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Confirm the Efficacy of Brain-Penetrant Candidate NLRP3 Inhibitors using the Human iPSC-Derived Microglia Inflammasome Assay

Introduction

Inflammasomes are large intracellular protein complexes that assemble in response to danger signals. Different types of inflammasomes respond to different activating stimuli and once assembled, they activate Caspase-1, triggering the secretion of IL-1β and IL-18 and the induction of pyroptosis, a lytic and inflammatory form of cell death, via cleavage of Gasdermin D (Figure 1). While inflammasomes are essential for innate host defence, their unrestrained activation is a key contributor to the chronic inflammation that underlies neurodegenerative disorders. Among inflammasomes, NLRP3 is a well-validated target for neurodegenerative diseases.

Use our Human iPSC-derived Microglia Inflammasome Assay to confirm that your candidate NLRP3 inhibitors are active in microglia, the key drivers of neuroinflammation, and fit for Central Nervous System (CNS) indications.

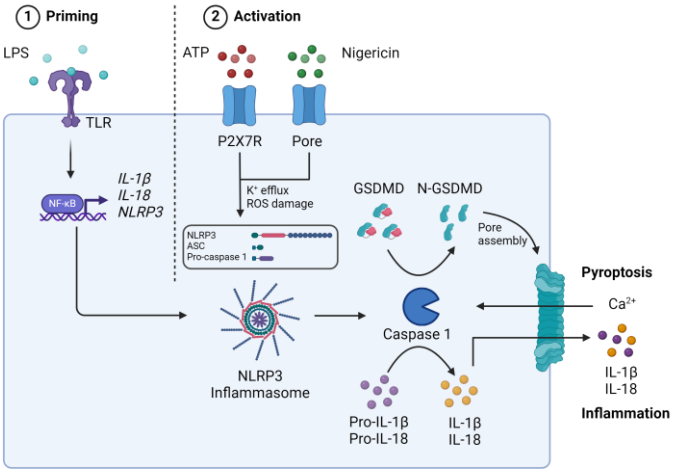

Figure 1. NLRP3 activation requires a priming stimulus and an activation stimulus. The priming stimulus activates NF-kB signalling, which in turn induces the precursor proteins of IL-1β and IL-18, as well as inflammasome component NLRP3, providing the elements for inflammasome complex formation and downstream signalling. Both endogenous (e.g. ATP released from damaged cells) or exogenous (e.g. the ionophore Nigericin) signals can act as activating stimuli, triggering the cytosolic assembly of a complex comprising the sensor protein NLRP3, the adaptor protein ASC and the effector protein Caspase 1. The proteolytic activity of Caspase-1 leads to the generation of biologically active IL-1β and IL-18, as well as Gasdermin D. The latter forms pores in the plasma membrane, thus releasing IL-1β and IL-18 and triggering a specialised form of cell death known as pyroptosis.

Methods

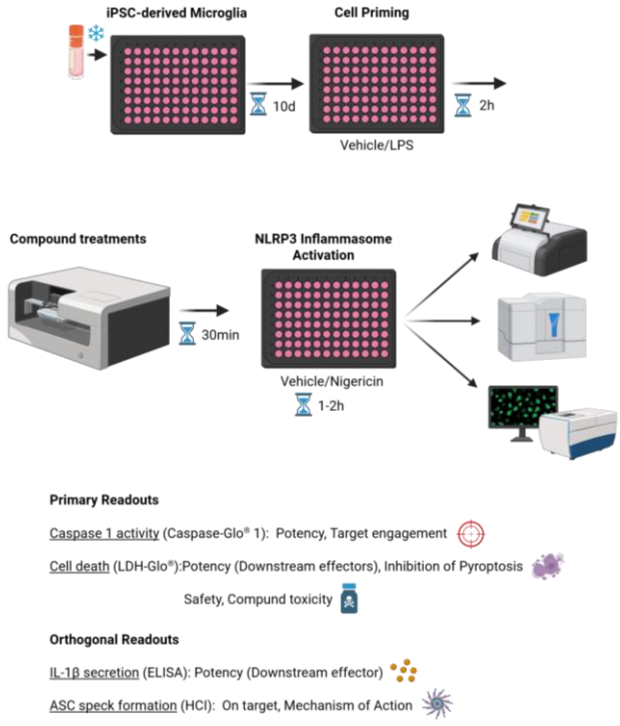

Figure 2. Human iPSC-derived microglia (microglia) were recovered from cryopreserved stocks and stabilised in culture for 10 days before being primed with either vehicle or LPS for 2 hours. Next, the cells were pre-treated with reference or test compounds for 30 minutes, followed by NLRP3 activation with either vehicle or Nigericin for up to 2 hours. Cell culture supernatants were collected and used to quantify Caspase-1 activity, as well as LDH and IL-1β concentration. The cells were then fixed and stained with a fluorescently labelled anti-ASC antibody and CellMask™, with nuclei counterstained with DAPI. Stained cells were analysed by high content imaging (HCI).

Results

LPS priming followed by Nigericin induces strong inflammasome activation in microglia.

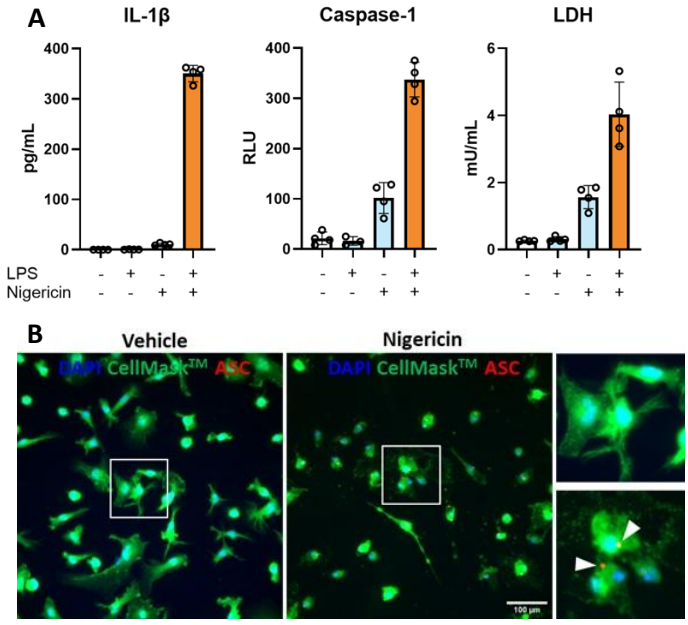

Figure 3. (A) IL-1β, Caspase-1 activity and LDH (an indicator of pyroptosis) were quantified in cell culture supernatants from microglia exposed to the indicated stimuli. **(B)** Microglia were immunostained with a fluorescently labelled anti-ASC antibody (red), CellMask™ (green), and DAPI (blue) after treatment with vehicle or LPS + Nigericin. Arrowheads point to ASC specks. **(C)** ASC specks were quantified by HCI in microglia exposed to the indicated stimuli. All data shown represents the mean (± SEM) of four technical replicates.

Concentration-dependent inhibition of microglial inflammasome activation by the selective NLRP3 inhibitor MCC950

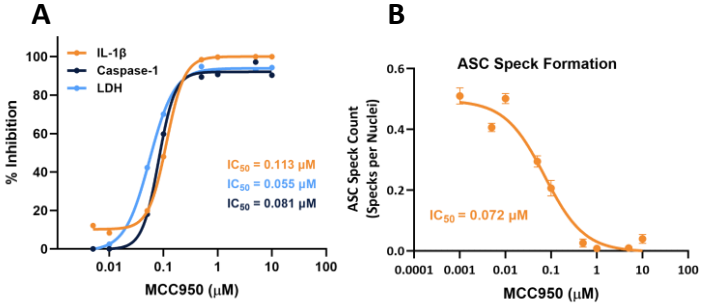

Figure 4. (A) Positive control validation. Concentration-dependent inhibition of IL-1β release, Caspase-1 activity and LDH by reference NLRP3 inhibitor MCC950. The calculated IC_{50} is indicated for each readout. **(B)** ASC specks were immunolabelled and quantified by HCI in microglia stimulated with LPS and Nigericin in the presence or absence of increasing concentrations of NLRP3 inhibitor MCC950. The calculated IC_{50} is shown.

Conclusions

NLRP3 inflammasome activation is not only a result of CNS tissue injury in neurodegenerative diseases but is also considered a major driver of the neuroinflammatory cascades that fuel their pathogenesis. Consequently, CNS-targeted NLRP3 inhibitors may offer therapeutic benefit across multiple neurodegenerative conditions, such as Alzheimer's and Parkinson's disease. Our human iPSC-derived microglia inflammasome assay delivers robust functional data on candidate CNS-targeting NLRP3 inhibitors, giving you decision-ready human evidence to confidently de-risk your progression into the clinic.

Key Assay Features:

- ✓ Physiological relevance for CNS indications: confirm compound efficacy in the key drivers of inflammation in human CNS.
- ✓ Disease relevance: assess compound effects in microglia carrying relevant disease-associated mutations.
- ✓ Industry standard Caspase-1 activation readout: quickly and effectively confirm target engagement and measure drug potency.
- ✓ Gain additional confidence by measuring IL-1β (effector function) and LDH release (pyroptosis).
- ✓ ASC speck formation: visualise and directly quantify the assembly of the inflammasome complex.
- ✓ Multiplex up to four readouts for maximal efficiency and insight.
- ✓ Off-target toxicity can be measured in untreated microglia
- ✓ Statistical analysis and IC_{50} calculations as standard
- ✓ Seamlessly integrates with our THP-1 NLRP3 screening assay and our Organotypic Brain slice NLRP3 assay to build a screening cascade.