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Ex-Vivo Human Whole Blood Assay for Evaluating the Pharmacodynamics of HT-6184: Reduction in NLRP3 Inflammasome Mediated Cytokine Production

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The NLRP3 inflammasome is an intracellular multiprotein complex that plays a crucial role in the innate immune response by mediating the maturation and release of pro-inflammatory cytokines IL-1 beta, and IL-18. The release of these cytokines leads to the downstream release of TNF-alpha, IL-6, which propagate a robust inflammatory response. Dysregulation of the NLRP3 inflammasome pathway has been linked to various inflammatory diseases, suggesting the possibility for novel therapeutic strategies targeting NLRP3 activation. NLRP3 has been linked to regulating hematopoietic stem progenitor cell (HSPCs) development, expansion, release, and mobility. In addition, various hematological disorders such as myelodysplastic syndrome, myeloproliferative neoplasms, acute and chronic leukemias, and graft-versus-host disease (GvHD) have all been shown to involve NLRP3 inflammasome activation. Given its significant role in these conditions, inhibiting the NLRP3 inflammasome may be a novel therapeutic strategy in hematology and hemato-oncology

This study utilized an ex-vivo human whole blood assay to investigate the pharmacodynamics of HT-6184, an allosteric Nek7 inhibitor. We used LPS and ATP to prime and activate the NLRP3 inflammasome and examined the effects of pre-treatment with various concentrations of HT-6184 (starting at 1 mM) on the production of critical pro-inflammatory cytokines, such as IL-1b, IL-18, IL-6 and TNF-a. Notably, preincubation with HT-6184 at concentrations as low as 4.1 nM led to a significant reduction in cytokine production ($p<0.0001$). Specifically, TNF-α and IL-6 were reduced by 38% and 58%, respectively, while IL-1β and IL-18 were decreased by 44% and 53% respectively.

These results provide further evidence of the ability of HT-6184 to modulate the activity of the NLRP3 inflammasome in a complex biological milieu. Our study suggests that HT-6184 has significant therapeutic potential in treating a broad spectrum of inflammasome-mediated diseases. In addition, this assay has the potential to be used to measure the pharmacodynamic properties and the efficacy of HT-6184 and other inflammasome inhibitors in future healthy volunteer clinical trials. In conclusion, this study highlights the importance of innovative and physiologically relevant assay systems in identifying and evaluating potential therapeutic agents for inflammatory diseases.

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