Review Article

Novel European Asiatic Clinical, Laboratory, Molecular and Pathobiological (2015-2020 CLMP) criteria for JAK2<sup>V617F</sup> trilinear polycythemia vera (PV), JAK2<sup>exon12</sup> PV and JAK2<sup>V617F</sup>, CALR and MPL<sup>515</sup> thrombocythemias: From Dameshek to Constantinescu-Vainchenker, Kralovics and Michiels

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Keywords: Myeloproliferative neoplasms; Essential thrombocythemia; Polycythemia vera; Primary megakaryocytic granulocytic myeloproliferation; Myelofibrosis; JAK2<sup>V617F</sup> mutation; MPL<sup>515</sup>mutation; CALRmutation; JAK2<sup>exon12</sup> mutation; CALR mutation; JAK2 wild type; Bone marrow histology
Novel European Asiatic Clinical, Laboratory, Molecular and Pathobiological (2015-2020 CLMP) criteria for JAK2V617F trilinear polycythemia vera (PV), JAK2V617F PV and JAK2V617F, CALR and MPL151 thrombocythemias: From Dameshek to Constantinescu-Vainchenker, Kralovics and Michiels

Abstract

The Myeloproliferative Neoplasms (MPN) of trilinear polycythemia vera (PV) and megakaryocytic leukemia (ML = primary megakaryocytic granulocytomatosis: PMGM) and Essential Thrombocythemia (ET) in the studies of Dameshek and Michiels are caused by the MPN driver mutations JAK2V617F, JAK2V617F, CALR and MPL151 discovered by Constantinescu-Vainchenker, Green and Kralovics. The JAK2V617F mutated trilinear myeloproliferative neoplasms (MPN) include a broad spectrum of clinical laboratory and bone marrow features in essential thrombocythemia (ET), promyelocytic and erythrocytotic PV, classical PV and advanced stages of marked PV and PV complicated by splenomegaly and secondary myelofibrosis (MF). Heterozygous JAK2V617F mutated ET is associated with low JAK2 allele and MPN disease burden and normal life expectancy. In combined heterogeneous and homozygous or homozygous JAK2V617F mutated trilinear PV, the JAK2 mutation load increases from less than 50% in promyelocytic PV and classical PV to above 50% up to 100% in hypercellular PV, advanced PV and PV with MF. Bone marrow histology show diagnostic features of erythremic, megakaryocytic and granulocytic (EMG) myeloproliferation in JAK2V617F mutated myeloproliferative neoplasms (MPN), which clearly differs from monolinear myeloproliferation (M) myeloproliferation of megakaryocyte (MG) myeloproliferation and CALR mutated thrombocythemia and dual megakaryocytic granulocytic (MG) myeloproliferation in CALR mutated thrombocythemia. The morphology of clustered large pleomorphic megakaryocytes with hyperlobulated nuclei are similar in JAK2V617F thrombocythemia, promyelocytic PV and classical PV patients. Monolinear megakaryocytic (M) myeloproliferation of large to giant megakaryocytes with hyperlobulated staghorn-like nuclei is the hallmark of MPL151 mutated normocellular thrombocythemia. CALR mutated thrombocythemia usually presents with high platelet count around 1000×10⁹/L and normocellular megakaryocytic (MG) proliferation of immature megakaryocytes with cloud-like hyperchromatic nuclei followed by dual megakaryocytic granulocytic (MG) myeloproliferation followed by various degrees of bone marrow fibrosis. Natural history and life expectancy of MPN patients are related to the response to treatment and the degree of anemia, splenomegaly, myelofibrosis and constitutional symptoms. The acquisition of epigenetic mutations at increasing age on top of MPN disease burden independently predict unfavorable outcome in JAK2V617F, MPL151 and CALR mutated myeloproliferative neoplasms (MPNs, which mutually exclude each other).

Introduction

The combination of plethoric appearance, splenomegaly, erythrocyte count above 6×10¹²/L, elevated platelet count and the presence of large megakaryocytes and pancytopenia in the bone marrow is diagnostic for trilinear polycythemia vera [1,2]. Venesection aiming at haematocrit of 0.40 is the first choice life saving treatment option in newly diagnosed PV that prevents major thrombosis and controls hypervolemic symptoms during long-term follow-up in the majority of PV patients [2-7]. PV is a trilinear myeloproliferative, thrombocythemic and granulocytic (EMG) myeloproliferation caused by either one unknown bone marrow stimulation factor or the lack of one inhibitory factor, [2,8,9]. Megakaryocyte leukemia (ML) is distinct from PV [10] and has been recognized by Georgii, et al. [11,12], as hypercellular thrombocythemia due to dual chronic or primary megakaryocytic granulocytic myeloproliferation (CMGM/PMGM) without features of PV [13-15].

The Hannover Bone Marrow criteria proposed by Georgii, et al. [11], translated the PVSG criteria in the Hannover BM criteria for ET, PV and PMGM and stages of each by grading of myelofibrosis (MF) as a secondary event in advanced stages of MPDs complicated by anemia, splenomegaly and fibrosis in the bone marrow [11,14,16]. Michiels drew attention to the importance of bone marrow histology as a pathognomonic clue to each of the MPDs ET, PV and PMGM [14-17]. The number and size of mature megakaryocytes in bone marrow biopsies are typically increased in ET and PV. Large megakaryocytes with mature cytoplasm and multilobulated nuclei and the tendency to cluster in small groups close to the sinuses represent the hallmark feature of ET (Figure 2). The histologic background of hematopoiisis in ET at platelet counts above 400×10⁹/L is one of normal cellularity in the early stage [14,17] (Table 3). A slight to moderate increased cellularity due to increased erythropoiesis may be seen in ET with increasing platelet counts between 400 to above 1000×10⁹/L against a background of normally maturing granulopoiesis and erythropoiesis comparable with the early stage of PV [18-20]. Increase in number and size of clustered large megakaryocytes comparable to ET and a moderate to marked increased cellularity due to increased erythropoiesis/megakaryopoiesis (EM) and erythro-megakaryo-granulopoiesis (EMG) are the diagnostic features of untreated PV [14,17] (Figure 3, Table 4). Increase of large megakaryocytes with mature cytoplasmas and multilobulated nuclei in a hypercellular bone marrow is even more conspicuously altered in PV than in ET or early promyelocytic PV. The megakaryocytes in PV usually have a pleomorphic appearance with a wide range of megakaryocyte sizes including small, medium sized and large forms (Tables 3,4) as can be demonstrated in immune stained bone marrow biopsies using monoclonal antibodies against platelet glycoprotein. The characteristic increase and clustering of large megakaryocytes and proliferation of erythropoiesis with hyperplasia of dilated sinuses are the diagnostic hallmark of untreated PV to distinguish it from secondary erythrocytosis [12,15,17,18], from Ph+ chronic granulocytic leukemia and Ph+ ET [13,15] and most importantly from PMGM [12,15,16]. Bone marrow histology in PMGM is dominated by atypical immature megakaryocytes, which are conspicuously large due to increase of nuclear as well as cellular size. The nuclei of megakaryocytes in PMGM are bulky with lobuli becoming clumsy. The lightly stained chromatin and irregular roundish nuclear forms give rise to the so-called cloud-like nuclei, which are almost never seen in ET and PV [11,12,14,16,21].

Within the European Working Group on myeloproliferative Disorders (EWG MPD founded by Dr. Michiels in 1994). Michiels, et al. [14], translated the Hannover Bone Marrow Classification of Georgii, et al. [11] in a new set of the Rotterdam Clinical and Pathological (RCP) criteria for the diagnosis of ET and PV and chronic, essential or primary
megakaryocytic granulocytic myeloproliferation PMGM as the third distinct MPD [11,12,14-17,22] (Table 2). The present appraisal of the myeloproliferative neoplasms (MPN) from Dameshek to Michiels review the clinical laboratory molecular and pathological (CLMP) characteristic of the MPNs caused by the driver mutations JAK2V617F, MPL515, JAK2 exon12, CALR and MPL515 discovered by Constantinescu-Vainchenker, Pardani, Green and Kralovics respectively (Tables 1,2, Figure 1).

### Diagnostic differentiation of ET and PV by erythrocyte count and BM histology

According to Dameshek, [2], Georgii, et al. [11,12], and Michiels, et al. [6,7,14-16,21,23-36], the diagnosis of ET according to PVSG and WHO criteria is one of exclusion. Bone marrow histology using hematoxylin and eosin is then essential to make the diagnosis of ET. The diagnosis of ET is based on a combination of clinical and laboratory findings. The diagnosis of ET is made when patients have at least two of the following criteria: an erythrocyte count above 5.8x10^12/L, a platelet count above 450x10^9/L, and at least one of the following: an increase in red cell mass of 10% or more, a decrease in red cell mass of 10% or more, and a decrease in red cell mass of 5% or more. The diagnosis of ET is supported by the presence of at least one of the following laboratory findings: an increase in the spontaneous release of hyperactive granulocytes, an increase in the spontaneous release of hyperactive platelets, an increase in the spontaneous release of hyperactive megakaryocytes, an increase in the spontaneous release of hyperactive platelets and megakaryocytes, an increase in the spontaneous release of hyperactive megakaryocytes, and an increase in the spontaneous release of hyperactive platelets and megakaryocytes.

### Figure 1: Upper part: The discovery by Constantinescu & Vainchenker of the JAK2V617F somatic mutation as the cause of trilinear myeloproliferative neoplasms and the sequential occurrence of heterozygous JAK2V617F mutation in essential thrombocythemia (ET) and homozygous JAK2V617F mutation in trilinear polycythemia vera (PV) which does explain the occurrence of three sequential phenotypes of ET, PV and myelofibrosis (MF) during life long follow-up in the studies of Dameshek and Michiels.

### Lower part: Concept of Michiels & De Raeve on the dynamics of the JAK2V617F disease processes in trilinear MPN ranged from normocellular ET and prodromal PV mimicking ET with normal erythrocyte count below 5.8x10^12/L to definitive increase in peripheral blood erythrocytes above 5.8x10^12/L in PV followed by masked PV, advanced PV complicated by fibrosis and splenomegaly, spentphase PV and blast transformation of post- PV myelofibrosis. Designed by Michiels 2020.

### Figure 2: The heterozygous JAK2V617F mutated acquired thrombocythemia and germline TPO and JAK2V617F or JAK2Q534R mutated hereditary thrombocythemia as well as acquired MPL515 and congenital hereditary MPL515 mutated thrombocythemia (ET) are driven by indirect cytokine activation heterozygous JAK2V617F+STATS, germline JAK2 or TPO→TpoR→MPL. Direct binding of TPO to the D3D4 domain of TpoR or direct cytokine activation (MPL 515 and MPL 505) cytokine receptor activation induce an ET phenotype of MPN without features of PV (ECC negative) Michiels, et al. 2014 [27]. CALR mutants Type 1 and 2, but not wild type CALR, did induce STATS activation via TpoR (MPL) and GCSFR, but not via EpoR. The STATS activation via GCSFR was much weak theran via TpoR (MPL). The extracellular domain of TpoR (MPL), but not of EpoR, was indispensable for CALR mutant induced activity and the D1D2 distal part of the extracelluar TpoR domain and its associated N-glycosylation sites but not the TPO binding site in the D3D4 domain Akaki, et al. Vainchenker and Kralovics 2017 [65].

### Figure 3: According to Constantinescu-Vainchenker low V617F constitutional kinase activity in heterozygous mutated JAK2V617F mutated patients is enough to produce the ET phenotype via the MPL signalling pathway and that higher V617F constitutional kinase activity in JAK2V617F mutated, heterozygous/homozygous or homozygous mutated patients is needed to produce the sequential stages of prodromal, classical and advanced (masked) PV phenotypes via activation of both the EPO and pathways of hematopoietic progenitors cells (Table 1). The JAK2V617F dosage hypothesis has been confirmed at the bone marrow hematopoietic stem cell level by the demonstration that endogenous erythroid colonies (ECC) from ET patients are mainly heterozygous for the JAK2V617F mutation, whereas all PV patients are either hetero/homozygous or mainly homozygous for the JAK2V617F mutation (Figure 8).

### Table 1: The 2006 concept of Michiels, et al. [2006a,b] [8,9], Belfucci & Michiels, 2006 [31] on the molecular etiology of JAK2V617F mutated hyperactivated platelets and platelet-mediated arteriolar erythromelalgic arterial (Michiels, et al. 1984 [86], 1985, 1993) in heterozygous essential thrombocythemia (ET) and major microvascular thrombosis in mixed heterozygous and homozygous thrombocythemia and polycythemia vera (TPV) complicated by splenomegaly due to myeloid metaplasia of the spleen and secondary bone marrow fibrosis according to the dosage hypothesis of Constantinescu & Vainchenker (James, et al. 2005 [57], Vainchenker & Constantinescu, 2005 [59], Villeval, et al. 2006 [60]).

### Table 2: The 2006 concept of Michiels, et al. [2006a,b] [8,9], Belfucci & Michiels, 2006 [31] on the molecular etiology of JAK2V617F mutated hyperactivated platelets and platelet-mediated arteriolar erythromelalgic arterial (Michiels, et al. 1984 [86], 1985, 1993) in heterozygous essential thrombocythemia (ET) and major microvascular thrombosis in mixed heterozygous and homozygous thrombocythemia and polycythemia vera (TPV) complicated by splenomegaly due to myeloid metaplasia of the spleen and secondary bone marrow fibrosis according to the dosage hypothesis of Constantinescu & Vainchenker (James, et al. 2005 [57], Vainchenker & Constantinescu, 2005 [59], Villeval, et al. 2006 [60]).
marrow and erythrocyte count were not used in the PVSG/WHO classification as a specific clue to ET in various MPNs [6,7,15-17,31,32]. Wassermann, et al. [37], introduced crude inclusion criteria for the PVSG/01 randomized clinical trial to be sure that patients do have PV because they were subjected to potential leukemogenic agents P32 and chlorambucil as compared phlebotomy aiming at a hematocrit to below 0.50 [37]. These crude criteria are used by the PVSG since 1975 as diagnostic criteria for PV [38]. The PVSG [38] and 2008 and 2016 WHO criteria for PV [39-41] did not measure erythrocyte counts and MCV and did not use bone marrow histology features and persisted to use only crude cut-off levels for hemoglobin and hematocrit (Hb> 18.5 g/dl and Ht> 0.60 in men and Hb> 16.5 and Ht> 0.56 in women) as surrogate measures of red cell mass (RCM) to separate ET from PV.

Michiels, Thiele & De Raev used bone marrow histology and erythrocyte and platelet counts as pathognomonic clue to distinguish all variants of MPN from reactive thrombocytosis, BCR/ABL positive thrombocythemia in chronic myeloid leukemia (CML), and thrombocythemia in myelodysplastic syndromes (MDS, 5q minus syndrome) by demonstrating that clustered mature large megakaryocytes occur in MPN, small monolobulated megakaryocytes in CML and dysmorphic megakaryocytes in MDS [6-8,14,15,23,31,32,34,42].

Megakaryocytes are identical large, mature and pleiomorphic in prefibrotic JAK2V617F positive ET and PV patients (Tables 2-5) and clearly different from the large giant mature megakaryocytes in MPL thrombocythemia (Table 6) and from the large immature megakaryocytes with ‘cloud-like’ nuclei in CALR positive thrombocythemia (Table 7).

Erythrocyte count above the upper limit of normal (> 5.8 x10^{12}L in males and > 5.6 x10^{12}L in females) on top of characteristic bone marrow histology obviates the need to measure RCM [5,16,27,31,32,43,44] (Figure 2, Table 3). Bone marrow histology of sequential stages in prodromal, overt and advanced PV is typically featured by increased cellularity due to increase erythroid megakaryocytic (EM), erythroidic, megakaryocytic granulocytic (EMG), and predominant megakaryocytic granulocytic (MG) myeloproliferation (Figures 5, 6 and Tables 4, 5). PV is frequently preceded by ET or

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**Table 2:** The spectrum of JAK2V617F mutated ET, PV and MF versus JAK2 wild type normocellular ET and hypercellular ET associated with prefibrotic PMGM [11,12,15] according to ECP criteria [8] by interpreting the PVSG/WHO bone marrow features into the ECP and ECMF criteria of myeloproliferative Disorders [15,17], MPD Doctor’s Brochure 2004, Michiels, et al. [8,9].

| Clinical, laboratory, and molecular (CLM) criteria | Bone marrow Pathology (P) criteria |
|--------------------------------------------------|----------------------------------|
| **Prefibrotic ET**                               | Normocellular ET                 |
| 1. Platelet count of > 350 x10^{11}              | Normocellular bone marrow (< 60%), Megakaryocytic (M) proliferation of clustered of medium sized to large (pleomorphic) mature megakaryocytes in anormocellular bone marrow (< 60%), no proliferation of erythropoiesis and granulopoiesis. |
| 2. Heterozygous JAK2V617F mutation, and low JAK2 allele mutation load | Reticulin fibrosis (RF) 0 or 1 |
| 3. Normal erythrocytes < 5.8x10^{12}/L males, < 5.6 x10^{12}/L females |                                         |
| 4. Hemoglobin (Hb) and hematocrit (Ht) normal or upper range of normal |                                         |
| **Prefibrotic prodomal PV**                      | ET with bone marrow features of PV |
| 1. Platelet count of > 350 x10^{11}              | Increased cellularity (60%-80%) due to increased erythroidic, megakaryocytic (EM) proliferation or trilinear erythroidic, megakaryocytic, granulocytic (EMG) proliferation. |
| Hb and Ht in upper range of normal, but erythrocyte count < 5.8x10^{12}/L males, < 5.6x10^{12}/L females |                                         |
| 2. Presence of JAK2V617F mutation and variable JAK mutation load | Increased of clustered medium sized to large (pleiomorphic) mature megakaryocytes. |
| 3. Low serum EPO, increased LAP score             | Spontaneous EEC. |
| **Prefibrotic hypercellular ET**                 | RF 0 or 1                        |
| 1. Platelet count of > 350 x10^{11}              | Hypercellular due to increased erythroid megakaryocytic and granulocytic myeloproliferation (EMG, masked PV, prefibrotic) or increased megakaryocytic, granulocytic (MG, fibrotic) proliferation with relative reduced erythroid precursors. |
| Hb and Ht in lower range of normal: h< 12 g/dl, low LDH and CD34+ | Loose to dense clustering of more pleiomorphic megakaryocytes with hyperploid or clumpsy nuclei |
| 2. Presence of JAK2V617F mutation and high JAK2 mutation load | Grading of reticulin fibrosis (RF, [113]) and myelofibrosis (MF, [11,12,109]) |
| 3. Moderate myeloid neoplasia of the spleen      | -Prefibrotic RF: RF-0/1 = MF-0, no/minor splenomegaly |
| →splenomegaly                                   | -Early fibrotic EMG: RF 2 → MF 1 and minor or moderate splenomegaly |
| 4. No preceding or allied CML, PMGM, RARS-T or MDS | -Fibrotic EMG: RF3, RCF = MF2 and overt splenomegaly |
|                                               | Post-ET MF: RF3/4 = MF-2/3 (WHO criteria) |

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**Table 3:** Clinical, Laboratory, Molecular and Pathobiology (2015-2020 CLMP) criteria for diagnosis of JAK2V617F mutated essential thrombocythemia (ET).

| Clinical, laboratory, and molecular (CLM) criteria | Bone marrow Pathology (P) criteria |
|--------------------------------------------------|----------------------------------|
| **Clinical stage 1:** Hb > 12 g/dl, normal LDH and CD34+ |                                         |
| **Clinical stage 2:** anemia Hb < 10 g/dl, normal LDH, CD34+ and splenomegaly |                                         |
| **Clinical stage 3:** severe anemia, Hb < 10 g/dl, LDH↑↑, CD34+, leukoerythroblastose, tear drop erythrocytes, and large spleen |                                         |
Table 4: Clinical, Laboratory, Molecular and Pathobiology (2015-2020 CLMP) criteria for the diagnosis of prodromal, masked and classical JAK2 mutated polycythemia vera (PV) versus primary or secondary erythrocytoses

**Clinical, laboratory, molecular (CLM) criteria**

**Bone marrowPathology (P) criteria**

**Major criteria for PV**

A 1. Erythrocytes > 5.8x10^12/L males and > 5.6x10^12/L females. Hemoglobin and hematocrit upper range of normal or increased.

A 2. Heterozygous and/or homozygous JAK2V617F or JAK2exon12 mutation.

A 3. Low serum Epo level.

**Confirmative criteria**

B 1. Persistent increase of platelet count x10^9/L: grade I: 400-1500, grade II: > 1500.

B 2. Granulocytes > 10x10^9/L or Leukocytes > 12x10^9/L and raised LAP-score or increased CD11b expression in the absence of fever or infection.

B 3. Myeloid neoplasia of the spleen → splenomegaly on ultrasound echogram (> 12 cm length in diameter) or on palpation.

B 4. Spontaneous endogenous erythroid colony (EEC) formation (optional).

**Clinical, Laboratory, Molecular and Pathobiology (2015-2020 CLMP) criteria for JAK2V617F trilinear polycythemia vera (PV), JAK2exon12 PV and JAK2V617F, CALR and MPL515 thrombocythemias: From Dameshek to Constantinescu-Vainchenker, Kralovics and Michiels**

**Table 5:**

| Clinical, laboratory, molecular (CLM) criteria | Bone marrowPathology (P) criteria |
|-----------------------------------------------|----------------------------------|
| Major criteria for PV                         |                                  |
| A 1. Erythrocytes > 5.8x10^12/L males and > 5.6x10^12/L females. Hemoglobin and hematocrit upper range of normal or increased. |                                  |
| A 2. Heterozygous and/or homozygous JAK2V617F or JAK2exon12 mutation. |                                  |
| A 3. Low serum Epo level.                    |                                  |
| Confirmative criteria                         |                                  |
| B 1. Persistent increase of platelet count x10^9/L: grade I: 400-1500, grade II: > 1500. |                                  |
| B 2. Granulocytes > 10x10^9/L or Leukocytes > 12x10^9/L and raised LAP-score or increased CD11b expression in the absence of fever or infection. |                                  |
| B 3. Myeloid neoplasia of the spleen → splenomegaly on ultrasound echogram (> 12 cm length in diameter) or on palpation. |                                  |
| B 4. Spontaneous endogenous erythroid colony (EEC) formation (optional). |                                  |

**Table 6:**

| 2015-2020 Clinical Laboratory, Molecular and Pathobiology (CLMP) criteria for the diagnosis of normocellular ET carrying one of the MPL515 mutations. |
|---------------------------------------------------------------------------------------------------------------------------------------------|
| **Clinical, laboratory, molecular (CLM) criteria** | **Bone marrow Pathology (P) criteria** |
| 1. Platelet count > 350x10^9/L and presence of large platelets in blood smear. | Megakaryocytic (M) proliferation in a normocellular (< 60%) bone marrow featured by large to giant mature megakaryocyte with hyperlobulated, staghorn-like nuclei. |
| 2. Normal Hemoglobin, haematocrit and erythrocyte count. | No increase of erythropoiesis, and granulopoiesis. |
| 3. Presence of MPL515 mutation. | No or slight increase in reticulin RF 0/1. |
| 4. Normal serum EPO. | Grading of bone marrow fibrosis: reticulin fibrosis (RF), Wilkins, et al. [113] and myelofibrosis (MF),Georgii. et al. [11,12,19]. |
| 5. Normal LAP score (CD11b). | |
| 6. No or slight splenomegaly. | |
| 7. No preceding or allied CML, PV, PMGM, RAS-T or MDS. | |

Clinical staging similar as in CALR thrombocythemia based on the degree of anemia, splenomegaly and myelofibrosis.
In newly diagnosed PV patients, the state of PV in remission (MCV below 70 fl) caused by iron deficiency [2,10] (Figure 4). The CLMP defined ET and PV patients [33] (Table 4). The CLMP defined ET had normal RCM and erythrocyte counts below 5.8x10^{12}/L with hematocrit values ranging from 0.40 to 0.45 (Figure 1, Tables 2-4). At erythrocytes above 5.8x10^{12}/L, diagnostic for PV, (Table 4), the hematocrit values ranged from 0.46 to 0.72 and Hb values ranged from 15.0 to 20.9 g/dL, which are clearly below the 2008/2016 WHO-defined criteria for PV [39,41,45]. Seven ET patients had normal RCM at erythrocyte counts between 4.4 to 5.3 x10^{12}/L of whom 4 had normocellular (< 60%) ET and 3 had hypercellular (60%-80%) prodromal PV bone marrow histology. Erythrocyte counts remain above 5.8x10^{12}/L in phlebotomy induced PV in hematological remission due to microcytic erythropoiesis (MCV below 70 fl) caused by iron deficiency [2,10] (Figure 4). In newly diagnosed PV patients, the state of PV in remission by phlebotomy due to iron deficiency induced microcytosis of erythrocytes is reached after about 1 to 2 years of repeated venesections (Figures 3,4). This is associated with the relief of hypervolemic symptoms in PV patients during long-term or even life-long follow-up [2,4,31,32].

In the late 1970s, the London PV study Group of Pearson, Messinezy, Thomas and Weitherley-Mein demonstrated that on top of the microvascular disease of thrombocythemia, the incidence of major arterial and venous episodes in PV correlated positively with increased haematocrit level [46-49]. A mean haematocrit of 0.60 and a mean platelet count of 512x10^{9}/L at time of diagnosis of PV were associated with microvascular ischemic events and major thrombosis in 49%. The risk of major vascular episodes were the lowest at hematocrits below 0.44, higher at hematocrits above 0.45 and the highest at hematocrits above 0.50 as was the case in the PVSG 01 study when not on low dose aspirin for the prevention of platelet-mediated microvascular ischemic disturbances, TIAs and acute coronary syndromes [36,50,51]. Low dose aspirin at hematocrits of around 0.40 in JAK2^{V617F} mutated ET and PV significantly reduces the incidences of both microvascular as well as major vascular events as compared to not using aspirin in randomized clinical trials [30,36,52]. Phlebotomy on top of low dose aspirin (40 to 80 mg OD) is the cornerstone of treatment of newly diagnosed PV patients with low, intermediate and high MPN disease burden [34-36,53].

### JAK2^{V617F} mutated trilinear MPN

EPO-independent progenitor colony-forming unit-erythroid (CFU-E) and burst forming unit-erythroid (BFU-E) labelled as spontaneous endogenous erythroid colony formation (EEC) became the hallmark of PV [54]. Analysis of about 500 PV patients from 26 studies indicated that EEC in expert hematological laboratories has a near 100% diagnostic specificity for overt and masked PV and ET mimicking PV or latent PV [54,55].

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**Table 7:** Clinical Laboratory, Molecular and Pathobiology (2015-2020 CLMP) criteria for hypercellular thrombocythemia associated with primary megakaryocytic, granulocytic myeloproliferation (PMGM) caused by calreticulin (CALR) mutations.

| CLM criteria | CALR thrombocythemia | Bone marrow Pathology (P) criteria |
|--------------|-----------------------|----------------------------------|
| A1 | No preceding or allied other subtype of myeloproliferative neoplasms PV, CML, MDS. The main presenting features is pronounced isolated thrombocythemia with platelet count around or above 1000x10^9/L. | Megakaryocytic (M) myeloproliferation of dense clustered atypical large immature megakaryocytes with hypolobulated nuclei in a normocellular bone marrow. |
| A2 | CALR mutation and JAK2 wild type. | Prethrombotic dual megakaryocytic granulocytic (MG) myeloproliferation and relative or absolute reduction of erythropoiesis and erythroid precursors. Abnormal dense clustering and increase in atypical medium sized, large to giant immature megakaryocytes containing bulbous (cloud-like) hypolobulated nuclei and definitive maturation defects. |
| C | Clinical stages of CALR Thrombocythemia. | No features of PV in blood and bone marrow. |
| C1 | Early clinical stage: Hb > 12g/dL, slight to moderate splenomegaly, thrombocytoysis around or above 1000x10^9/L, normal LAP score. | Grading reticulin fibrosis (RF), Wilkins, et al. [113], and myelofibrosis (MF) Georgii, et al. [11,12], Theile, et al. [109]. |
| C2 | Intermediate clinical stage: slight anemia Hb < 12 to 10 g/dL, decreasing platelet count, splenomegaly, increased LDH and definitive tear drop erythrocytes. | MF 0 | Prefibrotic CALR MG, no reticulin fibrosis RF 0/1. |
| C3 | Advanced stage: anemia Hb < 10 g/dL, tear drop erythrocytes, increased LDH, increased CD34+ cells, pronounced splenomegaly, normal or decreased platelet counts, leucocytosis or leukopenia. | MF 1 | Early fibrotic CALR MG slight reticulin fibrosis RF 2. |
| C4 | MF 2 | Fibrotic CALR MG increase RF grade 3 and slight to moderate collagen fibrosis. |
| C5 | MF 3 | Advanced fibrotic CALR MG with collagen fibrosis-osteosclerosis. |

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**Figure 4:** Megakaryocytic (M) proliferation in a normocellular bone marrow (60%) with myeloproliferation (PMGM) caused by calreticulin (CALR) mutations. The Megakaryocytic (M) proliferation of dense clustered atypical large immature megakaryocytes with hypolobulated nuclei in a normocellular bone marrow. The Pathology Laboratory Lam Rotterdam and De Raeve, Brussels.
The 9p loss of heterogeneity (9pLOH) due to mitotic recombination of chromosome 9p is the most frequent chromosomal lesion described in PV (∼33%) not detectable by cytogenetic analysis [56]. Kralovics, et al. sequenced 19 candidate genes mutations within the 9pLOH region and no mutations were found in the Janus kinase (JAK) gene, but Kralovics did not screen the JH2 pseudokinase gene thereby overlooking the JAK2V617F in the JH2 pseudokinase gene (Figure 1). Constantinescu & Vainchenker searched for a mutation in the 9pLOH region of the complete JAK2 gene and did found the JAK2V617F in the JAK2 pseudogene of 3 PV and 2 controls by detection of a G-to-T mutation at nucleotide 1849 in exon 12 leading a substitution of valine to phenylalanine at position 617 (V617F) in the JAK2 pseudo-gene. This V617F substitution of the JAK2V617F mutation was present in 40 of 45 PV patients, in 9 of 21 ET patients and in 3 of 7 MF patients [57], (Figure 1). The JAK2V617F substitution was absent in patients with secondary erythrocytosis (N = 35) and 15 controls [57]. Thirty percent of PV patients are homozygous for JAK2V617F mutation without so-called 9pLOH due to mitotic recombination, whereas heterozygous JAK2V617F mutated ET and PV patients showed the presence of 9pLOH [57,58] (Table 1, Figure 1).

Figure 5: Typical trilinear bone marrow histology [2,18] in classical polycythemia vera (PV) with increased cellularity (100%) due to increased erythrocytic, megakaryocytic (EM) proliferation or trilinear erythrocytic, megakaryocytic and granulocytic (EMG) proliferation. Increase of clustered medium to large (pleomorph) megakaryocytes with hyperlobulated nuclei (Pathology Laboratory Rotterdam Lam and Brussels, Dr De Raev).

Figure 6: State of the art treatment according to Dameshek [2,4] of a newly diagnosed PV patient (Ht 0.63, Hb 12.9 mmol/L, erythrocytes 7.1 x1012/L and MCV 89 fL) with repeated venesections as confirmed by Pearson & Wetherley-Mein [48] and Messinazy, et al. [49] of the London PV Study Group [46,47]. Repeated venesections for more than 1 year (from August 2012 to September 2013) was needed to induce a complete hematological remission (CHR) reaching the desired plateau of Ht 0.45, Hb 9.0 mmol/L and MCV of 66 fL. While on low dose aspirin neither microvascular nor major thrombosis did occur. Once the iron deficiency state is reached the erythrocytes remain microcytic and reach values of 7.0 to 7.2x10¹²/L without further need of phlebotomy due to the persistence of the iron deficient state [2,30]. Correction of the Ht from 0.63 to below 0.45 is associated with reduction of major venous and arterial thrombotic events [89,94,51], but the microvascular thrombotic syndrome of associated thrombocythemia persisted. Low dose aspirin in ET in the Dutch Collaborative Low dose Aspirin In Thrombocytemia (Dutch CLAT) Van Genderen studies [89-91,99] and low dose aspirin on top of phlebotomy or hydroxurea in the European Collaborative Low dose Aspirin In PV (ECLAP, [50]) reduced the incidences of microvascular disturbances and major thrombosis from above 50% per 100 pt/yr to less than 3% per 100 pt/yr in both ET and PV [91,94], but does not prevent the progression of JAK2 mutated MPN disease in terms of JAK2 mutation load. MPN disease burden like progressive leukocytosis, thrombocythemia, splenomegaly and constitutional symptoms [6,7,34].
Constantinescu & Vainchenker, [59] demonstrated that the acquisition of heterozygous, hetero-homzygous and homozygous due to mitotic recombination JAK2V617F mutation on chromosome 9p are the driver causes of sequential megakaryocytic (M), erythrocytic megakaryocytic (EM) and erythro-megakaryo-granulocytic (EMG) myeloproliferations seen in normocellular ET, promodal PV and classical and advanced PV in trilinear MPN (Figures 5, 6). [21,57,60], (Table 1, Figure 1). The JAK2V617F mutation as the driver cause of trilinear MPN was immediately confirmed in three large groups of ET, PV and MF patients due to inside information during the peer review process in 2004 [58,59,61]. JAK2V617F mutation induces a loss of inhibitory activity of the JAK2 pseudokinase part on the JAK2 JH1 kinase activity, which makes the TPO, EPO and granulocyte growth factor receptors on the hematopoietic progenitor cells hypersensitive to their growth factors TPO, EPO and granulocyte growth factor (Figures 5, 6). The JAK2V617F mutated hematopoietic cells produce Constitutively activated platelets and leukocytes (increased leukocyte alkaline phosphatase: LAP) and quantitative increase of platelets erythrocytes and granulocytes (Table 1). The sequential occurrence of low heterozygous, combined heterozygous and homozygous JAK2V617F allele load can readily explain the sequential occurrence of ET, promodal PV, classical PV and advanced PV followed by secondary MF in trilinear MPN during lifelong follow-up. JAK2V617F trilinear MPN is clearly different from JAK2 wild type hypercellular ET associated with PMGM [11,12,14,16] as the third distinct entity of MPD without features of PV (Figure 2, Tables 12-15 in Michiels, et al. [8]). According to 2006 ECMP criteria the sequential transitional states of JAK2V617F disease entity ranged in WHO-defined advanced PV and post-PV myelofibrosis(MF) patients ranged from 50% to 100% [63,67,69-71]. According to the Vainchenker’s “dosage” concept, heterozygosity for the JAK2V617F mutation in acquired ET and autosomal dominant JAK2 or TPO mutated hereditary ET (HET) is enough to activate megakaryocytes to induce the ET clinical phenotype [8,21,27,28,36,60,72] (Figure 5, Table 1). Patients with dominant hereditary ET (HET) heterozygous for the JAK2V617F and JAK2V617F germ line mutations have a clinical ET phenotype with normal values for Hb, Ht, erythrocytes, thrombopoietin (TPO), and erythropoietin (EPO) levels. The response to EPO in the EEC assay was normal in congenital JAK2V617F and JAK2V617F [73-75], but increased in acquired JAK2V617F mutated ET and PV (Figure 5, Table 1) [8]. According to the JAK2V617F dosage hypothesis the JAK2V617F mutation load is low in heterozygous mutated ET and increases from below to above 50% inpatients with homozygous JAK2V617F mutated PV, advanced PV and post-PV myelofibrosis [8,58,64,76] (Figure 6). According to Vainchenker’s “dosage” concept the higher intracellular levels of JAK2V617F kinase activity in homozygous mutated progenitor stem cells preferentially activate the erythropoietin receptor (EPOR) and generate a PV-like phenotype with erythrocytes above 5.8x10^12/L and increased activated platelet and leukocyte counts (Figures 6, Table 1) [8,9,24,35,36,60,70,72].

The JAK2V617F allele burden was directly correlated with increased levels of hematocrit, neutrophil count, LDH and neutrocyte alkaline phosphatase (LAP) score, spleen size on echogram [8] (Table 1) and with decreased values for platelets, serum ferritin, and erythropoietin, with higher relative risks for aquagenic pruritus, spleen size on echogram, total thrombosis and the need for myelosuppressive treatment [69]. The JAK2V617F allele burden in granulocytes in a prospective study of 175 PV patients could be quantified as 1%-25%, 25% to 50%, 50%-75% and 75%-100% in 57, 50, 34...
and 32 PV patients respectively [69]. Prefibrotic heterozygous JAK2V617F mutated ET usually runs a benign course with low JAK2 and MPN burden and a normal to near normal life expectancy (Table 1) [8,9,21] Figure 7, [76]. Prefibrotic heterozygous JAK2V617F mutated prodromal and classical PV usually have a low mutation burden associated with microvascular and major thrombosis at time of presentation (Table 1). Homozygous JAK2V617F mutated trilinear PV result in high JAK2V617F and hypercellular MPN burden associated with progressive extramedullary myeloid neoplasia of the spleen (MNS), splenomegaly and cytokine mediated MF during long-term follow-up [70,76] (Table 1, Figure 6). Transition of heterozygous into homozygous JAK2V617F mutation due to mitotic recombination of chromosome 9p (9pLOH) is strongly correlated with progression into advanced PV and masked PV with splenomegaly and associated with high JAK2 allele burden [6,7,24,34,63,70,76].

The UK MPN Study Group [71,78] elegantly confirmed the Vainchenker’s “dosage” concept at the biological EEC level by studying the genotype of individual BFU-E in a crosssectional cohort of 29 JAK2V617F mutated ET and 30 JAK2V617F mutated PV patients (Figure 8). The JAK2 mutation load was expressed as a percentage (%) of EEC colonies genotyped as homozygous (red), heterozygous (purple) or wild type [78] (Figure 8). All 29 JAK2V617F positive ET patients have heterozygous JAK2 mutated EEC colonies: 9 of them have a low percentage (< 10%) of homozygous JAK2 mutated colonies. Out of 30 JAK2V617F positive PV patients, 8 have heterozygous JAK2 mutated EEC, 13 have homozygous EEC colonies of more than 50% and 7 of less than 50%. Homozygous EEC colonies were absent or rare in heterozygous ET, but prevalent in JAK2V617F-positive PV [78] (Figure 6). These observations are completely in line with Vainchenker’s “dosage” concept (Figures 5, 6, Table 1) [8,9,21,60,72]. Additional cytogenetic [79], genetic or epigenetic alterations in PV and MF patients are of huge importance to the understanding of differences in biology, prognosis and outcome of MPN patients [81-84]. Using next generation sequencing (NGS) on top of the JAK2, MPL and CALR driver mutations of MPN is associated with impaired prognosis in MPN, MDS and other myeloid malignancies as well. The targeted search for epigenetic factors will become hugely important to the understanding of differences in biology, prognosis and outcome of MPN patients [81-84]. Using next generation sequencing (NGS) on top of the JAK2 or CALR mutation, the Swiss MPN investigators in Basel found one, two or more epigenetic somatic mutations in 65 (33%) of 197 WHO defined MPN patients (94 PV, 69 ET, 34 MF) [82]. Seventeen of 69 (25%) ET patients, 11 of 34 (32%) MF and none (0%) of 94 PV patients carried mutations in CALR. In addition to JAK2V617F and CALR, the most frequently observed epigenetic somatic mutations affecting the biology and natural history of MPN disease included TET2, ASXL1, DNMT3A, EZH2, and IDH1 [82-84]. Rare epigenetic mutations were NF1, NEF2, and RUNX1. The presence of one, two or more somatic mutations appeared to impair prognosis in JAK2 and CALR mutated MPN [82]. Tefferi, et al. [83,84], confirmed the Lundberg observations in large scale retrospective studies in WHO defined ET, PV and MF patients demonstrating that epigenetic somatic mutation detection on top of the JAK2, CALR and MPL mutational load and subtype MPN characterization is far superior to classify the distinct MPN diseases as compared to the crude WHO classification, that cannot clearly distinguish between ET, prodromal overt and masked PV and PV with MF. Dr. Green, addressed the key question whether the sequence of acquisition of somatic mutations can be inferred from the genotypes of detectable subclones [85]. For instance, if some tumor cells have JAK2V617F, and others from the same patient bear JAK2V617F with an additional somatic mutation, then this indicates that JAK2V617F came first. Genotyping individual hematopoietic colonies has shown that the order of acquisition of JAK2V617F, relative to mutations in TET2 or DNMT3A, influences subclonal composition within HSPCs and mature cell compartments, disease presentation, and clinical outcome. In JAK2-first patients, the HSC compartment is dominated by double-mutant cells, and such patients present at a younger age, often with PV. Conversely, in TET2-first patients, the HSC compartment is dominated by single mutant cells, and such patients present at an older age, usually with ET. JAK2-first patients had a greater likelihood of presenting with PV than with ET, had an increased risk of thrombosis, and an increased sensitivity of JAK2 mutant progenitors to ruxolitinib in vitro.

**Erythromelalgic microvascular circulation disturbances or platelet thrombophilia in PV and ET: From Dameshek to Michiels & Van Vliet**

Dameshek & Henthel, [1] described the presenting clinical manifestations in 20 newly diagnosed PV patients including quite severe headaches in 17, attacks of migraine in 14, visual disturbances, particularly spots before the eyes and coloured scotomas in 6, paresthesias numbing and tingling.
Figure 8: Proportions of JAK2 genotypes in BFU-Es from 59 patients with JAK2V617F-mutated essential thrombocythemia (ET) and polycythemia vera (PV) [78]. Each vertical bar represents 1 patient, divided according to the proportion of wild-type, heterozygous, and homozygous-mutant colonies obtained, with the absolute colony numbers shown above: (wild type white), heterozygous (purple) homozygous (red). Results of EEC colony genotypes are presented for 29 JAK2V617F-positive ET (B) patients (total 2277 colonies; mean 79 per patient) and for 30 JAK2V617F-positive PV (A) patients (total 2287 colonies; mean 76 colonies per patient). All 29 JAK2V617F positive ET patients have heterozygous JAK2 mutated EEC colonies and less than 10% homozygous colonies in 9 and 20% in 1 of them. Out of 30 JAK2V617F positive PV patients 8 have heterozygous JAK2 mutated EEC, 13 have homozygous EEC colonies of more than 50% and 7 of less than 50%.

A. In total 29 PV patients: 5 were heterozygous, 13 heterozygous/homozygous and 11 predominant homozygous (high allele burden) for the JAK2V617F mutation.

B. In total 30 ET patients: all are predominant heterozygous (low allele burden) for the JAK2V617F mutation but half of them do have a minor clone of homozygous mutated BFU-Es.

C and D. EEC colony genotypes for 18 patients with JAK2 exon 12 mutated PV (total 1931 colonies; mean 107 per patient). D show example sequence traces for patients with patients with homozygous JAK2 exon12 mutations in colonies. In total, 16 patients (5 “heterozygous-only” JAK2V617F-positive PV patients, 4 JAK2V617F positive PV patients with homozygous and heterozygous clones, 3 JAK2V617F positive ET patients with small homozygous clones, and 4 JAK2V617F mutated PV patients with homozygous clones) were assessed in this way (mean time between experiments, 13 months; range, 2-32 months) and showed reproducibility of proportions of heterozygous and homozygous-mutant colonies.

Interpretation. The JAK2V617F dosage concept of Constantinescu & Vainchenker is in line with the EEC bone marrow findings in the UK study in Figures A and B [78]. A low level of JAK2V617F kinase activity only activate the MPL (TPO) receptor and favors the ET phenotype in acquired heterozygous (Figure 2, Table 1), [8,27,60,76] and in dominant heterozygous JAK2 or TPO mutated ET (Figure 2, HET), [28]. A high level of JAK2V617F activity in heterozygous/homozygous or homozygous mutated trilinear MPN is needed to activate the erythropoietin receptor (EPOR) and generate a PV-like phenotype (Figure 3), [8,21,31,32,57,59]. Similarly, high levels of JAK2V617F activity of long duration in homozygous mutated trilinear MPN is needed to activate the granulocyte clony-stimulating factor receptor (C-GCSF, Figure 3) leading to EMF or MG bone marrow phenotype and progressive secondary myelofibrosis (MF) [31,32,69,70]. Other mechanisms do occur in the pathobiologies of myeloid metaplasia and myelofibrosis in advanced stage of trilinear MPNs.
in toes and fingers in 12 and various types of transient major thrombosis (cerebral, coronary, venous) in 9 cases. Dameshek & Henthel, et al. [1] noted that the lack of large vessel involvement in PV and the associated high platelet counts suggested the possibility of ‘platelet thrombophilia’ as the cause of multiple small peripheral vascular thromboses in the peripheral cerebral and coronary circulation similar to aspirin-responsive platelet-dependent thrombophilia in JAK2V617F mutated thrombocytosis in ET and PV patients first discovered and described by Michiels Ten Kate & Van Vliet in [86] 1984 1985 and subsequently confirmed between 1985 and 2018 [20-22,35,36,86].

The broad spectrum of acroparesthesia, erythromelalgic and acrocyanotic ischemia or gangrene together with the episodic and transient neurologic symptoms of migraine accompaniments, attacks of amnesia, dysasia, dysphasia, TIA, hemipareisis and acute coronary ischemic syndromes all are the consequence of one underlying disorder: arterial thrombophilia caused by JAK2V617F mutated platelet-mediated and MPL515 platelet-mediated arteriolar inflammation and thrombosis in acquired thrombocytopenias [20-22,27,28,35,36,86]. Platelet-mediated erythromelalgic microvascular disturbances also occur in dominant hereditary ET (HET) caused by heterozygous germ line gain of function mutation in the TPO, JAK2 and MPL genes [34,73-75] (Figure 5). Erythromelalgia is rare CALR mutated thrombocytemia and has never been observed in reactive thrombocytosis [35,36], Platelet-mediated inflammatory and thrombotic processes in the end-arterial microcirculation typically respond to aspirin, but not to platelet ADP inhibitors and anticoagulation with vitamin K antagonists [20-22,35,36].

JAK2V617F mutated platelets are constitutively activated, hypersensitive (sticky) and cause aspirin responsive platelet-mediated microvascular circulation disturbances, (Table 1). [20,86-94] has recently been discovered by Michiels as the novel Aspirin-responsive Sticky Platelet Syndrome in JAK2V617F, MPL and TPO mutated thrombocythemias [35,36].

**JAK2v617f mutations as cause of Isolated Erythrocythemia and PV**

The finding of the JAK2v617f mutations in the 5% PV patients negative for JAK2V617F, usually present with early stage PV or isolated erythrocythemia (IE, Figure 8) with increased red cell mass but normal leukocytes and platelets and no palpable spleen [95-98]. The frequency of JAK2v617f mutations among all PV patients is estimated around 3% [95,98]. JAK2 N542-E543del is the most frequent among the different reported exon 12 mutations. JAK2v617f mutated MPN patients with increased erythrocytes above 6×10^{12}/L and a typical PV bone marrow histology are diagnosed as benign IE or PV with a favourable outcome and normal life expectancy [95,96,98,99].

Pre-treatment bone marrow histology in JAK2v617f mutated PV or IE showed characteristic erythroid hyperplasia with minor and distinct histology changes of the megakaryocytic lineage, which are not seen in primary or secondary erythrocytoses (PE and SE) [95]. Cases of JAK2v617f mutated IE or PV have erythrocytes above 6×10^{12}/L [100], normal platelet and leucocyte counts, no or palpable spleen and a typical hypercellular bone histopathology predominantly due to erythroid hyperplasia and clusters of large megakaryocytes with hyperploid nuclei [95,98] (Figure 8). Bone marrow histology in 7 cases (4 IE, 3 PV) of JAK2v617f mutated MPN in the pathology study of Lakey, et al. [97], showed prominent hyperplasia of erythropoiesis and atypical small to medium-sized large megakaryocytes (Figure 8). A low percentage of homozygosity was found for the JAK2K539L-type and E543del-type exon 12 mutations (Figure 8) [78]. Godfrey, et al. [78], assessed the colony phenotypes for 18 patients with JAK2v617f- mutated PV in a total of 1931 colonies; mean 107 per patient (Figure 8C).

**Example sequence traces for patients with homozygous JAK2v617f mutations in colonies are shown in figure 8D.** In total, 16 patients (5 “homozygous-only” JAK2v617f-positive PV patients, 4 JAK2V617F-positive PV patients with homozygous and heterozygous clones, 3 JAK2V617F-positive ET patients with small homozygous clones, and 4 JAK2v617f mutated PV patients with homozygous clones showed reproducibility of proportions of heterozygous and homozygous-mutant colonies (Figure 8D).

**Acquired MPL515 mutated normocellular ET**

The prevalence of the MPL515 mutated ET range from 3% of MPN to 8.5% of JAK2 wild type ET and MF [101-103]. The clinical presentation in 30 MPL515 mutated ET patients (9 males and 21 females, age 22-84, mean 56 years) was featured major arterial thrombosis in 23%, venous thrombosis in 10%, aspirin responsive microvessel disturbances in 60%, and major hemorrhage in 7% [101]. The clinical, laboratory, molecular and pathological (CLMP) findings in MPL515 mutated ET were increased platelet count, 956+331×10^9/L in all, slight splenomegaly in 5 (17%), and no or palpable spleen and bone marrow in all table 6, [31,32,34]. Pretreatment bone marrow histology at the time of diagnosis in MPL515 mutated ET features large and giant megakaryocytes with hyperlobulated staghorn-like nuclei (Figure 10, Table 6), clearly different from JAK2v617f PV (Figure 9), and distinct from JAK2V617F ET and prodromal PV (Figures 2,3,9) and distinct from CALR thrombocytosis (Figures 11,12).

**Megakaryocyte Leukemia (ML) and CALR mutated Thrombocytopenia: From Dameshek 1951 to Kralovics 2013 and Michiels 2015**

According to Dameshek, [10] megakaryocyte leukemia (ML) is defined by platelet counts around and above 1000x10^9/L without features of PV in blood and bone marrow smear and biopsy. The traditional classification of the myeloproliferative disorders (MPD) by the PVSG and used in textbooks was revised in the Hannover Bone Marrow classification to include PV, primary thrombocytopenia (PTh), and hypercellular thrombocytosis related to primary megakaryocytic

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myeloproliferation (PMGM, Table 7) without features of PV [11,12,14,16,104]. The discovery of the calreticulin (CALR) as the main cause of JAK2/MPL515 wild type thrombocytopenia and PMF by Kralovics and his team [105] was identified by Michiels & De Raeve [31,32] as the driver cause of prefibrotic and fibrotic stages of PMGM without features of PV. This led to the second ground breaking event in the molecular landscape of the MPNs that induced a complete revision of all

MPN classifications of the PVSG, WHO into the current Clinical Laboratory, Genetic and Pathobiological (2018 CLMP) criteria for JAK2V617F trilinear MPN (Tables 3 and 4), and JAK2exon12 PV as compared to two distinct MPL515 (Table 6) and CALR thrombocytopenias and myelofibrosis (Table 7) without features of PV.

Kralovics performed targeted whole-exome sequencing in 6 cases of WHO defined JAK2/MPL wild type PMF patients and found somatic calreticulin (CALR) mutations of 52-bp deletion in 1, of 1bp deletion in 1 and recurrent 5-bp insertion in 4
The CALR somatic mutation was subsequently discovered as the driver cause of thrombocythemia in 78 of 311 (25%) ET patients and in 72 of 203 (35%) MF patients [105]. The CALR mutation was detected in none of 382 PV, 45 CML, 73 MDS, and 64 chronic myelomonocytic leukemia (CMML) patients. Three (12%) of 24 RARS-T cases were positive for both the SF3B1 and CALR mutation. A subsequent Italian-Austrian study of 1235 WHO-defined ET and MF patients detected the JAK2V617F, MPL515 and CALR mutation in 63.3%, 23.5% and 4.4% respectively with 8.8% being negative for all three mutations [76] (Figure 6). Evolution into MF during follow up was as high in CALR mutated ET as in JAK2V617F mutated PV (about 20% after 20 years). CALR mutated MPN patients lacked features of PV (normal erythrocytes and hematocrit), had higher platelet counts and a lower incidence of major thrombosis compared to JAK2V617F positive ET [76,105]. The large UK study confirmed the presence of the somatic CALR driver mutations in 80 of 112 (70%) JAK2/MPL wild type ET patients, and in 18 of 32 (56%) JAK2/MPL wild type MF patients and in none of 120 (70%) JAK2V617F or MPL515 mutated MF patients [106]. CALR mutations were detected in 10 of 120 (8%) MDS patients (RA in 5 of 53, RARS in 3 of 27 and RAEB-T in 2 of 27), and in one patient each with CMLML and atypical CML. CALR mutations were not found in control samples, lymphoid cancers, solid tumors, or cell lines [106]. A third large Italian study found CALR mutations in 15.5% of 576 WHO-defined ET and in 48.9% of JAK2/MPL wild type ET patients [107]. The distribution of the JAK2V617F, CALR and MPL515 mutations or triple negative cases in 254 WHO-defined MF patients was 58%, 25%, 8.3% and 8.7% with median overall survival of 8.2, 4.1, 4.3 and 2.5 years respectively reflecting advanced or end stage MPN disease [39].

The biological and clinical features of WHO-defined ET carrying the JAK2V617F and CALR mutation ET clearly differ [76]. The mutant allele burden was lower in JAK2V617F mutated than in CALR mutated ET (Figure 7). JAK2V617F ET patients were older, had higher hemoglobin and white blood cell counts but lower platelet counts. Serum erythropoietin levels are lower and frequently decreased in JAK2V617F ET but normal in CALR thrombocythemia. The cumulative risk of WHO-defined ET carrying the JAK2V617F mutation to transform into WHO-defined PV was 29% after 15 years but transformation into PV was never observed in CALR thrombocythemia. With the advent of the CALR mutation as the main driver cause of JAK2/MPL wild type ET, hypercellular ET associated with PMGM [8,11,12,14,15] and CALR thrombocythemia and myelofibrosis appeared to be the same distinct MPN entity without features of PV (Table 7) [31-33]. JAK2V617F mutated ET and PV patients had a similar two times higher risk of major thrombosis than that of CALR mutated thrombocythemia patients. CALR-mutated ET patients were more frequently male, had higher platelet counts, lower hemoglobin and leukocyte count and showed a lower risk of major thrombosis than JAK2 mutated ET patients in two large studies [107,108].

Bone marrow histology findings in 59 WHO-defined JAK2V617F positive ET and 44 JAK2 wild ET cases in the study of Pich, et al. [66], (Figure 11) revealed PV-like hypercellular morphological bone marrow changes of pleomorphic enlarged megakaryocytes in JAK2V617F mutated ET similar as described previously (Figure 9) [8,15]. Various stages erythropoiesis and or myelopoiesis with megakaryocyte proliferation as well as LDH and spleen size are more pronounced in PV-like phenotype in JAK2V617F mutated ET in particular at higher JAK2 mutation load (Figure 9), [66]. WHO defined JAK2V617F positive ET showing increased cellularity due to increased erythropoiesis is consistent with prodomal PV [62]. The prognosis of JAK2V617F mutated ET and prodomal PV is favorable and to be treated with low aspirin and additional phlebotomy in early PV to maintain ht below 0.45 in men and below 0.42 in women. This concept based on prospective clinical observations are completely in line with the present study of patients with JAK2V617F mutated ET, prodomal PV and PV.

The European Asiatic collaboration between Michiels & De Raeve from the Vannucchi’s study on WHO defined MPL515 mutated ET revealed that clustered large to giant mature megakaryocyte with staghorn nuclei and platelet count increase in a normocellular bone marrow are characteristic for JAK2 wild type ET carrying the MPL515 mutation [31-33]. JAK2/CALR wild type ET carrying the MPL515 mutation indeed displayed clustered large and giant mature megakaryocytes with a greater number of large deeply lobulated ‘staghorn’ nuclei in a normocellular bone marrow as the hallmark of MPL515 thrombocythemia (Figure 10) [6,7,31,32].

Between 2015 and 2018 Michiels & De Raeve found typical PMGM pictures in 15 CLMP defined consecutive newly diagnosed CALR mutated ET (Figures 11,12) and MF patients [6,7,31-33]. CALR thrombocythemia patients appeared to be phenotypically identical to JAK2 wild type PMGM defined by the Hannover Bone Marrow Classification and in retrospect surely belong to the original description by Dameshek of megakaryocyte leukemia (ML) without features of PV [10]. CALR mutated thrombocythemia and MF are clearly distinct from MPL515 normocellular thrombocythemia (Figure 10). JAK2V617F ET, prodomal PV and PV cases with regard to clinical, hematological and bone marrow features at presentation and during follow-up (Figure 9).

The European Asiatic collaboration between Michiels & De Raeve (Rotterdam-Brussels) and Yongoo and Myungshin Kim (Seoul, Korea) translated the laboratory, molecular and pathological characteristics in a large cross sectional study of 407 WHO defined MPN patients into the 2015-2020 CLMP classification (Tables 2-7). The Large cohort of 407 MPN

Novel European Asiatic Clinical, Laboratory, Molecular and Pathobiological (2015-2020 CLMP) criteria for JAK2V617F trilinear polycythemia vera (PV), JAK2V617F PV and JAK2V617F, CALR and MPL515 thrombocythemia: From Dameshek to Constantinescu-Vainchenker, Kralovics and Michiels
patients included PV in 111 (29%), ET in 179 (44%) and PMF in 117 (29%). The three driver mutations were detected in 82.6% of 407 MPN patients with a mutation distribution of JAK2 in 275 (67.5%), CALR in 55 (13.7%), MPL in 6 (1.5%) [100]. In this report we analyzed the CLMP characteristics of 337 Korean evaluable WHO defined MPN patients subdivided into JAK2V617F in 268 (80%), JAK2exon12 in 7 (2.1%, CALC in 56 (17%) and MPL in 6 (1.8%) [6,100]. The values of hemoglobin (Hb), hematocrit (Ht) and erythrocytes in JAK2V617F mutated trilinear MPN ranged from anemic to polycythemic values with mean values of Hb 14.7 g/dL, Ht 0.44 and erythrocytes 5.0x10^{12}/L (Table 8). The bone marrow (BM) lineage proliferation class in MPN including 101 PV, 95 ET and 78 PMF WHO defined patients MPN consisted of M (WHO-ET) in 80; EM and EMG in 116 consistent with prodromal and classical PV; and GM myelofibrosis in 72. The mean JAK2V617F mutation load was high 69 to 80% in EM, EMG and 69% in MG bone marrow class, but low (37%) in M class ET patients (Table 8).

JAK2exon12 patients in the study of Kim, et al. [100], are featured by idiopathic erythrocythemia (IE) not meeting WHO-defined PV with normal platelet and leukocyte counts, no or palpable spleen and a hypercellular bone marrow predominantly due to erythroid hyperplasia (EM, Table 8) [71,95-98]. JAK2exon12 mutated MPN in the study of Kim et al presented with erythrocyte counts above 5.8x10^{12}/L, normal platelet counts of less than 350x10^{9}/L and no anemia consistent with the diagnosis of erythrocythemic PV (Table 8). Increased erythropoiesis in bone marrow was absent in all cases of CALR and MPL mutated MPN (Table 8). Bone marrow histology in 56 cases of CALR mutated MPN typically featured predominant increased monolinar megakaryopoiesis M in two thirds and increased granulopoiesis and megakaryopoiesis (GM) in one third (Table 8).

The grade of bone marrow (BM) fibrosis in the study of

Table 8: Change of 2008/16 WHO into European Asiatic 2015-2020 CLMP characteristics in 337 patients with Myeloproliferative Neoplasms (MPN) caused by the somatic driver mutations in the JAK2V617F, JAK2exon12, and CALR [100].

| 337 MPN patients | JAK2V617F | JAK2exon12 | CALR |
|------------------|-----------|------------|------|
| Patients N       | 268       | 7          | 56   |
| % of 337         | 80%       | 2.1%       | 16.6%|
| Age yrs          | 66        | 66         | 56   |
| Range            | 22-89     | 46-76      | 20-89|
| Males (%)        | 45.5%     | 28.6%      | 41.1%|

| Hemoglobin g/dL  | 14.7      | 18.3       | 12.6  |
|------------------|-----------|------------|------|
| Range            | 6.2-22.6  | 13.7-21.1  | 7.5-16.1|
| Hematocrit (%)   | 43.9      | 49.9       | 38.4  |
| Range            | 19.7-69.1 | 46.2-59.3  | 22.9-47.0|

| Red Blood cells  | 5.010^{12}/L | 6.910^{12}/L | 4.2 10^{12}/L |
|------------------|---------------|--------------|---------------|
| Range            | 1.89-9.72     | 5.83-8.50    | 2.25-5.32     |

| Platelets 10^9/L | 650           | 281          | 898           |
|------------------|---------------|--------------|---------------|
| Range            | 13-3268       | 58-310       | 49-1979       |
| Leukocytes 10^9/L| 12.0          | 8.2          | 8.6           |
| Range            | 2.2-177       | 6.2-22       | 4.8-31        |

| 2008/16 WHO Class | JAK2V617F |
|-------------------|-----------|
| PV N              | 101       |
| ET N              | 95        |
| PMF N             | 78        |

| BM CLMP Class     |          |
|--------------------|----------|
| EM N               | 32 PV    |
| EGM N              | 84 PV    |
| MET N              | 80       |
| GM MF              | 72       |

| Mutation burden   |          |
|--------------------|----------|
| Total-Range        | 67% (1.8-99) |
| EM + EGM           | 85% (13-99) |
| M (ET)             | 37%ET     |
| GM (MF)            | 69%ET PV  |

Abbreviations: PV: Polycythemia Vera; ET: Essential Thrombocythemia; PMF: Primary Myelofibrosis; E: Erythroid; M: Megakaryocytic, G: Granulocytic myeloproliferation of increased bone marrow proliferation lineage. N: Number; Red = JAK2V617F mutated PV, ET or MF. JAK2 wild type either CALR (blue) or MPL (black) mutated. Type 1 revealed higher mutant allele burden (53%) compared to CALR type 2 (38%) MPN. JAK2exon12 mutated ‘forme fruste’ (prodromal PV) and early PV, and exon 12 PV patients presented with E(M) bone marrow proliferation without fibrosis.
Kim, et al. was divided into minimal fibrosis MF 0/1 and overt fibrosis MF 2/3 [7,11,12,21,109]. The frequency of overt fibrosis in JAK2V617F- and CALR-mutated and triple-negative MPN patients was 22.2%, 27.1% and 29.3%, respectively. JAK2-GM and CALR-GM showed a high rate of overt fibrosis (46.0 and 42.1%), followed by JAK2-M (17.5%), CALR-M (17.2%) and JAK2-EMG (10.4%; p < 0.001). None of the JAK2-EM (‘forme fruste’, early and overt PV and exon 12 PV) patients presented overt fibrosis.

The overall bone marrow histology findings of erythroid, granulocytic and/or megakaryocytic hyperplasia in JAK2V617F mutated MPN, and of granulocytic and/or megakaryocytic hyperplasia in CALR mutated MPN patients in the Seoul study are completely in line with the 2015-2020 CLMP classification of six distinct MPN disease entities and transitional MPN states. Comparing the survival curves of 2008/2016 WHO defined PV, ET and PMF versus the 2015-2020 CLMP defined JAK2V617F, JAK2 exon12, CALR and MPL515 defined MPN without fibrosis versus with fibrosis strongly suggest that bone marrow fibrosis (BMF) grade MF 0/1 versus grade 2/3 appeared to be main adverse prognostic factor when associated with JAK2V617F and triple negative MPN disease (Figures 13,14), [100].

Conclusion

The present insight review is a strenuous joint effort by a multicentre MPN European Asiatic collaborative study group to demonstrate that scrutinized and integral clinical, laboratory, genetic and pathological (2015-2020 CLMP) approaches and intense communications amongst clinicians, scientist, molecular biologists, and pathologists are warranted to more precisely diagnose and treat each MPN patient before avoidable major complications had occurred. The change of 2008/2016 WHO into the 2015-2020 CLMP criteria in table 8 incorporating the established 1975 PVSG and 2001/2008/2016 WHO classifications. The novel 2015-2020 CLMP criteria for at least five distinct clonal MPNs are in urgent need of validation in well designed large clinical prospective unmet need (PUN) studies within the context of the International Collaborations and Academic Research on MPN (ICAR.MPN 2015 founded and chaired by Dr. Michiels Europe and Dr. Shuvaev, Russia) to even better define improved standards for diagnosis, classification, natural history and novel treatment options of JAK2V617F, JAK2 exon12, CALR and MPL515 mutated myeloproliferative neoplasms [7,35,36,110,111-122].

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References

1. Dameshek W, Henstall HH. The diagnosis of polycythemia. Ann Intern Med. 1940; 13: 1360-1387.

2. Dameshek W. Physiopathology and course of polycythemia vera as related to therapy. JAMA. 1950; 142: 790-797. Q PubMed: https://www.ncbi.nlm.nih.gov/pubmed/15405984

3. Videbak A. Polycythemia vera. Course and prognosis. Acta Med Scand. CXCVIII. 179-197.

4. Dameshek W. The treatment of Polycythemia. Blood. 1946; 1: 256.

5. Michiels JJ. Physiopathology, etiologic factors, diagnosis and course of polycythemia vera as related to therapy according to William Dameshek 1940-1950. Turk J Hematol. 2013; 30: 102-110. PubMed: https://www.ncbi.nlm.nih.gov/pubmed/24385771

6. Michiels JJ, De Rave H, Valster F, Potters V, Kim Y, et al. Extension of 2016 World Health Organization (WHO) classification and a new set of clinical, laboratory, molecular and pathological criteria for the diagnosis of myeloproliferative neoplasms: from Dameshek to Vainchenker, Green and Kralovics. EMJ. 2017a; 2: 72-81.

7. Michiels JJ, Berneman Z, Gadiisseur A, Raeye HD, Schroyens W, et al. Myelofibrosis is a Secondary Event in JAK2 Trilinear Myeloproliferative Neoplasm (MPN) and in CALR and MPL Thrombocythemia: Implications for Novel Treatment Options of Prefibrotic MPN. J Hematol Thrombembolic Dis. 2017b; 5: 5.

8. Michiels JJ, Hendrik De Raeye, Berneman Z, Van Bockstaele D, Hebeda K, et al. The 2001 world health organization and updated European clinical and pathological criteria for the diagnosis classification and staging of the Philadelphia-negative chronic myeloproliferative disorders. Sem Thromb Hemost. 2006a; 32: 307-340. PubMed: https://www.ncbi.nlm.nih.gov/pubmed/16673274

9. Michiels JJ, Berneman Z, Van Bockstaele D, Van Der Planken M, De Raeye H, et al. Clinical and laboratory features, pathobiology of platelet-mediated thrombosis and bleeding complications and the molecular etiology of essential thrombocythemia and polycythemia vera: therapeutic implications. Sem Thromb Hemost. 2006b; 32: 174-207. PubMed: https://www.ncbi.nlm.nih.gov/pubmed/14820991

10. Dameshek W. Some speculations on the myeloproliferative syndromes. Blood. 1951; 6: 372-375. PubMed: https://www.ncbi.nlm.nih.gov/pubmed/14820991

11. Georgii A, Vykoupil KF, Buhr T, Choritz H, Döhler U, et al. Chronic myeloproliferative disorders in bone marrow biopsies. Path Res Pract. 1990; 186: 3-27.

12. Georgii A, Buhr T, Buesche G, Kreft A, Choritz H. Classification and staging of Ph-negative myeloproliferative disorders by histopathology from bone marrow biopsies. Leuk Lymphoma. 1996; 22: 15-29. PubMed: https://www.ncbi.nlm.nih.gov/pubmed/8951769

13. Michiels JJ, Prins MEF, Hagemeijer A, Brederoo P, van der Meulen J, et al. Philadelphia chromosome positive essential thrombocythemia and megakaryoblast leukemia. Am J Clin Pathol. 1987; 88: 645-752. PubMed: https://www.ncbi.nlm.nih.gov/pubmed/3479003

14. Michiels JJ. Diagnostic criteria of the myeloproliferative disorders (MPD): essential thrombocythemia (ET), polycythemia vera (PV) and chronic megakaryocytic granulocytic metaplasia (CMGM). Neth J Med. 1997; 51: 57-64. PubMed: https://www.ncbi.nlm.nih.gov/pubmed/9286142

15. Michiels JJ, Thiele J. Clinical and pathological criteria for the diagnosis of essential thrombocythemia, polycythemia vera and idiopathic myelofibrosis (agnogenic myeloid metaplasia). Int J Hematol. 2002; 76: 133-145. PubMed: https://www.ncbi.nlm.nih.gov/pubmed/12215011

16. Michiels JJ, Kutti J, Stark P, Bazzan M, Gugliotta L, et al. Diagnosis, pathogenesis and treatment of the myeloproliferative disorders essential thrombocythemia, polycythemia vera and essential megakaryocytic granulocytic myeloproliferation and myelofibrosis. Neth J Med. 1999; 54: 46-62. PubMed: https://www.ncbi.nlm.nih.gov/pubmed/10079679

17. Michiels JJ, Juvonen E. Proposal for revised diagnostic criteria of essential thrombocythemia and polycythemia vera by the Thrombocythemia Vera Study Group. Sem Thromb Hemost. 1997; 23: 339-347. PubMed: https://www.ncbi.nlm.nih.gov/pubmed/9263350

18. Kurnick JE, Ward HP, Block MH. Bone marrow sections in the differential diagnosis of polycythemia. Arch Pathol. 1972; 94: 489-499. PubMed: https://www.ncbi.nlm.nih.gov/pubmed/5086062

19. Ellis JT, Silver RT, Coleman M, Geller SA. The bone marrow in polycythemia vera. Sem Hematol. 1975; 12: 433-444. PubMed: https://www.ncbi.nlm.nih.gov/pubmed/1198128

20. Michiels JJ, Abels J, Stekete J, van Vliet HH, Vuzebvski VD. Erythromelalgia caused by platelet-mediated arterial inflammation and thrombosis in thrombocythemia. Ann Intern Med. 1985; 102: 466-471. PubMed: https://www.ncbi.nlm.nih.gov/pubmed/3977194

21. Bellucci S, Michiels JJ. The role of JAK2V617F mutation, spontaneous erythropoiesis and megakaryopoiesis, hypersensitive platelets, activated leukocytes, and endothelial cells in the etiology of thrombotic manifestations in polycythemia vera and essential thrombocythemia. Sem Thromb Hemost. 2006; 32: 381-398.

22. Michiels JJ. Platelet-dependent and aspirin-responsive arterial thrombophilia in essential thrombocythemia. The Thoraxcentre J. 1996; 8: 1-4.

23. Michiels JJ. Bone marrow histopathology and biological markers as specific clues to the differential diagnosis of essential thrombocythemia, polycythemia vera and prefibrotic or fibrotic myeloid metaplasia. Hematol J. 2004; 5: 93-102. PubMed: https://www.ncbi.nlm.nih.gov/pubmed/15048058

24. Michiels JJ, De Raeye H, Hebeda K, Lam KH, Bot F, et al. Biology Diagnosis and Classification of MPD. Furst International Lymphoma-Leukemia-Myeloma (LLM) Congress. Turk J Hematol. 2007; 24: 37-53.

25. Michiels JJ, Berneman Z, Schroyens W, De Raeye H. PVSG and WHO vs European Clinical, Molecular and Pathological (ECMP) criteria for prefibrotic myeloproliferative neoplasms. World J Hematol. 2013b; 2: 71-88.

26. Michiels JJ, Ten Kate FWJ, Koudstaal PJ, Van Genderen PJJ. Aspirin responsive platelet thrombophilia in essential thrombocythemia and polycythemia vera. World J Hematol. 2013c; 2: 20-43.

27. Michiels JJ, Ten Kate F, Lam KH, Schroyens W, Berneman Z, et al. The European clinical, Molecular and Pathological (ECMP) criteria and the 2007/2008 revision of the World Health Organization for the diagnosis, classification and staging of prefibrotic myeloproliferative neoplasms carrying the JAK2V617F mutation. Turk J Hematol. 2014a; 31: 239-254.

28. Michiels JJ, Staikos J, Kubish P, Pich A, De Raeye H. Autosomal dominant hereditary essential thrombocythemia due to a gain of function mutation in the thrombopoietin (TPO) of JAK2 gene as the cause of dominant congenital aspirin sticky platelet syndrome. J Hematol Thromb Dis. 2014b; 2: 6.

29. Michiels JJ, Pich A, De Raeye H, Campr V, Schwarz J. WHO clinical molecular and pathological (WHO-CMP) features of congenital MPL515K and the acquired MPL515L mutated essential thrombocythemia and myelofibrosis. J Hematol Thromb Dis. 2014c; 2: 6.

30. Michiels JJ. Myeloproliferative and thrombotic burden and treatment outcome in thrombocthyemia and polycythemia patients. World J Crit Care Med. 2015; 4: 230-239. PubMed: https://www.ncbi.nlm.nih.gov/pubmed/26261774
31. Michie尔斯 JJ, Bernetzn Z, Schroyens W, De Rave H. Changing concepts of diagnostic criteria of myeloproliferative disorders and the molecular etiology and classification of myeloproliferative neoplasms: From Dameshek 1950 to Vainchenker 2005 and beyond. Acta Haematol. 2015a; 133: 71-86. PubMed: https://www.ncbi.nlm.nih.gov/pubmed/25116092

32. Michiels JJ, Valster M, Wielenga F, Schelfout K, Potters V, et al. Increased erythrocyte count on top of bone marrow histology, but not by EPO level or JAK2V617F mutation load discriminates between JAK2V617F mutated essential thrombocythemia and polycythemia vera. J Hematol Thromb Dis. 2015c: 4: 16-53. PubMed: https://www.ncbi.nlm.nih.gov/pubmed/28465746

35. Michiels JJ. Aspirin-responsive erythromelalgia in JAK2-thrombocythemia and incurable inherited erythromelalgia in neuropathic Naav1.7 sodium channelopathy: From Mitchel 1878 to Michiels 2017. Exp Opi-nion Orphan Drug. 2017c.

36. Michiels JJ. Aspirin cures erythromelalgia and cerebrovascular disturbances in JAK2-thrombocythemia. World J Hematol. 2017d; 6: 32-54.

37. Wasserman LR. The management of polycythemia vera. Br J Haematol. 1971; 21: 371-376. PubMed: https://www.ncbi.nlm.nih.gov/pubmed/4941523

38. Berlin NI. Diagnosis and classification of the polycythemias. Sem Hematol. 1975; 12: 339-351.

39. Tefferi A, Pandaranani A. Mutation screening for JAK2V617F: when to order the test and how to interpret the results. Leuk Res. 2006; 108: 3472-3476. PubMed: https://www.ncbi.nlm.nih.gov/pubmed/16460800

40. Tefferi A, Pandaranani A. Mutation screening for JAK2V617F: when to order the test and how to interpret the results. Leuk Res. 2006; 108: 3472-3476. PubMed: https://www.ncbi.nlm.nih.gov/pubmed/16460800

41. Arber DA, Orazi A, Hasserjian R, Thiele J, Borowitz MJ, et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. Blood. 2016; 127: 2391-2405. PubMed: https://www.ncbi.nlm.nih.gov/pubmed/31659364

42. Michiels JJ. The Myeloproliferative Disorders. An historical appraisal and personal experiences. Leuk Lymphoma. 1996; 22: 1-14. PubMed: https://www.ncbi.nlm.nih.gov/pubmed/8951768

43. Messinezy M, WestwoodNB, Woodstock SP, Strong RM, Pearson TC. Low seru, erythropoietin: a strong diagnostic criterion of primary polycythemia even et normal hemoglobin levels. Clin Lab Haematol. 1995; 17: 217-220.

44. Messinezy M, Westwood NB, El-Hemaida I, Marsden JT, Sherwood RS, et al. Serum erythropoietin values in erythrocytoses and in primary thrombocythemia, Br J Haematol. 2002; 117: 47-53. PubMed: https://www.ncbi.nlm.nih.gov/pubmed/11918532

45. 2008 WHO criteria for polycythemia vera, primary myelofibrosis and essential thrombocythemia. Thiele, et al. In: Swerdlow SH, Campo E, Harris NL, et al. WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues. Lyon France IARC. 2008; 40-50.

46. Thomas DJ, du Boulay GH, Marshall J, Pearson TC, Ross Russell RW, et al. Cerebral blood-flow in polycythemia. Lancet. 1977; 2: 161-163. PubMed: https://www.ncbi.nlm.nih.gov/pubmed/69781

47. Thomas DJ, Marshall J, Russell RW, Wetherley-Mein G, du Boulay GH, et al. Effect of haematocrit on cerebral blood-flow in man. Lancet. 1977; 2: 941-943. PubMed: https://www.ncbi.nlm.nih.gov/pubmed/72286

48. Pearson TC, Wetherley-Mein. Vascular occlusive episodes and venous haematocrit in primary proliferative polycythemia. Lancet. 1978; 2: 1219-1222. PubMed: https://www.ncbi.nlm.nih.gov/pubmed/82733

49. Messinezy M, Pearson TC, Prochazka A, Wetherley-Mein G. Treatment of primary proliferative polycythemia by venesection and low dose busulphan: retrospective study from one centre. Br J Haematol. 1985; 61: 657-666. PubMed: https://www.ncbi.nlm.nih.gov/pubmed/4084455

50. Landolf M, Marchioli R, Kutti J, Gisslinger H, Tognoni G, et al. Efficacy and safety of low-dose aspirin in polycythemia vera. N Engl J Med. 2004; 350: 114-124. PubMed: https://www.ncbi.nlm.nih.gov/pubmed/14719110

51. Marchioli R, Finazzi G, Vannucchi AM, Barbui T for the CTYO-PV Collaborative Group. Cardiovascular events and intensivity of treatment in polycythemia vera. N Engl J Med. 2013; 368: 22-33.

52. Van Genderen PJJ, Michiels JJ. Hydroxyurea in essential thrombocytosis. N Engl J Med. 1995; 333: 802-803. PubMed: https://www.ncbi.nlm.nih.gov/pubmed/7643898

53. Lengfelder E, Merx K, Hehlmann R. Diagnosis and therapy of polycythemia vera. Sem Thromb Hemost. 2006; 32: 267-275. PubMed: https://www.ncbi.nlm.nih.gov/pubmed/16673281

54. Prchal JF, Axelrad AA. Bone marrow responses in polycythemia vera. Blood. 2016; 127: 2391-2405. PubMed: https://www.ncbi.nlm.nih.gov/pubmed/28465746

55. Westwood NB, Person TC. Diagnostic applications of haematopietic progenitor culture techniques in polycythemias andthrombocythemias. Leu Lymphoma. 1996; 22: 95-103. PubMed: https://www.ncbi.nlm.nih.gov/pubmed/8951779

56. Kralovics R, Guan Y, Prchal JT. Acquired uniparetal disomy of chromosome 9p is a frequent stem cell defect in polycythemia vera. Exp Hematol. 2002; 30: 229-236. PubMed: https://www.ncbi.nlm.nih.gov/pubmed/11882360

57. James C, Ugo V, Le Couedic JP, Staerk J, Delhommeau F, et al. A unique clonal JAK2 mutation leading to constitutive signaling causes polycythemia vera. Nature. 2005; 434: 1144-1148. PubMed: https://www.ncbi.nlm.nih.gov/pubmed/15793561

58. Kralovics R, Passamonti F, Buser AS, Teo SS, Teitl R, et al. A gain-offunction mutation of JAK2 in myeloproliferative disorders. N Engl J Med. 2005; 352: 1779-1790. PubMed: https://www.ncbi.nlm.nih.gov/pubmed/15858187

59. Vainchenker W, Constantinescu SN. A unique activating mutation in JAK2V617F is at the origin of polycythemia vera and allows a new classification of myeloproliferative diseases. Hematology Am Soc Hematol Educ Program. 2005; 195-200. PubMed: https://www.ncbi.nlm.nih.gov/pubmed/16304380

60. Villeval JL, James C, Pisani DF, Casadevall N, Vainchenker W. New insights into the pathogenesis of JAK2V617F-positive myeloproliferative disorders and consequences for the management of patients. Sem Thromb Hemost. 2006; 32: 341-351.

61. Baxter EJ, Scott LM, Campbell PJ, East C, Fourouclas N, et al. Acquired mismatch repair deficiency in sporadic colorectal cancers. Nature. 2005; 434: 67-73. PubMed: https://www.ncbi.nlm.nih.gov/pubmed/15858187

https://doi.org/10.29328/journal.ijbmr.1001011

https://www.heighpubs.org/hbmr

017
mutation of the tyrosine kinase in human myeloproliferative disorders. Lancet. 2005; 365: 1054-1061. 
PubMed: https://www.ncbi.nlm.nih.gov/pubmed/15781101

62. Campbell PJ, Baxter EJ, Beer PHA, Scott LM, Bench AJ, et al. Mutation of JAK2 in the myeloproliferative disorders: timing, clonality studies, cytogenetic associations, and the role in leukemic transformation. Blood. 2006; 108: 3548-3555. 
PubMed: https://www.ncbi.nlm.nih.gov/pubmed/16873677

63. Moliterno AR, Williams DM, Isaacs MA, Spivak JL. Phenotypic variability within the JAK2V617F-positive MPD: roles of progenitor cell and neutrophil allele burden. Exp Hematol. 2008; 36: 1480-1486. 
PubMed: https://www.ncbi.nlm.nih.gov/pubmed/18723264

64. Vainchenker W, Delhommeau F, Constantinescu SN, Bernard OH. New mutations and pathogenesis of myeloproliferative neoplasms. Blood. 2011; 118: 1723-1735. 
PubMed: https://www.ncbi.nlm.nih.gov/pubmed/21653328

65. Vainchenker W, Kralovic S. Genetic basis and molecular pathogenesis of classical myeloproliferative neoplasms. Blood. 2017; 129: 667-679. 
PubMed: https://www.ncbi.nlm.nih.gov/pubmed/28028029

66. Pich A, Riera L, Beggio E, Nicolino B, Godio L, et al. JAK2V617F mutation and allele burden are associated with distinct clinical and morphological subtypes in patients with essential thrombocythemia. J Clin Pathol. 2012; 65: 953-954. 
PubMed: https://www.ncbi.nlm.nih.gov/pubmed/22718845

67. Antonioli E, Guglielmelli P, Pancrazzi A, Bogani C, Verrucci M, et al. Clinical implications of the JAK2 V617F mutation in essential thrombocythemia. Leukemia. 2005; 19: 1847-1849. 
PubMed: https://www.ncbi.nlm.nih.gov/pubmed/16079890

68. Gale RE, Allen AJR, Nash MJ, Linch DC. Log-term serial analysis of X-chromosome inactivation patterns and JAK2 V617F mutant levels in patients with essential thrombocythemia show that minor mutant-positive clones can remain stable for many years. Blood. 2007; 109: 1241-1243. 
PubMed: https://www.ncbi.nlm.nih.gov/pubmed/17023581

69. Vannucchi AM, Antonioli E, Guglielmelli P, Longo G, Pancrazzi A, et al. Prospective identification of high-risk polycythemia vera patients based on JAK2V617F allele burden. Leukemia. 2007; 21: 1952-1959. 
PubMed: https://www.ncbi.nlm.nih.gov/pubmed/17625606

70. Passamonti F, Rumi E, Pietra D, Della Porta MG, Boveri E, et al. Relation between JAK2V617F mutation status, granulocyte activation, and constitutive mobilization of CD34+ cells into peripheral blood in myeloproliferative disorders. Blood. 2006; 107: 3676-3682. 
PubMed: https://www.ncbi.nlm.nih.gov/pubmed/16373657

71. Scott LM, Scott MA, Campbell PJ, Green AR. Progenitors homozygous for the V617F JAK2 mutation occur in most patients with polycythemia vera, but not essential thrombocythemia. Blood. 2006; 108: 2435-2437. 
PubMed: https://www.ncbi.nlm.nih.gov/pubmed/17672604

72. Delhommeau F, Pisani DF, James C, Casadevall N, Constantinescu S, et al. Oncogenic mechanism in myeloproliferative disorders. Cell Mol Life Sci. 2006; 63: 2939-2953. 
PubMed: https://www.ncbi.nlm.nih.gov/pubmed/16373657

73. Mead AJ, Rugless MJ, Jacobsen SE, Schuh A. Germline JAK2 mutation in a family with hereditary thrombocythiosis. N Eng J Med. 2012; 366: 967-969. 
PubMed: https://www.ncbi.nlm.nih.gov/pubmed/22397670

74. Mead AJ, Chowdhury O, Pecquet C, Dusa A, Wolf P, et al. Impact ofisolated germline JAK2V617F mutation on human hematopoiesis. Blood. 2013; 121: 4156-4165. 
PubMed: https://www.ncbi.nlm.nih.gov/pubmed/23535062

75. Etheridge SL, Cosgrove ME, Sangkhae V, Corbo LM, Roh ME, et al. A novel activating, germ line JAK2 mutation, JAK2(V644G), causes familialessential thrombocytosis. Blood. 2014; 123: 1059-1068. 
PubMed: https://www.ncbi.nlm.nih.gov/pubmed/24381227

76. Rumi E, Pietra D, Ferretti V, Klampfl T, Harutyunyan AS, et al. Jak2 or CALR mutation status defines subsets of essential thrombocythemia with substantially different clinical course and outcome. Blood. 2014; 123: 1552-1515. 
PubMed: https://www.ncbi.nlm.nih.gov/pubmed/24366362

77. Lamy T, Devillers A, Bernard M, Moisan A, Grulois I, et al. In apparent polycythemia vera: an unrecognized diagnosis. Am J Med. 1997; 102: 14-20. 
PubMed: https://www.ncbi.nlm.nih.gov/pubmed/9209196

78. Godfrey AL, Chen E, Pagano F, Ortmann CA, Silber Y, et al. JAK2V617F homozygosity arises commonly and recurrently in PV and ET, but PV is characterized by expansion of a dominant homozygous subclone. Blood. 2012; 120: 2704-2707. 
PubMed: https://www.ncbi.nlm.nih.gov/pubmed/22898600

79. Bench AJ, Nacheva EP, Champion KM, Green AR. Molecular genetics and cytogenetics of myeloproliferative disorders. Baillieres Clin Haematol. 1998; 11: 819-848. 
PubMed: https://www.ncbi.nlm.nih.gov/pubmed/10664019

80. Vannucchi A, Lasho TL, Guglielmelli P, Bianmonte F, Pardani A, et al. Mutations and prognosis in primary myelofibrosis. Leukemia. 2013; 27: 1861-1869. 
PubMed: https://www.ncbi.nlm.nih.gov/pubmed/23619563

81. Guglielmelli P, Lasho TL, Rotunno G, JScore J, Mannarelli C, et al. The number of prognostically detrimental mutations and prognosis in primary myelofibrosis: an international study of 797 patients. Leukemia. 2014; 28: 1494-1500. 
PubMed: https://www.ncbi.nlm.nih.gov/pubmed/24549259

82. Lundberg P, Karov A, Nienbold R, Looser R, Hao-Shen H, et al. Clonal evolution and clinical correlates of somatic mutations in myeloproliferative neoplasms. Blood. 2014; 123: 2220-2228. 
PubMed: https://www.ncbi.nlm.nih.gov/pubmed/24478400

83. Tefferi A, Lasho TL, Guglielmelli P, Finke CM, Rotunno G, et al. Targeted deep sequencing in polycythemia vera and essential thrombocythemia. Blood Adv. 2016; 1: 21-30. 
PubMed: https://www.ncbi.nlm.nih.gov/pubmed/29296692

84. Tefferi A, Lasho TL, Finke CM, Elala Y, Hanson CA, et al. Targeted deep sequencing in primary myelofibrosis. Blood Adv. 2016; 1: 105-111. 
PubMed: https://www.ncbi.nlm.nih.gov/pubmed/29296803

85. Ortmann CA, Kent DG, Nangalia J, Silber Y, Wedge DC, et al. Effect of mutation order on myeloproliferative neoplasms. Blood. 2014; 123: 2118-2128. 
PubMed: https://www.ncbi.nlm.nih.gov/pubmed/25546289

86. Michiels JJ, Ten Kate FWJ, Vuzevski VD, Abels J. Histopathology of erythromelalgia in thrombocythemia. Histopathology. 1984; 8: 669-678. 
PubMed: https://www.ncbi.nlm.nih.gov/pubmed/29263352

87. Michiels JJ, Berneman Z, Schroyens W, Koudstaal PJ, Lindemans J, et al. Platelet-mediated thrombotic complications in patients with essential thrombocythemia and polycythemia vera: a distinct aspirin-responsive and coumadin-resistant arterial thrombophilia. Platelets. 2006; 17: 528-544. 
PubMed: https://www.ncbi.nlm.nih.gov/pubmed/17127481

88. Michiels JJ, Ten Kate FWJ, Vuzevski VD, Abels J. Histopathology of erythromelalgia in thrombocythemia. Histopathology. 1984; 8: 669-678. 
PubMed: https://www.ncbi.nlm.nih.gov/pubmed/29263352

89. Michiels JJ, Berneman Z, Schroyens W, Koudstaal PJ, Lindemans J, et al. Platelet-mediated thrombotic complications in patients with essential thrombocythemia and polycythemia vera: a distinct aspirin-responsive and coumadin-resistant arterial thrombophilia. Platelets. 2006; 17: 528-544. 
PubMed: https://www.ncbi.nlm.nih.gov/pubmed/17127481

90. Novo European Asiatic Clinical, Laboratory, Molecular and Pathobiological (2015-2020 CLMP) criteria for JAK2V617F trilinear polycythemia vera (PV), JAK2(V617F) PV and JAK2V617F, CALR and MPL515thrombocythemia: From Dameshek to Constantinescu-Vainchenker, Kralovic and Michiels

https://doi.org/10.29328/journal.ijbmr.1001011

https://www.heighpubs.org/hbmr
89. Van Genderen PJJ, Michiels JJ, Van Strik R, Lindemans J, Van Vliet HDMM. Platelet consumption in thrombocythemia complicated by erythromelalgia: reversal by aspirin. Thromb Haemost. 1995; 73: 210-214. PubMed: https://pubmed.ncbi.nlm.nih.gov/7792731/

90. Van Genderen PJJ, Lucas IS, Van Strik R, Vuzevski VD, Prins FJ, et al. Erythromelalgia in essential thrombocythemia is characterized by platelet activation and endothelial cell damage but not by thrombin generation. Thromb Haemost. 1996; 76: 333-338. PubMed: https://pubmed.ncbi.nlm.nih.gov/8883266/

91. Van Genderen PJJ, Michiels JJ. Erythromelalgia: a pathognomonic microvascular thrombotic complication in essential thrombocythemia and polycythemia vera. Sem Thromb Hemost. 1997; 23: 357-363. PubMed: https://pubmed.ncbi.nlm.nih.gov/9263352/

92. Van Genderen PJJ, Mulder PGH, Waleboer M, Moedskijl D, Michiels JJ. Prevention and treatment of thrombotic complications in essential thrombocythemia: efficacy and safety of aspirin. Brit J Haematol. 1997a; 97: 179-184. PubMed: https://www.ncbi.nlm.nih.gov/pubmed/9136963

93. Van Genderen PJJ, Leenknegt H, Michiels JJ. The paradox of bleeding and thrombosis in thrombocythemia: is von Willebrand factor the link? Sem Thromb Hemost. 1997b; 23: 385-389. PubMed: https://pubmed.ncbi.nlm.nih.gov/16977569/

94. Van Genderen PJJ, Prins F, Michiels JJ, Schroer K. Thromboxane-dependent platelet activation in vivo precedes arterial thrombosis in thrombocythemia: A rationale for the use of low-dose aspirin as an antithrombotic agent. Brit J Haematol. 1999; 104: 438-441. PubMed: https://pubmed.ncbi.nlm.nih.gov/10086775/

95. Scott LM. The JAK2 exon 12 mutants, a comprehensive review. Amer J Hematol. 2011; 86: 668-676. PubMed: https://pubmed.ncbi.nlm.nih.gov/21674578/

96. Pardani A, Lasho TL, Finke C, Hanson CA, Tefferi A. Prevalence and clinicopathologic correlates of JAK2 exon 12 mutations in JAK2(formula)exon12, negative polycythemia vera. Leukemia. 2007; 21: 1960-1963. PubMed: https://www.ncbi.nlm.nih.gov/pubmed/17597810

97. Lakey MA, Pardani A, Hoyer JD, Nguyen PL, Lasho TL, et al. Bone marrow morphologic features in polycythemia vera with JAK2 exon 12 mutations. Am J Clin Pathol. 2010; 133: 942-948. PubMed: https://pubmed.ncbi.nlm.nih.gov/20472853/

98. Passamonti F, Elena C, Schnittger S, Skoda R, Anthony R Green et al. Molecular and clinical features of the myeloproliferative neoplasms associated with JAK2 exon 12 mutations. Blood. 2011; 117: 2813-2816. PubMed: https://www.ncbi.nlm.nih.gov/pubmed/21224469

99. Scott LM, Tong W, Levine RL, Scott MA, Beer PA, et al. JAK2 exon 12 mutations in polycythemia vera and idiopathic erythrocytosis. N Eng J Med. 2007; 356: 459-468. PubMed: https://pubmed.ncbi.nlm.nih.gov/17267906/

100. Kim Y, Park J, Jo I, Lee GD, Kim J, et al. Genetic-pathologic characterization of myeloproliferative neoplasms. Exp Mol Med. 2016; 48: 247. PubMed: https://pubmed.ncbi.nlm.nih.gov/27444979

101. Vannuccchi AM, Antonioli E, Guglielmelli P, Pancrazzi A, Guerini V, et al. Characteristics and clinical correlates of MPLS15W-L/K mutation in essential thrombocythemia. Blood. 2008; 112: 844-847. PubMed: https://pubmed.ncbi.nlm.nih.gov/18519816/

102. Beer PA, Campbell PJ, Scott LM. MPL mutations in myeloproliferative disorders: analysis of the PT-1 cohort. Blood. 2008; 112: 141-149. PubMed: https://www.ncbi.nlm.nih.gov/pubmed/18451306

103. Jones AV, Campbell PJ, Beer PA, Schnittger, Vannuccchi AM, et al. The JAK2 46/1 haplotype predisposes to MPL-mutated myeloproliferative neoplasms. Blood. 2010; 115: 4517-4523. PubMed: https://pubmed.ncbi.nlm.nih.gov/20304805/

104. Michiels JJ, Berneman Z, Schroyns W, Kuttji, Swollin B, et al. Philadelphia (Ph) chromosome positive thrombocythemia without features of chronic myeloid leukemia in peripheral blood and bone marrow: natural history and diagnostic differentiation from Ph-negative essential thrombocythemia. Ann Hematol. 2004; 83: 504-512. PubMed: https://pubmed.ncbi.nlm.nih.gov/15164229/

105. Klampfl T, Gisslinger H, Harutyunyan AS, Nirvathi H, Rumi E, et al. Somatic mutations od calreticulin in myeloproliferative neoplasms. N Eng J Med. 2013; 369: 2379-2387. PubMed: https://pubmed.ncbi.nlm.nih.gov/24325356/

106. Nangalia J, Massie CE, Baxter J, Nice FL, Gundem G, et al. CALR vs JAK2 vs MPL-mutated or triple-negative myelofibrosis: clinical, cytogenetic and molecular comparisons. Leukemia. 2014; 28: 1472-1477. PubMed: https://pubmed.ncbi.nlm.nih.gov/24402162/

107. Rotunno G, Mannarelli C, Guglielmelli P, Pacilli A, Pancrazzi A, et al. Impact of calreticulin mutations on clinical and haematological phenotype and outcome in essential thrombocthyemia. Blood. 2014; 123: 1552-1555. PubMed: https://www.ncbi.nlm.nih.gov/pubmed/24371211

108. Andrikovics H, Krahling T, Balassa K, Halm G, Bors A, et al. Distinct clinical characteristics of myeloproliferative neoplasms with calreticulin mutations. Haematologica. 2014; 99: 1184-1190. PubMed: https://pubmed.ncbi.nlm.nih.gov/24895366/

109. Thiele J, Kvasknicka HM, Facchetti F, Franco V, Van Der Walt J Orazi A. European consensus for grading bone marrow fibrosis and assessment of cellularity in myeloproliferative disorders. Haematologica. 2005; 90: 1128-1132. PubMed: https://pubmed.ncbi.nlm.nih.gov/16079113/

110. Kiladjian JJ, Cassinat B, Turlube P, Cambier N, Roussel M, et al. High molecular response rate of polycythemia vera treated with pegylated interferon-alpha2a. Blood. 2006; 108: 2037-2040. PubMed: https://pubmed.ncbi.nlm.nih.gov/16709929/

111. Larssen TS, Moeller MB, de Striker K, Peter Nørgaard, Jan Samuelsson, et al. Minimal residual disease and normalization of the bone marrow after longterm treatment with alfa-interferon2b in polycythemia vera. A report on molecular responses in seven patients in sustained complete hematological remission. Hematology. 2009; 14: 331-334. PubMed: https://pubmed.ncbi.nlm.nih.gov/19941739/

112. Verger E, Cassinat B, Chauveau A, Dosquet C, Giradier S, et al. Clinical and moelucular response to interferon-alpha therapy in essential thrombocythemia patients with CALR mutations. Blood. 2015; 126: 2585-2691. PubMed: https://pubmed.ncbi.nlm.nih.gov/26486786/

113. Wilkins BS, Erber WN, Bareford D, Buck G, Wheatley K, et al. Bone marrow pathology in essential thrombocythemia: interobserver reliability and utility for identifying disease subtypes. Blood. 2008; 111: 60-70. PubMed: https://pubmed.ncbi.nlm.nih.gov/17885079/

114. James C, Delhommeau F, Marzar C, Teysandier I, Le Couédic JP, et al. Detection of JAK2 V617F as a first intention diagnostic test for erythrocytosis. Leukemia. 2006; 20: 350-353. PubMed: https://pubmed.ncbi.nlm.nih.gov/16341032/

115. Cassinat B, verger E, Kiladjian JJ. Interferon alpha therapy in CALR-mutated essential thrombocythemia. N Eng J Med. 2014; 371: 188-189. PubMed: https://www.ncbi.nlm.nih.gov/pubmed/25006741
116. Juvonen E, Ikkala E, Fyrquist F, Ruutu T. Autosomal dominant erythrocytosis caused by increased sensitivity to erythropoietin. Blood. 1991; 78: 3066-3069. 
PubMed: https://pubmed.ncbi.nlm.nih.gov/1954391/  
117. De La Chapelle A, Traskelin AL, Juvonen E. Truncated erythropoietin receptor causes dominantly inherited benign erythrocytosis. Proc Natl Acad Sci USA. 1993; 90: 4495-4499. 
PubMed: https://pubmed.ncbi.nlm.nih.gov/8506290/  
118. Lacout C, Pisani DF, Tulliez M, Gachelin FM, Vainchenker W, et al. JAK2V617F expression in murine hematopoietic cells leads to MPD mimicking human PV with secondary myelofibrosis. Blood. 2006; 108: 1652-1660. 
PubMed: https://pubmed.ncbi.nlm.nih.gov/16670266/  
119. Laszlo J. Myeloproliferative disorders (MPD): myelofibrosis, myelosclerosis, extramedullary hematopoiesis, undifferentiated MPD and hemorrhagic thrombocytopenia. Semin Hematol. 1975; 12: 409-432. 
PubMed: https://www.ncbi.nlm.nih.gov/pubmed/1105793  
120. Quintas-Cardama A, Kantarjian H, Manshouri T, Rajyalakshmi Luthra, Zeev Estrov, et al. Pegylated interferon alfa-2a yields high rates of hematological and molecular response in patients with advanced essential thrombocythemia and polycythemia vera. J Clin Oncol. 2009; 27: 5418-5424. 
PubMed: https://pubmed.ncbi.nlm.nih.gov/19826111/  
121. Vannucchi AM, Papaemmanuil E, Campbell PJ, and Green AR. Somatic CALR Mutations in Myeloproliferative Neoplasms with Nonmutated JAK2. N Eng J Med. 2013; 369: 2391-2405. 
PubMed: https://pubmed.ncbi.nlm.nih.gov/24325359/  
122. 2001 WHO classification of the chronic myeloproliferative diseases (CMPD) polycythemia vera, chronic idiopathic myelofibrosis essential thrombocythemia and cMPD unclassifiable. In: Jaffe SS, Harris NL, Stern A, Vardiman JW eds. WHO classification of Tumours of haematopoietic and lymphoid tissues. Lyon, France IARC; 2001; 31-42.