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POSTER ABSTRACTS

602.MYELOID ONCOGENESIS: BASIC

Multiomic Characterization of Myelodysplastic Neoplasms (MDS) with Micromegakaryocytes Highlights the Role of *EZH2-RUNX1* deregulation in Disease Physiopathology and Response to Targeted Therapies

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Micromegakaryocytes (micro-MKs), an unequivocal myeloid dysplastic feature in adults, are present in 15-20% of myelodysplastic neoplasms (MDS). We recently associated the detection of these small megakaryocytes with a mono- or bi-lobated nucleus in bone marrow (BM) smears of MDS patients with an unfavorable prognosis. The aim of the present study was to elucidate the molecular bases of micro-MK formation and its impact on MDS response to targeted therapies.

We selected retrospectively a cohort of 40 MDS patient samples subjected to NGS myeloid panel and RNA sequencing and carried out a morphological re-evaluation that included micro-MKs recounting. Cases with isolated del(5q) or *SF3B1* mutation were excluded. MDS cases were selected according to the following morphological findings: no dismegakaryopoiesis, dismegakaryopoiesis without micro-MK, low rate of micro-MK (<10%; micro-MK ^{low}) and high rate of micro-MK (>40%; micro-MK ^{high}). No patient harbored an intermediate (10-40%) rate of micro-MK. A comparative characterization of 8 representative patients was carried out subjecting diagnosis BM samples to Tapestri and Chromium single cell proteogenomic and transcriptomic platforms, gene set enrichment analysis, and deep cytogenetic characterization by optical genome mapping.

Functional validation studies were performed using the megakaryoblastic cell line MEG01 differentiated to a mature MK phenotype by exposure to phorbol myristate acetate (PMA), as well as using the CRISPR-Cas9 tool for gene editing. Finally, activity of investigational drugs including 5-azacytidine, lenalidomide, venetoclax, rigosertib and pevonedistat, was evaluated in MEG01-activated cells and *in vivo* using a chicken embryo chorioallantoic membrane (CAM) model of MDS patient-derived xenograft (PDX).

The most frequently mutated genes across all cohorts were *ASXL1* (58%), *SRSF2* (38%), *RUNX1* (30%), *TET2* (30%), *STAG2* (20%), *EZH2* (18%), *DDX41* (10%), *U2AF1* (10%), and *ZRSR2* (10%) . Interestingly, *EZH2* and *RUNX1* mutations were enriched in the micro-MK ^{high} group (*EZH2:* 0%, 10%, 14.3% vs. 66.7%, *p 0.006; RUNX1: 0%, 20%, 35.7% vs. 83.3%, p 0.003*), and both mutations co-occurred in 85.7% (6/7) of the *EZH2* mutated cases. Single cell analyses revealed an enrichment of *RUNX1* alterations in three populations within micro-MK ^{high} BM samples, including hematopoietic stem and progenitor cells, non-classical monocytes and immature erythroid cells. These samples were also characterized by a 5-fold increase in MK progenitor abundance and by the upregulation of a set of 10 *EZH2*-repressed genes in neutrophil-like cells and myeloid progenitors, with 7q loss presumably affecting the *EZH2* locus in 50% of the cases with micro-MK. Using the *in vitro* MK differentiation model, we further demonstrated that *EZH2* downregulation hampered the acquisition of MK surface markers CD41 and CD61, and that venetoclax was the sole agent retaining notable cytotoxic activity in differentiated cells when compared to undifferentiated megakaryoblasts. Accordingly, in a first-in-kind *in vivo* CAM-MDS transplant model, venetoclax was able to impair BM infiltration by human cells in a PDX model of micro-MK+ MDS.

In summary, supporting the notion that *EZH2* and *RUNX1* exert a crucial role in the regulation of megakaryopoiesis, our present findings suggest that deregulation of *EZH2-RUNX1* activity by loss-of-function mutation and/or genetic deletion affecting myeloid precursors, impairs a proper megakaryocytic differentiation process in MDS. Thus, this phenomenon may be considered as a characteristic feature of MDS cases with an elevated proportion of micro-MK. Of note, preliminary *in vitro* and *in vivo* results support the use of venetoclax for the treatment of this subgroup of patients with dismal outcome.

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