IRX4204: An RXR Nuclear Receptor Agonist as a Potential Treatment for Stroke

Io Therapeutics, Inc.

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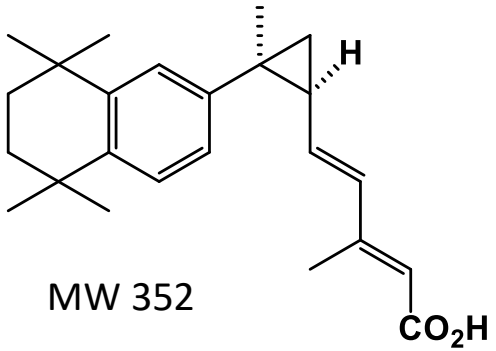
Pharmacologic Profile of IRX4204 as a Potential Treatment for Stroke

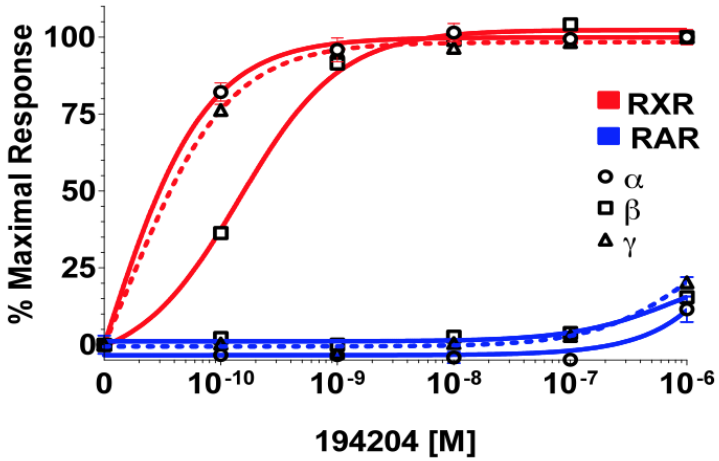
- IRX4204 is a potent and highly selective, brain penetrant, RXR nuclear receptor agonist compound which has demonstrated anti-neuroinflammatory effects, myelin protection, remyelination, and recovery of functional activities *in vivo* in multiple CNS models, including:
  - Middle cerebral artery occlusion stroke models with 3-day or 10-day delayed treatment;
  - MOG-induced EAE models manifesting neuroinflammation and demyelination;
  - Transfer of MOG-sensitized T-cells into naïve mice that develop neuroinflammatory demyelination;
  - Cuprizone-induced acute axonal damage and chronic demyelination/remyelination models.
- IRX4204 has multiple stroke protective and reparative activities *in vitro and in vivo*:
  - Inhibits stroke promoting mouse microglia and macrophage inflammatory responses;
  - Promotes human Treg proliferation and differentiation; Tregs are known to be stroke reparative;
  - Inhibits stroke promoting Th17 cell differentiation and pro-inflammatory cytokine production;
  - Promotes stroke reparative oligodendrocyte differentiation, myelin production, and remyelination;
  - Promotes cortical neuron survival and restorative neurite outgrowth;
  - Promotes cortical neuron protective mitochondrial respiration.
- IRX4204 has been safely administered orally to 100 humans for up to 20 months. Side effects include class effects of mild to moderate, reversible and treatable, dose-dependent increased triglycerides, decreased thyroid hormone, and transiently decreased leukocytes.
- IRX4204 can be formulated for oral, intravenous, or nose to CNS inhaled acute administration.

IRX4204: A Highly Potent and Highly Selective RXR Agonist Compound

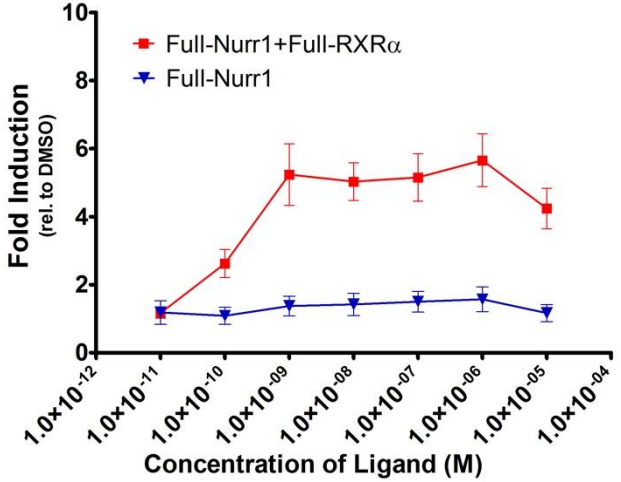


IRX4204 was discovered by Dr. Sagar Vuligonda at Allergan Pharmaceuticals in a diabetes drug discovery program. It is effective in the db/db mouse model of T2DM and is an insulin sensitizer in the brain.

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

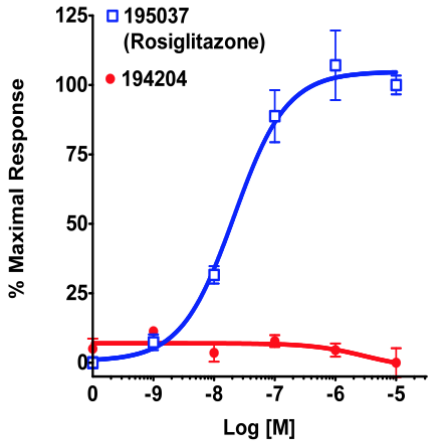


IRX4204 Transactivates Nurr1/RXR Heterodimers through RXR

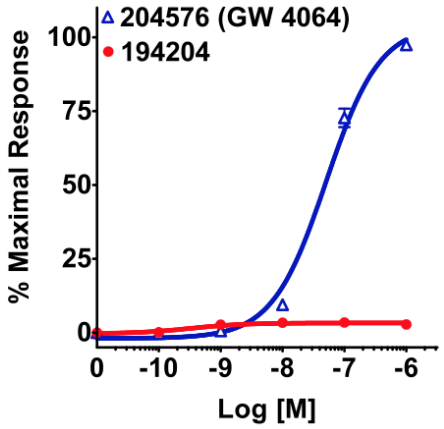


Wang et al, Oncotarget 2016

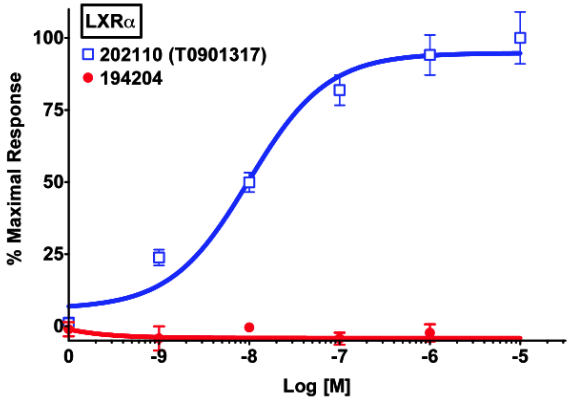
IRX4204 is Highly Selective for RXRs
It Does Not Directly Transactivate PPARγ, FXR, or LXRs



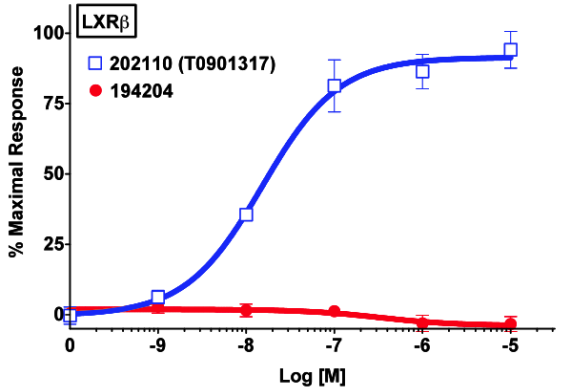
PPARγ/RXR



FXR/RXR



LXR/RXR



LXR/RXR

Wang et al,
Oncotarget 2016

IRX4204 is More Potent, More Selective, and Less Toxic than Bexarotene, which Produced Durable Functional Remyelination in MS Patients in a Placebo-controlled Clinical Trial

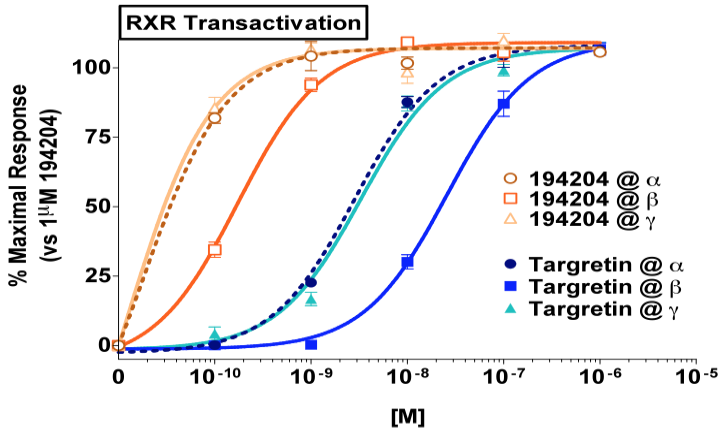
Bexarotene

- 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)
- Bexarotene treatment of MS patients in a controlled clinical trial produced durable functional reversal of demyelination:

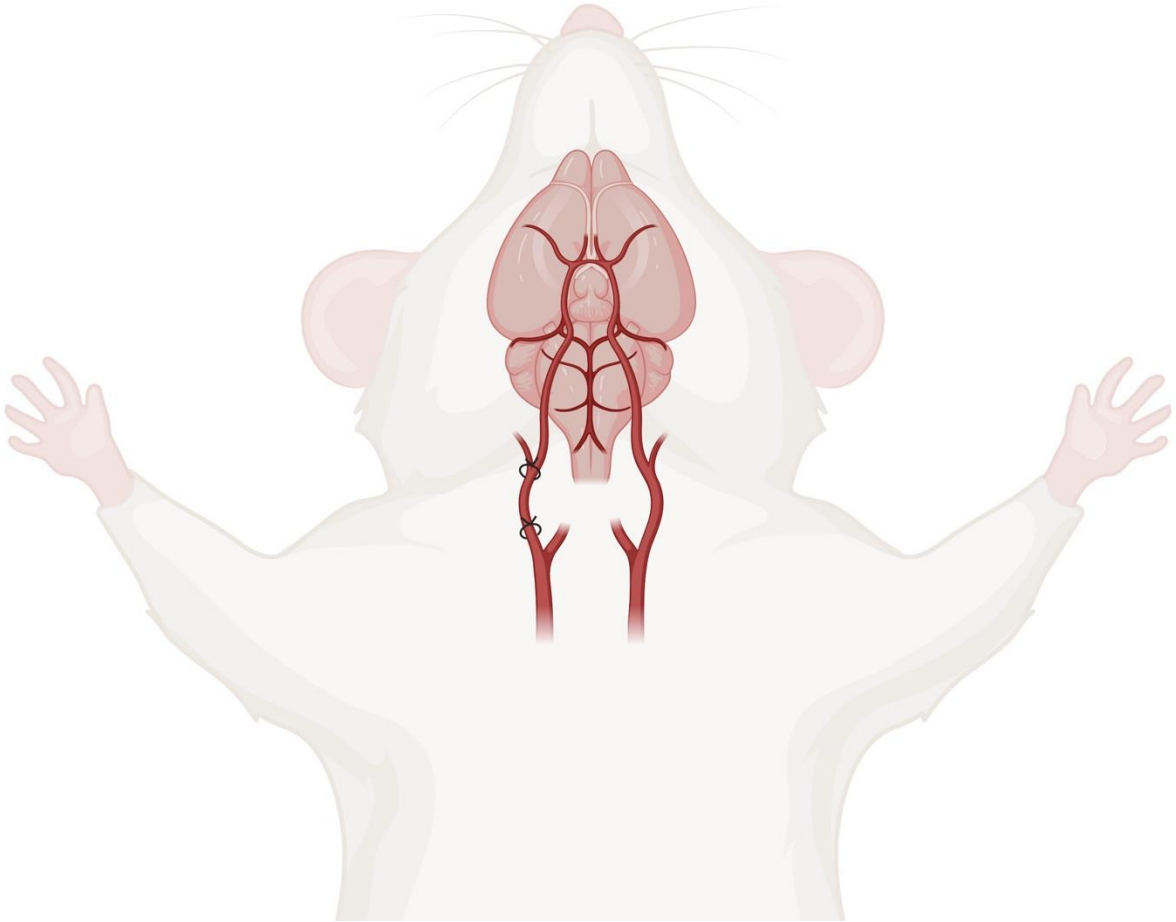
IRX4204

- 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
- Substantial preclinical data support that IRX4204 also has *in vivo* myelin protective and myelin reparative activities.

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



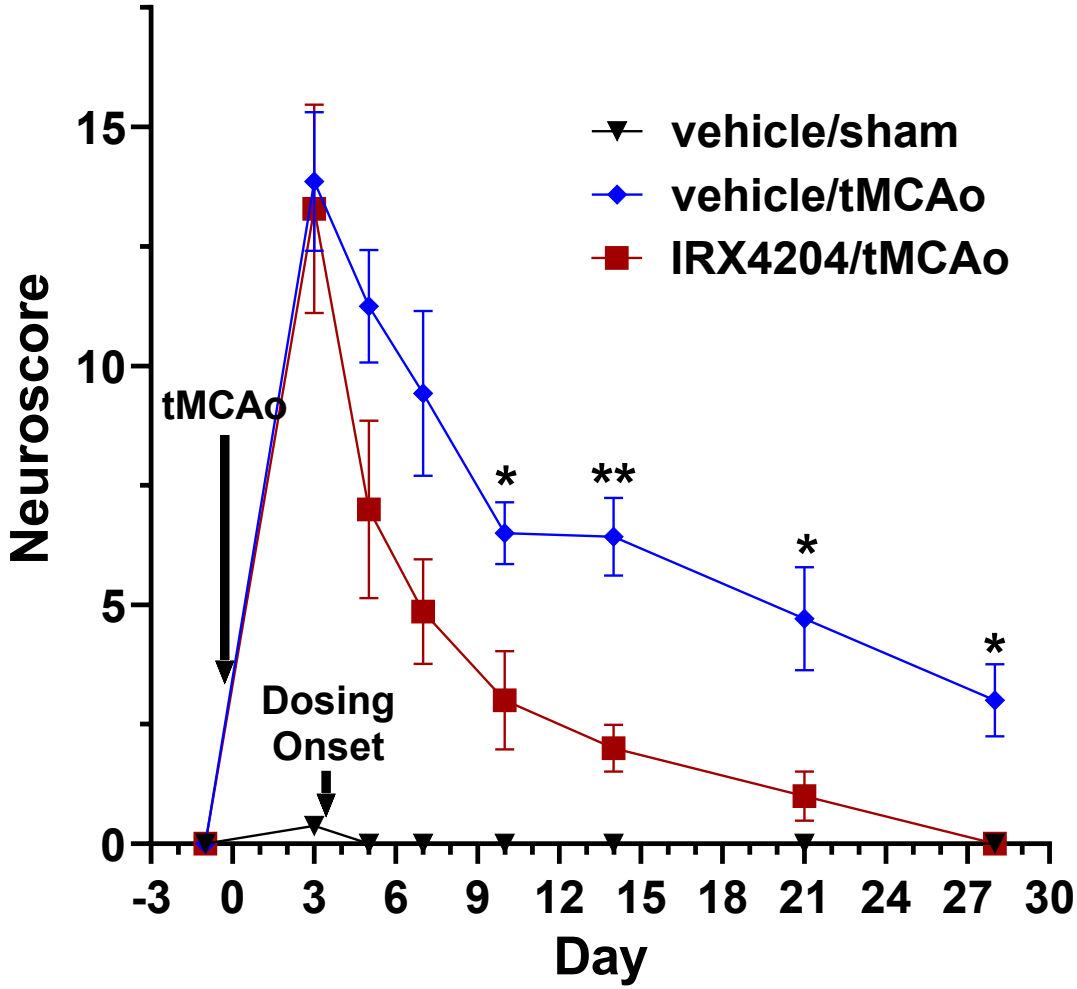
Mouse Middle Cerebral Artery Occlusion (MCAO) Stroke Model



SB: Sensorimotor Battery

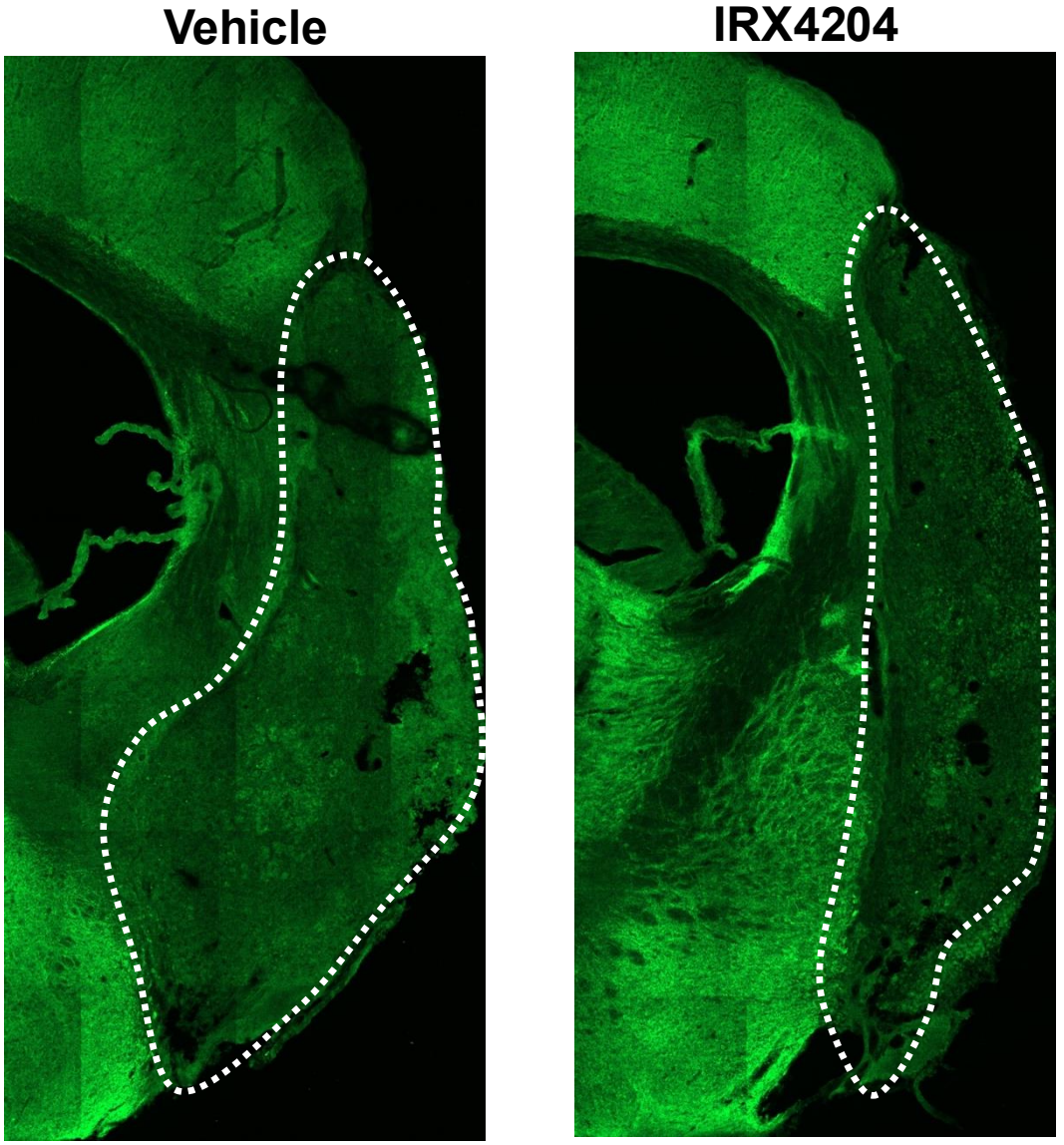
General behavioural deficits	Focal behavioural deficits
1. Hair	1. Body symmetry
2. Ears	2. Gait
3. Eyes	3. Circling behaviour
4. Posture	4. Forelimb symmetry
5. Spontaneous activity	5. Compulsory circling
6. Epileptic behaviour	6. Whisker response

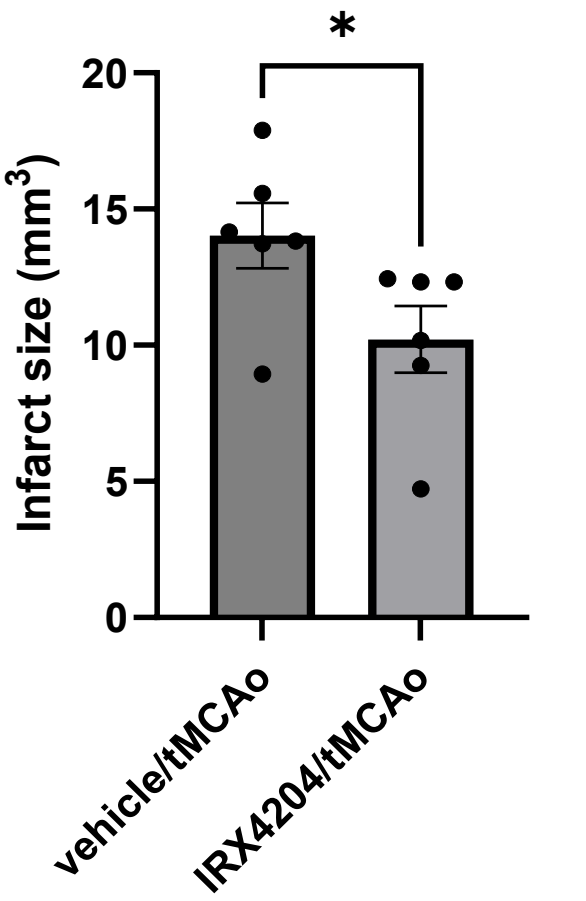
IRX4204 Completely Resolves Functional CNS Deficits in Mouse MCAO Model Even When Administration Starts 3 days after Stroke



IRX4204: 12 mg/kg, p.o., qd

IRX4204 Reduces Infarct Size in Mouse MCAO Stroke Model

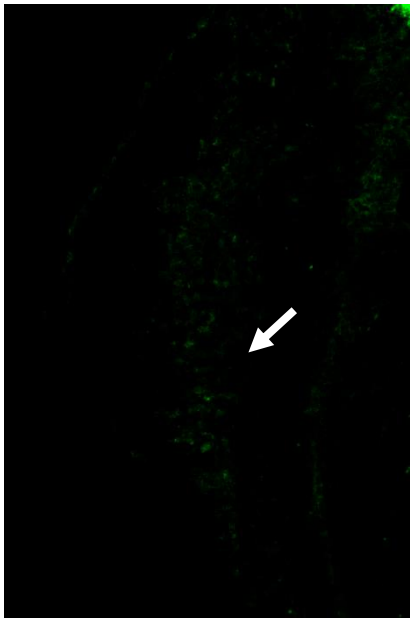




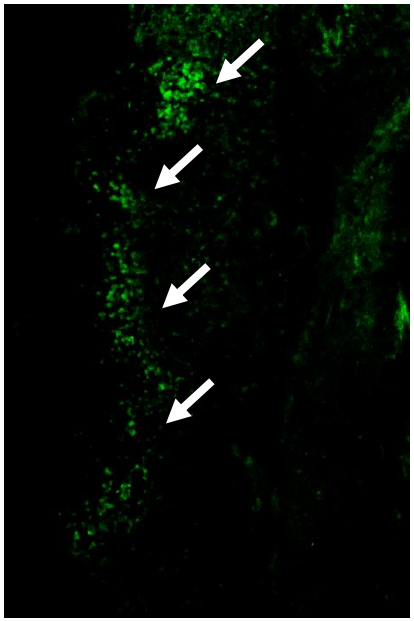
IRX4204 Promotes Myelin Repair in Mouse MCAO Stroke Model

Remyelination

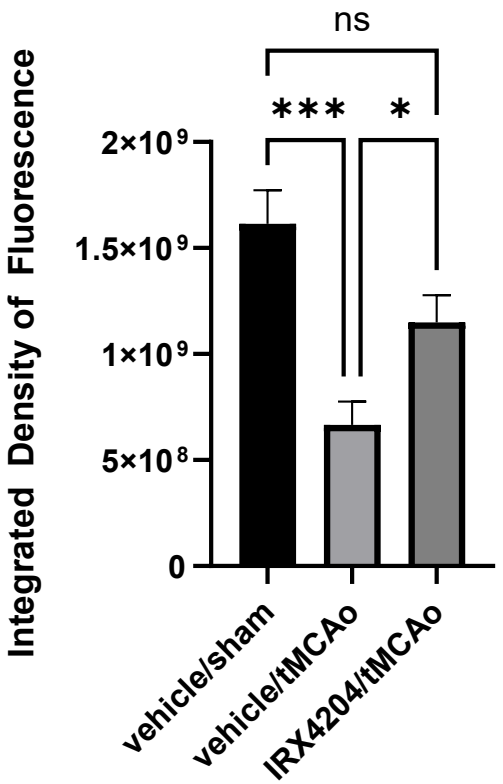
Vehicle



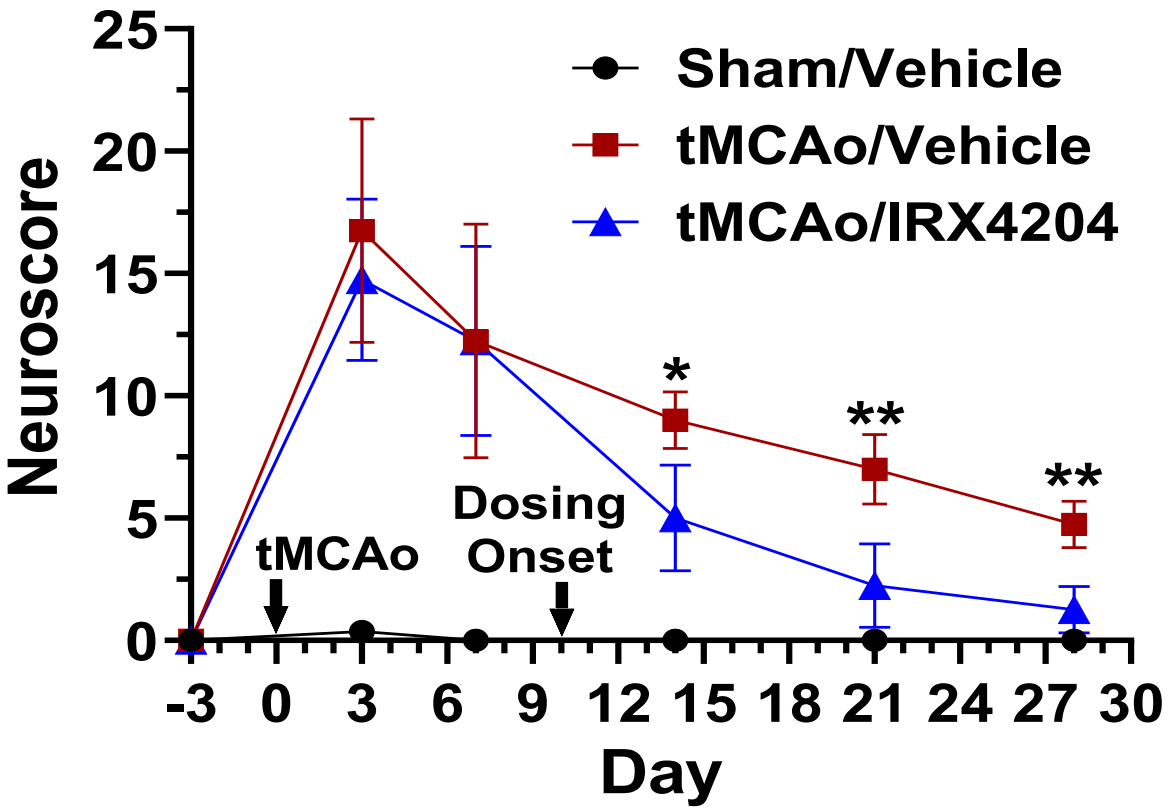
IRX4204



Myelin (MBP)



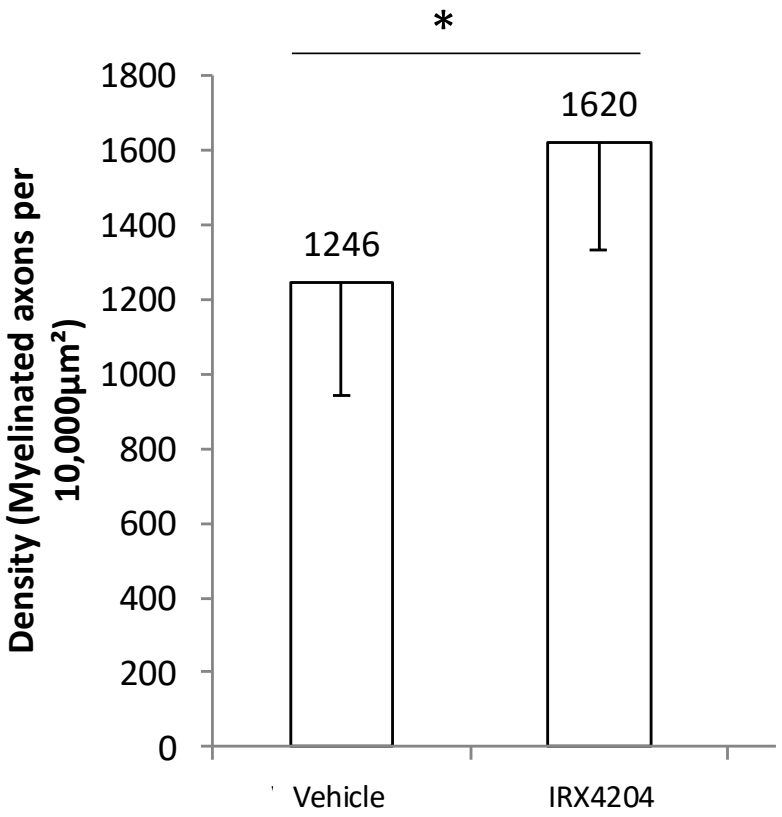
IRX4204 Nearly Completely Resolves CNS Deficits in Mouse MCAO Model Even When Administration is Delayed 10 days



Oral IRX4204 (12 mg/kg/day) starting 10 days after tMCAo reduces sensorimotor deficits in young male C57Bl/6 mice (12 weeks old). Data show mean ± SD (n=4/group). *p<0.05, **p<0.01 relative to tMCAo/Vehicle mice.

Data Provided by
George Robertson
Dalhousie University

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

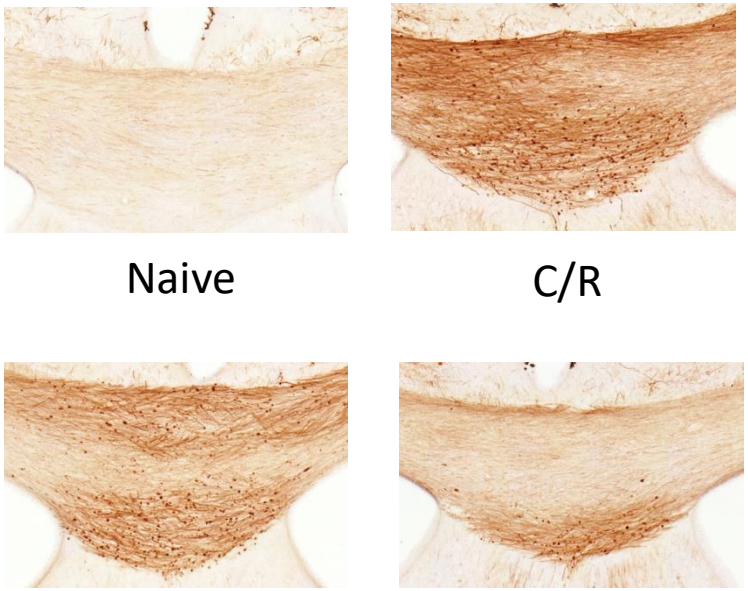


IRX4204 treatment resulted in a 30% statistically significant increase in myelin relative to vehicle ($p<0.05$).

Data provided by Renovo Neural, Inc.

IRX4204 + Thyroid Hormone Have Combination Acute Axonal Protective Effects in Murine Model of Cuprizone-induced Axonal Transection

SMI-32+ Ovoids are Transected Axons – Representative Images of Corpus Callosum



Naive

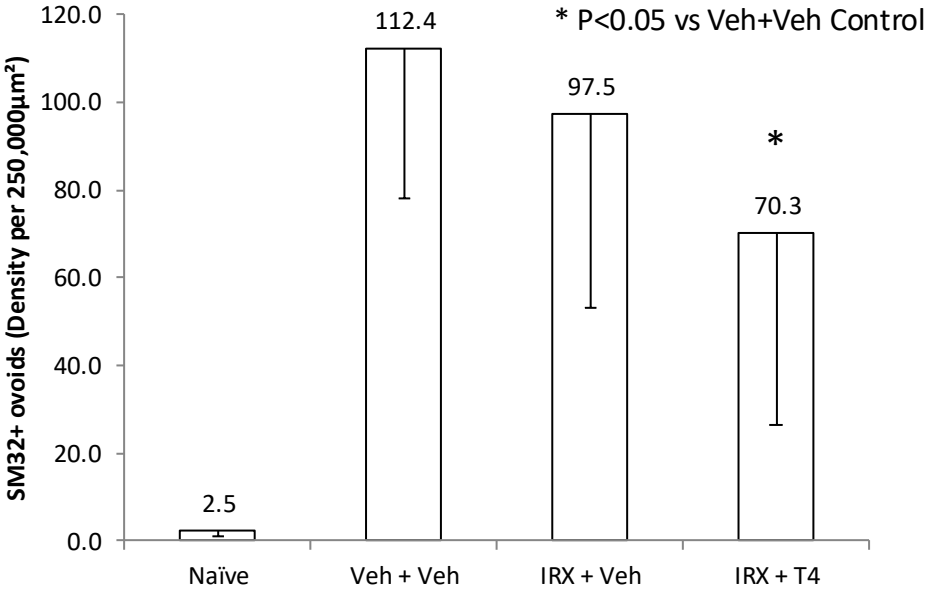
C/R

C/R IRX4204

C/R IRX4204, T4

Axonal Transection Induced by Cuprizone (C) plus Rapamycin (R)

SMI-32 Quantification of Transected Axons

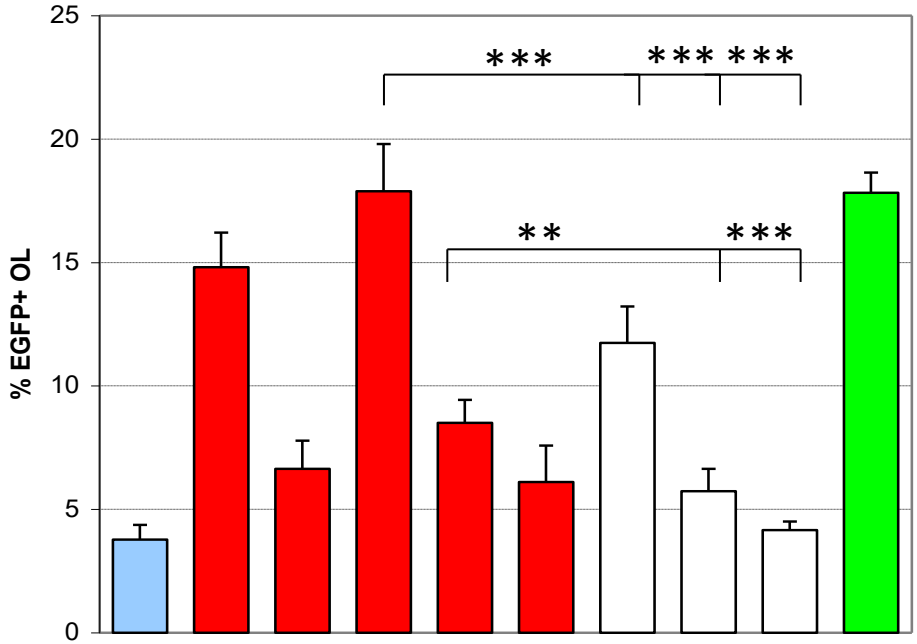


There was a significant reduction in corpus callosal axonal transection as shown by the number of SMI-32 positive ovoids in the IRX4204+T4 treatment group when compared to vehicle.

Data provided by Renovo Neural, Inc.

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

Percent of EGFP+ Oligodendrocytes



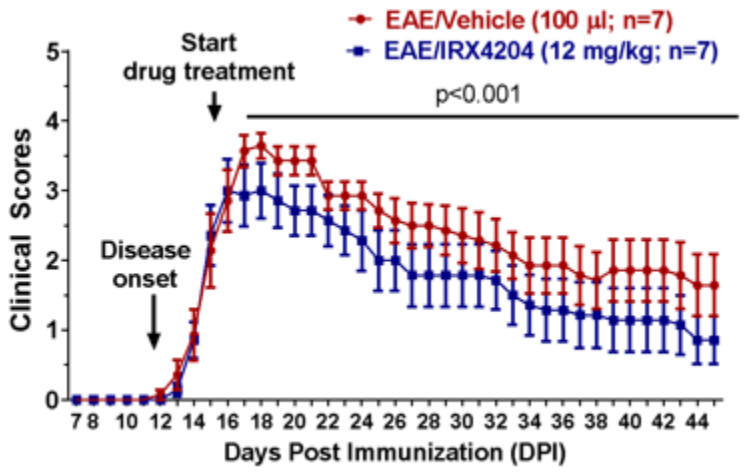
** P = < 0.01
*** P = < 0.0001

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

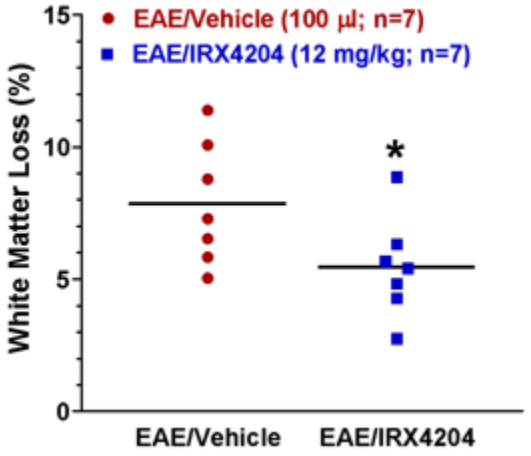
Data Provided by Renovo Neural, Inc.

IRX4204 Reduces Neuroinflammation-induced Myelin Loss in MOG Initiated EAE

IRX4204 Reduces EAE Disease Severity

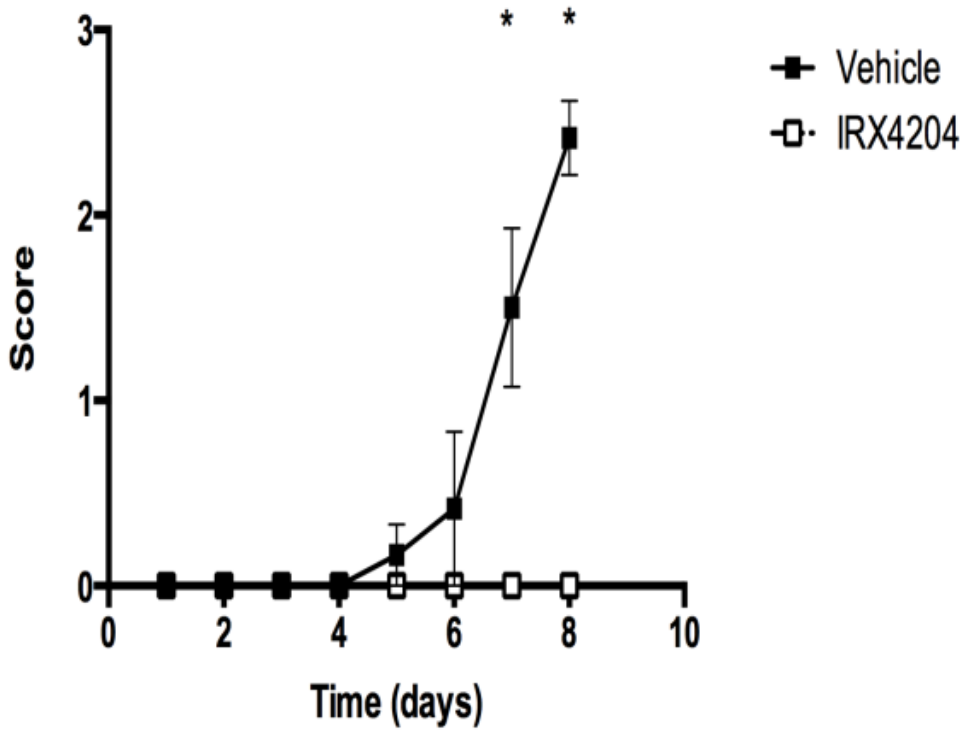


IRX4204 Reduces Spinal Cord White Matter Loss in EAE Mice



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

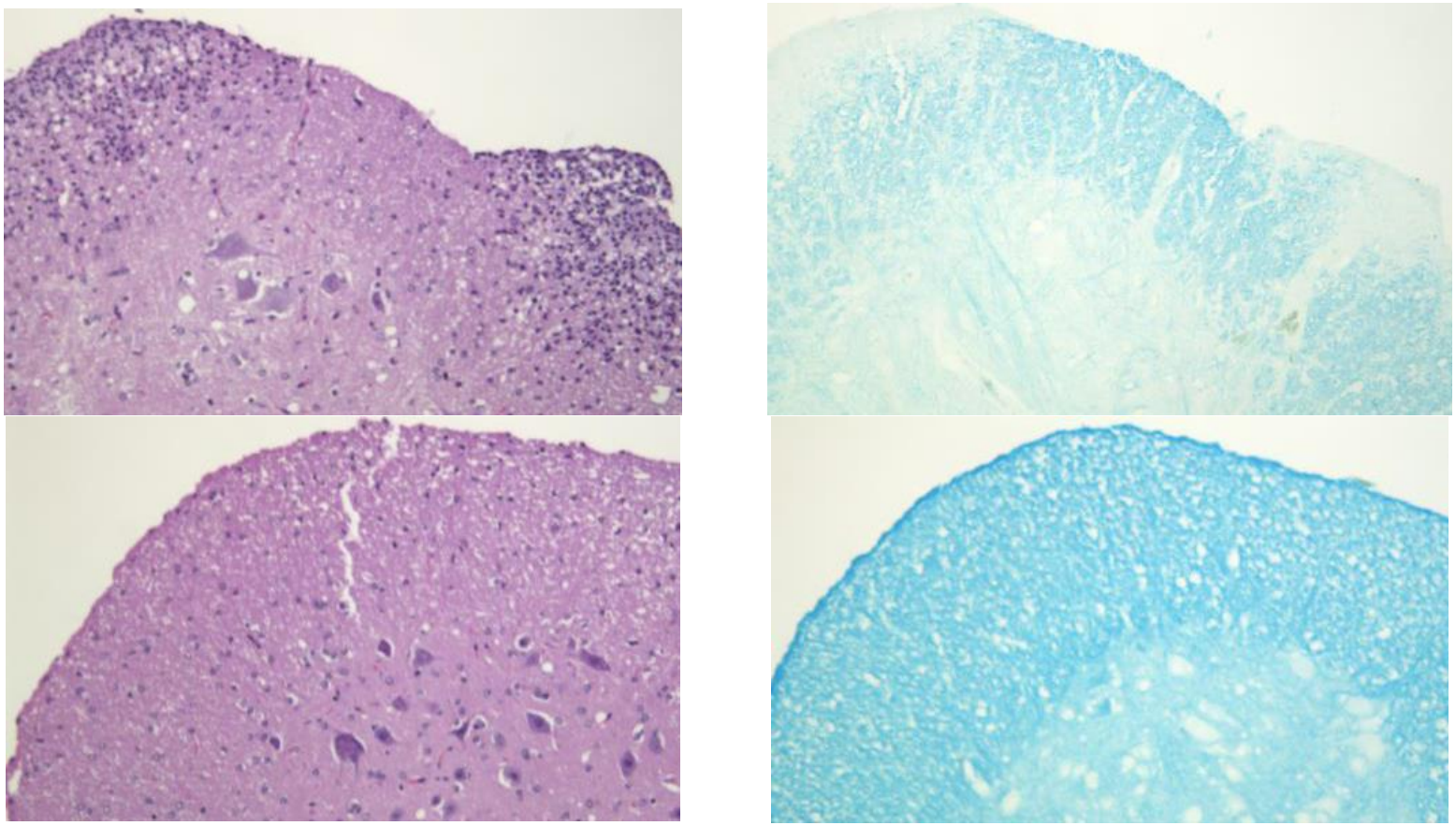
IRX4204 100% Prevented Th17-Mediated Autoimmune Disease *In Vivo* Mediated by Adoptively Transferred Antigen-sensitized Th17 cells in a Mouse Model of Neuroinflammation



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.

Chandraratna, et al.
Am J Transl Res, 2016

IRX4204 Inhibits CNS Infiltration of Leukocytes and Loss of Myelin in Th17 Adoptive Transfer EAE Model



Brain tissues from the Th17 adoptive transfer EAE model were stained for detection of infiltrating leukocytes (with hematoxylin and eosin, left), and integrity of myelin (with Luxol Fast Blue, right); IRX4204 treated animals (lower), vehicle treated animals (upper).

Chandraratna, et al.
Am J Transl Res, 2016

IRX4204 Increases Differentiation of Adult Human Oligodendrocytes

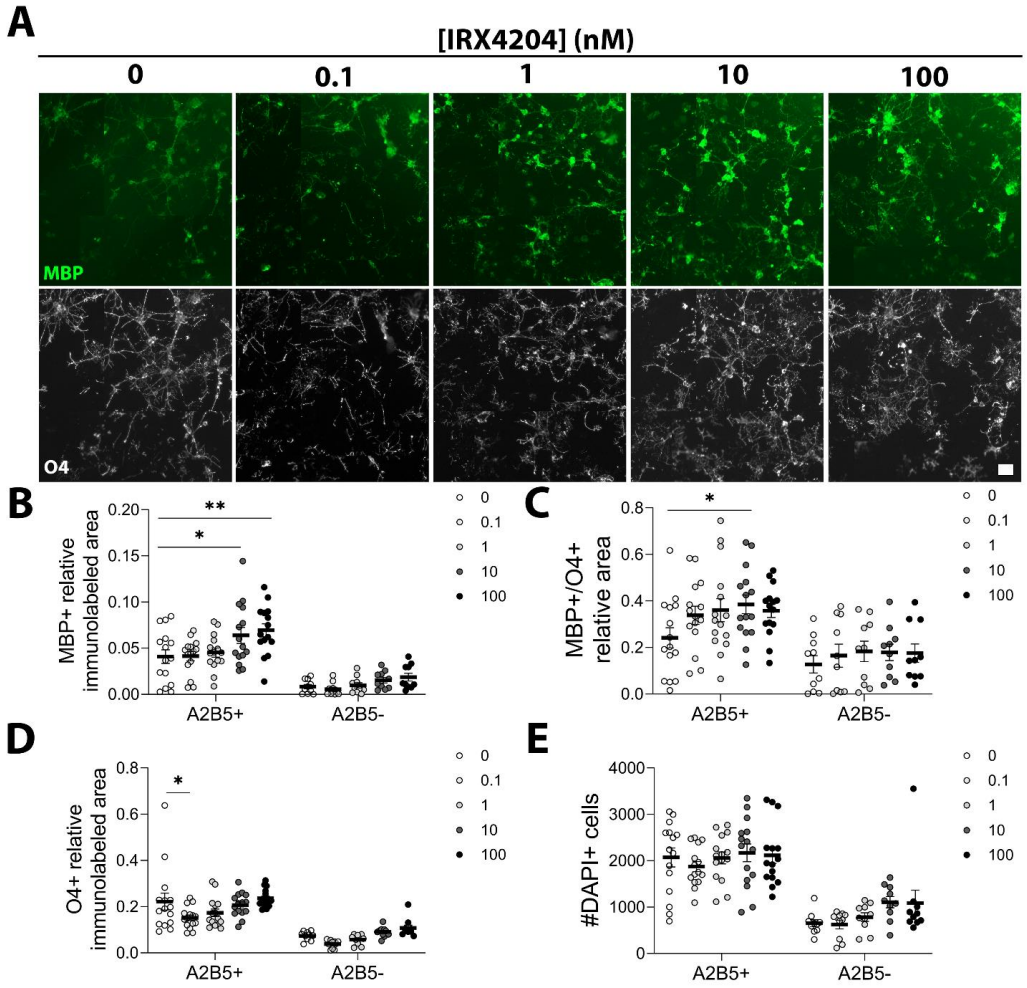
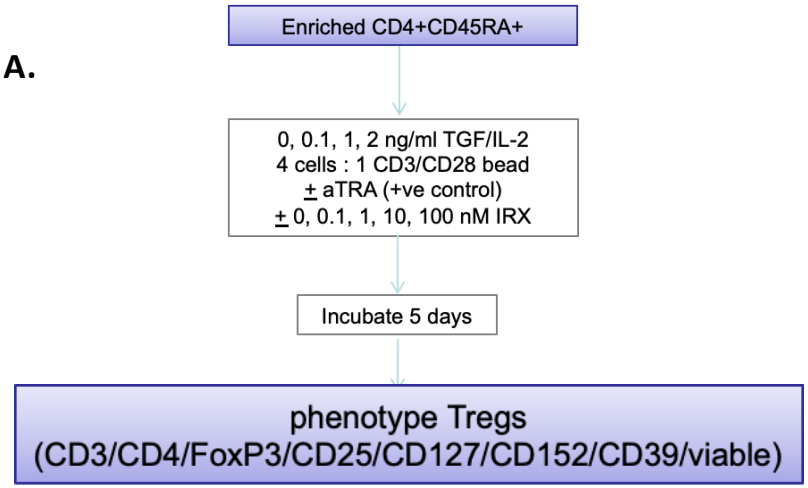


Fig A-E IRX4204 increases the differentiation of adult human A2B5+ oligodendrocytes. Data show the mean values ± SEM for A2B5+ cells (3 replicates; n=3) and A2B5- cells (n=2) with 5 wells/condition/replicate. *p<0.05; **p<0.01 between bars.

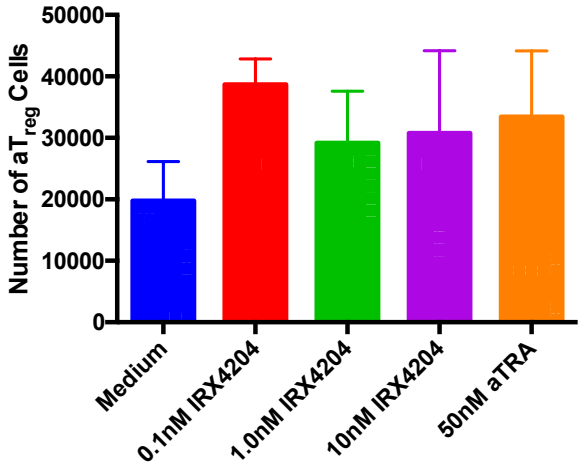
Data Provided by
Timothy Kennedy
McGill University

IRX4204 Promotes Potentially Stroke Reparative Human Treg Differentiation and Proliferation

A.



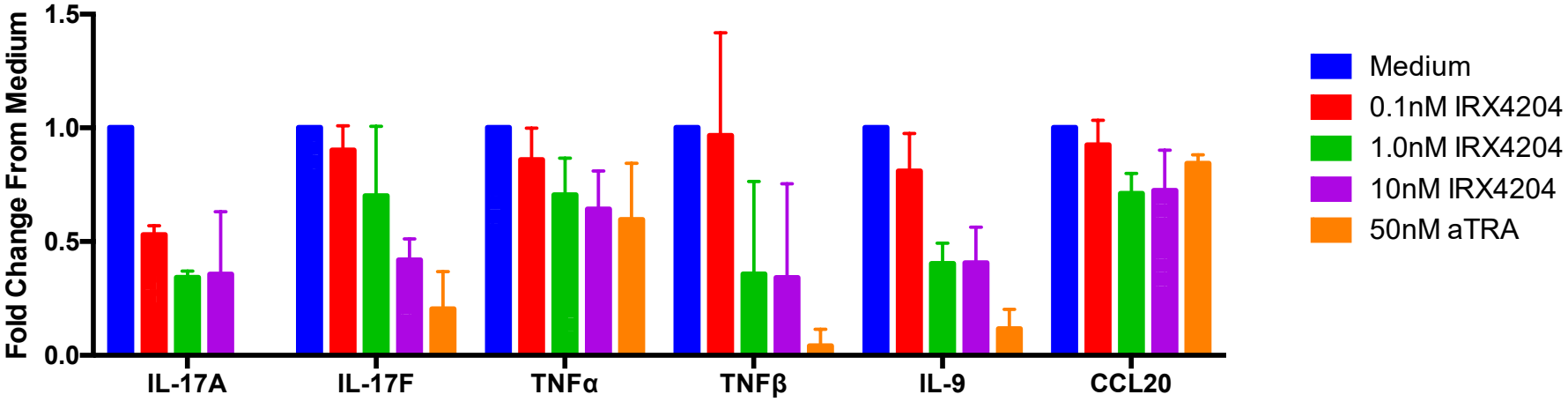
B.



Tregs are reported in many publications from many investigators to have stroke reparative activities. IRX4204 increases Treg conversion and proliferation of naïve CD4+ T cells in Treg skewing conditions, providing an additional potential mechanism for stroke recovery. **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 Pro-neuroinflammatory Cytokine Secretion by Human T-cells Cultured in Th17 Skewing Conditions

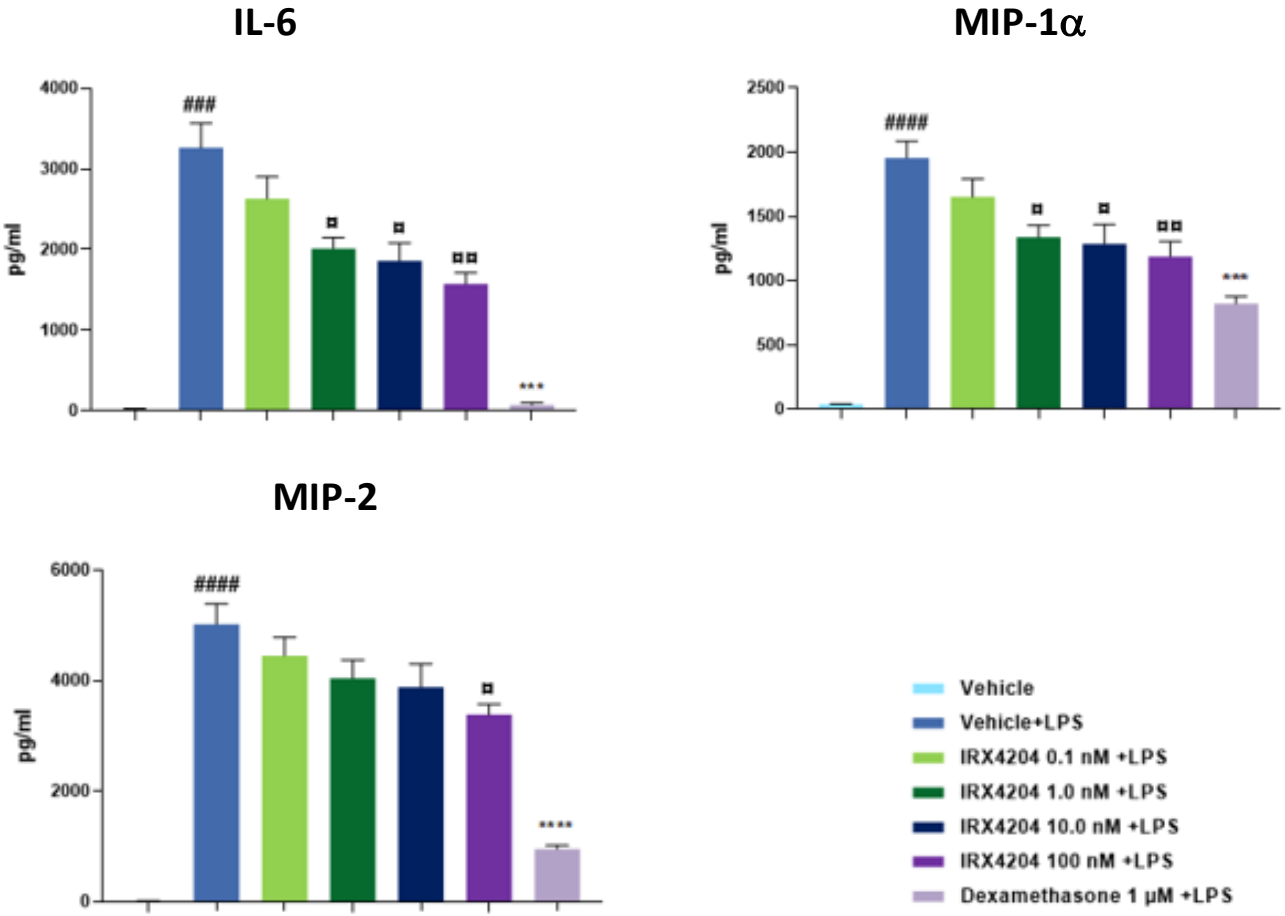


Normal Human PBMCs were isolated from whole blood, then magnetically separated to enrich a ~95% pure CD4+CD45RA+ naïve T cell fraction. Cells were cultured under Th17 skewing conditions for 5 days and stimulated anti-CD3/CD28 beads. Supernatants were collected and assayed following 5 days in culture.

Th17-derived pro-inflammatory cytokines including IL-17s, TNFs, IL-9, and others are reported in many publications from many investigators to have neuroinflammatory CNS damage promoting activities in acute stroke, though they may contribute to stroke recovery in the chronic post-stroke recovery phase. However, multiple therapeutic monoclonal antibodies which are bioactivity neutralizing for IL-17 or TNF-α are approved for chronic treatment of various inflammatory diseases, including psoriasis, psoriatic arthritis, rheumatoid arthritis, ankylosing spondylitis, hidradenitis, Crohn's disease, ulcerative colitis, and uveitis; validating IL-17 and TNF-α as useful therapeutic targets for multiple types of inflammatory diseases, both acute and chronic.

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

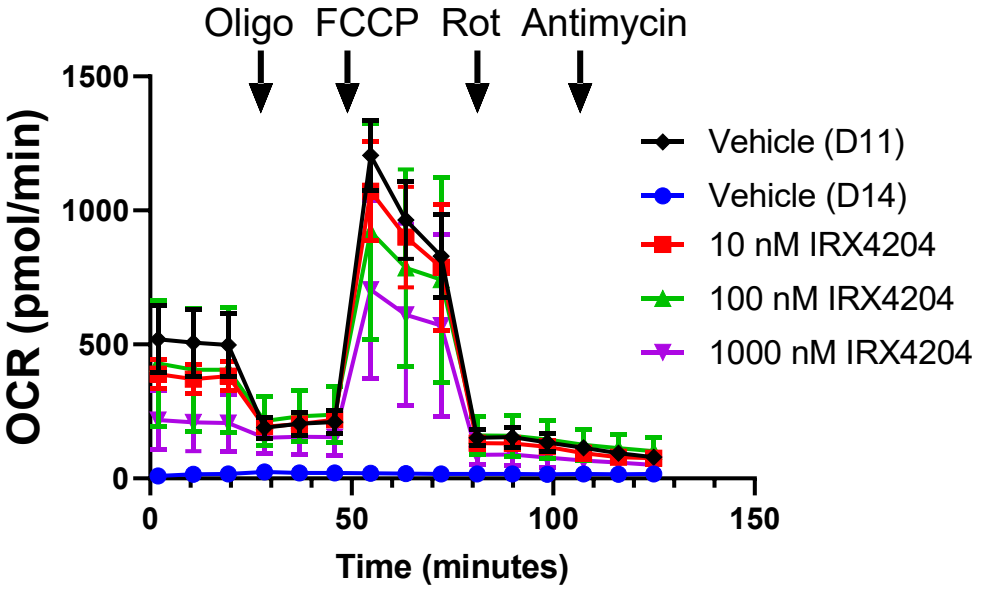
IRX4204 Inhibits LPS-induced Rat Cortical Microglial Production of Acute Stroke Damage Promoting IL-6, MIP-1α, and MIP-2

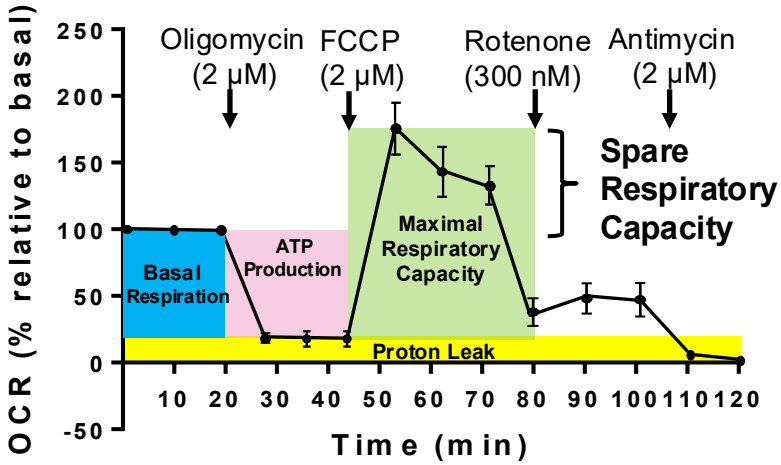


Cultures were 80% purified microglia, 20% astrocytes

Data provided by
Charles River Laboratories

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

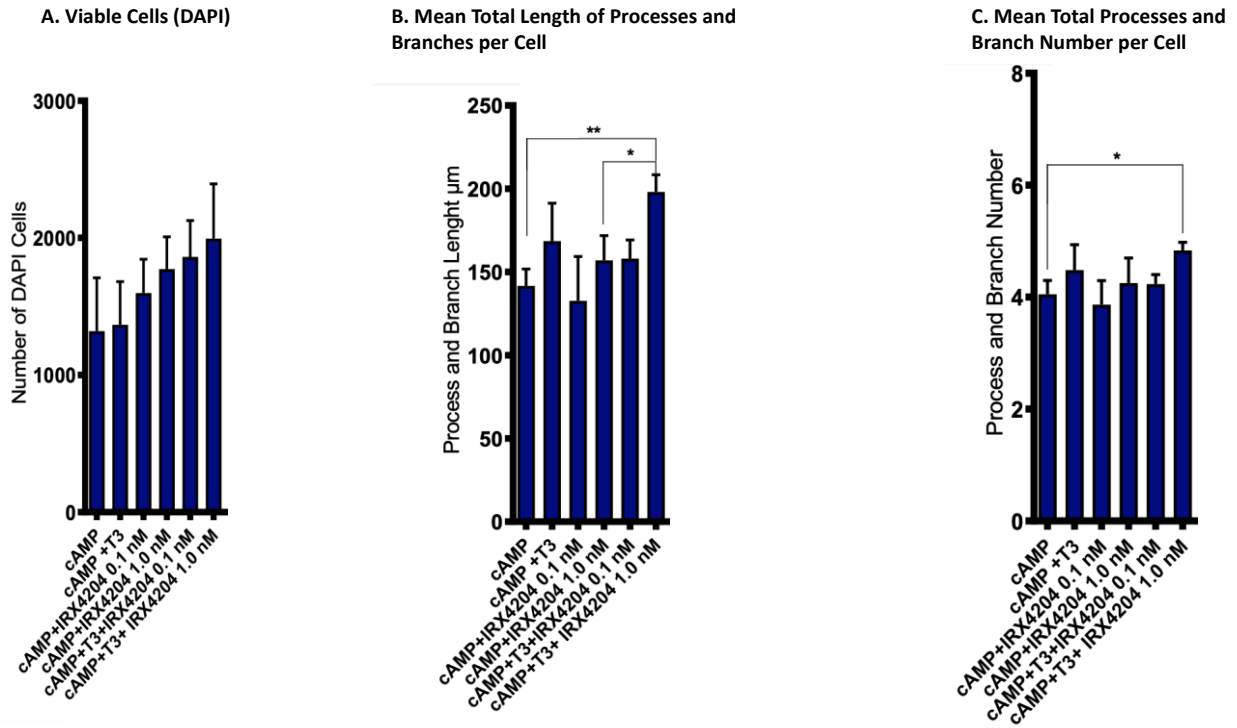




IRX4204 prevented the loss of mitochondrial respiration caused by culturing cortical neurons for a prolonged time (left panel). Relative to *in vitro* day 11 (D11) primary cultures of mouse cortical neurons treated with vehicle (0.01% DMSO), D14 cortical neurons showed a profound loss of mitochondrial 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

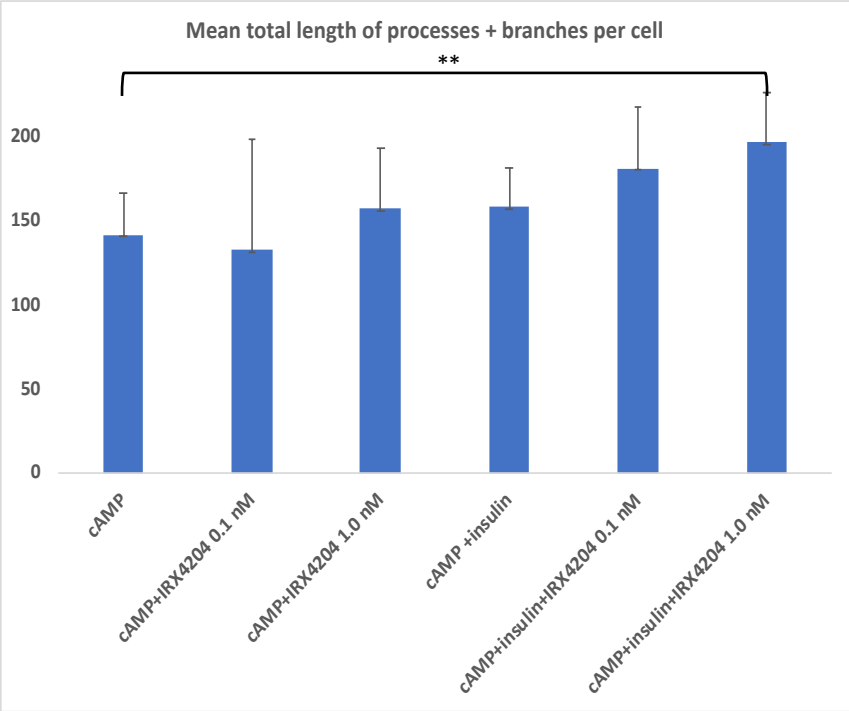
IRX4204 + T3 Have Combination Effects on Rat Cortical Neuron Survival and Neurite Outgrowth *in Vitro*

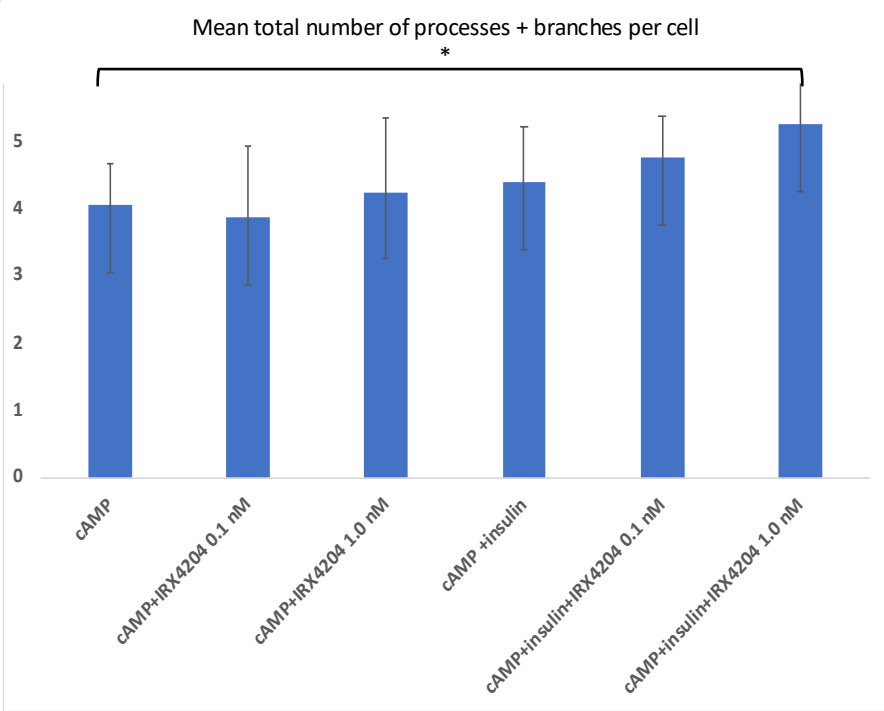


Data from 48-hour cultures of rat cortical neurons, supplemented with cyclic adenosine monophosphate (cAMP) as a metabolic energy source. DAPI is a stain that identifies viable cells. Panel A shows that IRX4204 alone, and in combination with thyroid hormone, increased mean survival of treated cortical 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).

Data provided by
Charles River Laboratories

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: 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 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 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 for Phase II clinical trials completed.
- Novel biomarker plasma and CSF assays for brain RXR activation have been developed and patented for neurodegenerative diseases clinical trials

IRX4204 Intellectual Property (Expiration Dates)

Issued US Patents for Neuroinflammatory Demyelinating Diseases, Neurodegenerative Diseases, and Aging-related Neurodegeneration

1. Monotherapy treatment with IRX4204 of any neurologic condition, either central or peripheral, which involves both neuroinflammation and demyelination (**2/11/37)**
2. Combination treatments of IRX4204 with thyroid hormone, and optionally, any neurotrophic factor including insulin, for treatment of Stroke, MS, ALS, CNS injury, aging related neurodegeneration, AD, PD, schizophrenia, depression, and autism **(10/31/36)**

Issued and Pending International Patents for Neuroinflammatory Demyelinating or Neurodegenerative Diseases (10/31/36)

The company owns issued and pending patents for treatment ALS, MS, aging related neurodegeneration, stroke, CNS injury, as well as most of the other neuroinflammatory indications listed above, in most of the following countries: Europe, Japan, 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 proprietary methods of synthesis of high chiral purity IRX4204 in the US and 40+ countries.

Salts/Polymorphs (03/23/43) The company owns issued US patents for compositions of matter for New Chemical Entities of various salts and polymorphs of IRX4204 that have substantially increased aqueous solubility; these patents are issued or pending in 50+ other countries to provide durable composition of matter patent protection.

Collaborators and Acknowledgements

George Robertson, Dalhousie University; Timothy Kennedy, McGill University; Studies of effects of IRX4204 in stroke models, differentiation of human oligodendrocytes, effects on mitochondrial respiration in cortical neurons, inhibition of neuroinflammation and myelin loss, and promotion of functional recovery in murine EAE models

Amanda Janesick, Bruce Blumberg; University of California, Irvine; RXR, Nurr1, and other nuclear receptor reporter assays with IRX4204

Elizabeth Nowak, Randy Noelle; Dartmouth University Geisel School of Medicine; Studies of IRX4204 on murine Treg and Th17 differentiation and in EAE models

Daniel Mielcarz, Jacqueline Channon; DARTLab, Dartmouth University Geisel School of Medicine; Studies of IRX4204 effects on human Treg and Th17 differentiation; and production of IL-17s, TNFs and other cytokines by human T-cells

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