Regulating the Inflammasome Pathway for the Treatment of Chronic Inflammatory Ophthalmic Disease
Ocunexus Drug Target: CONNEXIN43 HEMICHANNEL

- **Gap Junctions** are made up of intercellular channels that let cells “talk” to each other.

- Each **Gap Junction Channel** is formed by head-to-head docking of two **hemichannels** which are made up of “**connexin**” proteins, of which “**connexin43**” is most ubiquitous and crucial to normal physiological function.

- Hemichannels are normally closed prior to docking. **Open Undocked Hemichannels** occur as a result of chronic disease/injury, and their numbers are increased by the overexpression of “**connexin43**”.

- The open hemichannel forms a “**pathological pore**” exposing the cell cytoplasm to the extracellular space **promoting ATP release, inflammasome (NLRP3) activation multiple pro-inflammatory cytokine release** leading to a cycle of **inflammation, vessel leak, dropout, ischemia, edema, and ultimately fibrosis**

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Ocunexus’s technologies **maintain the closed hemichannel, to block the ongoing cycle of inflammation** and subsequent downstream pathology.
Connexin43 Hemichannel Block Inhibits ATP Release to Prevent Formation of the Inflammasome Complex

- The inflammasome is a multimeric oligomer which can be immunohistochemically labelled (NLRP3)
- Connexin hemichannel block prevents ATP release and so inflammasome assembly
- Exogenously added ATP retriggers assembly, proving the mode of action

(Mugisho et al. Biochim Biophys Acta. 2018; 1862:385-393)
Methodology
ARPE19 Cells challenged with 10 ng/mL TNF-α + 10 ng/mL IL-1β and/or high glucose (HG)

IL-6 (a pro-inflammatory cytokine)
IL-8 (a neutrophil chemotactic factor)
MCP-1 (a monocyte chemotactant)
ICAM-1 (leukocyte-endothelial cell adhesion factor)

Connexin43 Hemichannel Block Reduces Inflammatory Cytokine Release
By Blocking ATP Release and Inflammasome Activation - Retinal Pigment Epithelial

Key:
- Black: Basal
- Blue: HG (Hyperglycemic)
- Red: Cytokines (IL-1β / TNF-α)
- Green: HG + Cytokines
- Purple: Connexin hemichannel block reduces cytokine release
- Yellow: Exogenously added ATP increases cytokine release
Cx43 Hemichannel Inhibition in a Retinal Ischemia-Reperfusion Model
(Diabetic Retinopathy / Retinal Vein Occlusion)

Methodology
• Rat left anterior eye chamber cannulated using a stereotaxic manipulator arm with 30 gauge needle
• Intraocular pressure of the cannulated eye raised to 120 mmHg for 60 minutes
• Retinal ischemia confirmed by pallor of the posterior segment
• Reperfusion of the retinal vessels confirmed by ophthalmoscopy

GFAP (glial fibrillary acidic protein)
Cx43 labelling (green)
Arrows = vessel wall

Confocal Z stack analysis reveals astrocytic processes crossing the vessel lumen indicating endothelial cell disruption

Cx43 Levels Increase in Retinal Tissue After Ischemic Injury

Connexin43 Levels

<table>
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<th></th>
<th>0</th>
<th>500</th>
<th>1000</th>
<th>1500</th>
<th>2000</th>
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<th>3000</th>
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<td>Time after injury (Hours)</td>
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<td>4</td>
<td>8</td>
<td>24</td>
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* Significant differences compared to control
Administration of Cx43 Inhibitor Reduces Vessel Leak and Loss of Retinal Ganglion Cells

Dye leak reduced 86% at peak 4 hours post-injury vs controls (p<0.05)

Highly significant sparing of retinal ganglion cells at 7 and 21 days post-ischemia (p<0.05)

Upstream (at Cellular Level) Inhibition of Inflammasome (NLRP3) Pathway Activation by Inhibiting Cx43 Hemichannel Opening

Pathology = OVEREXPRESSION OF CONNEXIN43 HEMICHLANNELS IN THE CELL MEMBRANE

OPEN HEMICHLANNELS

ATP RELEASE INTO EXTRACELLULAR SPACE

Inflammasome (NLRP3) Pathway Activated

PERPETUATED RECYCLING OF NLRP3 INFLAMMASOME

Multiple pro-Inflammatory Cytokine Release

INFLAMMATION
VESSEL LEAK
EDEMA
ISCHEMIA
FIBROSIS
Lead Compound
Xiflam
(Oral Small Molecule)
Stage:
Phase 2B for Geographic Atrophy and Diabetic Retinopathy
Xiflam: Phase 2B Ready Orally Administered Cx43 Inhibitor for Geographic Atrophy (GA) and Diabetic Retinopathy (DR)

Phase 2B ready with full tox package and human safety database – IND to be filed for ophthalmology indications

Phase 2B Clinical trial design:
(1) Oral administration
(2) Randomized, controlled, dose ranging studies

Initial Indications:
(1) GA
(2) DR (Diabetic Macular Edema)

Mechanism of Action (MOA)
• Blocks premature Cx43 Hemichannel opening
• Stops resultant ATP release and Inflammasome (NLRP3) activation
• Inhibits release of multiple pro-inflammatory cytokines
• Ends the Perpetuated Cycle of Inflammation
• Addresses loss of vascular integrity, vascular leak, ischemia and edema
Xiflam Orally Dosed in Bright Light Model of GA

Methodology

- 2700 Lux, 30 cm above cage
- 24 hours exposure
- Xiflam vs Placebo treatment 2 hours into and at end of light exposure period
- Retinal Thickness Measured by OCT
- Visual Function Measured by ERG
- Follow-up Measurements at 2 weeks and 3 months
Orally Administered Xiflam Preserves Retinal Structure (OCT) and Function (ERG) in the Bright Light Preclinical Model of GA
Methodology

• An isolated strain of Sprague Dawley (SD) rats which develop diabetes within 4 weeks of birth (MSD rats)
• Glucose levels 14 – 21 mmol/L compared to 5.6 – 5.8 mmol/L in Normal SD rats
• MSD rats show clinical signs of diabetic retinopathy within 4 weeks of birth
• OCT imaging shows micro- and macroaneurysms
Micro aneurysms and macro aneurysms were specifically in the INL and ONL

Micro aneurysms - 20-30 µm diameter  Macro aneurysms - 140-160 µm diameter

OCT revealed approximately 5-8 abnormalities per eye (likely to be an underestimate)

Genotyping underway

Normal SD

Diabetic MSD – arrows indicate microaneurysms

Diabetic MSD – arrow indicates macroaneurysm
Xiflam Treated MSD rats with Diabetic Retinopathy have Fewer and Smaller Aneurysms and Significantly Improved Retinal Function (Week 8)

- Low dose 0.28mg/kg, once daily 14 days

Retinal OCT Imaging showing resolution of macro and microaneurysms

ERG Normal SD vs MSD (5 weeks)

ERG MSD vs Xiflam Treated MSD (8 weeks)
Connexin43 Expression Increases in DR around Blood Vessels (Human Donor Retina)

Region of neovascularization shows high Cx43 levels

DAPI = blue
Cx43 = Red
Isolectin (endothelial cell marker = green)

Xiflam - Orally Administered for GA and DR
Both Significant Unmet needs

- GA (Dry AMD) – no currently approved treatment – 80% of the AMD population
- Wet AMD is 20% and Tx is intravitreal anti VEGF generating $6B globally
- DR/DME – First line Tx intravitreal anti VEGF - 50% or more pts do not respond
- Ocunexus Patent Life (out to 2040) – Phase 2B ready compound
- Well tolerated with no drug related SAE’s – in over 1000 patients for migraine
- Orally dosed and bio-available – crosses both blood brain and retinal barrier
- Plan to file IND by Q3

Oral drug places the Optometric Community at the front line of Treatment for GA and DR