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POSTER ABSTRACTS

201.GRANULOCYTES, MONOCYTES, AND MACROPHAGES

Modulation of NLRP3 Inflammasome in Monocytes By HT-6184 in a Single-Cell Proteomic Study

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Background
The NLRP3 inflammasome is associated with the activation of monocytes and plays a crucial role in the innate immune response. The NLRP3 inflammasome is a multi-protein complex expressed primarily in monocytes and other immune cells. When monocytes encounter danger signals, such as pathogen-associated molecular patterns (PAMPs) and danger-associated molecular patterns (DAMPs), the NLRP3 inflammasome is assembled, leading to the maturation and secretion of potent pro-inflammatory cytokines, interleukin-1β (IL-1β), and interleukin-18 (IL-18). These cytokines, in turn, induce a robust inflammatory response to clear infections, promote tissue repair, and coordinate adaptive immunity. However, dysregulated NLRP3 inflammasome activation has been associated with various inflammatory disorders, such as autoimmune diseases, metabolic diseases, and neurodegenerative conditions.

Purpose
This study investigated the effects of a potent, allosteric NLRP3 inflammasome modulator, HT-6184, on cytokine release response from the activation of NLRP3 in human monocytes.

Methods
Human peripheral blood mononuclear cells (PBMCs) were utilized, and monocytes were isolated using a Miltenyi pan monocyte isolation kit. Subsequently, these isolated monocytes were exposed to different concentrations of HT-6184 (300 nM and 100 nM) before being stimulated with lipopolysaccharide (LPS) at a concentration of 10 ng/mL, a potent activator of the NLRP3 inflammasome. After 24 hours, the secreted cytokines were analyzed at the single-cell level using the Isospark, with data analysis performed using the Isospeak software.

Results
The results demonstrated that monocytes treated with LPS alone exhibited high levels of pro-inflammatory cytokines, including effector and stimulatory cytokines, and chemoattractive chemokines such as IL-8, MIF, MIP-1b, and TNF-alpha. In contrast, monocytes treated with HT-6184 and LPS showed reduced cytokine secretion, approaching levels observed in the control group. Notably, the HT- **6184**-treated group displayed a lower incidence of polyfunctionality (2%) than the LPS-only group (11%), indicating fewer cells capable of simultaneously secreting multiple cytokines. The 3D-TSNE graph analysis revealed that the HT-6184 and LPS-treated groups clustered with the control group, signifying similar cytokine secretion profiles. In contrast, the LPS-only group exhibited a distinct pattern of cytokine secretion.

Conclusions
These findings indicate that HT-6184 effectively inhibits the release of pro-inflammatory cytokines upon NLRP3 activation in monocytes. Further research should explore the characteristics of HT-6184 and its ability to modulate the inflammasome in diverse in vitro and in vivo models. The modulation of the NLRP3 inflammasome by HT-6184 holds potential therapeutic implications for managing inflammatory disorders associated with dysregulated NLRP3 activation.

Disclosures Bursey: *Halia Therapeutics:* Current holder of *stock options* in a privately-held company. **Bearss:** *Halia Therapeutics:* Current Employment, Current holder of *stock options* in a privately-held company. **Avei:** *Halia Therapeutics:* Current holder of *stock options* in a privately-held company. **Mollard:** *Halia Therapeutics:* Current Employment, Current holder of *stock options* in a privately-held company. **Bearss:** *Halia Therapeutics:* Current Employment, Current holder of *stock options* in a privately-held company. **Janat-Amsbury:** *Halia Therapeutics:* Current Employment, Current holder of *stock options* in a

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