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

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Submitted: 09 March 2020
Approved: 30 March 2020
Published: 03 April 2020

How to cite this article: Michiels JJ, Lam KH, Kate FT, Kim DW, Kim M, et al. Novel European Asiatic Clinical, Laboratory, Molecular and Pathobiological (2015-2020 CLMP) criteria for JAK2^{exon12} trilinear polycythemia vera (PV), JAK2^{V617F} PV and JAK2^{V617F}, CALR and MPL^{515} thrombocythemias: From Dameshek to Constantinescu-Vainchenker, Kralovics and Michiels. Int J Bone Marrow Res. 2020; 3: 001-020.

DOI: 10.29328/journal.ijbmr.1001011

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Keywords: Myeloproliferative neoplasms; Essential thrombocythemia; Polycythemia vera; Primary megakaryocytic granulocytic myeloproliferation; Myelofibrosis; JAK2^{V617F} mutation; MPL^{515} mutation; Calreticulin mutation; JAK2 wild type; Bone marrow histology
Abstract

The Myeloproliferative Neoplasms (MPN) of trilinear polycythemia vera (PV) and megakaryocytic leukemia (ML = primary megakaryocytic granulocytic myeloproliferation: PMGM) and Essential Thrombocytopenia (ET) in the studies of Dameshek and Michiels are caused by the MPN driver mutations JAK2V617F, JAK2exon12, CALR and MPL515 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 thrombocytopenia (ET), prodromal PV and erythroid myeloproliferation, PV and advanced stages of masked 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 or homozygous JAK2V617F mutated trilinear PV, the JAK2 mutation load increases from less than 50% in prodromal 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 erythrogenic, megakaryocytic and granulocytic (EMG) myeloproliferation in JAK2V617F mutated trilinear MPN, which clearly differs from monolinear megakaryocytic (M) myeloproliferation in MPL and CALR thrombocytopenia and dual megakaryocytic granulocytic (MG) myeloproliferation in CALR mutated thrombocytopenia. The morphology of clustered large pleomorphic megakaryocytes with hyperlobulated nuclei are similar in JAK2V617F thrombocytopenia, prodromal PV and classical PV patients. Monolinear megakaryocytic (M) myeloproliferation of large to giant megakaryocytes with hyperlobulated staghorn-like nuclei is the hallmark of MPL515 mutated normocellular thrombocytopenia. CALR mutated thrombocytopenia usually presents with high platelet count around 1000x10^9/L and normocellular megakaryocytic (M) 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, MPL515 and CALR mutated myeloproliferative neoplasms (MPNs, which mutually exclude each other).

Introduction

The combination of plethoric appearance, splenomegaly, erythrocyte count above 6x10^12/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 a 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 erythropoietic, thrombocytopenic and granulocytic 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 thrombocytopenia 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 hematopoesis in ET at platelet counts above 400x10^9/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 1000x10^9/L against a background of normally maturing granulopoesis 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/megakaryopoesis (EM) and erythro-megakaryo-granulopoesis (EMG) are the diagnostic features of untreated PV [14,17] (Figure 3, Table 4). Increase of large megakaryocytes with mature cytoplasm and multilobulated nuclei in a hypercellular bone marrow is even more conspicuously altered in PV than in ET or early prodromal stage 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 clumpy. 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].

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, JAK2exon12, 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

![Image](https://www.heighpubs.org/hbmr)

Table 1: The 2006 concept of Michiels, et al. [26]ab [89], Belfucci &Michiels, 2006 [31] on the molecular etiology of JAK2V617F mutated hypersensitive platelets and platelet-mediated arteriole erythromelalgic arterial (Michiels, et al. [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]).
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 Raeve used bone marrow histology and erythrocyte and platelet counts as pathognomic clue to distinguish all variants of MPN from reactive thrombocytosis, BCR/ABL positive thrombocytosis in chronic myeloid leukemia (CML), and thrombocytosis 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 x1012/L in males and > 5.6 x1012/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 erythrocytic megakaryocytic (EM), erythrocytic, 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 intergrating the PVSG/WHO bone marrow features into the ECP and ECFP criteria of myeloproliferative Disorders [15,17], MPD Doctor’s Brochure 2004, Michiels, et al (8,10).

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

<table>
<thead>
<tr>
<th><strong>Prefibrotic ET</strong></th>
<th><strong>Normocellular ET</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Platelet count of &gt; 350 x10^9/L</td>
<td>Normocellular bone marrow (&lt; 60%), Megakaryocytic (M) proliferation of clustered medium sized to large (pleomorphor) mature megakaryocytes in anormocellular bone marrow (&lt; 60%), no proliferation of erythrocytic and granulopoiesis.</td>
</tr>
<tr>
<td>2. Heterozygous JAK2V617F mutation, and low JAK2 allelic mutation load</td>
<td>Reticuline fibrosis (RF) 0 or 1</td>
</tr>
<tr>
<td>3. Normal erythrocytes &lt; 5.8x10^12/L males, &lt; 5.6 x10^12/L females</td>
<td></td>
</tr>
<tr>
<td>4. Hemoglobin (Hb) and hematocrit (Ht) normal or upper range of normal</td>
<td></td>
</tr>
</tbody>
</table>

**Prefibrotic prodromal PV**

<table>
<thead>
<tr>
<th><strong>ET with bone marrow features of PV</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Platelet count of ≥ 350 x10^9/L.</td>
</tr>
<tr>
<td>HH and Ht in upper range of normal, but erythrocyte count &lt; 5.8x10^12/L males, &lt; 5.6x10^12/L females.</td>
</tr>
<tr>
<td>2. Presence of JAK2V617F mutation and variable JAK2 mutation load</td>
</tr>
<tr>
<td>3. Low serum EPO, increased LAP score</td>
</tr>
</tbody>
</table>

**Prefibrotic hypercellular ET**

<table>
<thead>
<tr>
<th><strong>EMG, masked PV and MG fibrotic stages</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Platelet count of ≥ 350 x10^9/L.</td>
</tr>
<tr>
<td>2. Presence of JAK2V617F mutation and high JAK2 mutation load</td>
</tr>
<tr>
<td>3. Moderate myeloid neoplasia of the spleen</td>
</tr>
<tr>
<td>→ splenomegaly</td>
</tr>
<tr>
<td>4. No preceding or allied CML, PMGM, RARS-T or MDS.</td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>

**Clinical stage 1:** HH and Ht in lower range of normal: Hb> 12 g/dl, normal LDH and CD34+ |
**Clinical stage 2:** anemia Hb <12 to > 10 g/dl, LDH↑, and splenomegaly |
**Clinical stage 3:** severe anemia, Hb <10 g/dl, LDH↑↑, CD34+, leuкоerythroblastose, tear drop erythrocytes, and large spleen

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

<table>
<thead>
<tr>
<th><strong>Clinical, laboratory, and molecular (CLM) criteria</strong></th>
<th><strong>Bone marrow Pathology (P) criteria</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Platelet count of &gt; 350 x10^9/L.</td>
<td>Normocellular bone marrow (&lt; 60%), Megakaryocytic (M) proliferation of clustered medium sized to large (pleomorphor) mature megakaryocytes in anormocellular bone marrow (&lt; 60%), no proliferation of erythrocytic and granulopoiesis.</td>
</tr>
<tr>
<td>2. Heterozygous JAK2V617F mutation, and low JAK2 allelic mutation load</td>
<td>Reticuline fibrosis (RF) 0 or 1</td>
</tr>
<tr>
<td>3. Normal erythrocytes &lt; 5.8x10^12/L males, &lt; 5.6 x10^12/L females.</td>
<td></td>
</tr>
<tr>
<td>4. Hemoglobin (Hb) and hematocrit (Ht) normal or upper range of normal</td>
<td></td>
</tr>
</tbody>
</table>

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

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https://www.heighpubs.org/hbmr
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.

<table>
<thead>
<tr>
<th>Major criteria for PV</th>
<th>Bone marrow Pathology (P) criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>A 1. Erythrocytes &gt; 5.8x10^{12}/L males and &gt; 5.6x10^{12}/L females. Hemoglobin and hematocrit upper range of normal or increased.</td>
<td>PV. Increased cellularity (60% - 100%) due to increased erythroid, megakaryocytic (EM) proliferation or trilineal proliferation. Increase of clustered medium to large (pleomorph) megakaryocytes with hyperlobulated nuclei.</td>
</tr>
<tr>
<td>A 2. Heterozygous and/or homozygous JAK2V617F or JAK2exon12 mutation.</td>
<td>Hypercellular (90% - 100%) due to EMG prefibrotic advanced or masked PV with predominant increased fibrotic megakaryocytic, granulocytic (MG) proliferation with relative reduced erythroid precursors.</td>
</tr>
</tbody>
</table>

Confirmative criteria

| B 1. Persistent increase of platelet count x10^{12}/L grade I: 400-1500, grade II: > 1500. | Grading of secondary reticulin fibrosis (RF,[113]) and myelofibrosis (MF) [11,12,19]. |
| 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. | Prefibrotic: RF-0/1 = MF-0; Early fibrotic: RF-2 = MF-1; Fibrotic: RCF 3 = MF-2; Post-PV MF: RF 4 = MF-3. |
| 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 5: 2015-2020 CLMP staging of JAK2V617F positive prodromal PV, erythrocythemic PV, classical PV, masked PV and MF, inapparent PV, spent phase PV and post-PV myelofibrosis (MF).

<table>
<thead>
<tr>
<th>PV: CLMP stage</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical Diagnosis</td>
<td>Prodromal PV</td>
<td>Erythrocythemia (E)</td>
<td>Early PV</td>
<td>Classical PV</td>
<td>Masked PV</td>
<td>Inapparent PV</td>
<td>Advanced PV</td>
</tr>
<tr>
<td>LAP-score, CD11B</td>
<td>†</td>
<td>†</td>
<td>†</td>
<td>†</td>
<td>†/††</td>
<td>Variable</td>
<td>Variable</td>
</tr>
<tr>
<td>Red Cell Mass</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+/††</td>
<td>Variable</td>
<td>Variable</td>
</tr>
<tr>
<td>Erythrocytes x10^{12}/L</td>
<td>&lt; 5.8</td>
<td>&gt; 5.8</td>
<td>&gt; 5.8</td>
<td>&gt; 5.8</td>
<td>&gt; 5.8</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>Leukocytes x10^9/L</td>
<td>&lt; 10</td>
<td>&lt; 10</td>
<td>10 - 12</td>
<td>12 - 15</td>
<td>&gt; 15</td>
<td>†† or ††</td>
<td>&gt; 20</td>
</tr>
<tr>
<td>Platelets x10^9/L</td>
<td>&gt; 400</td>
<td>&lt; 400</td>
<td>&lt; or &gt; 400</td>
<td>&gt; 400</td>
<td>+1000</td>
<td>†† or †† Variable</td>
<td></td>
</tr>
<tr>
<td>CLMP bone marrow histology</td>
<td>EM</td>
<td>EM</td>
<td>EM</td>
<td>EMG</td>
<td>EMG</td>
<td>EMG-MF</td>
<td>MF</td>
</tr>
<tr>
<td>BM cellularity (%)</td>
<td>50-80</td>
<td>50-80</td>
<td>50-80</td>
<td>60-100</td>
<td>80-100</td>
<td>80-100</td>
<td>60-100</td>
</tr>
<tr>
<td>Grading MF 515</td>
<td>MF 0</td>
<td>MF 0</td>
<td>MF 0</td>
<td>MF 0</td>
<td>MF 1</td>
<td>MF 1/2</td>
<td>MF 2/3</td>
</tr>
<tr>
<td>Spleen size: On echogram below MCL</td>
<td>&lt; 12-15</td>
<td>&lt; 13</td>
<td>12-15</td>
<td>12-16</td>
<td>18 &gt; 20</td>
<td>16 &gt; 20</td>
<td>&gt; 20 cm</td>
</tr>
<tr>
<td>JAK2V617Fload Granulocytes %</td>
<td>Low</td>
<td>low</td>
<td>Moderate &lt; 50%</td>
<td>High &gt; 50% ++</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Risk stratification</td>
<td>Low Aspirin</td>
<td>Low Phlebot Aspirin</td>
<td>Low Phlebot Aspirin</td>
<td>Intermediate IFN</td>
<td>JAK2 inhibitor</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Therapeutic implications</td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

Table 6: 2015-2020 Clinical Laboratory, Molecular and Pathobiology (CLMP) criteria for the diagnosis of normocellular ET carrying one of the MPL515 mutations.

<table>
<thead>
<tr>
<th>Clinical, laboratory, molecular (CLM) criteria</th>
<th>Bone marrow Pathology (P) criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Platelet count &gt; 350x10^{12}/L and presence of large platelets in blood smear.</td>
<td>Megakaryocytic (M) proliferation in a normocellular (&lt; 60%) bone marrow featured by large to giant mature megakaryocytes with hyperlobulated, staghorn-like nuclei.</td>
</tr>
<tr>
<td>2. Normal Hemoglobin, haematocrit and erythrocyte count.</td>
<td>No increase of erythropoiesis, and granulopoiesis.</td>
</tr>
<tr>
<td>3. Presence of MPL515 mutation.</td>
<td>No or slight increase in reticulin RF 0/1.</td>
</tr>
<tr>
<td>5. Normal LAP score (CD11b).</td>
<td></td>
</tr>
<tr>
<td>6. No or slight splenomegaly.</td>
<td></td>
</tr>
<tr>
<td>7. No preceding or allied CML, PV, PMGM, RAS-T or MDS.</td>
<td></td>
</tr>
</tbody>
</table>

Clinical staging similar as in CALR thrombocytocemia based on the degree of anemia, splenomegaly and myelofibrosis.
below 5.8 x10^{12}/L with hematocrit values ranging from 0.40 to 0.45 (Figure 1, Tables 2-4). A mean haematocrit of 0.60 and a mean platelet count of 512 x10^{9}/L at time of diagnosis of PV were associated with 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 512 x10^{9}/L at time of diagnosis of PV were associated 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, TIA's 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].
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).
Constantinescu & Vainchenker, [59] demonstrated that the acquisitions of heterozygous, hetero-homozygous 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, prodomal 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 JH kinase part leading to enhanced activated of the normal 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 Contitutively 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, prodomal 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 from heterozygous normocellular ET and latent PV mimicking ET labelled as prodomal PV or forme fruste PV followed by heterozygous/homozygous mutated erythrocythemic and early PV, and homozygous mutated advanced PV and post-PV myelofibrosis (Figures 1,2, Table 1), [8,21,62]. The JAK2V617F mutated trilinear MPN phenotypic expression includes normocellular ET, prodomal PV, erythrocythemic PV with normal platelet and leukocyte count, classical PV, masked PV, and various degrees of splenomegaly and myelofibrosis (MF) [57,59] (Figure 1, Table 1). The quantitative JAK2V617F allele burden in neutrophils and in CD34+ cells from the same blood sample in 96 JAK2-positive MPN patients (17 ET, 64 PV and 15 MF mean follow-up 7 years) were below 50% in the majority of ET and about half of the PV patients [63]. The JAK2V617F, CD34+ allele burden were above 50% in about half of the PV patients and the majority of MF indicating advanced trilinear MPN disease burden. The neutrophil JAK2V617F allele burden is usually below 50% in ET and above 50% in PV and MF. The CD34+ allele burden is much lower than the neutrophil JAK2V617F allele burden in ET and early stage PV with no splenomegaly [64]. This is completely in line with the concept that some maturation of JAK2V617F mutated hematopoietic stem cells is needed to neoproliferate because of hypersensitivity megakaryopoesis to TPO and erythropoesis to EPO [64,65] (Figures 5,6, Table 1). The neutrophil JAK2V617F allele burden alone can overestimate the MPN disease burden at the bone marrow progenitor cell level in early stage ET and PV neoproliferative disease [63,64].

Bone marrow morphology of clustered medium to large mature megakaryocytes is similar in heterozygous-mutated JAK2V617F ET and homozygous-mutated JAK2V617F PV patients at time of diagnosis (Figures 2,3, Tables 3-5), [8,9,13,24,25,66]. The bone marrow in heterozygous JAK2V617F ET is normocellular with increased clustered large megakaryocytes (M) proliferation and no or slight increase of erythropoiesis [8,9,24,61,66-68]. The bone marrow in JAK2V617F PV patients with less than 50% mutation load is hypercellular (60-80%) due to increased erythropoiesis and megakaryopoesis in prodomal PV. Classical, masked and advanced stages of PV typically show a trilinear 90-100% hypercellular bone marrow [2] due to increased erythro-megakaryo-granulocytic (EMG, [15,17,18]). The JAK2V617F allele burden 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 mutation in acquired ET and autosomal dominant JAK2 or TPO mutated hereditary ET (HET) 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 5.8x1012/L and increased activated platelet and leukocyte counts (Figures 6, Table 1) [8,9,24,35,36,57,60,70,72].

The JAK2V617F allele burden was directly correlated with increased levels of hematocrit, neutrophil count, LDH and leukocyte 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 in figures 1 and 2 [77], Table 4, [31,32] followed by post-PV myelofibrosis 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 genotypes 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 prognostic significance [80-84]. The presence of epigenetic factors like TET2 or ASXL1 etc 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, NFE2, 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 distinct 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 & 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, paraesthesias 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 exon 12 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 JAK2 exon 12 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 colony-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 pathophysiologies of myeloid metaplasia and myelofibrosis in advanced stage of trilinear MPN.
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 JAK2exon12 mutated thrombocytopenia 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 acrocyanosis, erythromelalgia and acrocyanotic ischemia or gangrene together with the episodic and transient neurologic symptoms of migraine accompaniments, attacks of amnesia, dysasia, dysphasia, TIAs, hemiparesis and acute coronary ischemic syndromes all are the consequence of one underlying disorder: arterial thrombophilia causally linked to JAK2exon12 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 thrombocytopenia 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 [20-22,27,28,35,36,86]. JAK2exon12 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 JAK2exon12, MPL and TPO mutated thrombocytopenias [35,36].

**JAK2exon12 mutations as cause of Isolated Erythrocytosis and PV**

The finding of the JAK2exon12 mutations in the 5% PV patients negative for JAK2exon12 usually present with early stage PV or isolated erythrocytosis (IE, Figure 8) with increased red cell mass but normal leukocytes and platelets and no palpable spleen [95-98]. The frequency of JAK2exon12 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. JAK2exon12 mutated MPN patients with increased erythrocytes above 6x10^12/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 JAK2exon12 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 JAK2exon12 mutated IE or PV have erythrocytes above 6x10^12/L [100], normal platelet and leukocyte 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 JAK2exon12 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 JAK2 K539L-type and E543del-type exon 12 mutations (Figure 8) [78]. Godfrey, et al. [78], assessed the colony genotypes for 18 patients with JAK2exon12-mutated PV in a total of 1931 colonies; mean 107 per patient (Figure 8C).

Example sequence traces for patients with homozygous JAK2exon12 mutations in colonies are shown in figure 8D. In total, 16 patients (5 “heterozygous-only” JAK2exon12-positive PV patients, 4 JAK2exon12-positive PV patients with homozygous and heterozygous clones, 3 JAK2exon12-positive ET patients with small homozygous clones, and 4) JAK2exon12-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 splanomegaly in 5 (17%), and no PV features in blood 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 JAK2exon12 PV (Figure 9), and distinct from JAK2exon12 ET and prodromal PV (Figures 2,3,9) and distinct from CALR thrombocytopenia (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 hyper celluar thrombocytopenia related to primary megakaryocytic
myeloiplofication (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 thrombocytemia and PMF by Kralovics and his team [105] was identified by Michiels & De Ravee [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 thrombocythemia 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, 1bp deletion in 1 and recurrent 5-bp insertion in 4
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

MF patients. 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 JAK2V617F or MPL515 mutated MPN 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].

Incorporation of 2008 WHO into 2020 clinical, laboratory, molecular and bone marrow pathology (CLMP) classification of myeloproliferative neoplasms

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 prodromal PV [62]. The prognosis of JAK2V617F mutated ET and prodromal PV is favorable and to be treated with low aspirin and additional phlebotomy in early PV to maintain hct 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, prodromal PV and PV.

Bone marrow histology analysis of bone marrow biopsies by Michiels & De Raeve from the Vannucchi’s study on WHO defined MPL515 mutated ET revealed that clustered large to giant maure 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, promdromal 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
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 5.0x1012/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 5.8x1012/L, normal platelet counts of less than 350x109/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 monolinear 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].

<table>
<thead>
<tr>
<th>337 MPN patients</th>
<th>JAK2V617F</th>
<th>JAK2exon12</th>
<th>CALR</th>
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<tr>
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<td>268</td>
<td>7</td>
<td>56</td>
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<tr>
<td>% of 337</td>
<td>80%</td>
<td>2.1%</td>
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<td>Range</td>
<td>22-89</td>
<td>46-76</td>
<td>20-89</td>
</tr>
<tr>
<td>Males (%)</td>
<td>45.5%</td>
<td>28.6%</td>
<td>41.1%</td>
</tr>
<tr>
<td>Hemoglobin g/dL</td>
<td>14.7</td>
<td>18.3</td>
<td>12.6</td>
</tr>
<tr>
<td>Range</td>
<td>6.2-22.6</td>
<td>13.7-21.1</td>
<td>7.5-16.1</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>43.9</td>
<td>49.9</td>
<td>38.4</td>
</tr>
<tr>
<td>Range</td>
<td>19.7-69.1</td>
<td>46.2-59.3</td>
<td>22.9-47.0</td>
</tr>
<tr>
<td>Red Blood cells</td>
<td>5.01012/L</td>
<td>6.91012/L</td>
<td>4.2 1012/L</td>
</tr>
<tr>
<td>Range</td>
<td>1.89-9.72</td>
<td>5.83-8.50</td>
<td>2.25-5.32</td>
</tr>
<tr>
<td>Platelets 109/L</td>
<td>650</td>
<td>281</td>
<td>898</td>
</tr>
<tr>
<td>Range</td>
<td>13-3268</td>
<td>58-310</td>
<td>49-1795</td>
</tr>
<tr>
<td>Leukocytes 109/L</td>
<td>12.0</td>
<td>8.2</td>
<td>8.6</td>
</tr>
<tr>
<td>Range</td>
<td>2.2-177</td>
<td>6.2-22</td>
<td>4.8-31</td>
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2008/16 WHO Class

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<thead>
<tr>
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<tbody>
<tr>
<td>PV N</td>
</tr>
<tr>
<td>ET N</td>
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<tr>
<td>PMF N</td>
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<th>BM CLMP Class</th>
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<tr>
<td>MET N</td>
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<table>
<thead>
<tr>
<th>Mutation burden</th>
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<tr>
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<tr>
<td>EM + EGM</td>
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<tr>
<td>M (ET)</td>
</tr>
<tr>
<td>GM (MF)</td>
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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. *CALR type 1 revealed a higher mutant allele burden (53%) compared to CALR type 2 (38%) MPN. JAK2V617F 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 MPL 515 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 JAK2 V617F and triple negative MPN disease (Figures 13,14), [100].

**Figure 13:** Comparing the survival curves of WHO defined PV, ET and PMF (upper curve) versus CLMP defined MPN without fibrosis (MF grade 0/1) versus with fibrosis (MF grade 2/3) in JAK2, CALR and triple negative MPN demonstrates that fibrosis is a measurable and objective adverse prognostic factor when associated with JAK2V617F and triple negative MPN disease (bottom). [Kim, et al. 2016] [100].

**Figure 14:** Clinical Laboratory Molecular and Pathobiological (2015-2020 CLMP) translational states of JAK2 mutated Myeloproliferative Neoplasms: MPN versus EPO receptor dominant congenital erythrocytosis caused by erythrocytosis (E); Transition of heterozygous into homozygous JAK2V617F mutation due to mitotic recombination is associated with a change of clinical ET phenotype into PV and MF phenotype of trilinear myeloproliferative neoplasm (MPN) during life long follow-up (Dameshek 1950, [2,57,58,60,21,31,32] James, et al. 2005, Kralovics, et al. 2005, Villeval, et al. 2006, Michiels, et al. 2006, Bellucci & Michiels, 2006, Michiels, et al. 2015ab). CALR or MPL515 thrombocythemia and bone marrow fibrosis (BMF) are distinct MPNs that mutually exclude each other and do not have PV features seen in JAK2V617F trilinear MPN and JAK2exon 12 E or PV.

### 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 incorporate 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 (ICARM.PN 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].

### Acknowledgement

The authors are grateful to Alexander Georgii, Juergen Thiele, Ayalew Tefferi, Alessandro Venucchi, Tiziano, Barbui, Stefan Constantinescu, William Vainchenker, Anthony Green, Radek Skoda, Robert Kralovics, Martin Griesshammer, Heinz Gisslinger, Hans Hasselbalch, Jiri Schwarz and Jean Jacques Kiladjian for their original and expert contributions to our current understanding of the JAK2V617F, JAK2 exon12, CALR and MPL515 mutated myeloproliferative neoplasms (MPNs).
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Novel European Asiatic Clinical, Laboratory, Molecular and Pathobiological (2015-2020 CLMP) criteria for JAK2*V617F trilinear polycythemia vera (PV), JAK2*V617F PV and JAK2*V617F, CALR and MPL*515 thrembocythemia: From Dameshek to Constantinescu-Vainchenker, Kralovics and Michiels


