The highly potent and selective third generation RXR agonist compound IRX4204 is a potential treatment for normal aging-related neurodegeneration, Parkinson's disease (PD), and Alzheimer's disease (AD)

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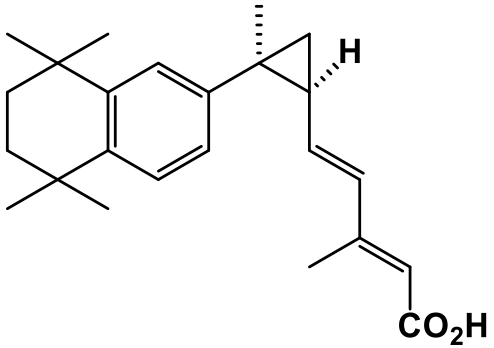
First and Second Generation RXR Agonists

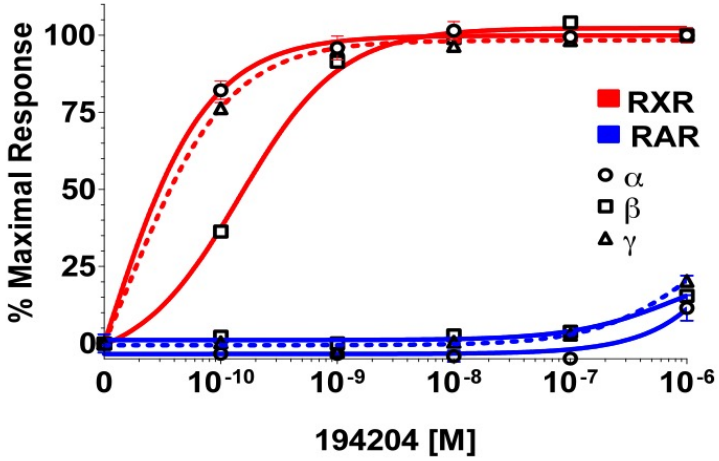
- First Generation RXR Agonist: 9-cis Retinoic Acid
  - Marketed as Alitretinoin™ for topical treatment of AIDS related Kaposi's Sarcoma.
  - Non-selective, nearly equal RAR and RXR agonism.
  - Promotes survival of dopaminergic neurons, forms Nurr1 heterodimers.
  - Promotes differentiation of oligodendrocyte precursor cells.

- Second Generation RXR Agonist: bexarotene
  - Marketed as Targretin™ for oral or topical treatment of CTCL.
  - Semi-selective for RXR, has substantial RAR, LXR, PPAR gamma agonist activities and related side effects.
  - Deemed too toxic for chronic systemic use due to RAR rashes and lipids.
  - Effective in PD, AD, ALS, and stroke models.
  - Promotes Treg differentiation, inhibits Th17 differentiation.
  - Effective orally and topically in clinical trials in psoriasis patients.
  - Effected durable myelin repair in a phase II RCT in relapsing/remitting MS.
  - Effected removal of brain amyloid without cognitive improvement in an RCT but only in the ApoE4 non-carrier subset of AD patients.

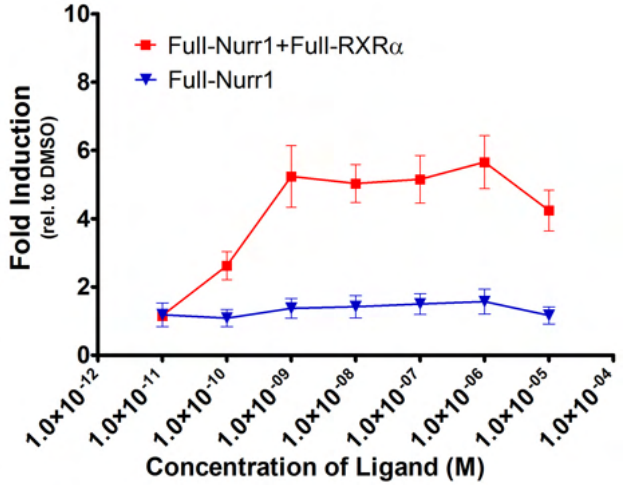
IRX4204, A Third Generation RXR Agonist Compound Activates RXR and Nurr1, But Not RAR Nuclear Receptors at Nanomolar Concentrations



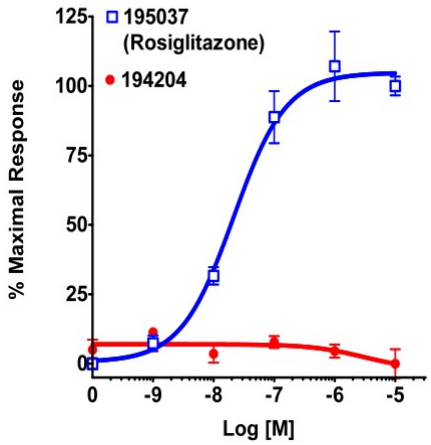
IRX4204 – A Potent RXR Homodimer Agonist with no RAR Activity at nM Concentrations



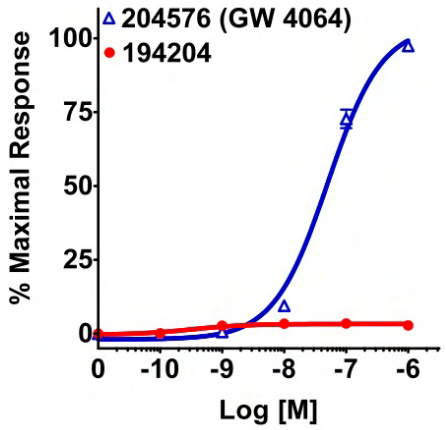
IRX4204 Transactivates Nurr1/RXR Heterodimers through RXR



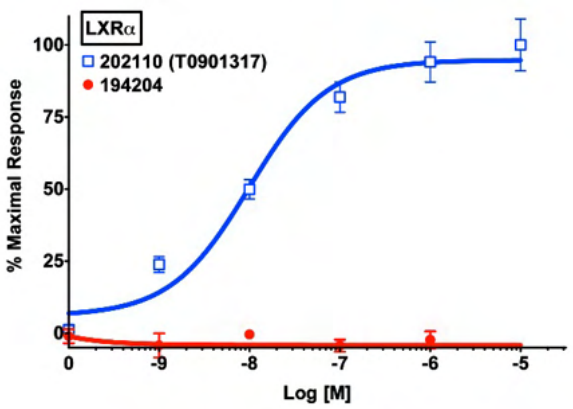
IRX4204 is highly selective for RXR
It does not transactivate PPARγ, FXR, or LXR



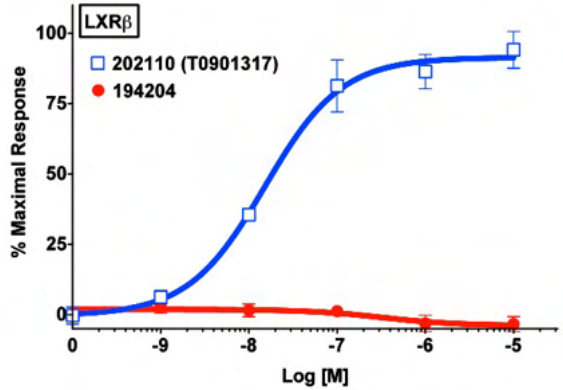
PPARγ/RXR



FXR/RXR

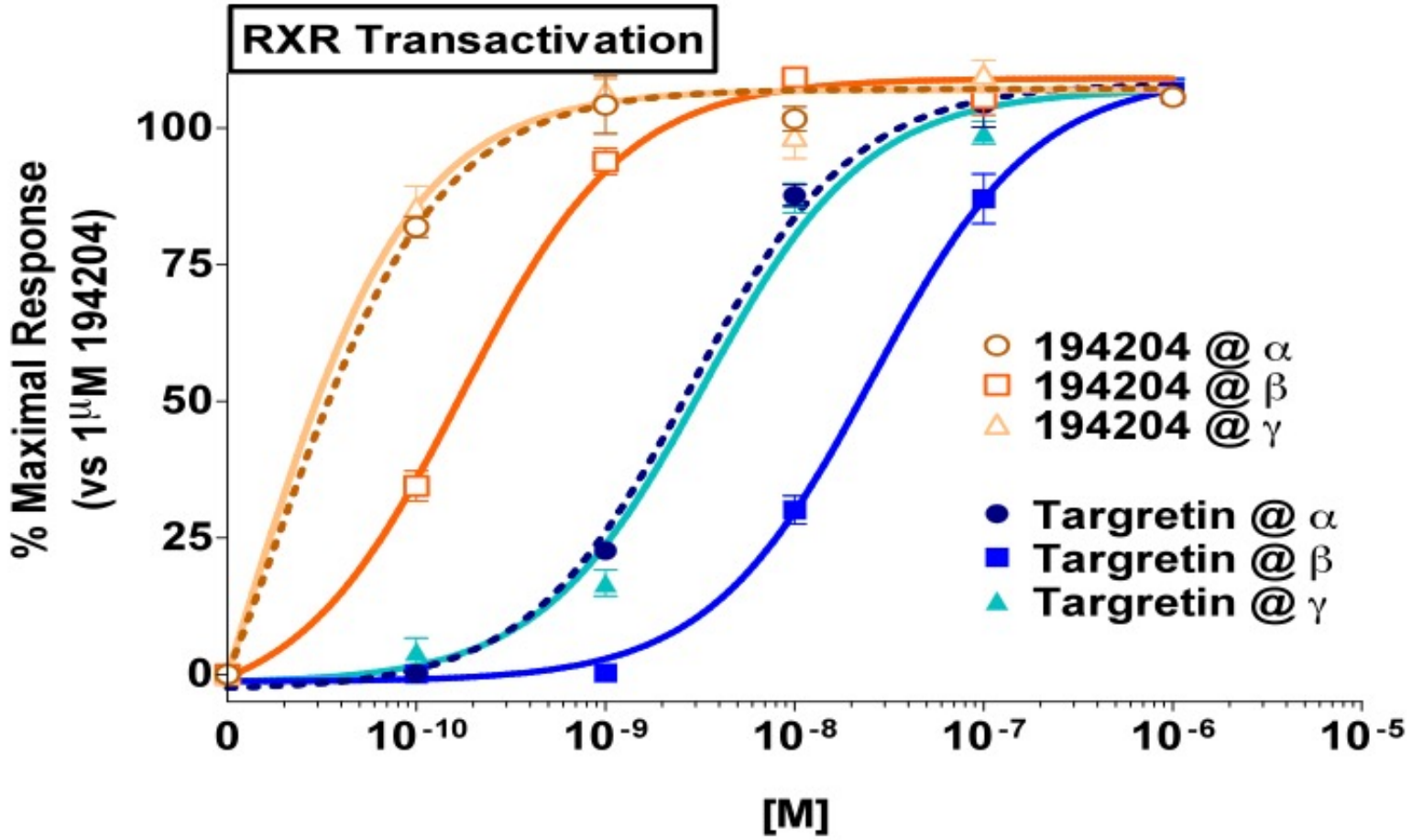


LXR/RXR



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.

Brain Pathology of Normal Aging-related Neurodegeneration

- Normal aging-related neurodegeneration is deemed to be related to chronic low-grade inflammation in the brain, so called neuro-inflammaging.
- Neuro-inflammaging is deemed to result in chronic age-related loss of myelinated nerve fibers in otherwise healthy non-human primates and humans.
- Loss of myelinated nerve fibers causes disruption of functional neuro-electrical connections between neurons in various parts of the brain.
- Chronic neuro-inflammaging and loss of myelin leads to death of neurons.
- These neurodegenerative processes produce loss of cognitive functions, memory loss, and other functional disabilities with normal brain aging.
- Similar aging brain pathologies underlie other neurological conditions, including PD and AD, which also have disease specific brain pathologies.

Neuro-inflammaging in PD and AD

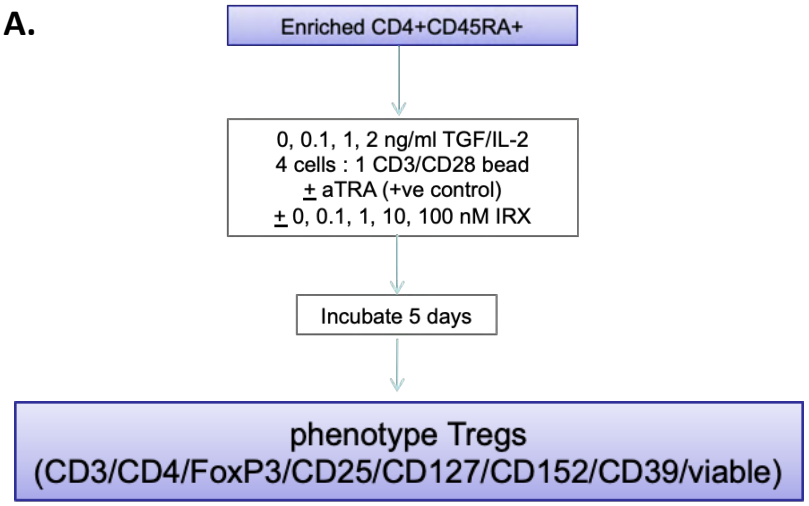
- **Neuro-inflammaging is related to imbalance of immunosuppressive T-regulatory cells (Treg) with pro-inflammatory cytokine interleukin-17 (IL-17) overproducing T-cells (Th17) in the brain.**

- **Neuro-inflammaging also is related to overactivity of brain microglia, which produce the pro-inflammatory cytokine interleukin-6 (IL-6), and other pro-inflammatory factors.**

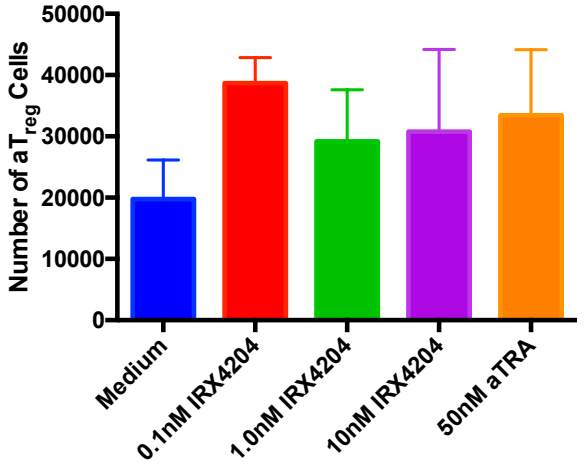
- **Parkinson's disease (PD) and Alzheimer's disease (AD) brains manifest similar neuro-inflammaging pathologies to those observed in normal brain aging, but also have distinct pathologies related to deposition of abnormal misfolded proteins such as alpha synuclein in PD, and beta amyloid in AD.**

IRX4204 Promotes Human Treg Differentiation and Proliferation In Vitro

A.

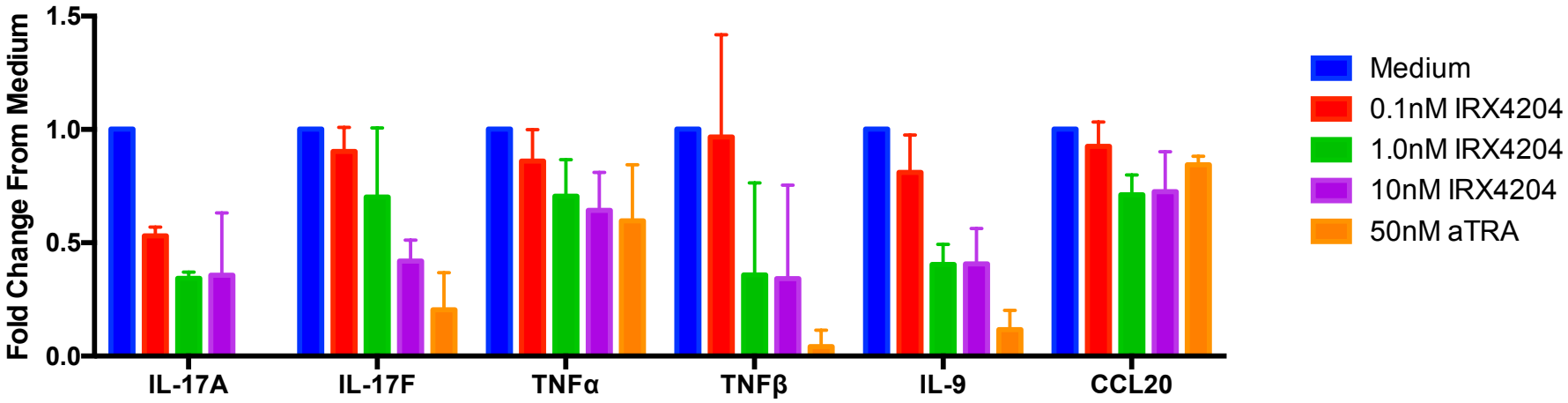


B.



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 1ng/ml TGFβ. IRX4204 increased the number of Treg cells. The mean and SD of three normal healthy individuals are shown.s

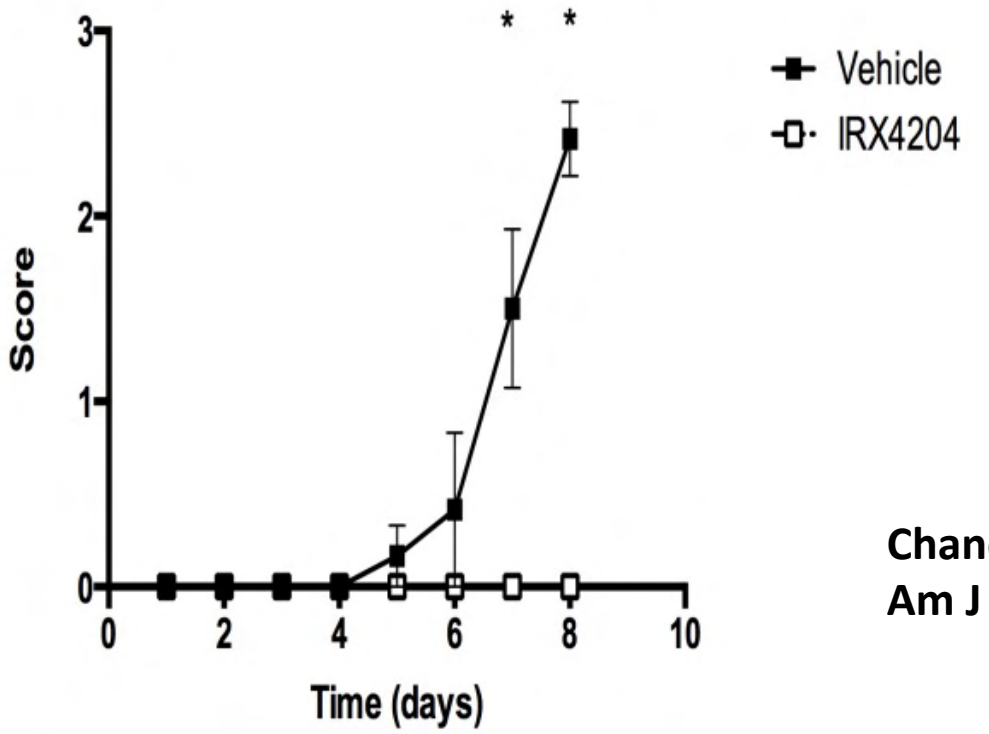
IRX4204 inhibits Th17-associated cytokine secretion by human T-cells cultured in Th17 skewing conditions



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 are 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 clinical utility of IRX4204 for treatment of these same and other IL-17 and TNF-α mediated diseases.

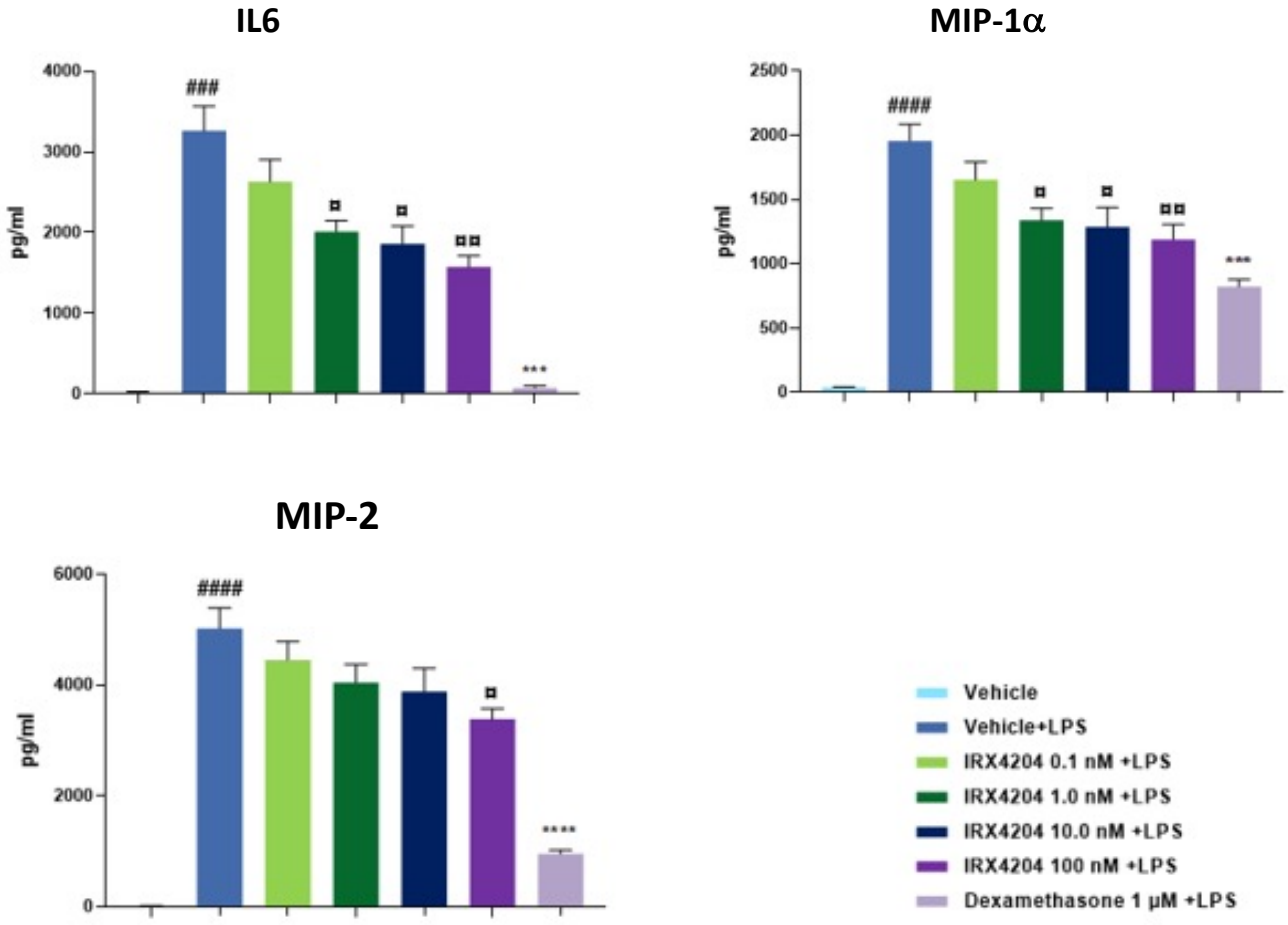
IRX4204 100% Prevented Th17-Mediated Autoimmune Disease *In Vivo* Mediated by Adoptively Transferred Antigen-sensitized Purified Th17 cells in a Mouse Model of Autoimmune Encephalomyelitis



Chandraratna, et al.
Am J Transl Res, 2016

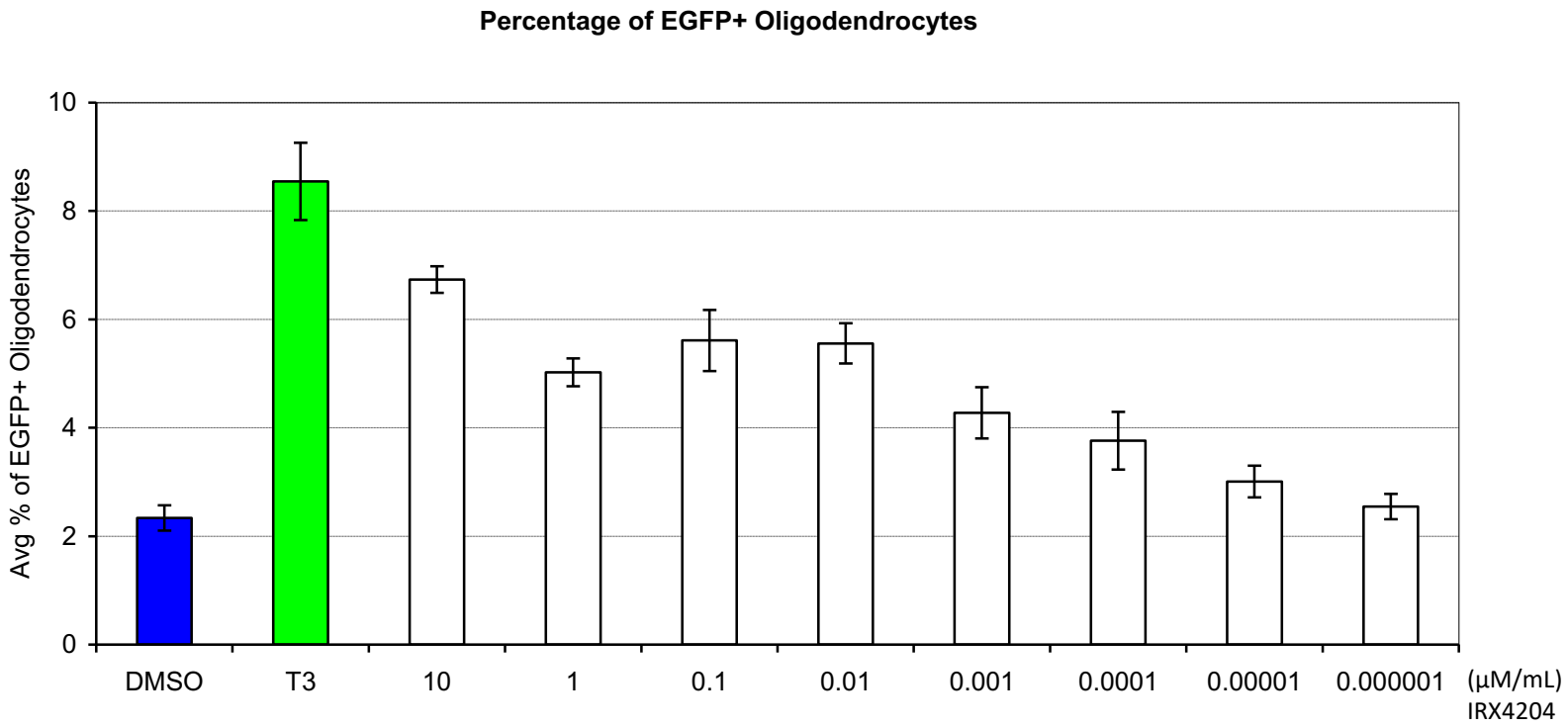
Mice were sensitized with Myelin Oligodendrocyte Glycoprotein. Purified Th17 cells were transferred to other mice that developed autoimmune encephalomyelitis.
Disease activity was scored using a standard assessment of limb motor functions.
IRX4204 statistically significantly inhibited in vivo transmission of autoimmunity by Th17 cells.

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



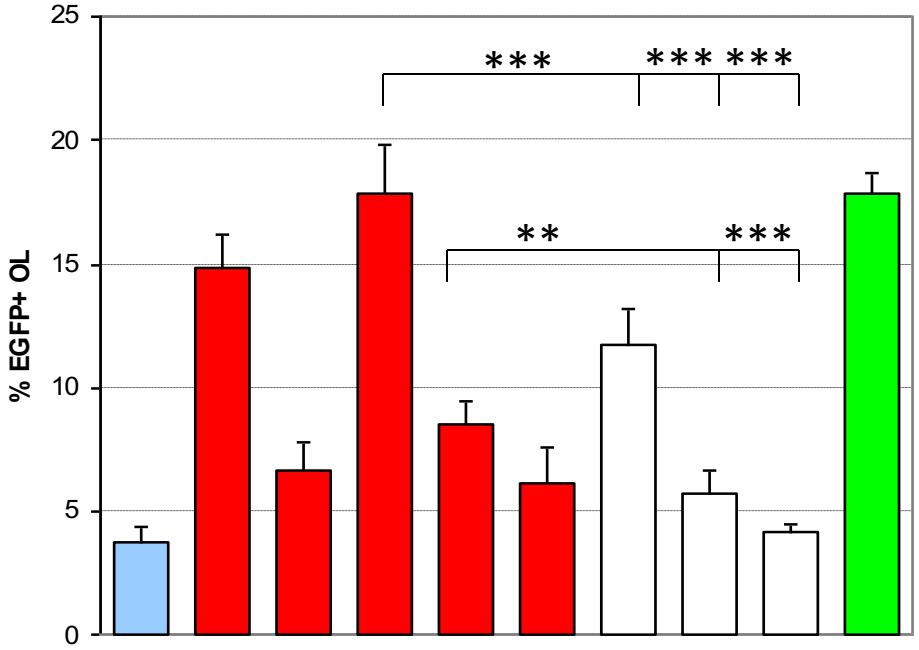
Cultures were 80% purified microglia, 20% astrocytes

IRX4204 Promotes Differentiation of Oligodendrocyte Precursor Cells into Myelin Producing Oligodendrocytes



IRX4204 and Thyroid Hormone T3
Have Additive or Synergistic Effects on OPC Differentiation in Vitro

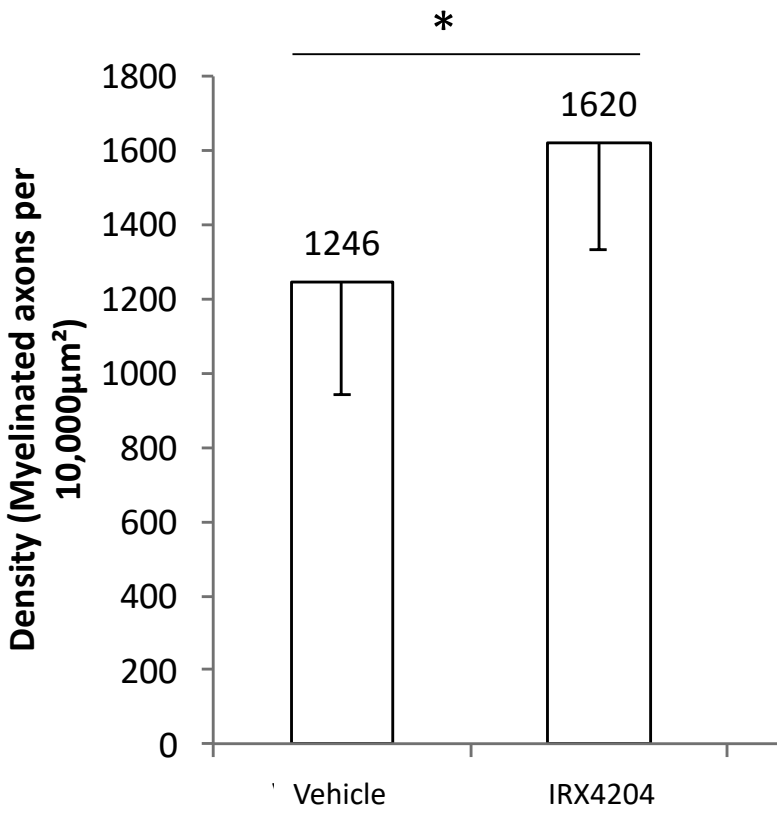
Percent of EGFP+ Oligodendrocytes

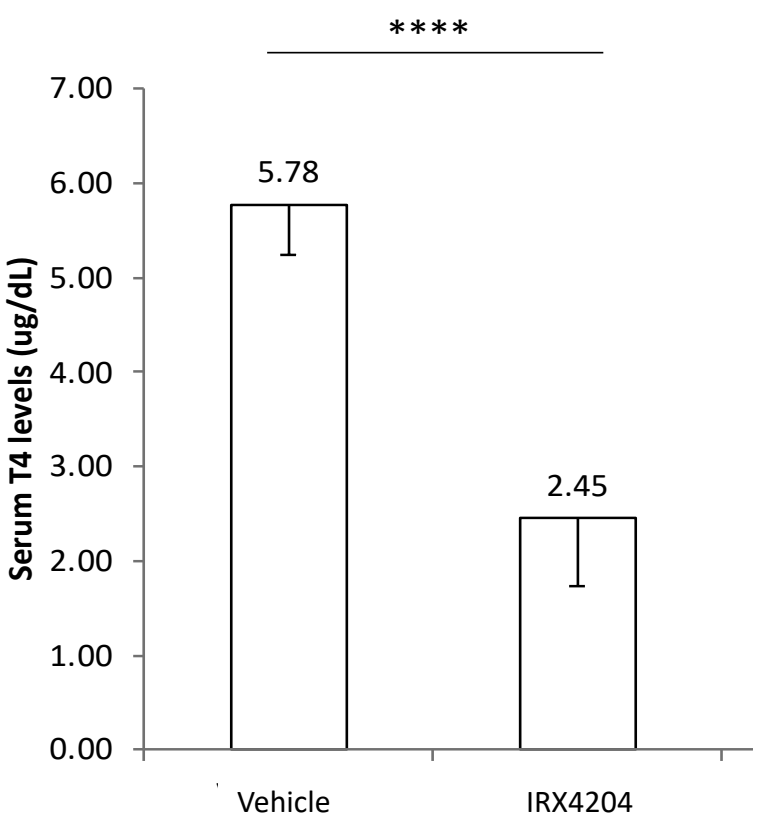


** P = < 0.01
*** P = < 0.0001

Col	2	3	4	5	6	7	8	9	10	11	
IRX	0	10uM	1nM				0	0	0		
T3	0	0	0	0.1	0.01	0.001	0.1	0.01	0.001	10	ng/ml

IRX4204 Promotes Remyelination in Mouse Model of Cuprizone-induced Chronic Demyelination

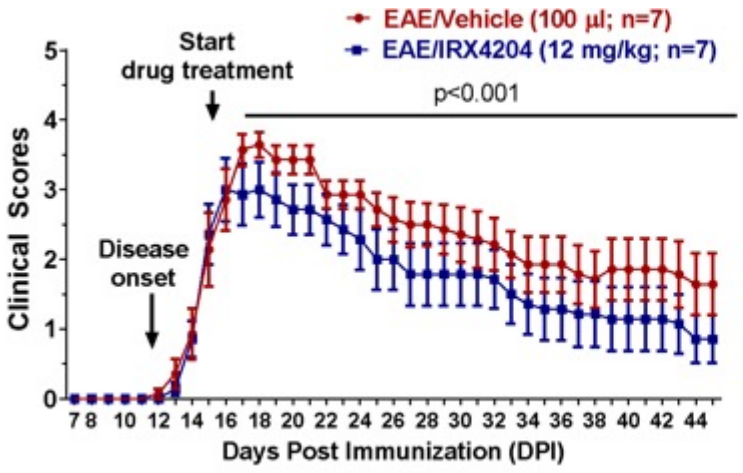




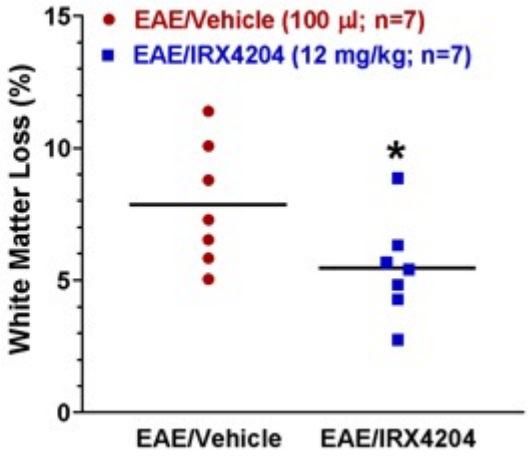
IRX4204 treatment resulted in a 30% statistically significant increase in myelin relative to vehicle ($p<0.05$), notwithstanding inducing highly significant hypothyroidism.
Study done at Renovo Neural, Inc.

IRX4204 Reduces Myelin Loss in MOG-Induced EAE

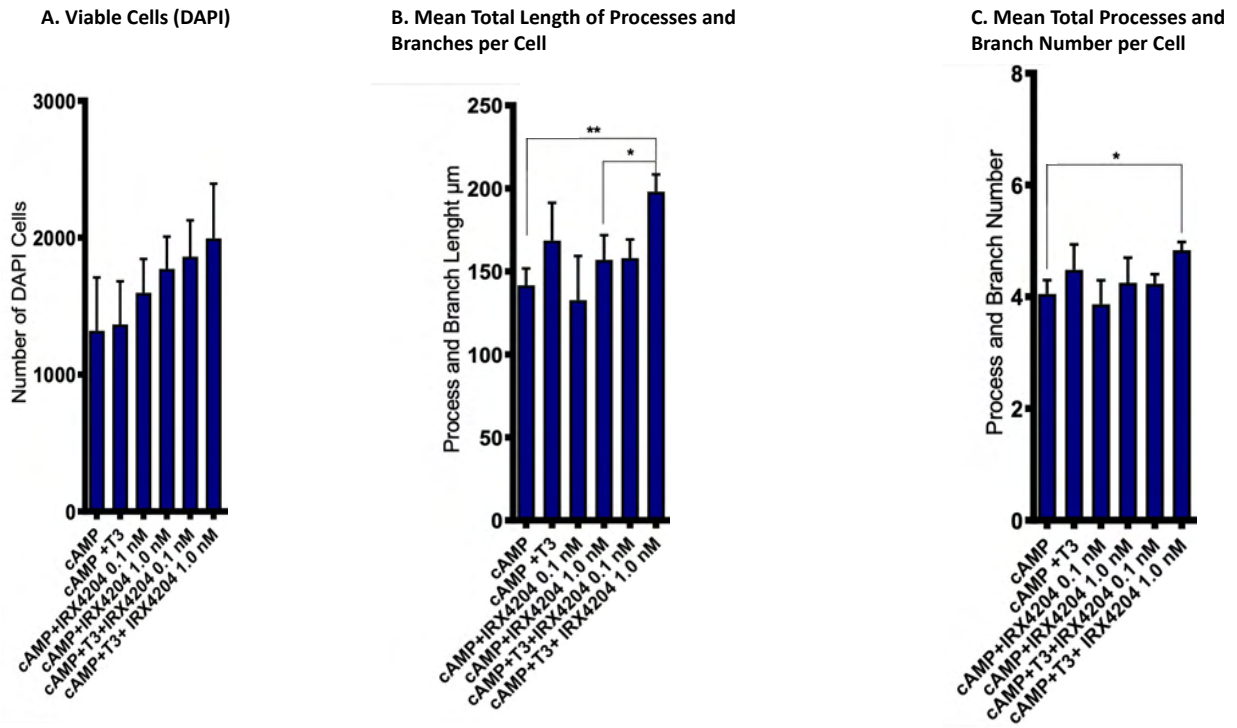
IRX4204 Reduces EAE Disease Severity



IRX4204 Reduces Spinal Cord White Matter Loss in EAE Mice

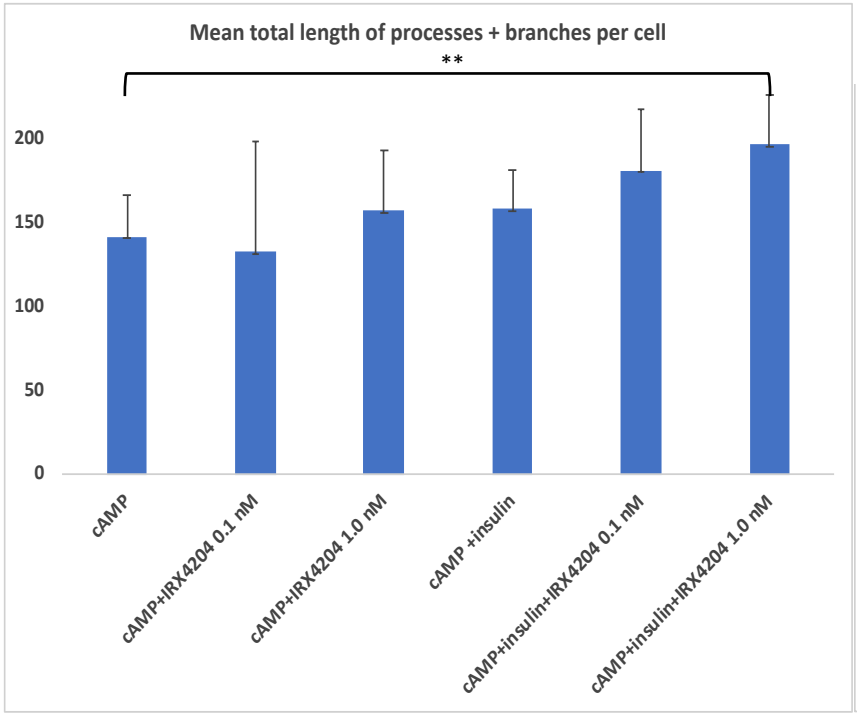


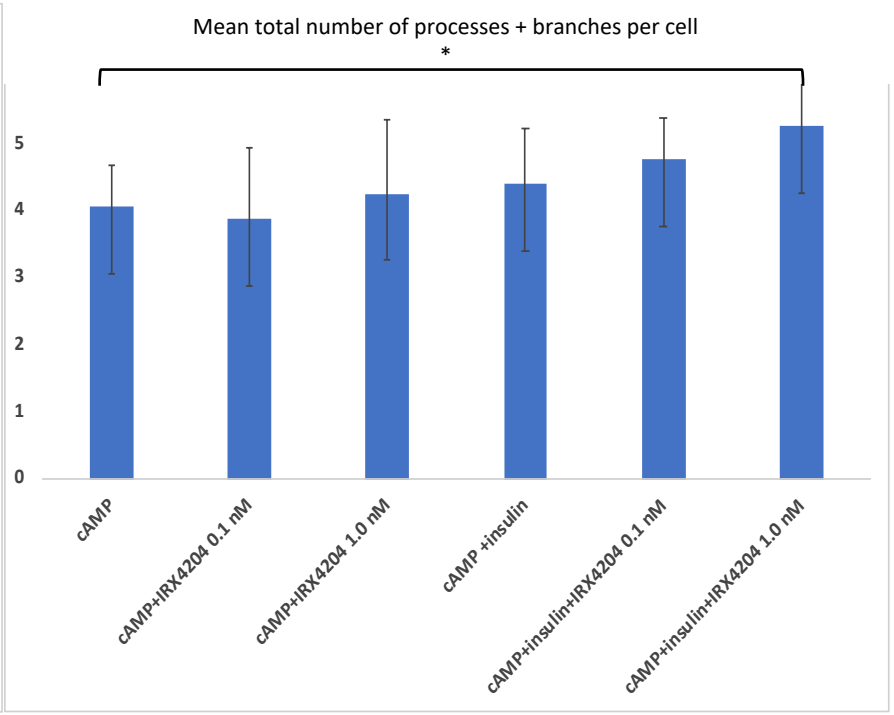
IRX4204 + T3 Have Combination Effects on Rat Cortical Neuron Survival and Neurite Outgrowth in Vitro



Data from 48 hour cultures of rat cortical neurons, supplemented with cyclic adenosine monophosphate (cAMP) as a metabolic energy source. DAPI is a stain that identifies viable cells. Panel A shows that IRX4204 alone, and in combination with thyroid hormone, increased mean survival of treated cortical neurons. Panels B and C show data from an automated imaging and quantitation system for assessing outgrowth of neurites. The combination of IRX4204 plus thyroid hormone statistically significantly increased the length of neurites relative to cAMP alone (**p<.01); and IRX4204 alone (*p<.05); and increased the total number of neurites per cell relative to cAMP alone (*p<.05).

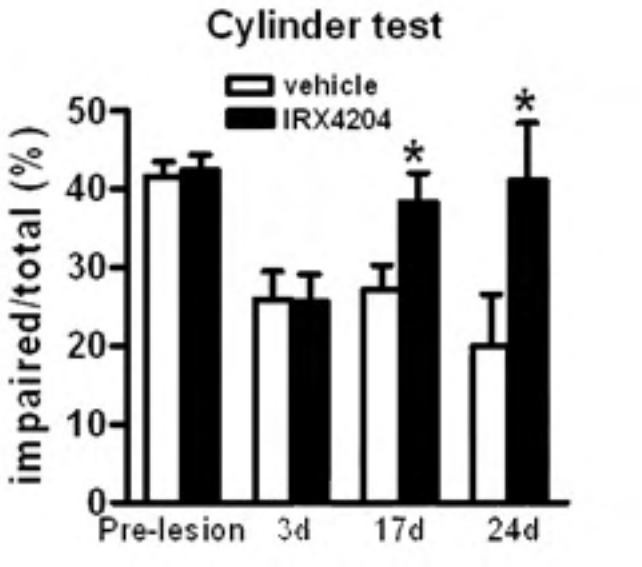
IRX4204 + Insulin Have Combination Effects on Rat Cortical Neuron Neurite Outgrowth in Vitro





Data are means ± SEM, N=6 per group.
Statistical significance (Welch's t-test): ** p< 0.01, * p< 0.05.

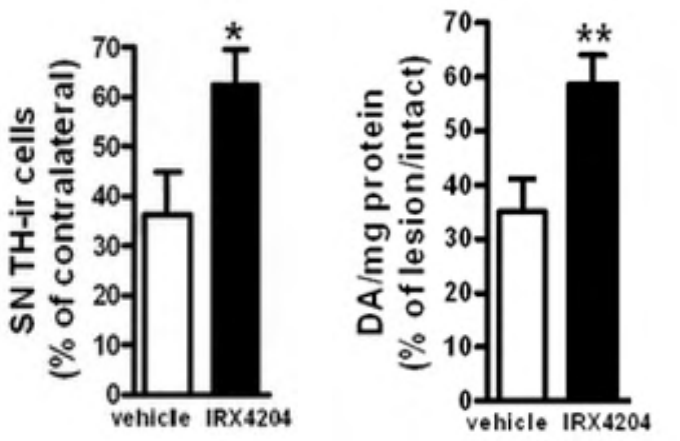
IRX4204 Reverses Asymmetric Impairment in Limb Usage Induced by 6-OHDA Lesions in Rat PD Model



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

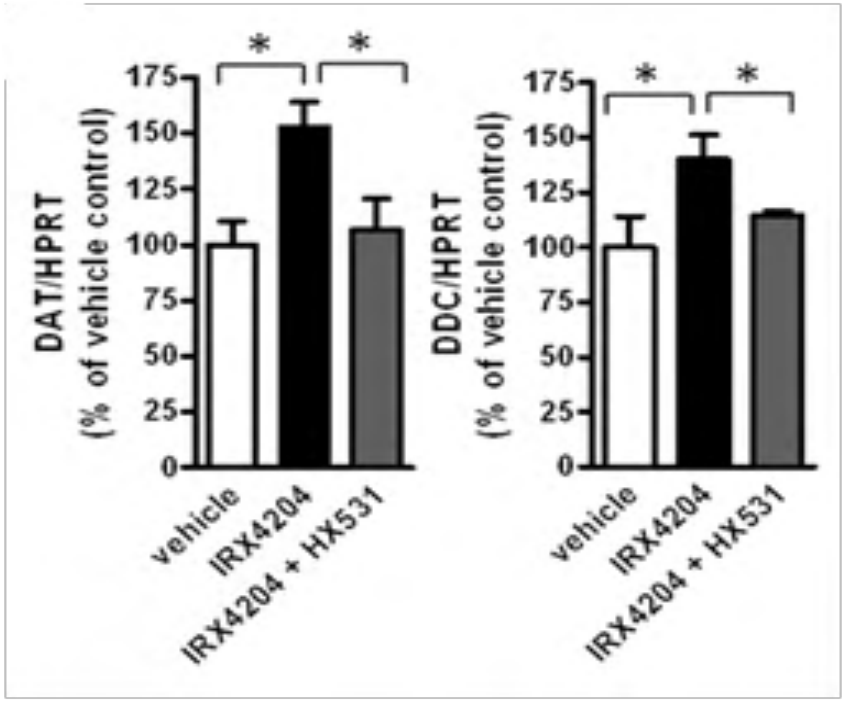
Detects forelimb impairments, specifically asymmetry in limb use for weight bearing movements when leaning on walls of glass cylinder during exploration of a novel environment. Compares use of the affected side to the unaffected side, therefore each animal serves as its own control.

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

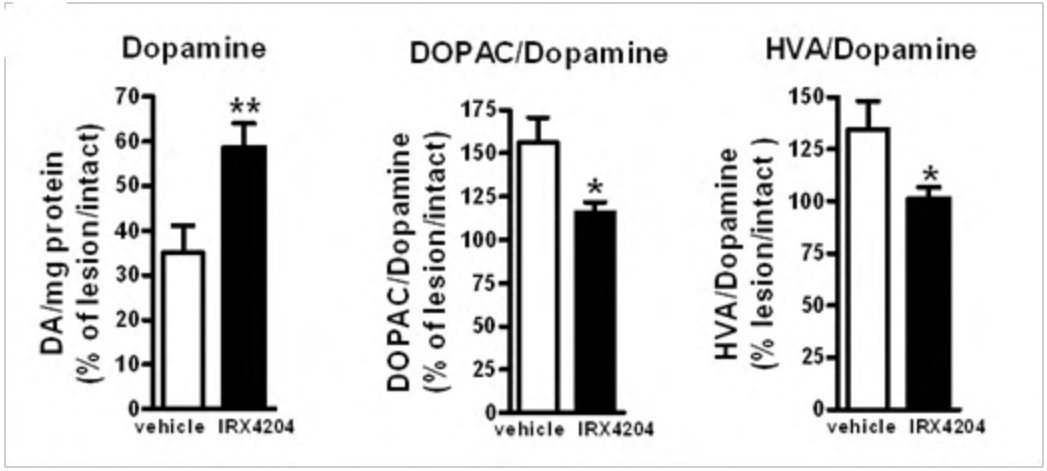


Rats were stereotaxically injected with 6-OHDA (40µg in 2µl) into right striatum. Rats were treated with IRX4204 (10mg/kg/d) or vehicle by oral gavage for 21 days starting 3 days post-lesion.

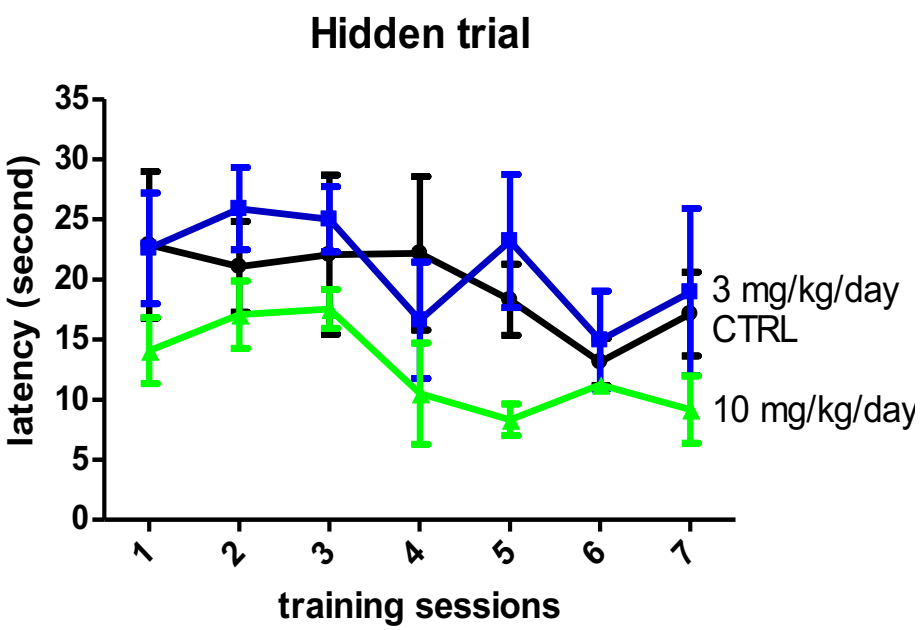
IRX4204 Induces Expression of Nurr1/RXR Regulated Genes DAT and DDC in Cultured Primary Ventral Midbrain Neurons in an RXR Dependent Manner

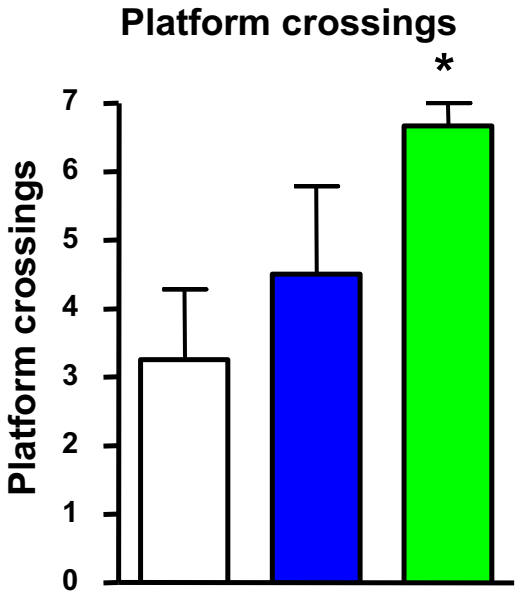


IRX4204 Treatment Reduces Loss of Dopamine and Reduces Dopamine Catabolism in 6-OHDA Lesioned Rats

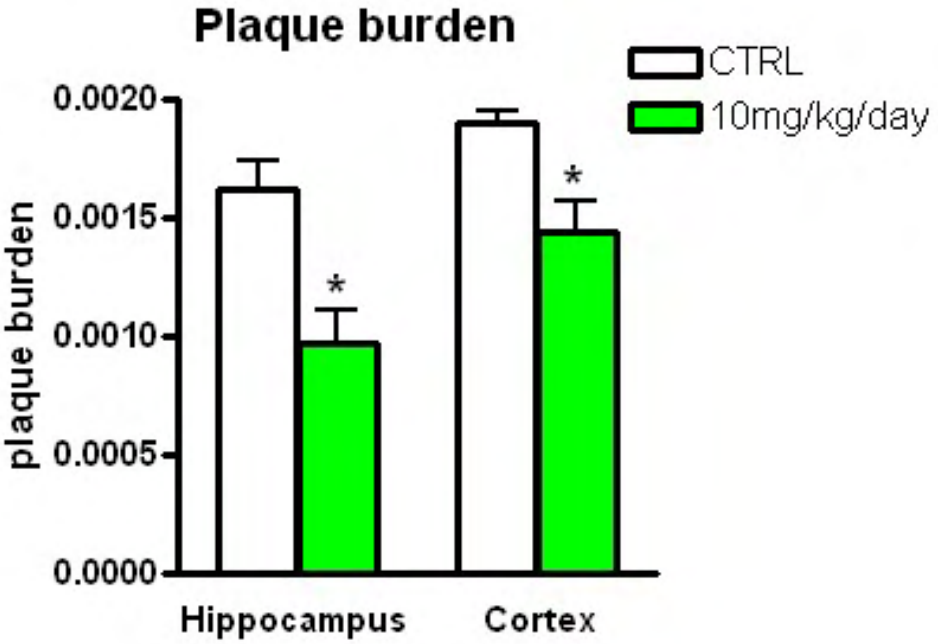


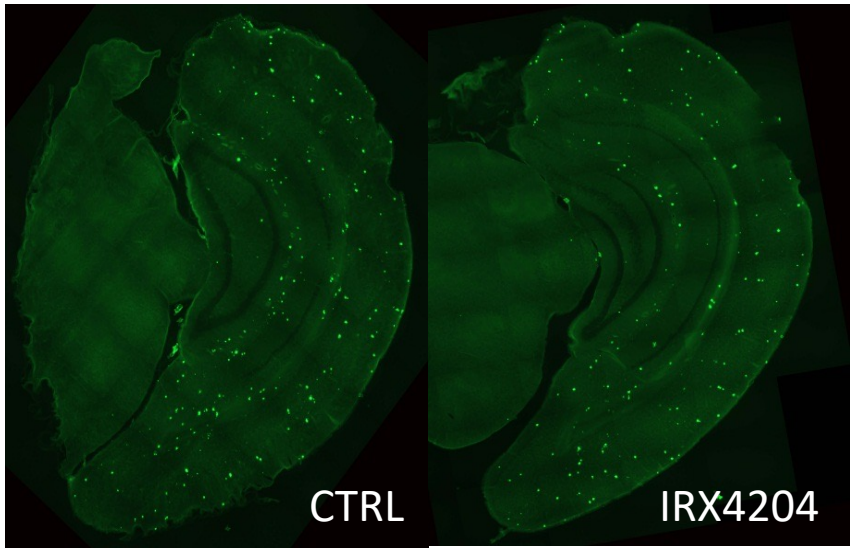
Oral Administration of IRX4204 Attenuates Cognitive Deterioration in TgCRND8 AD Model in Mice as Assessed by Morris Water Maze Test





Oral Administration of IRX4204 Attenuates Amyloid Neuropathology in TgCRND8 AD Model in Mice





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 clinical trial drug supply API is completed. Manufacture of drug and placebo capsules for Phase II trials completed.
- Planning phase II clinical trials in PD, psoriasis, and Her2+ breast cancer.

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

- **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.**

Conclusions: IRX4204 for Treatment of Normal Aging-related Neurodegeneration

- **IRX4204 promotes differentiation and growth of human Tregs, and inhibits differentiation of human Th17 cells, while reducing production of IL-17 and TNF.**
- **IRX4204 inhibits production of IL-6 and other pro-inflammatory factors by microglia.**
- **IRX4204 promotes differentiation and growth of myelin-producing oligodendrocytes *in vitro* and promotes myelinated nerve fiber protection and repair (remyelination) in mouse models of acute axonal loss and chronic demyelination.**
- **IRX4204 has direct effects *in vitro* on cortical neurons of promoting neurite outgrowth, alone and in combination with insulin, or thyroid hormone.**
- **These findings support the potential for IRX4204 protecting against or even reversing neuro-inflammaging-induced chronic loss of myelinated nerve fibers and neuronal damage in normal aging-related neurodegeneration.**
- **Nose to brain administration of IRX4204 in combination with insulin or other neurotrophic factors, and thyroid hormone may be a useful embodiment for prevention or treatment of normal aging-related neurodegeneration.**

Conclusions: IRX4204 for Treatment of PD or AD

- **IRX4204 demonstrated beneficial effects *in vivo* studies in rodent models of PD, and AD.**
- **IRX4204 reduced motor deficits in PD models and increased dopaminergic neuron survival.**
- **IRX4204 decreased deposition of new beta amyloid in the brains of beta amyloid transgenic mice while preserving memory functions.**
- **IRX4204 demonstrated safety and tolerability of oral dosing in phase I and II clinical trials in 85 patients with various cancers and 15 patients with PD for up to 20 months.**
- **IRX4204 demonstrated brain penetrance, and improvement of motor functions of 13/15 PD patients in an open-label phase I/II clinical trial.**
- **The company is planning to initiate a placebo-controlled phase II RCT of IRX4204 in PD patients in Q4 of 2024.**
- **IRX4204 provides opportunities for future development of combination treatments with other generally well-tolerated and already available agents, such as insulin, GLP-1 agonists, other neurotrophic factors, thyroid hormone, or anti-beta amyloid monoclonal antibodies.**

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

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