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A multiplex functional *in vitro* high throughput assay to measure compound effects on the inflammasome

White Paper

Introduction

Inflammasomes are multimeric protein complexes that act as part of the innate immune response to induce inflammation upon recognition of pathogenic or damage signals. They are therefore key regulators of the inflammatory response. Nucleotide-binding oligomerization domain-like receptors family pyrin domain containing 3 (NLRP3) is one such inflammasome component that has been well characterised in neuroinflammation with links to chronic pain[1] and neurodegenerative diseases[2], such as Parkinson's and Alzheimer's Disease, as well as a number of other diseases such as cancer[3] and diabetes[4].

Upon activation of the NLRP3 inflammasome, inflammatory cytokines, such as interleukin-1β (IL-1β), are released in a caspase-1 dependent manner, and act to recruit immune cells to the site of infection/damage[5].

The NLRP3 inflammasome can be primed by lipopolysaccharides (LPS), through activation of toll-like receptors (TLR), and subsequently activated by nigericin, or adenosine triphosphate (ATP) (Figure 1). Compounds can therefore be tested for NLRP3 inhibition *in vitro*, with reduction of readouts, such as IL-1β and pyroptosis, used as markers of inflammasome inhibition. Domainex has established a robust assay using THP-1 cells (Figure 2). MCC950 is a known NLRP3 inhibitor and was therefore tested in concentration-response format to demonstrate the validity of the assay (see Figure 3).

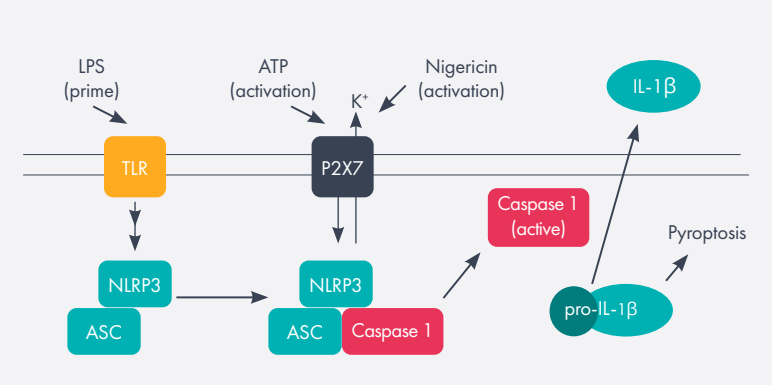

Figure 1: Schematic representation of inflammasome activation

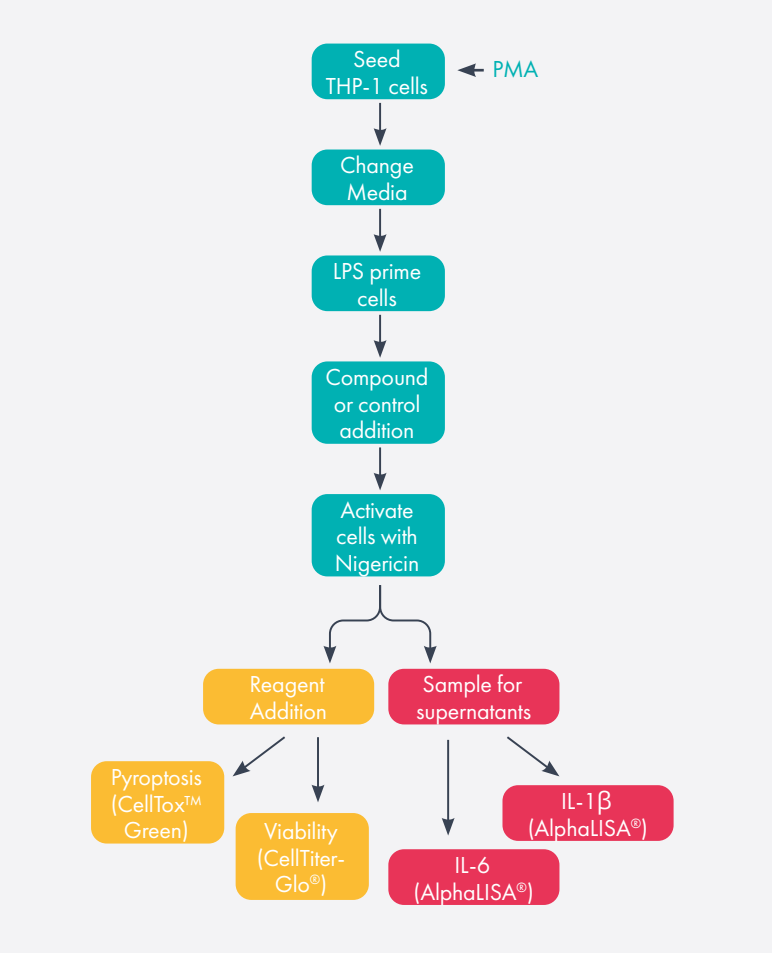

Figure 2: Inflammasome assay method schematic

Method

THP-1 cells are plated into 384 well format and differentiated to a macrophage phenotype using Phorbol 12-myristate 13-acetate (PMA). Cells are primed with LPS before incubation with the test and control compounds. Following this incubation, cells are activated with nigericin and incubated for a further period after which supernatants are collected and stored. CellTiter-Glo® or CellTox™ Green are added to the remaining assay volume and the plate read for luminescence. IL-1β and IL-6 AlphaLISAs are performed on stored supernatants.

CellTiter-Glo® and CellTox™ Green results are reported in relative light units (RLU). Cytokine measurements (IL-1β and IL-6) are reported as concentration (pg/ml). pIC_{50} results for MCC950 are given for all readouts.

Domainex also routinely performs the assay using human whole blood (data not shown) in a 96 well plate assay format, with reduction of IL-1β used as a marker of inflammasome inhibition.

Example Data

As demonstrated in Figure 3, the CellTiter-Glo® and CellTox™ Green readouts (indicating viability and pyroptosis respectively) are inversely related. Pyroptosis, a highly inflammatory form of lytic programmed cell death, is triggered upon activation of the NLRP3 inflammasome, therefore a concentration-dependent reduction in CellTox™ Green or an increase in CellTiter-Glo® demonstrate the inhibitory effect of MCC950 on the NLRP3 inflammasome.

A concentration-response effect is also seen with IL-1β secretion, demonstrating inhibition of responses at higher MCC950 concentrations due to the suppressive action of MCC950 on the NRLP3 inflammasome. IL-6 acts as a control with no MCC950 mediated effected on its production.

Conclusion

Domainex has established a robust activity assay for screening compounds for inhibition of NLRP3 inflammasome, which can be performed in THP-1 cells or human whole blood. A range of assay readouts can be measured in a multiplexed manner. Domainex can develop the assays in further cell lines, on behalf of clients, as required.

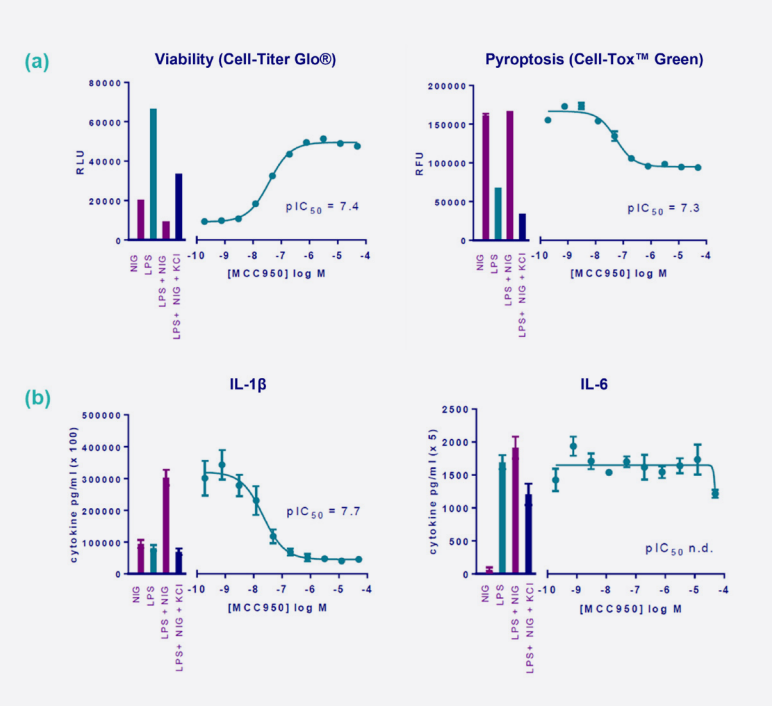

Figure 3: MCC950 inhibition of inflammasome markers. Panel (a) shows luminescence-based readouts for viability (CellTiter-Glo®) and Pyroptosis (CellTox™ Green). Panel (b) shows AlphaLISA based readouts for IL-1β and IL-6 measurement. Nigericin (Nig) only, LPS only, LPS and Nigericin and LPS, Nigericin and KCl controls (extracellular potassium to demonstrate the role of K^+ efflux as in figure 1) are found on the left-hand panel of each figure. pIC_{50} values are also shown.

References

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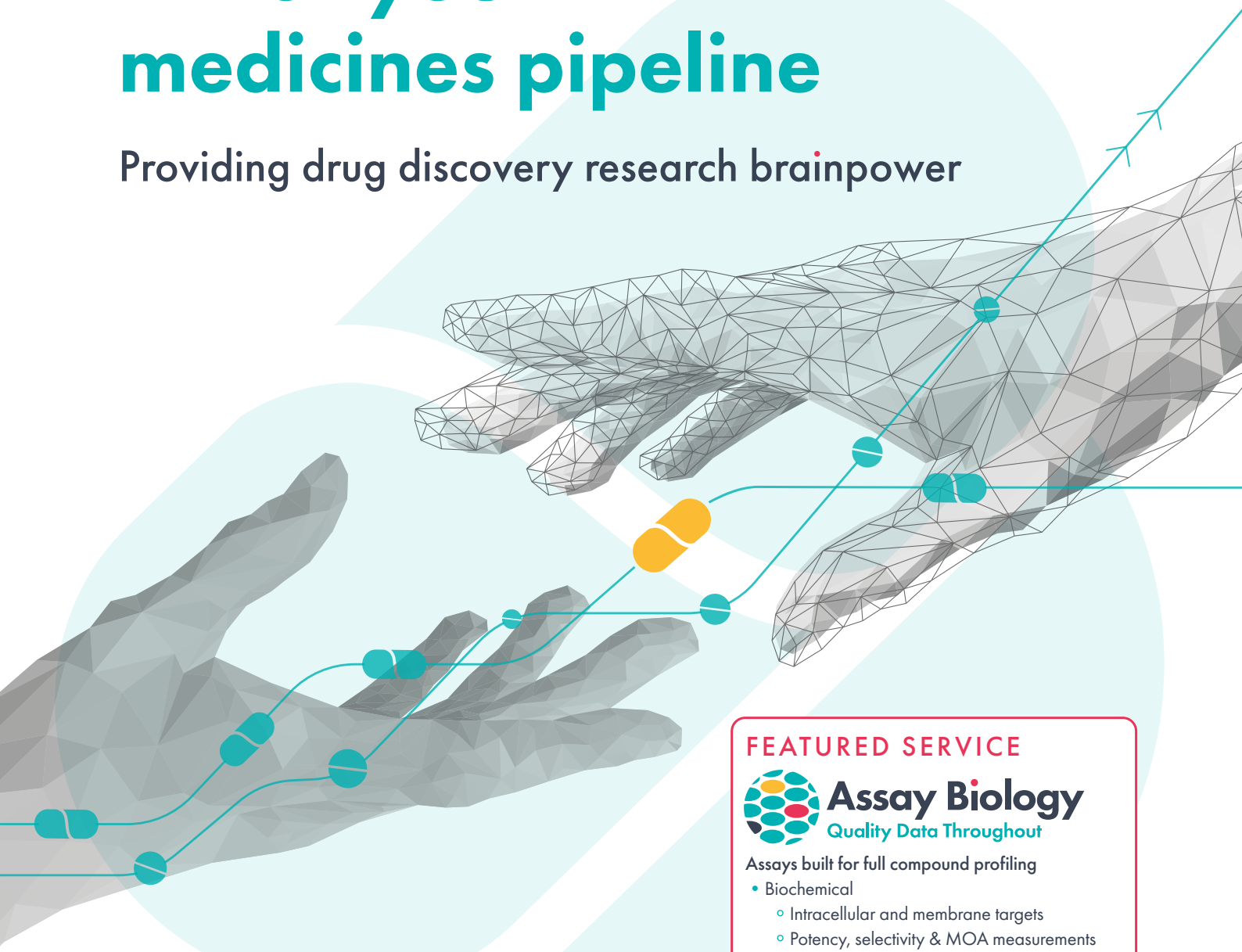

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