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POSTER 19: Cooperativity of Tet2, Srsf2, and Nras mutations to promote aberrant mRNA expression and splicing in chronic myelomonocytic leukemia

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Background: Chronic myelomonocytic leukemia (CMML) is a hematologic malignancy defined by persistent monocytosis and overlapping features of myelodysplastic and myeloproliferative neoplasms. The lack of representative cell models of CMML has limited the understanding of the disease and the development of novel therapeutic strategies. In this context, we generated Hoxb8 cellular models mimicking 3 main recurrent mutations in CMML, namely *TET2*, *SRSF2* and *NRAS* mutations.

Methods: Kit+ cells from different transgenic mice (Scl-Cre, Tet2-/-, Tet2-/- x Srsf2P95H/WT) were conditionally immortalized using the ER-Hoxb8 system. The NrasG12D mutation was introduced as a third genetic event by lentiviral transduction. The 14 Hoxb8 progenitor cell lines generated are therefore: 5 wild-type (Scl-Cre) controls (WT), 3 Tet2-/- (single mutants), 3 Tet2-/- x Srsf2P95H/WT (double mutants), and 3 Tet2-/- x Srsf2P95H/WT x NrasG12D (triple mutants).

Results: RNA-sequencing data identified 1831 deregulated genes in simple mutants *versus* WT, including myeloid differentiation genes such as *Gata2*, whose downregulation has been previously reported in *Tet2*-/- mice. *Cebpe* was also downregulated in the simple mutants as compared to WT, a finding that we validated in CD34+ cells from CMML patients and healthy individuals. 573 genes were downregulated in double mutant *versus* WT cell lines, some of which are involved in DNA repair and G2M checkpoint. The addition of NrasG12D resulted in significant overexpression of 1669 genes, such as genes in the Mtorc1 and Myc pathways,

consistent with the proliferative advantage of the triple mutant cell lines. Splicing analysis revealed 1661 misspliced targets in the simple mutants as compared to WT, while 1339 misspliced targets were identified in the double mutant compared to simple mutant cell lines. The most common splicing event was single exon skipping for both *Tet2* loss and *Srsf2*P95H/WT. Some targets were found misspliced by both *Tet2* loss and *Srsf2*P95H/WT. 168 misspliced targets found in CMML patients were also identified in our Hoxb8 models, of which 24 were previously described in *Srsf2*P95H/WT mouse models.

Conclusion: Our Hoxb8 models recapitulate previous findings of abnormal mRNA expression and splicing both in mouse and patient data. Our results highlight that *Tet2* loss significantly contribute to mRNA splicing alterations, which raises the hypothesis of how TET2 cooperates, or not, with SRSF2P95H/WT to modulate mRNA splicing in CMML.