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# RIPK1 activation mediates disease progression in multiple sclerosis by driving neuroinflammatory signaling in microglia and astrocytes

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## INTRODUCTION

- Multiple sclerosis (MS) is a chronic neurodegenerative disease of the central nervous system (CNS) characterized by neuroinflammation, demyelination, and axonal degeneration.
- Microglia and astrocytes are altered to an activated, pro-inflammatory state during MS but current therapies target peripheral immunity rather than focusing on neuroinflammation directly in the CNS.
- Receptor interacting protein kinase 1 (RIPK1) mediates inflammatory and cell death signaling downstream of death and Toll-like receptors, and aberrant RIPK1 activation has been implicated in various inflammatory and neurodegenerative diseases[1-3].

## METHODS

We utilize the Meso Scale Discovery (MSD) platform to assess RIPK1 activation and expression levels in cells and mouse and human tissue samples

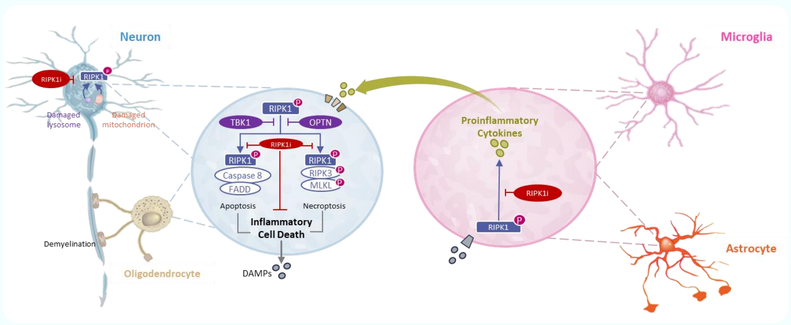
We establish a human induced pluripotent stem cell (iPSC)-derived tri-culture system containing neurons, astrocytes, and microglia

Bulk and single cell RNA-sequencing approaches are used to interrogate the transcriptional effect of RIPK1 activation in vitro and in the experimental autoimmune encephalomyelitis (EAE) mouse model of MS

## OBJECTIVES

- To investigate the role of RIPK1 kinase activity in various CNS cell types and its relation to MS pathogenesis.
- To assess RIPK1 kinase activation and expression in post-mortem brain samples from MS patients relative to control

**Proposed mechanism of RIPK1 inhibition in the CNS**

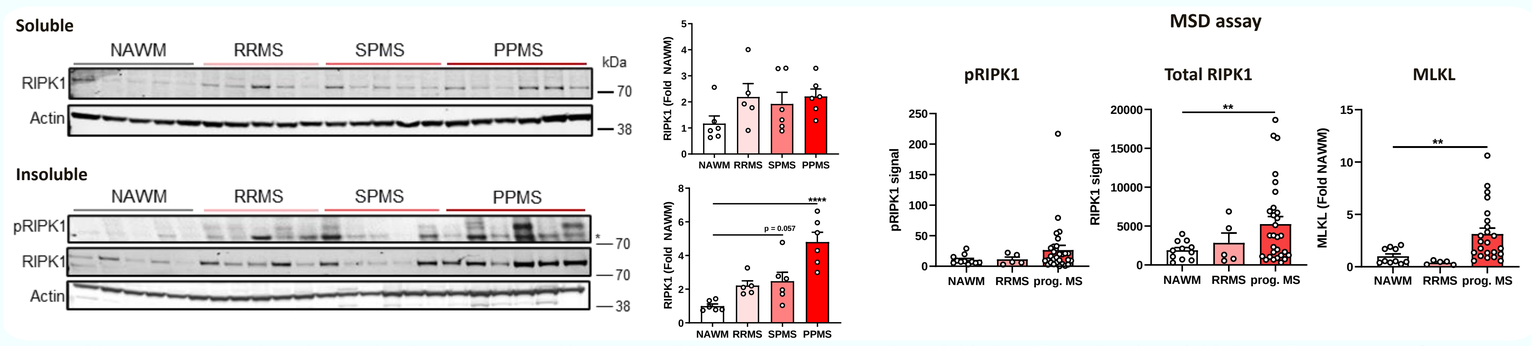



DAMPs, damage-associated molecular patterns; FADD, fas-associated protein with death domain; MLKL, mixed lineage kinase domain-like protein; OPTN, optineurin; RIPK3, receptor-interacting serine/threonine-protein kinase 3; TBK1, tank-binding kinase 1.

Main takeaway: RIPK1 activation in microglia and astrocytes induces a detrimental neuroinflammatory program that contributes to the neurodegenerative environment in progressive MS.

## RESULTS

**Figure 1: RIPK1 activity is elevated in post-mortem brain samples from humans with progressive MS**




Activated (S166 pRIPK1) and total RIPK1 levels were assessed by Western blot and MSD assay in the soluble (TBS/Triton) and insoluble (RIPA/urea) protein fractions derived from MS white matter lesions and normal-appearing white matter (NAWM). RRMS, relapsing remitting multiple sclerosis; SPMS, secondary progressive multiple sclerosis; PPMS, primary progressive multiple sclerosis

## CONCLUSIONS

RIPK1 activation and expression is elevated in humans with progressive MS

RIPK1 activation in microglia and astrocytes drives a deleterious inflammatory immune-related gene signature and impairs OPC myelination

Therapeutic RIPK1 inhibition in EAE mouse model attenuates disease progression, reduces neurofilament and TSPO levels, and suppresses deleterious astrocyte and microglia signaling

Identified RIPK1-dependent and MS-relevant biomarkers in CNS tissue in inflammatory mouse models

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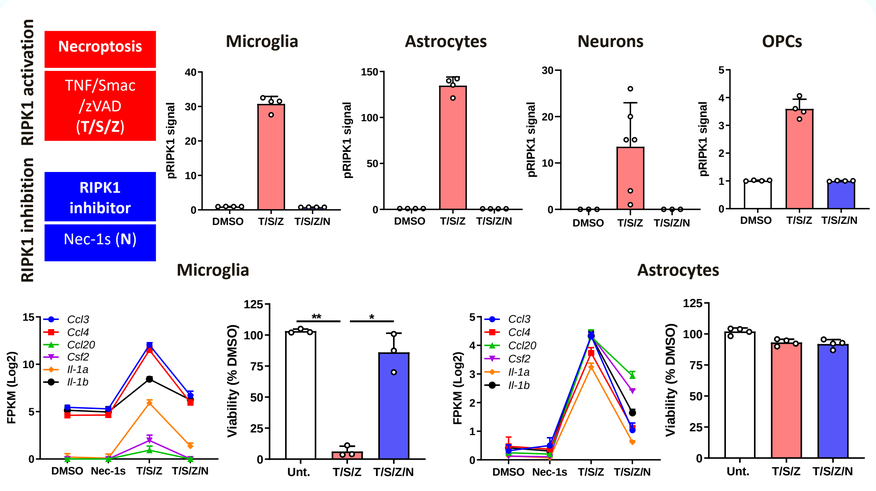
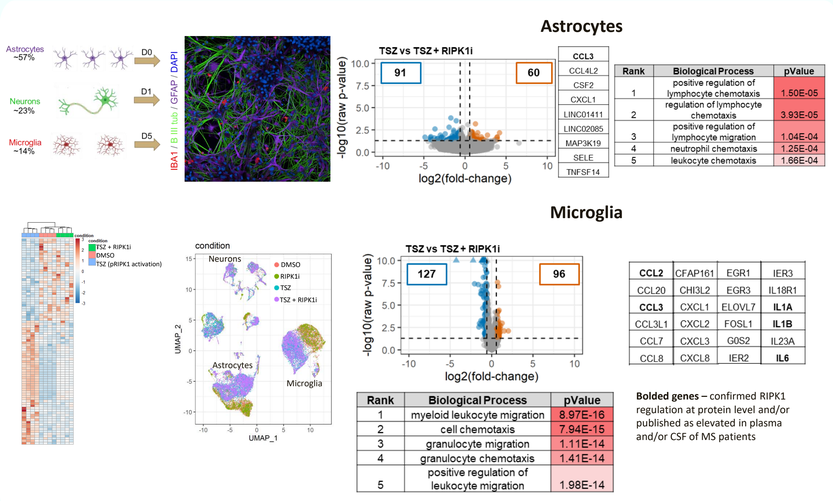
**Figure 2: RIPK1 activation occurs in all murine CNS cells and can modulate inflammatory and/or cell death signaling**



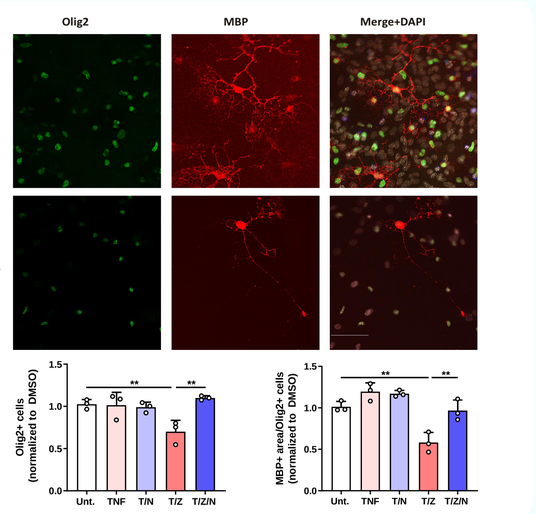

MSD assay depicts pRIPK1 signal activation upon stimulation with TNF/Smac/zVAD (T/S/Z), normalized to DMSO control. RIPK1 activation in mouse microglia but not astrocytes induces necroptosis in vitro. OPCs, oligodendrocyte progenitor cells.

**Figure 3: Astrocytes and microglia drive RIPK1-dependent neuroinflammatory changes in human iPSC tri-culture**



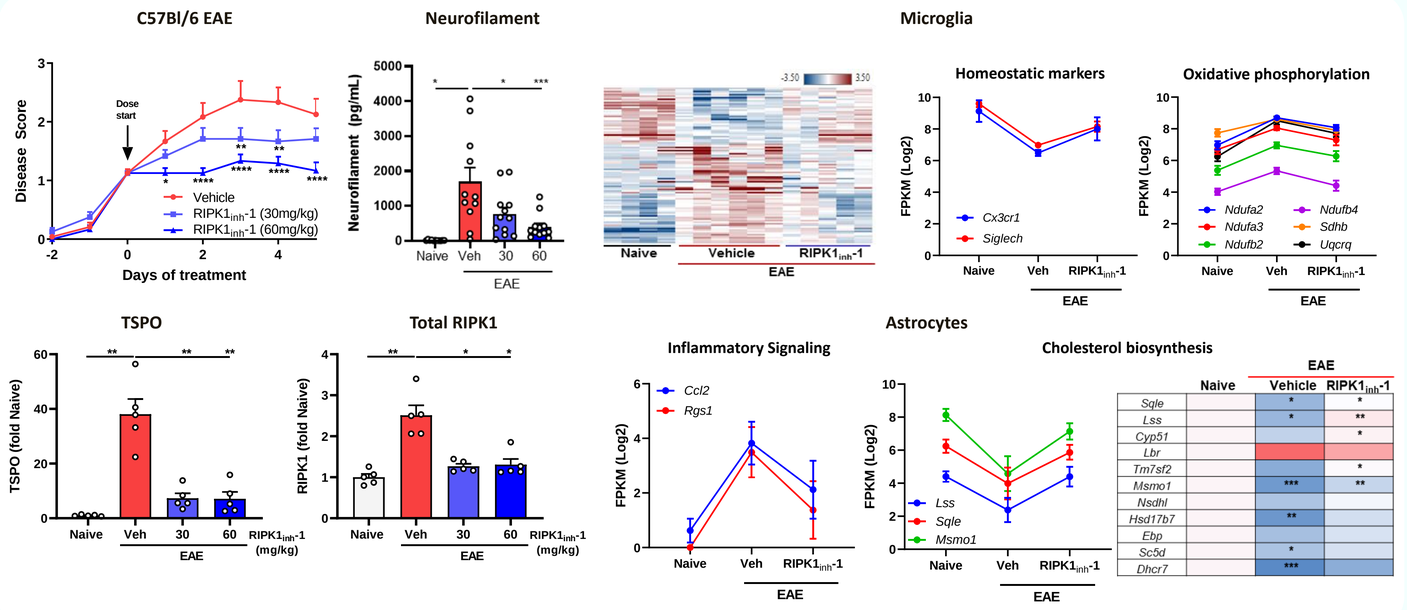

Single cell RNA-sequencing of a human iPSC-derived tri-culture demonstrates that RIPK1 activation drives inflammatory and immune-related gene activation in microglia and astrocytes. RIPK1i, RIPK1 inhibitor.

**Figure 4: RIPK1 activation in microglia and astrocytes mediates deleterious non-cell autonomous signaling**



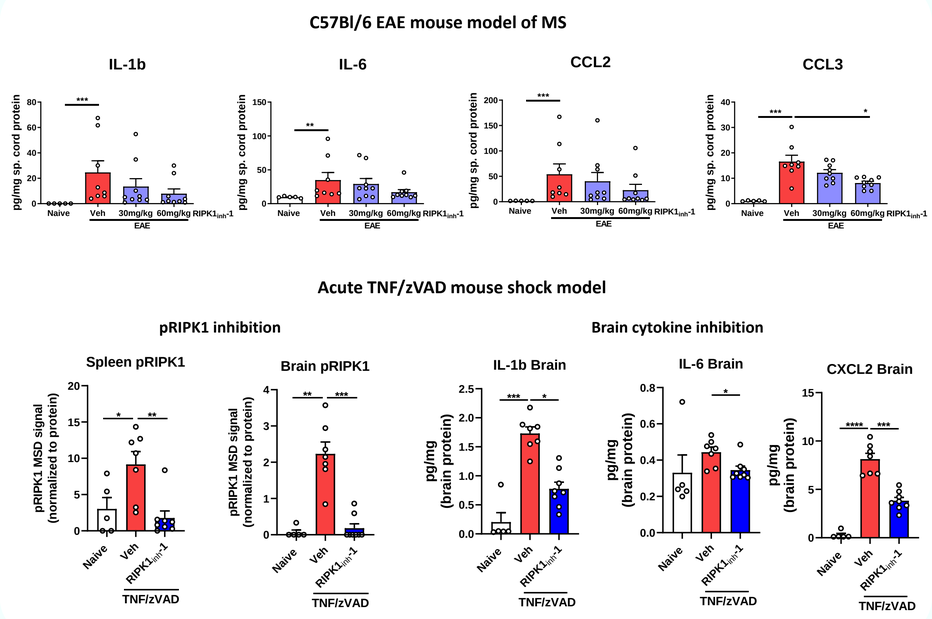

RIPK1 activation with TNF/zVAD (T/Z) in murine mixed glial culture with OPCs reduces OPC maturation and myelination capacity. Direct cell-cell contact with both microglia and astrocytes is required for these deleterious non-cell autonomous effects. MBP, myelin basic protein; OPC, oligodendrocyte progenitor cell; T, TNF.

**Figure 5: RIPK1 inhibition attenuates disease progression and neuroinflammation in EAE mouse model**




Therapeutic RIPK1 inhibition with a CNS-penetrant tool compound ameliorated EAE disease severity and plasma neurofilament levels in a dose-dependent manner. Neuroinflammation as assessed by TSPO and total RIPK1 levels were reduced in the EAE spinal cords treated with RIPK1 inhibitor. RNA sequencing of sorted spinal cord microglia and astrocytes demonstrated RIPK1 inhibition could restore homeostatic microglial markers and attenuate alterations in mitochondrial dysfunction and oxidative phosphorylation genes. RIPK1 inhibition in astrocytes suppressed inflammatory signaling, including *Ccl2*, and reversed the down-regulation of cholesterol biosynthesis genes observed in EAE vehicle-treated mice. EAE, experimental autoimmune encephalomyelitis; RIPK1inh-1, RIPK1 inhibitor; TSPO, translocator protein; Veh, vehicle;.

**Figure 6: RIPK1 inhibition reduces expression of multiple cytokines in CNS tissue of inflammatory mouse models**




Pro-inflammatory cytokines were assessed in the spinal cord of EAE mice with an MSD panel. RIPK1 inhibition reduced the elevation of multiple cytokines in a dose-dependent manner in the EAE mouse model. pRIPK1 levels in the spleen and brain were reduced to baseline with RIPK1 inhibitor treatment in the acute TNF/zVAD shock model. Brain cytokine levels were ameliorated with RIPK1 inhibition in the TNF/zVAD model.

**Disclosure**
MZ, FP, AB, LW, CZ, AM, YR, ML, SR, AC, TH and DO were Sanofi employees at the time this research was conducted, and may hold stock and/or stock options in the company. AD and AK have nothing to disclose. **Contact**: Matija Zelic; Sanofi, Cambridge, MA, USA. Matija.Zelic@sanofi.com

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