**Introduction:** Activation of the NLRP3 inflammasome induces maturation of IL-1β and IL-18, both validated targets for treating acute and chronic inflammatory diseases. Although the activation of NLRP3 is well characterized, effective and safe therapies that inhibit NLRP3 are needed.

**AIM:** To investigate the effects and mechanism of action of OLT1177, a beta-sulfonyl nitrile molecule which is safe and orally bioavailable in humans.

**Methods and Results:**

- Human monocytes-derived macrophages (HMDM) were generated from peripheral blood mononuclear cells (PBMCs) by differentiation for 7d in presence of hGM-CSF (5ng/ml).
- Neutrophils were isolated from the freshly obtained peripheral blood of healthy donors using Polymorphoprep™.
- *In vitro* inflammasome formation was induced with 1µg/ml LPS (E. coli) for 4 hours followed by ATP (5mM) or nigericin (10µM) for 1h, Flagellin (S.typhimurium) overnight (3.75 µg/ml) and Poly(dA:dT) overnight (5µg/ml) for the NLRP3, NLRC4 and AIM2 inflammasome, respectively.
- Fluorescent Resonance Energy Transfer (FRET) analysis was used to determine the distance between NLRP3 and caspase-1 in HMDM following LPS/ATP stimulation in presence of OLT1177.
- Electrophysiological recording was performed in U937 cells stimulated with LPS and ATP using whole cell patch-clamp.
- *In vivo* study was conducted in C57BL/6J male mice. Animals were treated with OLT1177 (5 doses BID of 200 mg/kg) or matching volume of vehicle (saline) IP. Mice were then injected with LPS (5mg/kg) and sacrificed after 2h.
- The clinical trial was a single-center, placebo-controlled, dose escalation study of the safety and PK of OLT1177 Caps (100, 300 and 1000mg) in 35 healthy subjects in single-dose, and multiple-dose regimens. The PK parameters of OLT1177 and area under the concentration time curve (AUC) were used to characterize systemic drug exposure.

**OLT1177 reduces IL-1β and IL-18 release and prevents NLRP3 inflammasome formation in HMDM**

**OLT1177 reduces inflammasome activation in human neutrophils**

**OLT1177 has no effect on TNFα, priming and acts downstream of P2X7R**

**Conclusion**

OLT1177 is a novel, safe and specific inhibitor of the NLRP3 inflammasome, with the potential to be a lead compound for the treatment of IL-1β and IL-18-mediated diseases.