IRX4204 is a Phase II Clinical Stage Candidate Treatment for Disease Modification of Parkinson's Disease (PD)

Io Therapeutics, Inc.

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Rationale for IRX4204 Treatment of PD

- RXR agonist IRX4204 and an earlier generation 100-x less potent RXR agonist bexarotene, reduce motor deficits and increase dopaminergic neuron survival and functions *In vitro and in vivo* in rat PD models.

- Inhibiting misfolded α-synuclein deposition may be a disease course modifying activity of IRX4204 for PD. IRX4204 inhibits α-synuclein oligomer formation; and pathologic fibril formation in dopaminergic neurons. Multiple RXR agonists including bexarotene have been reported to promote lysosomal autophagy of α-synuclein by RXR-dependent mechanisms in human dopaminergic neurons (Tipathi, A,. *et. al.,* Cell Mol Life Sci, 2024).

- PD has multiple neuroinflammatory pathologies. IRX4204 has multiple anti-inflammatory, and immune suppressive mechanisms of action. IRX4204 promotes differentiation of human Tregs, inhibits human Th17 production of IL-17s and TNFs; inhibits rat cortical microglia production of IL-6, MIP-1α, MIP-2; and inhibits mouse microglia and astrocyte production of IL-1β, TNF-α, and iNOS. (Kasheke, G., *et. al*, Acta Neuropathol Comm; 2024).

- White matter disease and demyelination are known components of PD pathology. IRX4204 promotes differentiation of human oligodendrocyte precursor cells and promotes anatomic and functional remyelination in autoimmune, cuprizone toxicity, and hypoxic mouse models of demyelination.

- IRX4204 demonstrated brain penetrance, and rapid improvement of UPDRS motor functions in 13 of 15 early PD patients in an open-label phase I/II clinical trial in early-stage PD.

- Oral dosing of IRX4204 was safe and well tolerated in 100 cancer and PD patients for up to 20 months.

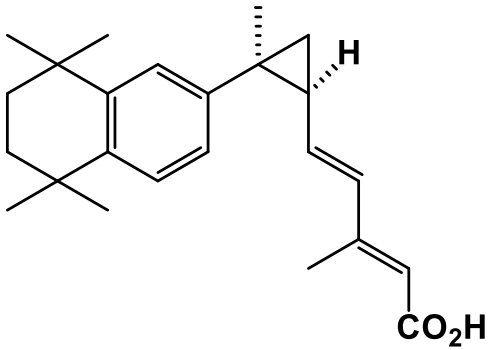
Planned Phase II Double-blind, Placebo-controlled Clinical Trial of IRX4204 for Treatment of Parkinson's Disease

The company is preparing to file an IND with the FDA for conduct of a phase II double-blind placebo-controlled clinical trial of IRX4204 in 20 patients with Parkinson's disease at a premier academic medical center that has substantial expertise in PD clinical trials.

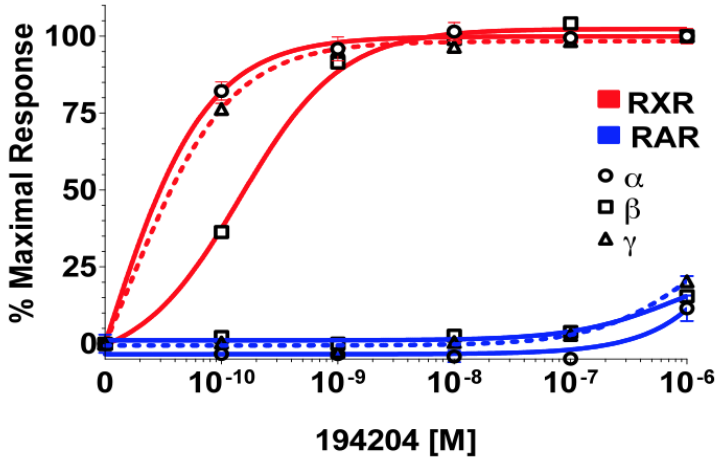
The trial will be of a crossover design, with each patient receiving 28 days of IRX4204 and 28 days of placebo in a blinded sequence. There will be a 14-day washout period between the two treatment periods. Following the double-blind crossover treatment periods, the company will seek FDA allowance for all patients to be allowed rollover into an open-label extension trial of treatment with IRX4204 for at least 6 months, possibly longer, to obtain further evaluation of safety of IRX4204 in chronic dosing of PD patients, and its longer-term effects on biomarkers and clinical outcome measures.

The patients will have standard safety assessments, and will be evaluated for efficacy using a novel PD health inventory instrument, the standard UPDRS Parkinson's rating scale assessments, and a wearable digital device to monitor motion, tremor, balance, sleep, and walking speed. An extensive panel of biomarkers will be collected from blood and CSF. Biomarkers will include brain derived exosomes for quantitation of RXR activation, cytokine neurofilament light chain, GFAP; α-synuclein and tau proteins and fragments and mass spectroscopic proteomics analyses of plasma and CSF.

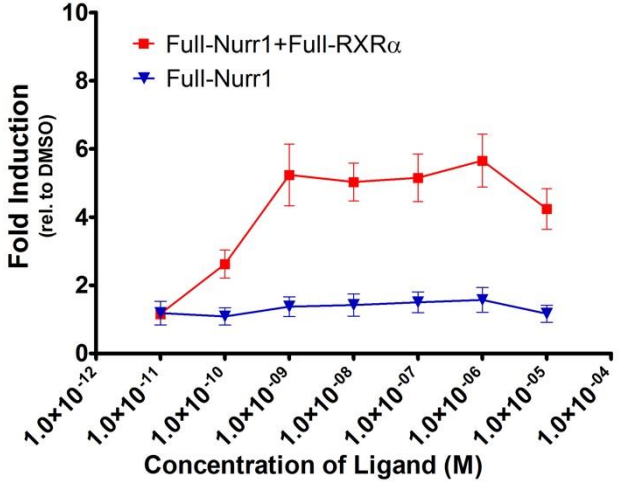
IRX4204, A Potent RXR Agonist Compound Activates RXR and Nurr1, But Not RAR Nuclear Receptors at Low Nanomolar Concentrations



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



IRX4204 Transactivates Nurr1/RXR Heterodimers through RXR



IRX4204 is >100-Fold More Potent, More RXR-Selective, and Less Toxic than Bexarotene, an FDA-approved Earlier Generation RXR Agonist which has been Effective in Clinical Trials in CTCL, Psoriasis, and Multiple Sclerosis

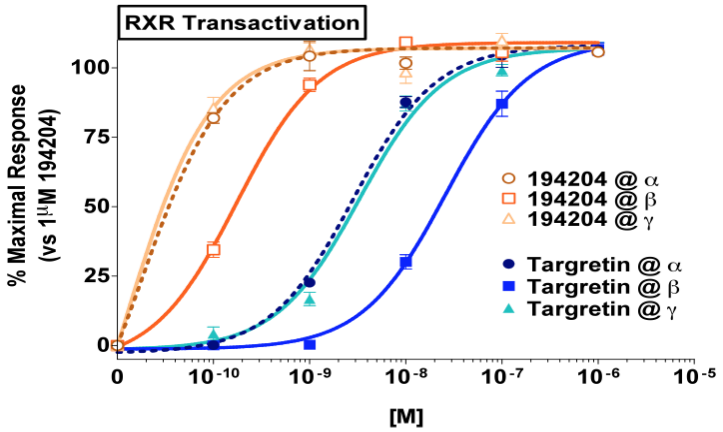
Bexarotene

- Bexarotene is a relatively less selective, less potent RXR agonist than IRX4204. It is approved by FDA as an oral and topical drug for treatment of T-Cell Lymphoma .
- Bexarotene transactivates RXRs, RARs, PPARγ, and LXRs.
- Bexarotene is more toxic in humans than IRX4204 (rashes related to RAR activation, and greater lipid elevations).
- Like IRX4204, bexarotene promotes Treg differentiation and inhibits Th17 differentiation, and is neuroprotective and myelin reparative.
- Bexarotene demonstrated efficacy for treatment of psoriasis patients in clinical trials as either an oral or topical drug and produced durable remyelination and reversed CNS aging on MRI given orally in a placebo-controlled multiple sclerosis phase II clinical trial.

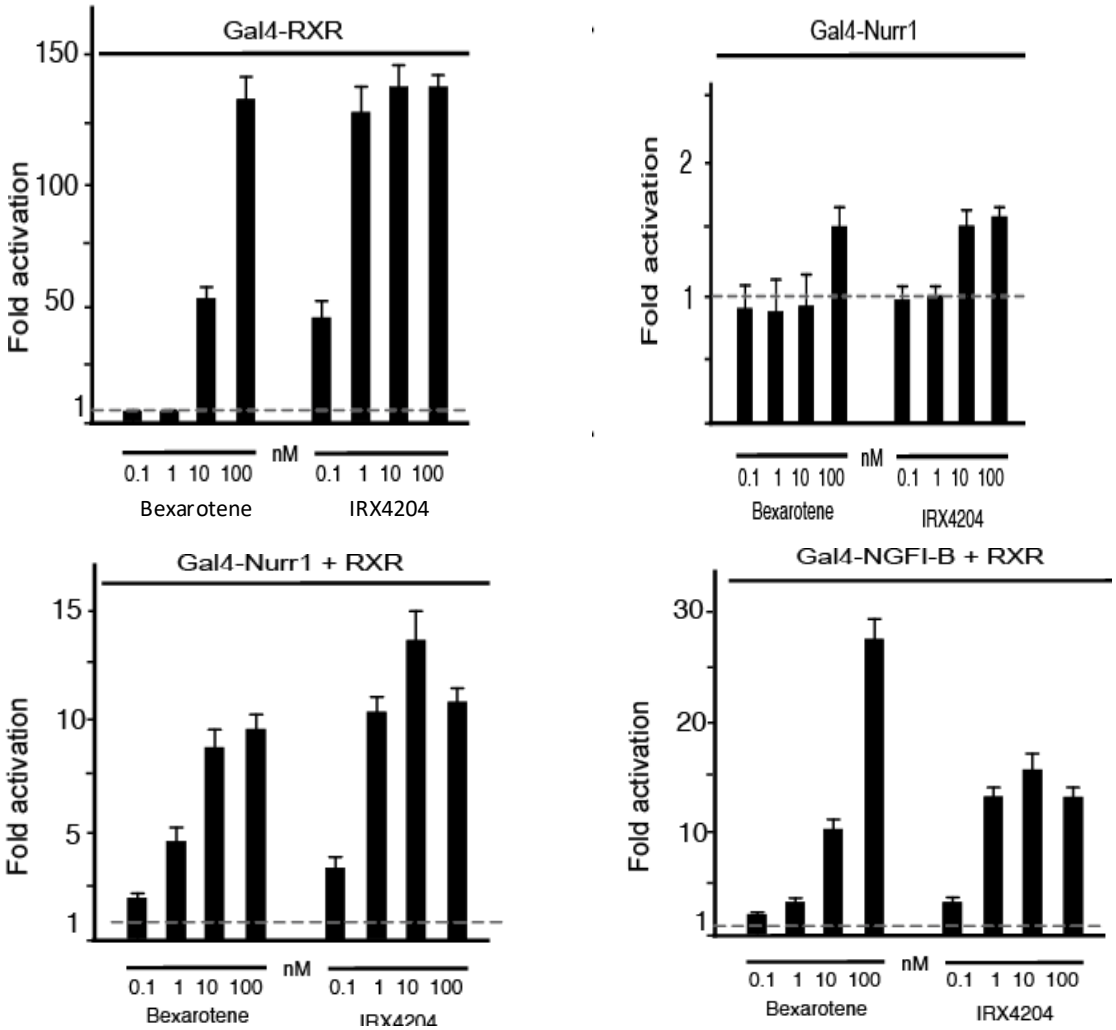
IRX4204

- IRX4204 is a highly selective RXR agonist, 100x more potent than bexarotene.
- IRX4204 is highly selective for RXRs; not directly active on RARs, PPARγ, LXRs, and FXR.
- Like bexarotene, IRX4204 promotes Treg differentiation and inhibits Th17 differentiation, is neuroprotective and myelin reparative.
- IRX4204 was well tolerated as an oral drug in 100 humans in Phase I/II clinical trials; no RAR cutaneous rash and less lipid elevations than bexarotene. It can be formulated for oral, IV, inhaled, or topical use.
- Substantial preclinical data support that IRX4204 may be effective in humans with psoriasis, MS, other forms of T-cell autoimmunity, PD, AD, other diverse neurodegenerative diseases, and cancers.

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

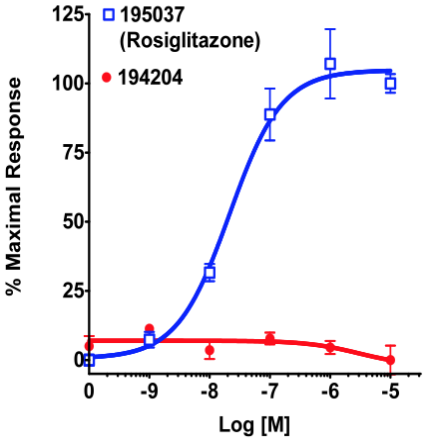


Reporter Assays with RXR Ligands Demonstrate IRX4204 is More Potent Than Bexarotene for Activation of RXR, Nurr1 (NR4A2), and Nur77 (NR4A1, NGFI-B)

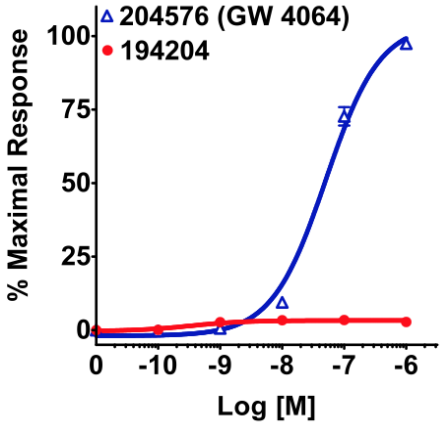


Data provided by Thomas Perlmann
Karolinska Institute, Stockholm, Sweden

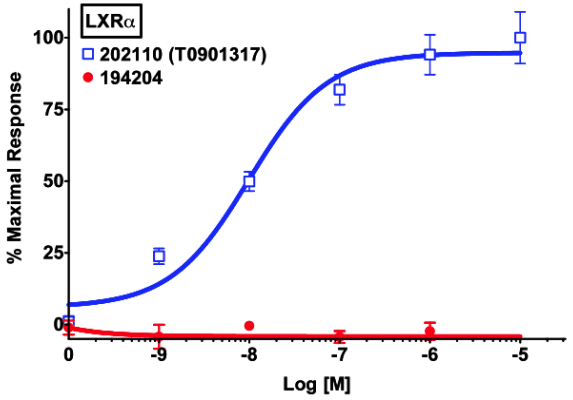
IRX4204 is Highly Selective for RXRs
It Does Not Transactivate PPARγ, FXR, or LXRs



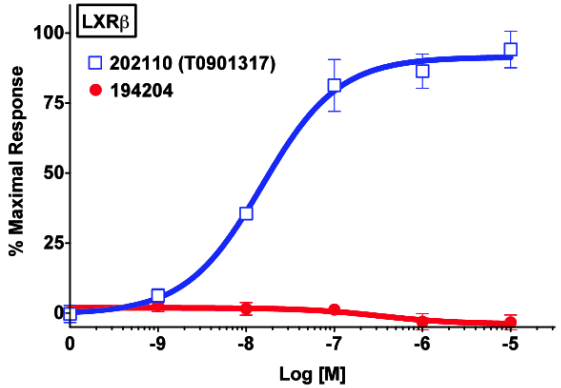
PPARγ/RXR



FXR/RXR



LXR/RXR



LXR/RXR

Wang et al,
Oncotarget 2016

Phase IIA Clinical Trial in Early Parkinson's Disease

Mean Changes from Baseline to Week 2 in Total Motor Score, Total UPDRS, and TSH Indicate CNS Effects of IRX4204 and Support Potential for Clinical Efficacy of IRX4204 in PD

Dose	Total Motor Change*^	Total UPDRS Change*^	Changes in TSH
20 mg/day	-14.5%	-6.6%	-98.8%
10 mg/day	-11.7%	-13.6%	-36.6%
5 mg/day	-31.4%	-18.7%	-28.9%

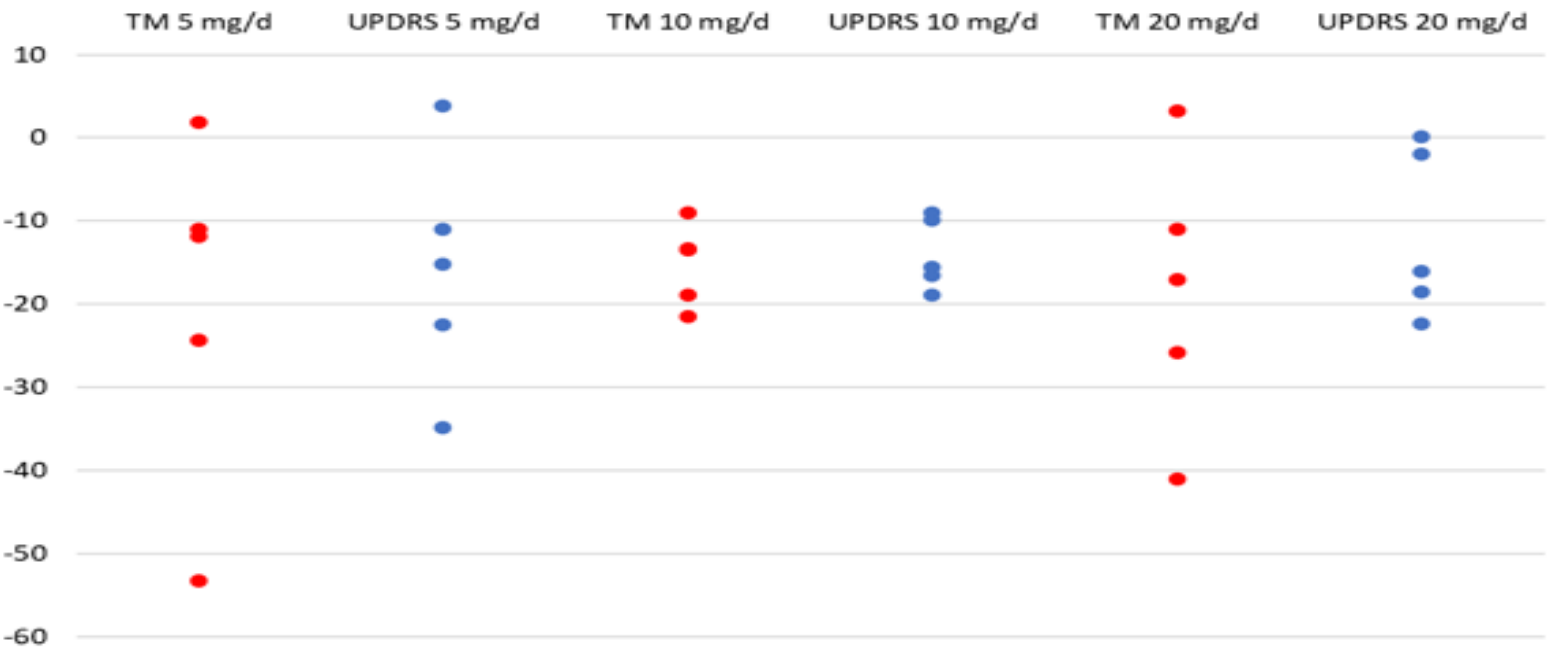
*Reduction in UPDRS and Total Motor scores represent clinical improvement

^Mean changes, n=5 per group

- **13 of 15 Patients demonstrated improvement in UPDRS Total Motor Score in open label assessments.**
- **TSH Reduction is a class effect of RXR agonists, caused by inhibition of hypothalamic TRF synthesis; and is a biomarker of brain penetrance of IRX4204. Hypothyroidism can be easily monitored and treated with thyroid hormone supplementation if necessary, however in the 5 mg/day group only 1 of 5 subjects became mildly hypothyroid.**

IRX4204 Improved Total Motor (TM) and UPDRS Scores in 13 of 15 Early PD Patients in a 28-Day Phase IIA Open Label Clinical Trial

Percent Change from Baseline



Mean % Change	-19.8	-16.0	-15.3	-14.1	-18.4	-11.9
Improved	4/5	4/5	5/5	5/5	4/5	4/5

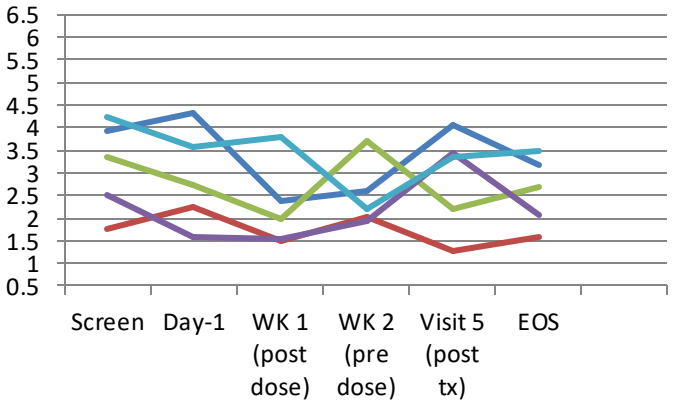
Decrease in Total Motor or Unified Parkinson's Disease Rating Scale scores indicates improvement.

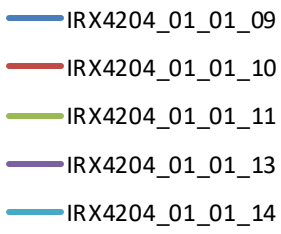
Patients in the 5 mg/day and 10 mg/day cohorts received 28 days of IRX4204; 20 mg/day patients received 17 days of IRX4204. 20 mg/day cohort was stopped early for transient decreases in absolute neutrophil counts in all 5 patients, to a nadir of 870/µl in one patient, all of which rapidly reversed with discontinuation of IRX4204 and were not associated with infections.

Clinical trial conducted by
Ken Marek and Dana Jennings
Molecular Neuroimaging, Inc.

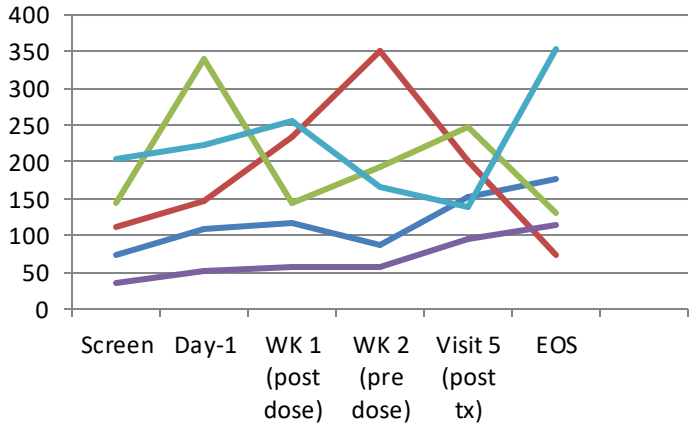
Phase IIA Clinical Trial of IRX4204 in Early PD Patients
Clinical Safety Laboratories for 5 mg/day Cohort

Absolute Neutrophils

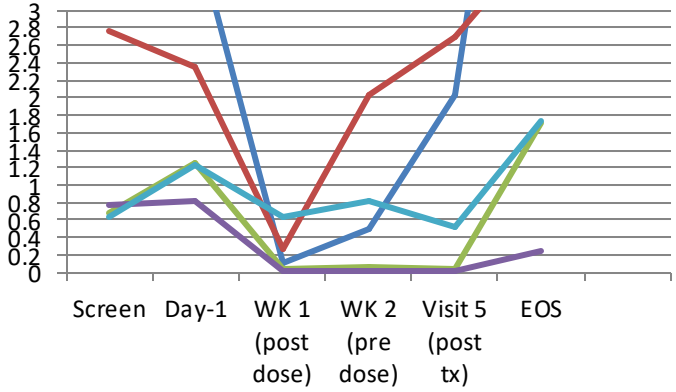




Triglycerides (non-fasting)

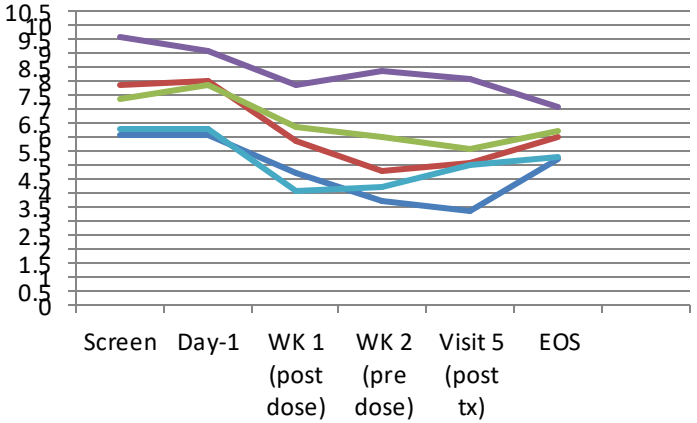


TSH



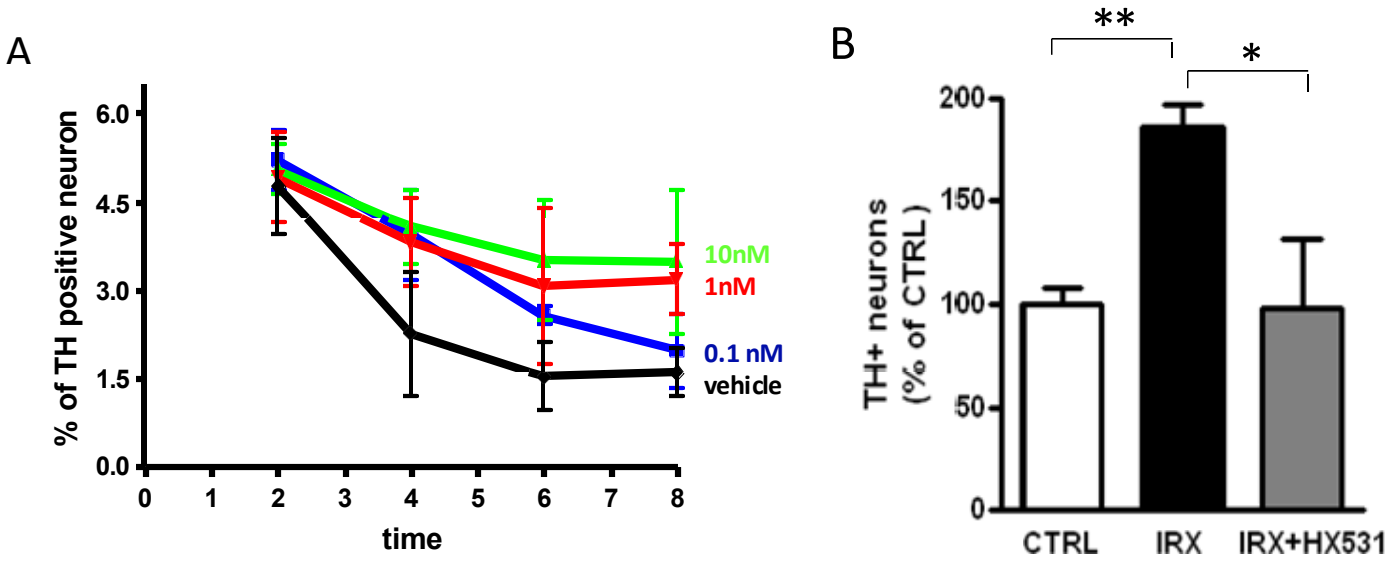
TSH Reduction is a class effect of RXR agonists, caused by inhibition of hypothalamic TRF synthesis, and is a biomarker of brain penetrance of IRX4204; T4 is reduced secondary to decreased TSH

T4



Clinical trial conducted by
Ken Marek and Dana Jennings
Molecular Neuroimaging, Inc.

IRX4204 Improves DA Neuron Survival in Enriched Mesencephalic DAergic Cultures through RXR



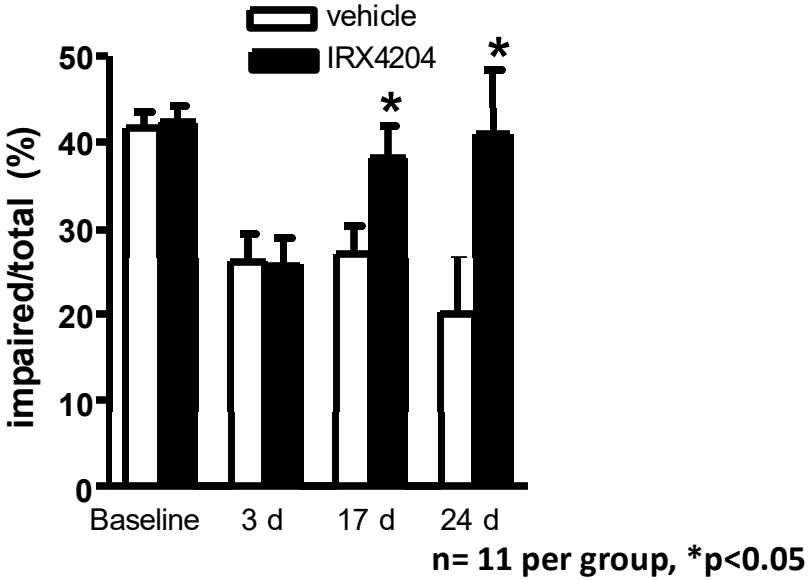
Mesencephalic cultures from mouse ventral midbrain were isolated at Explant 13.5-14 days and incubated for 8 days in the presence or absence of IRX4202; (A) time and dose effect of IRX4204 on DA neuron survival; (B) The number of TH positive neurons in the presence of 1 nM IRX4204 with or without 100 nM of RXR antagonist HX531.

Wang et al,
Oncotarget 2016

Oral Administration of IRX4204 Improves Motor Function in 6-OH Dopamine PD Rat Model

Cylinder Test (paw placement test, rearing test, limb use asymmetry test)

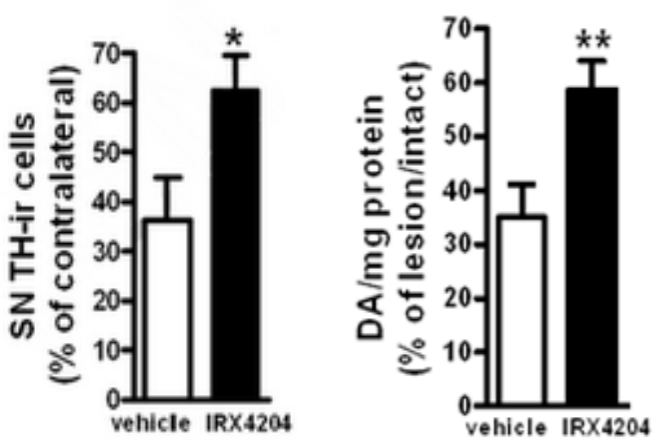
- Detects forelimb impairments, specifically, asymmetry in limb use for weight bearing movements when leaning on walls of glass cylinder during exploration of a novel environment in animals unilaterally lesioned in the striatum with 6-OH dopamine.
- Compares use of the affected side to the unaffected side, therefore each animal serves as its own control.



n= 11 per group, *p<0.05

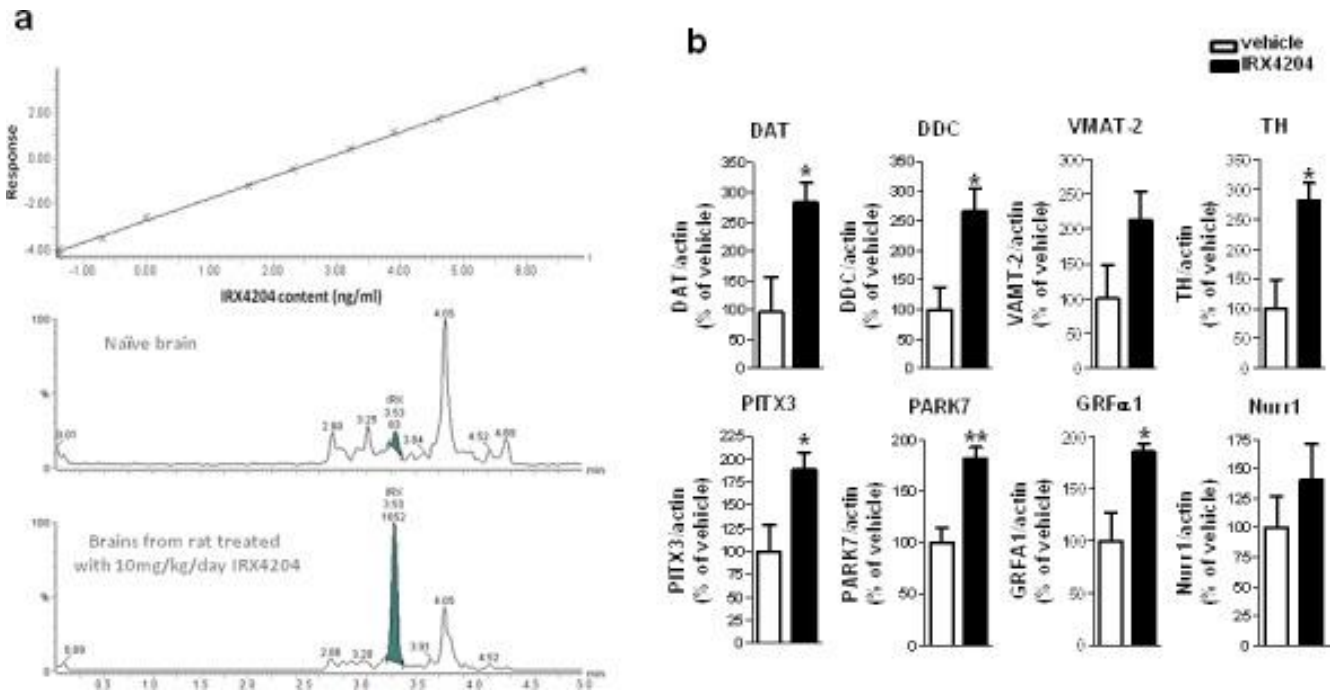
Wang et al,
Oncotarget 2016

IRX4204 Treatment Protects Against Loss of TH-Positive Midbrain Neurons and Reduces Loss of Dopamine in 6-OHDA Lesioned Rat PD Model



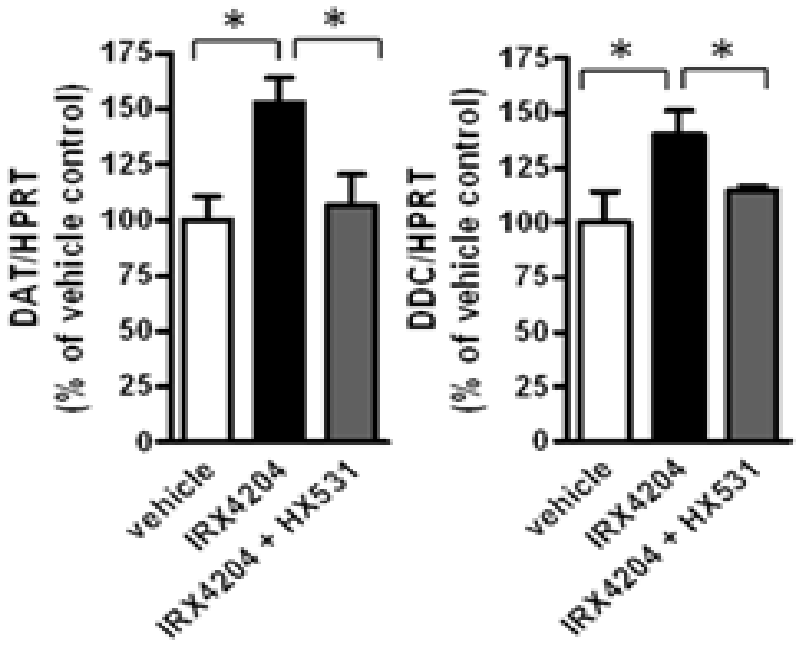
Rats were stereotaxically injected with 6-OHDA (40µg in 2µl) into right striatum. Rats were treated with IRX4204 (10mg/kg/d) or vehicle by oral gavage for 21 days starting 3 days post-lesion.

IRX4204 is Bioactive in Rat Brains Following Oral Administration and Activates Nurr1 Downstream Genes in the Substantia Nigra

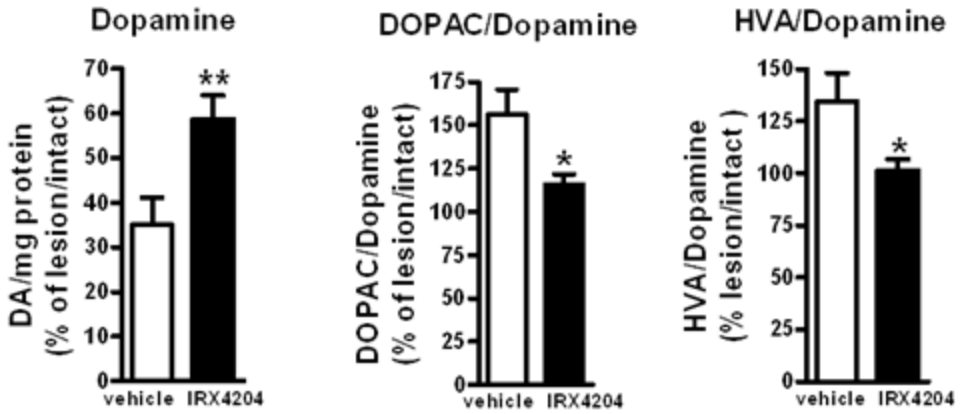


a. Detection of IRX4204 in the brains: Dose-response calibration curve of IRX4204 detection in the brain matrix with detection limit of 0.25nM (top panel); Representative IRX4204 LC/MS/MS chromatograms from brains of vehicle and IRX4204 treated rats (middle and bottom panel) **b.** Expression of Nurr1 downstream genes in the SN following 7 days oral treatment with 10 mg/kg/day IRX4204. Real-time PCR data are expressed as the percentage of vehicle treated controls.

IRX4204 Induces Expression of Nurr1/RXR Regulated Genes DAT and DDC in Cultured Primary Ventral Midbrain Neurons in an RXR Dependent Manner

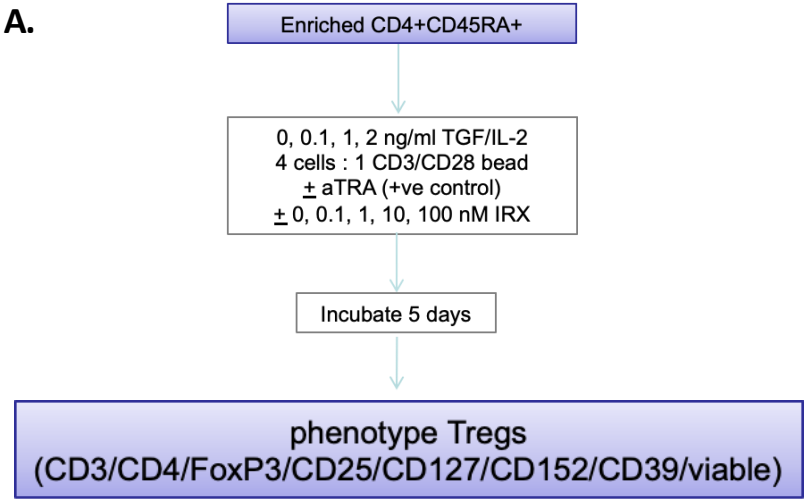


IRX4204 Treatment Reduces Loss of Dopamine and Reduces Dopamine Catabolism in 6-OHDA Lesioned Rats

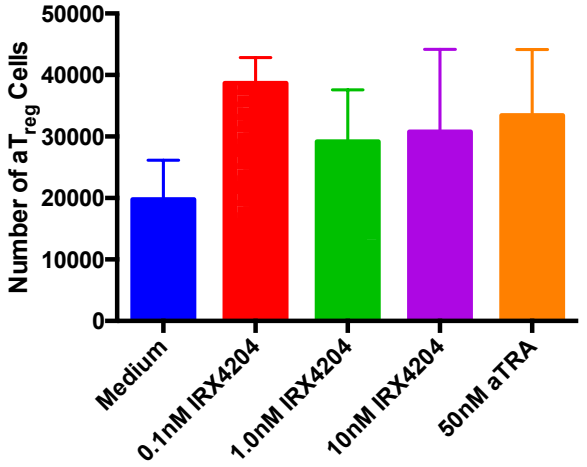


IRX4204 Promotes Human Treg Differentiation and Proliferation In Vitro

A.

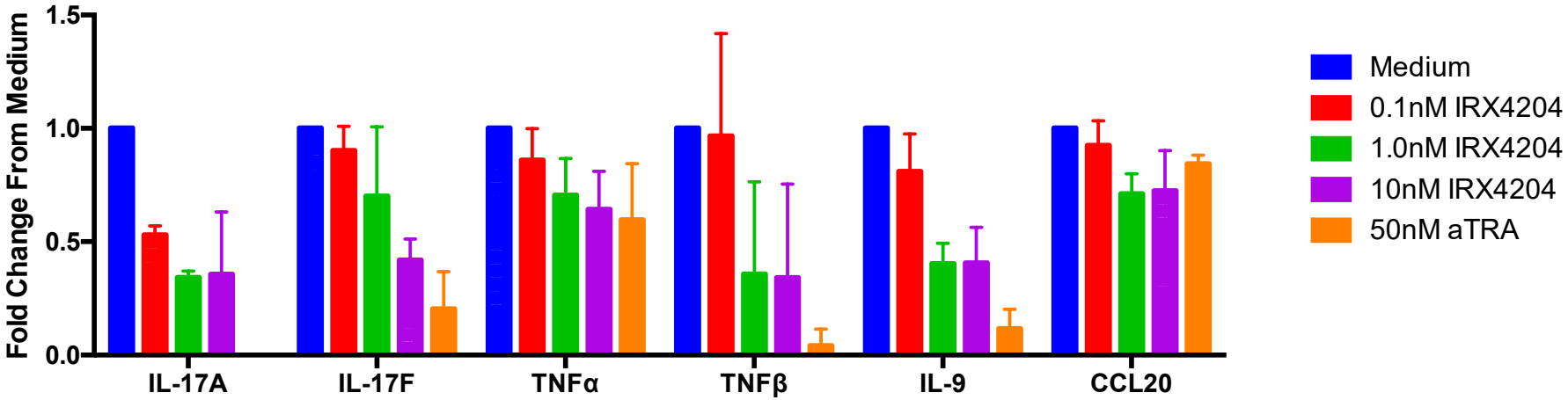


B.



IRX4204 increases Treg conversion and proliferation of naïve CD4+ T cells in Treg skewing conditions. **A.** Methods: PBMCs were isolated from whole blood, then magnetically separated to enrich a ~95% pure CD4+CD45RA+ naïve T cell fraction. Cells were stimulated as indicated and analyzed via flow cytometry after 5 days in culture. Flow cytometry gating strategy: Non-aggregate, then live, then singlet cells were gated. The CD3+CD4+ cells were gated and analyzed for the frequency of CD127loCD25+ cells. These cells were then analyzed for FoxP3, CD39, and CD152. **B.** Results: Absolute number of Treg cells from cells cultured in skewing conditions with 1 ng/ml TGFβ. IRX4204 increased the number of Treg cells. The mean and SD of three normal healthy individuals are shown.

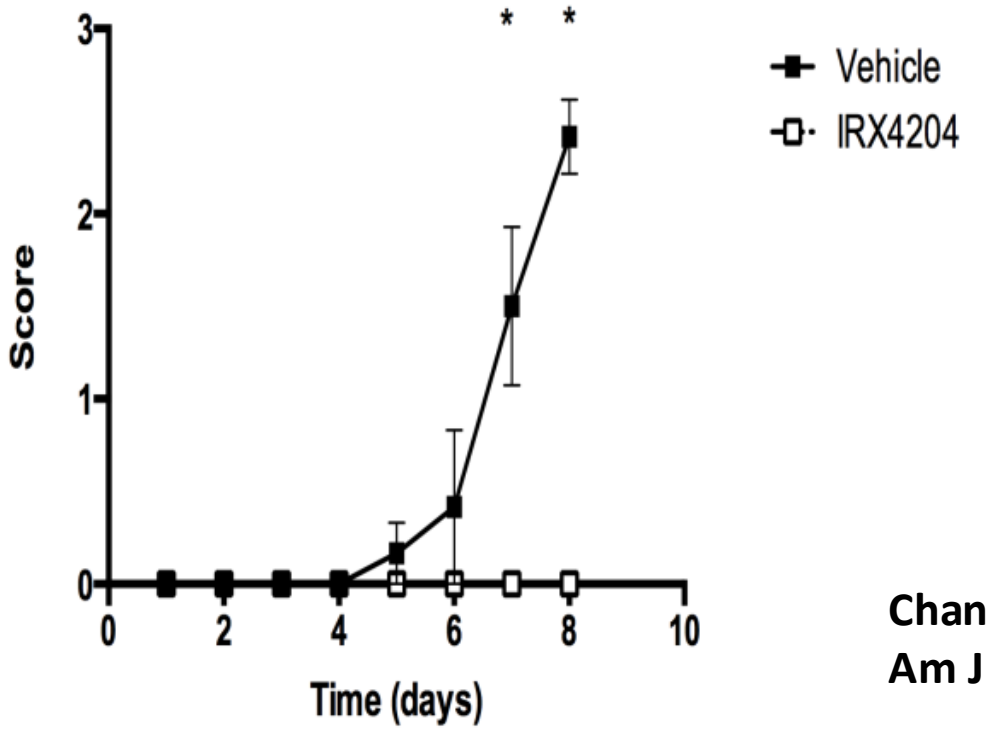
IRX4204 inhibits Th17-associated cytokine secretion by human T-cells cultured in Th17 skewing conditions



Normal Human PBMCs were isolated from whole blood, then magnetically separated to enrich a ~95% pure CD4+CD45RA+ naïve T cell fraction. Cells were cultured under Th17 skewing conditions for 5 days and stimulated anti-CD3/CD28 beads. and supernatants collected following 5 days in culture.

Treg and Th17 imbalance occurs in multiple forms of neurodegeneration including PD and AD. Therapeutic monoclonal antibodies which are bioactivity neutralizing for IL-17 and TNF-α are effective in randomized controlled clinical trials, and FDA approved for treatment of diverse autoimmune diseases including rheumatoid arthritis, psoriasis, psoriatic arthritis, ankylosing spondylitis, and Crohn's disease; validating IL-17 and/or TNF-α as therapeutic targets for these autoimmune diseases. Monoclonal antibodies are limited in their brain penetrance, making use of a brain penetrant compound with similar anti-autoimmune activities desirable. The preclinical data shown above, using human T-cells *in vitro*, provides strong support for clinical utility of IRX4204 for treatment of Treg/Th17 imbalanced autoimmune diseases of the brain.

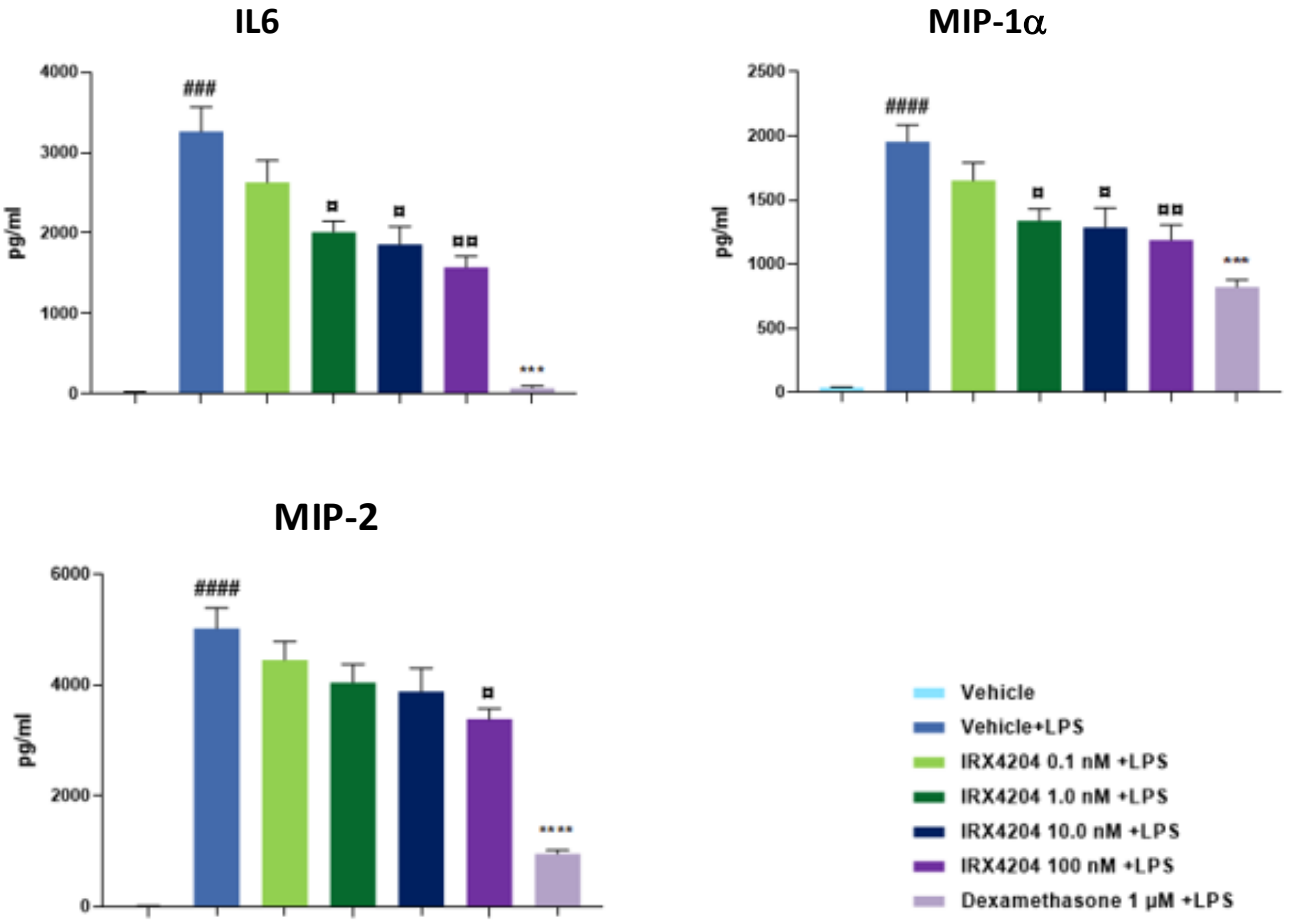
IRX4204 Demonstrated 100% Inhibitory Activity on Th17s *In Vivo*



Chandraratna, et al.
Am J Transl Res, 2016

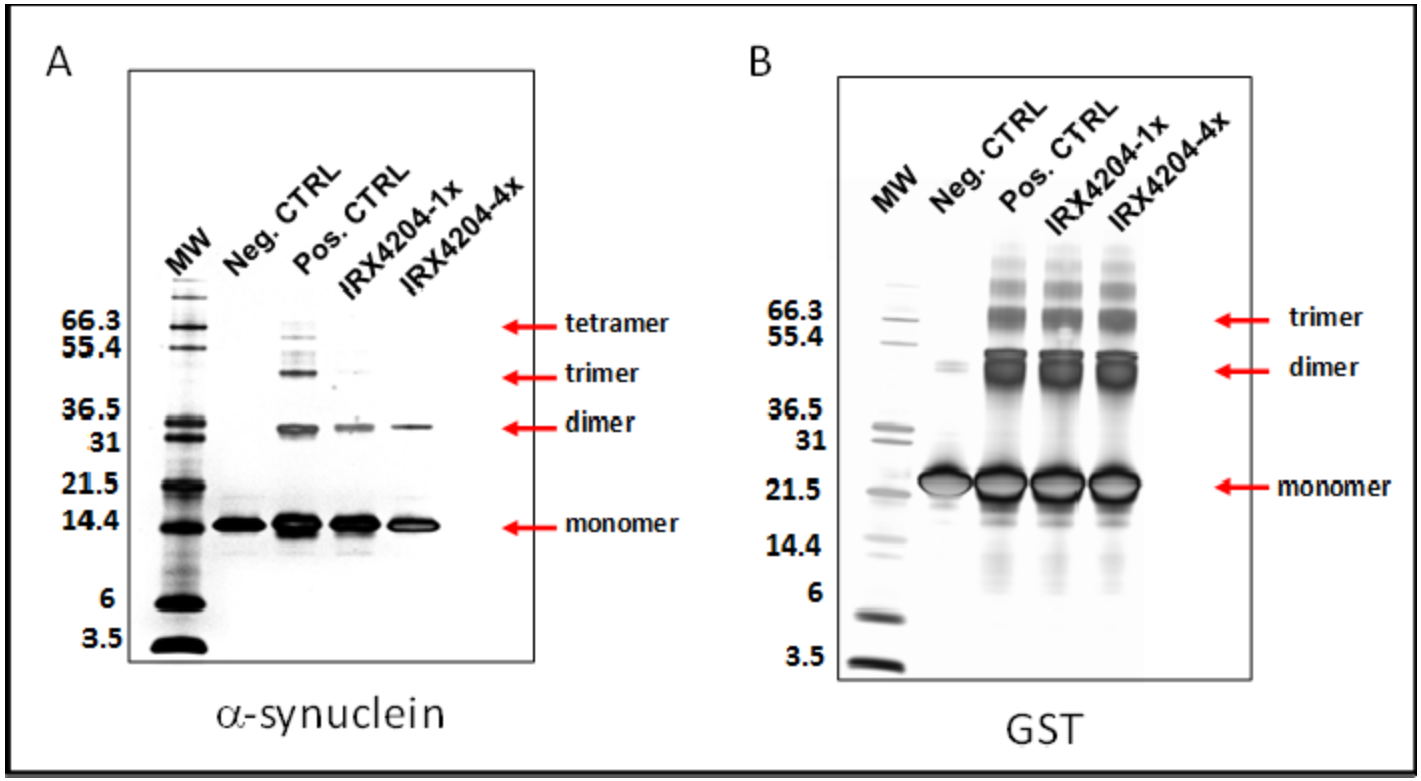
IRX4204 100% prevented Th17-mediated autoimmune disease mediated by adoptively transferred antigen-sensitized purified Th17 cells in a mouse model of autoimmune encephalomyelitis. Mice were sensitized with myelin oligodendrocyte glycoprotein. Purified Th17 cells were transferred to other mice that developed autoimmune encephalomyelitis. Disease activity was scored using a standard assessment of limb motor functions.

IRX4204 inhibits LPS-induced rat cortical microglial production of IL-6 and pro-inflammatory chemokines



Cultures were 80% purified microglia, 20% astrocytes

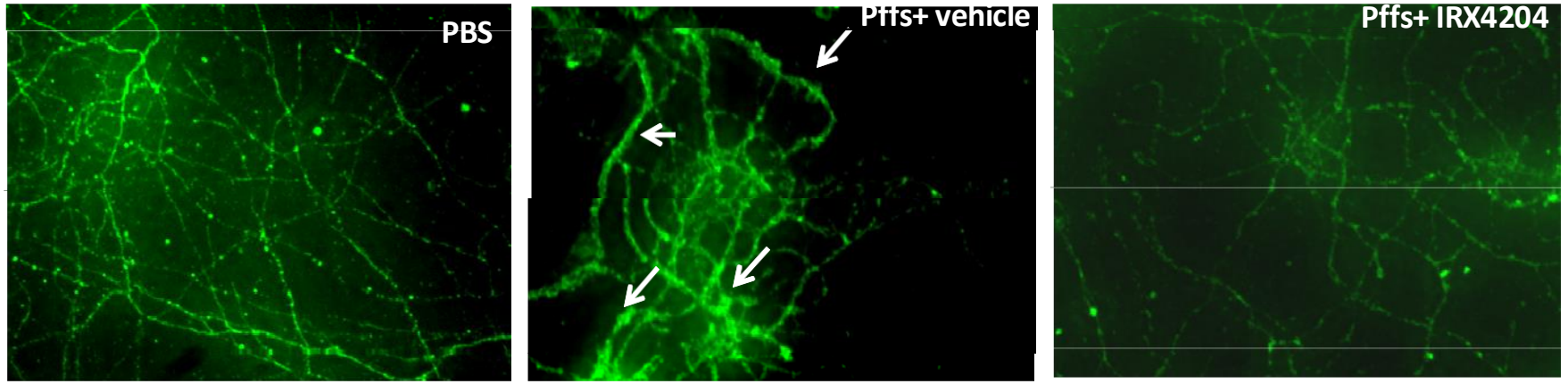
IRX4204 Interferes with Alpha-Synuclein Oligomerization in Photo-induced Cross-Linking of Unmodified Proteins (PICUP) Assay



SDS-PAGE of (A) alpha synuclein and (B) glutathione synthetase (GST) crossed-linked in the presence or absence of 4X molar excess of the indicated compounds. Negative controls are non-cross-linked alpha-synuclein or GST. Positive control is aggregated alpha-synuclein or GST.

Data provided by Giulio Pasinetti
Icahn School of Medicine at Mount Sinai

IRX4204 Prevents α-synuclein Pre-formed Fibrils (Pffs)-induced Fibrillar-like Structure Formation



Primary neurons were treated with human α-synuclein pre-formed fibrils (Pffs) for 14 days and fixed with 4% PFA+1% triton 100. The cells were immunostained with anti-α-synuclein antibody.

In PBS treated neurons, endogenous mouse α-synuclein localized to presynaptic puncta while in pffs-treated neurons, α-synuclein formed fibrillar-like structures. Treatment of IRX4204 prevented the formation of fibrillar-structure induced by Pffs.

Data provided by Giulio Pasinetti
Icahn School of Medicine at Mount Sinai

RXR nuclear receptor signaling modulates lipid metabolism and triggers lysosomal clearance of alpha-synuclein in neuronal models of synucleinopathy.

Arati Tripathi, *et.al.,* Cell Mol Life Sci; 2024: Ann Romney Center for Neurologic Diseases, Brigham and Women's Hospital and Harvard Medical School, Boston, MA

Abstract: "Disease-modifying strategies for Parkinson disease (PD), the most common synucleinopathy, represent a critical unmet medical need. Accumulation of the neuronal protein alpha-synuclein (αS) and abnormal lipid metabolism have each been implicated in PD pathogenesis."

"With regard to αS proteostasis, our study reveals that RXR agonism stimulates lysosomal clearance of αS." "Our findings show that RXR-activating ligands can modulate fatty acid metabolism and αS turnover to confer benefit in cellular models of PD, including patient neurons."

"We offer a new paradigm to investigate nuclear receptor ligands as a promising strategy for PD and related synucleinopathies."

IRX4204: Product Development Status

- Company owns US INDs for IRX4204 for treatment of cancers, Parkinson's disease, and psoriasis
- GLP 6-month rat, 9-month dog toxicology completed under NIH SBIR Phase II grant; dose related ocular cataracts noted in rats and dogs
- PK in rats, dogs and humans consistent with once daily oral dosing
- Brain penetrance demonstrated in mice, rats, and PD patients
- Binding to K^+ hERG channel negative
- Binding to Panlabs panel of brain neurotransmitter receptors negative
- GLP gene toxicology panel negative
- GLP *in vivo* safety pharmacology panel (CNS, CV, Respiratory) negative
- GMP manufacturing on multi-kg scale; 99+% chemical and chiral purity,
- Multi-year compound stability demonstrated
- Formulated in oral gel capsules for Phase II clinical trials
- Patented salt/polymorph NCE can be formulated for oral, parenteral, inhaled nose to brain, inhaled mouth to lung, dermal, and ocular administration

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 well-tolerated for up to 20 months of continuous oral treatment.
- 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 API and manufacture of GMP drug and placebo capsules for Phase II clinical trials has been completed.

IRX4204 Intellectual Property for PD

Io Therapeutics owns a portfolio of issued patents for use of IRX4204 for treatment of Parkinson's disease, in the US, Canada, Mexico, Europe (16 countries), Israel, Japan, South Korea, Singapore, Hong Kong, People's Republic of China, Australia, New Zealand, and South Africa. Most of these patents are valid until 10/31/2036.

Most of the above countries also have issued patents with claims variously for use of IRX4204 for treatment of Alzheimer's disease, normal aging-related neurodegeneration, multiple sclerosis, amyotrophic lateral sclerosis, stroke, traumatic or hypoxic CNS injury, schizophrenia, depression, autism; and autoimmune skin, arthritic, and gastrointestinal diseases. Most of these patents are valid until 10/30/2036.

The company owns issued and pending patents for proprietary methods of synthesis of high chiral purity IRX4204, and compositions of matter of proprietary intermediates in all the above listed countries plus India, Brazil, Chile, Vietnam, Malaysia, Philippines, Thailand, Indonesia, and Saudi Arabia. The patents are valid until 11/16/2038.

The company owns an extensive portfolio of issued and pending patents with claims for compositions of matter of salts and polymorphs of IRX4204 that have enhanced solubility in aqueous solutions, including methods of synthesis of these compositions, and their specific X-ray crystallographic structures. These patents are issued or pending in the US, EP, Canada, Mexico, Japan, People's Republic of China, Hong Kong, India, South Korea, Singapore, Taiwan, Thailand, Malaysia, Philippines, Vietnam, Indonesia, Australia, New Zealand, Israel, South Africa, Algeria, Egypt, Nigeria, Brazil, Chile, Argentina, Columbia, Peru, Saudi Arabia, United Arab Emirates, Qatar, Bahrain, Kuwait, and Oman. These patents are valid until 03/14/2043. The company is seeking additional patents for nose to brain inhaled formulations.