



**QUEEN'S  
UNIVERSITY  
BELFAST**

## **A guideline for the diagnosis and management of polycythaemia vera. A British Society for Haematology Guideline**

BSH Committee (2018). A guideline for the diagnosis and management of polycythaemia vera. A British Society for Haematology Guideline. *British Journal of Haematology*. <https://doi.org/10.1111/bjh.15648>

**Published in:**  
British Journal of Haematology

**Document Version:**  
Peer reviewed version

**Queen's University Belfast - Research Portal:**  
[Link to publication record in Queen's University Belfast Research Portal](#)

**Publisher rights**  
© 2018 John Wiley & Son.  
This work is made available online in accordance with the publisher's policies. Please refer to any applicable terms of use of the publisher.

**General rights**  
Copyright for the publications made accessible via the Queen's University Belfast Research Portal is retained by the author(s) and / or other copyright owners and it is a condition of accessing these publications that users recognise and abide by the legal requirements associated with these rights.

**Take down policy**  
The Research Portal is Queen's institutional repository that provides access to Queen's research output. Every effort has been made to ensure that content in the Research Portal does not infringe any person's rights, or applicable UK laws. If you discover content in the Research Portal that you believe breaches copyright or violates any law, please contact [openaccess@qub.ac.uk](mailto:openaccess@qub.ac.uk).

1 **Title**

2 A guideline for the diagnosis and management of polycythaemia vera

3 A British Society for Haematology Guideline

4 **Authors**

5 McMullin MF<sup>1</sup>, Harrison CN<sup>2</sup>, Ali S<sup>3</sup>, Cargo C<sup>4</sup>, Chen F<sup>5</sup>, Ewing J<sup>6</sup>, Garg M<sup>7</sup>, Godfrey

6 A<sup>8</sup>, Knapper S<sup>9</sup>, McLornan D<sup>2</sup>, Nangalia J<sup>10</sup>, Sekhar M<sup>11</sup>, Wadelin F<sup>12</sup>, Mead AJ<sup>13</sup>.

7 Authors' affiliations

8 1, Centre for Medical Education, Queen's University, Belfast.

9 2. Guy's and St Thomas' NHS Foundation Trust, London

10 3. [Castle Hill Hospital, Hull and East Yorkshire Hospitals NHS Trust](#)

11 4. Leeds Teaching Hospitals NHS Trust, Leeds.

12 5. The Royal London Hospital, Bart's Health NHS Trust, London.

13 6. Birmingham Heart of England NHS Foundation Trust, Birmingham

14 7. University Hospital of Leicester NHS Trust, Leicester (BSH representative)

15 8. Department of Haematology and Haematopathology and Oncology Diagnostic

16 Service, Cambridge University Hospitals NHS Foundation Trust, Cambridge.

17 9. Cardiff University School of Medicine

18 10. Wellcome Trust Sanger Institute, Hinxton, Cambridge, CB10 1SA.

19 11. Royal Free London NHS Foundation Trust, London.

20 12. Nottingham University Hospital, Nottingham.

21 13. MRC Weatherall, Institute of Molecular Medicine, University of Oxford.

1 Correspondence to

2 BSH Administrator, British Society for Haematology, 100 White Lion Street, London  
3 N1, 9PF, UK. Email: [bsguidelines@b-s-h.org.uk](mailto:bsguidelines@b-s-h.org.uk)

4 **Keywords:** Diagnosis, management, polycythaemia vera, risk stratification,  
5 cytoreductive therapy

## 6 **Methodology**

7 This guideline was compiled according to the BSH process at [b-s-h.org.uk](http://b-s-h.org.uk). The  
8 Grading of Recommendation Assessment, Development and Evaluation (GRADE)  
9 nomenclature was used to evaluate levels of evidence and to assess the strength of  
10 the recommendations. The GRADE criteria can be found at  
11 <http://www.gradeworkinggroup.org>.

## 12 *Literature review details*

13 The literature review was conducted on 2<sup>nd</sup> March 2017. Databases searched  
14 include MEDLINE(OVID), Embase (OVID) and CENTRAL(The Cochrane library)  
15 using search terms (and relevant MESH terms) polycythaemia vera, erythrocytosis,  
16 familial, high oxygen affinity haemoglobin, defects of oxygen sensing pathway,  
17 diagnosis, investigation, molecular, mutation, *JAK2,MPL, CALR*, bone marrow, red  
18 cell mass, erythropoietin, risk, management, treatment, cytoreduction, venesection,  
19 hydroxyurea, interferon, busulfan, pipobroman, radioactive phosphorus, aspirin,  
20 anagrelide, ruxolitinib, thrombosis, haemorrhage, pregnancy, pruritus, surgery and  
21 management. The search covered the period from 2005 the date of last version of  
22 the guideline, to February week 3 2017. Exclusions included articles not in English,  
23 studies not in humans, single case reports and case series of under 5 cases. A total

1 of 6062 articles were identified which with exclusions and duplications resulted in  
2 1215 articles which were reviewed.

### 3 *Review of manuscript*

4 Review of the manuscript was performed by the British Society for Haematology  
5 (BSH) Guidelines Committee General Haematology Task Force, the BSH Guidelines  
6 Committee and the General Haematology Sounding Board of BSH. It was also on  
7 the members section of the BSH website for comment. A patient representative from  
8 MPN-Voice ([www.mpnvoice.org.uk](http://www.mpnvoice.org.uk)) participated in the guideline writing meeting. The  
9 guideline has been reviewed by MPN-Voice; this organisation does not necessarily  
10 approve or endorse the contents.

### 11 **Introduction**

12 The previous guideline was published in 2005 (McMullin, Bareford et al. 2005) with  
13 an amendment in 2007 (McMullin, Reilly et al. 2007) to update the diagnostic criteria  
14 following the discovery of the *JAK2* mutation in patients with polycythaemia vera  
15 (PV). Since that time there has been a considerable amount of research in the area  
16 concerning diagnostics, risk stratification, new agents and reinvestigation of existing  
17 agents. It was therefore decided to evaluate the literature to formulate guidance on  
18 the diagnostic pathway for erythrocytosis, risk stratification of polycythaemia vera  
19 (PV), management of PV including specific situations and the management of  
20 secondary erythrocytosis. Here we provide evidence-based guidance on diagnosis,  
21 risk stratification and management of PV. Our review of the evidence led us to some  
22 differences in diagnostic criteria and risk stratification than have been proposed by  
23 other international organisations. We discuss the reasons for this. An accompanying

1 guideline looks at management of specific situations in PV and management of  
2 secondary erythrocytosis.

### 3 **The diagnostic pathway for investigation of an erythrocytosis**

4 Patients with a persistently raised venous haematocrit (Hct) (>0.52 males, >0.48  
5 females) should be investigated. As suggested in our previous guideline and  
6 confirmed in recent literature, Hct has been consistently shown to perform better in  
7 identifying patients with a raised red cell mass than haemoglobin concentration  
8 (Alvarez-Larrán, Pereira et al. 2012, Ancochea, Alvarez-Larrán et al. 2014).

9 Patients should be investigated according to the proposed algorithm (Fig. 1).

10 Investigation requires knowledge of diagnostic criteria for both PV and potential  
11 secondary causes of erythrocytosis (Tables I and II). A detailed history, examination  
12 and stage 1 investigations (listed below) should identify a potential cause in the  
13 majority of patients, although a proportion will require more extensive testing and in  
14 some a cause cannot be found (idiopathic erythrocytosis). The potential for dual  
15 pathology should also be considered.

### 16 **Initial assessment**

#### 17 *Clinical History and Examination*

18 A detailed clinical history and examination are essential and, in the absence of a  
19 molecular marker of disease, will determine further investigations and management.  
20 Particular attention should be paid to the drug history (prescribed and recreational),  
21 smoking, alcohol consumption and body habitus. Systematic questioning should  
22 elicit symptoms related to other potential secondary causes of erythrocytosis (see  
23 Table II). A proportion of patients, who have a clear secondary cause for their  
24 erythrocytosis, may not need any further investigations.

## 1 **Stage 1 Investigations**

### 2 *Full blood count / blood film*

3 The full blood count analysis will not only confirm a raised Hct but will also identify  
4 neutrophilia and thrombocytosis, which are common in *JAK2* V617F-positive PV and  
5 part of the criteria for *JAK2*-negative PV (Table 1). As smokers have a significantly  
6 higher neutrophil count than non-smokers (Whitehead, Robinson et al. 1995) a  
7 neutrophilia is defined as  $>12.5 \times 10^9/l$  in this patient group.

8 A blood film should be reviewed in all patients to look for any atypical features. In  
9 those with confirmed PV, abnormalities such as circulating blasts, leucoerythroblastic  
10 features and monocytosis would be indications for bone marrow assessment.

### 11 *Renal and Liver Function*

12 A number of renal and hepatic diseases can cause erythrocytosis. Serum calcium  
13 levels should also be determined to exclude a parathyroid adenoma/carcinoma,  
14 which rarely causes secondary erythrocytosis.

### 15 *Arterial oxygen saturation (SaO<sub>2</sub>) / carboxyhaemoglobin*

16 Identifying tissue hypoxia, a cause of secondary erythrocytosis, can be achieved  
17 most simply by using pulse oximetry in the clinic. An SaO<sub>2</sub> of <92% has been  
18 shown to be associated with an absolute erythrocytosis (Berlin 1975). Clinicians  
19 should however be aware of three situations of hypoxic erythrocytosis where this  
20 testing is unreliable and will give a normal result. These are carbon monoxide  
21 poisoning, high oxygen affinity haemoglobins and sleep apnoea syndrome. Those  
22 with suspected high oxygen affinity haemoglobins should undergo genetic testing as  
23 described below. In those with suspected sleep apnoea (heavy snoring with daytime

1 somnolence or increased body mass index  $>30 \text{ kg/m}^2$ ), referral should be made to a  
2 respiratory or sleep physician.

3 Carboxyhaemoglobin (COHb) levels are significantly higher in smokers compared  
4 with non-smokers and cigarette consumption has been shown to be directly related  
5 to COHb levels (Castleden and Cole 1975). Testing can therefore be performed at  
6 baseline where smoking is suspected.

### 7 *Serum Ferritin*

8 Low serum ferritin levels are common in PV patients and iron deficiency can mask  
9 the presentation of PV giving a misleadingly low Hct because iron deficiency limits  
10 erythropoiesis and hypochromic microcytosis develops.

### 11 *Serum Erythropoietin*

12 Erythrocyte production is controlled by the hormone erythropoietin (EPO).  
13 Measurement of serum EPO can provide information on potential causes of  
14 erythrocytosis and help stratify further testing (see Fig.1). EPO levels are commonly  
15 high in hypoxic conditions or when erythrocytosis is secondary to exogenous  
16 administration or endogenous overproduction. In contrast EPO levels are typically  
17 low in PV, although their diagnostic utility in this setting is limited in the era of *JAK2*  
18 mutation testing (Ancochea, Alvarez-Larrán et al. 2014).

### 19 *JAK2 V617F mutational analysis*

20 The identification of *JAK2* mutations in almost all PV patients has revolutionised the  
21 diagnosis of PV. The *JAK2 V617F* mutation can be found in over 95% of PV  
22 patients (James, Ugo et al. 2005) and an exon 12 mutation in most remaining  
23 patients (Scott, Tong et al. 2007). Testing for *JAK2 V617F* in peripheral blood is

1 sensitive and bone marrow samples are not required to identify this (Takahashi,  
2 Patel et al. 2013). Testing for *JAK2 V617F* is advised as a stage 1 investigation and  
3 should confirm the diagnosis the vast majority of PV patients. Separate guidance is  
4 available for assays used for detection of *JAK2* mutations (Bench, White et al. 2013).

### 5 **Further Investigations in *JAK2 V617F*-negative erythrocytosis**

6 Further investigations are warranted in those patients with a persistent, significant  
7 erythrocytosis if *JAK2 V617F* studies are negative and a secondary cause is not  
8 immediately apparent (See Figure 1). Secondary causes must be considered since  
9 PV is rare in the absence of a *JAK2 V617F* mutation.

#### 10 *Red Cell Mass studies*

11 Patients with Hct >0.60 (males) or >0.56 (females) can be assumed to have an  
12 absolute erythrocytosis, but in others red cell mass (RCM) studies can be helpful to  
13 confirm an absolute erythrocytosis. A RCM more than 25% above the mean  
14 predicted value is diagnostic of an absolute erythrocytosis (Pearson, Guthrie et al.  
15 1995). Those with a raised Hct but a RCM within the normal range have an apparent  
16 erythrocytosis. A relative erythrocytosis, found in states of dehydration, can be  
17 confirmed when the RCM is within the normal range and plasma volume is below  
18 normal. Patients with a relative or apparent erythrocytosis require no further  
19 investigation. It is noted however that due to the many drawbacks of this test  
20 including cost and labour, access to RCM studies is variable nationally.

#### 21 *Abdominal Ultrasound*

22 Radiological splenomegaly is a minor criterion for *JAK2 V617F*-negative PV (Table  
23 1) and ultrasound is the simplest method for detection. Abdominal ultrasound can



1 also exclude secondary causes of erythrocytosis, particularly renal and hepatic  
2 pathology including hepatocellular carcinoma. .

3 Further testing can be stratified according to the EPO level measured during stage 1  
4 investigations.

#### 5 **Normal or low EPO level**

##### 6 *JAK2 exon 12 analysis*

7 Compared with *JAK2* V617F, patients with exon 12 mutated- PV tend to be younger,  
8 with higher haemoglobin concentrations, lower white blood cell (WBC) and platelet  
9 counts, and isolated an isolated increase in erythropoiesis without granulocytic or  
10 megakaryocytic morphological abnormalities (Scott, Tong et al. 2007, Passamonti,  
11 Elena et al. 2011). In contrast to *JAK2* V617F testing, a discrepancy between exon  
12 12 mutant allele burden in bone marrow and peripheral blood has occasionally been  
13 described (Kjær, Westman et al. 2012).

##### 14 *Bone marrow biopsy*

15 Bone marrow histology may be helpful in distinguishing PV from secondary  
16 erythrocytosis (Thiele, Kvasnicka et al. 2005). Bone marrow aspiration in PV typically  
17 reveals markedly increased erythropoiesis with moderate to marked increase in  
18 granulopoiesis and megakaryopoiesis; widely variable megakaryocyte size, including  
19 large forms with hyperlobated nuclei; and absent iron stores. The bone marrow  
20 trephine biopsy sections show hypercellularity; trilineage expansion of haemopoiesis  
21 (rarely preferentially erythroid) and normoblastic erythropoiesis. Granulocytic  
22 maturation may be left-shifted and disorderly; megakaryocytes show increased  
23 variation in size, often with a predominance of large forms with uneven or reduced

1 nuclear lobulation, and megakaryocyte clusters are common. Reticulin is increased  
2 in a minority of patients (up to WHO grade 1 in most cases).

3 Presence of an acquired genetic abnormality is a major criterion for *JAK2*- negative  
4 PV and the presence of an abnormal karyotype can therefore support this diagnosis.  
5 An acquired *LNK* mutation would also support the diagnosis. More recently  
6 mutations in a number of other genes, most commonly *TET2* and *DNMT3A*, have  
7 been reported in PV (Delic, Rose et al. 2016) These gene mutations are not,  
8 however, disease specific and have also been reported in healthy individuals  
9 (Genovese, Kähler et al. 2014, Jaiswal, Fontanillas et al. 2014) limiting their  
10 application in the diagnostic setting, particularly when found in isolation.

### 11 **High EPO level**

12 A raised EPO level should lead to a thorough search for secondary causes of  
13 erythrocytosis (Table 2), which may require additional supplementary investigations  
14 (Figure 1).

### 15 *Imaging*

16 Further imaging (e.g. CT head and neck) is indicated in this setting if a cause for the  
17 high EPO has not been identified. Imaging should aim to exclude rare tumours such  
18 as a cerebellar haemangioblastoma, pheochromocytoma, meningioma or a  
19 parathyroid tumour, all of which can rarely cause erythrocytosis.

### 20 **Gene sequencing for congenital erythrocytosis**

21 A number of germline mutations in genes involved in oxygen sensing, erythropoiesis  
22 and oxygen transport have now been implicated in patients with otherwise  
23 unexplained erythrocytosis. These include mutations in the *EPO* receptor and genes

1 in the oxygen sensing pathway (*VHL*, *EGLN1*, *EPAS1*), high oxygen affinity  
2 haemoglobinopathies caused by mutations in the globin genes *HBA1*, *HBA2*, *HBB*,  
3 and 2,3-bisphosphoglycerate deficiency as a result of *BPGM* mutations (Bento,  
4 Percy et al. 2014). These patients can present with low, normal or high EPO levels;  
5 high affinity haemoglobins and 2,3-BPG deficiency also cause a left shift of the  
6 oxygen dissociation curve. In the past this group of mutations were detected by  
7 Sanger sequencing of individual genes, in an order directed by EPO levels and P50  
8 analysis, but this approach is labour-intensive and time-consuming (Bento, Percy et  
9 al. 2014). More recently next generation sequencing-based targeted panels have  
10 been developed to assess established and novel genes implicated in erythrocytosis  
11 (Camps, Petousi et al. 2016), negating the need for P50 testing. A targeted panel  
12 should be performed in patients in whom congenital erythrocytosis is suspected,  
13 particularly young patients or those with a family history.

#### 14 **Diagnostic criteria for polycythaemia vera**

##### 15 ***Presentation***

16 Polycythaemia vera (PV) presents at a median age of 60 years with a slight male  
17 predominance. Patients can present with arterial or venous vascular occlusive  
18 events, microvascular disturbances, or occasionally, haemorrhage. Splenic pain  
19 and/or enlargement, pruritus, gout and constitutional symptoms such as fatigue may  
20 be present. Alternatively, asymptomatic patients may be identified incidentally  
21 following a full blood count. All patients who are newly diagnosed with PV should be  
22 discussed in a multidisciplinary team setting.

23 The recommended diagnostic criteria for *JAK2*-positive and the very rare *JAK2*-  
24 negative PV are given in full in table I.

1 **Role of the bone marrow biopsy in *JAK2 V617F*-positive patients**

2 Although the WHO classification considers histology to be useful in distinguishing PV  
3 from other myeloproliferative neoplasms (MPNs) (Arber, Orazi et al. 2016), several  
4 studies have reported high rates of non-consensus or failure to reach a histological  
5 diagnosis in patients with PV (Koopmans, Bot et al. 2011, Madelung, Bondo et al.  
6 2013) (Alvarez-Larrán, Ancochea et al. 2014) Given the uncertain utility of bone  
7 marrow histology in the diagnosis of uncomplicated PV, it is not mandatory in all  
8 patients, but should be considered if there are atypical features such as marked  
9 splenomegaly or a history of splanchnic vein thrombosis where it is necessary to  
10 establish if there is an occult myeloproliferative neoplasm. The degree of baseline  
11 fibrosis can also be ascertained, which as discussed below may have a prognostic  
12 role. Bone marrow biopsy may nonetheless be useful in those patients likely to have  
13 a long disease history, as a baseline sample for comparison in the event of  
14 suspected disease transformation. Abnormal karyotype and other molecular  
15 abnormalities (e.g. *TET2* mutations) have been reported in PV and some may have  
16 prognostic value (Delic, Rose et al. 2016, Cerquozzi, Barraco et al. 2017) but these  
17 tests are not required routinely at diagnosis.

18 **Differentiation of *JAK2 V617F*-positive PV from other MPNs**

19 In patients with a *JAK2 V617F* mutation, haemoglobin and/or Hct are currently used  
20 as a surrogate for RCM to distinguish between PV and essential thrombocythaemia  
21 (ET) (Arber, Orazi et al. 2016), but Hct has the better accuracy in predicting red cell  
22 mass (Alvarez-Larrán, Pereira et al. 2012, Ancochea, Alvarez-Larrán et al. 2014).  
23 Concerns have however been raised that distinguishing PV from ET based on blood

1 count thresholds alone may fail to identify a subgroup of patients with “masked” PV,  
2 who may be better managed as PV rather than ET.

3 The definition of masked PV has been inconsistent across studies. When using a  
4 raised RCM to define an erythrocytosis, studies have shown that a Hct threshold of  
5 0.52 in males will fail to identify approximately 20% of male patients with a raised  
6 RCM, whilst the threshold of 0.48 in women is more sensitive (Alvarez-Larrán,  
7 Pereira et al. 2012). These “masked” PV patients were reported to have similar  
8 outcomes to those with “overt” PV when managed equivalently (Alvarez-Larrán,  
9 Angona et al. 2016).

10 By contrast, other studies have defined masked PV as those patients who did not  
11 meet the haemoglobin-based thresholds for PV but did meet the other WHO criteria,  
12 mainly bone marrow histology and JAK2 status. Patients meeting this definition had  
13 poorer outcomes in terms of myelofibrotic or leukaemic transformation and survival,  
14 but no difference in rate of thrombosis. Hct thresholds of 0.48/0.49 (females/males)  
15 were subsequently proposed to discriminate ET from PV in the WHO 2016 revision  
16 (Barosi, Tefferi et al. 2015).

17 The proposed BSH Hct-based thresholds have good specificity but will miss a  
18 minority of patients with a raised RCM. By lowering the Hct threshold it may be  
19 possible to identify patients with histology more typical of PV who may have certain  
20 adverse outcomes, but these findings have not yet been reproduced independently.  
21 It is unknown whether management of any of these patients using a strict Hct target  
22 benefits their vascular risk. However, in patients with a *JAK2* V617F mutation and  
23 borderline Hct levels (especially males with Hct 0.48-0.52), the possibility of true  
24 erythrocytosis should be considered, especially if the patient is at high risk of

1 vascular events. Options in this group include performing a RCM study to clarify the  
2 diagnosis or, pragmatically, managing the patient with a Hct target as for PV.

3 It should also be noted that Hct is a poor surrogate of red cell mass in patients who  
4 have had splanchnic vein thrombosis (Lamy, Devillers et al. 1997), and these high-  
5 risk patients are best managed with standard blood count targets regardless of blood  
6 count parameters at diagnosis. Hct is also reduced by pregnancy and gestation-  
7 specific ranges should be used when considering the distinction between PV and ET  
8 in a patient presenting during pregnancy.

### 9 ***JAK2 V617F* allele burden**

10 Quantitative assessments of the *JAK2 V617F* allele burden in peripheral blood  
11 granulocytes have shown that this parameter tends to be higher in PV than ET. A  
12 higher mutant allele burden correlates with certain clinical features at presentation  
13 including higher haemoglobin levels, higher WBCs, lower platelet counts, lower  
14 mean cell volume (MCV), lower serum ferritin and EPO, more splenomegaly and  
15 more pruritus (Dupont, Massé et al. 2007, Tefferi, Strand et al. 2007, Vannucchi,  
16 Antonioli et al. 2007, Passamonti, Rumi et al. 2010). However, there is no validated  
17 threshold at which *JAK2 V617F* allele burden can confirm or refute a diagnosis of PV  
18 and this investigation is not recommended routinely.

### 19 **Low level *JAK2 V617F* allele burden**

20 A low level *JAK2 V617F* mutation (allele burden <1-3%) should be interpreted in the  
21 context of clinical, haematological and other laboratory findings (Bench, White et al.  
22 2013). If the result is reproducible and does not represent a false positive, this  
23 finding may provide support for a diagnosis of a PV in a patient with otherwise  
24 unexplained, significant erythrocytosis (Perricone, Polverelli et al. 2017). However,

1 the *JAK2* V617F mutation has been identified in normal individuals, often at a low  
2 allele burden, with a frequency that increases with age (Genovese, Kähler et al.  
3 2014, Jaiswal, Fontanillas et al. 2014). Caution is therefore warranted and  
4 comprehensive investigations should exclude an alternative secondary or congenital  
5 cause of erythrocytosis. The test should preferably be repeated within 3-6 months,  
6 and clinical assessment for other features of an MPN e.g. splenomegaly, bone  
7 marrow histological features, and screening for an additional mutation in *JAK2* exon  
8 12 may be helpful.

#### 9 **Recommendations:**

- 10 • **In patients with persistent, significant and unexplained erythrocytosis,**  
11 **testing for *JAK2* V617F is recommended, using a peripheral blood**  
12 **sample and an assay sufficiently sensitive to detect a mutant allele**  
13 **burden as low as 1-3%.**

14 **(GRADE 1B)**

#### 15 **Risk stratification in PV**

16 The principal aims of risk stratification in PV are a) to select patients at higher risk of  
17 thrombosis for consideration of cytoreductive therapy and b) to provide the most  
18 accurate information to patients on the risks and implications of a diagnosis of PV.

#### 19 **Thrombosis and Bleeding Risk**

20 At diagnosis, in the largest prospective study to date the European Collaboration on  
21 Low-Dose Aspirin in Polycythaemia Vera (ECLAP), age  $\geq$  65 years and a prior  
22 history of thrombosis were found to be the most important predictors of  
23 cardiovascular events (Marchioli, Finazzi et al. 2005). A baseline WBC of  $>15.0 \times$

1  $10^9/l$  is a significant predictor of thrombosis, particularly an increased risk of  
2 myocardial infarction (Landolfi, Di Gennaro et al. 2007); however, prognostic models  
3 including leucocytosis have not been prospectively validated. Cardiovascular risk  
4 factors (smoking, diabetes mellitus, arterial hypertension, hypercholesterolaemia)  
5 also contribute to thrombotic risk in PV (Barbui, Vannucchi et al. 2017) (Landolfi, Di  
6 Gennaro et al. 2007) (Gangat, Strand et al. 2007). Once treatment is initiated,  
7 cardiovascular events occur more frequently in patients with less stringent Hct  
8 control (Marchioli, Finazzi et al. 2013) and when the WBC remains elevated  $>11 \times$   
9  $10^9/l$  (Barbui, Masciulli et al. 2015). A relationship between thrombocytosis (either at  
10 diagnosis or follow-up) and thrombotic risk has not been established in PV (Di Nisio,  
11 Barbui et al. 2007), but extreme thrombocytosis ( $\geq 1500 \times 10^9/l$ ) is associated with  
12 increased risk of bleeding due to acquired von Willebrand disease and should be  
13 considered an indication for initiation of cytoreductive therapy (Budde and van  
14 Genderen 1997).

## 15 **Survival and Transformation Risk**

16 The impact of age, degree of leucocytosis and prior history of venous thrombosis on  
17 long term prognosis are well-established. In the ECLAP study, age  $>65$  years was  
18 associated with inferior survival and age  $>70$  years was associated with increased  
19 incidence of leukaemia/myelodysplasia (Marchioli, Finazzi et al. 2005). In the IWG-  
20 MRT study, age  $>61$  years was associated with inferior overall and leukaemia-free  
21 survival (Tefferi, Rumi et al. 2013). Longer disease duration has been associated  
22 with increased risk of myelofibrotic (MF) transformation (Marchioli, Finazzi et al.  
23 2005). Leucocytosis with  $WBC \geq 15 \times 10^9/l$  is associated with an inferior leukaemia-  
24 free survival. A prior history of venous thrombosis also impacts negatively on overall  
25 survival. The IWG-MRT Prognostic Score uses these three parameters (age, WBC



1 and thrombotic history) to delineate distinct risk groups for overall survival (Tefferi,  
2 Rumi et al. 2013), but this prognostic score has not been independently validated in  
3 prospective studies.

4 Several other clinical and laboratory variables have been reported to influence  
5 overall survival and/or risk of disease transformation. The presence of splenomegaly  
6 in PV patients has been associated with shorter overall survival and increased risk of  
7 transformation to both MF and AML (Abdulkarim, Ridell et al. 2011). The presence of  
8 an abnormal karyotype adversely impacts overall and leukaemia-free survival  
9 (Tefferi, Rumi et al. 2013). A raised lactate dehydrogenase (LDH) and the presence  
10 of reticulin fibrosis at diagnosis predict a higher rate of transformation to  
11 myelofibrosis (MF) but not to acute myeloid leukaemia (AML) (Alvarez-Larrán,  
12 Bellosillo et al. 2009) (Barbui, Thiele et al. 2012). Prospective analysis indicates that  
13 a *JAK2* mutant allele burden of >50% is also associated with increased risk of MF  
14 (but not of AML or thrombosis) (Passamonti, Rumi et al. 2010) but the clinical utility  
15 of this measurement is not yet well-established. Although *JAK2* exon12-mutated  
16 disease has a subtly different clinical phenotype to *JAK2* V617F-driven PV (higher  
17 haemoglobin concentration, lower WBC), there appears to be no difference in long-  
18 term prognosis (Passamonti, Elena et al. 2011). Targeted gene sequencing is a  
19 rapidly advancing area; approximately 15% of PV patients have mutations of ≥1 of  
20 *ASXL1*, *SRSF2* and *IDH2* and these patients have a reduced rate overall survival in  
21 univariate analysis (Tefferi, Lasho et al. 2016).

## 22 **Recommendations: Risk stratification**

- 23 • **Age and thrombotic history should be used to define risk groups for**  
24 **thrombosis in PV (GRADE 1A).**

- 1 • **‘High risk’**: age  $\geq 65$  years and/or prior PV-associated arterial or venous  
2 **thrombosis (GRADE1A)**
- 3 • **‘Low risk’**: age  $< 65$  years and no PV-associated thrombotic history  
4 **(GRADE 1A)**
- 5 • **Some ‘low risk patients’ may be to be considered at higher risk in the**  
6 **presence of cardiovascular risk factors, elevated WBC, extreme**  
7 **thrombocytosis or Hct uncontrolled with venesection (GRADE 1B)**
- 8 • **A number of variables including age, prior thrombosis, the presence of**  
9 **splenomegaly, serum LDH level, degree of reticulin staining, presence of**  
10 **an abnormal karyotype and *JAK2* mutant allele burden may be utilised**  
11 **when counselling the patient on longer term prognosis including overall**  
12 **survival and disease transformation risk (GRADE 2B).**
- 13 • **Deep sequencing for ‘high risk mutations’ e.g. *ASXL1*, *SRSF2*, *IDH1/2* is**  
14 **not yet ‘standard of care’ but may be considered in selected cases**  
15 **where their presence may influence management. (GRADE 2B).**

## 16 **Management of polycythaemia vera**

17 Patients with PV may present with thrombosis or cardiovascular disease. Disease-  
18 related PV symptoms such as microvascular disturbance, pruritus (which may be  
19 excruciating), migraine-type headache and fatigue may also be presenting features  
20 which can significantly impact on quality of life (Harrison, Koschmieder et al. 2017).  
21 However, patients may be asymptomatic at presentation.

22 The goals of treatment are to reduce complications and therefore improve survival.  
23 (Table III). Mortality is chiefly related to thromboembolic events and the principal aim  
24 of therapy is to reduce this risk. Targeted assessment and management of

1 cardiovascular risk factors such as hypertension, hypercholesterolaemia and  
2 diabetes mellitus and smoking is essential. Reduction in symptom burden is also a  
3 valid target for treatment. There is evidence that patients with inadequately  
4 controlled PV as determined by hydroxycarbamide (HC) use, splenomegaly and  
5 venesection requirements have a significantly higher symptom score measured by  
6 the MPN-SAF (Geyer, Scherber et al. 2016). Frequent need for concurrent  
7 venesection may indicate need for dose alteration and or change of treatment.

### 8 **Haematocrit target**

9 The target for Hct control in PV was originally based on data from assessment of  
10 numbers of vascular events at different Hct levels and it was estimated that a target  
11 below 0.45 should be maintained (Pearson and Wetherley-Mein 1978). This target  
12 has now been validated in a randomised clinical trial by the investigators of the  
13 CYTO-PV group who assessed the impact of stringent Hct reduction to <0.45  
14 compared with a more liberal target range of 0.45-0.50. Patients with a Hct target of  
15 < 0.45 had a significantly lower rate of cardiovascular death and major thrombosis  
16 than those with a target of 0.45-0.50 (Marchioli, Finazzi et al. 2013). It was noted  
17 that the median WBC was significantly lower in the low Hct group, which may have  
18 been related to variation in the use of cytoreductive therapy between the groups. The  
19 impact of this parameter on the difference in outcome between the groups has been  
20 debated (McMullin, Harrison et al. 2013).

21 The European LeukemiaNet (ELN) has by consensus recommended response  
22 criteria for PV . There is, however, little evidence that stringent achievement of these  
23 contributes to improved outcomes apart from the Hct target. These are a valuable

1 set of measures to assess treatment outcome with consistency across clinical trials  
2 but are not as useful in clinical practice (Barosi, Tefferi et al. 2015).

### 3 **Platelet and leucocyte target**

4 There is considerable published evidence that there is an association between  
5 increased WBC and thrombosis risk in PV (Barbui, Carobbio et al. 2009,  
6 Caramazza, Caracciolo et al. 2009) ((De Stefano, Za et al. 2010, Barbui, Masciulli et  
7 al. 2015) (Cerquozzi, Barraco et al. 2017). In contrast, one prospective study did not  
8 find such an association (Passamonti, Rumi et al. 2010). An analysis of long-term  
9 outcome of patients enrolled into the ECLAP study demonstrated that in patients  
10 with WBC  $>15 \times 10^9/l$  there was increased incidence of thrombosis in comparison  
11 with those with WBC  $<10 \times 10^9/l$ , largely related to an increase in myocardial  
12 infarction (Landolfi, Di Gennaro et al. 2007). In a retrospective study of PV to  
13 determine whether blood counts influenced the complication rate and survival, older  
14 age and elevated lactate dehydrogenase at diagnosis were found to be risk factors  
15 for vascular complications. When the vascular complication occurred, 41% of the  
16 patients with a complication had elevated WBC compared with 20% of patients  
17 without a complication (Enblom-Larsson, Girodon et al. 2017). The CYTO-PV study  
18 treatment arms which showed a lower thrombotic risk in those intensively managed  
19 to Hct  $<0.45$  showed a comparatively lower WBC which may have contributed to the  
20 lower rate of thrombotic events (Marchioli, Finazzi et al. 2013) (McMullin, Harrison et  
21 al. 2013). There is no evidence from randomised trials to determine whether  
22 treatment targeted at reducing leucocyte count impacts on overall outcome and  
23 therefore no recommendation to target WBC as a treatment goal can be made.  
24 Indeed, no evidence for improved survival or lower thrombosis risk was seen in

1 patients achieving complete or partial response according to ELN criteria in an  
2 analysis of PV patients treated with HC whereas a better prognosis was seen when  
3 there was a white cell and platelet response (Alvarez-Larrán, Pereira et al. 2012).  
4 There is evidence that at extremes of platelet count there is a risk for bleeding and  
5 haemorrhage which may necessitate cytoreductive treatment in those with high  
6 counts.

### 7 **Allele burden reduction**

8 There is currently no indication to monitor allele burden sequentially outside the  
9 clinical trial setting. Whilst many studies have used allele burden reduction to assess  
10 impact of treatment there is currently no clear clinical impact of this as a target. Allele  
11 burden over 50% may correlate with progression to myelofibrosis (Passamonti, Rumi  
12 et al. 2010) but there is no evidence that this alters outcome and no evidence that  
13 lowering allele burden alters outcome.

### 14 **Bone marrow response**

15 There is no indication that serial monitoring of bone marrow morphology or fibrosis  
16 grade is of value but this should be undertaken if there is suspected progression  
17 from blood counts or symptomatology.

### 18 **Venesection**

19 Randomised trial data supports venesection of 200 – 500 mls blood at intervals  
20 suitable for patient size/tolerability should be used to achieve and maintain Hct of  
21 <0.45 (Marchioli, Finazzi et al. 2013). In low risk patients this is usually adequate to  
22 maintain target Hct. Where frequent venesection is needed to achieve this target  
23 then an alternative approach using a cytoreductive agent may need to be  
24 considered. High levels of venesection requirement have been reported to have an

1 association with higher thrombosis risk in patients on HC, specifically in those  
2 patients requiring 3 or more venesections per year (Alvarez-Larrán, Pérez-Encinas  
3 et al. 2017).

4 No study has explicitly defined a gender difference in Hct target. A different target  
5 Hct in males and females is not recommended

6 Iron deficiency may result from venesection. Whilst generally this is asymptomatic,  
7 restless legs, concentration problems, impaired cognitive function, dizziness, fatigue,  
8 headaches and inactivity and other symptoms may warrant a different treatment  
9 approach. Iron administration must be undertaken with extreme caution and with  
10 close supervision and monitoring of blood counts. Severe symptoms may warrant an  
11 alternative approach such as cytoreductive therapy.

## 12 **Aspirin**

13 The value of low-dose aspirin in patients with PV was demonstrated in the ECLAP  
14 study. In this double blind, placebo-controlled randomised trial those randomised to  
15 aspirin 100 mg daily had significantly fewer vascular events at 3 years compared to  
16 placebo. There was a 60% decrease in the risk of the combined primary end-point  
17 which was of thrombotic events and death from cardiovascular causes. Major  
18 bleeding events were not significantly increased (Landolfi, Marchioli et al. 2004).

## 19 **Cytoreductive therapy**

20 High-risk patients should be considered for cytoreductive therapy. Low-risk patients  
21 who may benefit from cytoreduction include those with progressive splenomegaly,  
22 progressive leucocytosis (e.g. WBC  $>15 \times 10^9/l$ ) thrombocytosis (e.g. platelets  $>1500$   
23  $\times 10^9/l$ ) and poor tolerance of venesection.

## 1 *Hydroxycarbamide*

2 Hydroxycarbamide is a cytoreductive agent, a non-alkylating antimetabolite which  
3 acts through inhibition of ribonucleotide reductase thereby regulating the rate of DNA  
4 synthesis. HC has a dose-dependent effect and needs to be individually titrated to  
5 achieve optimal count control. The efficacy of HC in controlling blood counts and  
6 preventing thrombosis has been extrapolated from the evidence in ET (Cortelazzo,  
7 Finazzi et al. 1995) and HC has been used in the management of PV. A recent  
8 retrospective study of PV demonstrated that patients treated with HC experienced  
9 significantly fewer vascular complications (11%) than patients treated with  
10 venesection only with a survival advantage for patients treated with HC when  
11 adjusted for variables supporting the use of this agent in first-line treatment (Enblom-  
12 Larsson, Girodon et al. 2017).

## 13 *Side effects*

14 HC is generally well tolerated. Macrocytosis is expected. Myelosuppression is seen  
15 in some patients. Mucocutaneous side effects occur including ulceration in  
16 perimalleolar areas, oral aphthous ulceration, actinic keratosis, squamous cell  
17 cancer and other skin lesions. Gastrointestinal side effects have been reported.

## 18 *Leukaemogenic risk and risk of secondary malignancy*

19 There has been much debate and concern over the potential leukaemogenic risk of  
20 treatments used for PV and also the potential for secondary malignancies. The  
21 natural history of PV is that a proportion of patients will experience progression to  
22 acute leukaemia and myelofibrosis. There is currently no conclusive evidence that  
23 this risk is exacerbated by the use of HC alone. A recent large study European ET  
24 trial, the EXELS study compared anagrelide treated patient versus those treated with

1 other cytoreductive therapies and found there was a higher incidence of leukaemia  
2 and increased incidence of other cancers in those treated with other cytoreductive  
3 therapies including HC alone (Besses, Kiladjian et al. 2013) (Birgegård, Besses et al.  
4 2018). A Swedish population-based study with a nationwide MPN cohort, identified  
5 those who developed AML/myelodysplastic (MDS) and matched controls,  
6 retrospective case record analysis was undertaken. Whilst the risk of AML/MDS was  
7 increased in patients exposed to high doses of <sup>32</sup>P and alkylators or 2 or more  
8 cytoreductive agents, this was not seen in those patients treated exclusively with HC  
9 (Björkholm, Derolf et al. 2011). A long-term assessment from the ECLAP study  
10 showed no increased MDS/AML in those treated with HC alone (Finazzi, Caruso et  
11 al. 2005) and a retrospective analysis showed no association between HC or  
12 busulfan and AML (Tefferi, Rumi et al. 2013).

13 A higher incidence of second malignancies has been seen in a small cohort of  
14 patients treated with HC compared to interferon (INF) alone (Hansen, Sørensen et  
15 al. 2017), particularly non-melanoma skin cancers, and this has been seen in  
16 population-based studies with increased risk of non-melanotic skin cancer in  
17 patients treated with HC, especially in older patients of male sex (Gómez, Guillem et  
18 al. 2016). Another study looked at treatment characteristics of a large number of  
19 patients with ET, diagnosed and followed during a 30-year period. The different  
20 therapies administered, comprising HC and alkylating agents, did not appear to have  
21 any impact on the development of secondary malignancy with a similar rate of  
22 secondary malignancies in untreated patients. Male gender and age >60 years were  
23 the only factors that were correlated with higher risk (Santoro, Sperduti et al. 2017).

24 HC is recommended as a first line cytoreductive treatment option for all patients for  
25 whom this is required. The risk benefit profiles need to be discussed with patients.



1 HC is not safe in pregnancy and it is recommended that it be stopped 3 months prior  
2 to intended conception. Adequate contraception should be used by patients  
3 receiving this medication.

#### 4 *Hydroxycarbamide intolerance and resistance*

5 There has been an attempt to define the criteria to suggest failure of HC as first line  
6 therapy for PV. The ELN have by consensus suggested a unified definition of  
7 resistance to intolerance or hydroxycarbamide. This classification identifies a group  
8 of patients who have a poorer prognosis who may require or benefit from a change  
9 of treatment (Barosi, Birgegard et al. 2010) (Table IV). In retrospective studies  
10 resistance is associated with worse survival, with development of anaemia or  
11 cytopenias identifying a group with poorer outcome (Alvarez-Larrán, Pereira et al.  
12 2012, Alvarez-Larrán, Pérez-Encinas et al. 2017).

#### 13 *Interferons*

14 Numerous single centre studies have observed that interferon- $\alpha$  (IFN- $\alpha$ ) can be  
15 successfully to normalise blood counts, reduce splenomegaly and prevent  
16 thrombosis in PV (Silver 2006) It is also effective in many patients in reducing  
17 pruritus (Taylor, Dolan et al. 1996). This agent is of particular interest due to its anti-  
18 clonal activity as demonstrated by molecular (as assessed by mutation burden of  
19 *JAK2* V617F) and histological remissions (Larsen, Møller et al. 2009) (Stauffer  
20 Larsen, Iversen et al. 2013). No leukaemogenic effect has been identified. However,  
21 side effects often limit use and most commonly include flu-like symptoms and mood  
22 changes. In a minority of patients, endocrine and autoimmune disorders also occur  
23 warranting regular monitoring of thyroid function and additional investigations where

1 indicated. Treatment with IFN- $\alpha$  is usually continuous but occasionally it can be  
2 stopped for prolonged periods of time.

3 Longer acting pegylated IFN- $\alpha$ -2a (PEG- $\alpha$ -2a) requires less frequent administration  
4 and is generally better tolerated. Two Phase II studies of PEG- $\alpha$ -2a demonstrated  
5 complete responses of 70-95% as well as complete molecular remissions of 14 –  
6 24% with treatment discontinuation due to side-effects observed in 8-10% of  
7 patients only (Kiladjian, Cassinat et al. 2006) (Quintás-Cardama, Kantarjian et al.  
8 2009) Comparable results have been noted in single-centre studies (Crisà, Cerrano  
9 et al. 2017, Gowin, Jain et al. 2017) A Phase III study of PEG- $\alpha$ -2a versus HC as  
10 first-line treatment for high-risk PV is underway and interim analysis shows no  
11 significant advantage for PEG- $\alpha$ -2a over HC (Mascarenhas, Prchal et al. 2016  
12 )

13 Pegylated interferon- $\alpha$ -2b (PEG- $\alpha$ -2b) has also been assessed in two studies which  
14 included PV patients and whilst it has been shown to be effective in controlling  
15 disease, clinical use has been limited by high discontinuation rates due to side  
16 effects (Samuelsson, Hasselbalch et al. 2006) (Jabbour, Kantarjian et al. 2007).

17 Recently, interim analysis from a phase III study of proline-PEG- $\alpha$ -2b  
18 (Ropeginterferon) has demonstrated complete haematological responses in 71% of  
19 PV patients, sustained reductions in mutation burden of *JAK2* V617F, good  
20 tolerability and confirmed non-inferiority (or no significant advantage) to HC as first  
21 line treatment for patients with high risk PV (Gisslinger, Klade et al. 2017)

## 22 *Ruxolitinib*

23 Following early studies of the JAK1 and 2 inhibitor ruxolitinib in MF this agent was

1 tested in PV and ET, in patients resistant or intolerant to HC. The majority of PV  
2 patients became phlebotomy-independent and had an improvement in symptoms  
3 and splenomegaly (>50%) (Verstovsek, Passamonti et al. 2014).

4 Following this a phase III trial RESPONSE evaluated the efficacy and safety of  
5 ruxolitinib in a specific subgroup of PV patients who were both refractory to, or  
6 intolerant of HC and who required ongoing phlebotomy and had splenomegaly  
7 (Vannucchi 2015). Patients were randomized between ruxolitinib and best available  
8 therapy (BAT), which could include any therapy, and crossover was permitted.  
9 Patients on ruxolitinib achieved good Hct control and spleen response, although only  
10 21% of patients achieved both. Improvements in disease-related symptoms were  
11 described. Anaemia and thrombocytopenia were the main haematological adverse  
12 events. Herpes zoster infection was described in patients on ruxolitinib. Non-  
13 melanoma skin cancer was reported in both arms. Thromboembolic events were  
14 more frequent on BAT arm but this was not a pre-determined outcome. Data  
15 published from this study also suggested that molecular responses can occur  
16 perhaps to the same extent as with interferon (Pieri, Pancrazzi et al. 2015,  
17 Vannucchi, Verstovsek et al. 2017) and that even profoundly iron deficient patients  
18 can normalise their iron parameters with ruxolitinib therapy (Verstovsek, Harrison et  
19 al. 2017). Disease transformation occurred and there is no information to suggest  
20 that ruxolitinib therapy impacts these events.

21 However, the RESPONSE study had some inherent bias: first, the population of the  
22 study is highly selected as they had to be venesection-dependent. Second, patients  
23 on the BAT arm were allowed to receive HC and many did so reflecting the lack of  
24 therapy options in this setting. Finally, as patients received other treatments before  
25 ruxolitinib and crossed between the treatment arms it is difficult to establish if the

1 events such as skin cancer, or disease transformation could be an effect of the  
2 ruxolitinib or occur as an accumulative effect of other cytoreductive therapies.

3 Following RESPONSE, a second randomized open label phase 3b study  
4 (RESPONSE-2) was developed to determinate the efficacy of ruxolitinib versus BAT  
5 in a similar population of PV patients as RESPONSE but the patients were not  
6 required to have splenomegaly (Passamonti, Griesshammer et al. 2017). Here  
7 ruxolitinib showed good responses in controlling Hct and PV-related symptoms.  
8 However, the follow-up for the study is short, the majority of patients had received  
9 HC previously and HC was part of BAT options.

10 RELIEF was a randomized study focusing on PV-related symptoms for patients on a  
11 stable dose of HC (Mesa, Vannucchi et al. 2017) with crossover to ruxolitinib allowed  
12 after week 16. The primary endpoint, the percentage of patients with  $\geq 50\%$  reduction  
13 in symptoms, was seen in significantly more patients in the ruxolitinib arm. A  
14 statistically significant reduction in itching was also noted in the ruxolitinib. This study  
15 was perhaps underpowered but showed that ruxolitinib improves symptoms in  
16 patients with controlled PV.

17 The evidence from these trials suggests ruxolitinib has a role in the treatment of HC-  
18 resistant or intolerant PV.

### 19 *Other treatments*

20 There are several cytotoxic agents that are effective in controlling blood counts but  
21 which have been associated with increased rates of leukaemic transformation.

22 Busulfan, a cell cycle non-specific alkylating agent, has such an association.

23 However, retrospective studies show that it is an effective therapy for MPNs (Begna,  
24 Abdelatif et al. 2016) although an actuarial probability of leukaemia transformation of

1 10% at 3 years was reported in one study (Alvarez-Larrán, Martínez-Avilés et al.  
2 2014). Busulfan is useful in treating PV in those with limited life expectancy. It can be  
3 given in dosing regimens of 2 – 4 mg daily until counts are controlled but patients  
4 need to be seen frequently to check for neutropenia or thrombocytopenia so that  
5 treatment can be interrupted. An alternative regimen is pulsed single 25 – 50 mg  
6 doses at intervals of approximately 6 weeks.

7 <sup>32</sup>P is has a leukaemogenic potential but a single intravenous dose can be effective  
8 for long term control. One retrospective study showed its efficacy with remission  
9 rates of 90% (Lawless, McMullin et al. 2016). Doses can be repeated if the response  
10 is lost but the leukaemogenic risk increases with the cumulative exposure. <sup>32</sup>P is a  
11 suitable treatment for those with limited life expectancy who are self-caring (so that  
12 there is no risk for carers).

13 Pipobroman a bromide derivative of piperazine similar to alkylating agents has been  
14 compared to HC in randomised trials and shown to be effective but has continuing  
15 leukaemogenic potential (Kiladjian, Chevret et al. 2011) and therefore should only be  
16 used in those with limited life expectancy.

17 Anagrelide, a megakaryocyte differentiation inhibitor, is licensed as second line  
18 therapy in ET. Retrospective reports of anagrelide used in combination with HC in  
19 PV have shown that it is effective at lowering the platelet count and it may be useful  
20 in combination when an elevated platelet count is an issue (Ahn, Natelson et al.  
21 2013).

22 A number of other agents have been used for cytoreduction in PV with varying  
23 efficacy. A small study investigated the use of imatinib. The complete response rate

1 was 30% with frequent side effects. This has not been studied further and is not  
2 recommended for the treatment of PV (Silver, Bourla et al. 2012)

3 Histone-deacetylase inhibitors (HDACi) inhibit proliferation of cells with a *JAK2*  
4 V617F mutation. Two HDACis have been tested in PV in phase 2 trials. Vorinostat in  
5 a trial of PV and ET achieved a response rate of 35% but with a very high drop-out  
6 rate because of adverse events (Andersen, McMullin et al. 2013). Givinostat was  
7 assessed in a phase 2 study of PV unresponsive to HC monotherapy with response  
8 rates in the order of 50% and with high rates of improvement in pruritus (Finazzi,  
9 Vannucchi et al. 2013). HDACis need to be assessed further in trials before they can  
10 be recommended for clinical use.

11

12 Following evaluation of all recent evidence it is recommended that all patients  
13 including those stratified as low-risk should be venesected to a Hct target of 0.45 and  
14 given low dose aspirin if there are no specific contraindications. High-risk patients  
15 should be treated with cytoreductive therapy in addition. However, low-risk patients  
16 with any of the criteria listed below, may also need to be considered for cytoreductive  
17 therapy

18

19 **RECOMMENDATIONS: Management options for ALL PV including low-risk**  
20 **patients**

- 21 • **Target haematocrit of <0.45 in all patients (GRADE 1A)**
- 22 • **Low dose aspirin (75 – 100 mg) in all patients (GRADE 1A)**
- 23 • **Targeted intervention to reduce cardiovascular risk factors**

1 **Consider cytoreductive therapy in low-risk patients with:**

- 2 • **History of treated arterial hypertension, ischaemia heart disease or**
- 3 **diabetes mellitus**
- 4 • **Persistent leucocytosis (e.g. WBC  $>15 \times 10^9/l$ )**
- 5 • **Uncontrolled haematocrit (or poor tolerability of venesection)**
- 6 • **Extreme / progressive thrombocytosis (e.g.  $\geq 1500 \times 10^9/l$ ) and/or**
- 7 **haemorrhagic symptoms**
- 8 • **Progressive / symptomatic splenomegaly**
- 9 • **Uncontrolled or progressive disease-related symptoms e.g. weight loss,**
- 10 **sweats**

11 **(GRADE 1B)**

12 **Recommendations: Management options in high-risk patients**

- 13 • **First Line: hydroxycarbamide or interferon (preferably pegylated**
- 14 **interferon)**
- 15 • **Second line: In patients treated with hydroxycarbamide 1<sup>st</sup> line**
- 16 **interferon as second line treatment or where treated with interferon 1<sup>st</sup>**
- 17 **line recommend hydroxycarbamide as second line treatment**
- 18 • **Consider pegylated interferon as second line in those patients who have**
- 19 **had non-pegylated interferon 1<sup>st</sup> line and could not tolerate it**
- 20 • **Ruxolitinib 2<sup>nd</sup> /3<sup>rd</sup> line in HC resistant or intolerant**

21 **(GRADE 1A)**

22 **Third-line or further treatment options**

- 1 • **Busulfan or <sup>32</sup>P or pipobroman in those with limited life expectancy**
- 2 **(GRADE 1B).**
- 3 • **Anagrelide in combination with hydroxycarbamide may be helpful in**
- 4 **those where platelet control is difficult (GRADE 2C)**

## 5 **Acknowledgements**

6 The authors would like to the BSH task forces and the BSH sounding boards for their  
7 support in preparing this guideline. They also wish to thank Jacky Wilson for help in  
8 undertaking the initial literature review. All authors reviewed the literature search,  
9 formulated and agreed guidance and contributed to drafting writing and editing of the  
10 manuscript.

## 11 **Declaration of Interest**

12 The BSH paid the expenses incurred during the writing of this guidance. All authors  
13 have made a declaration of interests to the BSH and Task Force Chairs which may  
14 be reviewed on request.

## 15 **References**

- 16 Abdulkarim, K., B. Ridell, P. Johansson, J. Kutti, S. Safai-Kutti and B. Andréasson (2011). "The impact  
17 of peripheral blood values and bone marrow findings on prognosis for patients with essential  
18 thrombocythemia and polycythemia vera." *Eur J Haematol* **86**(2): 148-155.
- 19 Ahn, I. E., E. Natelson and L. Rice (2013). "Successful long-term treatment of Philadelphia  
20 chromosome-negative myeloproliferative neoplasms with combination of hydroxyurea and  
21 anagrelide." *Clin Lymphoma Myeloma Leuk* **13 Suppl 2**: S300-304.
- 22 Alvarez-Larrán, A., A. Ancochea, M. García, F. Climent, F. García-Pallarols, A. Angona, A. Senín, C.  
23 Barranco, L. Martínez-Avilés, S. Serrano, B. Bellosillo and C. Besses (2014). "WHO-histological criteria  
24 for myeloproliferative neoplasms: reproducibility, diagnostic accuracy and correlation with gene  
25 mutations and clinical outcomes." *Br J Haematol* **166**(6): 911-919.
- 26 Alvarez-Larrán, A., A. Angona, A. Ancochea, F. García-Pallarols, C. Fernández, R. Longarón, B.  
27 Bellosillo and C. Besses (2016). "Masked polycythaemia vera: presenting features, response to  
28 treatment and clinical outcomes." *Eur J Haematol* **96**(1): 83-89.
- 29 Alvarez-Larrán, A., B. Bellosillo, L. Martínez-Avilés, S. Saumell, A. Salar, E. Abella, E. Gimeno, S.  
30 Serrano, L. Florensa, B. Sánchez, C. Pedro and C. Besses (2009). "Postpolycythaemic myelofibrosis:  
31 frequency and risk factors for this complication in 116 patients." *Br J Haematol* **146**(5): 504-509.



1 Alvarez-Larrán, A., L. Martínez-Avilés, J. C. Hernández-Boluda, F. Ferrer-Marín, M. L. Antelo, C.  
2 Burgaleta, M. I. Mata, B. Xicoy, A. Martínez-Trillos, M. T. Gómez-Casares, M. A. Durán, B. Marcote, A.  
3 Ancochea, A. Senín, A. Angona, M. Gómez, V. Vicente, F. Cervantes, B. Bellosillo and C. Besses  
4 (2014). "Busulfan in patients with polycythemia vera or essential thrombocythemia refractory or  
5 intolerant to hydroxyurea." *Ann Hematol* **93**(12): 2037-2043.

6 Alvarez-Larrán, A., A. Pereira, F. Cervantes, E. Arellano-Rodrigo, J. C. Hernández-Boluda, F. Ferrer-  
7 Marín, A. Angona, M. Gómez, B. Muiña, H. Guillén, A. Teruel, B. Bellosillo, C. Burgaleta, V. Vicente  
8 and C. Besses (2012). "Assessment and prognostic value of the European LeukemiaNet criteria for  
9 clinicohematologic response, resistance, and intolerance to hydroxyurea in polycythemia vera."  
10 *Blood* **119**(6): 1363-1369.

11 Alvarez-Larrán, A., M. Pérez-Encinas, F. Ferrer-Marín, J. C. Hernández-Boluda, M. J. Ramírez, J.  
12 Martínez-López, E. Magro, Y. Cruz, M. I. Mata, P. Aragües, M. L. Fox, B. Cuevas, S. Montesdeoca, J. A.  
13 Hernández-Rivas, V. García-Gutiérrez, M. T. Gómez-Casares, J. L. Steegmann, M. A. Durán, M.  
14 Gómez, A. Kerguelen, A. Báez, M. C. García, C. Boqué, J. M. Raya, C. Martínez, M. Albors, F. García,  
15 C. Burgaleta, C. Besses and G. E. d. N. M. F. Negativas (2017). "Risk of thrombosis according to need  
16 of phlebotomies in patients with polycythemia vera treated with hydroxyurea." *Haematologica*  
17 **102**(1): 103-109.

18 Ancochea, A., A. Alvarez-Larrán, C. Morales-Indiano, F. García-Pallarols, L. Martínez-Avilés, A.  
19 Angona, A. Senín, B. Bellosillo and C. Besses (2014). "The role of serum erythropoietin level and JAK2  
20 V617F allele burden in the diagnosis of polycythaemia vera." *Br J Haematol* **167**(3): 411-417.

21 Andersen, C. L., M. F. McMullin, E. Ejerblad, S. Zweegman, C. Harrison, S. Fernandes, D. Bareford, S.  
22 Knapper, J. Samuelsson, E. Löfvenberg, O. Linder, B. Andreasson, E. Ahlstrand, M. K. Jensen, O. W.  
23 Bjerrum, H. Vestergaard, H. Larsen, T. W. Klausen, T. Mourits-Andersen and H. C. Hasselbalch (2013).  
24 "A phase II study of vorinostat (MK-0683) in patients with polycythaemia vera and essential  
25 thrombocythaemia." *Br J Haematol* **162**(4): 498-508.

26 Arber, D. A., A. Orazi, R. Hasserjian, J. Thiele, M. J. Borowitz, M. M. Le Beau, C. D. Bloomfield, M.  
27 Cazzola and J. W. Vardiman (2016). "The 2016 revision to the World Health Organization  
28 classification of myeloid neoplasms and acute leukemia." *Blood* **127**(20): 2391-2405.

29 Barbui, T., A. Carobbio, A. Rambaldi and G. Finazzi (2009). "Perspectives on thrombosis in essential  
30 thrombocythemia and polycythemia vera: is leukocytosis a causative factor?" *Blood* **114**(4): 759-763.

31 Barbui, T., A. Masciulli, M. R. Marfisi, G. Tognoni, G. Finazzi, A. Rambaldi and A. Vannucchi (2015).  
32 "White blood cell counts and thrombosis in polycythemia vera: a subanalysis of the CYTO-PV study."  
33 *Blood* **126**(4): 560-561.

34 Barbui, T., J. Thiele, F. Passamonti, E. Rumi, E. Boveri, M. L. Randi, I. Bertozzi, F. Marino, A. M.  
35 Vannucchi, L. Pieri, G. Rotunno, H. Gisslinger, B. Gisslinger, L. Müllauer, G. Finazzi, A. Carobbio, A.  
36 Gianatti, M. Ruggeri, I. Nichele, E. D'Amore, A. Rambaldi and A. Tefferi (2012). "Initial bone marrow  
37 reticulin fibrosis in polycythemia vera exerts an impact on clinical outcome." *Blood* **119**(10): 2239-  
38 2241.

39 Barbui, T., A. M. Vannucchi, A. Carobbio, E. Rumi, G. Finazzi, H. Gisslinger, M. Ruggeri, M. L. Randi,  
40 M. Cazzola, A. Rambaldi, B. Gisslinger, L. Pieri, J. Thiele, A. Pardani and A. Tefferi (2017). "The  
41 effect of arterial hypertension on thrombosis in low-risk polycythemia vera." *Am J Hematol* **92**(1):  
42 E5-E6.

43 Barosi, G., G. Birgegard, G. Finazzi, M. Griesshammer, C. Harrison, H. Hasselbalch, J. J. Kiladjan, E.  
44 Lengfelder, R. Mesa, M. F. Mc Mullin, F. Passamonti, J. T. Reilly, A. M. Vannucchi and T. Barbui  
45 (2010). "A unified definition of clinical resistance and intolerance to hydroxycarbamide in  
46 polycythaemia vera and primary myelofibrosis: results of a European LeukemiaNet (ELN) consensus  
47 process." *Br J Haematol* **148**(6): 961-963.

48 Barosi, G., A. Tefferi, C. Besses, G. Birgegard, F. Cervantes, G. Finazzi, H. Gisslinger, M.  
49 Griesshammer, C. Harrison, R. Hehlmann, S. Hermouet, J. J. Kiladjan, N. Kröger, R. Mesa, M. F. Mc  
50 Mullin, A. Pardani, F. Passamonti, J. Samuelsson, A. M. Vannucchi, A. Reiter, R. T. Silver, S.  
51 Verstovsek, G. Tognoni and T. Barbui (2015). "Clinical end points for drug treatment trials in BCR-

1 ABL1-negative classic myeloproliferative neoplasms: consensus statements from European  
2 LeukemiaNET (ELN) and International Working Group-Myeloproliferative Neoplasms Research and  
3 Treatment (IWG-MRT)." Leukemia **29**(1): 20-26.

4 Begna, K., A. Abdelatif, S. Schwager, C. Hanson, A. Pardanani and A. Tefferi (2016). "Busulfan for the  
5 treatment of myeloproliferative neoplasms: the Mayo Clinic experience." Blood Cancer J **6**: e427.

6 Bench, A. J., H. E. White, L. Foroni, A. L. Godfrey, G. Gerrard, S. Akiki, A. Awan, I. Carter, A. Goday-  
7 Fernandez, S. E. Langabeer, T. Clench, J. Clark, P. A. Evans, D. Grimwade, A. Schuh, M. F. McMullin, A.  
8 R. Green, C. N. Harrison, N. C. Cross and B. C. f. S. i. Haematology (2013). "Molecular diagnosis of the  
9 myeloproliferative neoplasms: UK guidelines for the detection of JAK2 V617F and other relevant  
10 mutations." Br J Haematol **160**(1): 25-34.

11 Bento, C., M. J. Percy, B. Gardie, T. M. Maia, R. van Wijk, S. Perrotta, F. Della Ragione, H. Almeida, C.  
12 Rossi, F. Girodon, M. Aström, D. Neumann, S. Schnittger, B. Landin, M. Minkov, M. L. Randi, S.  
13 Richard, N. Casadevall, W. Vainchenker, S. Rives, S. Hermouet, M. L. Ribeiro, M. F. McMullin, H.  
14 Cario, A. Chauveau, A. P. Gimenez-Roqueplo, B. Bressac-de-Paillerets, D. Altindirek, F. Lorenzo, F.  
15 Lambert, H. Dan, S. Gad-Lapiteau, A. Catarina Oliveira, C. Fraga, G. Taradin, G. Martin-Nuñez, H.  
16 Vitória, H. Diaz Aguado, J. Palmblad, J. Vidán, L. Relvas, M. Luigi Larocca, M. Luigia Randi, M. Pedro  
17 Silveira, M. Percy, M. Gross, R. Marques da Costa, S. Beshara, T. Ben-Ami, V. Ugo and ECE-  
18 Consortium (2014). "Genetic basis of congenital erythrocytosis: mutation update and online  
19 databases." Hum Mutat **35**(1): 15-26.

20 Berlin, N. I. (1975). "Diagnosis and classification of the polycythemia." Semin Hematol **12**(4): 339-  
21 351.

22 Besses, C., J. J. Kiladjian, M. Griesshammer, L. Gugliotta, C. Harrison, R. Coll, J. Smith, B. Abhyankar  
23 and G. Birgegård (2013). "Cytoreductive treatment patterns for essential thrombocythemia in  
24 Europe. Analysis of 3643 patients in the EXELS study." Leuk Res **37**(2): 162-168.

25 Birgegård, G., C. Besses, M. Griesshammer, L. Gugliotta, C. N. Harrison, M. Hamdani, J. Wu, H.  
26 Achenbach and J. J. Kiladjian (2018). "Treatment of essential thrombocythemia in Europe: a  
27 prospective long-term observational study of 3649 high-risk patients in the Evaluation of Anagrelide  
28 Efficacy and Long-term Safety study." Haematologica **103**(1): 51-60.

29 Björkholm, M., A. R. Derolf, M. Hultcrantz, S. Y. Kristinsson, C. Ekstrand, L. R. Goldin, B. Andreasson,  
30 G. Birgegård, O. Linder, C. Malm, B. Markevärn, L. Nilsson, J. Samuelsson, F. Granath and O.  
31 Landgren (2011). "Treatment-related risk factors for transformation to acute myeloid leukemia and  
32 myelodysplastic syndromes in myeloproliferative neoplasms." J Clin Oncol **29**(17): 2410-2415.

33 Budde, U. and P. J. van Genderen (1997). "Acquired von Willebrand disease in patients with high  
34 platelet counts." Semin Thromb Hemost **23**(5): 425-431.

35 Camps, C., N. Petousi, C. Bento, H. Cario, R. R. Copley, M. F. McMullin, R. van Wijk, P. J. Ratcliffe, P.  
36 A. Robbins, J. C. Taylor and W. Consortium (2016). "Gene panel sequencing improves the diagnostic  
37 work-up of patients with idiopathic erythrocytosis and identifies new mutations." Haematologica  
38 **101**(11): 1306-1318.

39 Caramazza, D., C. Caracciolo, R. Barone, A. Malato, G. Saccullo, V. Cigna, S. Berretta, L. Schinocca, G.  
40 Quintini, V. Abbadessa, F. Di Raimondo and S. Siragusa (2009). "Correlation between leukocytosis  
41 and thrombosis in Philadelphia-negative chronic myeloproliferative neoplasms." Ann Hematol  
42 **88**(10): 967-971.

43 Castleden, C. M. and P. V. Cole (1975). "Carboxyhaemoglobin levels of smokers and non-smokers  
44 working in the City of London." Br J Ind Med **32**(2): 115-118.

45 Cerquozzi, S., D. Barraco, T. Lasho, C. Finke, C. A. Hanson, R. P. Ketterling, A. Pardanani, N. Gangat  
46 and A. Tefferi (2017). "Risk factors for arterial versus venous thrombosis in polycythemia vera: a  
47 single center experience in 587 patients." Blood Cancer J **7**(12): 662.

48 Cortelazzo, S., G. Finazzi, M. Ruggeri, O. Vestri, M. Galli, F. Rodeghiero and T. Barbui (1995).  
49 "Hydroxyurea for patients with essential thrombocythemia and a high risk of thrombosis." N Engl J  
50 Med **332**(17): 1132-1136.

1 Crisà, E., M. Cerrano, E. Beggiato, G. Benevolo, G. Lanzarone, P. M. Manzini, A. Borchiellini, L. Riera,  
2 M. Boccadoro and D. Ferrero (2017). "Can pegylated interferon improve the outcome of  
3 polycythemia vera patients?" J Hematol Oncol **10**(1): 15.

4 De Stefano, V., T. Za, E. Rossi, A. M. Vannucchi, M. Ruggeri, E. Elli, C. Micò, A. Tieghi, R. R. Cacciola, C.  
5 Santoro, G. Gerli, P. Guglielmelli, L. Pieri, F. Scognamiglio, F. Rodeghiero, E. M. Pogliani, G. Finazzi, L.  
6 Gugliotta, G. Leone, T. Barbui and G. C. M. N. W. Party (2010). "Leukocytosis is a risk factor for  
7 recurrent arterial thrombosis in young patients with polycythemia vera and essential  
8 thrombocythemia." Am J Hematol **85**(2): 97-100.

9 Delic, S., D. Rose, W. Kern, N. Nadarajah, C. Haferlach, T. Haferlach and M. Meggendorfer (2016).  
10 "Application of an NGS-based 28-gene panel in myeloproliferative neoplasms reveals distinct  
11 mutation patterns in essential thrombocythaemia, primary myelofibrosis and polycythaemia vera."  
12 Br J Haematol **175**(3): 419-426.

13 Di Nisio, M., T. Barbui, L. Di Gennaro, G. Borrelli, G. Finazzi, R. Landolfi, G. Leone, R. Marfisi, E.  
14 Porreca, M. Ruggeri, A. W. Rutjes, G. Tognoni, A. M. Vannucchi, R. Marchioli and E. C. o. L.-d. A. i. P.  
15 V. E. Investigators (2007). "The haematocrit and platelet target in polycythemia vera." Br J Haematol  
16 **136**(2): 249-259.

17 Dupont, S., A. Massé, C. James, I. Teyssandier, Y. Lécluse, F. Larbret, V. Ugo, P. Saulnier, S. Koscielny,  
18 J. P. Le Couédic, N. Casadevall, W. Vainchenker and F. Delhommeau (2007). "The JAK2 617V>F  
19 mutation triggers erythropoietin hypersensitivity and terminal erythroid amplification in primary  
20 cells from patients with polycythemia vera." Blood **110**(3): 1013-1021.

21 Enblom-Larsson, A., F. Girodon, M. Bak, D. Hersby, V. Jooste, H. Hasselbalch, P. Johansson and B.  
22 Andreasson (2017). "A retrospective analysis of the impact of treatments and blood counts on  
23 survival and the risk of vascular events during the course of polycythaemia vera." Br J Haematol  
24 **177**(5): 800-805.

25 Finazzi, G., V. Caruso, R. Marchioli, G. Capnist, T. Chisesi, C. Finelli, L. Gugliotta, R. Landolfi, J. Kutti, H.  
26 Gisslinger, R. Marilus, C. Patrono, E. M. Pogliani, M. L. Randi, A. Villegas, G. Tognoni, T. Barbui and E.  
27 Investigators (2005). "Acute leukemia in polycythemia vera: an analysis of 1638 patients enrolled in a  
28 prospective observational study." Blood **105**(7): 2664-2670.

29 Finazzi, G., A. M. Vannucchi, V. Martinelli, M. Ruggeri, F. Nobile, G. Specchia, E. M. Pogliani, O. M.  
30 Olimpieri, G. Fioritoni, C. Musolino, D. Cilloni, P. Sivera, G. Barosi, M. C. Finazzi, S. Di Tollo, T.  
31 Demuth, T. Barbui and A. Rambaldi (2013). "A phase II study of Givinostat in combination with  
32 hydroxycarbamide in patients with polycythaemia vera unresponsive to hydroxycarbamide  
33 monotherapy." Br J Haematol **161**(5): 688-694.

34 Gangat, N., J. Strand, C. Y. Li, W. Wu, A. Pardanani and A. Tefferi (2007). "Leucocytosis in  
35 polycythaemia vera predicts both inferior survival and leukaemic transformation." Br J Haematol  
36 **138**(3): 354-358.

37 Genovese, G., A. K. Kähler, R. E. Handsaker, J. Lindberg, S. A. Rose, S. F. Bakhom, K. Chambert, E.  
38 Mick, B. M. Neale, M. Fromer, S. M. Purcell, O. Svantesson, M. Landén, M. Höglund, S. Lehmann, S.  
39 B. Gabriel, J. L. Moran, E. S. Lander, P. F. Sullivan, P. Sklar, H. Grönberg, C. M. Hultman and S. A.  
40 McCarroll (2014). "Clonal hematopoiesis and blood-cancer risk inferred from blood DNA sequence."  
41 N Engl J Med **371**(26): 2477-2487.

42 Geyer, H., R. Scherber, H. Kosiorek, A. C. Dueck, J. J. Kiladjian, Z. Xiao, S. Slot, S. Zweegman, F.  
43 Sackmann, A. K. Fuentes, D. Hernández-Maraver, K. Döhner, C. N. Harrison, D. Radia, P. Muxi, C.  
44 Besses, F. Cervantes, P. L. Johansson, B. Andreasson, A. Rambaldi, T. Barbui, K. Bonatz, A. Reiter, F.  
45 Boyer, G. Etienne, J. C. Ianotto, D. Ranta, L. Roy, J. Y. Cahn, N. Maldonado, G. Barosi, M. L. Ferrari, R.  
46 P. Gale, G. Birgegard, Z. Xu, Y. Zhang, X. Sun, J. Xu, P. Zhang, P. A. te Boekhorst, S. Commandeur, H.  
47 Schouten, H. L. Pahl, M. Griesshammer, F. Stegelmann, T. Lehmann, Z. Senyak, A. M. Vannucchi, F.  
48 Passamonti, J. Samuelsson and R. A. Mesa (2016). "Symptomatic Profiles of Patients With  
49 Polycythemia Vera: Implications of Inadequately Controlled Disease." J Clin Oncol **34**(2): 151-159.

50 Gisslinger, H., C. Klade, P. Georgiev, D. Krochmalczyk, L. Gercheva, M. Egyed, V. Rossiev, P. Dulicek,  
51 A. Illes, H. Pylypenko, L. Sicheva, J. Mayer, V. Yablokova, K. Krejcy, B. Grohman-Izay, H. Hasselbalch,

1 R. Kralovica and J. Kiladjan (2017). Ropoginterferon alfa-2b induces high rates of clinical,  
2 hematological and molecular responses in polycythemia vera: Two-year results from the first  
3 prospective randomized controlled trial. *Blood*. **130**: 320.

4 Gowin, K., T. Jain, H. Kosiorek, R. Tibes, J. Camoriano, J. Palmer and R. Mesa (2017). "Pegylated  
5 interferon alpha - 2a is clinically effective and tolerable in myeloproliferative neoplasm patients  
6 treated off clinical trial." *Leuk Res* **54**: 73-77.

7 Gómez, M., V. Guillem, A. Pereira, F. Ferrer-Marín, A. Álvarez-Larrán, A. Kerguelen, N. Estrada, J.  
8 Martínez-López, A. Angona, P. Amat, B. Navarro, C. Besses and J. C. Hernández-Boluda (2016). "Risk  
9 factors for non-melanoma skin cancer in patients with essential thrombocythemia and polycythemia  
10 vera." *Eur J Haematol* **96**(3): 285-290.

11 Hansen, I. O., A. L. Sørensen and H. C. Hasselbalch (2017). "Second malignancies in hydroxyurea and  
12 interferon-treated Philadelphia-negative myeloproliferative neoplasms." *Eur J Haematol* **98**(1): 75-  
13 84.

14 Harrison, C. N., S. Koschmieder, L. Foltz, P. Guglielmelli, T. Flindt, M. Koehler, J. Mathias, N. Komatsu,  
15 R. N. Boothroyd, A. Spierer, J. Perez Ronco, G. Taylor-Stokes, J. Waller and R. A. Mesa (2017). "The  
16 impact of myeloproliferative neoplasms (MPNs) on patient quality of life and productivity: results  
17 from the international MPN Landmark survey." *Ann Hematol* **96**(10): 1653-1665.

18 Jabbour, E., H. Kantarjian, J. Cortes, D. Thomas, G. Garcia-Manero, A. Ferrajoli, S. Faderl, M. A. Richie,  
19 M. Beran, F. Giles and S. Verstovsek (2007). "PEG-IFN-alpha-2b therapy in BCR-ABL-negative  
20 myeloproliferative disorders: final result of a phase 2 study." *Cancer* **110**(9): 2012-2018.

21 Jaiswal, S., P. Fontanillas, J. Flannick, A. Manning, P. V. Grauman, B. G. Mar, R. C. Lindsley, C. H.  
22 Mermel, N. Burt, A. Chavez, J. M. Higgins, V. Moltchanov, F. C. Kuo, M. J. Kluk, B. Henderson, L.  
23 Kinnunen, H. A. Koistinen, C. Ladenvall, G. Getz, A. Correa, B. F. Banahan, S. Gabriel, S. Kathiresan, H.  
24 M. Stringham, M. I. McCarthy, M. Boehnke, J. Tuomilehto, C. Haiman, L. Groop, G. Atzmon, J. G.  
25 Wilson, D. Neuberg, D. Altshuler and B. L. Ebert (2014). "Age-related clonal hematopoiesis  
26 associated with adverse outcomes." *N Engl J Med* **371**(26): 2488-2498.

27 James, C., V. Ugo, N. Casadevall, S. N. Constantinescu and W. Vainchenker (2005). "A JAK2 mutation  
28 in myeloproliferative disorders: pathogenesis and therapeutic and scientific prospects." *Trends Mol*  
29 *Med* **11**(12): 546-554.

30 Kiladjan, J. J., B. Cassinat, P. Turlure, N. Cambier, M. Roussel, S. Bellucci, M. L. Menot, G. Massonnet,  
31 J. L. Dutel, K. Ghomari, P. Rousselot, M. J. Grange, Y. Chait, W. Vainchenker, N. Parquet, L.  
32 Abdelkader-Aljasseem, J. F. Bernard, J. D. Rain, S. Chevret, C. Chomienne and P. Fenaux (2006). "High  
33 molecular response rate of polycythemia vera patients treated with pegylated interferon alpha-2a."  
34 *Blood* **108**(6): 2037-2040.

35 Kiladjan, J. J., S. Chevret, C. Dosquet, C. Chomienne and J. D. Rain (2011). "Treatment of  
36 polycythemia vera with hydroxyurea and pipobroman: final results of a randomized trial initiated in  
37 1980." *J Clin Oncol* **29**(29): 3907-3913.

38 Kjær, L., M. Westman, C. Hasselbalch Riley, E. Høgdall, O. Weis Bjerrum and H. Hasselbalch (2012).  
39 "A highly sensitive quantitative real-time PCR assay for determination of mutant JAK2 exon 12 allele  
40 burden." *PLoS One* **7**(3): e33100.

41 Koopmans, S. M., F. J. Bot, K. H. Lam, A. M. van Marion, H. de Raeve and K. M. Hebeda (2011).  
42 "Reproducibility of histologic classification in nonfibrotic myeloproliferative neoplasia." *Am J Clin*  
43 *Pathol* **136**(4): 618-624.

44 Lamy, T., A. Devillers, M. Bernard, A. Moisan, I. Grulois, B. Drenou, L. Amiot, R. Fauchet and P. Y. Le  
45 Prise (1997). "Inapparent polycythemia vera: an unrecognized diagnosis." *Am J Med* **102**(1): 14-20.

46 Landolfi, R., L. Di Gennaro, T. Barbui, V. De Stefano, G. Finazzi, R. Marfisi, G. Tognoni, R. Marchioli  
47 and E. C. o. L.-D. A. i. P. V. (ECLAP) (2007). "Leukocytosis as a major thrombotic risk factor in patients  
48 with polycythemia vera." *Blood* **109**(6): 2446-2452.

49 Landolfi, R., R. Marchioli, J. Kutti, H. Gisslinger, G. Tognoni, C. Patrono, T. Barbui and E. C. o. L.-D. A. i.  
50 P. V. Investigators (2004). "Efficacy and safety of low-dose aspirin in polycythemia vera." *N Engl J*  
51 *Med* **350**(2): 114-124.

1 Larsen, T. S., M. B. Møller, K. de Stricker, P. Nørgaard, J. Samuelsson, C. Marcher, M. T. Andersen, O.  
2 W. Bjerrum and H. C. Hasselbalch (2009). "Minimal residual disease and normalization of the bone  
3 marrow after long-term treatment with alpha-interferon2b in polycythemia vera. A report on  
4 molecular response patterns in seven patients in sustained complete hematological remission."  
5 Hematology **14**(6): 331-334.

6 Lawless, S., M. F. McMullin, R. Cuthbert and R. Houston (2016). "(32)P in the treatment of  
7 myeloproliferative disorders." Ulster Med J **85**(2): 83-85.

8 Madelung, A. B., H. Bondo, I. Stamp, P. Loevgreen, S. L. Nielsen, A. Falensteen, H. Knudsen, M.  
9 Ehinger, R. Dahl-Sørensen, N. B. Mortensen, K. D. Svendsen, T. Lange, E. Ralfkiaer, K. Nielsen, H. C.  
10 Hasselbalch and J. Thiele (2013). "World Health Organization-defined classification of  
11 myeloproliferative neoplasms: morphological reproducibility and clinical correlations--the Danish  
12 experience." Am J Hematol **88**(12): 1012-1016.

13 Marchioli, R., G. Finazzi, R. Landolfi, J. Kutti, H. Gisslinger, C. Patrono, R. Marilus, A. Villegas, G.  
14 Tognoni and T. Barbui (2005). "Vascular and neoplastic risk in a large cohort of patients with  
15 polycythemia vera." J Clin Oncol **23**(10): 2224-2232.

16 Marchioli, R., G. Finazzi, G. Specchia, R. Cacciola, R. Cavazzina, D. Cilloni, V. De Stefano, E. Elli, A.  
17 Iurlo, R. Latagliata, F. Lunghi, M. Lunghi, R. M. Marfisi, P. Musto, A. Masciulli, C. Musolino, N.  
18 Cascavilla, G. Quarta, M. L. Randi, D. Rapezzi, M. Ruggeri, E. Rumi, A. R. Scortechini, S. Santini, M.  
19 Scarano, S. Siragusa, A. Spadea, A. Tieghi, E. Angelucci, G. Visani, A. M. Vannucchi, T. Barbui and C.-P.  
20 C. Group (2013). "Cardiovascular events and intensity of treatment in polycythemia vera." N Engl J  
21 Med **368**(1): 22-33.

22 Mascarenhas, J., J. Prchal, A. Rambaldi, R. Messi, D. Berenzon, A. Yacoub, C. Harrison, M. McMullin,  
23 A. Vannucchi, J. Ewing, C. O'Connell, J. Kiladjian, A. Mead, E. Winton, D. Leibowitz, V. De Stefano, M.  
24 Arcasoy, C. Kessler, R. Catchatorian, D. Rondelli, R. Silver, E. Ritchie, A. Nagler, M. Kremyanskaya, R.  
25 Schlenk, R. Weinberg, M. Salama, G. Tognoni, G. Prosperini, A. Di Lelio, E. Serone, L. Marfisi, J.  
26 Kieczko, H. Kosiorek, T. Barbui, D. AC and H. R (2016

27 ). Interim analysis of the myeloproliferative disorders research consortium (MPD-RC) 112 global  
28 phase III trial of front line pegylated interferon alpha-2a vs. hydroxyurea in high risk polycythemia  
29 vera and essential thrombocythemia, Blood. **128**.

30 McMullin, M. F., D. Bareford, P. Campbell, A. R. Green, C. Harrison, B. Hunt, D. Oscier, M. I. Polkey, J.  
31 T. Reilly, E. Rosenthal, K. Ryan, T. C. Pearson, B. Wilkins and G. H. T. F. o. t. B. C. f. S. i. Haematology  
32 (2005). "Guidelines for the diagnosis, investigation and management of  
33 polycythaemia/erythrocytosis." Br J Haematol **130**(2): 174-195.

34 McMullin, M. F., C. N. Harrison and J. J. Kiladjian (2013). "Treatment target in polycythemia vera." N  
35 Engl J Med **368**(16): 1554-1555.

36 McMullin, M. F., J. T. Reilly, P. Campbell, D. Bareford, A. R. Green, C. N. Harrison, E. Conneally, K.  
37 Ryan, M. e. D. S. National Cancer Research Institute and B. C. f. S. i. Haematology (2007).  
38 "Amendment to the guideline for diagnosis and investigation of polycythaemia/erythrocytosis." Br J  
39 Haematol **138**(6): 821-822.

40 Mesa, R., A. M. Vannucchi, A. Yacoub, P. Zachee, M. Garg, R. Lyons, S. Koschmieder, C. Rinaldi, J.  
41 Byrne, Y. Hasan, F. Passamonti, S. Verstovsek, D. Hunter, M. M. Jones, H. Zhen, D. Habr and B.  
42 Martino (2017). "The efficacy and safety of continued hydroxycarbamide therapy versus switching to  
43 ruxolitinib in patients with polycythaemia vera: a randomized, double-blind, double-dummy,  
44 symptom study (RELIEF)." Br J Haematol **176**(1): 76-85.

45 Passamonti, F., C. Elena, S. Schnittger, R. C. Skoda, A. R. Green, F. Girodon, J. J. Kiladjian, M. F.  
46 McMullin, M. Ruggeri, C. Besses, A. M. Vannucchi, E. Lippert, H. Gisslinger, E. Rumi, T. Lehmann, C. A.  
47 Ortmann, D. Pietra, C. Pascutto, T. Haferlach and M. Cazzola (2011). "Molecular and clinical features  
48 of the myeloproliferative neoplasm associated with JAK2 exon 12 mutations." Blood **117**(10): 2813-  
49 2816.

50 Passamonti, F., M. Griesshammer, F. Palandri, M. Egyed, G. Benevolo, T. Devos, J. Callum, A. M.  
51 Vannucchi, S. Sivgin, C. Bensasson, M. Khan, N. Mounedji and G. Saydam (2017). "Ruxolitinib for the

1 treatment of inadequately controlled polycythaemia vera without splenomegaly (RESPONSE-2): a  
2 randomised, open-label, phase 3b study." Lancet Oncol **18**(1): 88-99.

3 Passamonti, F., E. Rumi, D. Pietra, C. Elena, E. Boveri, L. Arcaini, E. Roncoroni, C. Astori, M. Merli, S.  
4 Boggi, C. Pascutto, M. Lazzarino and M. Cazzola (2010). "A prospective study of 338 patients with  
5 polycythemia vera: the impact of JAK2 (V617F) allele burden and leukocytosis on fibrotic or leukemic  
6 disease transformation and vascular complications." Leukemia **24**(9): 1574-1579.

7 Pearson, T. C., D. L. Guthrie, J. Simpson, S. Chinn, G. Barosi, A. Ferrant, S. M. Lewis and Y. Najean  
8 (1995). "Interpretation of measured red cell mass and plasma volume in adults: Expert Panel on  
9 Radionuclides of the International Council for Standardization in Haematology." Br J Haematol **89**(4):  
10 748-756.

11 Pearson, T. C. and G. Wetherley-Mein (1978). "Vascular occlusive episodes and venous haematocrit  
12 in primary proliferative polycythaemia." Lancet **2**(8102): 1219-1222.

13 Perricone, M., N. Polverelli, G. Martinelli, L. Catani, E. Ottaviani, E. Zuffa, E. Franchini, A. Dizdari, D.  
14 Forte, E. Sabattini, M. Cavo, N. Vianelli and F. Palandri (2017). "The relevance of a low JAK2V617F  
15 allele burden in clinical practice: a monocentric study." Oncotarget **8**(23): 37239-37249.

16 Pieri, L., A. Pancrazzi, A. Pacilli, C. Rabuzzi, G. Rotunno, T. Fanelli, P. Guglielmelli, R. Fjerza, C. Paoli, S.  
17 Verstovsek and A. M. Vannucchi (2015). "JAK2V617F complete molecular remission in polycythemia  
18 vera/essential thrombocythemia patients treated with ruxolitinib." Blood **125**(21): 3352-3353.

19 Quintás-Cardama, A., H. Kantarjian, T. Manshour, R. Luthra, Z. Estrov, S. Pierce, M. A. Richie, G.  
20 Borthakur, M. Konopleva, J. Cortes and S. Verstovsek (2009). "Pegylated interferon alfa-2a yields  
21 high rates of hematologic and molecular response in patients with advanced essential  
22 thrombocythemia and polycythemia vera." J Clin Oncol **27**(32): 5418-5424.

23 Samuelsson, J., H. Hasselbalch, O. Bruserud, S. Temerinac, Y. Brandberg, M. Merup, O. Linder, M.  
24 Bjorkholm, H. L. Pahl, G. Birgegard and N. S. G. f. M. Disorders (2006). "A phase II trial of pegylated  
25 interferon alpha-2b therapy for polycythemia vera and essential thrombocythemia: feasibility,  
26 clinical and biologic effects, and impact on quality of life." Cancer **106**(11): 2397-2405.

27 Santoro, C., I. Sperduti, R. Latagliata, E. Baldacci, B. Anaclerico, G. Avvisati, M. Breccia, F. Buccisano,  
28 M. Cedrone, G. Cimino, C. De Gregoris, M. De Muro, A. Di Veroli, S. Leonetti Crescenzi, M.  
29 Montanaro, E. Montefusco, R. Porrini, A. Rago, A. Spadea, F. Spirito, N. Villivà, A. Andriani, G.  
30 Alimena and M. G. Mazzucconi (2017). "Role of treatment on the development of secondary  
31 malignancies in patients with essential thrombocythemia." Cancer Med **6**(6): 1233-1239.

32 Scott, L. M., W. Tong, R. L. Levine, M. A. Scott, P. A. Beer, M. R. Stratton, P. A. Futreal, W. N. Erber,  
33 M. F. McMullin, C. N. Harrison, A. J. Warren, D. G. Gilliland, H. F. Lodish and A. R. Green (2007). "JAK2  
34 exon 12 mutations in polycythemia vera and idiopathic erythrocytosis." N Engl J Med **356**(5): 459-  
35 468.

36 Silver, R. T. (2006). "Long-term effects of the treatment of polycythemia vera with recombinant  
37 interferon-alpha." Cancer **107**(3): 451-458.

38 Silver, R. T., M. H. Bourla, K. Vandris, S. Fruchtman, J. L. Spivak, E. J. Feldman and A. J. Salvado (2012).  
39 "Treatment of polycythemia vera with imatinib mesylate." Leuk Res **36**(2): 156-162.

40 Stauffer Larsen, T., K. F. Iversen, E. Hansen, A. B. Mathiasen, C. Marcher, M. Frederiksen, H. Larsen, I.  
41 Helleberg, C. H. Riley, O. W. Bjerrum, D. Rønnev-Jessen, M. B. Møller, K. de Stricker, H. Vestergaard  
42 and H. C. Hasselbalch (2013). "Long term molecular responses in a cohort of Danish patients with  
43 essential thrombocythemia, polycythemia vera and myelofibrosis treated with recombinant  
44 interferon alpha." Leuk Res **37**(9): 1041-1045.

45 Takahashi, K., K. P. Patel, H. Kantarjian, R. Luthra, S. Pierce, J. Cortes and S. Verstovsek (2013). "JAK2  
46 p.V617F detection and allele burden measurement in peripheral blood and bone marrow aspirates  
47 in patients with myeloproliferative neoplasms." Blood **122**(23): 3784-3786.

48 Taylor, P. C., G. Dolan, J. P. Ng, B. Paul, R. Collin and J. T. Reilly (1996). "Efficacy of recombinant  
49 interferon-alpha (rIFN-alpha) in polycythaemia vera: a study of 17 patients and an analysis of  
50 published data." Br J Haematol **92**(1): 55-59.

1 Tefferi, A., T. L. Lasho, P. Guglielmelli, C. M. Finke, G. Rotunno, Y. Elala, A. Pacilli, C. A. Hanson, A.  
2 Pancrazzi, R. P. Ketterling, C. Mannarelli, D. Barraco, T. Fanelli, A. Pardanani, N. Gangat and A. M.  
3 Vannucchi (2016). "Targeted deep sequencing in polycythemia vera and essential  
4 thrombocythemia." Blood Adv **1**(1): 21-30.

5 Tefferi, A., E. Rumi, G. Finazzi, H. Gisslinger, A. M. Vannucchi, F. Rodeghiero, M. L. Randi, R. Vaidya,  
6 M. Cazzola, A. Rambaldi, B. Gisslinger, L. Pieri, M. Ruggeri, I. Bertozzi, N. H. Sulai, I. Casetti, A.  
7 Carobbio, G. Jerczynski, D. R. Larson, L. Müllauer, A. Pardanani, J. Thiele, F. Passamonti and T.  
8 Barbui (2013). "Survival and prognosis among 1545 patients with contemporary polycythemia vera:  
9 an international study." Leukemia **27**(9): 1874-1881.

10 Tefferi, A., J. J. Strand, T. L. Lasho, R. A. Knudson, C. M. Finke, N. Gangat, A. Pardanani, C. A. Hanson  
11 and R. P. Ketterling (2007). "Bone marrow JAK2V617F allele burden and clinical correlates in  
12 polycythemia vera." Leukemia **21**(9): 2074-2075.

13 Thiele, J., H. M. Kvasnicka and V. Diehl (2005). "Bone marrow features of diagnostic impact in  
14 erythrocytosis." Ann Hematol **84**(6): 362-367.

15 Vannucchi, A. M. (2015). "Ruxolitinib versus standard therapy for the treatment of polycythemia  
16 vera." N Engl J Med **372**(17): 1670-1671.

17 Vannucchi, A. M., E. Antonioli, P. Guglielmelli, G. Longo, A. Pancrazzi, V. Ponziani, C. Bogani, P. R.  
18 Ferrini, A. Rambaldi, V. Guerini, A. Bosi, T. Barbui and M. R. Consortium (2007). "Prospective  
19 identification of high-risk polycythemia vera patients based on JAK2(V617F) allele burden." Leukemia  
20 **21**(9): 1952-1959.

21 Vannucchi, A. M., S. Verstovsek, P. Guglielmelli, M. Griesshammer, T. C. Burn, A. Naim, D.  
22 Paranagama, M. Marker, B. Gadbow and J. J. Kiladjan (2017). "Ruxolitinib reduces JAK2 p.V617F  
23 allele burden in patients with polycythemia vera enrolled in the RESPONSE study." Ann Hematol  
24 **96**(7): 1113-1120.

25 Verstovsek, S., C. N. Harrison, J. J. Kiladjan, C. Miller, A. B. Naim, D. C. Paranagama, D. Habr and A.  
26 M. Vannucchi (2017). "Markers of iron deficiency in patients with polycythemia vera receiving  
27 ruxolitinib or best available therapy." Leuk Res **56**: 52-59.

28 Verstovsek, S., F. Passamonti, A. Rambaldi, G. Barosi, P. J. Rosen, E. Rumi, E. Gattoni, L. Pieri, P.  
29 Guglielmelli, C. Elena, S. He, N. Contel, B. Mookerjee, V. Sandor, M. Cazzola, H. M. Kantarjian, T.  
30 Barbui and A. M. Vannucchi (2014). "A phase 2 study of ruxolitinib, an oral JAK1 and JAK2 Inhibitor,  
31 in patients with advanced polycythemia vera who are refractory or intolerant to hydroxyurea."  
32 Cancer **120**(4): 513-520.

33 Whitehead, T. P., D. Robinson, S. L. Allaway and A. C. Hale (1995). "The effects of cigarette smoking  
34 and alcohol consumption on blood haemoglobin, erythrocytes and leucocytes: a dose related study  
35 on male subjects." Clin Lab Haematol **17**(2): 131-138.

36