

## "ASXL1"-erating inflammation and bone marrow fibrosis in myeloproliferative neoplasms

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## Title: “ASXL1”-erating inflammation and bone marrow fibrosis in myeloproliferative neoplasms

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In this issue of *Haematologica*, Shi et al.<sup>1</sup> report on a critical role of ASXL1 mutations in driving bone marrow fibrosis via a EGR1-TNFA axis in both murine models and PMF patients.

Additional sex combs like 1 (ASXL1) mutations are one of the most common molecular biological abnormalities in patients with primary myelofibrosis (PMF), and the effect of these mutations on prognosis remains controversial. Recent reports demonstrated that ASXL1 mutations alone are not detrimental but confer a worse prognosis when associated with a mutation in TP53 or high-risk genes<sup>2</sup>. In line with these findings, it was demonstrated that ASXL1 mutations are early driver events in primary myelofibrosis (PMF) but might be acquired later in the disease course of secondary myelofibrosis (SMF)<sup>3</sup>. This raises the question of the effect of ASXL1 mutations on the hematopoietic stem and progenitor cells. Here, Shi et al. sought to shed light on the mechanism of aberrant lineage differentiation and transcription deregulation tied to ASXL1 mutations in MPNs, using patient biopsies and the hematopoietic-specific Vav-Cre-driven *Asx1*<sup>-/-</sup> JAK2VF murine model.

In their article, Shi and colleagues<sup>1</sup> once again confirm that ASXL1 mutations, regardless of the “MPN driver” mutation, are associated with a more severe disease phenotype (e.g. larger spleens higher fibrosis grades, lower hemoglobin) and higher monocyte frequency but do not specifically differentiate between PMF and SMF or additional mutations. The hematopoietic-specific JAK2VF murine model with deletion of *Asx1* represents a model for early acquisition of ASXL1 mutations comparable to ASXL1 being an early event in PMF<sup>3</sup>. In line with their own and with earlier clinical data, Shi et al. demonstrate that loss of *Asx1* triggers earlier fibrosis onset and a generally more severe phenotype and also induces a differentiation bias towards the monocyte/macrophage lineage. Monocytosis in patients with primary myelofibrosis was previously associated with inferior survival<sup>4</sup> and could be explained by a more severe inflammatory state. As ASXL1 mutations were associated with monocytosis in patients and the murine model, the authors explored the hypothesis of monocyte-derived fibrocytes contributing to more severe fibrosis. Fibrocytes are still only very broadly defined as spindle-shaped cells expressing markers of both hematopoietic cells (CD34, CD43, CD45, CD68, LSP-1, and major histocompatibility complex class II) and stromal cells (collagen I, collagen III, and fibronectin) and have been associated with primary myelofibrosis<sup>5</sup>. Shi et al. show an association of an increased frequency of fibrocytes in patients carrying an ASXL1 mutation when compared to controls but functional evidence of active ECM production of these cells contributing to fibrosis still remains to be demonstrated. Surprisingly, the authors show no significant difference in staining of Gli1- and LepR-positive in their relatively small cohort of patients (n=4 ASXL1MUT vs n=8 ASXL1WT) which were previously reported to expand as fibrosis-driving cells in response to an MPN clone<sup>6,7</sup>. This might be due to the fact that both are known to be expressed in low levels and difficult to be detected in immunofluorescence without signal amplification. Another critical point is the preparation of tissue, specifically fixation and decalcification, which significantly impacts stainings of the bone marrow. Recent work by van Egeren and colleagues<sup>8</sup> just described a population of CD34- bone marrow monocytes using single cell RNA sequencing and found that the JAK2 mutation increased expression of intermediate monocyte genes and the fibrocyte-associated surface protein

SLAMF7 in these cells. It would now be interesting to explore if there is also an association with ASXL1 co-mutations.

Shi et al. sought to dissect transcriptional differences upon co-mutation/loss of *Asx1* in their murine model. Using bulk-RNAseq of the heterogeneous population of *ckit*<sup>+</sup> hematopoietic stem and progenitor cells, the authors show that inflammation-related pathways such as *Nfkb*, *TNFA* and *IL-17*, are upregulated in *Asx1*<sup>-/-</sup> *JAK2VF* BM *ckit*<sup>+</sup> cells and confirmed higher serum levels of *TNF-a* in *ASXL1* mutant patients and *Asx1*<sup>-/-</sup> *JAK2VF* mice. Given the strong association they observe between the double mutants/co-mutations, it would have been also of particular interest to see the effect of the co-mutation on e.g. *CD14*<sup>+</sup> monocytes and not only progenitor cells. Interestingly, Shi and colleagues observed and validated the upregulation of *Egr1* in LSKs, GMPs and monocytes of *Asx1*<sup>-/-</sup> *JAK2VF* mice. This is an interesting link to fibrosis as *Egr1* expression was described in solid organ fibrosis to be induced by fibrogenic (pro-inflammatory) stimuli and to regulate the expression of extracellular matrix components, matrix remodeling enzymes and fibrogenic cytokines such as *TGF-β*, leading to myofibroblast differentiation. Shi et al. further leveraged RNAseq, ATACseq and CHIP-seq to investigate the transcriptional and epigenetic alterations in *Asx1*<sup>-/-</sup> *JAK2VF* double mutants and highlight increased chromatin accessibility associated with increased levels of histone marks on enhancers, also specifically on the *Egr1* locus. This is a strong point towards a role of *EGR1* in more advanced fibrosis.

Recent pivotal studies have transformed our understanding of mutation acquisition in MPN<sup>9,10</sup> and the timing of acquisition of an *ASXL1* mutation in MPN patients seems to be crucial for the phenotype. This raises the question what role the timing of *ASXL1* mutations in MPN has on disease and fibrosis initiation and progression, and if similar pathways and genes are activated. The “*ASXL1*-erating” effect on fibrosis kinetics in MPN was clearly demonstrated and it will be interesting to see in the future the functional effect of an *EGR1*/*TNFA* axis which could potentially act as a therapeutic intervention point.

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