*BCR::ABL*1-Negative MPN

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Contributor: Sophie Combaluzier, Julie Quessada, Norman Abbou, Robin Arcani, Antoine Tichadou, Jean Gabert, Régis Costello, Marie Loosveld, Geoffroy Venton, Yaël Berda-Haddad

Myeloproliferative neoplasms (MPN) are clonal hematopoietic stem cell-derived disorders characterized by uncontrolled proliferation of differentiated myeloid cells. Two main groups of MPN, *BCR::ABL1*-positive (Chronic Myeloid Leukemia) and *BCR::ABL1*-negative (Polycythemia Vera, Essential Thrombocytosis, Primary Myelofibrosis) are distinguished. For many years, cytomorphologic and histologic features were the only proof of MPN and attempted to distinguish the different entities of the subgroup *BCR::ABL1*-negative MPN. World Health Organization (WHO) classification of myeloid neoplasms evolves over the years and increasingly considers molecular abnormalities to prove the clonal hematopoiesis.

Keywords: myeloproliferative neoplasms ; cytomorphology ; molecular biology

1. Introduction

At a physiopathological level, *BCR::ABL1*-negative MPN (including PV, ET and PMF) are mainly characterized by a medullar hyperplasia with repercussions on one or more blood cells lines and by a trend to extramedullary hematopoiesis [1]. Conversely to AL, the myeloid maturation is still conserved, and no excess of blastic cells is found. Different hemogram perturbations point towards MPN, notably elevation of hematocrit (Hct), hemoglobin (Hb), red blood cells (RBC) count in PV, chronic thrombocytosis in ET, erythroleukocytosis [2], teardrop-shaped RBC in PMF, even thrombocytosis and leukocytosis in the initial phase of PMF. Within the same entity of *BCR::ABL1*-negative MPN, it still exists a real heterogeneity according to the disease stage but also the mutations found. Moreover, there is a continuum and thin borders among *BCR::ABL1*-negative MPN above all in *JAK2V617F*-positive neoplasms [3].

BCR::ABL1-negative MPN are rather older adult diseases with a median age at diagnosis at 65–67 years [4]. Nevertheless, *CALR*-mutated and “triple-negative” ET patients are diagnosed at a younger age (49 years) than other ET [5]. These diseases are more frequent in males than in females with a sex ratio comprised between 1.4–2.3, except ET in which a sex ratio is comprised between 0.5–0.7. Nowadays, PV is the most prevalent MPN with 9.2–30 per 100,000 [4] whereas PMF prevalence is the lowest (1.76–4.05 per 100,000) [6]. Various risk factors of MPN are described, such as autoimmune diseases, standard immunosuppressive therapies, and tobacco smoking. This last one shows an increased risk to develop PV but not ET [4]. Inflammation can play a role in the MPN progression principally in PMF [7][8].

2. Polycythemia Vera (PV)

Polycythemia vera (PV) is an MPN characterized by clonal erythrocytosis which can be composed of two phases: polycythemic phase and secondary myelofibrosis phase. PV diagnosis is defined thanks to the data of the hemogram, blood mass evaluation, BM biopsy and molecular biology.

In the case of polycythemia detection on blood count, it is necessary to distinguish relative erythrocytosis (increased Red Cell Mass (RCM) and decreased plasma volume, thus Total Blood Mass (TBM) unchanged), from absolute erythrocytosis (increased RCM and unchanged or increased plasma volume, thus elevated TBM) [1] and primitive (PV and congenital polycythemia with erythropoietin (EPO) receptor gene mutations) from secondary polycythemias (congenital origin with a more affine hemoglobin for dioxygen, or EPO hyperproduction by acquired causes (prolonged hypoxia, inappropriate EPO secretion)) [9].

In the polycythemic phase, neutrophilia and rarely basophilia may be present on PB with occasional immature granulocytes but circulating blasts are usually not detected. The BM aspiration shows a trilineage hyperplasia (panmyelosis) with especially erythroid hyperplasia but without dyserythropoiesis. There is also slight granulocytic hyperplasia with no dysgranulopoiesis signs.

Only erythrocytosis permits people to distinguish PV from other MPN; nevertheless, it is difficult to objectify this characteristic. To be sure that there is an absolute erythrocytosis, RCM must be above 125% [1]. As mentioned above, this criterion has been removed in 2022 WHO classification [10]. However, this characteristic is particularly interesting in the definition of the mPV, which is a sub-entity of PV able to mimic an ET. In mPV, also called “pre-polycythemic phase”, patients present obvious thrombocytosis and not really elevated Hb/Hct with lower EPO levels than in ET [11]. The examination of BM will be very useful to discriminate with ET. This particular entity, positive for *JAK2V617F* mutation, mimics an ET presentation and explains the reason why the 2016 WHO classification reduced the threshold values of Hb and Hct. In daily practice, complex cases or atypical presentations exist and require a multidisciplinary approach with a meticulous analysis of megakaryocytic lineage on both blood and medullar smears. The absence of platelets dystrophy nor large or giant size MKs in BM allows people to discuss a diagnosis of mPV rather than ET [12][13]. Reticulin staining is normal in 80% of cases but increased reticulin or mild to moderate collagen fibrosis can be observed, depending on the stage of disease [14]. At the diagnosis, the discovery of fibrosis grade 1 has a negative prognostic impact with the rapid evolution of the disease [15][16]. Therefore, BM biopsy should be realized whatever the levels of Hb, Hct and EPO for establishing the initial diagnosis.

Transformation into myelofibrosis can occur in 4.9–6% of PV at 10 years [16][17]. This stage is generally associated with cytopenia, extra-medullary hematopoiesis and hypersplenism. On the hemogram, this phase is characterized by a decreased RBC count, the presence of poikilocytosis with teardrop-shaped RBC and leukoerythroblastosis with neutrophilic on the blood smear ($\geq 13 \times 10^9$/L) [18][19]. The discovery of circulating blasts (≥10%) in PB indicates a transformation in AP.

The BM biopsy shows lower cellularity: erythropoiesis and granulopoiesis are decreased with possible fibrosis of grade 2–3. Dystrophic megakaryocytic clusters with hyperchromatic and very dysmorphic nuclei (hypolobulation) are prominent and an elevation of blast count is observed [16]. Osteosclerosis and the transformation into MDS or AL (more than 20% blast count in PB or BM) are around 10% [1].

The molecular analysis finds a gain of function *JAK2V617F* mutation in exon 14 in more than 95% of PV cases in a homozygous state after mitotic recombination but it is not specific for this entity and is found also in 50% of patients with ET (heterozygous state), PMF (homozygous after mitotic recombination) [20] or Refractory Anemia with Ring Sideroblasts and Thrombocytosis (RARS-T) [21] and at a lower frequency in other myeloid neoplasms (in 8% of Chronic MyeloMonocytic Leukemia (CMML) for example) [22][23]. *JAK2V617F* mutation have been reported in rare CML patients with *BCR::ABL1* fusion gene, in independent clones probably [24][25][26][27][28]. Studies have proved that the phenotype of these different MPN could be modified by the allelic burden of the *JAK2V617F* mutation. An increased *JAK2V617F* allelic burden (>50%) or homozygous mutation [3] correlates with an increase in Hb and leukocytes count and a lower platelet count [29][30]. It is associated with an increased risk of thrombosis and fibrotic evolution [1][31][32]. In mPV, *JAK2V617F* allele burden is lower with less elevated Hb level and leukocytes count than PV [11][14]. *JAK2* exon 12 mutation is present in approximately 3% of PV cases and correlates with variable leukocytosis [33] and lower platelet count than *JAK2V617F* mutated cases but with higher Hb level and a subnormal serum EPO concentration. Indeed, like the *JAK2V617F* mutation in exon 14, the exon 12 mutation also induces erythropoietin hypersensitivity [34]. In BM, erythroid preponderance is observed [29] and associated with an increase in MKs with variable nuclear lobulation (only one lobe or hyperlobulation) and a large spectrum of sizes but notably a predominance of small MKs. Cluster formation is also possible but rare and subtle compared to *JAK2V617F* mutated cases. The reticulinic fibrosis is normal or discreetly majored. Nevertheless, nearly half of *JAK2* exon 12 mutated PV does not show megakaryocytic cytomorphologic abnormalities [35]. Both mutations have similar prognosis. Finally, no *MPL* mutation is observed in PV and only very rare *CALR* mutation type I can be observed [36]. In these two described cases, erythropoiesis is elevated with irregular MKs but without clustering [16].

In laboratory practice, there are sometimes complex diagnoses when both cytology and molecular analysis (VAF < 1%) [29] are negative but with an evocative clinic for PV. With the study of karyotypes (abnormal in 20% of patients) [37], the development of both whole-genome analysis (CGH microarrays) and myeloid Next-Generation Sequencing (NGS) technologies, the molecular landscape of MPN becomes considerably more complex with the discovery of numerous acquired genetic abnormalities. These abnormalities have an impact on the evolution of the diseases and permit people to explain the heterogeneity of MPN phenotypes. In addition to the *JAK2* mutations, around 50% of patients with PV have additional mutations at diagnosis time and their presence is associated with a high risk of progression [14][38].

The heterogeneity of the MPN evolution is not only explained by the type of mutated genes but also by their chronology of appearance and their allelic load. Regarding the order of appearance, an additional mutation can appear prior to or after a driver mutation and modify the phenotype as demonstrated with *TET2* and *DNMT3A* in *JAK2*-positive MPN [20][39]. For example, if *TET2* mutation is the first event in HSC, an ET phenotype will be induced with the expansion of single mutant

cells in the HSC population limiting excess production of MKs and erythrocytes until the second hit with *JAK2V617F* mutation. In the case of "*JAK2*-first", a PV phenotype with excessive production of RBC and an expansion of double mutant cells in the HSC population is observed. Comparably to *TET2*, if *DMT3A* mutation is acquired before *JAK2V617F*, the MPN patients more commonly develop an ET phenotype whereas a PV phenotype will be observed in the case of "*JAK2*-first". Therefore, the mutational order affects both self-renewal of mutants in the HSC population and the proliferation driving to ET or PV phenotype [40].

3. Essential Thrombocythemia (ET)

Essential thrombocythemia (ET) is an MPN characterized by clonal thrombocytosis. Following the 2016 WHO classification, ET requires all four major criteria or the first three major and minor criteria [12]. In the 2022 WHO classification, nothing was changed in diagnostic criteria [10].

Compared to the 2008 WHO classification, the criteria are not really changed, except the definition of *MPL* and *CALR* mutations, which have been described in 2006 and 2013, respectively [41][42][43]. With the introduction of this molecular biology criterion, the criterion of reactional thrombocytosis moves to a minor criterion. However, the presence of reactional thrombocytosis does not formally exclude the hypothesis of underlying ET hence the importance of detecting the three driver mutations (*JAK2*, *MPL* and *CALR*) in persistent thrombocytosis. ET diagnosis is especially difficult because an isolated platelet elevation superior to 450 × 10^9/L can be found in many situations, such as post-splenectomia, post chirurgical or obstetrical stress, iron deficiency, inflammatory status, corticotherapy or cancers [44], and in contrast to PV, about 10% of ET patients are triple-negative genotypes [5]. Thus, ET remains an exclusion diagnosis. Moreover, most of the other myeloid neoplasms can mimic ET: CML with thrombocytosis and especially the pre-PMF. In the first case, the detection of *BCR::ABL1* permits the distinction whereas in the second case, molecular analysis will not permit it. In this case, some cytologic clues can help to distinguish the two entities: in ET there is typically no anemia nor abnormalities in RBC indices and there is no teardrop-shaped RBC on blood smear [32]. The morphologic analysis of BM will be determinant as seen below [45][46].

In ET, an elevated platelet count will be the main abnormality in routine hemogram. Sometimes, a moderate leukocytosis composed of neutrophils can be associated. Often, platelet can present morphologic abnormalities, observed on the blood smear: macroplatelets, giant platelets, strange shapes, pseudopods, agranular platelets or circulating micromegakaryocytes in post-ET PMF [47]. BM smears show normo- or hypercellularity with an important megakaryocytic hyperplasia [1]. Erythroblastic and granulocytic lineages are correctly represented [16].

On the myelogram, 20 to 49% of the total MKs are large to giant [48], with abundant, mature cytoplasm associated with hyperlobulated "staghorn-like" nuclei [16].

BM cytomorphology is crucial to differential diagnosis. In reactive thrombocytosis, MKs are increased but do not present morphological abnormalities, they are mature with sometimes a pleomorphism [49]. In CML, MKs are typically reduced in size with normal chromatin pattern [13] and in MDS/MPN with ring sideroblasts, the MKs have different sizes from small to giant with the Pearl staining highlighting the ring sideroblasts [32]. An increase in erythroblastic and granulocytic lineages should lead to a mPV in case of *JAK2V617F* mutation associated [16]. The presence of dense clusters of MKs and atypical MKs will be in favor of pre-PMF. Reticulin staining is usually normal at diagnosis in ET; grade 1 fibrosis can be observed only in less than 5% of cases [50]. This key point is very important in differential diagnosis with pre-PMF. Nowadays, standardized machine learning tools are developed and permit the improvement of the definition of reticulin fibrosis. This aspect is interesting to harmonize the quantitation of this fibrosis in different laboratories [51]. Indeed, ET and pre-PMF do not share the same risk of progression towards myelofibrosis (at 15 years, 9% versus 17% in ET and pre-PMF, respectively) and leukemic transformation (at 15 years, 2% versus 12% in ET and pre-PMF, respectively) [29][52]. Rare patients (less than 5% at 10 years) display an evolute form with indirect signs of myelofibrosis on the blood smear (poikilocytosis, teardrop-shaped RBC, leukoerythroblastosis), or circulating blasts (≥20%) in the case of transformation in AL [53]. In case of indirect myelofibrosis signs on the blood smear, the BM aspiration and BM biopsy can eventually show grade 2–3 fibrosis features [16].

On the molecular aspect, *JAK2V617F* mutant is present in approximately 50% of cases [35] with usually a VAF less than 25% whereas *JAK2* exon 12 mutation is not observed [16]. ET with a high *JAK2V617F* allelic burden is more susceptible to evolving into PV or PMF. Moreover, a "PV-like" phenotype is described in *JAK2V617F*-positive ET and not in *JAK2V617F*-negative ET [1]. Thrombosis is more frequent in *JAK2V617F*-positive ET patients [54]. *MPL* mutations are present in 3–8% of ET [5] and are associated with an increase in both platelet count and serum EPO level, and with both lower Hb level and marrow cellularity than in *JAK2V617F*-positive ET patients [55][56][57]. In a few patients, somatic *MPLS505N* mutations had

been reported although this mutation was initially associated with inherited thrombocytosis [56]. *MPL*-positive ET is more associated with an evolution towards PMF [58]. *CALR* mutants are present in 30% of ET and are more frequent in young patients with less thrombotic risk [59]. Moreover, *CALR* mutations are found in 67–71% of ET that are not mutated for *JAK2* and *MPL* [42][43]. They are exclusive and more frequent after *JAK2* mutation. Comparatively, with *JAK2V617F*-mutated patients, *CALR*-mutated ET are associated with higher platelet count, lower Hb level and leukocyte count [32]. Type 1 *CALR* mutation is more frequent in ET (50%) [60][61] and shows a higher risk to evolve towards myelofibrosis [3][32][62]. Type 2 *CALR* mutation is observed in young patients and is associated with higher platelet count but with a lower incidence of thrombosis at diagnosis and less myelofibrotic transformation.

On the morphologic axis, in *JAK2V617F* and *CALR*-mutated ET, the size of MKs is twice as large as the *MPL*-positive ET or triple-negative ET reported by experimented cytologists. In triple-negative ET (around 10% of ET), the platelet count is very elevated with specific morphologic abnormalities on MKs and is an indolent disease with less vascular events [5]. Therefore, in these cases, the myeloid NGS is very interesting to prove clonal hematopoiesis (with VAF ≥ 2%) above all in cases of mutations of genes implicated in epigenetic regulation (*TET2*, *DNMT3A*, *ASXL1*, *EZH2* and *IDH1/2*), in mRNA splicing (*SF3B1*, *SRSF2* and *U2AF1*) and in the regulation of cytokine signaling (*CBL* (casitas B lineage lymphoma protooncogene)) [20]. In PV, 53% of ET patients have one or more sequence variants/mutations revealed by NGS studies [38]. The *CALR*-positive MPN have a less complex molecular landscape than *JAK2*-positive MPN [63]. When *CALR* mutation is associated with an additional mutation like *ASXL1*, median Hb level is lower than only *CALR*-mutated ET. In *JAK2V617F*-positive ET, when patients are *TET2* or *DNMT3* mutated before the driver mutation, the ET phenotype is preponderant [39][40]. As in PV, the *SRSF2* mutation seems to be associated with a worse prognosis in ET, as well as the *SF3B1*, *U2AF1* and *TP53* mutations [37][38][64] The monitoring of *CALR* allele burden seems to be interesting to predict evolution [60] as for additional mutations, the study of the rate of the allele burden seems to be interesting for the leukemic transformation process [65].

4. Primitive Myelofibrosis (PMF)

PMF is the least common and most aggressive MPN manifested by BM fibrosis, extramedullary hematopoiesis (mainly in the spleen and/or liver) and inappropriate production of cytokines [6][66]. Myelofibrosis can be primitive or secondary to others, such as MPN or MDS, infection, inflammatory or autoimmune diseases, metabolic diseases and neoplastic disorders [45]. PMF evolves in two phases: the prefibrotic PMF/early PMF (pre-PMF) corresponding from 30 to 50% of PMF cases with no significant increase in reticulin or collagen (grade 0 or 1) and the fibrotic phase (overt PMF) with reticulin or collagen fibrosis of grade 2 or 3 [12]. Compared to the 2008 version, the 2016 WHO classification introduces the pre-PMF phase. The diagnosis of this phase requires three major criteria and at least one minor criterion confirmed in two consecutive determinations [12]. Molecular criteria take a preponderant place because besides the three well-known driver mutations, it is more and more necessary to search clonality markers as *ASXL1*, *DNMT3A, EZH2*, *TET2*, *IDH1/2*, *SRSF2* and *SF3B1* because they are more frequent in PMF and in advanced MPN. The 2022 WHO classification insists on this molecular aspect because the presence of additional mutations (*ASXL1*, *EZH2*, *IDH1/2*, *SRSF2* and *U2AF1)* are associated with poor outcomes and called “High-Risk Mutations” (HRM) [10][31].

At the pre-PMF stage, hemogram usually shows anemia, moderate leukocytosis (>11 × 10^9/L) and/or thrombocytosis (25% of patients) with macroplatelets and giant platelets on blood smear.

Due to this thrombocytosis, it is necessary to differentiate pre-PMF from ET based on morphologic findings in the BM biopsy as detailed in WHO 2022 classification. Pre-PMF demonstrates hypercellular BM with granulocytic proliferation with often erythropoiesis hypoplasia without dysplasia [3] and without immature cells excess, nor maturation blockade. Megakaryocytic abnormalities which are better appreciated by the BM biopsy are the key factors for the diagnosis with analysis of topographical distribution in BM. There are numerous MKs with variable sizes (small to high), pleomorphic with aberrant nuclei–cytoplasmic ratio, hyperchromatic nuclei with variable lobulation (bulbous, cloud-like or balloon-shaped nuclei), and the presence of naked megakaryocytic nuclei [13][48]. These are often arranged in dense clusters adjacent to BM vascular sinuses and bone trabeculae [32]. With the new machine learning approach, it is possible to precisely distinguish the morphological features of MKs and MPN with high accuracy [67].

Compared with other MPN, in pre-PMF, MKs are more atypical and represent a strong argument to diagnose pre-PMF and not ET [46]. This cytological distinction is very important because pre-PMF will evolve more often and/or rapidly towards overt PMF than ET [52][68]. Lymphoid reactional nodules are present in about a quarter of cases [69]. BM aspiration shows the same cytologic abnormalities and it is complementary to the biopsy. At this stage, the BM biopsy does not highlight significative fibrosis, a maximum of grade 1 [70].

In the case of overt PMF, major criteria have not changed in 2016 compared to 2008 but details are noticed about grades of fibrosis [12]. In contrast, there is an additional minor criteria which is the presence of leukocytosis ≥ 11 × 10^9/L [71]. At this stage, hemogram usually shows anemia and a lower platelet count. It is also possible to observe leukopenia. On blood smear, there is often an erythroleukocytosis [12] and an anisopoikilocytosis with teardrop-shaped RBC which are characteristic features but not specific. Circulating immature cells and MKs of different shapes (high size, micromegakaryocytes, nude nuclei or classical MKs) may also be present. There is an increase in the number of circulating CD34+ cells in the later stages in association with extramedullary hematopoiesis, usually more than 15 per microliter [72]. Monocytosis (>1 × 10^9/L) is a sign of evolution in PMF [73]. To distinguish this evolution from CMML, it is important to be attentive to cytological indirect signs of the PB and BM biopsy, evoking overt PMF. Moreover, the molecular analysis should be performed for the research of MPN driver mutations to avoid a misdiagnosed CMML [10][74]. The main difference with the prefibrotic stage is the apparition of reticulinic and/or collagenic fibrosis > grade 1 [12]. Due to fibrosis, the BM aspirate is typically hypocellular and often unhelpful. It shows some dystrophic MKs and rare erythroblastic islets. BM biopsy shows heterogenic cellularity with the coexistence of both conserved hematopoietic (in vascular sinuses) and fibrotic clusters. There is also the presence of significant megakaryocytic dystrophia grouped together in clusters of variable size [69]. A redistribution of fat cells along the bone trabecules is observed in BM PMF [16]. Lymphocyte infiltration and reactional plasmacytosis are frequent. Immature CD34+ cells remain inferior to 10% but an evolution in AL is possible and happens for 5–30% of patients [69]. Due to phenotypic mimicry of a lot of diseases (malignant or not) [6], PMF diagnosis is difficult and BM biopsy has a major role in its diagnosis [1]. Guidelines were established to define semiquantitative BM fibrosis grading system or semiquantitative grading of collagen and osteosclerosis [75] in order to use reproductible tools (for example: machine learning approach to quantify reticulin fibrosis like continuous indexing of fibrosis) [51]. In the 2022 WHO classification, experts insist on this aspect for monitoring BM fibrosis in case of treatment by JAK1/2 inhibitors [10]. In myelofibrosis following pre-PMF, PV or ET, the level of reticulin fibrosis is the main component, followed by an increase in the number of collagen fibers [45]. Moreover, some links are now highlighted between cytomorphologic and molecular features.

In PMF (early or advanced stages), mutations of *JAK2V617F*, *JAK2* exon 12, *CALR* and *MPL* are found in, respectively, 50%, 2–3%, 25–30% and 13.5% of cases [6][16]. Moreover, *CALR* mutations are found in 56–88% of PMF that are not mutated for *JAK2* and *MPL* [42][43]. About 12% of PMF are triple-negative and correspond to difficult diagnosis [5]. *JAK2V617F*-positive PMF cases show a higher Hb level, white blood cell and platelet counts associated with an elevated allele burden. In these patients, an elevated CRP was observed, with an increased IL-1 receptor antagonist (RA), IL-10 and IL-2R. Elevated CRP was described as associated with a bad survival [76]. Due to TPO hypersensitivity, an *MPL* mutation, associated or not to *CALR* mutation, leads only to an increase in platelet count and megakaryocytopoiesis. *MPLW515L*-positive PMF present a more severe anemia [77]. Type 1 *CALR* mutation is associated with a higher platelet count and less incidence of anemia and leukocytosis [32] thus survival is favorable [62][78]. Among *CALR* mutations, *CALR del52* is more frequent in PMF (70%) than *CALR ins5* (13%) [60][61][79]. As it was shown that the rate of *JAK2* allele burden could modify the phenotype of MPN between ET, PV and PMF, the rate of *CALR* allele burden usually found in the initial clone, is more elevated in PMF than in ET [60]. In triple-negative myelofibrosis the prognostic is pejorative with a significantly higher risk of leukemic evolution [5]. A total of 40% of PMF patients have an abnormal karyotype with commonly 20q−, 13q−, +8, +9, 12p−, inv(3), −5/5q− and 11q23 rearrangements and abnormalities in chromosomes 1 and 7 [80][81]. From the 2016 WHO classification and with the development of myeloid NGS, molecular analysis takes a preponderant place. In PMF (primary or secondary), more than 80% of patients have genomic variants in myeloid genes: *ASXL1*, *TET2*, *SRSF2*, *EZH2* and *IDH1/2* that are the most involved genes and *TP53*, *EZH2*, *SRSF2* and *U2AF1* mutations are associated with adverse prognostic likewise for *IDH1/2*, *CBL* and *K/NRAS* [80][82]. Among the numerous studies published, the number of mutations can influence prognosis. For example, *EZH2*-mutated PMF patients have a higher leukocytes count with blastic cells and more important splenomegaly [78]. Stable MPN have either no additional mutations or one additional mutation at diagnosis while unstable MPN have around 4 additional mutations reflecting genetic instability during disease progression [63][65]. Mutations in *DNMT3A*, *IDH1/2*, *TP53* and *SRSF2* genes are more frequent in MPN evolving into AML [80]. *DNMT3A* and *TP53* mutations are more frequent in post PV/ET AML than in post-PMF AML [63] and mutations in *U2AF1* are more associated with anemia and short survival [6]. *TP53* mutations are considered as a late event because they are observed at the leukemic transformation in post-MPN AL although they may be present at diagnosis at a low frequency [65][82]. International prognostic scores involving additional mutations were developed for PMF, PV and ET to identify patients with the risk of progression. In PMF, additional mutations play a central role in the development of prognostic scores: IPSS, DIPSS, DIPSS-Plus, MIPSS70, MIPSS70+ version 2, GIPSS and MYSEC-PM for secondary myelofibrosis to categorize patients for the risk of evolution. GIPPS is exclusively based on genetic and cytogenetic data [37][52][78][83][84]. Therefore, in clinical practice, NGS is recommended to improve risk stratification even if it is not mandatory to establish the optimal treatment [64]. Mutation of *ASXL1* was initially associated with an unfavorable prognosis but in a recent French study, the impact on prognosis could be different if *ASXL1* is alone

or associated with *TP53* or high-risk mutations [82][85]. Thus, it becomes more and more complex to define the prognostic of patients.

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