Molecular diagnosis of the myeloproliferative neoplasms: UK guidelines for the detection of *JAK2* V617F and other relevant mutations

Anthony J. Bench,[1] Helen E. White,[2,3] Letizia Foroni,[4] Anna L. Godfrey,[5] Gareth Gerrard,[4] Susanna Akiki,[6] Abida Awan,[7] Ian Carter,[8] Andrea Goday-Fernandez,[1] Stephen E. Langabeer,[9] Tim Clench,[10] Jordan Clark,[11] Paul A. Evans,[12] David Grimwade,[13] Anna Schuh,[14] Mary F. McMullin,[15] Anthony R. Green,[5] Claire N. Harrison[16] and Nicholas C. P. Cross[2,3]

[1]Molecular Malignancy Laboratory and Haemato-Oncology Diagnostic Service, Cambridge University Hospitals NHS Foundation Trust, Cambridge, [2]National Genetics Reference Laboratory, Salisbury NHS Foundation Trust, Salisbury, [3]Faculty of Medicine, University of Southampton, Southampton, [4]Imperial Molecular Pathology, Imperial College Academic Health Science Centre, London, [5]Cambridge Institute for Medical Research, Department of Haematology, University of Cambridge, Cambridge, [6]West Midlands Regional Genetics Laboratory, Birmingham Women's NHS Foundation Trust, Birmingham, [7]Molecular Diagnostics Centre, Manchester Royal Infirmary, Manchester, [8]Nottingham University Hospitals NHS Trust, Nottingham, UK, [9]Cancer Molecular Diagnostics, St. James's Hospital, DublinIreland, [10]Bristol Royal Infirmary, Bristol, [11]UK NEQAS for Leucocyte Immunophenotyping, Sheffield, [12]HMDS, Leeds Institute of Oncology, St. James's University Hospital, Leeds, [13]Department of Medical and Molecular Genetics, King's College London School of Medicine, London, [14]Oxford Cancer and Haematology Centre, Churchill Hospital, Oxford, [15]Belfast City Hospital, Queen's University Belfast, Belfast, and [16]Guy's and St Thomas' NHS Foundation Trust, Guy's Hospital, London UK

Summary

Molecular genetic assays for the detection of the *JAK2* V617F (c.1849G>T) and other pathogenetic mutations within *JAK2* exon 12 and *MPL* exon 10 are part of the routine diagnostic workup for patients presenting with erythrocytosis, thrombocytosis or otherwise suspected to have a myeloproliferative neoplasm. A wide choice of techniques are available for the detection of these mutations, leading to potential difficulties for clinical laboratories in deciding upon the most appropriate assay, which can lead to problems with inter-laboratory standardization. Here, we discuss the most important issues for a clinical diagnostic laboratory in choosing a technique, particularly for detection of the *JAK2* V617F mutation at diagnosis. The *JAK2* V617F detection assay should be both specific and sensitive enough to detect a mutant allele burden as low as 1–3%. Indeed, the use of sensitive assays increases the detection rate of the *JAK2* V617F mutation within myeloproliferative neoplasms. Given their diagnostic relevance, it is also beneficial and relatively straightforward to screen *JAK2* V617F negative patients for *JAK2* exon 12 mutations (in the case of erythrocytosis) or *MPL* exon 10 mutations (thrombocytosis or myelofibrosis) using appropriate assays. Molecular results should be considered in the context of clinical findings and other haematological or laboratory results.

Keywords: myeloproliferative neoplasm, molecular diagnosis, *JAK2*, *MPL*.

Introduction

The classical *BCR-ABL1* negative myeloproliferative neoplasms (MPN) comprise polycythaemia vera (PV), essential thrombocythaemia (ET) and primary myelofibrosis (PMF). In 2005, an acquired mutation within *JAK2* exon 14 was identified (c.1849G>T), which results in a valine to phenylalanine substitution at codon 617 – p.Val617Phe, usually abbreviated to V617F (Baxter *et al*, 2005; James *et al*, 2005; Kralovics *et al*, 2005; Levine *et al*, 2005a). This codon lies in the JH2 pseudokinase domain of JAK2 and the mutation is thought to interfere with JH2-mediated autoinhibition leading to constitutive activation of the tyrosine kinase function. This results in activation of a number of downstream pathways including JAK/STAT, PI3K/AKT and MAPK/ERK. The *JAK2* V617F mutation has been observed in up to 98% of patients with PV and 50–60% of patients with ET and PMF. With the exception of the syndrome 'refractory anaemia with ringed sideroblasts associated with marked thrombocytosis' (RARS-T) where it is observed in approximately one half of patients (Szpurka *et al*, 2006; Schmitt-Graeff *et al*, 2008), the *JAK2* V617F mutation is uncommon in other myeloid disorders, such as myelodysplastic syndrome, chronic myelomonocytic

Correspondence: Dr Anthony J. Bench, Molecular Malignancy Laboratory and Haemato-Oncology Diagnostic Service, Box 234, Cambridge University Hospitals NHS Foundation Trust, Hills Road, Cambridge, CB2 0QQ, UK.
E-mail: anthony.bench@addenbrookes.nhs.uk

© 2012 Blackwell Publishing Ltd

First published online 11 October 2012
doi:10.1111/bjh.12075

leukaemia and acute myeloid leukaemia (Jones *et al*, 2005; Levine *et al*, 2005b).

In 2007, mutations within exon 12 of *JAK2* were described in some *JAK2* V617F negative PV patients (Scott *et al*, 2007a) as well as in patients previously categorized as idiopathic erythrocytosis, raising the suggestion that all patients with PV carry a mutation within *JAK2* (Scott *et al*, 2007b; McMullin, 2008; Wang *et al*, 2008). At least 17 different mutations have now been described within exon 12 (Passamonti *et al*, 2011). Although these mutations are not located within the JH2 domain, they are also thought to interfere with JH2-mediated autoinhibition. In 2006, mutations within exon 10 of the thrombopoietin receptor, *MPL*, were identified in ET and PMF patients (Pardanani *et al*, 2006) and at least five different pathogenetic mutations have been described (Chaligné *et al*, 2008; Schnittger *et al*, 2009a; Boyd *et al*, 2010) that affect codons S505 or W515. Other variants have also been described (Ma *et al*, 2011) but their pathogenicity is not known.

The demonstration of an acquired mutation within *JAK2* and/or *MPL* now forms part of the World Health Organization criteria for the diagnosis of MPN (Swerdlow *et al*, 2008). Patients presenting with erythrocytosis should be assessed for the presence of a *JAK2* mutation. The British Committee for Standards in Haematology (BCSH) guidelines state that the presence of a *JAK2* mutation (V617F or exon 12) and a raised haematocrit (>0·52 male; >0·48 female) or raised red cell mass (>25% above predicted) is sufficient to make a diagnosis of PV (McMullin *et al*, 2007; McMullin, 2008). Likewise, patients presenting with a persistent thrombocytosis should be assessed for the presence of *JAK2* V617F and, if negative, *MPL* exon 10 mutations (Harrison *et al*, 2010). The presence of an acquired pathogenetic mutation (i.e. *JAK2* V617F and/or *MPL* exon 10 mutation) and a sustained thrombocytosis (platelet count $> 450 \times 10^9$/l) in the absence of evidence for another myeloid malignancy is sufficient to make a diagnosis of ET (Harrison *et al*, 2010). For PMF, the demonstration of *JAK2* V617F and/or *MPL* exon 10 mutations is a major diagnostic criterion because it confirms the primary nature of the disorder (Swerdlow *et al*, 2008). The demonstration of a *JAK2* V617F mutation in samples from patients not otherwise meeting specific diagnostic criteria for a MPN, for example presenting with unexplained splanchnic vein thrombosis (Dentali *et al*, 2009), suggests an underlying MPN or, more rarely, another myeloid malignancy.

The aim of these guidelines is to provide information and suggestions for those diagnostic laboratories that perform or wish to perform assays for the detection of *JAK2* V617F, for which many different techniques are available. Diagnostic assays are also available for the detection of *JAK2* exon 12 and *MPL* exon 10 mutations and these are also discussed. A strategy for the efficient combination of *JAK2* V617F, *JAK2* exon 12 and *MPL* exon 10 mutations detection assays in suspected myeloproliferative neoplasms is discussed. These guidelines are broadly in line with the screening strategy proposed by Tefferi *et al* (2011). A step-wise algorithm for supplementary *JAK2* exon 12 or *MPL* exon 10 mutation analysis is both cost-effective and an efficient use of available material and may reduce the need for a bone marrow biopsy in some patients. Furthermore, these guidelines highlight the technical issues of relevance for diagnostic laboratories.

Detection of the *JAK2* V617F mutation at diagnosis

A number of different factors that contribute to the choice of assay for the detection of the *JAK2* V617F mutation at diagnosis will be discussed. Quantification of the *JAK2* V617F mutation, either at diagnosis for prognostic information or during treatment as a means of minimal residual disease assessment, is not discussed here although the use of such assays is entirely applicable in the diagnostic setting. Many assays for quantification of the *JAK2* V617F mutant burden have been developed which differ markedly in their performance with respect to specificity and sensitivity and these have been subject to a comprehensive comparison by the European LeukemiaNet and MPN&MPNr-EuroNet study groups (Jovanovic *et al*, 2011).

Type of sample

DNA extracted from peripheral blood or bone marrow is acceptable for *JAK2* V617F mutation analysis provided the nucleic acid obtained is of acceptable quality for the assay to be performed successfully. In most cases, peripheral blood is the preferred option and EDTA is the usual anticoagulant. Use of other anti-coagulants is acceptable although care should be taken in the case of Lithium heparin tubes to completely remove any anticoagulant during the nucleic acid extraction procedure because its presence may inhibit polymerase chain reaction (PCR) amplification (Yokota *et al*, 1999). A sample of sufficient volume to obtain a reasonable amount of nucleic acid should be taken (2–10 ml peripheral blood is usually fine although some centres have optimized routine DNA extraction from smaller volumes), although samples from neutropenic patients may yield less nucleic acid. There does not appear to be a major difference in the *JAK2* V617F allele burden between whole blood and bone marrow (Larsen *et al*, 2008). Therefore, a bone marrow aspirate may be assessed (taken into an EDTA tube or cytogenetic culture medium) but is generally not necessary if peripheral blood is available. The sample should be received within 24–48 h after being taken but, in our experience, for DNA analysis samples of up to 1 week old are usually acceptable for non-quantitative tests. Peripheral blood may be frozen until nucleic acid is extracted and it is often possible to extract DNA from stained or (preferably) unstained and unfixed slides if necessary (Jones *et al*, 2006). The use of DNA derived from plasma for the detection of the *JAK2* V617F mutation has been described (Ma *et al*, 2008). How-

© 2012 Blackwell Publishing Ltd

ever, this methodology has not been independently validated and does not offer any obvious advantage over DNA derived directly from peripheral blood.

Isolation of peripheral blood granulocytes

An important question often raised is whether isolation of peripheral blood granulocytes is necessary to perform *JAK2* V617F mutation detection assays. The *JAK2* V617F mutation arises in a haematopoietic progenitor cell but, in most patients, is restricted to the myeloid lineage. In addition, the proportion of myeloid cells carrying the *JAK2* V617F mutation can vary widely amongst patients. In general, patients with ET tend to carry a lower overall level of the *JAK2* V617F mutation compared to those with PV or PMF (Passamonti & Rumi, 2009) (due to lower proportion of *JAK2* V617F positive cells and the presence of a monoallelic mutation in most V617F positive cells). A further confounding factor is that patients who have been treated with hydroxycarbamide may exhibit lower levels of *JAK2* V617F (Girodon *et al*, 2008). Although other studies have found minimal changes in V617F levels on hydroxycarbamide (Antonioli *et al*, 2010), the possibility of a lower disease burden is worth bearing in mind if *JAK2* V617F testing is carried out after cytoreductive treatment. Overall, the quantitative level of the *JAK2* V617F mutation is about 15% lower in peripheral blood compared to purified granulocytes (Hermouet *et al*, 2007) due to the presence of *JAK2* V617F negative lymphocytes. If highly sensitive assays are used, there is no difference in detection rate between peripheral blood and granulocytes as a source of material (Hermouet *et al*, 2007; Goday-Fernandez *et al*, 2008; Cankovic *et al*, 2009). However, when a moderately sensitive assay, such as agarose gel-based allele-specific PCR (Baxter *et al*, 2005; sensitivity approximately 3%) was used, purification of granulocytes increased the detection rate from 92% to 97% for PV and from 57% to 61% for ET (Goday-Fernandez *et al*, 2008). Hence, isolation of granulocytes should not be required provided the assay is sufficiently sensitive (sensitivity of 1–3% or better). If the assay utilized has a lower sensitivity (see Table I), then enrichment of granulocytes (Asimakopoulos *et al*, 1996) may be necessary. An alternative approach is to prepare nucleic acid from erythropoietin (EPO) independent erythroid burst-forming unit (BFU-E) colonies, as such colonies should consist entirely of clonal malignant cells. However, this is time consuming, technically challenging, no quality assurance scheme is available and EPO-independent BFU-E are not observed in all patients.

Nucleic acid

Genomic DNA is the preferred choice of nucleic acid due to its stability although assays involving RNA/cDNA are also available. Analysis of RNA/cDNA also allows platelets to be interrogated, but this is not necessary on a routine basis. Commercial DNA purification kits, either manual or automatic, generally give reliable DNA of acceptable quantity and quality, as do many in-house purification methods. It is advisable to process both control samples and the sample under investigation using the same method to reduce variability. DNA concentration should also be calculated using the same method for all samples because different instruments may produce widely varying apparent concentrations (e.g. UV spectrophotometer; Nanodrop spectrophotometer (Thermo Scientific, Wilmington, DE, USA); Qubit fluorometer (Life Technologies, Paisley, UK)). These two points are probably more important for 'comparative' techniques, such as high resolution melt (HRM) analysis or denaturing high performance liquid chromatography (dHPLC). The amount of template nucleic acid required will depend on the particular assay chosen.

Table I. Diagnostic approaches for the detection of the *JAK2* V617F mutation.

Method	Approximate sensitivity* (%)	Example reference(s)
ARMS/allele-specific PCR†	0·1–5	Baxter *et al* (2005); Chen *et al* (2007); Jones *et al* (2005); McClure *et al* (2006); Tan *et al* (2007)
Real time allele-specific PCR	0·01–1	Cankovic *et al* (2009); Denys *et al* (2010); Kroger *et al* (2007); Larsen *et al* (2007); Lippert *et al* (2006)
Melting curve analysis	1–5	Cankovic *et al* (2009); James *et al* (2006); McClure *et al* (2006); Wu *et al* (2011)
High resolution melt analysis	1–5	Rapado *et al* (2009); Qian *et al* (2010)
Restriction enzyme digestion†	2–10	Campbell *et al* (2005); Cankovic *et al* (2009)
Direct sequencing	10–20	Lippert *et al* (2009)
Pyrosequencing	5	Jelinek *et al* (2005); Jones *et al* (2005)
dHPLC	1–20	Albiero *et al* (2008); Sattler *et al* (2006); Stevenson *et al* (2006)

ARMS, amplification-refractory mutation system; dHPLC, denaturing high performance liquid chromatography.
*Actual sensitivity will depend on exact protocol.
†Sensitivity usually greater when assessed by capillary gel electrophoresis rather than agarose gel electrophoresis.

© 2012 Blackwell Publishing Ltd

Choice of assay and sensitivity

A large number of different approaches for the detection of the *JAK2* V617F mutation have been described (Table I). For each type of technique, slightly different assays have been designed that vary with instrument, primer and/or probe sequence and detection method. The techniques described broadly fall into two main categories. Firstly, those assays that are designed to specifically target the c.1849G>T mutation (for example, allele-specific PCR) and, secondly, mutation scanning assays that target the region of exon 14 encompassing the c.1849G>T mutation (for example, direct sequencing, HRM analysis). For assays that specifically target the mutant allele, specificity is usually achieved through the use of a mutation-specific primer or probe. Commercial kits are available for detection of *JAK2* V617F and these are based on similar approaches.

Two main criteria are important in the choice of an assay. Firstly, it should be specific (i.e. no false negatives or a clearly defined background level such that *JAK2* V617F negative and positive cases can be readily distinguished). Secondly, the assay must be sensitive enough to be able to identify a *JAK2* V617F mutant allele with a burden as low as 1–3%. This threshold has been shown to be pathogenetically relevant and carry clinical significance (Wang *et al*, 2008; Mason *et al*, 2011). Consequently, direct sequencing is not recommended as the method of choice because it only has a sensitivity of 10–20%. Other assays that possess a sensitivity of 3–5%, such as restriction enzyme digestion, agarose gel-based allele-specific PCR and pyrosequencing, may also fail to identify a small number of patients who carry a pathogenetically important low level *JAK2* V617F mutation. Use of more sensitive assays does indeed increase the detection rate of *JAK2* V617F in both PV and ET patients particularly when unfractionated peripheral blood is assessed (Campbell *et al*, 2005; Goday-Fernandez *et al*, 2008; Wang *et al*, 2008; Cankovic *et al*, 2009; Lippert *et al*, 2009). Finally, to achieve a sensitivity of 1–3%, it is necessary to analyse at least 20 ng of genomic DNA, equivalent to 3030 diploid genomes.

False positives

False positive results may also occur due to cross-reactivity of primers or probes (Mason *et al*, 2011). Hence, particularly with highly sensitive assays, it is critically important to assess the false positive rate using a series of healthy control samples (see below). The assay should also be able to give an indication of the quality/quantity of the DNA to judge whether it carries sufficient sensitivity for each patient. Sample quality may be judged using absolute copy number of a control gene, the C_T value for a control gene, the strength of a band on a gel, the peak height of fragment, the height of the (pyro) sequence or other appropriate output. Specific criteria should be laid down to identify samples that are of poor quality. It is important to stress that all results of molecular investigations should be considered in the context of clinical, morphological, haematological and other laboratory findings.

Interpretation of low level JAK2 V617F at diagnosis

A result of <1–3% V617F should be interpreted in the context of clinical, morphological, haematological and other laboratory findings. Such considerations not only mitigate against occasional technical aberrations but it has been claimed that *JAK2* V617F may occasionally be found in haematologically normal individuals when highly sensitive assays are used (Sidon *et al*, 2006; Xu *et al*, 2007; Nielsen *et al*, 2011). Assuming the result does not represent a false positive, it is reproducible and the amplification is distinct from appropriate normal controls, such a result may well represent a true low level clone. In patients with other laboratory or clinical criteria suggestive of a MPN, this result provides objective evidence in support of the diagnosis. Low level *JAK2* V617F may occur for a number of reasons: (i) prior treatment with cytoreductive therapy may reduce the level of the *JAK2* V617F positive clone within the sample (Girodon *et al*, 2008); (ii) the presence of two MPN clones in the patient, only one of which is *JAK2* V617F positive (Beer *et al*, 2009). Mutation assessment of *JAK2* exon 12 or *MPL* exon 10 may reveal the existence of such second clones.

In a patient with a low level *JAK2* V617F mutation but with a normal full blood count, the clinical significance is less clear. Obviously, iron-deficient PV has to be excluded. It is still possible that this may reflect the presence of a chronic MPN and that the relevant blood parameters have risen above the individual's own baseline, but are not yet above the upper limit for the normal range of the appropriate population. Alternatively, a low level *JAK2* V617F positive clone may remain stable, or even occasionally disappear with time (for example, if it arises in a short-lived haematopoietic precursor), without significant clinical effects. Such patients may warrant further clinical surveillance. Whatever the cause and clinical situation pertaining to a low level *JAK2* V617F mutation, it is prudent to obtain a fresh sample (e.g. within 3–6 months if possible) to enable the assay to be repeated.

False low levels and false negatives

False low levels or even false negatives can occur due to the presence of an additional exon 14 mutation or inherited polymorphism. If these additional changes lie within one of the primer or probe binding sites, they may reduce the efficiency of V617F-specific PCR amplification. Rare instances of additional acquired mutations or constitutional variants have been reported (Table II). Depending on the assay utilized, these can lead to a false negative result for V617F or apparent low level amplification. Assessment of the c.1849G>T (V617F) mutation by an additional method that utilizes different primer/probe sets may be helpful in situations of apparent low level amplification.

© 2012 Blackwell Publishing Ltd

Table II. Non-V617F variants within *JAK2* exon 14.

Mutation	Amino acid	Reference
1831T>G/1849G>T	L611V/V617F	Cleyrat *et al* (2010)
1839T>C/1849G>T	Y613Y/V617F	T. Clench (unpublished observations)
1848T>C/1849G>T	C616C/V617F	Wong *et al* (2007)
1849G>T/1851C>T/1852T>C	V617F/C618R	Warshawsky *et al* (2010); A. Goday-Fernandez and A. J. Bench (unpublished observations)
1849G>T/1853G>T	V617F/C618F	Warshawsky *et al* (2010)
1860C>A	D620E	Schnittger *et al* (2006)
G1849G>T/1860C>A	V617F/D620E	Grunebach *et al* (2006)
1849G>A	V617I	Mead *et al* (2012)

Considerations for validation of a JAK2 V617F detection assay

Prior to introduction and as part of ongoing quality control, the assay should be appropriately validated. Particularly with the more sensitive assays, a large series of healthy control samples should be assessed to determine the false positive rate (Mattocks *et al*, 2010). Ideally at least 100 should be tested, which gives a lower confidence interval of 97·5% specificity assuming all results are negative (300 samples are necessary to give 99% specificity). These data should demonstrate lack of a 'positive' result for the healthy control panel or, as a minimum, identify a cut-off below which the result is defined as 'not detected'. If false positives are observed, consideration should be given to modification of the assay to reduce or prevent inappropriate amplification. The validation process should also determine the approximate sensitivity of the assay. This is more straightforward for assays that are able to quantify the absolute amount of mutant *JAK2* V617F burden through the use of standard curve reagents. In reality, comparison between laboratories is difficult to achieve given the absence of certified reference reagents that could be universally applied. However a suitable dilution series can be prepared using the *JAK2* V617F positive cell lines, such as UKE-1 or HEL and used to determine assay sensitivity. Of these, UKE-1 may be preferable because HEL has multiple copies of mutant *JAK2* (Quentmeier *et al*, 2006). As described above, achievement of a sensitivity of 1–3% is desirable and such an assay would be expected to identify the vast majority of patients with a pathogenetically relevant level of *JAK2* V617F mutant clone. Whatever the sensitivity achieved, it is important to indicate the assay sensitivity when reporting results.

Ongoing internal quality control should be performed and appropriate controls should be included on each run. Such controls would include a known *JAK2* V617F positive (>5% V617F), a *JAK2* V617F positive at a level of 1–3% (or other percentage <5% to assess sensitivity) and normal control(s). As described above, a result that appears to be lower than the 1–3% positive control may still be valid but should be interpreted carefully and in the clinical context. Finally, participation in an appropriate external quality assessment programme for *JAK2* V617F detection (e.g. www.ukneqasli.org.uk) provides an independent assessment of test quality. Participation in such a programme is required for laboratory accreditation in the United Kingdom. It is noteworthy that a recent international study from the United Kingdom National External Quality Assessment Service for Leucocyte Immunophenotyping found that 20% of testing laboratories failed to detect mutant *JAK2* in a sample with 2% V617F and 9% of laboratories failed to detect the mutation at 5% V617F (Clark *et al*, 2012).

Supplementary tests for *JAK2* V617F negative cases

As described earlier, *JAK2* V617F negative MPN patients may carry mutations at other loci including *JAK2* exon 12, *MPL*, *TET2*, *ASXL1*, *CBL*, *SH2B3* (also termed *LNK*) and *EZH2* (Tefferi & Vainchenker, 2011). Diagnostic assays are available for the detection of mutations within *JAK2* exon 12 and *MPL* exon 10. Assessment of the other loci is not currently performed in a diagnostic capacity in the UK. Changing technologies will facilitate more comprehensive and cost effective mutation screening in the near future, however despite the fact that mutations in some genes, e.g. *EZH2*, have been associated with a poor prognosis (Guglielmelli *et al*, 2011), it remains uncertain if the routine detection of mutations in these genes is of any real value. As exclusion of chronic myeloid leukaemia (CML) is one of the criteria for the diagnosis of ET and PMF (Swerdlow *et al*, 2008), many laboratories also screen for the *BCR-ABL1* fusion gene by reverse transcription polymerase chain reaction (RT-PCR) or fluorescence *in situ* hybridization (FISH). Diverse rearrangements of *PDGFRA* or *PDGFRB* are generally associated with eosinophilic MPN or MDS/MPN and should not be routinely screened for in patients with classical MPN without eosinophilia (Jones & Cross, 2004; Reiter *et al*, 2007).

JAK2 exon 12 mutation

Mutations within exon 12 of *JAK2* have, so far, only been reported in patients with polycythaemia vera, some of which

© 2012 Blackwell Publishing Ltd

were classified as idiopathic erythrocytosis (Percy *et al*, 2007; Passamonti *et al*, 2011). *JAK2* exon 12 mutation positive patients tend to be characterized by isolated erythrocytosis, erythroid hyperplasia and low serum EPO (Percy *et al*, 2007; Scott *et al*, 2007a). At least 17 different mutations have been described, often as a result of a six base pair deletion (Cazzola, 2007; Passamonti *et al*, 2011). These mutations fall into three main groups – those that result in a deletion of glutamic acid at codon 543 (E543del); those that lead to a lysine to leucine substitution at codon 539 (K539L) and duplications that lead to substitution of the phenylalanine at codon 547.

Given that the presence of a *JAK2* exon 12 mutation in a patient with erythrocytosis is diagnostic for PV (McMullin *et al*, 2007), one strategy is to screen all patients presenting with unexplained erythrocytosis who are *JAK2* V617F negative for mutations within *JAK2* exon 12 (Fig 1). Alternatively, as most cases of *JAK2* V617F negative erythrocytosis turn out not to carry an exon 12 mutation (Fig 1), other tests, such as measurement of serum erythropoietin (EPO), isolation of EPO-independent BFU-E colonies or examination of the bone marrow trephine biopsy, could be performed to exclude cases unlikely to be true PV.

Due to the large number of possible mutations, techniques that target specific mutations, such as allele-specific PCR, are of limited value for the detection of *JAK2* exon 12 mutations. Direct sequencing remains an option but the level of disease in peripheral blood is often even lower than for *JAK2* V617F mutation due to the erythroid lineage specificity. Direct sequencing would probably require analysis of the bone marrow aspirate or EPO-independent BFU-E colonies to be of sufficient sensitivity (Cazzola, 2007). Highly sensitive mutation scanning methods have been developed for the identification of *JAK2* exon 12 mutations. The most commonly used for are HRM analysis (Jones *et al*, 2008; Rapado *et al*, 2009; Ugo *et al*, 2010), melting curve assay (Schnittger *et al*, 2009b) and dHPLC. The sensitivity of these assays range from 1% to 10% depending on the mutation present. More sensitive assays, such as PCR clamping assays (Laughlin *et al*, 2010), would enable low level *JAK2* exon 12 positive clones to be identified in the peripheral blood.

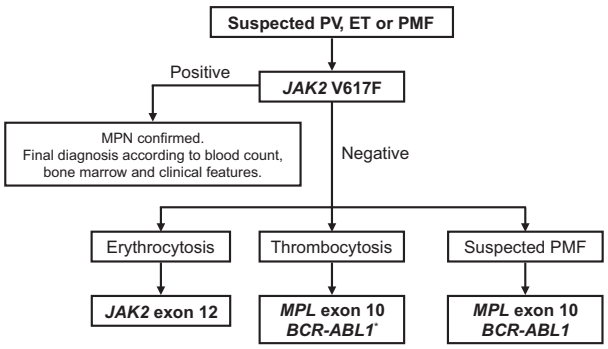

Fig 1. Molecular diagnostic algorithm for the classical myeloproliferative neoplasms. Approximately 2% of *JAK2* V617F negative cases that present with erythrocytosis carry a *JAK2* exon 12 mutation and approximately 8% of *JAK2* V617F negative cases presenting with thrombocytosis or myelofibrosis carry a *MPL* exon 10 mutation. *If blood film/count suggestive of chronic myeloid leukaemia (Harrison *et al*, 2010). Cytogenetic analysis may also be helpful if no molecular genetic abnormality is detected.

MPL exon 10 mutations

Mutations within *MPL* exon 10 have been reported in 5–10% of patients with ET and PMF patients but not in any PV patients (Pardanani *et al*, 2006, 2011; Beer *et al*, 2008). At least 5 pathogenetic mutations within *MPL* exon 10 have been described (Pardanani *et al*, 2006; Beer *et al*, 2008; Chaligné *et al*, 2008; Schnittger *et al*, 2009a; Boyd *et al*, 2010) (W515L; W515K; W515R; W515A; S505N). Other mutations within *MPL* have been observed although the pathogenetic significance of some of these mutations is not clear (Williams *et al*, 2007; Chaligné *et al*, 2008; Pardanani *et al*, 2011).

Because of the positive diagnostic value of demonstration of a *MPL* exon 10 mutation, especially for patients presenting with unexplained thrombocytosis, screening for *MPL* exon 10 mutations has been recommended in cases of suspected ET or PMF that are *JAK2* V617F negative (Swerdlow *et al*, 2008; Harrison *et al*, 2010) (Fig 1). Bone marrow examination to assess megakaryocyte morphology may not be necessary in patients with ET for whom a *JAK2* V617F or *MPL* exon 10 mutation has been demonstrated (Harrison *et al*, 2010).

In contrast to *JAK2* exon 12 mutations, the repertoire of *MPL* exon 10 mutations is relatively restricted. Therefore, two main approaches have been applied for the detection of *MPL* exon 10 mutations:

- An allele-specific PCR approach for each known mutation in a similar fashion to *JAK2* V617F mutation detection. As for detection of *JAK2* V617F, pyrosequencing (Beer *et al*, 2008), allele-specific PCR (Beer *et al*, 2008) or allele-specific real time PCR assays are available (Laurent *et al*, 2007; Ghaderi *et al*, 2008; Pancrazzi *et al*, 2008), with real time PCR assays generally possessing higher sensitivity (up to 0·1%). Real time PCR thus enables detection of low level *MPL* W515L/K mutations in the peripheral blood as for *JAK2* V617F real time PCR assays. The disadvantage of such an approach is that multiple PCR assays are required to detect all possible mutations. Furthermore, allele-specific real time PCR and pyrosequencing assays are only available for detection of W515L and W515K mutations.
- Whole exon mutation scanning approach. The most frequently applied approaches are HRM (Boyd *et al*, 2010) and melting curve analysis (Pardanani *et al*, 2006, 2011; Schnittger *et al*, 2009a). These approaches offer the advantage of quickly assessing patients for all W515 mutations and S505 mutations. The sensitivity for these assays is approximately 2–5% – i.e. less sensitive than real time allele-specific PCR but substantially better than direct sequencing. Whether low frequency *MPL* exon 10 mutation positive clones are missed by these assays is not

© 2012 Blackwell Publishing Ltd

known. However, given that low level *JAK2* V617F mutation is common in ET, it would be expected that some ET patients possess low level *MPL* exon 10 mutations. The combination of mutation scanning methods with PCR methods that preferentially amplify the mutant allele could improve sensitivity.

BCR-ABL1 assessment

Exclusion of CML is a requirement in the diagnostic criteria of both ET and PMF but not PV (Swerdlow *et al*, 2008). Despite the rare occurrences of *JAK2* V617F positive/*BCR-ABL1* positive cases (Hussein *et al*, 2008; Pieri *et al*, 2011) the demonstration of a *BCR-ABL1* fusion in a patient with thrombocytosis or myelofibrosis indicates a diagnosis of CML and excludes a diagnosis of ET or PMF. Guidelines for investigation of thrombocytosis (Harrison *et al*, 2010) indicate that screening for the *BCR-ABL1* fusion gene should only be necessary if atypical features, such as basophilia or left shift of neutrophils, are present within the blood irrespective of the *JAK2* V617F status. Whether assessment for the *BCR-ABL1* fusion gene needs to be carried out for *JAK2* V617F or *MPL* exon 10 positive PMF is unclear but may be useful if these mutations are not detected to exclude a diagnosis of CML (Swerdlow *et al*, 2008).

Other causes of erythrocytosis and thrombocytosis

A number of non-malignant causes of erythrocytosis and thrombocytosis may be investigated and an increasing panel of genes have been identified that are implicated in familial erythrocytosis and thrombocytosis. Congenital causes of erythocytosis include mutations in globin genes giving rise to high oxygen affinity haemoglobin, *BPGM* mutation resulting in bisphosphoglycerate mutase deficiency, mutations in components of EPO signalling pathway (*EPOR*) and mutations within components of oxygen sensing pathways such as within *VHL*, *EGLN1* (also termed *PHD2*) and *EPAS1* (*HIF2A*). Especially in younger patients, mutations within such genes may identify the cause of the erythrocytosis (McMullin, 2008). Inherited forms of thrombocythaemia may be caused by mutations within the 5′ untranslated region of *THPO* (also termed *TPO*) or within the *MPL* locus itself, including K39N (MPL-Baltimore), P106L and S505N mutations (Skoda, 2010). Recently, two families with inherited thrombocytosis and activating mutations within *JAK2* (V617I and R564Q) have been reported (Etheridge *et al*, 2011; Mead *et al*, 2012). By contrast, the V617F mutation itself has not been reported to be inherited in familial cases although family members may acquire the mutation independently (Cario *et al*, 2005).

Acknowledgements

DG and NCPC gratefully acknowledge support from the Minimal Residual Disease Workpackage (WP12) of the European LeukemiaNet. AJB, AGF and TC carried out experiments. AJB, HEW, LF, ALG, GG, SA, AA, IC, SEL, TC, JC, PAE, DG, AS, MFM, ARG, CNH and NCPC wrote the manuscript.

References

Albiero, E., Bernardi, M., Madeo, D., Ruggeri, M. & Rodeghiero, F. (2008) A new TMHA-DHPLC assay for the rapid mutation screening of JAK2 exon 14 in myeloproliferative disorders. *American Journal of Hematology*, **83**, 603–604.

Antonioli, E., Carobbio, A., Pieri, L., Pancrazzi, A., Guglielmelli, P., Delaini, F., Ponziani, V., Bartalucci, N., Tozzi, L., Bosi, A., Rambaldi, A., Barbui, T. & Vannucchi, A.M. (2010) Hydroxyurea does not appreciably reduce JAK2 V617F allele burden in patients with polycythemia vera or essential thrombocythemia. *Haematologica*, **95**, 1435–1438.

Asimakopoulos, F.A., Gilbert, J.G.R., Aldred, M.A., Pearson, T.C. & Green, A.R. (1996) Interstitial deletion constitutes the major mechanism for loss of heterozygosity on chromosome 20q in polycythemia vera. *Blood*, **88**, 2690–2698.

Baxter, E.J., Scott, L.M., Campbell, P.J., East, C., Fourouclas, N., Swanton, S., Vassiliou, G.S., Bench, A.J., Boyd, E.M., Curtin, N., Scott, M.A. & Erber, W.N.; The Cancer Genome Project & Green AR (2005) Acquired mutation of the tyrosine kinase JAK2 in human myeloproliferative disorders. *Lancet*, **365**, 1054–1061.

Beer, P.A., Campbell, P.J., Scott, L.M., Bench, A.J., Erber, W.N., Bareford, D., Wilkins, B.S., Reilly, J.T., Hasselbalch, H.C., Bowman, R., Wheatley, K., Buck, G., Harrison, C.N. & Green, A.R. (2008) MPL mutations in myeloproliferative disorders: analysis of the PT-1 Cohort. *Blood*, **112**, 141–149.

Beer, P.A., Jones, A.V., Bench, A.J., Goday-Fernandez, A., Boyd, E.M., Vaghela, K.J., Erber, W.N., Odeh, B., Wright, C., McMullin, M.F., Cullis, J., Huntly, B.J., Harrison, C.N., Cross, N.C. & Green, A.R. (2009) Clonal diversity in the myeloproliferative neoplasms: independent origins of genetically distinct clones. *British Journal of Haematology*, **144**, 904–908.

Boyd, E.M., Bench, A.J., Goday-Fernandez, A., Anand, S., Vaghela, K.J., Beer, P., Scott, M.A., Bareford, D., Green, A.R., Huntly, B. & Erber, W.N. (2010) Clinical utility of routine MPL exon 10 analysis in the diagnosisof essential thrombocythaemia and primary myelofibrosis. *British Journal of Haematology*, **149**, 250–257.

Campbell, P.J., Scott, L.M., Buck, G., Wheatley, K., East, C.L., Marsden, J.T., Duffy, A., Boyd, E.M., Bench, A.J., Scott, M.A., Vassiliou, G.S., Milligan, D.W., Smith, S.R., Erber, W.N., Bareford, D., Wilkins, B.S., Reilly, J.T., Harrison, C.N. & Green, A.R. (2005) Definition of subtypes of essential thrombocythaemia and relation to polycythaemia vera based on JAK2 V617F mutation status: a prospective study. *Lancet*, **366**, 1945–1953.

Cankovic, M., Whiteley, L., Hawley, R.C., Zarbo, R.J. & Chitale, D. (2009) Clinical performance of JAK2 V617F mutation detection assays in a molecular diagnostics laboratory. *American Journal of Clinical Pathology*, **132**, 713–721.

Cario, H., Goerttler, P.S., Steimle, C., Levine, R.L. & Pahl, H.L. (2005) The JAK2 V617F mutation is acquired secondary to the predisposing alteration in familial polycythaemia vera. *British Journal of Haematology*, **130**, 795–805.

Cazzola, M. (2007) Somatic mutations of JAK2 exon 12 as a molecular basis of erythrocytosis. *Haematologica*, **92**, 1585–1589.

Chaligné, R., Tonetti, C., Besancenot, R., Roy, L., Marty, C., Mossuz, P., Kiladjian, J.J., Socié, G., Bordessoule, D., Le Bousse-Kerdilès, M.C., Vainchenker, W. & Giraudier, S. (2008) New mutations of MPL in primitive myelofibrosis: only the MPL W515 mutations promote a G1/S-phase transition. *Leukemia*, **22**, 1557–1566.

Chen, Q., Lu, P., Jones, A.V., Silver, R.T. & Wang, Y.L. (2007) Amplification refractory mutation system, a highly sensitive and simple polymerase

© 2012 Blackwell Publishing Ltd

chain reaction assay, for the detection of JAK2 V617F mutation in chronic myeloproliferative disorders. *The Journal of Molecular Diagnostics*, **9**, 272–276.

Clark, J.R., Jack, A.L., Barnett, D. & Reilly, J.T. (2012) JAK2 V617F false negative rate in the UK NEQAS LI programme. *British Journal of Haematology*, **157**(Suppl 1), 56.

Cleyrat, C., Jelinek, J., Girodon, F., Boissinot, M., Ponge, T., Harousseau, J.L., Issa, J.P. & Hermouet, S. (2010) JAK2 mutation and disease phenotype: a double L611V/V617F in cis mutation of JAK2 is associated with isolated erythrocytosis and increased activation of AKT and ERK1/2 rather than STAT5. *Leukemia*, **24**, 1069–1073.

Dentali, F., Squizzato, A., Brivio, L.,Appio, L., Campiotti, L., Crowther, M., Grandi, A.M. & Ageno, W. (2009) JAK2 V617F mutation for the early diagnosis of Ph- myeloproliferative neoplasms in patients with venous thromboembolism: a meta-analysis. *Blood*, **113**, 5617–5623.

Denys, B., El Housni, H., Nollet, F., Verhasselt, B. & Philippé, J. (2010) A real-time polymerase chain reaction assay for rapid, sensitive, and specific quantification of the JAK2 V617F mutation using a locked nucleic acid-modified oligonucleotide. *The Journal of Molecular Diagnostics*, **12**, 512–519.

Etheridge, L., Corbo, L.M., Kaushansky, K., Chan, E. & Hitchcock, I.S. (2011) A novel activating JAK2 mutation, JAK2R564Q, causes familial essential thrombocytosis (fET) via mechanisms distinct from JAK2 V617F. *Blood*, **118**, 123. (ASH Annual Meeting Abstracts).

Ghaderi, M., Stromberg, O. & Porwit, A. (2008) Rapid real-time PCR assay for detection of MPL W515L mutation in patients with chronic myeloproliferative disorders. *International Journal of Laboratory Hematology*, **32**, 122–126.

Girodon, F., Schaeffer, C., Cleyrat, C., Mounier, M., Lafont, I., Santos, F.D., Duval, A., Maynadié, M. & Hermouet, S. (2008) Frequent reduction or absence of detection of the JAK2-mutated clone in JAK2 V617F-positive patients within the first years of hydroxyurea therapy. *Haematologica*, **93**, 1723–1727.

Goday-Fernandez, A., Boyd, E., Bench, A. & Erber, W. (2008) Real time detection of JAK2 V617F in the myeloproliferative disorders. *Haematologica*, **93**(s1), 302. Abs.0754.

Grunebach, F., Bross-Bach, U., Kanz, L. & Brossart, P. (2006) Detection of a new JAK2 D620E mutation in addition to V617F in a patient with polycythemia vera. *Leukemia*, **20**, 2210–2211.

Guglielmelli, P., Biamonte, F., Score, J., Hidalgo-Curtis, C., Cervantes, F., Maffioli, M., Fanelli, T., Ernst, T., Winkelman, N., Jones, A.V., Zoi, K., Reiter, A., Duncombe, A., Villani, L., Bosi, A., Barosi, G., Cross, N.C. & Vannucchi, A.M. (2011) EZH2 mutational status predicts poor survival in myelofibrosis. *Blood*, **118**, 5227–5234.

Harrison, C.N., Bareford, D., Butt, N., Campbell, P., Conneally, E., Drummond, M., Erber, W., Everington, T., Green, A.R., Hall, G.W., Hunt, B.J., Ludlam, C.A., Murrin, R., Nelson-Piercy, C., Radia, D.H., Reilly, J.T., Van der Walt, J., Wilkins, B. & McMullin, M.F. (2010) Guideline for investigation and management of adults and children presenting with a thrombocytosis. *British Journal of Haematology*, **149**, 352–375.

Hermouet, S., Dobo, I., Lippert, E., Boursier, M.C., Ergand, L., Perrault-Hu, F. & Pineau, D. (2007) Comparison of whole blood vs purified blood granulocytes for the detection and quantitation of JAK2 V617F. *Leukemia*, **21**, 1128–1130.

Hussein, K., Bock, O., Theophile, K., Seegers, A., Arps, H., Basten, O., Grips, K.H., Franz-Werner, J., Büsche, G. & Kreipe, H. (2008) Chronic myeloproliferative diseases with concurrent BCR-ABL junction and JAK2 V617F mutation. *Leukemia*, **22**, 1059–1062.

James, C., Ugo, V., Le Couédic, J.P., Staerk, J., Delhommeau, F., Lacout, C., Garçon, L., Raslova, H., Berger, R., Bennaceur-Griscelli, A., Villeval, J.L., Constantinescu, S.N., Casadevall, N. & Vainchenker, W. (2005) A unique clonal JAK2 mutation leading to constitutive signalling causes polycythaemia vera. *Nature*, **434**, 1144–1148.

James, C., Delhommeau, F., Marzac, C., Teyssandier, I., Couédic, J.P., Giraudier, S., Roy, L., Saulnier, P., Lacroix, L., Maury, S., Tulliez, M., Vainchenker, W., Ugo, V. & Casadevall, N. (2006) Detection of JAK2 V617F as a first intention diagnostic test for erythrocytosis. *Leukemia*, **20**, 350–353.

Jelinek, J., Oki, Y., Gharibyan, V., Bueso-Ramos, C., Prchal, J.T., Verstovsek, S., Beran, M., Estey, E., Kantarjian, H.M. & Issa, J.P. (2005) JAK2 mutation 1849G>T is rare in acute leukemias but can be found in CMML, Philadelphia chromosome–negative CML, and megakaryocytic leukemia. *Blood*, **106**, 3370–3373.

Jones, A.V. & Cross, N.C. (2004) Oncogenic derivatives of platelet-derived growth factor receptors. *Cellular and Molecular Life Sciences*, **61**, 2912–2923.

Jones, A.V., Kreil, S., Zoi, K., Waghorn, K., Curtis, C., Zhang, L., Score, J., Seear, R., Chase, A.J., Grand, F.H., White, H., Zoi, C., Loukopoulos, D., Terpos, E., Vervessou, E.C., Schultheis, B., Emig, M., Ernst, T., Lengfelder, E., Hehlmann, R., Hochhaus, A., Oscier, D., Silver, R.T., Reiter, A. & Cross, N.C. (2005) Widespread occurrence of the JAK2 V617F mutation in chronic myeloproliferative disorders. *Blood*, **106**, 2162–2168.

Jones, A.V., Silver, R.T., Waghorn, K., Curtis, C., Kreil, S., Zoi, K., Hochhaus, A., Oscier, D., Metzgeroth, G., Lengfelder, E., Reiter, A., Chase, A.J. & Cross, N.C. (2006) Minimal molecular response in polycythemia vera patients treated with imatinib or interferon alpha. *Blood*, **107**, 3339–3341.

Jones, A.V., Cross, N.C.P., White, H.E., Green, A.R. & Scott, L.M. (2008) Rapid identification of JAK2 exon 12 mutations using high resolution melting analysis. *Haematologica*, **93**, 1560–1564.

Jovanovic, J.V., Vannucchi, A.M., Lippert, E., Oppliger Leibundgut, E., Maroc, N., Hermouet, S., Nickless, G., Cassinat, B., Guglielmelli, P., van der Reijden, B., Bench, A., Tobal, K., Wilkins, B., Cuthill, K., Ivey, A., Yeoman, K., Percy, M.J., Schwemmers, S., Gruender, A., Kelley, T.W., Reading, S., Pancrazzi, A., McMullin, M.F., Pahl, H.L., Harrison, C.N., Prchal, J.T., Chomienne, C., Kiladjian, J.J., Barbui, T. & Grimwade, D. (2011) Systematic evaluation of DNA-based quantitative-polymerase chain reaction (Q-PCR) assays to track treatment response in patients with JAK2-V617F associated myeloproliferative neoplasms: a Joint European LeukemiaNet/MPN&MPNr-EuroNet study. *Blood*, **118**, 2812. (ASH Annual Meeting Abstracts).

Kralovics, R., Passamonti, F., Buser, A.S., Teo, S.S., Tiedt, R., Passweg, J.R., Tichelli, A., Cazzola, M. & Skoda, R.C. (2005) A gain-of-function mutation of JAK2 in myeloproliferative disorders. *New England Journal of Medicine*, **352**, 1779–1790.

Kroger, N., Badbaran, A., Holler, E., Hahn, J., Kobbe, G., Bornhäuser, M., Reiter, A., Zabelina, T., Zander, A.R. & Fehse, B. (2007) Monitoring of the JAK2-V617F mutation by highly sensitive quantitative real-time PCR after allogeneic stem cell transplantation in patients with myelofibrosis. *Blood*, **109**, 1316–1321.

Larsen, T.S., Christensen, J.H., Hasselbalch, H.C. & Pallisgaard, N. (2007) The JAK2 V617F mutation involves B- and T-lymphocyte lineages in a subgroup of patients with Philadelphia-chromosome negative chronic myeloproliferative disorders. *British Journal of Haematology*, **136**, 745–751.

Larsen, T.S., Pallisgaard, N., Møller, M.B. & Hasselbalch, H.C. (2008) Quantitative assessment of the JAK2 V617F allele burden: equivalent levels in peripheral blood and bone marrow. *Leukemia*, **22**, 194–195.

Laughlin, T.S., Moliterno, A.R., Stein, B.L. & Rothberg, P.G. (2010) Detection of exon 12 mutations in the JAK2 gene: enhanced analytical sensitivity using clamped PCR and nucleotide sequencing. *The Journal of Molecular Diagnostics*, **12**, 278–282.

Laurent, C., Demas, V., Delabesse, E. & Brousset, P. (2007) Detection of the MPL W515L mutation in bone marrow core biopsy specimens with essential thrombocythemia using the TaqMan assay. *Human Pathology*, **38**, 1581–1582.

Levine, R.L., Wadleigh, M., Cools, J., Ebert, B.L., Wernig, G., Huntly, B.J., Boggon, T.J., Wlodarska, I., Clark, J.J., Moore, S., Adelsperger, J., Koo, S., Lee, J.C., Gabriel, S., Mercher, T., D'Andrea, A., Fröhling, S., Döhner, K., Marynen, P., Vandenberghe, P., Mesa, R.A., Tefferi, A., Griffin, J.D., Eck, M.J., Sellers, W.R., Meyerson, M., Golub, T.R., Lee, S.J. & Gilliland, D.G. (2005a) Activating mutation in the tyrosine kinase JAK2 in polycythemia vera, essential thrombocythemia, and myeloid metaplasia with myelofibrosis. *Cancer Cell*, **7**, 387–397.

Levine, R.L., Loriaux, M., Huntly, B.J.P., Loh, M.L., Beran, M., Stoffregen, E., Berger, R., Clark, J.J., Willis, S.G., Nguyen, K.T., Flores, N.J., Estey, E., Gattermann, N., Armstrong, S., Look, A.T., Griffin, J.D., Bernard, O.A., Hein-

© 2012 Blackwell Publishing Ltd

rich, M.C., Gilliland, D.G., Druker, B. & Deininger, M.W. (2005b) The JAK2 V617F activating mutation occurs in chronic myelomonocytic leukemia and acute myeloid leukemia, but not in acute lymphoblastic leukemia or chronic lymphocytic leukemia. *Blood*, **106**, 3377–3379.

Lippert, E., Boissinot, M., Kralovics, R., Girodon, F., Dobo, I., Praloran, V., Boiret-Dupré, N., Skoda, R.C. & Hermouet, S. (2006) The JAK2-V617F mutation is frequently present at diagnosis in patients with essential thrombocythemia and polycythemia vera. *Blood*, **108**, 1865–1867.

Lippert, E., Girodon, F., Hammond, E., Jelinek, J., Reading, N.S., Fehse, B., Hanlon, K., Hermans, M., Richard, C., Swierczek, S., Ugo, V., Carillo, S., Harrivel, V., Marzac, C., Pietra, D., Sobas, M., Mounier, M., Migeon, M., Ellard, S., Kröger, N., Herrmann, R., Prchal, J.T., Skoda, R.C. & Hermouet, S. (2009) Concordance of assays designed for the quantification of JAK2 V617F: a multicenter study. *Haematologica*, **94**, 38–45.

Ma, W., Kantarjian, H., Zhang, X., Sun, W., Buller, A.M., Jilani, I., Schwartz, J.G., Giles, F. & Albitar, M. (2008) Higher detection rate of *JAK2* mutation using plasma. *Blood*, **111**, 3906–3907.

Ma, W., Zhang, X., Wang, X., Zhang, Z., Yeh, C.H., Uyeji, J. & Albitar, M. (2011) MPL mutation profile in JAK2 mutation-negative patients with myeloproliferative disorders. *Diagnostic Molecular Pathology*, **20**, 34–39.

Mason, J., Akiki, S. & Griffiths, M.J. (2011) Pitfalls in molecular diagnosis in haemato-oncology. *Journal of Clinical Pathology*, **64**, 275–278.

Mattocks, C.J., Morris, M.A., Matthijs, G., Swinnen, E., Corveleyn, A., Dequeker, E., Müller, C.R., Pratt, V. & Wallace, A. (2010) A standardized framework for the validation and verification of clinical molecular genetic tests. *European Journal of Human Genetics*, **18**, 1276–1288.

McClure, R., Mai, M. & Lasho, T. (2006) Validation of two clinically useful assays for evaluation of JAK2 V617F mutation in chronic myeloproliferative disorders. *Leukemia*, **20**, 168–171.

McMullin, M.F. (2008) The classification and diagnosis of erythrocytosis. *International Journal of Laboratory Hematology*, **30**, 447–459.

McMullin, M.F., Reilly, J.T., Campbell, P., Bareford, D., Green, A.R., Harrison, C.N., Conneally, E. & Ryan, K. (2007) Amendment to the guideline for diagnosis and investigation of polycythaemia/erythrocytosis. *British Journal of Haematology*, **138**, 821–822.

Mead, A.J., Rugless, M.J., Jacobsen, S.E. & Schuh, A. (2012) Germline JAK2 mutation in a family with hereditary thrombocytosis. *The New England Journal of Medicine*, **366**, 967–969.

Nielsen, C., Birgens, H.S., Nordestgaard, B.G., Kjaer, L. & Bojesen, S.E. (2011) The JAK2 V617F somatic mutation, mortality and cancer risk in the general population. *Haematologica*, **96**, 450–453.

Pancrazzi, A., Guglielmelli, P., Ponziani, V., Bergamaschi, G., Bosi, A., Barosi, G. & Vannucchi, A.M. (2008) A sensitive detection method for MPLW515L or MPLW515K mutation in chronic myeloproliferative disorders with locked nucleic acid-modified probes and real-time polymerase chain reaction. *The Journal of Molecular Diagnostics*, **10**, 435–441.

Pardanani, A.D., Levine, R.L., Lasho, T., Pikman, Y., Mesa, R.A., Wadleigh, M., Steensma, D.P., Elliott, M.A., Wolanskyj, A.P., Hogan, W.J., McClure, R.F., Litzow, M.R., Gilliland, D.G. & Tefferi, A. (2006) MPL515 mutations in myeloproliferative and other myeloid disorders: a study of 1182 patients. *Blood*, **108**, 3472–3476.

Pardanani, A., Guglielmelli, P., Lasho, T.L., Pancrazzi, A., Finke, C.M., Vannucchi, A.M. & Tefferi, A. (2011) Primary myelofibrosis with or without mutant MPL: comparison of survival and clinical features involving 603 patients. *Leukemia*, **25**, 1834–1839.

Passamonti, F. & Rumi, E. (2009) Clinical relevance of JAK2 (V617F) mutant allele burden. *Haematologica*, **94**, 7–10.

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

Percy, M.J., Scott, L.M., Erber, W.N., Harrison, C.N., Reilly, J.T., Jones, F.G., Green, A.R. & McMullin, M.F. (2007) The frequency of JAK2 exon 12 mutations in idiopathic erythrocytosis patients with low serum erythropoietin levels. *Haematologica*, **92**, 1607–1614.

Pieri, L., Spolverini, A., Scappini, B., Occhini, U., Birtolo, S., Bosi, A., Albano, F., Fava, C. & Vannucchi, A.M. (2011) Concomitant occurrence of BCR-ABL and *JAK2* V617F mutation. *Blood*, **118**, 3445–3446.

Qian, J., Lin, J., Yao, D.M., Chen, Q., Xiao, G.F., Ji, R.B., Li, Y., Yang, J. & Qian, Z. (2010) Rapid detection of JAK2 V617F mutation using high-resolution melting analysis with LightScanner platform. *Clinica Chimica Acta*, **411**, 2097–2100.

Quentmeier, H., MacLeod, R.A., Zaborski, M. & Drexler, H.G. (2006) *JAK2* V617F tyrosine kinase mutation in cell lines derived from myeloproliferative disorders. *Leukemia*, **20**, 471–476.

Rapado, I., Grande, S., Albizua, E., Ayala, R., Hernández, J.A., Gallardo, M., Gilsanz, F. & Martinez-Lopez, J. (2009) High resolution melting analysis for JAK2 exon 14 and exon 12 mutations – A diagnostic tool for myeloproliferative neoplasms. *The Journal of Molecular Diagnostics*, **11**, 155–161.

Reiter, A., Grimwade, D. & Cross, N.C. (2007) Diagnostic and therapeutic management of eosinophilia-associated chronic myeloproliferative disorders. *Haematologica*, **92**, 1153–1158.

Sattler, M., Walz, C., Crowley, B.J., Lengfelder, E., Jänne, P.A., Rogers, A.M., Kuang, Y., Distel, R.J., Reiter, A. & Griffin, J.D. (2006) A sensitive high-throughput method to detect activating mutations of Jak2 in peripheral-blood samples. *Blood*, **107**, 1237–1238.

Schmitt-Graeff, A.H., Teo, S.S., Olschewski, M., Schaub, F., Haxelmans, S., Kirn, A., Reinecke, P., Germing, U. & Skoda, R.C. (2008) JAK2 V617F mutation status identifies subtypes of refractory anemia with ringed sideroblasts associated with marked thrombocytosis. *Haematologica*, **93**, 34–40.

Schnittger, S., Bacher, U., Kern, W., Schröder, M., Haferlach, T. & Schoch, C. (2006) Report on two novel nucleotide exchanges in the JAK2 pseudokinase domain: D620E and E627E. *Leukemia*, **20**, 2195–2197.

Schnittger, S., Bacher, U., Haferlach, C., Beelen, D., Bojko, P., Bürkle, D., Dengler, R., Distelrath, A., Eckart, M., Eckert, R., Fries, S., Knoblich, J., Köchling, G., Laubenstein, H.P., Petrides, P., Planker, M., Pihusch, R., Weide, R., Kern, W. & Haferlach, T. (2009a) Characterization of 35 new cases with four different MPLW515 mutations and essential thrombocytosis or primary myelofibrosis. *Haematologica*, **94**, 141–144.

Schnittger, S., Bacher, U., Haferlach, C., Geer, T., Müller, P., Mittermüller, J., Petrides, P., Schlag, R., Sandner, R., Selbach, J., Slawik, H.R., Tessen, H.W., Wehmeyer, J., Kern, W. & Haferlach, T. (2009b) Detection of JAK2 exon 12 mutations in 15 patients with JAK2 V617F negative polycythemia vera. *Haematologica*, **94**, 414–418.

Scott, L.M., Tong, W., Levine, R.L., Scott, M.A., Beer, P.A., Stratton, M.R., Futreal, P.A., Erber, W.N., McMullin, M.F., Harrison, C.N., Warren, A.J., Gilliland, D.G., Lodish, H.F. & Green, A.R. (2007a) JAK2 exon 12 mutations in polycythemia vera and idiopathic erythrocytosis. *New England Journal of Medicine*, **356**, 459–468.

Scott, L.M., Beer, P.A., Bench, A.J., Erber, W.N. & Green, A.R. (2007b) Prevalance of JAK2 V617F and exon 12 mutations in polycythaemia vera. *British Journal of Haematology*, **139**, 511–512.

Sidon, P., El Housni, H., Dessars, B. & Heimann, P. (2006) The JAK2 V617F mutation is detectable at very low level in peripheral blood of healthy donors. *Leukemia*, **20**, 1622.

Skoda, R.C. (2010) Hereditary myeloproliferative disorders. *Haematologica*, **95**, 6–8.

Stevenson, W.S., Hoyt, R., Bell, A., Guipponi, M., Juneja, S., Grigg, A.P., Curtis, D.J., Scott, H.S., Szer, J., Alexander, W.S., Tuckfield, A. & Roberts, A.W. (2006) Genetic heterogeneity of granulocytes for the JAK2 V617F mutation in essential thrombocythaemia: implications for mutation detection in peripheral blood. *Pathology*, **38**, 336–342.

Swerdlow, S.H., Campo, E., Harris, N.L., Jaffe, E.S., Pileri, S.A., Stein, H., Thiele, J. & Vardiman, J.W. (2008) WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues. IARC Press, Lyon, France.

Szpurka, H., Tiu, R., Murugesan, G., Aboudola, S., Hsi, E.D., Theil, K.S., Sekeres, M.A. & Maciejewski, J.P. (2006) Refractory anemia with ringed sideroblasts associated with marked thrombocytosis (RARS-T), another myeloproliferative con-

© 2012 Blackwell Publishing Ltd

dition characterized by JAK2 V617F mutation. *Blood*, **108**, 2173–2181.

Tan, A.Y., Westerman, D.A. & Dobrovic, A. (2007) A simple, rapid, and sensitive method for the detection of the JAK2 V617F mutation. *American Journal of Clinical Pathology*, **127**, 977–981.

Tefferi, A. & Vainchenker, W. (2011) Myeloproliferative neoplasms: molecular pathophysiology, essential clinical understanding, and treatment strategies. *Journal of Clinical Oncology*, **29**, 573–582.

Tefferi, A., Noel, P. & Hanson, C.A. (2011) Uses and abuses of *JAK2* and *MPL* mutation tests in myeloproliferative neoplasms. *The Journal of Molecular Diagnostics*, **13**, 461–466.

Ugo, V., Tondeur, S., Menot, M.L., Bonnin, N., Le Gac, G., Tonetti, C., Mansat-De Mas, V., Lecucq, L., Kiladjian, J.J., Chomienne, C., Dosquet, C., Parquet, N., Darnige, L., Porneuf, M., Escoffre-Barbe, M., Giraudier, S., Delabesse, E. & Cassinat, B. (2010) Interlaboratory development and validation of a HRM method applied to the detection of JAK2 exon 12 mutations in polycythemia vera patients. *PLoS ONE*, **5**, e8893.

Wang, Y.L., Vandris, K., Jones, A., Cross, N.C., Christos, P., Adriano, F. & Silver, R.T. (2008) JAK2 mutations are present in all cases of polycythemia vera. *Leukemia*, **22**, 1289.

Warshawsky, I., Mularo, F., Hren, C. & Jakubowski, M. (2010) Failure of the Ipsogen MutaScreen kit to detect the JAK2 617V>F mutation in samples with additional rare exon 14 mutations: implications for clinical testing and report of a novel 618C>F mutation in addition to 617V>F. *Blood*, **115**, 3175–3176.

Williams, D.M., Kim, A.H., Rogers, O., Spivak, J.L. & Moliterno, A.R. (2007) Phenotypic variations and new mutations in JAK2 V617F–negative polycythemia vera, erythrocytosis, and idiopathic myelofibrosis. *Experimental hematology*, **35**, 1641–1646.

Wong, C.L., Ma, E.S., Wang, C.L., Lam, H.Y. & Ma, S.Y. (2007) JAK2 V617F due to a novel TG-CT mutation at nucleotides 1848–1849: diagnostic implication. *Leukemia*, **21**, 1344–1346.

Wu, Z., Yuan, H., Zhang, X., Liu, W., Xu, J., Zhang, W. & Guan, M. (2011) Development and inter-laboratory validation of unlabeled probe melting curve analysis for detection of JAK2 V617F mutation in polycythemia vera. *PLoS ONE*, **6**, e26534.

Xu, X., Zhang, Q., Luo, J., Xing, S., Li, Q., Krantz, S.B., Fu, X. & Zhao, Z.J. (2007) JAK2 (V617F): prevalence in a large Chinese hospital population. *Blood*, **109**, 339–342.

Yokota, M., Tatsumi, N., Nathalang, O., Yamada, T. & Tsuda, I. (1999) Effects of heparin on polymerase chain reaction for blood white cells. *Journal of Clinical Laboratory Analysis*, **13**, 133–140.

© 2012 Blackwell Publishing Ltd