Does the order of mutational acquisition in myeloproliferative neoplasms matter? Evidence from *JAK2* exon 12 and *DNMT3A* co-mutant polycythemia vera

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Myeloproliferative neoplasms (MPN) have in common an abnormal clonal expansion of differentiated hematopoietic cells in the bone marrow (BM), usually accompanied by excessive production of progenies leading to numeric blood cell increase. Three classical MPN types are recognized—polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF).

Characteristic somatic mutations of *JAK2*, *MPL*, and *CALR*, considered “drivers” directly linked to the MPN phenotype, were discovered in MPN [1]. The somatic *JAK2*-V617F gain-of-function mutation is found in >95% of PV patients [1], while most of the small proportion of *JAK2*-V617F-negative PV individuals bear *JAK2* exon 12 mutations [2]. Both *JAK2* mutations lead to constitutive activation of the JAK-STAT signaling pathway supporting the neoplastic outgrowth of the respective clone [3]. Other accompanying non-driver mutations such *ASXL1*, *EZH2*, and/or *TET2* are implicated too, especially in PMF, and are able to considerably affect individual clinical course [1, 4]. These latter mutations have major impact on epigenetic modulation, enhance myeloid tumorigenesis in general, and are encountered in a variety of hematological neoplasms. Another recurrently mutated

gene, also encoding for an epigenetic modifier, is *DNMT3A*, and its loss-of-function mutations are supposed to abrogate hematopoietic cell differentiation and increase self-renewal, thus promoting tumor growth. The incidence of *DNMT3A* mutations in MPN is 3% (ET) to 15% (PMF) [5, 6].

In December 2016 in a peripheral hospital, a 39-year-old male patient presented with visual disturbance and bilateral congestion of the orbital/periorbital veins and a slight splenomegaly of 14.5 cm. He had peripheral blood anisopoikilocytosis and anisochromia and hemoglobin of 244 g/L, hematocrit of 75%, and erythropoietin level <1 U/L. Empirical therapy with regular phlebotomies was started because of the risk of hyperviscosity complications, and after several courses, his values changed to 149 g/L, 47%, and 2.7 U/L (lower lab range 4.3 U/L), respectively. Noticeably, the white blood cell count was in the normal range (8.78 g/L) but with a slight neutrophilia (6.73 g/L; upper lab range 6.7 g/L), while monocytes (0.29 g/L) and platelets (266 g/L) were within normal ranges. Three months later, after rheological stabilization, a diagnostic BM biopsy was performed showing hypercellularity and a loose network of reticulin fibers with some intersections, especially in perivascular areas, corresponding to reticulin fibrosis grade 1 (Fig. 1a, b). All hematopoietic lineages were increased with groups of pleomorphic to small, at occasions, dysplastic megakaryocytes around sinuses forming just a few loose clusters. BM aspirate showed considerably erythroid predominance without dysplasia; some maturation disturbances in <10% of the myelopoietic cells and pleomorphic, at occasions (<10%), hypolobated megakaryocytes; and depleted iron stores. Conventional karyotypization yielded an unremarkable male karyotype 46,XY, as did array comparative genomic hybridization, additionally ruling out acquired uniparental disomies (aUPD). High-throughput sequencing analysis applying a customized NGS panel for “myeloid” mutations in 38 genes detected two mutations: *JAK2* H538-539delinsQL (allelic burden 26%) and

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Fig. 1 Bone marrow biopsy three months after several courses of phlebotomy showing hypercellularity (packed marrow), erythroid hyperplasia and increased amounts of atypical but loosely clustered megakaryocytes (**a**, HE staining), and a loose network of reticulin fibers, corresponding to reticulin fibrosis grade 1 (**b**, reticulin staining)

DNMT3A R771X (allelic burden 11%). The integrative diagnosis of a chronic-phase MPN with myelofibrosis grade I and *JAK2* exon 12 mutation, consistent with phlebotomy-pretreated PV, was established. The patient was put on acetylsalicylic acid 100 mg for six months with the need for regular phlebotomies, and then on ruxolitinib 10 mg bid finally leading to clinical course stabilization (hematocrit 42%, spleen size normalization) as per his last clinical visit in November 2019. The majority of the *JAK2*-V617F-negative *JAK2* exon 12 mutant patients are found to harbor one of the following four mutations: N542-E543del, F537-K539delinsL, K539L, and H538QK539L. Such exon 12 mutant alleles induce cytokine-independent/-hypersensitive proliferation in erythropoietin receptor-expressing cell lines by constitutive

activation of the JAK-STAT signaling pathway [2, 3]. *JAK2* exon 12 mutations are only observed in PV and are specific to patients with isolated erythrocytosis without concomitant leukocytosis or thrombocytosis. Several other exon 12 mutational variants have been identified [3, 7]; the mutation in our present case, i.e., H538-539delinsQL, also leads to substitution of lysine for leucine at codon 539, analogously to K539L, joining the above list.

In addition, our patient harbored a subclonal (since trisomies and aUPD for chromosomes 2 and 9 have been excluded) *DNMT3A* R771X mutation. Importantly, Jacquelin et al. recently provided insights into how *JAK2*-V617F and *DNMT3A* mutations collaborate to accelerate disease progression in PV-like models by upregulating self-renewal

programs: double-mutant cells show changes in chromatin accessibility with decreased repressive H3K27me3 marks replaced by activating H3K27ac marks [8].

Furthermore, the order of acquisition of *JAK2* and *DNMT3A* mutations may influence the MPN phenotype [9]. Mostly, *DNMT3A* mutations precede the *JAK2* mutation, which was not the case in our patient. Indeed, such order of mutational acquisition, i.e., “*DNMT3A* first,” is rather associated with an ET phenotype. By contrast, acquisition of *JAK2*-V617F prior to mutation of *DNMT3A*, i.e., “*JAK2* first,” is more likely associated with PV, as it was the case here. Finally, the co-occurrence of both mutations in our patient may explain the rather unusual neutrophilia, which is not common in exon 12 mutant PV, the presence of myelofibrosis, as suggested by observations of higher *DNMT3A* mutational frequencies in post-PV myelofibrosis [5], and possibly the occasional dysmegakaryopoiesis.

Subtle unusual details of the clinical history and the histopathology of our “*JAK2* first” exon 12 and *DNMT3A* co-mutant patient, such as his rather early age of presentation, initial affection by myelofibrosis, some dysmegakaryopoiesis, packed marrow, and neutrophilia, fit with and can be likely explained by the type and sequence of mutation acquisition [9]. We think that—along with analogous observations in other cases—we contribute evidence justifying prospective clinical studies with a closer look at the mutational order in MPN to address whether this information might contribute to diagnostic and therapeutic precision.

Author contributions RT wrote the manuscript and participated in establishing the diagnosis. JP held the clinical contact and treated the patient. PL performed molecular analysis. AT partially wrote the manuscript, established the diagnosis, and took microphotographs. All authors have read, edited, and approved the manuscript.

Compliance with ethical standards

Statement of ethics The study has been conducted in accordance with the World Medical Association Declaration of Helsinki. The patient has given a written consent to publish his case.

Conflict of interest The authors declare that they have no conflicts of interest.

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