IRX4204

A Phase II Clinical Stage Potent and Selective RXR Agonist Compound for Treatment of Parkinson's Disease

Presentation for the
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Sichuan Provincial Neuroscience Society
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Io Therapeutics, Inc.
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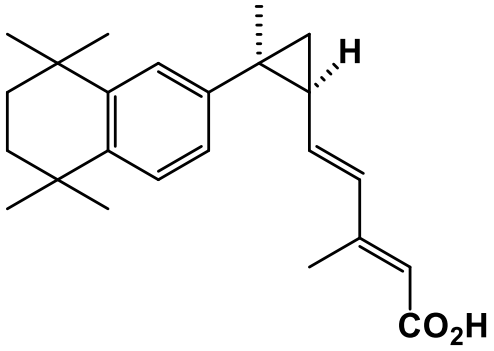
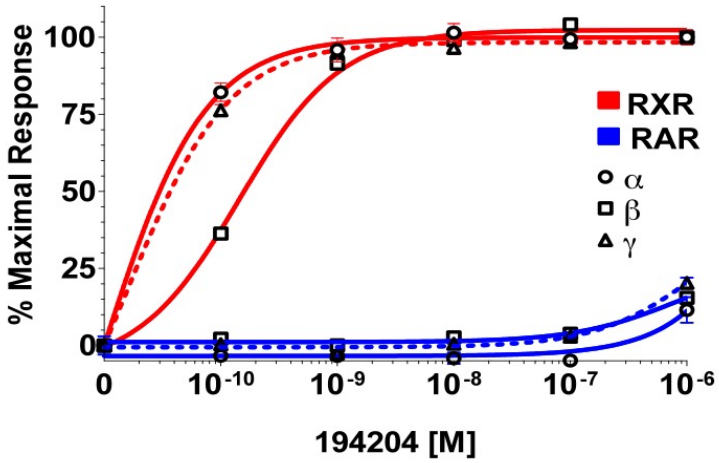
Chief Executive Officer

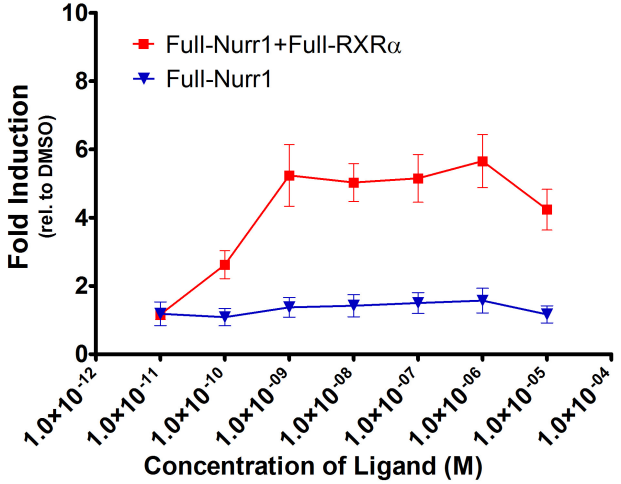
IRX4204 Activates RXR and Nurr1, But Not RAR Nuclear Receptors in Reporter Assays at Nanomolar Concentrations



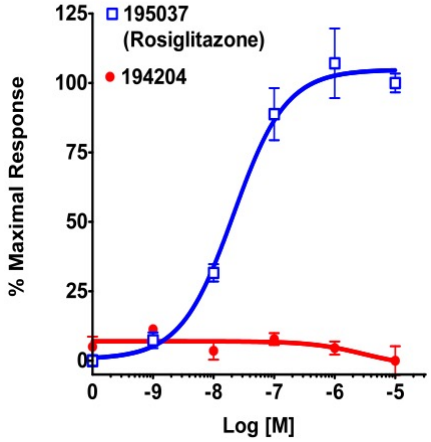
IRX4204 – A Potent RXR Homodimer Agonist with no RAR Activity at nM Concentrations



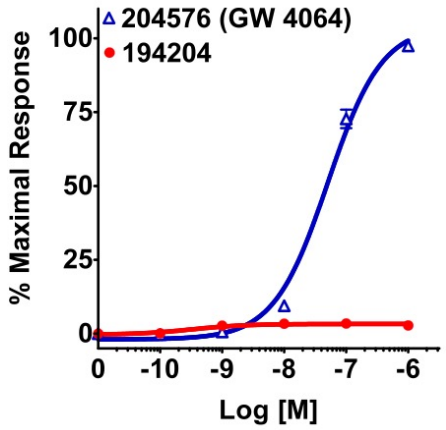
IRX4204 Transactivates Nurr1/RXR Heterodimers through RXR



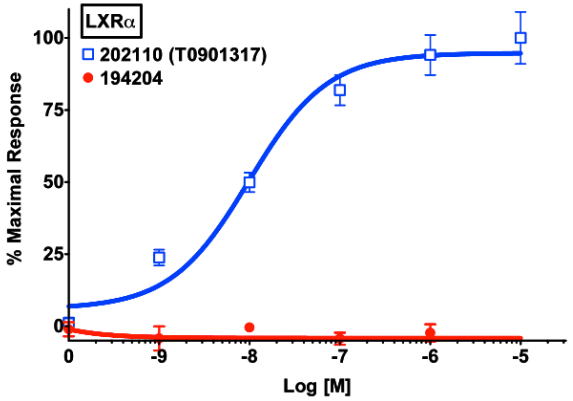
IRX4204 is highly selective for RXR
It does not transactivate PPARγ, FXR, or LXR



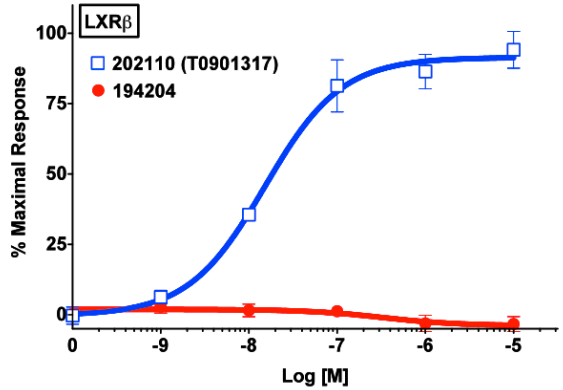
PPARγ/RXR



FXR/RXR

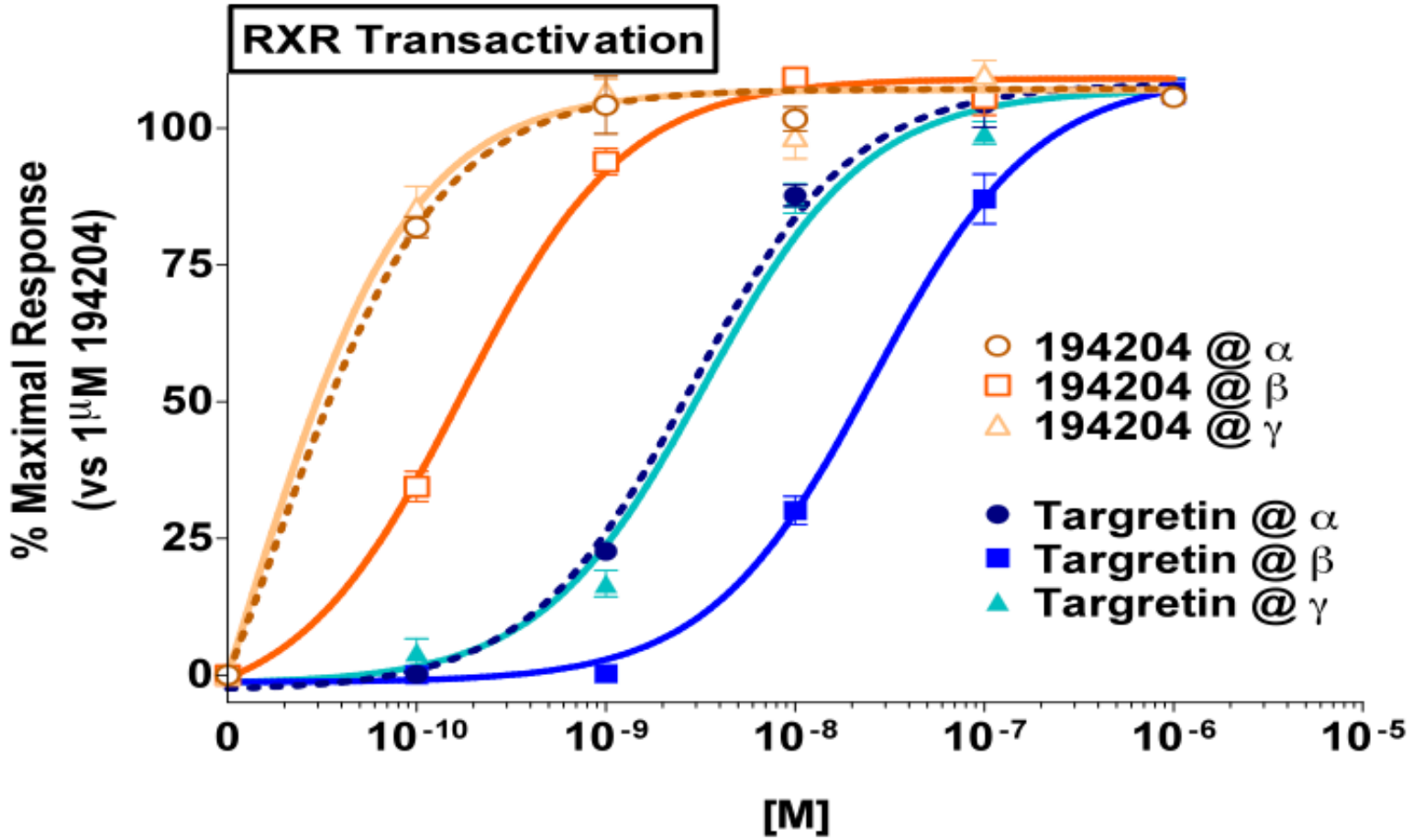


LXR/RXR



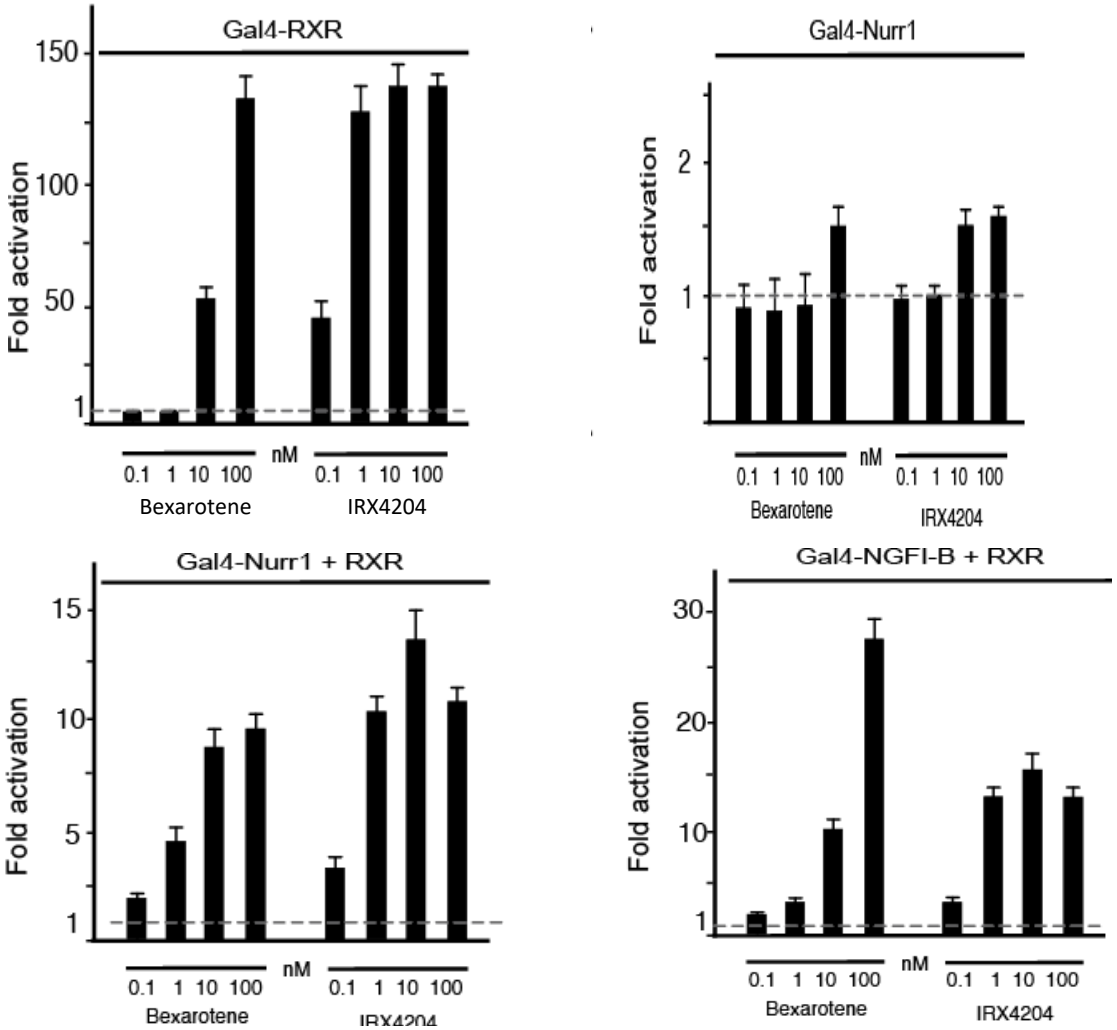
LXR/RXR

IRX4204 is Greater Than 100-Fold More Potent than Targretin (bexarotene) in Transactivation of RXR Homodimers



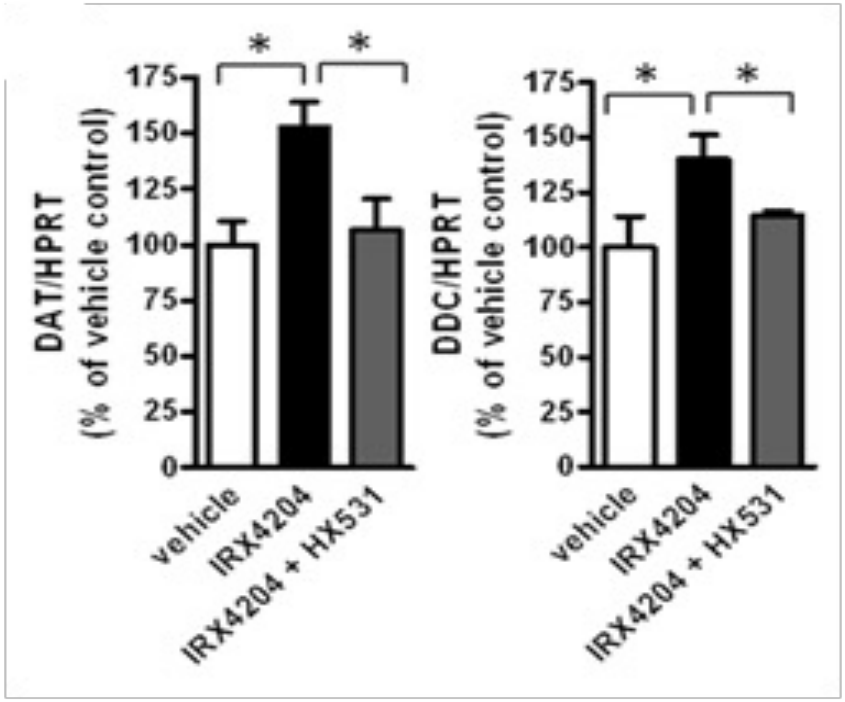
Bexarotene is a 100x less potent and far less selective RXR agonist than IRX4204. It is the only FDA approved RXR agonist. It has off target activation of RAR. It is approved only for treatment of CTCL. It has been effective orally and topically in clinical trials in psoriasis.

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



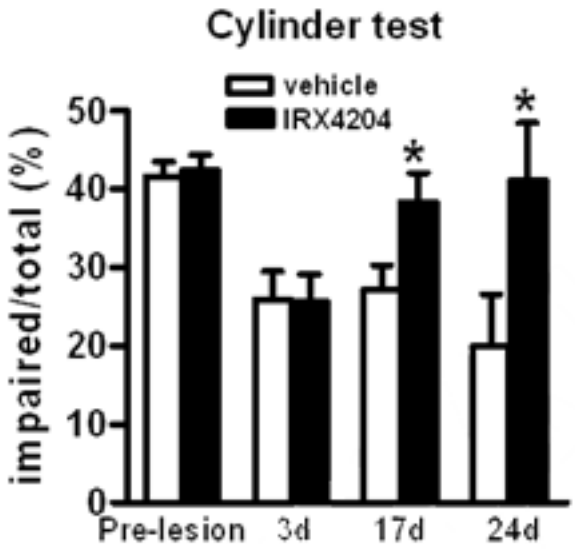
Data provided by
Thomas Perlmann
Karolinska Institute
Stockholm, Sweden

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



Jun Wang, Giulio Pasinetti;
Mount Sinai School of Medicine

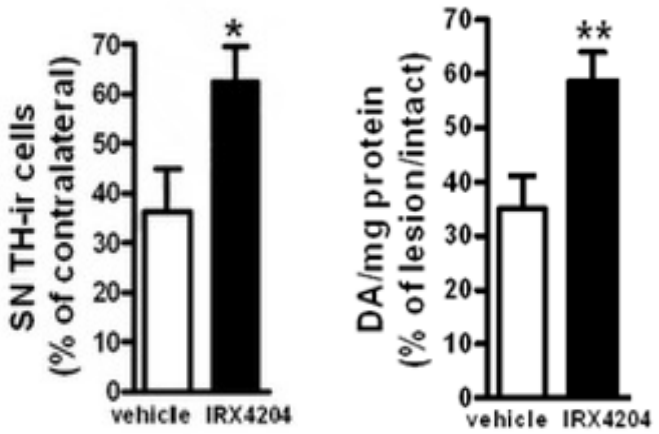
IRX4204 Reverses Asymmetric Impairment in Limb Usage Induced by 6-OHDA Lesions in Rat PD Model



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

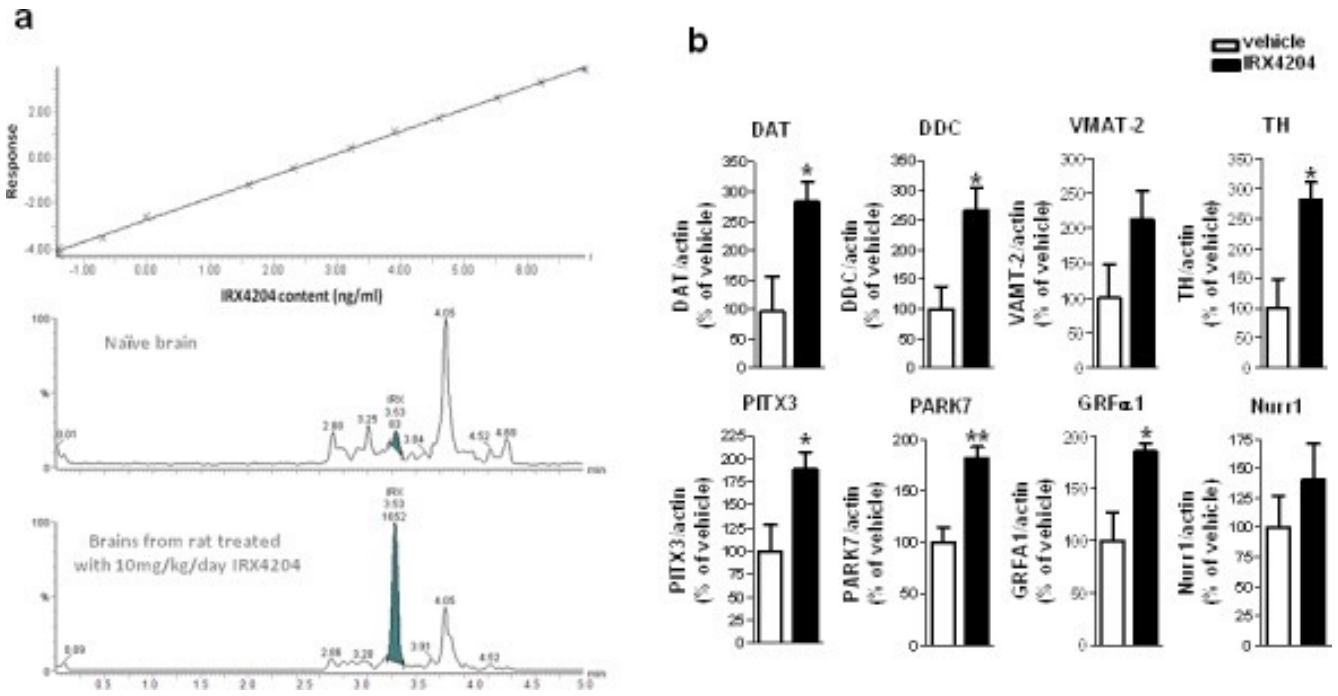
Detects forelimb impairments, specifically asymmetry in limb use for weight bearing movements when leaning on walls of glass cylinder during exploration of a novel environment. Compares use of the affected side to the unaffected side, therefore each animal serves as its own control.

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



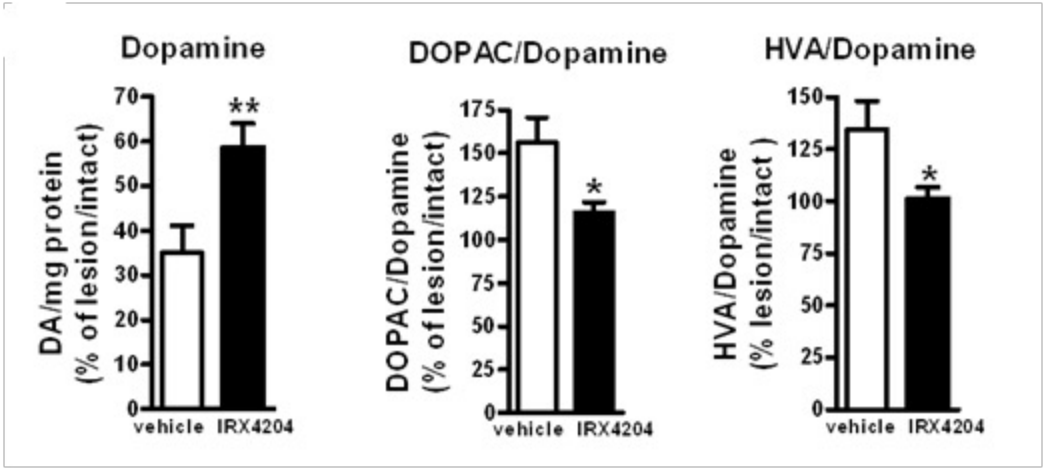
Rats were stereotaxically injected with 6-OHDA (40µg in 2µl) into right striatum. Rats were treated with IRX4204 (10mg/kg/d) or vehicle by oral gavage for 21 days starting 3 days post-lesion.

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

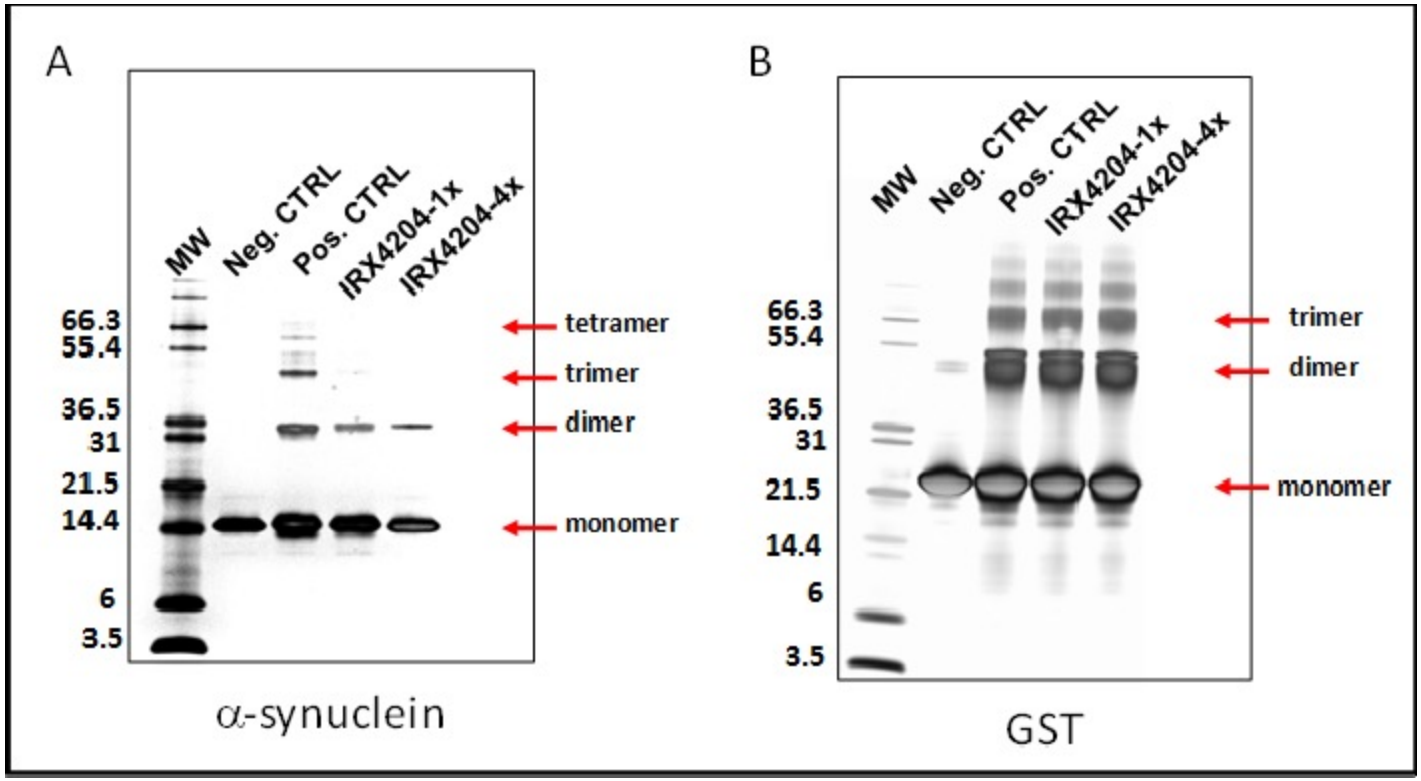


a. Detection of IRX4204 in the brains: Dose-response calibration curve of IRX4204 detection in the brain matrix with detection limit of 0.25nM (top panel); Representative IRX4204 LC/MS/MS chromatograms from brains of vehicle and IRX4204 treated rats (middle and bottom panel) **b.** Expression of Nurr1 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 Treatment Reduces Loss of Dopamine and Reduces Dopamine Catabolism in 6-OHDA Lesioned Rats

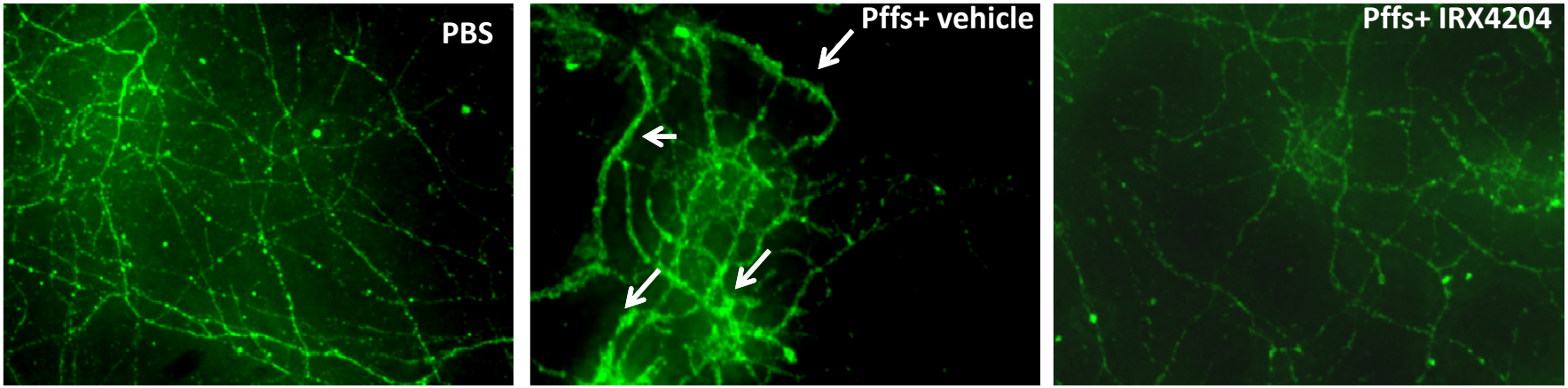


IRX4204 Interferes with Alpha-Synuclein Oligomerization in Photo-induced Cross-Linking of Unmodified Proteins (PICUP) Assay



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.

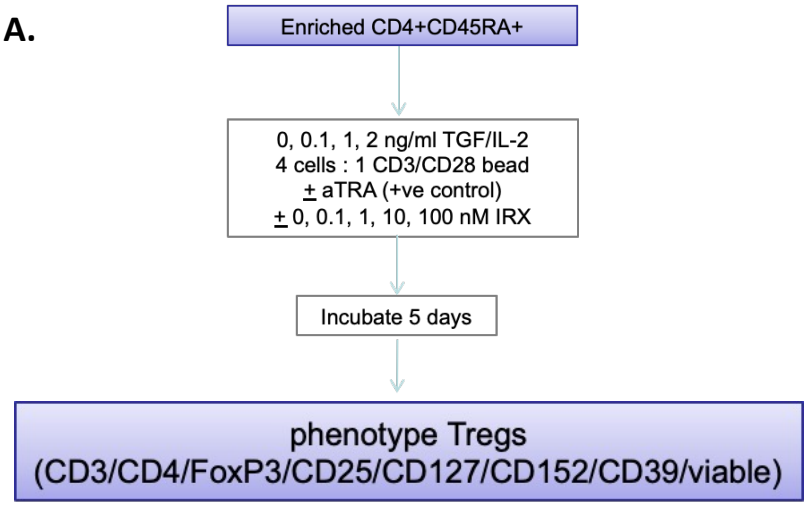
IRX4204 prevent α-synuclein pre-formed fibrils (Pffs)-induced fibrillar-like structure formation



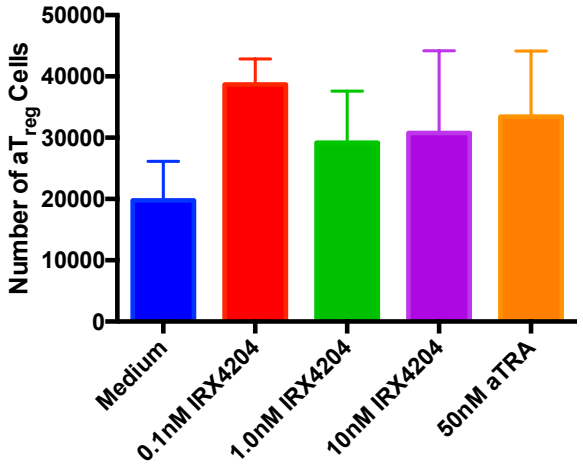
Primary neurons were treated with human α-synuclein pre-formed fibrils (Pffs) for 14 days and fixed with 4% PFA+1% triton 100. The cells were immunostained with anti-α-synuclein antibody. In PBS treated neurons, endogenous mouse α-synuclein localized to presynaptic puncta while in pffs-treated neurons, α-synuclein formed fibrillar-like structures. Treatment of IRX4204 prevented the formation of fibrillar-structure induced by Pffs.

IRX4204 Promotes Human Treg Differentiation and Proliferation In Vitro

A.



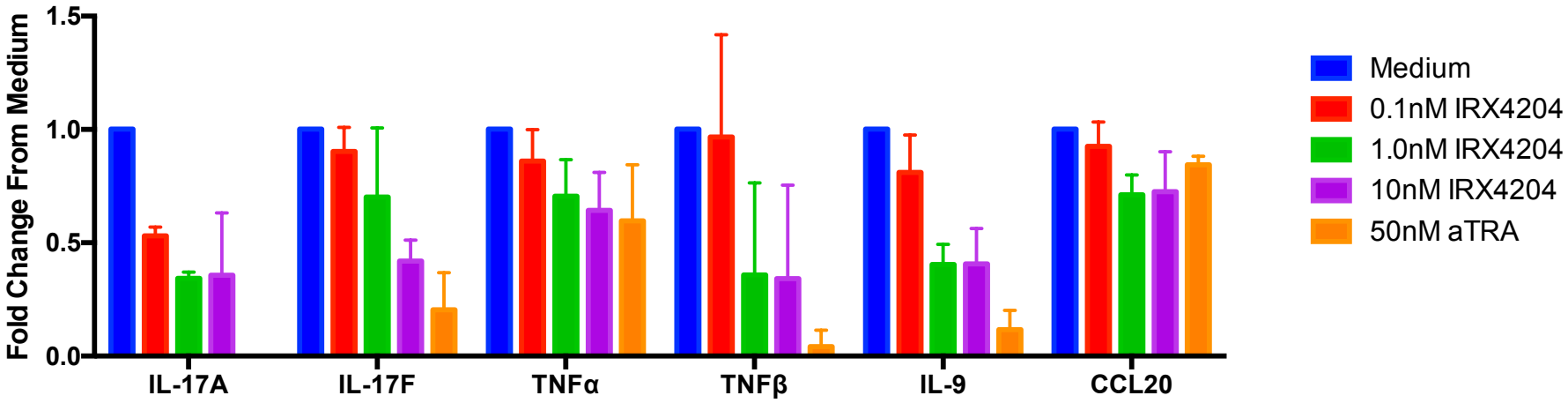
B.



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.

Data from Daniel Mielcarz, Jacqueline Channon; DARTLab, Randy Noelle, Dartmouth University

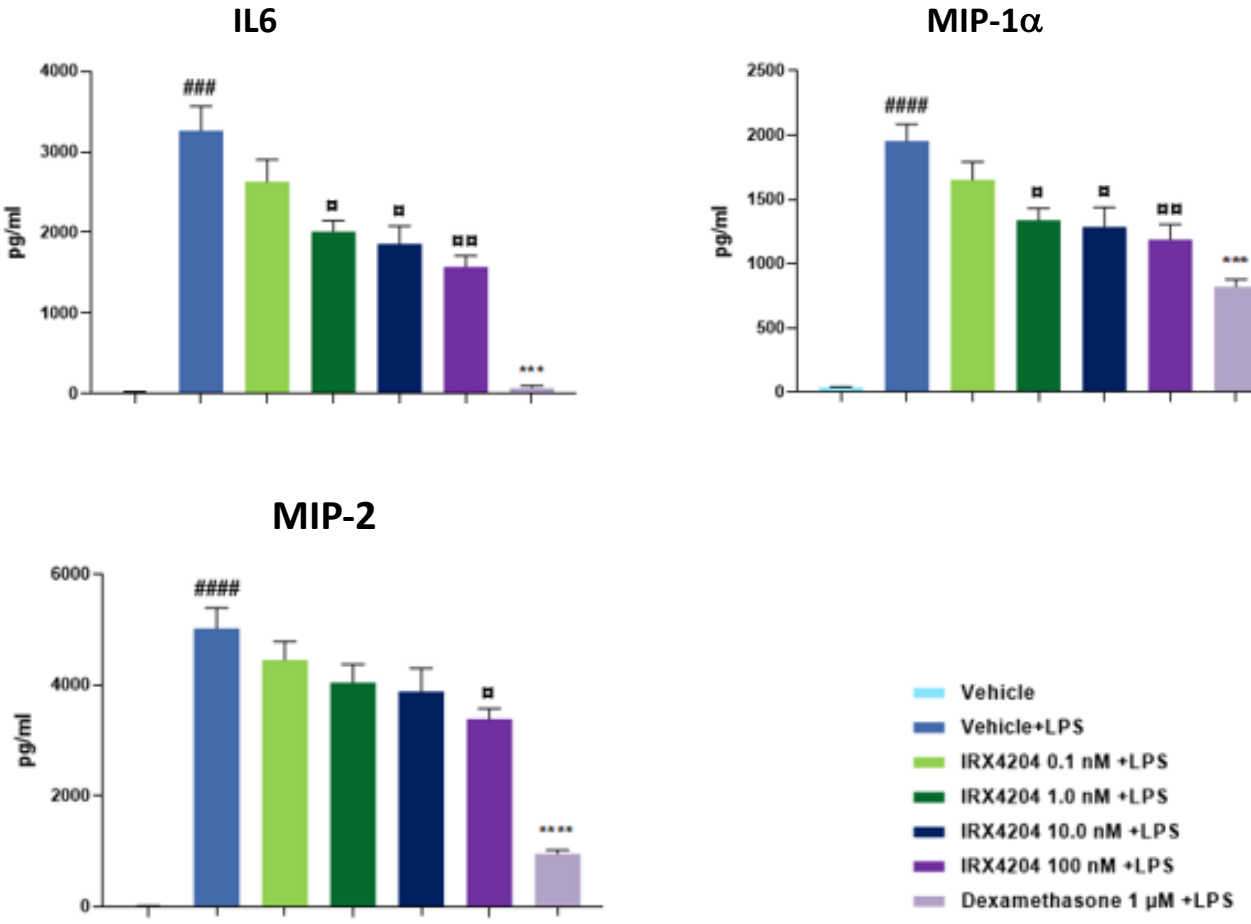
IRX4204 inhibits Th17-associated cytokine secretion by human T-cells cultured in Th17 skewing conditions



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.

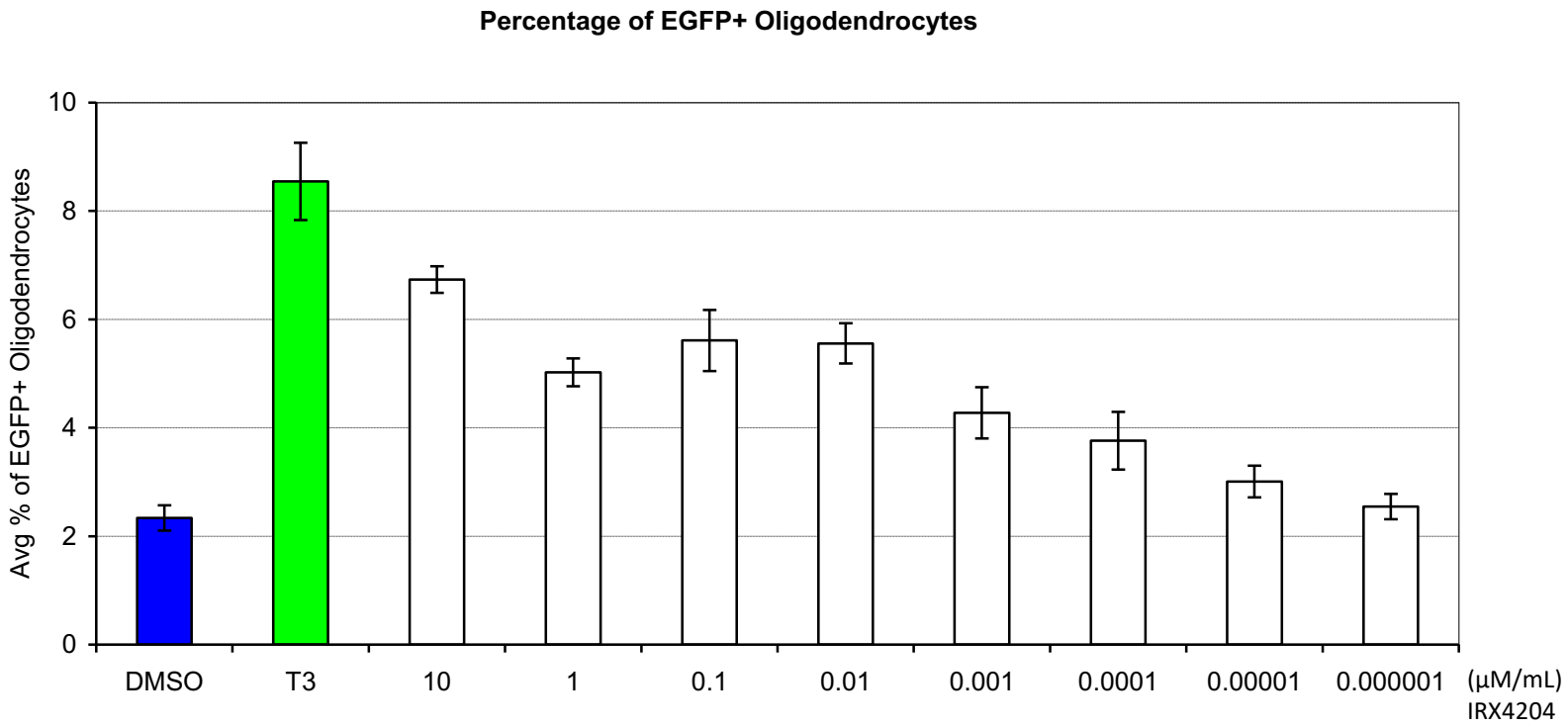
Therapeutic agents which are bioactivity neutralizing for IL-17 and TNF-α are effective in randomized controlled clinical trials, and FDA approved, for treatment of rheumatoid arthritis, psoriasis, psoriatic arthritis, ankylosing spondylitis, Crohn's disease, ulcerative colitis, and uveitis; validating IL-17 and TNF-α as therapeutic targets for diverse autoimmune diseases. The preclinical data shown above, using human T-cells in vitro, provides strong support for potential clinical utility of IRX4204 for treatment of these same IL-17 and TNF-α mediated diseases.

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



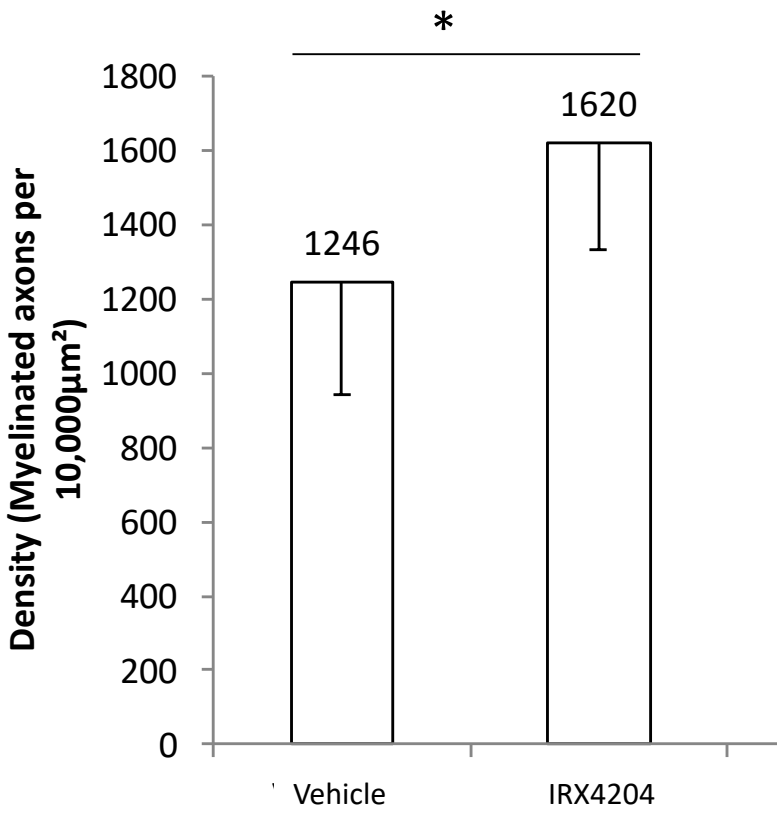
Cultures were 80% purified microglia, 20% astrocytes
Data from Charles River Laboratories

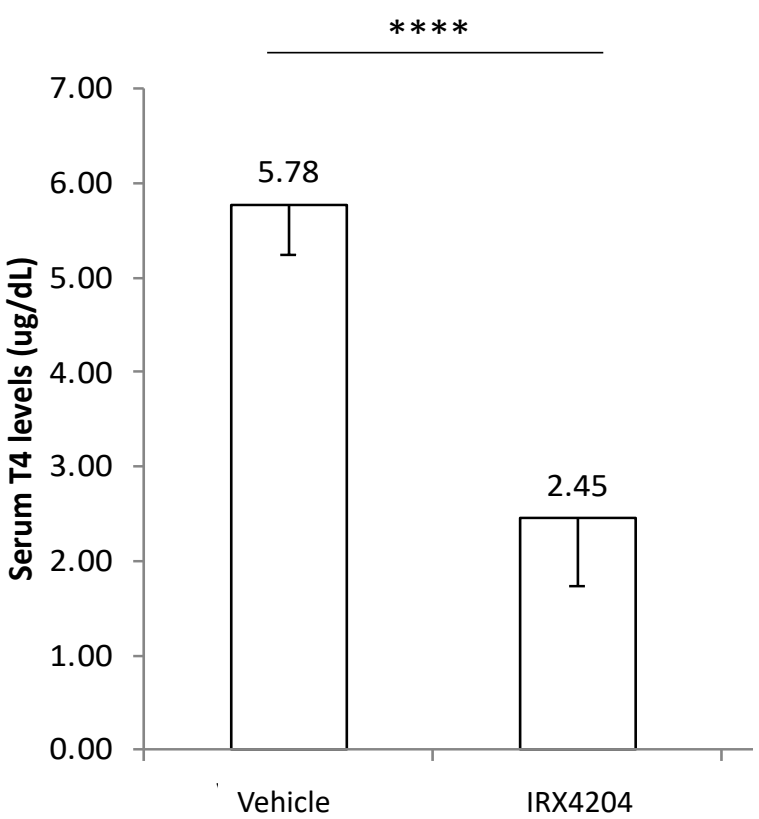
IRX4204 Promotes Differentiation of Oligodendrocyte Precursor Cells into Myelin Producing Oligodendrocytes



Data from Satish Medicetty, Bruce Trapp; Renovo Neural Inc.
Supported by US National Multiple Sclerosis Society

IRX4204 Promotes Remyelination in a Murine Model of Cuprizone-induced Chronic Demyelination





IRX4204 treatment resulted in a 30% statistically significant increase in myelin relative to vehicle ($p<0.05$), notwithstanding inducing highly significant hypothyroidism.

Study done at Renovo Neural, Inc.; Supported by US National Multiple Sclerosis Society

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 daily oral dosing.
- Common side effects include dose related reversible suppression of thyroid stimulating hormone, with resultant treatable hypothyroidism; mild to moderate reversible increased triglycerides; and mild to moderate reversible leukopenia. Oral dosing with 5 mg/day appears to be safe and potentially effective for PD.

Phase I/II Clinical Trial in Early Parkinson's Disease

Mean Changes from Baseline to Week 2 in Total Motor Score, UPDRS, and TSH Indicate CNS Effects 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

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.

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

Next Step for Clinical Development of IRX4204 for Treatment of Parkinson's Disease

- Conduct a Phase II randomized, double-blind, placebo-controlled clinical trial
- Approximately 20 patients
- Randomized 1:1 to IRX4204 at 5 mg/day vs placebo for 28 days
- Perform safety assessments for adverse events, clinical laboratories
- Perform clinical efficacy assessments with UPDRS, cognitive and emotional assessments, the University of Rochester PD Health Inventory, and a wearable device for digital quantification of motor functions
- Assay serial blood samples for quantitation of diverse biomarkers including brain derived exosomes for Nurr1 and Nur77, NF-kB, IkB, α-synucleins, neurofilaments, IL-17, TNF, IL-6, markers of OPC differentiation, and other markers

Collaborators and Acknowledgements

Elizabeth Nowak, Randy Noelle; Dartmouth University
Murine Treg and Th17 differentiation studies; EAE models; EAE model of combination treatment with IRX4204 + thyroid hormone

Daniel Mielcarz, Jacqueline Channon; DARTLab, Dartmouth University
Human Treg and Th17 differentiation and Th17 production of proinflammatory cytokines studies

George Robertson; Dalhousie University
EAE models; myelin protection in EAE; neuronal mitochondrial respiration studies

Jun Wang, Giulio Pasinetti; Mount Sinai School of Medicine
Alzheimer's and Parkinson's disease models; studies of IRX4204 effects on α–synuclein, β-amyloid, and tau proteins

Robin Avila, Satish Medicetty, Bruce Trapp; Renovo Neural, Inc.
OPC differentiation in vitro studies; axonal protection and remyelination studies in cuprizone-induced demyelination models

Raj Thangavelu, Bruce Blazar; University of Minnesota
GVHD studies

Thomas Perlmann; Karolinska Institute
RXR reporter assays with IRX4204 qnd bexarotene

Danna Jennings, Ken Marek; Molecular Neuroimaging, Inc.
Parkinson's disease phase I clinical trial

Charles River Laboratories
Cortical neuron neurite outgrowth studies; microglial production of proinflammatory cytokines and chemokines studies

MD Biosciences, Ltd.
PD model of combination treatment with IRX4204 + thyroid hormone