#Sa4063: Selective Degradation of NIMA-related kinase 7 (NEK7) via a Molecular Glue Degrader Inhibits IL-1 Downstream of NLRP3 Inflammasome Activation: A Novel Therapeutic Approach for Cardiovascular Inflammation

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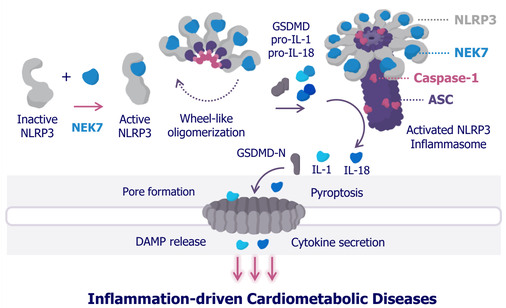
Introduction to NEK7 in NLRP3 Inflammasome Activation



Inflammation-driven Cardiometabolic Diseases
(selected examples)

The NLRP3 inflammasome is a multi-protein complex that triggers the generation of pro-inflammatory cytokines (IL-1, IL-18) in response to cell stress.

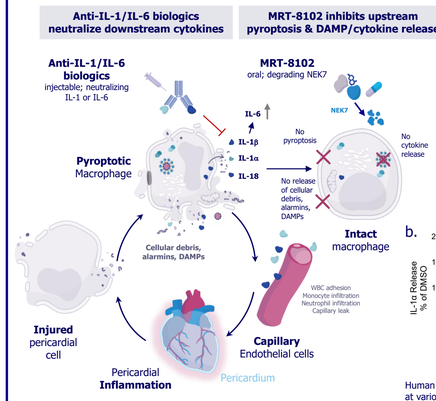
Activation of the NLRP3 inflammasome critically depends on **NIMA-related kinase 7, or NEK7**, a serine/threonine-protein kinase that facilitates assembly of the active NLRP3 inflammasome complex in a kinase-independent manner.

NEK7 degradation constitutes a novel approach to modulating the NLRP3 inflammasome, which may have broad therapeutic implications in a variety of inflammatory diseases, including several cardiovascular and cardiometabolic diseases.

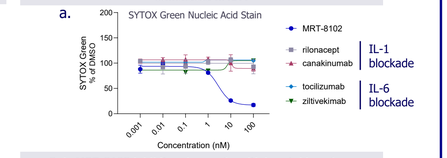
MRT-8102 is Differentiated from Anti-IL-1/IL-6 Therapies in Pericarditis

Anti-IL-1/IL-6 biologics neutralize downstream cytokines

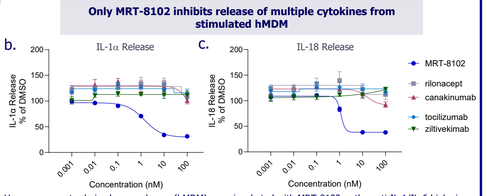
MRT-8102 inhibits upstream pyroptosis & DAMP/cytokine release



Only MRT-8102 inhibits pyroptotic membrane permeabilization in stimulated hMDM



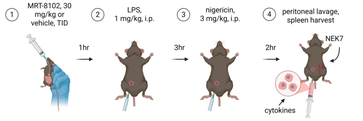
Only MRT-8102 inhibits release of multiple cytokines from stimulated hMDM

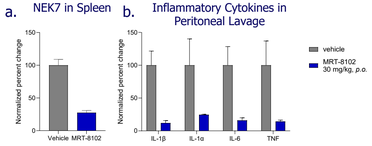


Human monocyte-derived macrophages (hMDM) were incubated with MRT-8102 or the anti-IL-1/IL-6 biologics at various concentrations for 20 h. Cells were then primed with LPS (1 µg/mL) for 3 h. SYTOX Green Nucleic acid stain was then added to the cells, along with 10 µM nigericin to activate NLRP3 inflammasomes. After 1 h, **(a)** membrane integrity was assessed using SYTOX Green fluorescence and **(b, c)** IL-1α/IL-18 levels were measured in supernatants by MSD multiplex assay. All data were normalized to the mean of the DMSO-treated control samples and represent the mean ± SEM.

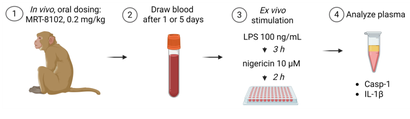
Oral Dosing of MRT-8102 Suppressed Cytokine Release in Mouse Peritonitis and Non-human Primate Whole Blood *Ex Vivo* Stimulation Models

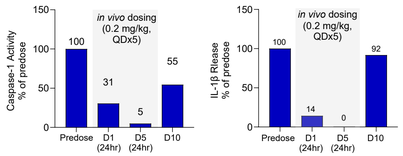
***In vivo CRBN*^{I391V} mouse peritonitis model**





***Ex vivo* stimulation of whole blood from cynomolgus monkeys dosed orally with MRT-8102**

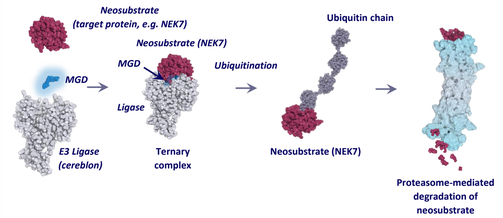


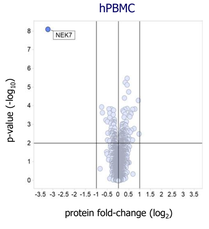


Crbn^{I391V} mice were dosed orally, TID, with vehicle or compound, starting 24 h prior to stimulation. On the stimulation day, animals received a final dose, followed 1 h later by LPS (1 mg/kg, i.p.), and nigericin (3 mg/kg, i.p.) 3 h after LPS. Two hours later, spleens and peritoneal lavage fluid were collected for analysis. **(a)** NEK7 protein levels in spleen lysates were quantified by JESS capillary immunoassay, normalized to the vehicle-treated group. **(b)** Cytokine concentrations in peritoneal lavage fluid were measured by MSD multiplex assay, normalized to the vehicle-treated group. Data represent mean ± SEM.

Non-human primates were dosed *in vivo* with MRT-8102 orally at 0.2 mg/kg for 5 days. **(a)** Caspase-1 activity and **(b)** IL-1β release in plasma from whole blood following *ex vivo* stimulation with LPS (100 ng/mL, 3 h) + nigericin (10 µM, 2 h). Catalytic activity of Caspase-1 was measured by luminescence; IL-1β release in plasma was measured by monkey-specific ELISA. Each time point was normalized to whole blood stimulated from predose sample from the same animal. Results expressed as mean of MRT 8102-treated group (n=2 per dose group, 1 male, 1 female).

MRT-8102 is an Oral, Highly Selective, NEK7 MGD





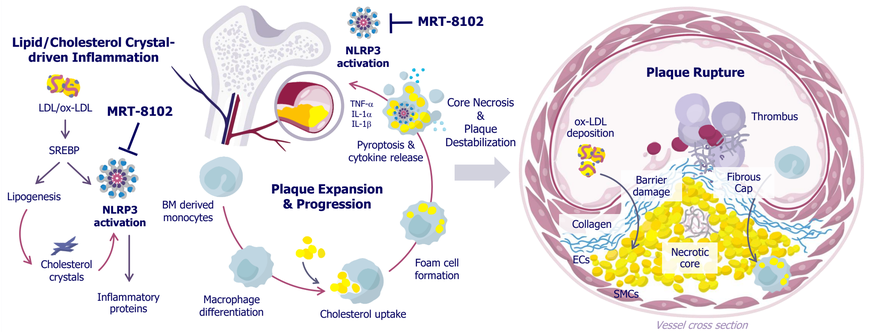
Molecular glue degraders (MGDs) create novel recognition interfaces on the surface of E3 ubiquitin ligases such as cereblon (CRBN) to recruit target proteins for degradation by the ubiquitin-proteasome system. MGDs are attractive not only for their ability to induce protein-protein interactions and eliminate their molecular targets, but also for their favorable physicochemical properties and associated developability compared to PROTACs.

The impact of MRT-8102 on global proteome modulation was assessed in human peripheral blood mononuclear cells (hPBMC). Cells were treated for 24 h with 10 µM MRT-8102 or DMSO and resulting protein modulation was assessed by quantitative tandem mass tag proteomics. Confidence p-value and protein fold-change plotted relative to DMSO control.

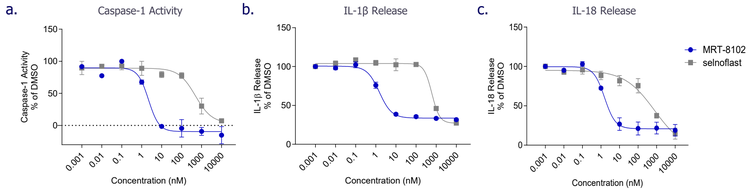
Summary and Future Development

- Activation of the NLRP3 inflammasome critically depends on NEK7.
- MRT-8102 is a selective, potent and durable NEK7 degrader that leads to inhibition of NLRP3 inflammasome *in vitro* and *in vivo* and subsequently inhibits production of cytokines including IL-1β, IL-1α, IL-6, and TNF.
- MRT-8102 is currently being investigated in a Phase 1 healthy volunteer study (MRT-8102-001; NCT07119125)
- Inflammasome modulation by degradation of NEK7 represents a novel and differentiated approach with potential therapeutic application in multiple cardiovascular and cardiometabolic diseases, including pericarditis and atherosclerosis.

NLRP3/NEK7-driven Inflammation Drives Atherosclerotic Plaque Pathogenesis

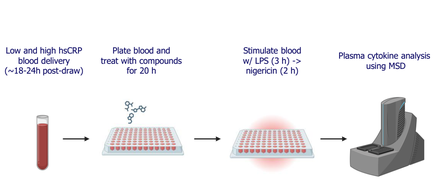


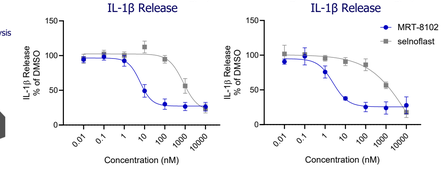
MRT-8102 inhibits cholesterol crystal-induced NLRP3 inflammasome activation



Human monocyte-derived macrophages (hMDM) were incubated with MRT-8102 or the NLRP3 inhibitor, selnoflast, for 20 h. Cells were then stimulated with LPS (1 µg/mL) and cholesterol crystals (1 mg/mL) for 24 h. **(a)** Caspase-1 activity was measured in the supernatants using the Promega Caspase-Glo® 1 Inflammasome Assay. **(b)** IL-1β and **(c)** IL-18 levels in the supernatant were measured by ELISA. All data are normalized to DMSO-treated control samples and represent mean ± SEM.

MRT-8102 Suppresses Cytokine Release in *Ex Vivo*-stimulated Whole Blood From Human Donors with High hsCRP Levels





Whole blood from low (< 2.0 mg/L) and high (≥ 2.0 mg/L) hsCRP donors was plated in 96-well plates and treated with various concentrations of MRT-8102 or the NLRP3 inhibitor, selnoflast, for 20 h. The blood was then stimulated with LPS (100 ng/mL) for 3 h, followed by nigericin (5 µM) for 2 h. Plasma was then isolated and analyzed for IL-1β using MSD multiplex assay. All data were normalized to the mean of the DMSO-treated control samples and represent the mean ± SEM.

MRT-8102-001 Phase 1 Study (Part 3): PoC in Subjects with Increased CVD Risk

Study population
- ~36 subjects
- Obesity
- Elevated CRP

Primary endpoint
- Safety and tolerability

Secondary endpoints
- Change in CRP levels
- Pharmacokinetics

Exploratory endpoints
- NEK7 degradation
- Inflammatory markers: IL-6, IL-1β, IL-18
- Body weight