IRX4204: A Phase II Clinical Stage Third Generation RXR Nuclear Receptor Agonist for Treatment of Alzheimer's Disease and Other Dementias

Io Therapeutics, Inc.

Martin E. Sanders, M.D.
Chief Executive Officer

Vidyasagar Vuligonda, Ph.D.
Chief Science Officer

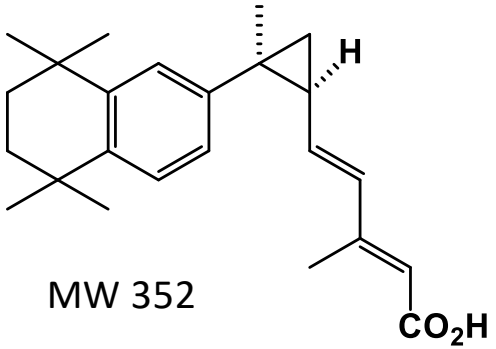
Executive Summary: IRX4204 for Treatment of AD and Other Dementias

- IRX4204 has multiple effects on CNS cells: neurons, microglia, oligodendrocytes, astrocytes, T-cells.
- IRX4204 activates RXR heterodimers with Nurr1 and Nur77, more potently than bexarotene.
- IRX4204 decreases beta amyloid expression in primary cultured mouse neurons more than bexarotene.
- IRX4204 Increases ApoE expression in primary cultured mouse neurons more than bexarotene.
- Like bexarotene, IRX4204 decreased deposition of beta amyloid in the brains of an Aβ transgenic mouse model of AD, while preserving cognitive functions.
- IRX4204 Increases mouse cortical neuron mitochondrial respiration.
- IRX4204 promotes *in vitro* cortical neuron survival and differentiation with increased neurite outgrowth, alone and in combination with insulin or thyroid hormone.
- IRX4204 decreases aggregation of beta amyloid *in vitro,* more than bexarotene.
- IRX4204 decreases beta amyloid expression in primary cultured mouse neurons more than bexarotene.
- IRX4204 inhibits rat microglia and mouse macrophage production of IL-6, nitric oxide, MIP-1α, MIP-2.
- IRX4204 Inhibits expression of IL-1β, TNFα, and iNOS *in vitro* in mouse astrocytes.
- IRX4204 promotes differentiation of human Tregs and inhibits differentiation of human Th17s *in vitro*.
- IRX4204 inhibits IL-17 A and F, TNF α and β, and IL-9 production *in vitro* by human Th17 cells.
- IRX4204 promotes oligodendrocyte differentiation *in vitro* and myelin repair *in vivo*.
- IRX4204 demonstrated safety and tolerability of oral dosing in phase I and II clinical trials. in 85 patients with various cancers for up to 20 months, and up to 28 days in 15 early PD patients.
- IRX4204 presents opportunities for combination treatments with other agents for AD and other dementias, including nasal inhaled insulin, GLP-1 agonists, and anti-β amyloid antibodies.

Bexarotene: A Second Generation Less Potent and Less Selective RXR Agonist Has Unacceptable Toxicity for Chronic Treatment of Neurodegenerative Diseases, But Provided Preliminary Clinical Evidence in RCTs Supporting Efficacy of RXR Agonism for Treatment of AD, MS, Brain Aging, and Schizophrenia

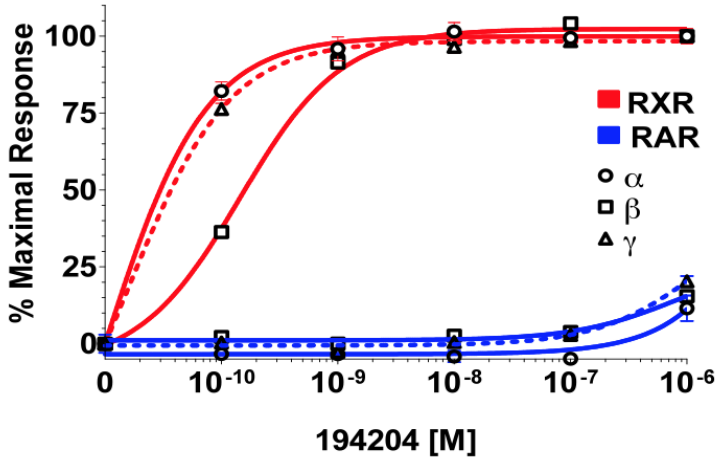
- Bexarotene is relatively selective for RXR, but has substantial RAR, LXR, and PPARγ off-target agonist activities associated with toxicities, including RAR skin rashes; and higher lipid elevations than observed with IRX4204.
- It is 100x less potent than IRX4204 as an RXR agonist.
- FDA approved for oral or topical treatment of CTCL, now available as a generic drug.
- Inhibits deposition and clears brain of β-amyloid in Aβ transgenic mice while preserving or improving cognitive functions.
- Reduced brain β-amyloid in PET scans in APOE4 non-carrier AD patients without cognitive improvement in a four-week RCT.
- Durably repaired visual tract demyelination in RRMS patients in a six-month RCT.
- Reversed MRI appearance of brain aging by 11 months over 6 months of treatment in the RRMS RCT.
- Decreased positive symptoms of schizophrenia in a six-week RCT.
- Like IRX4204, bexarotene has potent anti-inflammatory, anti-autoimmune, and myelin reparative activities.
- Bexarotene is too toxic for chronic treatment of AD but has provided clinical evidence supporting potential utility of a more potent, more selective RXR agonist, such as IRX4204, for treatment of AD and other chronic CNS diseases.

IRX4204: A Third Generation Highly Potent and Highly Selective RXR Agonist Compound

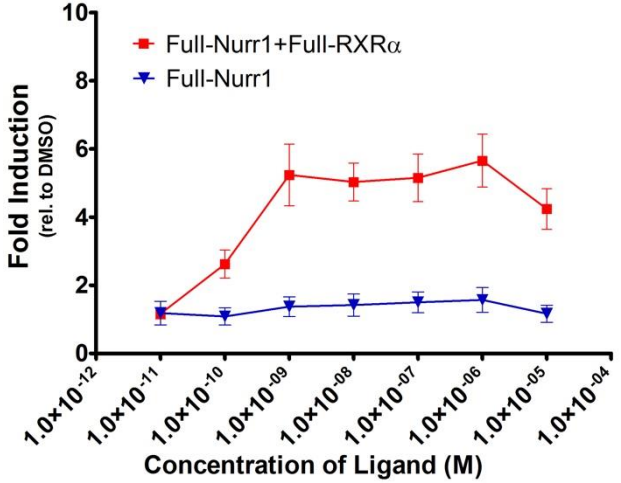


IRX4204 was discovered by Dr. Sagar Vuligonda at Allergan Pharmaceuticals in a diabetes drug discovery program. It is effective in the db/db mouse model of T2DM and is an insulin sensitizer in the liver and brain. It has two chiral centers and one pharmacologically active stereoisomer.

IRX4204 is a Potent RXR Homodimer Agonist with no RAR Activity at nM Concentrations

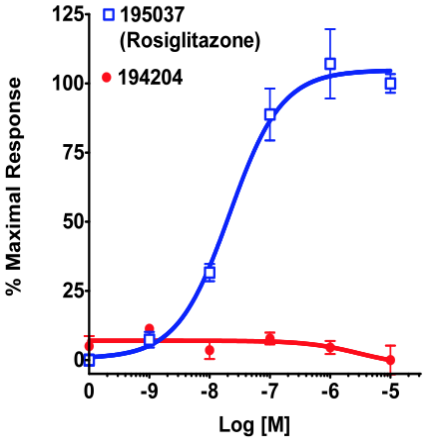


IRX4204 Transactivates Nurr1/RXR Heterodimers through RXR

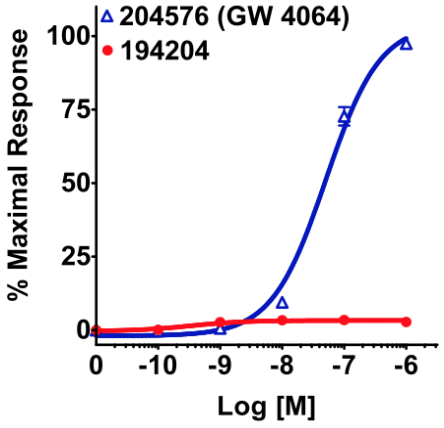


Wang et al,
Oncotarget 2016

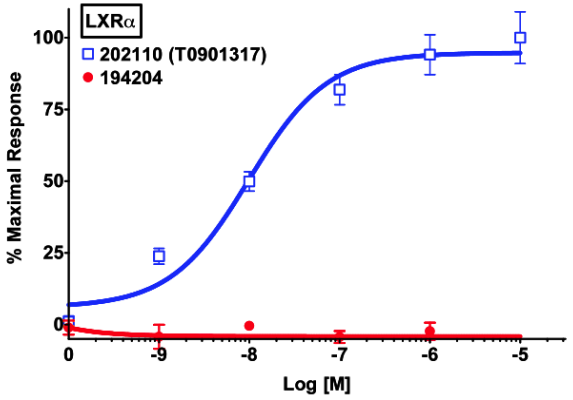
IRX4204 is Highly Selective for RXRs
It Does Not Transactivate PPARγ, FXR, or LXRs



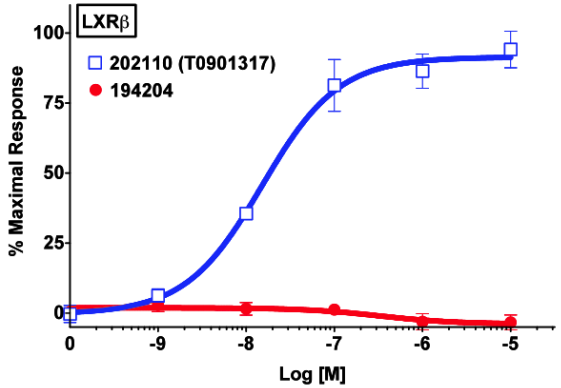
PPARγ/RXR



FXR/RXR



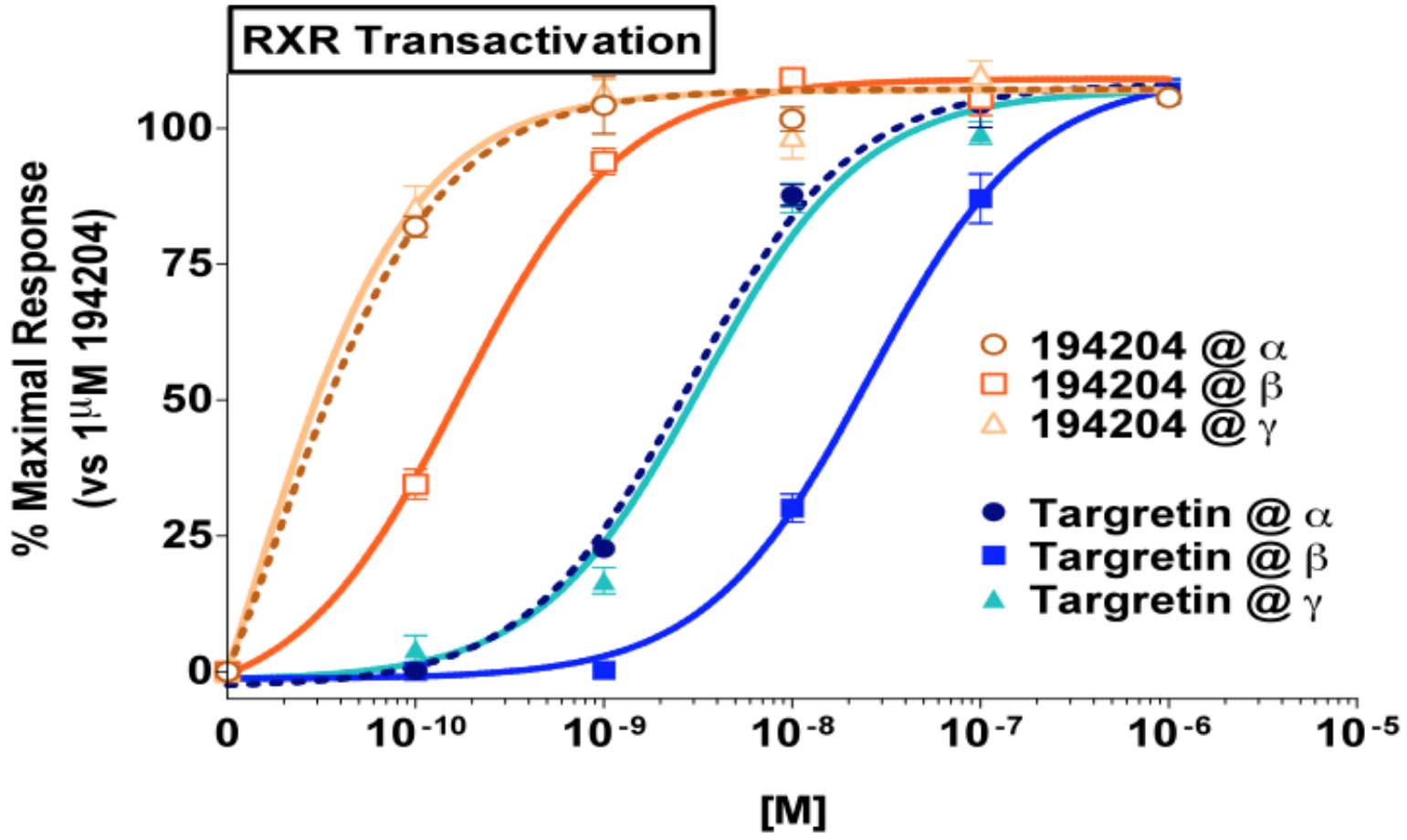
LXR/RXR



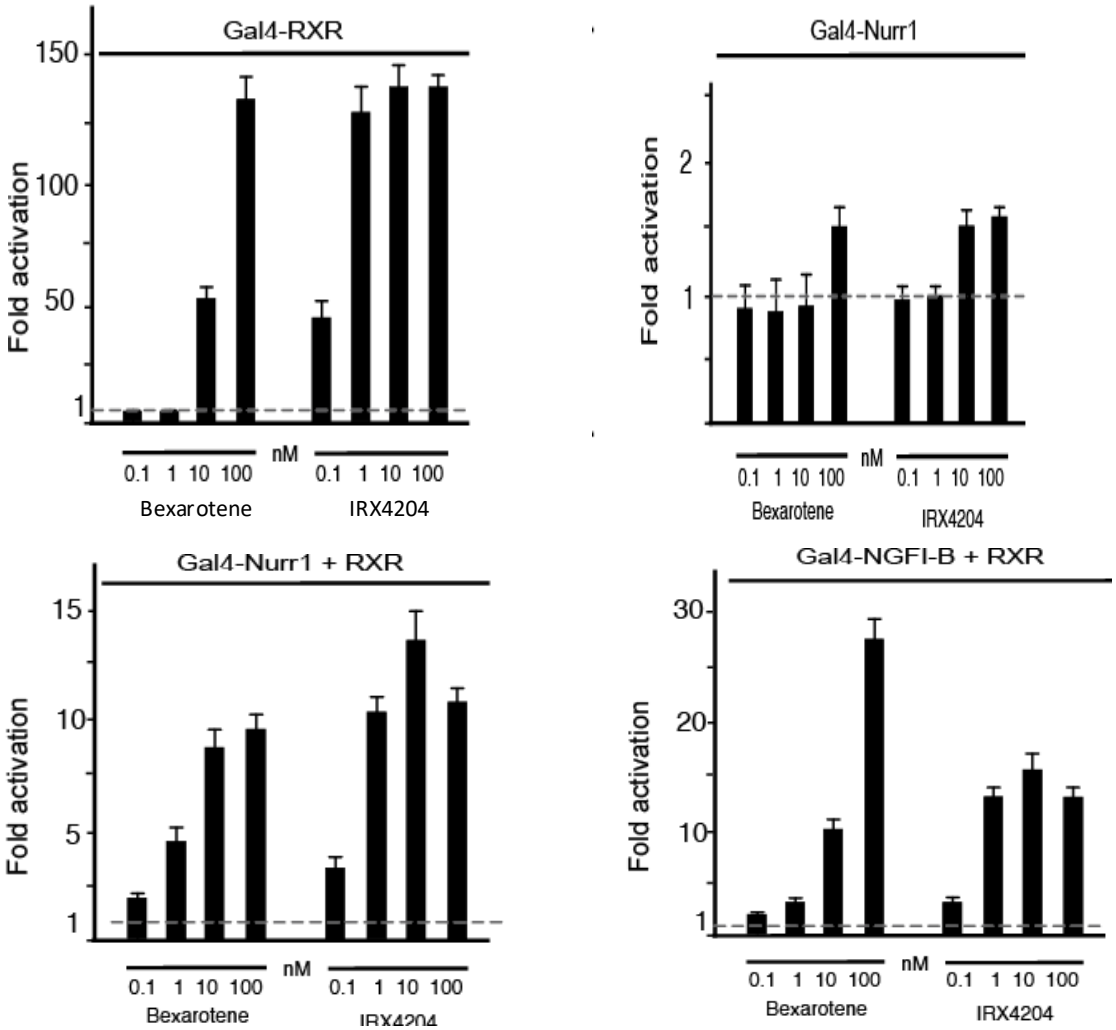
LXR/RXR

Wang et al,
Oncotarget 2016

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

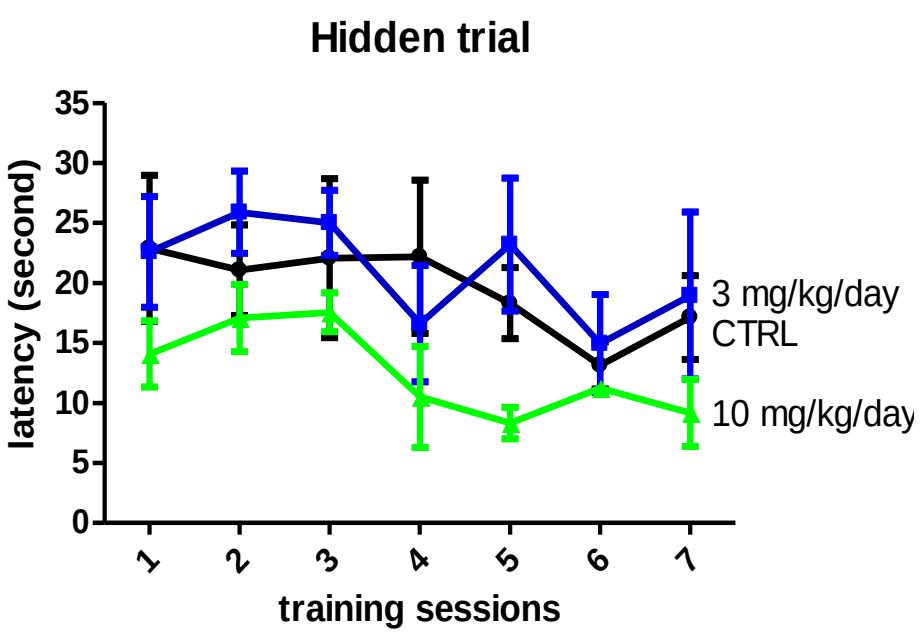


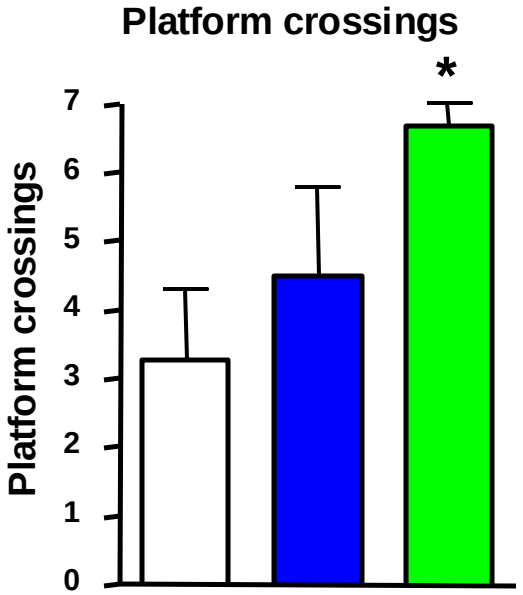
Reporter Assays with RXR Ligands Demonstrate IRX4204 is More Potent Than Bexarotene for Activation of RXR, Nurr1 (NR4A2), and Nur77 (NR4A1, NGFI-B)



Data provided by Thomas Perlmann
Karolinska Institute, Stockholm, Sweden

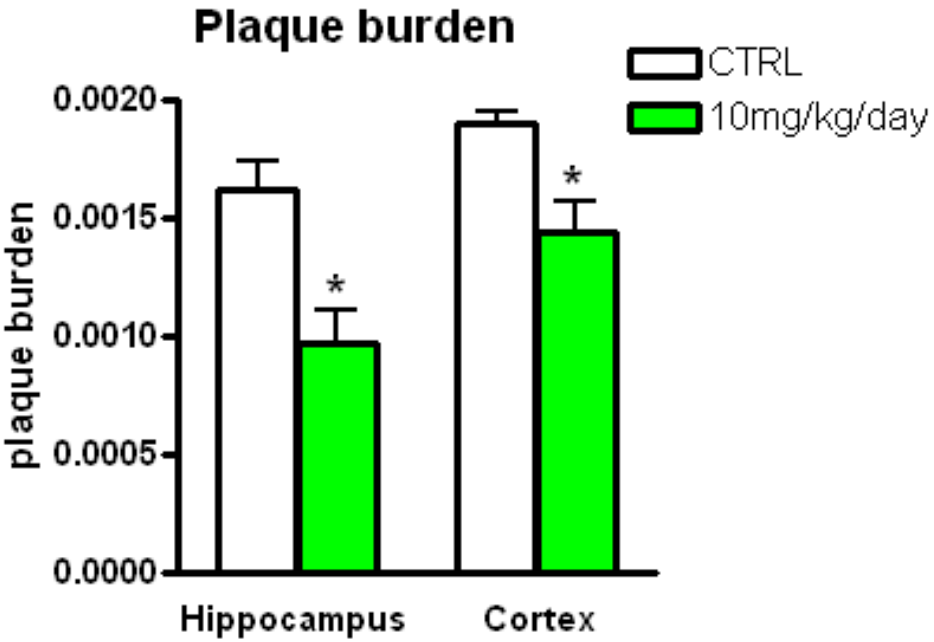
Oral Administration of IRX4204 Attenuates Cognitive Deterioration in TgCRND8 AD Model in Mice as Assessed by Morris Water Maze Test

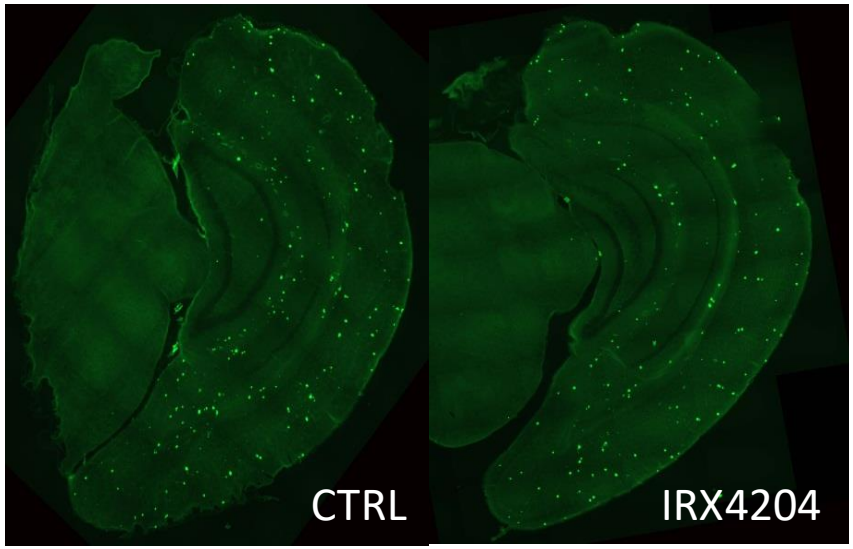




Data provided by Giulio Pasinetti, *et al*
Icahn School of Medicine at Mount Sinai

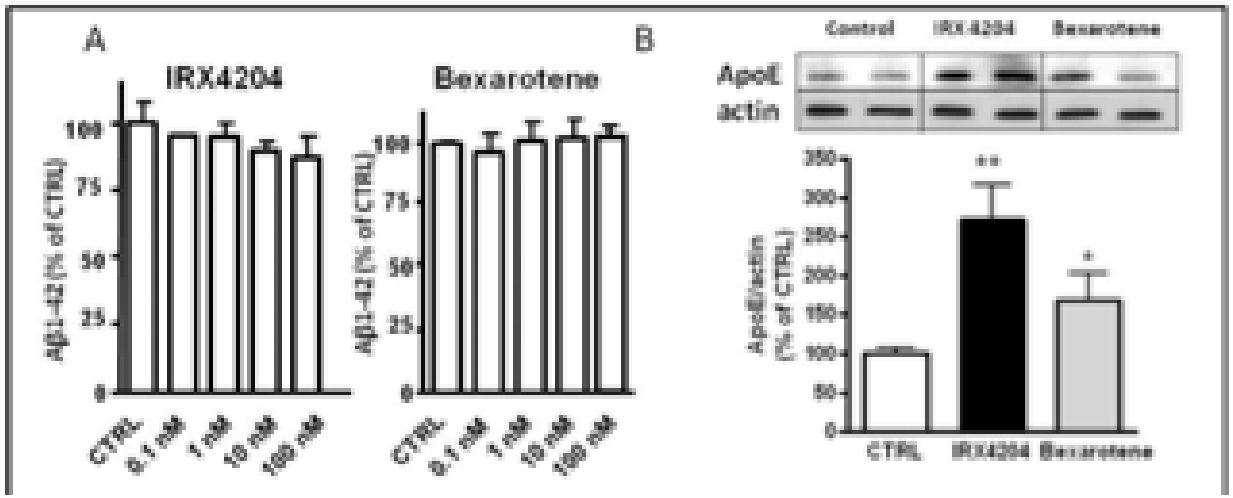
Oral Administration of IRX4204 Attenuates Amyloid Neuropathology in TgCRND8 AD Model in Mice





Data provided by Giulio Pasinetti, *et al*
Icahn School of Medicine at Mount Sinai

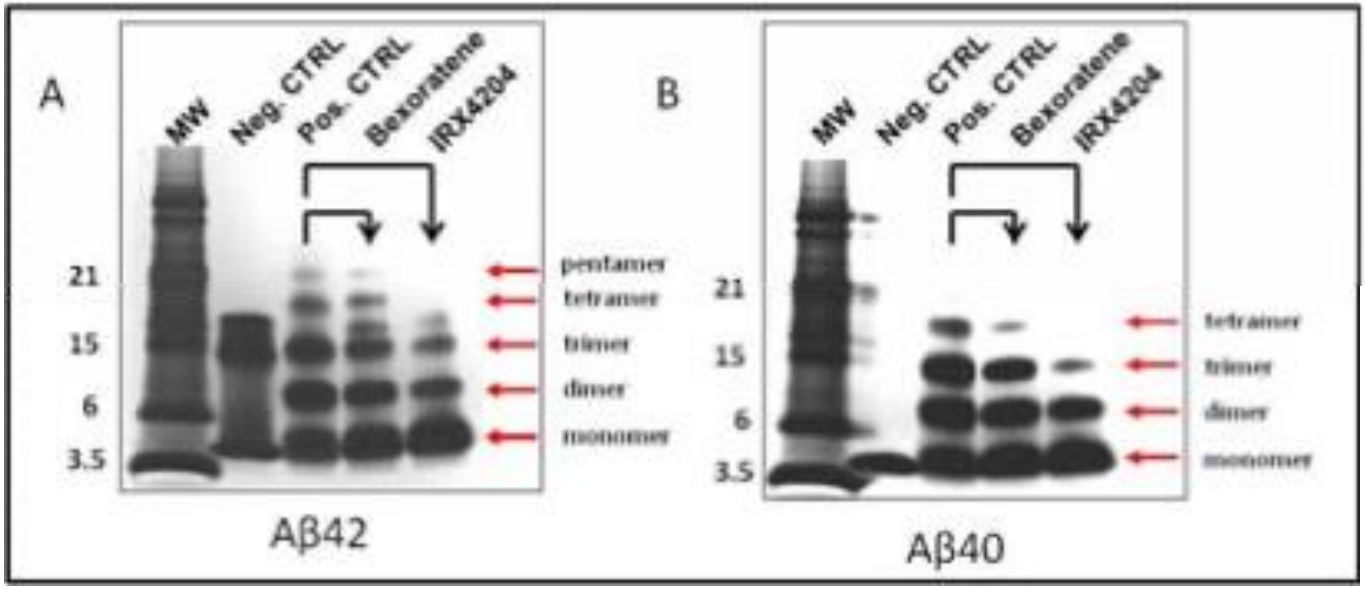
IRX4204 Decreased Beta Amyloid Expression and Increased ApoE Expression in Primary Cultured Mouse Neurons More Than Was Observed with Bexarotene



Beta Amyloid peptide was assayed in cultured cortico-hippocampal neurons from heterozygous TG2576 transgenic mouse primary neurons after 16 hours of culture in indicated concentrations of IRX4204 or bexarotene (Panel A). Primary cortico-hippocampal neurons from C57B6 wild type mice were cultured with 100 nM of IRX4204 or bexarotene, and total proteins were isolated and examined by Western Blot analysis for ApoE expression.

Data provided by Giulio Pasinetti , *et al*
Icahn School of Medicine at Mount Sinai

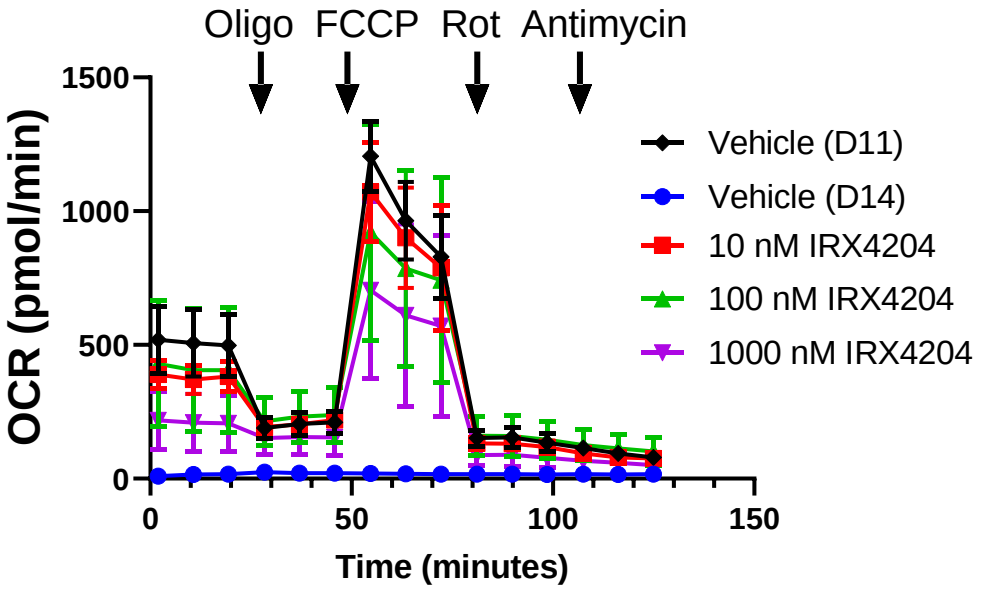
IRX4204 Interferes with Beta Amyloid Peptide Oligomerization in Photo-induced Crosslinking of Unmodified Proteins (PICUP) Assay

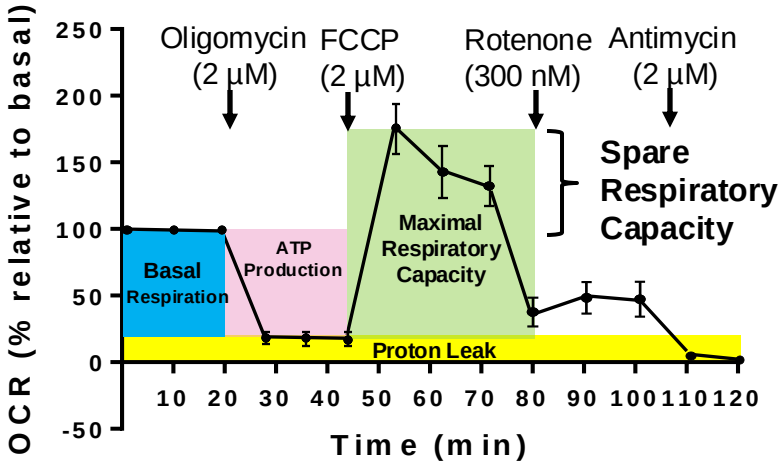


Silver stained SDS-PAGE of Beta Amyloid peptides photo cross-linked in the presence or absence of 4X molar excess of test compounds. Positive controls are cross-linked aggregated Beta Amyloid peptides. Negative controls are non-cross-linked Beta Amyloid peptides.

Data provided by Giulio Pasinetti, *et al*
Icahn School of Medicine at Mount Sinai

IRX4204 Preserves and Increases Mitochondrial Respiration in Cultured Murine Cortical Neurons *in Vitro* in Seahorse Assay

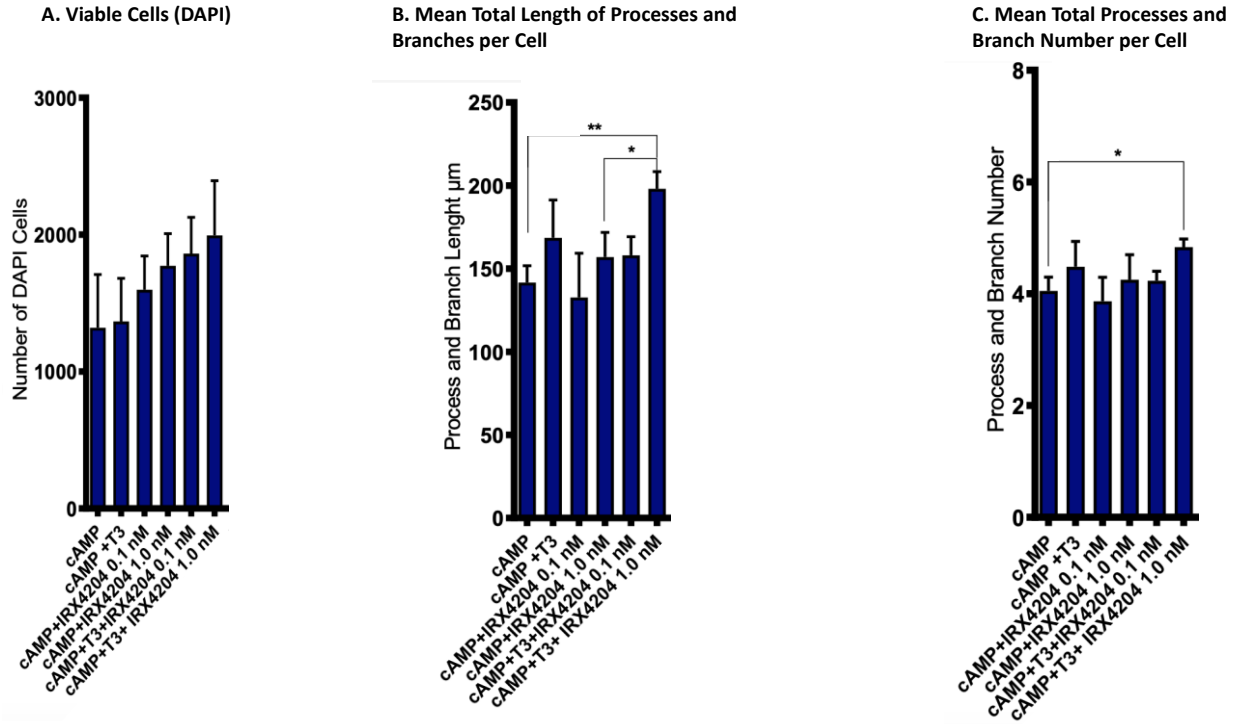




IRX4204 prevented the loss of mitochondrial respiration caused by culturing cortical neurons for a prolonged time (left panel). Relative to *in vitro* day 11 (D11) primary cultures of mouse cortical neurons treated with vehicle (0.01% DMSO), D14 cortical neurons showed a profound loss of mitochondrial respiration. Treatment with IRX4204 (10, 100 or 1000 nM) from D11-14 (72 hr drug treatment) prevented this loss of mitochondrial respiration. Relative to D14 vehicle treated cultures, IRX4204 elevated basal respiration, ATP production and spare respiratory capacity. Seahorse XF24 extracellular flux instrumentation was used to assess basal respiration, ATP production and spare respiratory capacity (right panel). Each data point and bar represent the mean ± SD for 6-8 biological replicates.

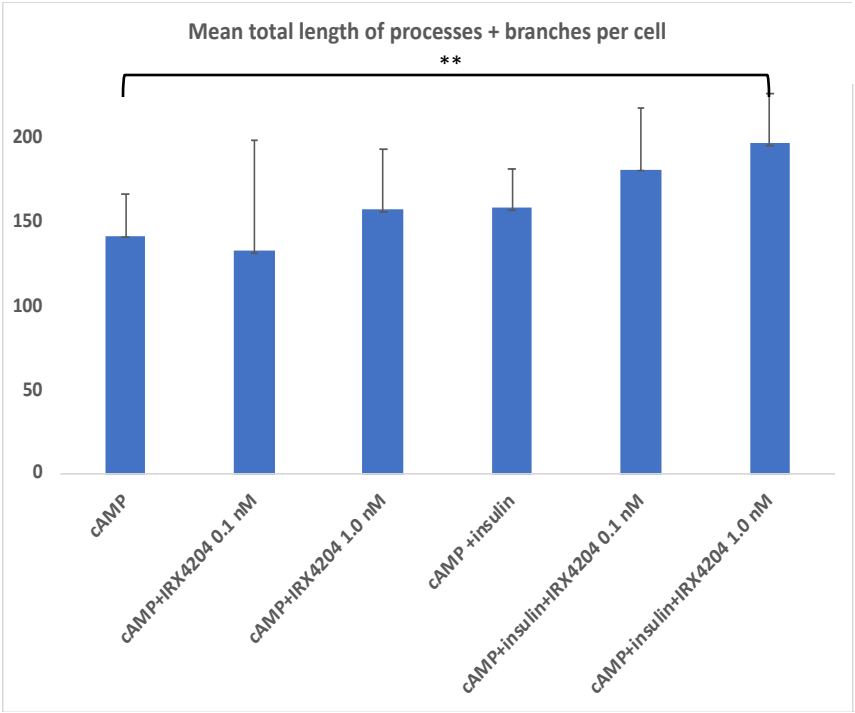
Data provided by George Robertson,
Dalhousie University, Halifax, Nova Scotia, Canada

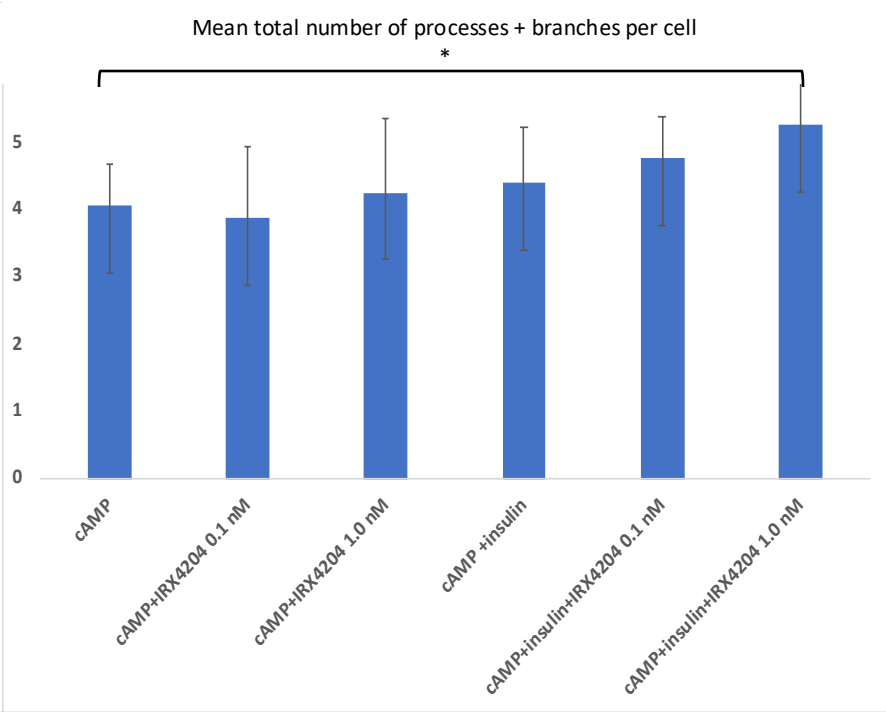
IRX4204 + T3 Have Combination Effects on Rat Cortical Neuron Survival and Neurite Outgrowth *in Vitro*



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).

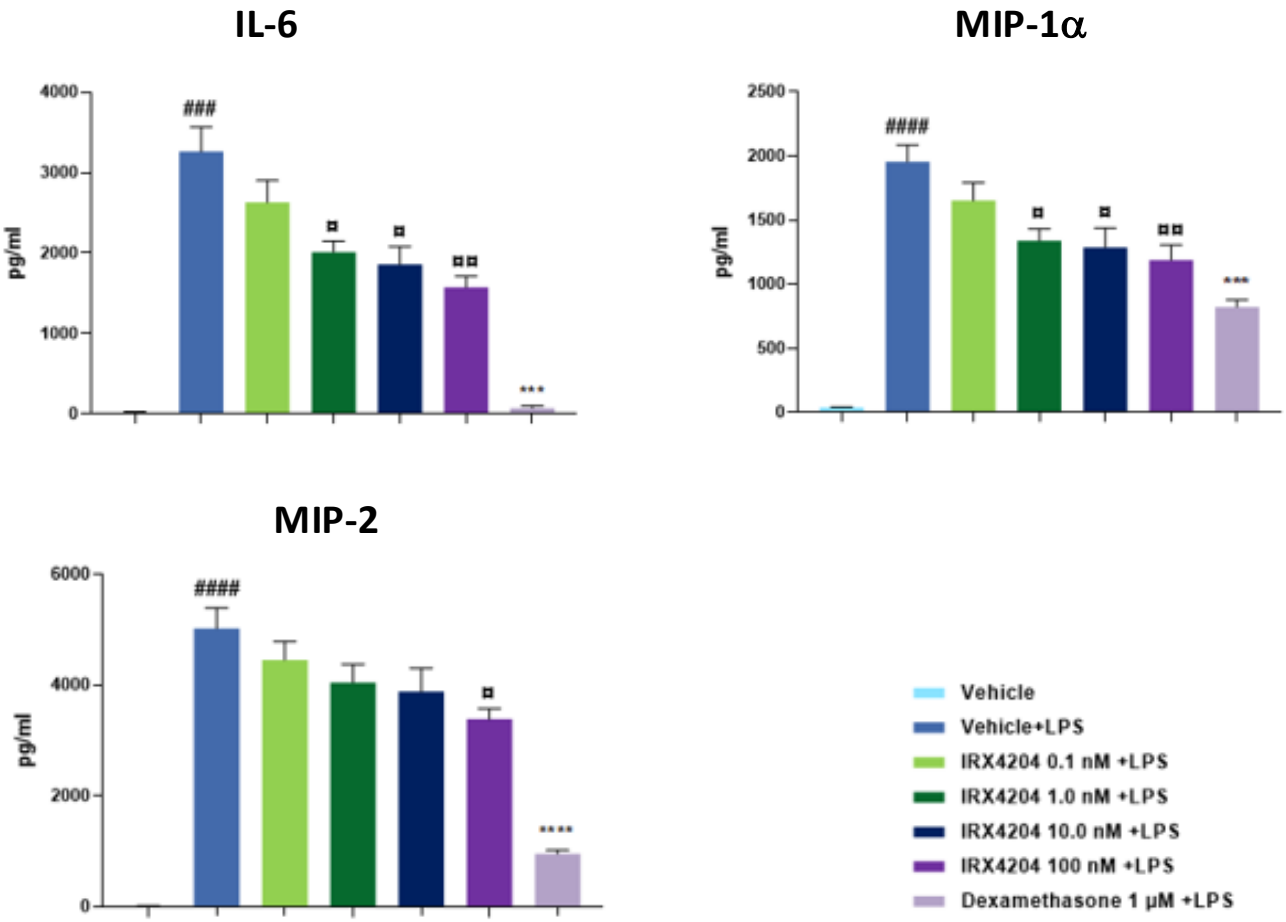
IRX4204 + Insulin Have Combination Effects on Rat Cortical Neuron Neurite Outgrowth *in Vitro*





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

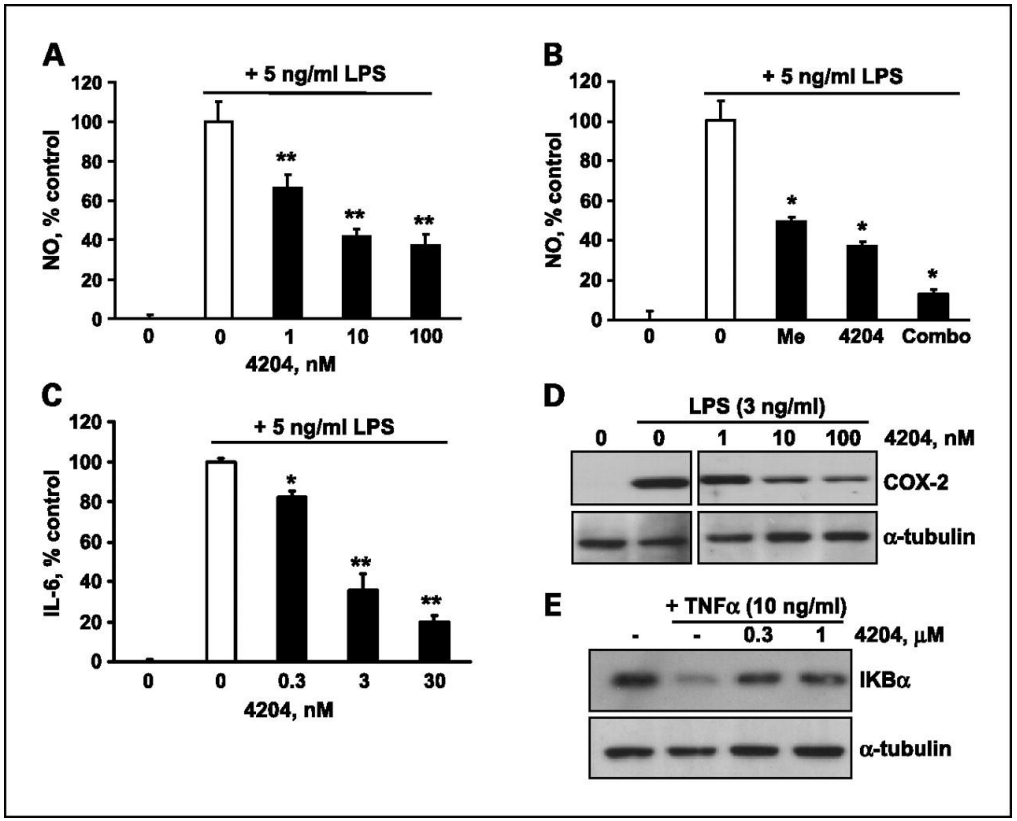
IRX4204 Inhibits LPS-induced Rat Cortical Microglial Production of IL-6 and Pro-inflammatory Chemokines



Cultures were 80% purified microglia, 20% astrocytes

Data provided by
Charles River Laboratories

IRX4204 Inhibits IL-6, Nitric Oxide, and COX-2 Production and Increases IKBα Production by a Murine Macrophage-like Cell Line

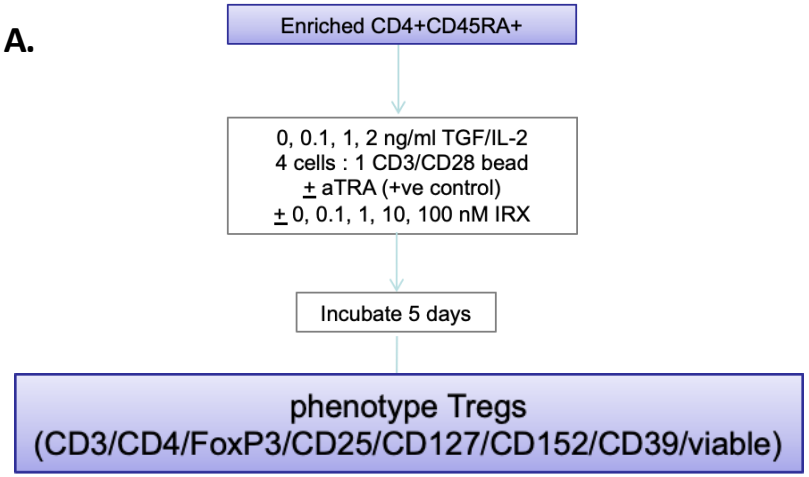


4204 inhibits the effects of lipopolysaccharide and tumor necrosis factor-α. RAW264.7 mouse macrophage–like cells were treated either with NRX194204 (4204) alone (A) or with 4204 (100 nmol/L) alone and in combination (B) with CDDO-Me (1 nmol/L) and then stimulated with lipopolysaccharide (LPS) for 24 h. The Griess reaction was used to measure nitric oxide in the medium. RAW cells were also treated with 4204 and lipopolysaccharide for 24 h (C and D), and either the amount of interleukin 6 released into the media was measured using an ELISA (C) or cell lysates were immunoblotted with cyclooxygenase-2 antibodies (D). E, RAW cells were treated with 4204 for 24 h, the cells were then stimulated with tumor necrosis factor-α for 15 min, and cell lysates were immunoblotted using IKBα antibodies. *, $P < 0.05$ versus control; **, $P < 0.001$ versus control.

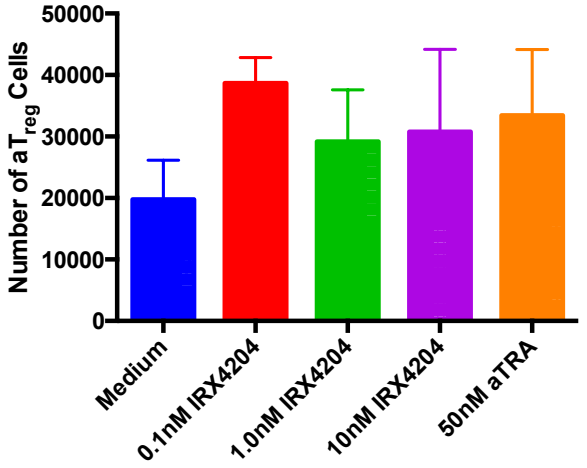
K Liby, et al.
Clin Cancer Res 2007

IRX4204 Promotes Human Treg Differentiation and Proliferation *In Vitro*

A.



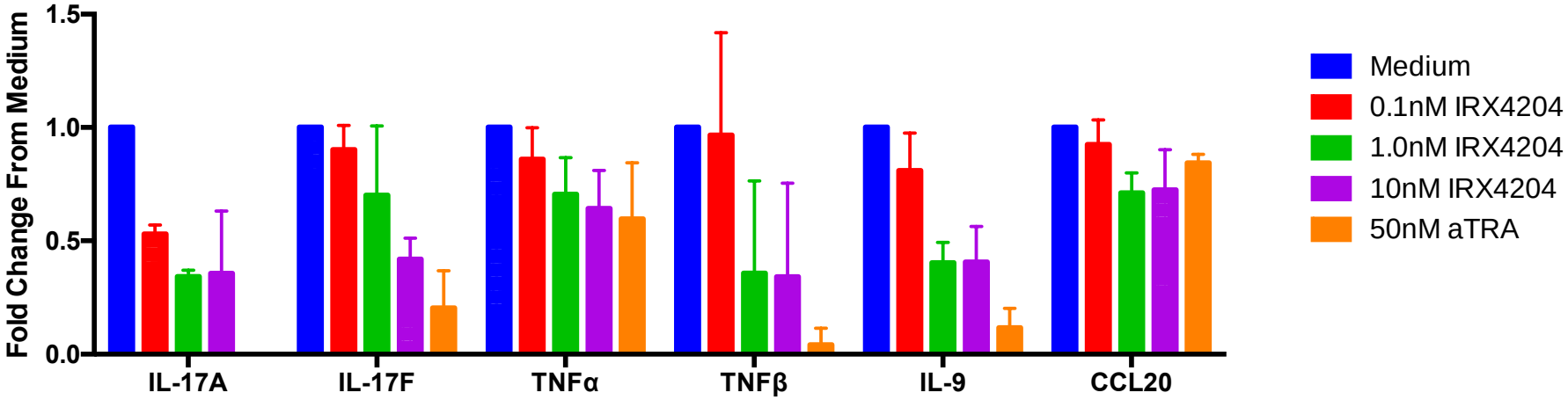
B.



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.

Data provided by Daniel Mielcarz, *et al*
DARTLab, Dartmouth University
Geisel School of Medicine

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



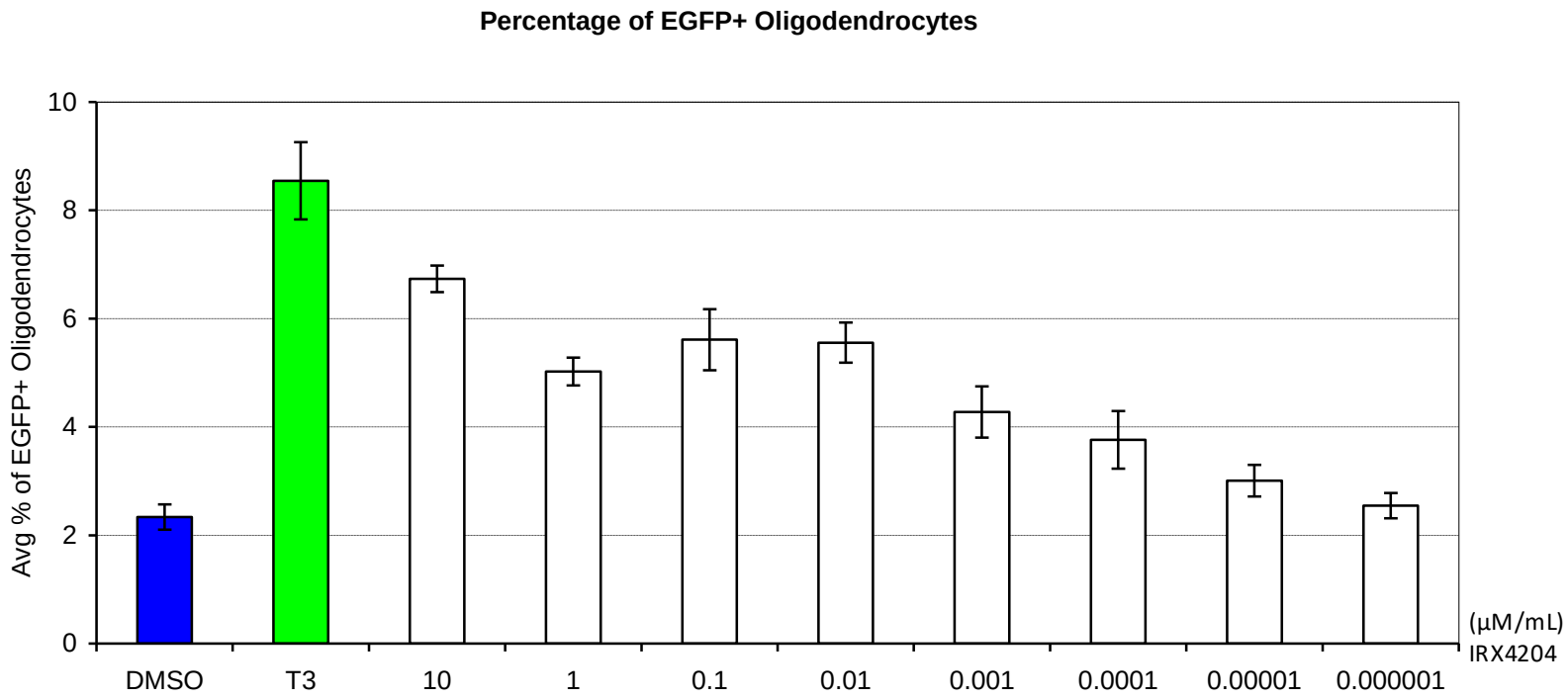
No effects seen on GM-CSF, IFN-γ, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-10, IL-12, IL13, IL15, IL-25, IL-21, IL-22, IL-23, IL-27, IL-28A, IL-31, IL-33

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.

Multiple therapeutic monoclonal antibodies which are bioactivity neutralizing for IL-17 or TNF-α are approved for treatment of various autoimmune diseases, including psoriasis, psoriatic arthritis, rheumatoid arthritis, ankylosing spondylitis, hidradenitis, Crohn's disease, ulcerative colitis, and uveitis; validating IL-17 and TNF-α as therapeutic targets for multiple autoimmune diseases.

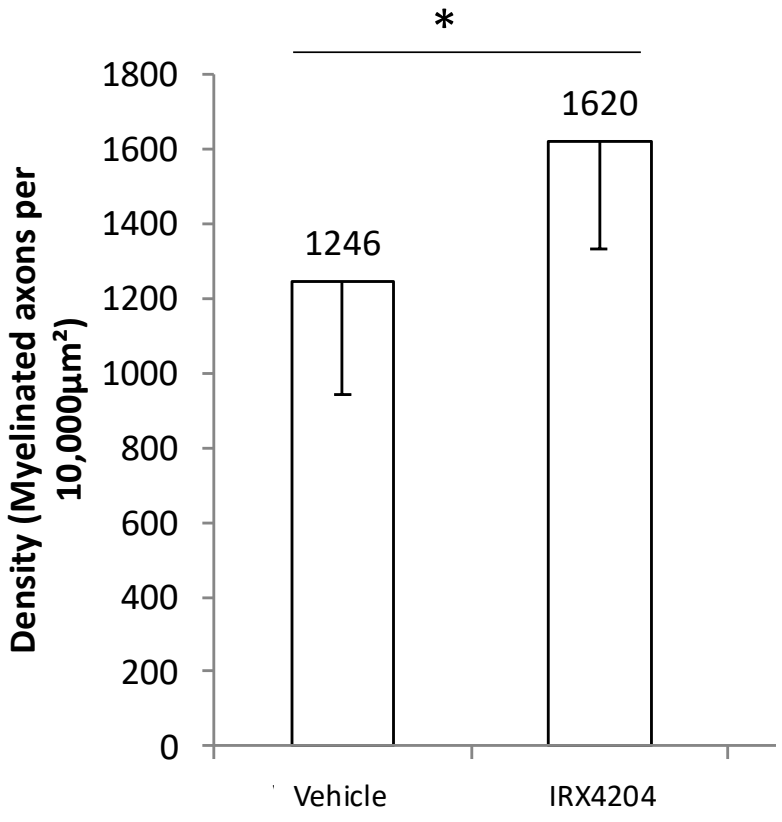
Data provided by Daniel Mielcarz, *et al*
DARTLab, Dartmouth University
Geisel School of Medicine

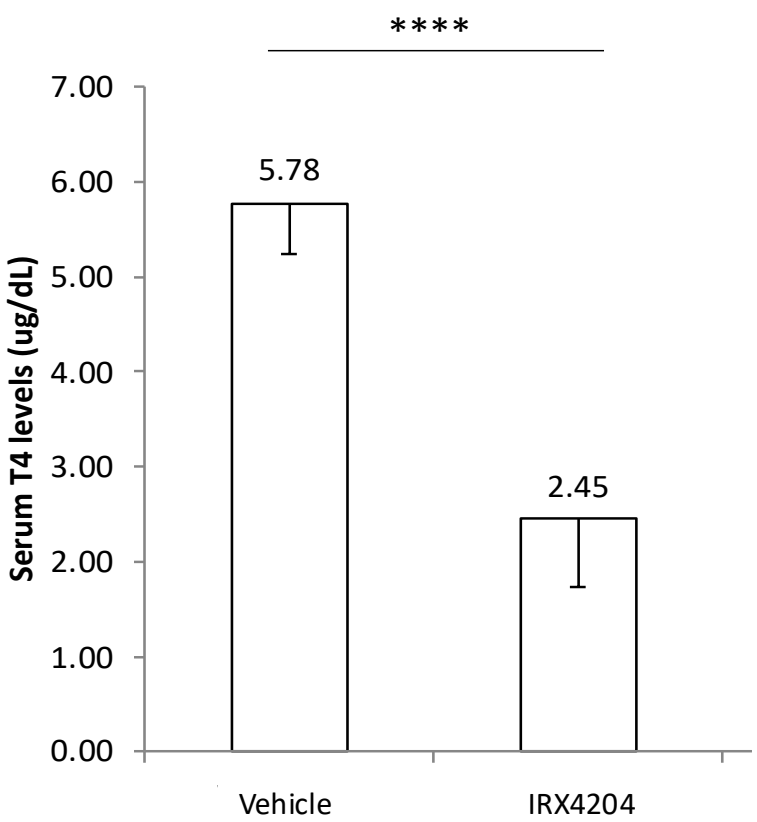
IRX4204 Promotes Differentiation of Murine Oligodendrocyte Precursor Cells into Myelin Producing Oligodendrocytes



Data provided by
Renovo Neural, Inc.

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

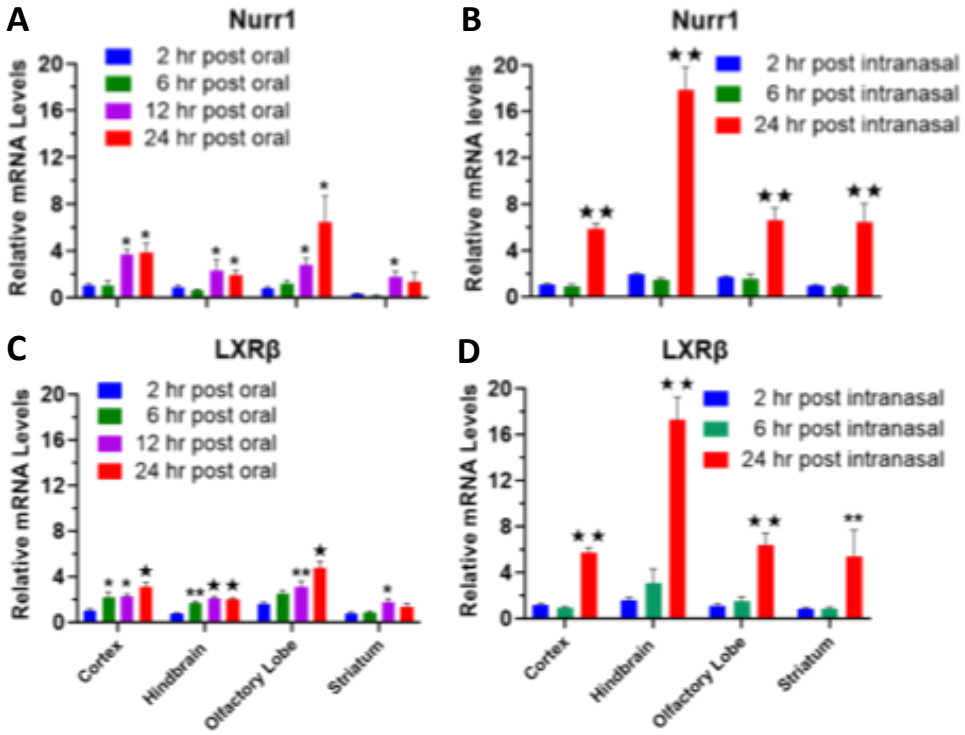




IRX4204 treatment resulted in a 30% statistically significant increase in myelin relative to vehicle ($p<0.05$), notwithstanding inducing highly significant hypothyroidism.
Study done at Renovo Neural, Inc.

Data provided by Renovo Neural, Inc.

Oral or Intranasal Delivery of IRX4204 Nanoparticles to Mice Produce Sustained Nurr1 and LXRβ Pharmacodynamic Actions in the CNS



Nurr1 and LXRβ mRNA levels at varying times after the oral administration of IRX4204 (12 mg/kg; A and C) or intranasal delivery of IRX4204 nanoparticles (0.12 mg/kg; B and D) in female C57Bl/6 mice. Nurr1 and LXRβ mRNA levels are expressed relative to mice that were treated orally with vehicle (NEOBEE, 100 µl; A and C) or intranasally with empty nanoparticles suspended in water (24 µl; two installations of 6 µl into each nostril; B and D). *p<0.05, **p<0.01,*p<0.001, and **p<0.0001 relative to the 2-hour time point

Data provided by George Robertson,
Dalhousie University, Halifax, Nova Scotia, Canada

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; milder findings than in bexarotene toxicology reported in SBA (which also showed ocular cataracts)
- 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 NCEs can be formulated for oral, parenteral, inhaled nose to brain, inhaled mouth to lung, dermal, and ocular administration
- Company has a small ongoing discovery program for back-up and fourth generation RXR agonist compounds

IRX4204 Clinical Development Status

- 100 patients have been treated with IRX4204 under US INDs, 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 RXR agonist class effects of dose-related reversible suppression of TSH with resultant treatable hypothyroidism; mild to moderate reversible increased triglycerides; and mild to moderate reversible neutropenia.
- One drug related SAE, rapid onset tumor lysis syndrome in a prostate cancer patient.
- 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 GMP IRX4204 and placebo capsules sufficient for multiple Phase II clinical trials completed.
- Novel biomarker plasma and CSF assays for brain RXR and heterodimers activation have been developed and patented for neurodegenerative diseases clinical trials.
- Preparing to conduct phase II placebo-controlled RCTs in PD and psoriasis patients.

IRX4204 Intellectual Property (Expiration Dates)

Issued US Patents for Neurodegenerative Diseases (12/13/32; 10/31/36; 2/11/37; 3/28/39)

1. Monotherapy treatment with IRX4204 of any neurologic condition, either central or peripheral, which involves both neuroinflammation and demyelination
2. Combination treatments of IRX4204 with thyroid hormone, and optionally, any neurotrophic factor, for aging related neurodegeneration, AD, PD, MS, ALS, Stroke, brain or spinal cord trauma, hypoxic brain injury, schizophrenia, depression, autism

Issued US Patents for Autoimmune Diseases (10/13/32; 10/30/36)

inflammatory arthritis (RA, OA, PSA, AS, Juvenile arthritis); skin diseases (psoriasis, hidradenitis, eczema, vitiligo, alopecia, others; GI diseases (Crohn's, UC, celiac disease); allergic diseases (asthma, allergic rhinitis)

International Patents for Neurodegenerative, Autoimmune Diseases (10/31/36; 10/30/36)

The company owns issued patents for treatment of Parkinson's disease in all of the following countries; as well as issued and pending patents for many of the other above listed neurodegenerative and autoimmune indications, in Europe, Japan, people's Republic of China, Hong Kong, South Korea, Singapore, Australia, New Zealand, Canada, Mexico, Israel, and South Africa

Methods of Synthesis (11/16/38) The company owns issued and pending patents for methods of synthesis of high chiral purity IRX4204 in the US and 40+ countries

Salts/Polymorphs (03/23/43) The company owns an issued US patents for NCE salts and polymorphs of IRX4204 with greater aqueous solubility; prosecuting in 50+ countries

Collaborators and Acknowledgements

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