THE EFFECT OF NOVEL ANTI-INFLAMMATORY DRUGS ON ALZHEIMER'S DISEASE

Leah Marut
Advisor: Giridhar Akkaraju
Department of Biology

Background: Alzheimer's Disease

Neurodegenerative Disease

Characterized by cognitive, function, and neuronal loss

Most Common Form of Dementia

Associated with memory loss, impaired judgment, vision/spatial issues, etc

Currently Affects Over 5 Million Americans

This also creates a financial burden, and this number is only expected to rise as more of the population ages

Alzheimer's Pathology

Beta-Amyloid Plaques

Accumulation of these natural proteins between neurons can disrupt cell function

Neurofibrillary Tangles

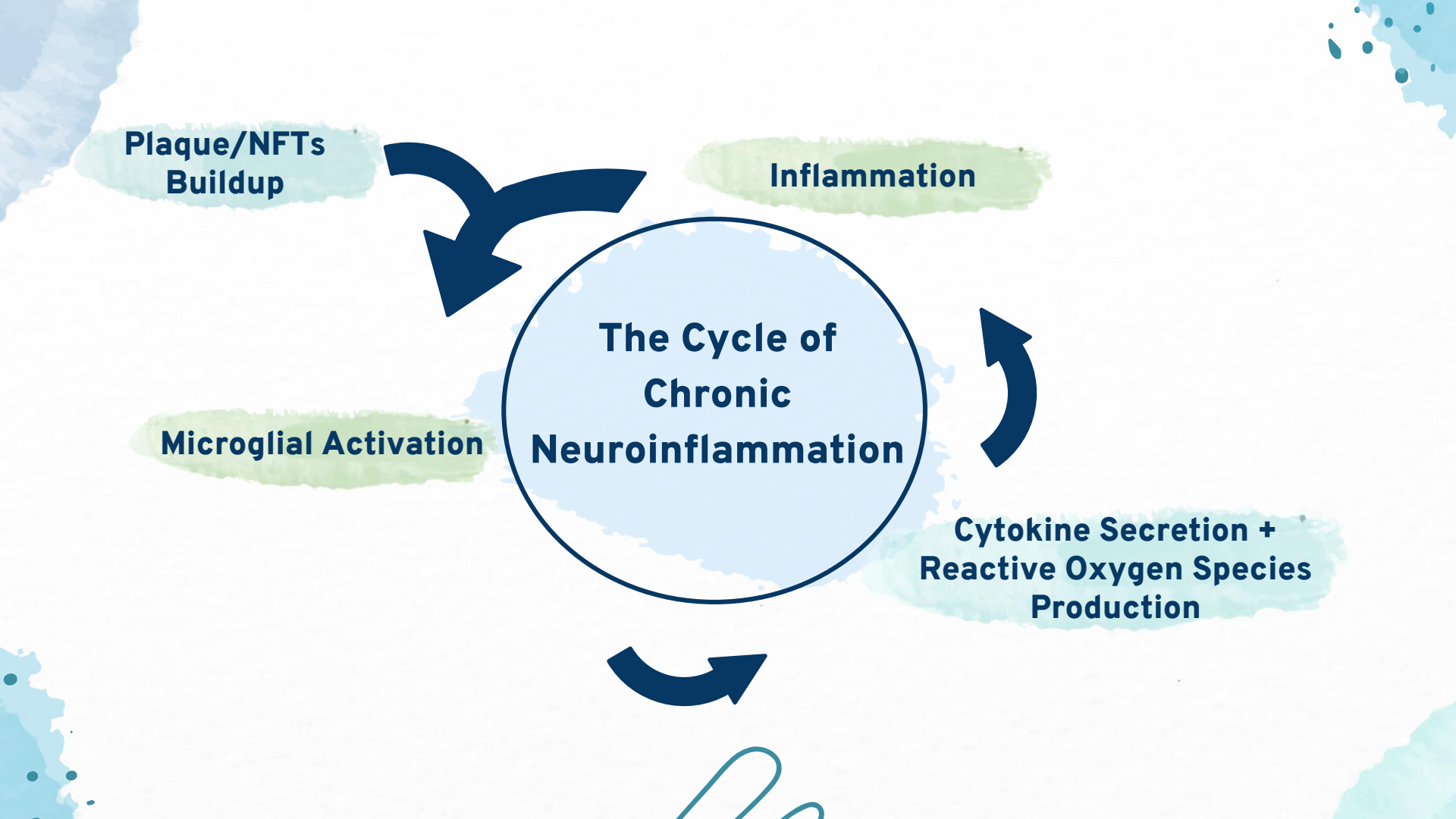
Hyperphosphorylation of tau proteins (stabilize microtubules) → tau proteins aggregate into NFTs, and microtubules collapse (compromises cell function)

Microglial Cell Activity

These cells usually clear away toxins and protein collections (e.g. Aβ proteins before they can form plaques), but have demonstrated a loss in this ability after prolonged activation

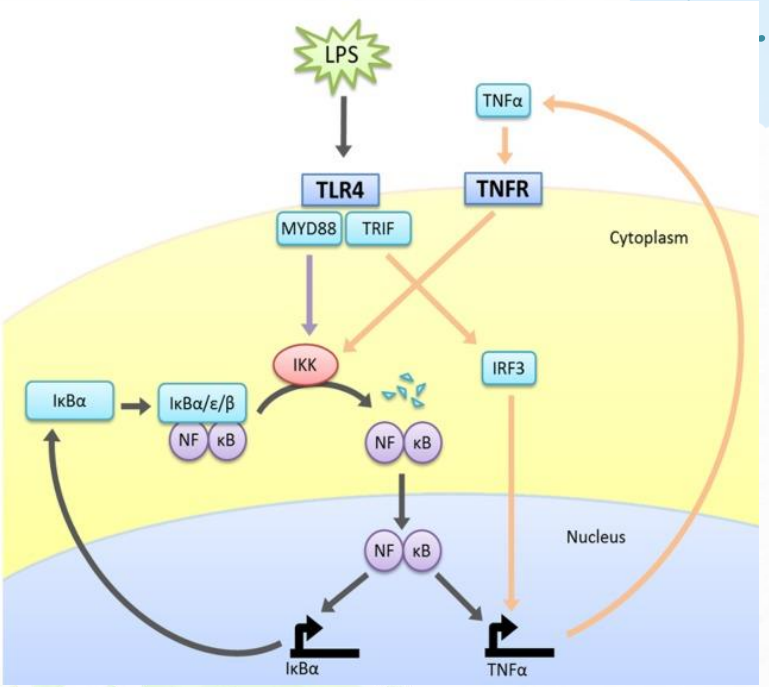
Chronic Inflammation

May provide a link between these known pathologies by facilitating or exacerbating them

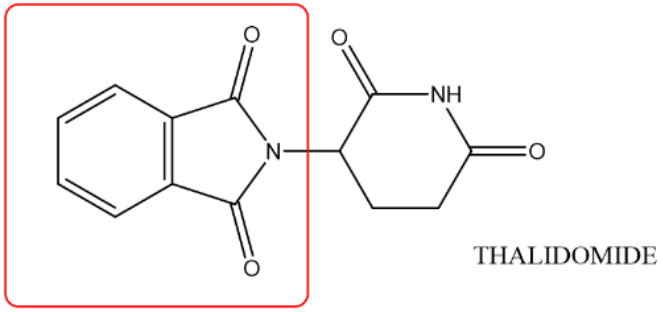

Plaque/NFTs Buildup
Inflammation
The Cycle of Chronic Neuroinflammation
Microglial Activation
Cytokine Secretion + Reactive Oxygen Species Production

Background: NF-κB + Inflammation Pathway

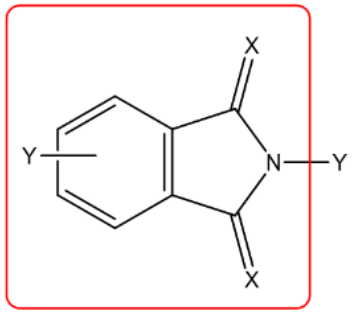
- Transcription factor that controls the expression of pro-inflammatory cytokines and IκBα subunit
- Constitutively bound to inhibitory protein IκBα
- When the inflammation pathway is stimulated, IκB Kinase (IKK) phosphorylates IκBα → induces its dissociation, ubiquitination, and subsequent degradation
- NF-κB is allowed to travel into the nucleus and upregulate its target genes



Structure of the Drugs Tested



THALIDOMIDE

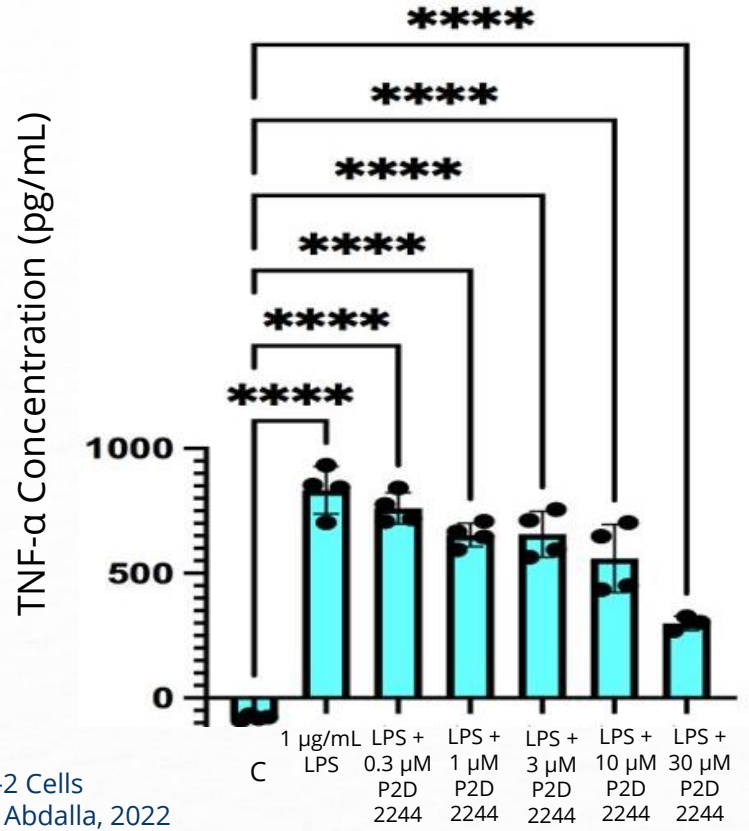


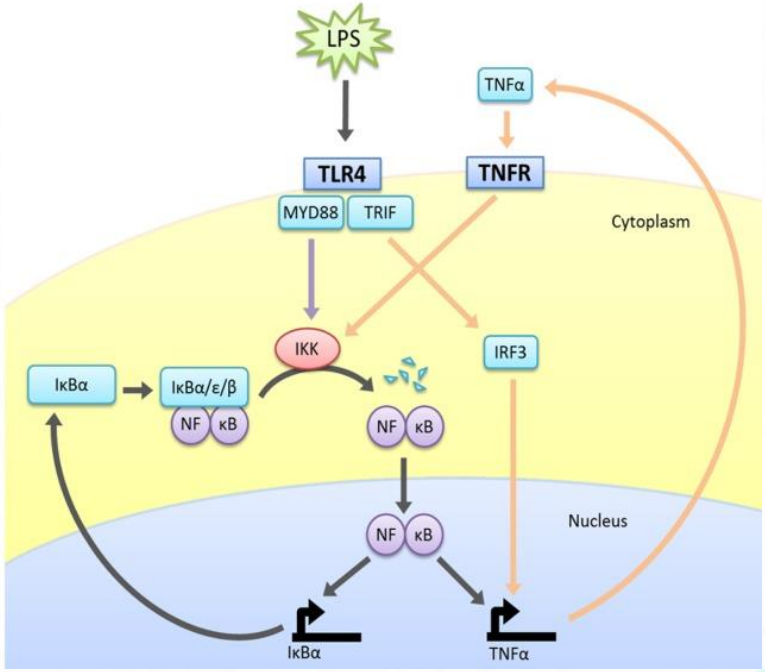
X= O, S,

Y= ALKYL, ARYL, H, NH_2, OH etc.

- The drugs tested are created and provided to us by P2D Biosciences
- These drugs are analogs of the isoindolinone moiety of thalidomide

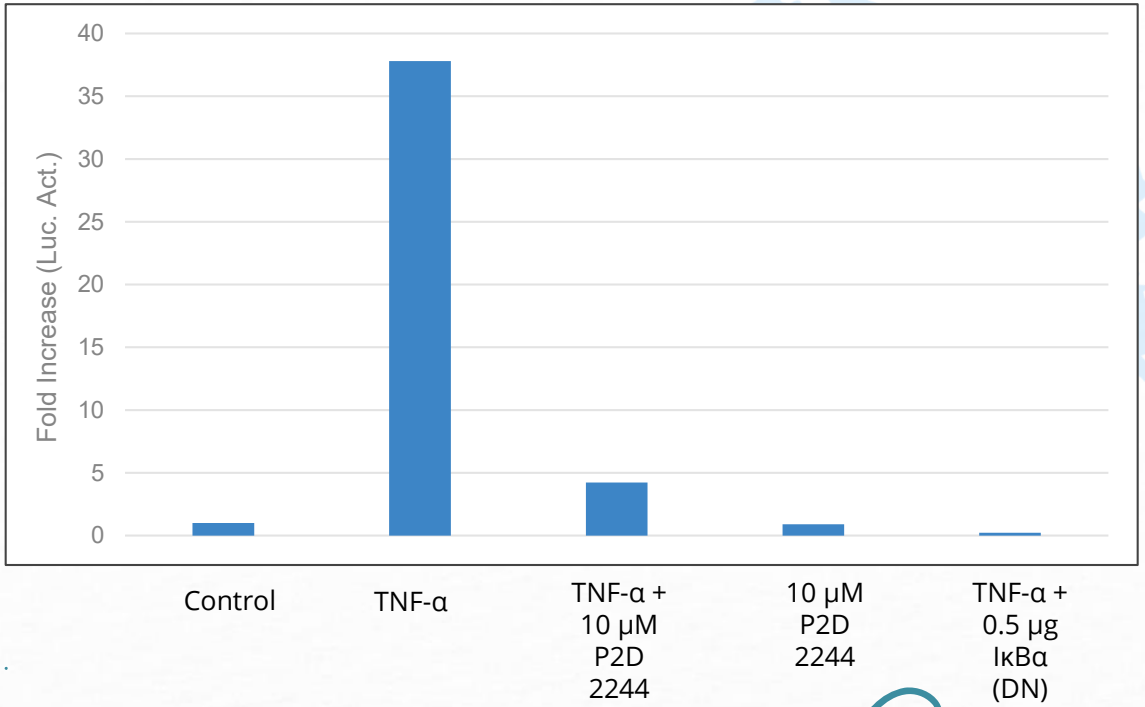
LPS-Induced TNF-α Secretion in the Presence of P2D 2244

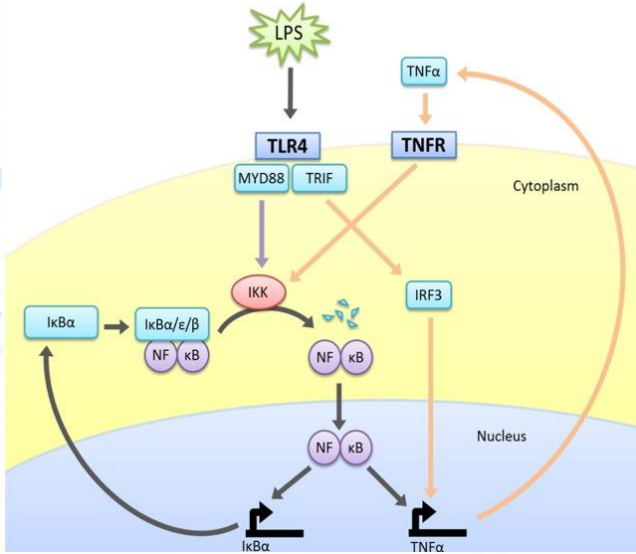




BV-2 Cells
Source: R. Abdalla, 2022

P2D 2244 Inhibits the TNF-α Induced Activation of NF-KB





HEK293 Cells
Source: K. Paugh, 2023

Hypothesis

The class of novel anti-inflammatory drugs we are testing inhibits NF-κB activation by inhibiting IκBα degradation.

Testing The Hypothesis

Procedure: Overview

Culture Cells

Grow BV-2 cells in incubator

Treat Cells

Perform a time course treatment with a P2D-treated set against a control set

Western Blot

Allow for detection of IκBα + visual representation of IκBα levels

Analyze Results

Visualize Western results and quanitfy IκBα levels across the samples

Procedure: Cell Treatment

Incubate with P2D

Allow P2D to be taken up by cells

LPS Time Course

Apply LPS in varying time increments ranging from 0-60 minutes to induce cell inflammation

Harvest All Cells

Remove cells from LPS to stop the time course; prepare them for further analysis

Procedure: Western Blot

Run a Gel

Separate all the proteins in the cell samples by size

Transfer to Membrane

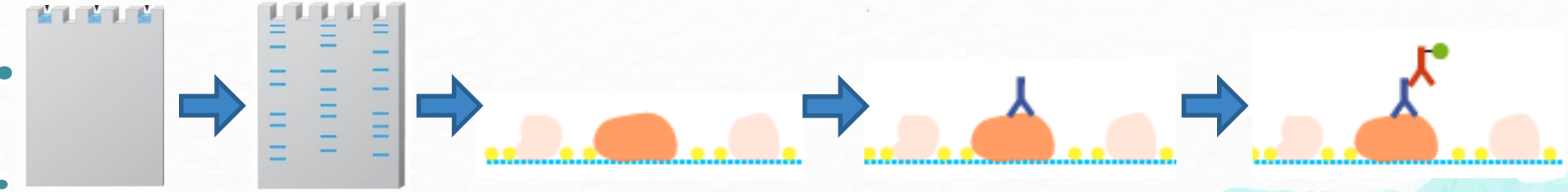
Transfer gel results to a more durable membrane

Primary Antibodies

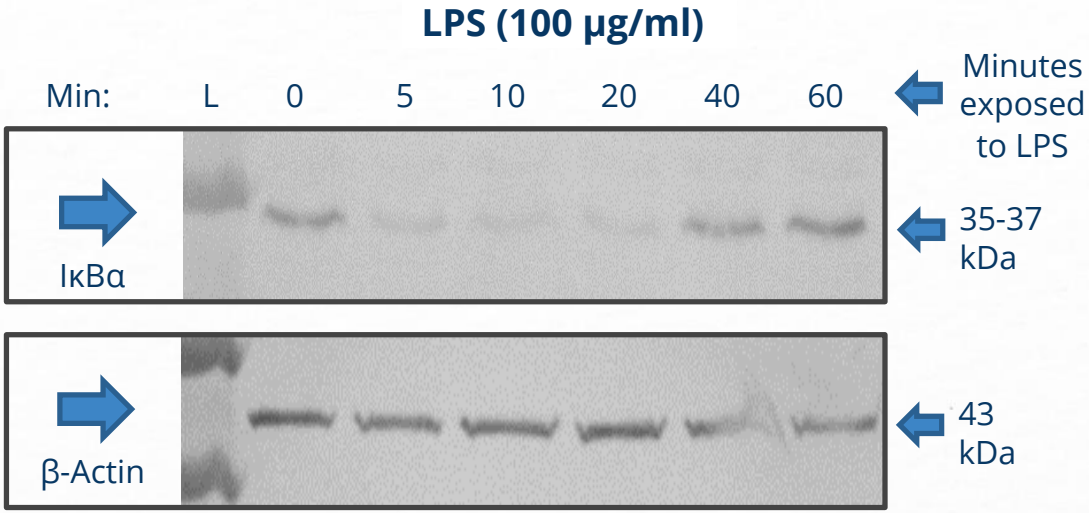
Incubate with primary antibodies that bind to IκBα

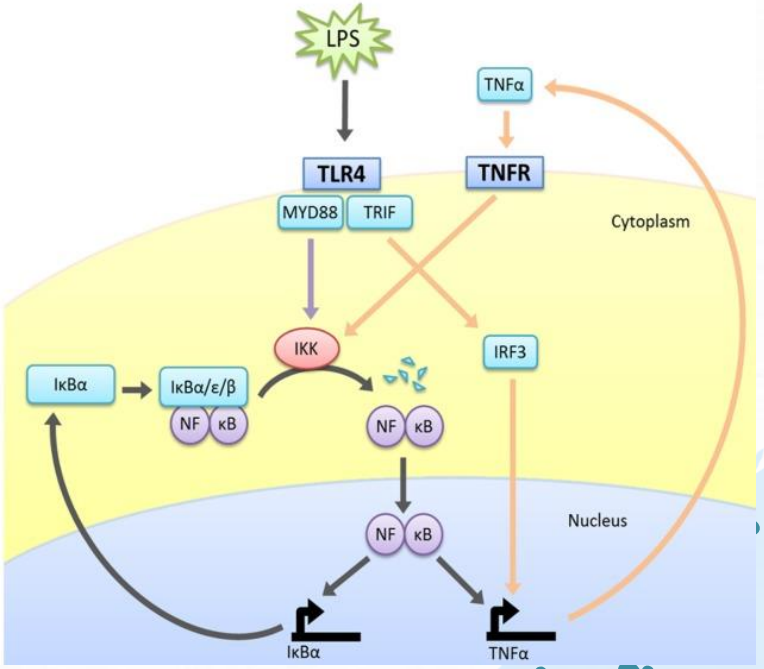
Secondary Antibodies

Incubate with secondary antibodies that bind to the primary antibodies and allow for detection



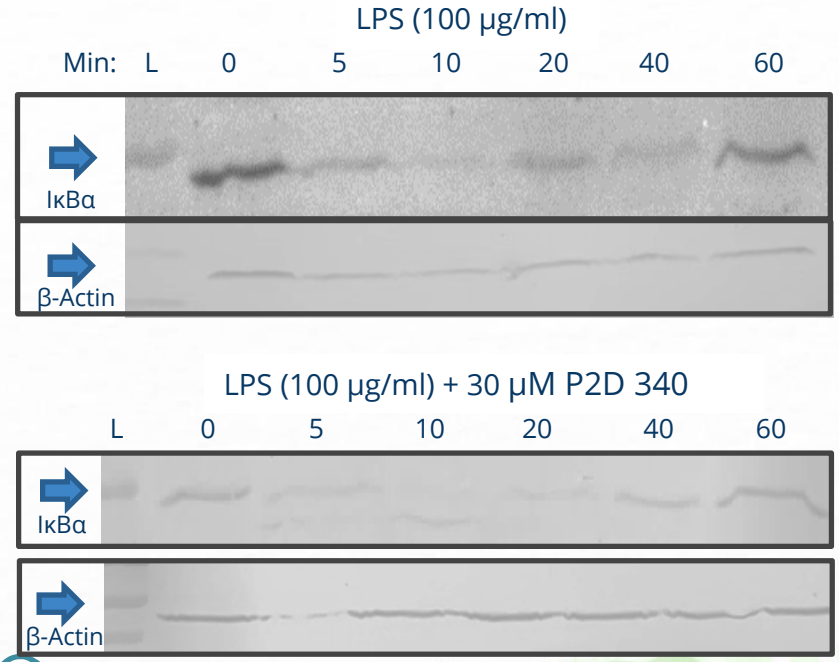
Interpreting Western Blot Results

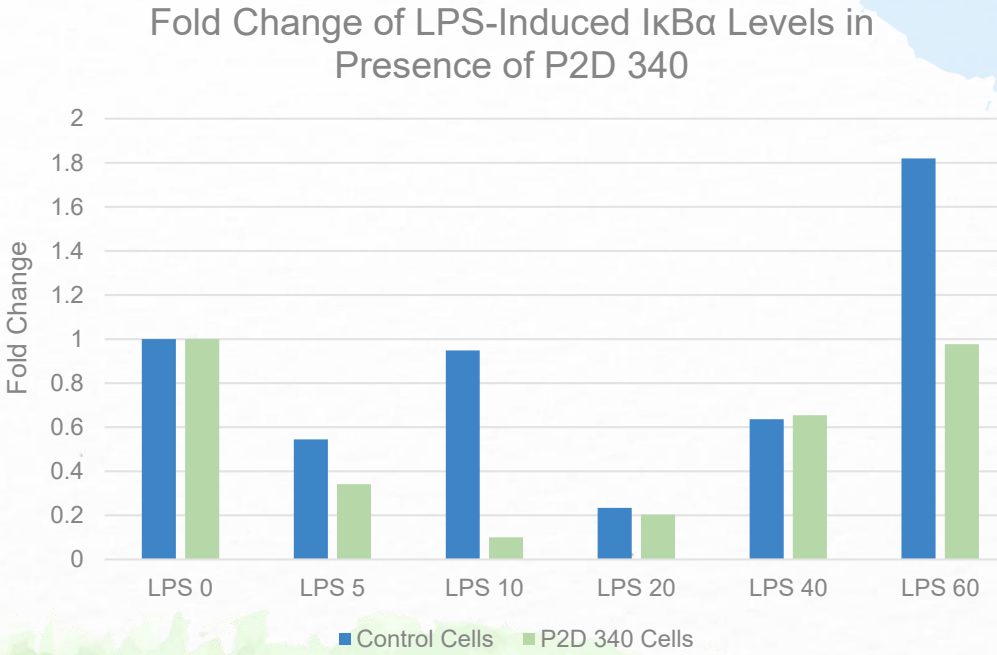




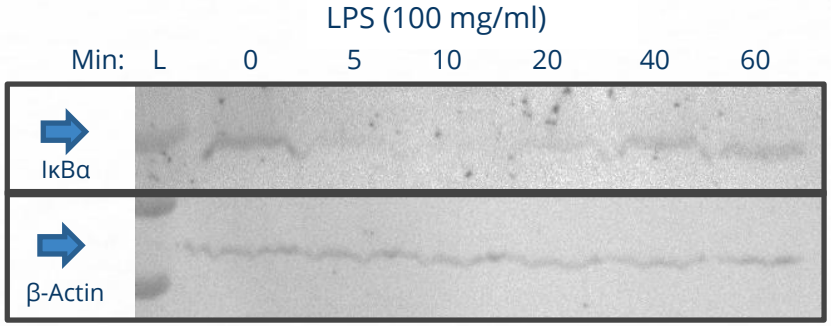
Results!

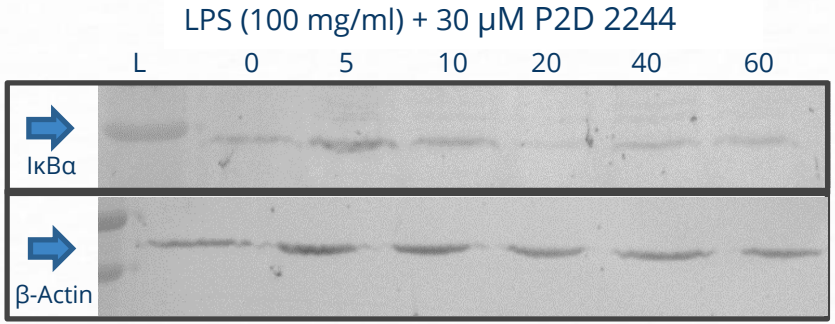
The Effect of P2D 340 on LPS-Induced Degradation of IκBα

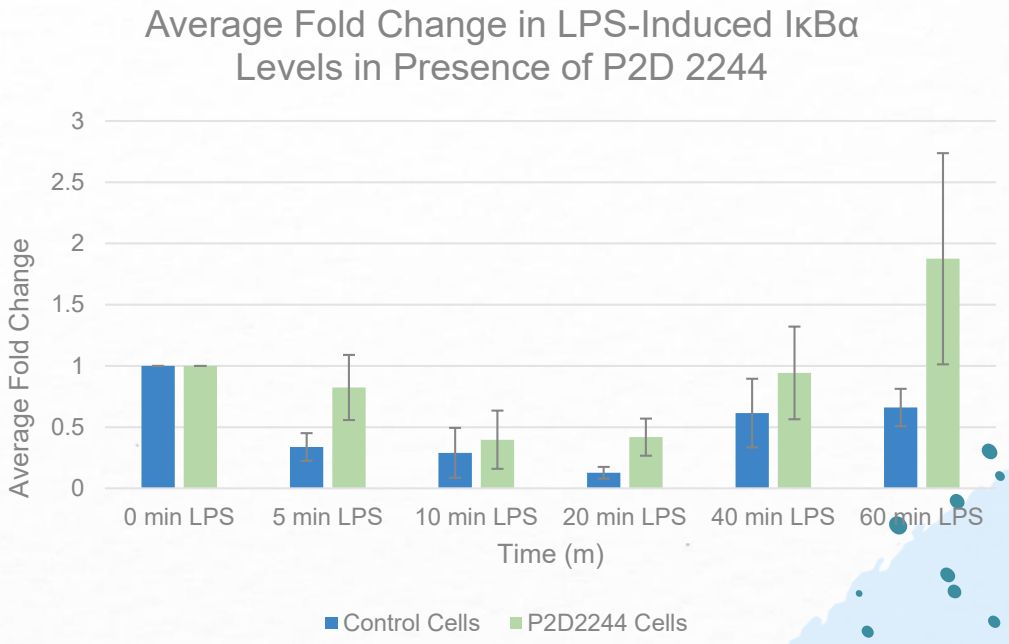




P2D 2244 May Inhibit the LPS-Induced Degradation of IκBα







Summary, Conclusions, & Next Steps

2 novel drug compounds were tested for their effect on IκBα degradation

P2D 340

Results did not suggest that P2D 340 inhibits IκBα degradation

→ This compound will not be further pursued for its ability to decrease NF-κB activation

P2D 2244

In prior mouse studies, P2D 2244 demonstrated greater ability to improve memory and cognition

→ Results suggest drug inhibits IκBα degradation at early time points

→ Further elucidate the compound's mechanism of action and the location of IκBα interference

Thank you!
Questions?