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ORAL ABSTRACTS

631.MYELOPROLIFERATIVE SYNDROMES AND CHRONIC MYELOID LEUKEMIA: BASIC AND TRANSLATIONAL

MYC-Alarmin Axis As a Novel Oncogenic Driver in a Subgroup of Triple Negative Myeloproliferative Neoplasms

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Primary Myelofibrosis (PMF) is an aggressive myeloproliferative neoplasm (MPN) characterized by constitutional symptoms, cytopenias, splenomegaly, extramedullary hematopoiesis, bone marrow (BM) fibrosis, and a propensity to transform to acute myeloid leukemia (AML). Despite advances in understanding the underlying genetic abnormalities in MPN and the development of JAK2 inhibitors to treat MF, there is an urgent need to devise new treatment strategies, particularly for triple negative myelofibrosis (TN-MF) cases that lack mutations in the JAK2 kinase pathway and who have inferior outcomes.

To identify potential oncogenic drivers in TN-MF, we performed cytogenetic analyses and targeted exome sequencing of 98 genes commonly mutated in myeloid malignancies in 584 MF patients identified from the Total Cancer Care database at Moffitt Cancer Center. Notably, there were no significant differences in the somatic mutation profiles (other than JAK2 activating mutations) between TN *vs. JAK2/CALR/MPL* mutant MF patients. However, trisomy of chromosome 8 occurs more frequently in TN-MF *vs.* other subtypes (26.7% *vs.* 6.2%, p=0.0001). To identify oncogenic drivers in trisomy 8+ TN-MF, we performed scRNA-seq analysis from 18,651 cells (*n*=15,850 from 3 normal donors and *n*=2,801 from a trisomy 8+ TN-MF patient) and identified a total of 1,260 genes that are differentially regulated in hematopoietic stem cells (HSCs) of trisomy 8+ TN-MF compared to normal donors. Among these, 131 genes were located on chromosome 8 and *MYC* was one of the top 5 genes that are significantly upregulated in trisomy 8+ HSCs. Additional immunohistochemistry staining demonstrated that MYC protein levels in BM cells were significantly higher in trisomy 8+ TN-MF patients *vs.* trisomy 8- patients.

To assess whether MYC might be a driver of MPN, we established an Mx1-Cre $^{+/-}$;Rosa26 $^{LSL\text{-}MYC/LSL\text{-}MYC}$ transgenic mouse model that inducibly overexpresses MYC in HSCs following treatment of polyinosinic-polycytidylic acid (pIpC). Forced MYC expression induced profound anemia, monocytosis, megakaryocytic atypia, splenomegaly, increased BM collagen deposition/fibrosis, extramedullary hematopoiesis in spleen and liver, and significantly reduced OS *vs.* Mx1-Cre $^{+/-}$ wild type controls (median OS 258 days *vs.* not reached (NR), p<0.0001). MYC also promoted expansion of HSCs, myeloid progenitors, and Gr-1 $^{+}$/CD11b $^{+}$ mature myeloid cells in BM and spleen; thus, MYC was sufficient to provoke MF-like disease independent of JAK2 pathway mutations. Subsequent scRNA-seq analysis of 25,232 cells (*n*=13,552 from control and *n*=11,680 from a

MYC transgenic mouse) revealed that *S100a9* mRNA levels were increased by MYC in most major hematopoietic cell types. Supporting oncogenic roles of S100A9 in MYC-driven MF, deletion of *S100a9* in Mx1-Cre $^{+/-}$;Rosa26 $^{LSL\text{-}MYC/LSL\text{-}MYC}$;S100a9 $^{-/-}$ mice abolished MYC-induced MF phenotypes and significantly improved OS (median OS NR *vs.* 225 days, p=0.0345).

To test whether inhibition of MYC can suppress MF disease progression *in vivo*, lethally irradiated CD45.1 $^{+}$/CD45.2 $^{+}$ WT mice were transplanted with BM cells harvested from CD45.2 $^{+}$ Mx1-Cre $^{+/-}$;Rosa26 $^{LSL\text{-}MYC/LSL\text{-}MYC}$ mice that were treated with pIpC 20 weeks prior to transplant. MYCi975, a small molecule that inhibits MYC, reduced MYC and S100a9 protein levels in BM and spleen cells. Further, MYCi975 effectively suppressed MYC-driven MF phenotypes and disease progression, and significantly improved OS (median OS NR *vs.* 252 days, p=0.0109).

In summary, our studies are the first to describe an oncogenic role of MYC in MF pathogenesis, where MYC provokes an alarmin-driven inflammatory circuit, and where the MYC-S100A9 axis represents a therapeutic vulnerability for TN-MF patients. Accordingly, our results provide a strong rationale for testing agents targeting MYC or S100A9 in early phase clinical trials in MPNs having increased MYC levels or activity.

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