATRA or IRX5183 (10 mg/Kg) for 3 weeks. **E:** Quantitative analysis of data presented in (**D**). Horizontal lines represent mean of bioluminescence fold change from D0 or treatment. Squares and diamonds represent individual mice.

Leukemia, 2020; DOI: 10.1038/s41375-020-0790-4

Data from G Ghiaur Laboratory
Johns Hopkins School of Medicine

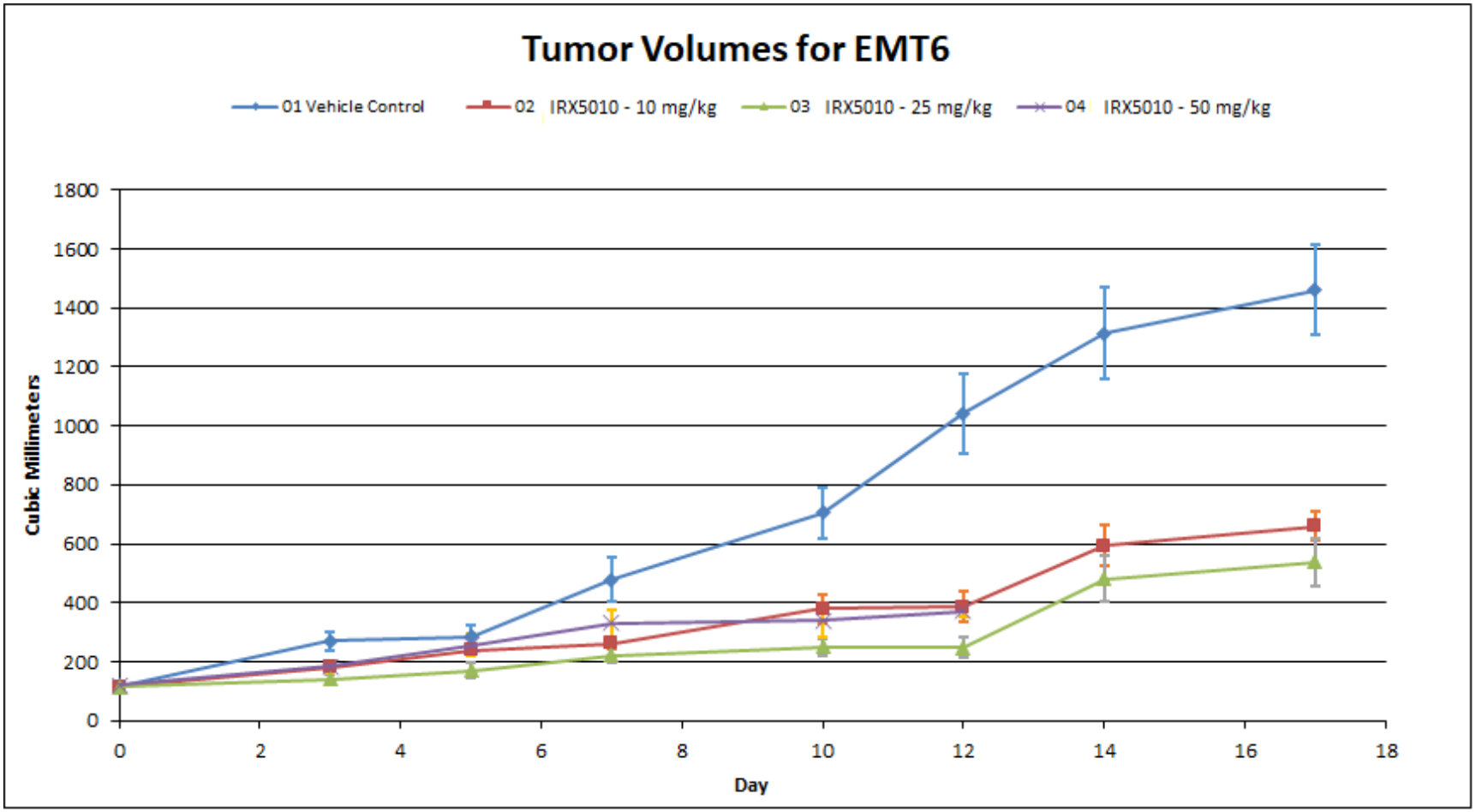
Selective RARγ Agonists
Drug Discovery and Preclinical Development

- Io Therapeutics has discovered RARγ agonist compounds which are effective as monotherapies in syngeneic mouse models of triple negative breast cancer, non-small cell lung cancers, colon cancer, and prostate cancer.

- These RARγ agonists promote tumor infiltrating T-lymphocytes which inhibit tumor growth in the in vivo models. They also inhibit tumor infiltration of myeloid derived suppressor cells. They have combination effects with an anti-PDL-1 checkpoint inhibitor monoclonal antibody. The RARγ agonists compounds have minimal direct in vitro activity on growth of cancer cells. Their efficacy against cancers appears to be mediated solely by promoting anti-cancer immune responses,

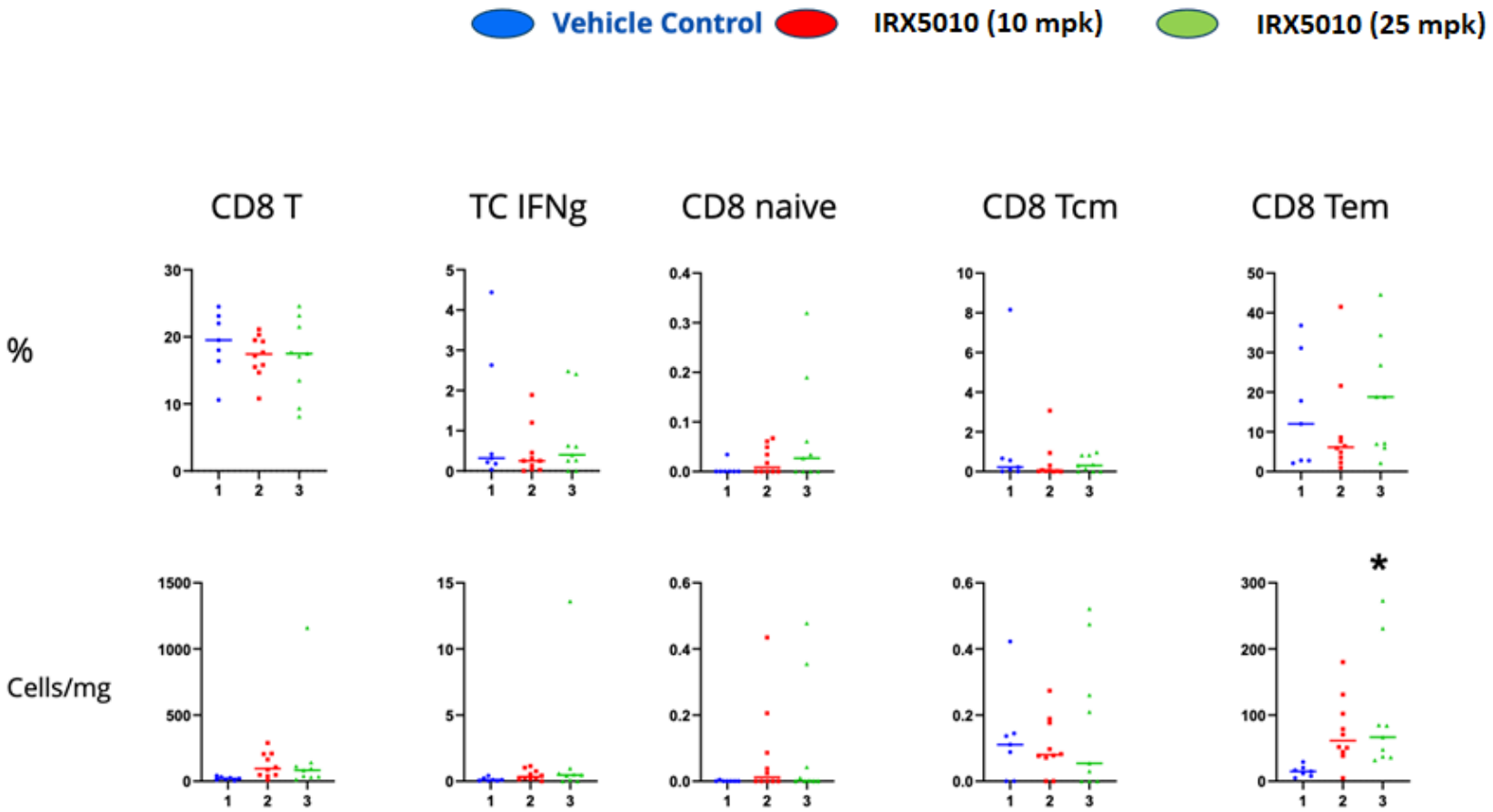
- The lead compound, IRX5010 is ready for IND enabling studies.

IRX5010: a Highly Selective RARγ Agonist Compound Inhibits In Vivo Tumor Growth of EMT-6 Triple Negative Breast Cancer in Syngeneic Mouse Model



Data from Champions Oncology, Inc.

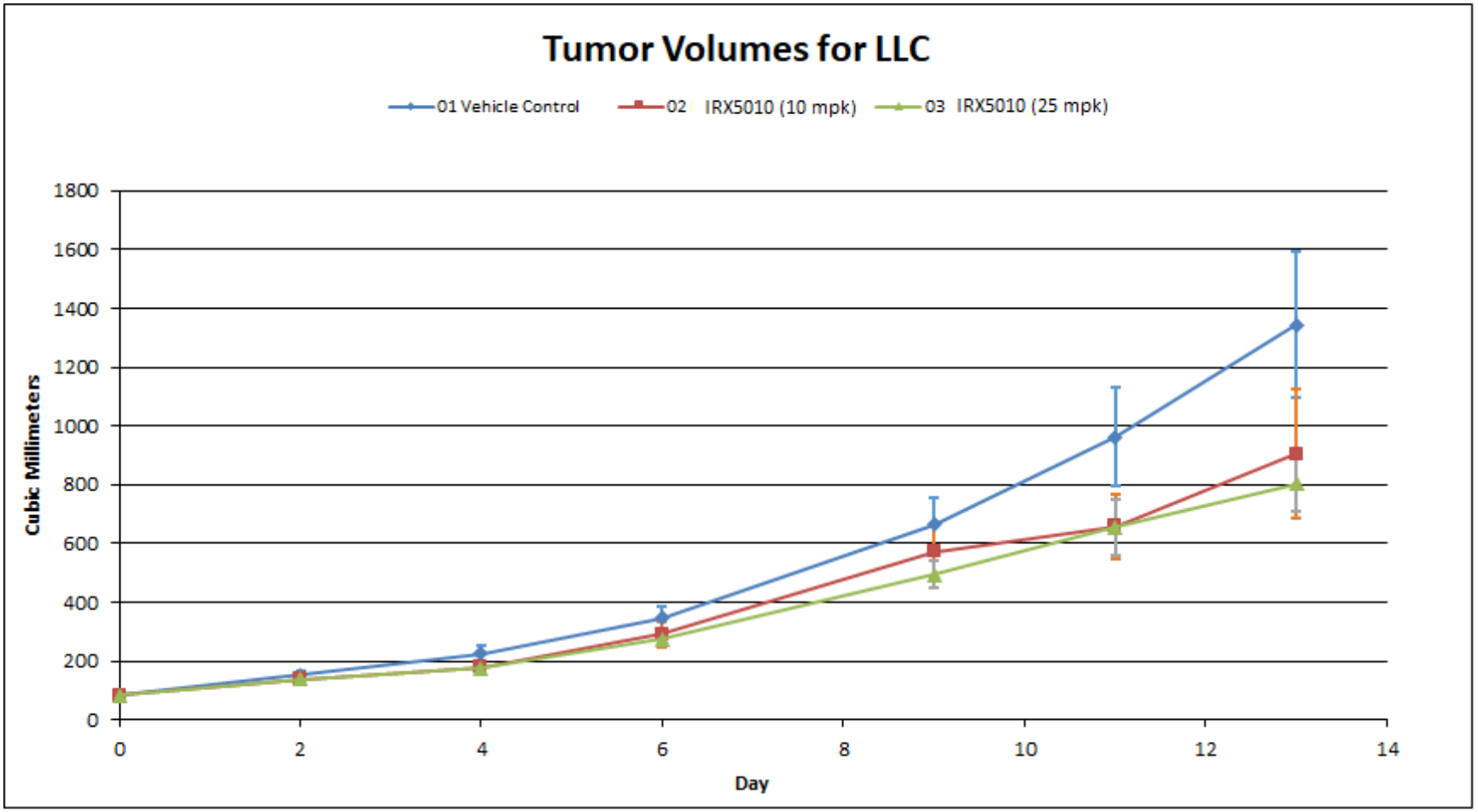
IRX5010: a Highly Selective RARγ Agonist Compound Induces Effector Memory Tumor Infiltrating Lymphocytes in EMT-6 Triple Negative Breast Cancer in Syngeneic Model



CD8 Tem = CD8 T effector memory (CD8+ CD44+ CD62L-)

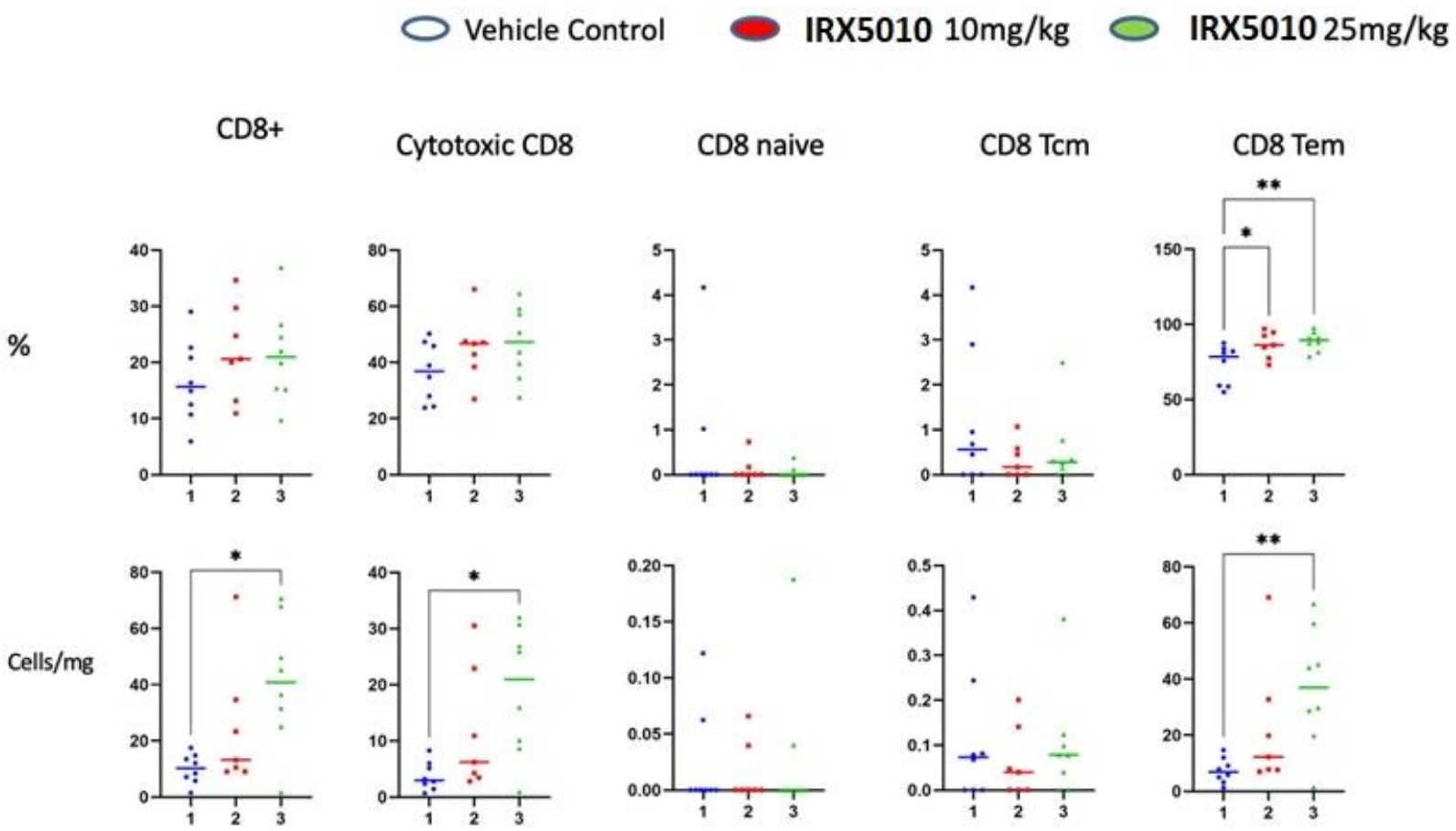
Data from Champions Oncology, Inc.

IRX5010: a Highly Selective RARγ Agonist Compound Inhibits In Vivo Tumor Growth of Lewis Lung Cancer in Syngeneic Mouse Model



Data from Champions Oncology, Inc.

IRX5010, a Highly Selective RARγ Agonist Compound Induces Effector Memory Tumor Infiltrating Lymphocytes in Syngeneic Lewis Lung Cancer Model

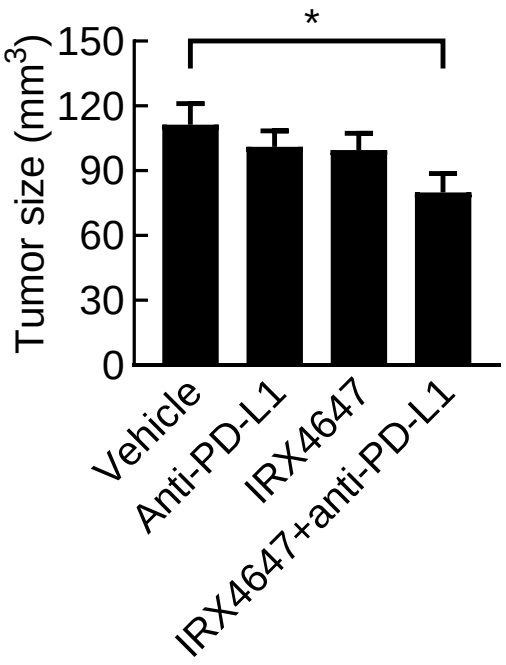


Cytotoxic CD8 = CD8+ IFNγ+
CD8 Tem = (CD8 T effector memory) CD8+ CD44+ CD62L-

Data from Champions Oncology, Inc.

Combined RARγ Agonist and Anti-PD-L1 Treatment Reduced Non-small Cell Lung Cancer Growth

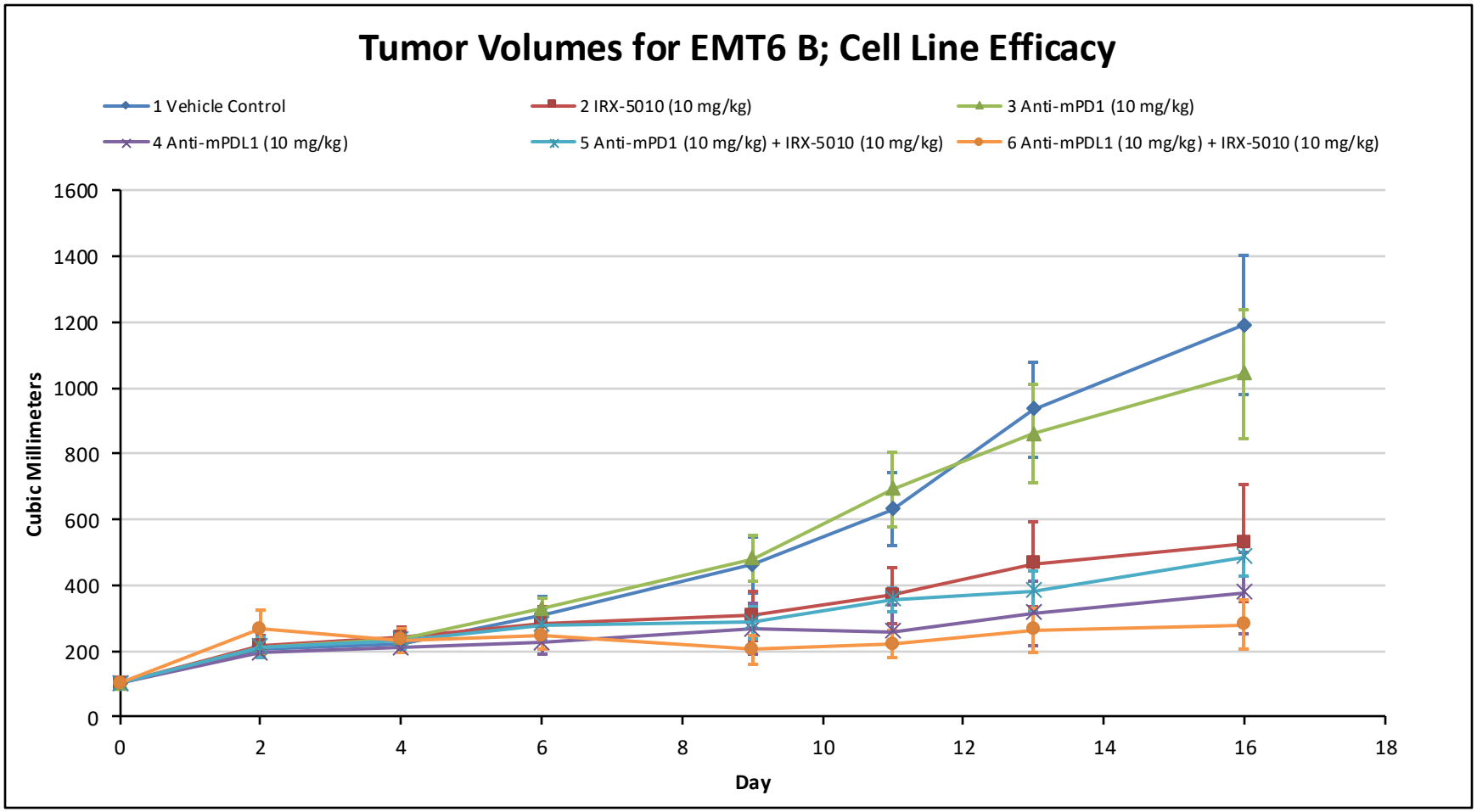
344SQ cell injection
(129/sv male mice)



Treatment day 14

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.
Frederick National Laboratory for Cancer Research

IRX5010 Effects on Triple Negative Breast Cancer in Combination with anti-PDL-1



Effects of IRX5010 *In Vivo* on EMT-6 Triple Negative Breast Cancer Growth, TILs, and MDSC Infiltration in Combination with Checkpoint Inhibitors

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%

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

Collaborators and Acknowledgements

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MD Anderson Cancer Research Center
RXR agonist IRX4204 in preclinical Her2+ breast cancer studies

Ethan Dmitrovsky, MD; Gina Wei, Xi Lui
Frederick National Laboratory for Cancer Research
RARγ agonist in preclinical in vitro and in vivo studies of non-small cell lung cancers and combination studies with checkpoint inhibitor

Gabriel Ghiaur, MD; Christian Gocke, MD, PhD; Richard Jones, MD
Johns Hopkins School of Medicine
RARα agonist IRX5183 in clinical and preclinical studies of multiple myeloma and AML

Mary Topalovski, PhD; and colleagues at Champions Oncology, Inc.,
preclinical studies of RARγ agonists in multiple types of cancers