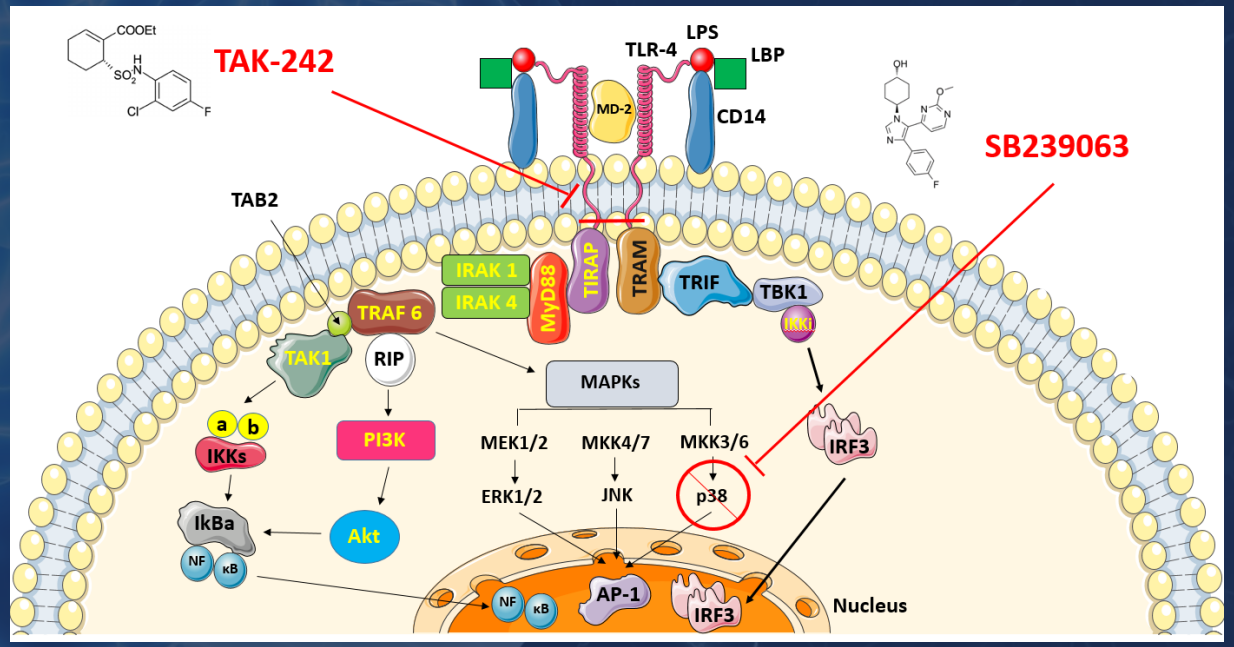
Introduction

- Alzheimer's disease (AD) - chronic, age-related neurodegenerative disorder
- Inflammation in AD is thought to accelerate neuronal cell degeneration, synapse loss and contribute to the worsening of disease severity
- Activation of microglial Toll-like receptor 4 (TLR4) by AD-specific DAMPs leads to the activation of the p38 MAPK pathway and upregulation of pro-inflammatory mediators
- In the AD brain, p38 MAPK activation is increased - potential therapeutic target



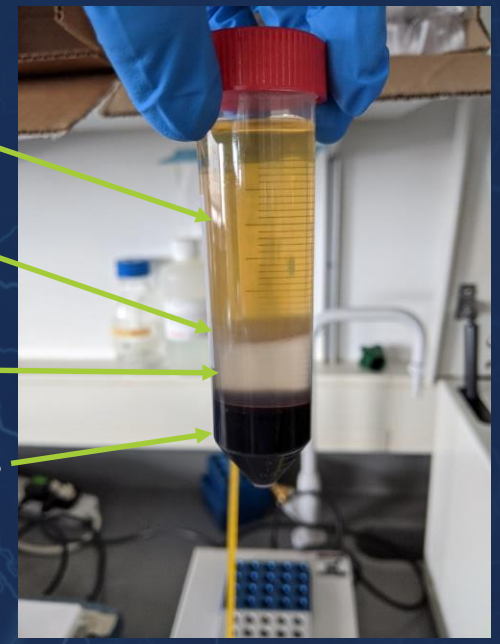
Aim:

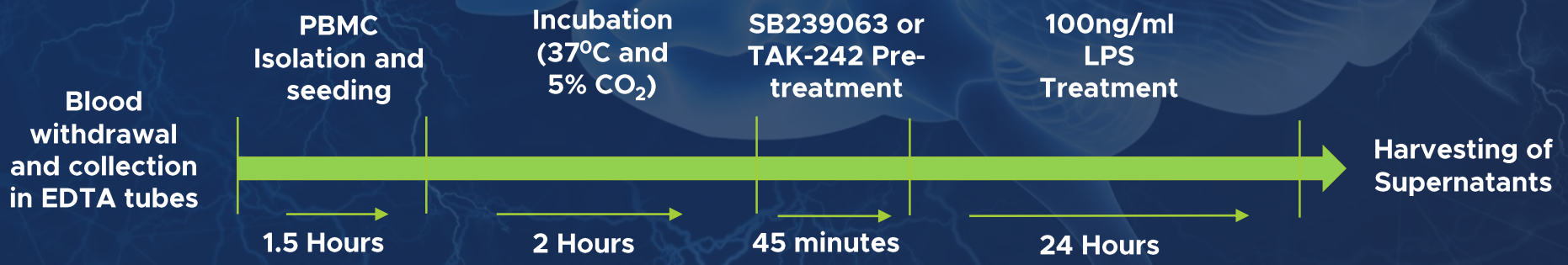
- ➢ To establish an *ex vivo* human peripheral blood mononuclear cell (PBMC) assay for screening novel p38 MAPK inhibitors

Methods

- Whole blood obtained from healthy donors:
  - Male (n=3) and female (n=2)
  - Aged between 18-60
  - Not taking any anti-inflammatory medications at the time of sampling

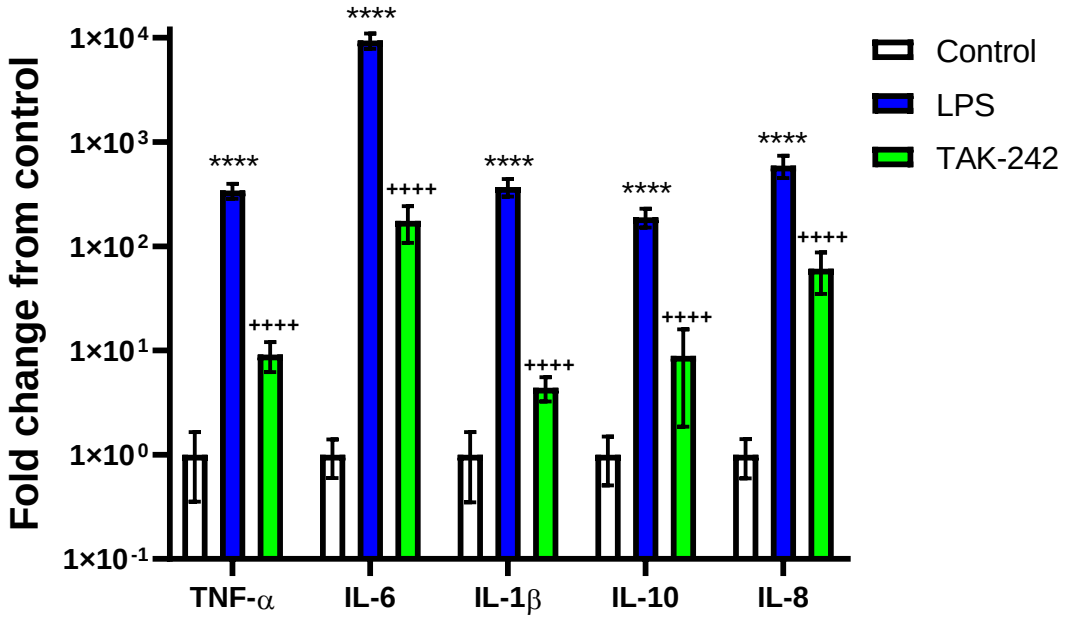
- Cytokines analysed using Meso Scale Discovery V-Plex multiplex assay (Data: Mean ± SEM, One-Way ANOVA, Fisher's LSD Post-hoc test)





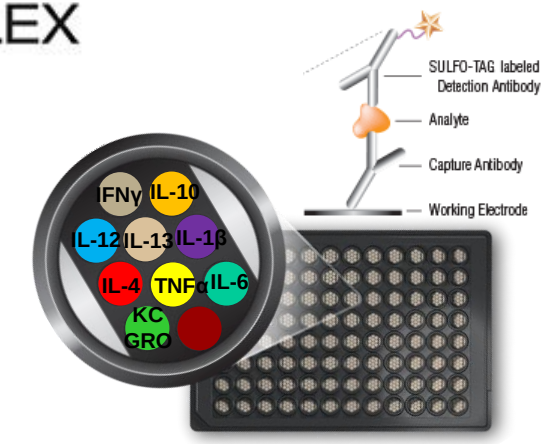
Results

- Initial assay validation – LPS ± TLR-4 antagonist TAK-242
- V-plex Meso Scale Discovery



Statistical Analysis
*****P*<0.0001 vs Control
$^{++++}$*P*<0.0001 vs LPS
One-way ANOVA (*P*<0.01); Fisher's LSD. Values expressed as protein concentration (pg/ml)

V-PLEX



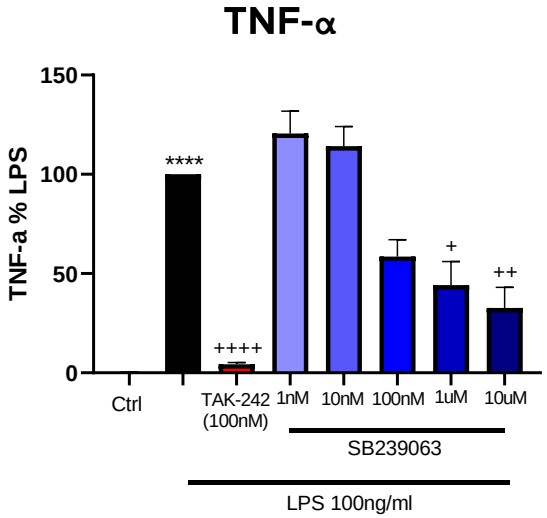
MSD Meso Scale Discovery

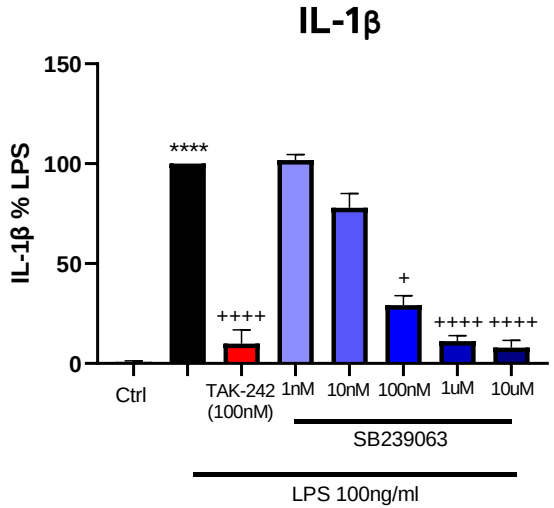
Pro-inflammatory Panel

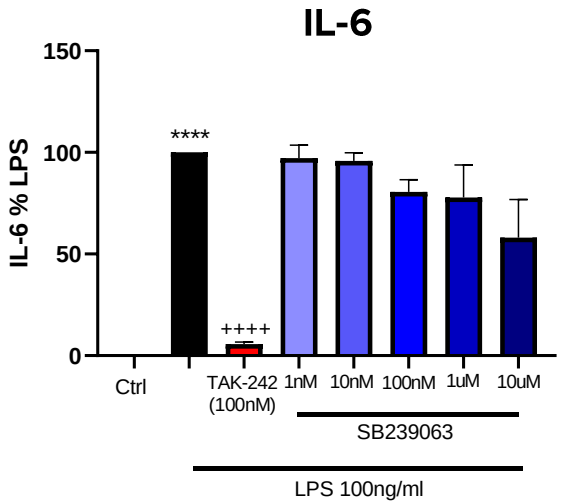
- ❑ IL-1β
- ❑ IL-6
- ❑ TNF-α
- ❑ IL-10
- ❑ IL-8

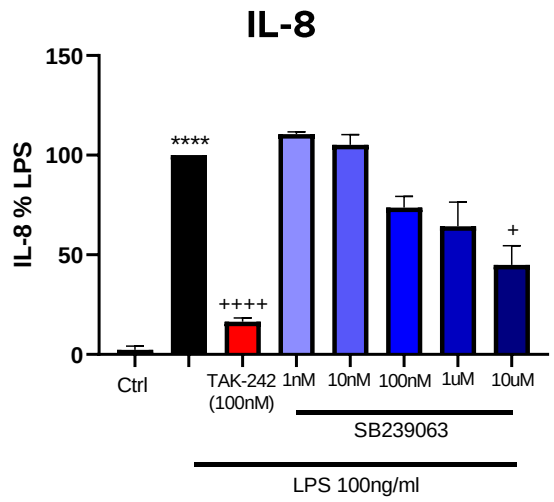
Results

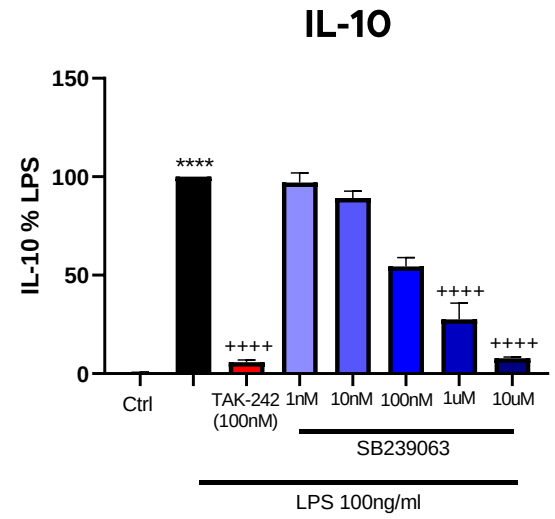
Figure 1: Effect of SB239063 (p38 MAPK inhibitor) on cytokine expression in human PBMC cell culture supernatants following LPS stimulation







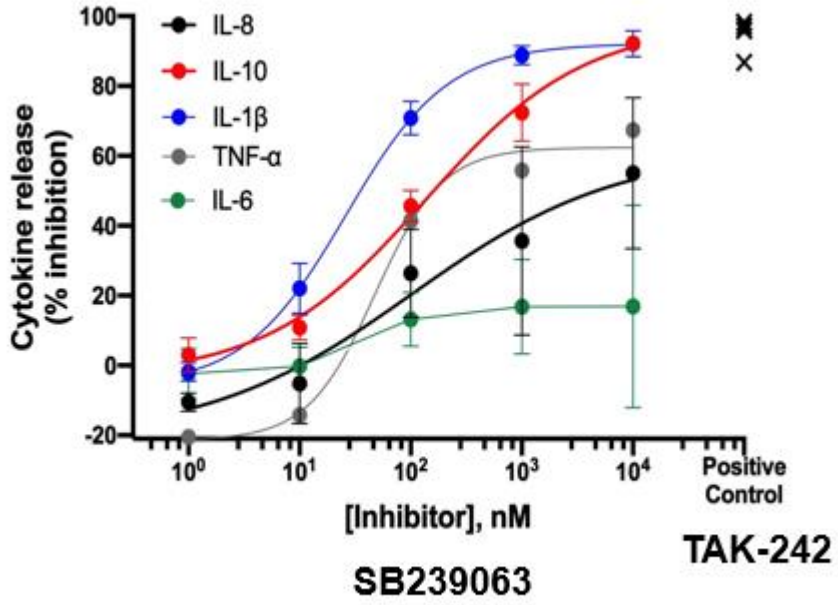




Statistical Analysis	
One-Way ANOVA (P<0.01); Fisher's LSD. Values expressed as % LPS	
****	*P*<0.0001 vs Control
+	*P*<0.05 vs LPS
++	*P*<0.01 vs LPS
++++	*P*<0.0001 vs LPS

Results

Figure 2: Maximum inhibition of SB239063 (%) and IC_{50} cytokine expression in human PBMC cell culture supernatants following LPS stimulation



Target	Maximum inhibition (%)	IC_{50} (nM)	Positive control inhibition (%)
IL-8	55.1	102.1	86.8
IL-10	92.2	135.0	95.9
IL-1β	92.1	26.1	98.2
TNF-α	67.4	47.8	95.9
IL-6	16.9	39.1	97.1

Conclusion/Summary

- LPS stimulation for 24 hours produced a significant increase in the expression of all cytokines measured, which was prevented by TAK-242 (all cytokines) and SB239063 in a concentration-dependent manner for all cytokines measured bar - IL-6 (conflicting evidence – macrophages Page et al. 2010).

- These data suggest that the p38 MAPK inhibitor SB239063 can prevent LPS-mediated cytokine production in PBMCs.

- PBMCs represent a cost effective, semi-high-throughput assay for testing novel p38 MAPK inhibitors under investigation for the treatment of AD-associated inflammation

- PBMCs isolated from AD patients are reported to exhibit altered innate immune activity in comparison to aged-matched controls, thus, future work aims to establish this assay in patient-derived PBMCs