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# POSTER ABSTRACTS

## 604.MOLECULAR PHARMACOLOGY AND DRUG RESISTANCE: MYELOID NEOPLASMS

### The Dual Inflammasome/Myddosome Inhibitor HT-6184 Restores Erythropoiesis in MDS/AML

*Emma Rabinovich, MD*[1,2], *Ariel Fromowitz*[2,3], *Opeyemi Ajibade, MD MS*[4], *Srabani Sahu*[5], *Divij Verma, PhD*[2], *Sarah Aminov, MSc*[2], *Nandini Ramachandra, MS*[5], *Christopher Selverian*[2], *Nailya Khalizova*[2], *Srinivas Aluri, PhD*[5], *Rahul Sanawar, PhD*[2], *Shanisha Gordon Mitchell*[2], *Milagros Carbajal*[2], *Jacqueline Boultwood, PhD*[6], *Andrea Pellagatti, PhD*[6], *Ulrich Steidl, MD PhD*[2], *Aditi Shastri, MD*[2], *Rongbao Zhao, PhD*[5], *Alexis Mollard, PhD*[7], *Margit Janat-Amsbury*[7], *David J. Bearss, PhD*[8], *Alan F. List, MD*[7], *Amit Verma, MD*[2]

[1] Department of Bone Marrow Transplant, Memorial Sloan Kettering Cancer Center, New York, NY
[2] Blood Cancer Institute, Department of Oncology, Albert Einstein College of Medicine, Bronx, NY
[3] Department of Stem Cell Transplantation and Cellular Therapy, The University of Texas MD Anderson Cancer Center, Houston, TX
[4] Blood Cancer Institute, Department of Oncology, Albert Einstein College of Medicine, Brooklyn, NY
[5] Montefiore Einstein Cancer Center, Blood Cancer Institute, Department of Oncology, Montefiore Medical Center, Albert Einstein College of Medicine, Bronx, NY
[6] Bloodwise Molecular Haematology Unit, Nuffield Division of Clinical Laboratory Sciences, University of Oxford, John Radcliffe Hospital, Oxford, United Kingdom
[7] Halia Therapeutics, Lehi, UT
[8] Halia Therapeutics, Alpine, UT

Background:
The NLRP3 inflammasome is one of the key biological drivers of ineffective hematopoiesis and inflammation in myelodysplastic syndrome (MDS). Inflammasome activation arises from both cell-extrinsic (e.g., Toll-like receptors) and cell-intrinsic signals (e.g., somatic mutations) that direct caspase-1 maturation leading to GATA-1 degradation, myeloid skewing, and pyroptotic cell death. HT-6184 is a potent allosteric inhibitor of the inflammasome scaffold protein NEK7 that blocks inflammasome assembly, promotes ASC speck dissolution, and inhibits myddosome activation of NF$\kappa\beta$. To investigate preclinical activity in MDS, we evaluated HT-6184 in MDS and acute myeloid leukemia (AML) models, including patient-derived sample cultures.
Results:
Expression of inflammasome pathway components was first assessed by gene expression profiling in a large cohort of MDS bone marrow CD34+-selected cells and healthy age-matched controls. Cellular expression of the inflammasome components, *caspase-1*, *gasdermin D*, *IL-18* and *Pycard* was significantly upregulated in MDS specimens compared to healthy controls. Furthermore, cases with the highest expression of inflammasome effectors had significantly inferior overall survival (Log Rank $P < 0.05$).
The novel inflammasome inhibitor, HT-6184, was designed to prevent NEK7 from mediating the oligomerization of NLRP3 monomers following an activation signal, ultimately preventing inflammasome assembly. Thus, the structure of HT-6184 was tailored to maximize its interactions with NEK7, rigidifying the protein, and locking it in a conformation that prevents interaction with NLRP3. The allosteric activity of HT-6184 was examined using nano differential scanning fluorimetry and confirmed with limited proteolysis assays, which demonstrated that HT-6184 disrupts NEK7 conformations at single-digit nanomolar concentrations. This was confirmed in cell-based assays showing its ability to inhibit IL-1$\beta$ release with a 50% inhibitory concentration (IC) of 46 nM in THP-1 cells. These data further correlate with HT-6184 blocking of ASC speck formation with an IC90 of 100 nM in THP-1 cells as well as speck dissolution. HT-6184 also affects the NF-$\kappa$B pathway, by blocking myddosome function and resulting in a decreased expression of an NF-$\kappa$B driven reporter gene, with an IC50 of 10.2 nM in transfected cells.
HT-6184 exerts its pharmacological activity by inhibiting the priming and activation steps of the NLRP3 inflammasome. It is pharmacologically active in a human whole blood assay, strongly downregulating the production of pro-inflammatory cytokines. Anti-inflammatory efficacy was also observed in vivo in a murine inflammation model of inflammatory bowel disease. HT-6184 incubation with human monocytes abrogated production of IL-1$\beta$ and IL-6, reverting human monocytes to a less

inflamed state as demonstrated by single cell proteomic studies (isoplexis platform). It similarly decreased IL-1$\beta$ production from monocytes derived from a TET2 -/+ mouse model that preferentially develops monocytic expansion. Finally, treatment initiation of primary MDS/AML bone marrow specimens (N=10) with HT-6184 in methylcellulose cultures markedly increased erythroid differentiation and reduced myelomonocytic colonies in the majority of samples. This was evidenced by the change in total number of colonies and by fluorescence activated cell sorting (FACS) assessment of differentiation markers (Glycophorin A, CD71, CD11b, CD14) after 14 days of incubation. Inflammasome-directed IL-1$\beta$ secretion in primary MDS samples was also abrogated by HT-6184 treatment.

Conclusions:

These findings demonstrate that the novel dual inflammasome/myddosome inhibitor, HT-6184 effectively suppresses myeloid skewing, restores erythroid commitment in MDS/AML specimens, and provides a rationale for clinical development in myeloid malignancies.

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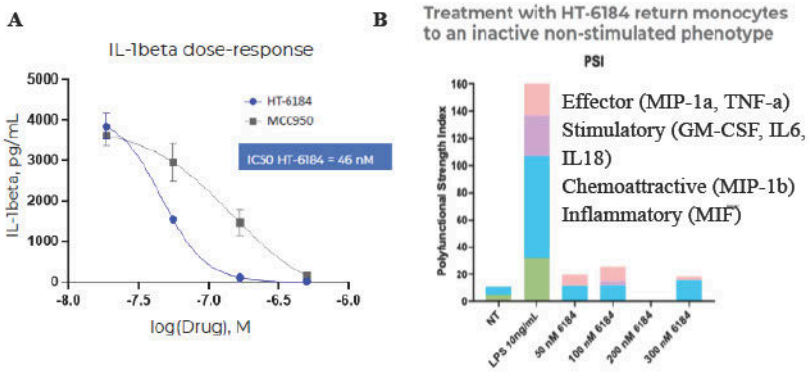


**Fig 1: HT-6184 is a potent inhibitor of inflammasome in monocytes**: HT-6184 inhibits IL-1b secretion from monocytes more effectively when compared to other inhibitors of the inflammasome pathway (A). HT-6184 reverts monocytes to a non-inflammatory phenotype when examined with single cell proteomics (Isoplexis system)

**Figure 1**

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