

**The use of genetic tests to diagnose and manage patients with
myeloproliferative and myeloproliferative/myelodysplastic neoplasms, and
related disorders**

A British Society for Haematology Good Practice Paper

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Methodology

This Good Practice Paper was compiled according to the BSH process at [<https://bsh.org.uk/media/16732/bsh-guidance-development-process-dec-5-18.pdf>]. The British Society for Haematology (BSH) produces Good Practice Papers to recommend good practice in areas where there is a limited evidence base but for which a degree of consensus or uniformity is likely to be beneficial to patient care. The Grading of Recommendations Assessment, Development and Evaluation (GRADE) nomenclature was used to evaluate levels of evidence and to assess the strength of recommendations. The GRADE criteria can be found at <http://www.gradeworkinggroup.org>.

Literature review details

Pubmed was searched from Jan 2018 – September 2020 using the terms (myeloproliferative OR polycythemia OR thrombocythemia OR myelofibrosis OR eosinophilia OR mastocytosis OR neutrophilia OR myelomonocytic OR eosinophilic CEL OR CNL or CMML or JMML) AND (mutation OR variant) AND (diagnosis OR prognosis). Summary information from the 1063 hits was manually reviewed to identify 135 relevant publications. Relevant studies prior to January 2018 were identified from reviews published during the literature search period.

Review of the manuscript

Review of the manuscript was performed by the BSH Guidelines Committee General Haematology Task Force, the BSH Guidelines Committee and the General Haematology sounding board of BSH. It was also on the members section of the BSH website for comment. It has also been reviewed by members of the National

Cancer Research Institute (NCRI) MPN subgroup, the Chair of the NCRI MDS subgroup and lead scientists from the Genomics Laboratory Hubs in England and representative genetic testing laboratories in Wales, Scotland and Northern Ireland; these organisations do not necessarily approve or endorse the contents.

Introduction

Genetics and genomics are playing an increasingly important role in the diagnosis and management of patients with haematological neoplasms. Next generation sequencing (NGS) panels are widely available and initiatives such as the National Genomic Test Directory (NGTD; www.england.nhs.uk/publication/national-genomic-test-directories) in England along with parallel developments in the devolved nations aim to facilitate a standardised approach to testing and provide equity of access. A key component of this approach is the definition of eligibility criteria for specific tests to ensure appropriate usage from both clinical and financial perspectives.

This good practice paper focuses on the use of genetic and genomic tests for adult chronic myeloid neoplasms as defined by the World Health Organization (1), including myeloproliferative neoplasms (MPN), myelodysplastic/myeloproliferative neoplasms (MDS/MPN), myeloid/lymphoid neoplasms with eosinophilia and rearrangement of *PDGFRA*, *PDGFRB*, or *FGFR1*, or with *PCM1-JAK2* (MLN-eo) and mastocytosis. We have not included chronic myeloid leukaemia (CML) as this has been covered recently elsewhere (2), as has the full spectrum of clinical and laboratory investigations for patients with abnormal blood counts and/or suspected myeloid neoplasia (1, 3-9).

Classical *BCR-ABL* 1-negative myeloproliferative neoplasms

Screening investigations for erythrocytosis, thrombocytosis, suspected

myelofibrosis and atypical thrombosis: Molecular screening investigations for the common MPN phenotype driver mutations (*JAK2*, *CALR*, *MPL*), usually performed on peripheral blood DNA, are shown in Table 1. These assays will identify a mutation in almost all patients with polycythaemia vera (PV) and 85–90% with essential thrombocythaemia (ET) and primary myelofibrosis (PMF). Single-target assays may be employed sequentially but multiplex assays, typically using NGS, sequence several targets in parallel and are more cost effective. Either approach is acceptable if laboratory turnaround times and assay sensitivity (10) are satisfactory (e.g. detection of 1–3% variant allele frequency (VAF) or lower for *JAK2* c.1849G>T (p.Val617Phe), usually referred to as *JAK2* V617F, and 5% VAF for *JAK2* exon 12, *CALR* exon 9 or *MPL* exon 10 variants). The use of broad myeloid NGS panels to screen cases with suspected MPN is unlikely to be cost effective, but if larger panels are used we recommend that the initial analysis and report should be limited to common MPN driver mutations (Table 1).

Universal reporting of mutant allele burden on diagnostic samples is not essential, although this should be considered where prognostically useful, e.g. suspected progression of PV to post-PV myelofibrosis (MF) (11), or where demonstration of molecular response will be relevant (see section 1.3). Low allele burden results (e.g. <1% *JAK2* V617F) should be reported as such, since the clinical significance may be less certain given the prevalence of low level *JAK2* V617F in the general population (see below). In patients with low level *JAK2* V617F and MPN phenotype, screening

for *CALR* and *MPL* mutations should be carried out as these mutations may coexist (12). *JAK2* V617F and *CALR* mutations may also coexist with *BCR-ABL1*, with such cases usually being identified following the persistence of thrombocytosis or other MPN features despite achievement of a good molecular response to tyrosine kinase inhibitor therapy for CML (13, 14). Specific *CALR* mutations (type 1, 52bp deletion; type 2, 5bp insertion; type 1-like and type 2-like) (15) have prognostic significance in PMF (Table 2) and should be reported routinely.

Clinical context must be considered prior to performing screening assays. In patients with erythrocytosis or thrombocytosis, molecular screening investigations (Table 1) are recommended in those with persistently and significantly elevated counts (haematocrit >0.52 l/l in males or >0.48 l/l in females; platelet count $\geq 450 \times 10^9/l$) (3, 4), after exclusion of secondary causes or where abnormalities are out of keeping with any possible secondary cause. Exclusion of *BCR-ABL1* is important for all patients with thrombocytosis lacking a *JAK2*, *CALR* or *MPL* mutation or with atypical features (e.g. basophilia, left-shifted granulocytes, small hypolobated megakaryocytes). *JAK2* V617F is also found in healthy individuals, at increasing prevalence with older age ("clonal haematopoiesis", CH) (16-20). Although CH is associated with increased risk of developing cardiovascular disease (21), there is no prospective evidence to guide management of most patients with normal or near-normal blood counts who harbour *JAK2* V617F but do not fulfil diagnostic criteria for MPN, even if there are also abnormalities on bone marrow histology. The *JAK2* 46/1 haplotype, and common polymorphisms in *TERT* and other genes only confer a weak predisposition to MPN and therefore there is no clinical value in screening for these in routine practice (22, 23).

In patients with normal blood counts and atypical thrombosis, molecular screening investigations are recommended where a positive result will inform aetiology and assist management. *JAK2* V617F is particularly associated with splanchnic vein thrombosis, whilst *CALR* mutations are uncommon, especially with normal blood counts. Both mutation types have been detected infrequently in patients with cerebral vein thrombosis without an MPN (*JAK2* more frequently than *CALR*), and in all of these settings there is a lack of evidence-based management guidelines (24-29). In patients with normal blood counts and other atypical sites of thrombosis, there is currently inadequate evidence to recommend molecular screening investigations since the significance of a positive result and consequences for management recommendations are uncertain. However, in patients with arterial or unprovoked venous thrombosis who have a mildly or variably elevated haematocrit or platelet count, not reaching the criteria above, screening may be considered to inform possible aetiology and to prompt close blood count surveillance if cytoreduction is not commenced immediately.

- **Molecular screening for *JAK2*, *CALR* and *MPL* variants as appropriate (Table 1) is recommended in patients with persistent erythrocytosis or thrombocytosis (GRADE 1B)**
- **Screening for *JAK2* V617F is recommended in cases with normal blood counts and unexplained splanchnic vein thrombosis (GRADE 1B) and may be considered in selected patients with unexplained cerebral vein thrombosis (GRADE 2C)**

- **Screening for *CALR* variants may be considered in patients with splanchnic vein thrombosis or cerebral vein thrombosis (GRADE 2C)**
- **Screening for *JAK2*, *CALR* and *MPL* variants should be considered for patients with arterial or unprovoked venous thrombosis who have a mildly or variably elevated haematocrit or platelet count that persists for 2–3 months (GRADE 2C)**
- ***BCR-ABL1* should be excluded in cases with persistent thrombocytosis negative for *JAK2*, *CALR* and *MPL* variants or with atypical features (GRADE 1B)**

Testing for additional somatic driver variants with myeloid gene small variant “panels” +/- cytogenetic analysis

Additional somatic mutations in cancer driver genes include small variants (single nucleotide substitutions or small insertions/deletions) in *TET2* (10-15% MPN), *ASXL1* (5-10%) and *DNMT3A* (5-10%) (30-32), all of which are also associated with CH (16-19). Mutations are found at lower prevalence in regulators of splicing (*SRSF2*, *SF3B1*, *U2AF1*, *ZRSR2*) and of chromatin structure, epigenetic functions and cellular signalling (e.g. *EZH2*, *IDH1*, *IDH2*, *CBL*, *KRAS*, *NRAS*, *STAG2*, *TP53*) (32). Frequencies are often higher in PMF, post-PV or post-ET MF, and/or blast phase of other MPN or MDS/MPN.

The improved cost effectiveness of NGS technologies now permits widespread testing for panels of such “myeloid gene” variants which, as a minimum for MPN, should include the genes listed under M85.2 in the NGTD 9the current version can be found at <https://www.england.nhs.uk/publication/national-genomic-test->

directories/). There is a general consensus that reporting abnormalities down to 5% variant allele frequency (VAF) is adequate for routine analysis, but standardised interpretation of panel results needs further development. For all myeloid neoplasms panel analysis can be performed with DNA extracted from peripheral blood, but DNA extracted from bone marrow is preferred if available. Running and reporting panels is relatively expensive, and in older populations can also identify incidental CH. Use of a panel for all MPN patients is therefore currently neither necessary nor easily deliverable, but panels can add useful supplementary information in specific situations, as detailed below.

Cytogenetic abnormalities are most often found in PMF or post-PV/post-ET MF, in which an abnormal karyotype is reported in up to 45% of patients (33, 34). Conventional karyotyping identifies the commoner copy number abnormalities and deletions (e.g. 20q-, 13q-, +8, +9, 1q+, -7/7q-) and less common balanced translocations (e.g. t(1;6)) (35), and has been incorporated into several prognostic scoring systems (36-38). Other genome-wide technologies such as large pan-cancer NGS panels and SNP (single nucleotide polymorphism) arrays identify the common copy number losses and gains with greater resolution than conventional cytogenetics but will not identify balanced translocations. However these assays may also detect regions of copy-number neutral loss of heterozygosity (LOH) that are not identified by conventional karyotyping but are included in some prognostic models (32). An abnormal karyotype is reported at diagnosis in 5–10% of patients with ET and ~15% with PV (39-41), and although such findings may have some prognostic significance, first line management is not generally altered as a result.

a) At presentation of a suspected MPN, with negative screening investigations

i) Erythrocytosis. Patients with unexplained erythrocytosis who lack *JAK2* V617F may be considered for a bone marrow biopsy and *JAK2* exon 12 mutation screening; most are diagnosed with “idiopathic” erythrocytosis if there is no apparent secondary cause (3). The rare entity of *JAK2*-unmutated PV is still recognised in patients with other myeloproliferative clinicopathological features and marrow histology (3) but its molecular aetiology is mostly undefined. A very small number of *JAK2*-unmutated cases with clonal erythrocytosis due to somatic mutations in the *SH2B3* gene have been reported, although the phenotype was of idiopathic erythrocytosis with suppressed erythropoietin rather than classic PV (42) and optimal management of such cases is unknown. There is currently insufficient evidence to recommend myeloid gene panel testing or cytogenetic analysis in the great majority of cases with *JAK2*-unmutated erythrocytosis. Testing may be considered in rare patients with true *JAK2*-unmutated PV, although there is no evidence to guide such practice. Other patients with *JAK2*-unmutated erythrocytosis may be considered for testing for congenital causes of erythrocytosis, discussed elsewhere (3).

ii) Thrombocytosis or suspected PMF. In the 10-15% of patients with ET and PMF who lack mutations in *JAK2*, *CALR* or *MPL*, the finding of an additional driver mutation in a myeloid gene panel can support the diagnosis of a clonal disorder, with the proviso that incidental CH could be found in older individuals. The likelihood of identifying a mutation in such patients depends on age, clinical presentation, and gene panel content. More than half of patients with “triple-negative” PMF do harbour additional mutations when screened with comprehensive genomic assays (32) and approximately a third have an abnormal karyotype (35). In patients with bone marrow

histology and clinical features consistent with PMF, myeloid gene panel testing in combination with conventional karyotyping (or SNP array) is recommended.

The diagnosis of triple-negative ET is made on bone marrow histology, although distinction from reactive causes can be challenging, especially in those with mild thrombocytosis. A small minority harbour a non-canonical mutation in *JAK2* or *MPL*, or in another driver gene (32, 43, 44). However in a large analysis of recurrent genomic abnormalities in myeloid neoplasms, no mutations or chromosomal abnormalities were found in over 80% of patients with “triple-negative” ET, including all those aged under 39 years (32). It remains possible that at least a subset of these patients may not have a clonal disorder (45). In older patients there is a higher likelihood of finding a driver mutation (or occasionally a chromosomal copy number abnormality or LOH, e.g. chromosome 20); however, the risk of incidental CH also increases. Other differential diagnoses including MDS/MPN should be considered in triple-negative patients with other “myeloid” mutations, through correlation with blood counts and marrow appearances.

In patients with thrombocytosis who test negative for MPN phenotype driver mutations, there is insufficient evidence to support unselected myeloid gene panel testing. Bone marrow histology remains the key investigation to confirm a diagnosis of MPN in such cases. Moreover in young patients with confirmed low-risk ET, there is no evidence to support cytoreduction (46) and low-dose aspirin therapy has a very limited evidence base (47), meaning that most patients can be managed expectantly. However myeloid gene panel testing and cytogenetic analysis or other techniques for

copy number abnormalities may be considered to look for a clonal marker in some situations:

- Younger patients (e.g. under 60 years) with bone marrow histology typical of ET (or MPN-U or suspected prefibrotic MF) where confirmation of a clonal disorder would be useful in view of the patient's likely long-term disease course and ideally where a broad panel that covers non-canonical variants in *JAK2* and *MPL* and a range of other driver genes is available.
- Patients with significant thrombocytosis (e.g. platelet count $>600 \times 10^9/l$), no reactive cause and borderline bone marrow histology, where cytoreduction would be indicated if there was convincing evidence of a clonal disorder. Examples would include those with an unexplained thrombotic event, particularly younger patients. For older patients without thrombosis, testing may be considered but results must be interpreted with caution in view of the possibility of incidental CH.

Testing is not indicated in patients with normal or reactive bone marrow histology. A myeloid gene panel and cytogenetic analysis is also indicated in patients with bone marrow features suggestive of MDS or MDS/MPN.

- **A myeloid gene panel and cytogenetic analysis (or equivalent) is recommended for patients with bone marrow histology and clinical features consistent with PMF (+/- suggestive features of MDS or MDS/MPN) who test negative for *JAK2/CALR/MPL* (GRADE 1B).**
- **A myeloid gene panel and cytogenetic analysis (or equivalent) is not recommended for most patients with *JAK2/CALR/MPL*-negative**

erythrocytosis or thrombocytosis but may be considered in individual cases (GRADE 2C).

b) Patients with a known *JAK2*, *CALR* or *MPL* mutation

In patients with a confirmed clonal disorder, a myeloid gene panel and/or cytogenetic analysis can add information about diagnosis or prognosis at presentation or at suspected transformation; in future it may add information about options for targeted therapy.

Supplementary molecular information may allow definition of an alternative diagnosis associated with *JAK2* V617F such as MDS/MPN. In patients with a clinical presentation suggestive of an MPN and a *JAK2*, *CALR* or *MPL* mutation, but with additional cytopenias(s) at diagnosis and unexplained ring sideroblasts or other morphological dysplasia, or with significant peripheral blood monocytosis (monocytes $\geq 1 \times 10^9/l$), myeloid gene panel testing and cytogenetic analysis are recommended. The finding of other driver mutations may either support an alternative diagnosis (e.g. *SF3B1* mutation in MDS/MPN with ring sideroblasts and thrombocytosis) or provide supportive information where the differential diagnosis is challenging (e.g. MPN with monocytosis vs CMML).

For patients with ET and PV who develop cytopenias during cytoreductive therapy, marrow assessment may show morphological dysplasia with a differential diagnosis of disease progression vs therapy-related morphological changes. In this context myeloid gene panel testing and cytogenetic analysis may be considered. However, the finding of additional driver mutations is not evidence of disease progression *per*

se in the absence of baseline molecular information. The number and nature of such variants must be considered in conjunction with a detailed clinical and drug history.

The presence of additional somatic driver mutations carries prognostic significance in PMF, PV and ET (37, 39, 48). Prognostic scoring models can incorporate molecular information in a variety of ways, from single gene information to comprehensive genomic and cytogenetic / copy number profiles (48). Several high molecular risk (HMR) genes are recognised, (Table 2), but mutations in other genes (e.g. *TP53*) are also important prognostic indicators (49). Myeloid gene panel testing and conventional karyotyping are of most utility, and are recommended, in patients with PMF or post-PV/post-ET MF who are candidates for allogeneic stem cell transplantation in whom decisions can be informed by accurate prognostic information (50). In all other patients, testing may be considered for prognostic purposes if the additional genomic data will guide clinical management, for example:

- 1) Younger patients who at diagnosis fulfil BSH criteria for ET, PV or myeloproliferative neoplasm, unclassifiable (MPN-U) but have atypical clinical features that warrant additional closer surveillance, e.g. marked splenomegaly, atypical bone marrow histology (including those meeting WHO criteria for prefibrotic MF) (1).

- 2) Patients with MPN who are not candidates for allogeneic transplantation but in whom comprehensive prognostic information would aid clinical management and discussion with the patient.

- 3) Patients requiring testing as part of entry to a clinical trial.

Targeted therapies are now available for patients with acute myeloid leukaemia (AML) who harbour specific driver mutations such as in *IDH1/2* (51, 52). The latter are found in chronic-phase MPNs, but such therapies have not yet been tested in this setting. In patients with blast phase MPNs, myeloid panel testing is recommended for prognostic risk stratification (53-55) or if knowledge of driver mutations could support eligibility for a targeted therapy or entry to a clinical trial. Repeat testing during chronic phase is rarely helpful, although additional mutations may emerge at transformation (31).

- **Myeloid gene panel testing is recommended for MPN cases who test positive for *JAK2/CALR/MPL* mutations and have additional cytopenias(s) at diagnosis, unexplained ring sideroblasts or other dysplasia, increased blasts (including blastic transformation), peripheral blood monocytosis or atypical clinical features (GRADE 1B)**
- **Myeloid gene panel testing and conventional karyotyping are recommended for all patients with PMF, post-PV or post-ET MF who are candidates for allogeneic stem cell transplant (GRADE 1B)**
- **Myeloid gene panel testing should be considered for other patients if the additional genomic data will guide clinical management (GRADE 2C)**

Disease monitoring: quantitative assays of clonal burden

Quantitative assays of *JAK2* V617F mutant allele burden have been employed to assess clonal dynamics in clinical trials, with molecular responses being reported

with drugs including pegylated interferon-alfa and ruxolitinib (56-61). A small number of patients taking pegylated interferon-alfa were reported to maintain complete molecular remissions for over a year off therapy (57). These studies have not yet confirmed that achieving a particular level of molecular response is associated with more favourable vascular or transformation risk and molecular response is therefore not currently considered a formal treatment target. At present there is therefore no evidence to recommend routine quantitative monitoring of clonal burden. However assessment using quantitative, high-sensitivity assays (e.g. real time quantitative PCR or digital PCR) of mutant allele burden may be considered in patients who are in haematological response on low-dose pegylated interferon alfa, where a confirmed molecular remission would support a further dose reduction or trial without therapy. These assays can also be used in patients following post-allogeneic stem cell transplant to monitor for residual disease and guide early intervention with donor-lymphocyte infusion prior to clinical relapse (62, 63). Fluorescence *in situ* hybridisation (FISH) for any known cytogenetic abnormalities can also be helpful to monitor disease following therapy, albeit with more limited sensitivity than most quantitative PCR assays.

- **High-sensitivity assays of mutant allele burden are recommended following post-allogeneic stem cell transplant to monitor for residual disease (GRADE 1C).**
- **Quantitative assays of mutant allele burden are not recommended for most MPN patients but may be considered where demonstration of molecular response would influence clinical management (GRADE 2C).**

Atypical myeloproliferative neoplasms

CEL and MLN-eo

Patients with persistent eosinophilia of at least $1.5 \times 10^9/l$ with no obvious secondary cause should be investigated for *FIP1L1-PDGFR*A on peripheral blood or bone marrow by FISH or nested reverse transcriptase polymerase chain reaction (nested RT-PCR) (7, 64). Either technique alone may miss occasional cases (65, 66) and so both, or other supplementary approaches (65, 67), should be considered in cases with a high index of suspicion.

Almost all tyrosine kinase (TK) gene fusions apart from *FIP1L1-PDGFR*A are associated with visible cytogenetic rearrangements and therefore bone marrow (BM) cytogenetic analysis should ideally be performed for cases with a suspected myeloid neoplasm if *FIP1L1-PDGFR*A is not detected (7, 64). The diversity of fusions precludes effective targeted RT-PCR analysis, although an increasing number of cases are being picked up by broad or targeted RNAseq screens. Although effective, this approach is currently too expensive to recommend as a general screening tool in all but exceptional cases. Break-apart FISH analysis for specific loci (*PDGFR*A, *PDGFR*B, *FGFR*1, *JAK*2 for MLN-eo; *ABL*1, *FLT*3, *ETV*6, other TK genes for CEL) may also be used to identify disruption of key loci. It is important that any suspected fusion (including *FIP1L1-PDGFR*A) identified by cytogenetics or FISH is confirmed by molecular methods to ensure that targeted therapy is used appropriately and to facilitate subsequent molecular monitoring, which is available for *FIP1L1-PDGFR*A and most other fusions in specialist centres. The timing of tests should follow that

recommended for CML, including more frequent tests for patients attempting treatment-free remission (2, 68, 69). Mastocytosis should be considered if serum tryptase is elevated in the absence of a TK gene fusion and examination of bone marrow histology is essential in this context. If negative for the markers above, a myeloid panel or targeted analysis should be considered to detect other markers of clonality associated with eosinophilia (Table 3) (70-74).

- **Patients with persistent eosinophilia should be investigated initially for *FIP1L1-PDGFRA* by FISH and/or nested RT-PCR (GRADE 1B).**
- **Bone marrow cytogenetics or FISH is recommended to screen for other fusion genes, which must then be confirmed by molecular methods (GRADE 1B).**
- **Myeloid gene panel and *KIT* D816V testing should be considered for patients with persistent unexplained eosinophilia who test negative for fusion genes (GRADE 2B).**

CNL, MPN-U

CSF3R mutations are strongly, but not exclusively, associated with chronic neutrophilic leukaemia (CNL (75, 76) and are a central diagnostic feature of this disorder (1). Wider genomic profiling indicates a significant overlap in the pattern of mutated genes between CNL and MDS/MPN (77) suggestive of a disease continuum. *ASXL1* mutations were associated with an adverse prognosis in CNL in one study (78), but did not influence response to ruxolitinib (79).

MPN-U is an uncommon subtype consisting largely of cases that fail to meet the diagnostic criteria for a specific MPN subtype, or present with features that overlap with two or more subtypes. As such, most cases test positive for *JAK2* V617F, *CALR* or other myeloid driver mutations (80).

- **Testing for *CSF3R* variants, preferably as part of wider myeloid panel, is recommended for all patients with suspected CNL (Grade 2B)**

Mastocytosis

Up to 90% of adult systemic mastocytosis (SM) cases across all subtypes test positive for *KIT* c.2447A>T; p.(Asp816Val), usually referred to as *KIT* D816V. Due to the nature of the disease the VAF is often too low for detection by NGS and thus targeted, sensitive methods such as real time quantitative PCR or digital PCR are often required for analysis of peripheral blood or bone marrow samples.

Alternatively, standard mutation analysis may be performed on purified mast cells (81, 82). In many cases *KIT* D816V is detectable in peripheral blood (83) but, if negative, analysis of a BM sample should be considered if there is a high index of suspicion. If *KIT* D816V is not detected in the marrow and there is a strong clinical suspicion of mastocytosis, a wider screen for D816 variants or other *KIT* mutations should be considered. In children with mastocytosis, *KIT* D816V is only seen in 30–50% of cases and other activating *KIT* mutations account for most of the remaining cases (81, 84).

- **Sensitive testing for *KIT* D816V is recommended for all patients with a clinical suspicion of mastocytosis (GRADE 1B).**

- **If negative for *KIT* D816V, screening for other *KIT* mutations should be considered for adults (but is recommended for children) (GRADE 1B).**

Additional somatic mutations are found in 70–90% of advanced SM patients. Most mutation-positive cases have SM with an associated haematological neoplasm (SM-AHN), with the AHN usually being a subtype of MDS/MPN. Mutations are less frequent (<20%) in patients with indolent SM (ISM) (85, 86). In advanced SM, mutations in *SRSF2*, *ASXL1*, *RUNX1*, *EZH2* and *NRAS* have been associated with an adverse prognosis and thus molecular profiling is useful to guide transplant decisions (82, 86-88). In ISM, high VAF ($\geq 30\%$) mutations in *ASXL1*, *RUNX1* and/or *DNMT3A* have been associated with an adverse prognosis (86) but the value of routine molecular profiling in this subtype remains to be established.

An abnormal karyotype is seen in a quarter of SM-AHN cases but is infrequent in other subtypes. An abnormal karyotype, and particularly a poor-risk karyotype (e.g. monosomy 7, complex karyotype) is associated with an adverse prognosis but there is disagreement as to whether or not this effect is independent of mutational status (89, 90).

- **Myeloid panel analysis is recommended for patients with advanced SM who are candidates for allogeneic stem cell transplantation (GRADE 1B).**
- **Myeloid panel analysis may be considered for other SM patients if the apparent aggressiveness of the disease might influence options for therapy (GRADE 2B).**

- **Myeloid panel and/or bone marrow cytogenetics should be considered to characterise the AHN component of SM-AHN (GRADE 2B)**

Myelodysplastic/myeloproliferative neoplasms

The diagnosis of the adult MDS/MPN overlap syndromes - chronic myelomonocytic leukaemia (CMML), atypical CML *BCR-ABL1*-negative (aCML), MDS/MPN with ring sideroblasts and thrombocytosis (MDS/MPN-RS-T) and MDS/MPN-unclassifiable (MDS/MPN-U) – remain heavily reliant on bone marrow morphology and clinical assessment. Molecular genetics can however provide key information to assist with diagnosis, sub-classification and prognostication across the spectrum of these disorders.

Initial investigations in suspected MDS/MPN

In patients with a suspected MDS/MPN it is essential to exclude *BCR-ABL1* in all cases and also exclude rearrangements of *PDGFRA*, *PDGFRB*, *FGFR1* or *PCM1-JAK2* in the rare cases with an associated eosinophilia (1). Cytogenetics should also be performed at the time of a confirmed diagnosis (8) due to its importance for both demonstrating clonality and informing prognosis. This should also exclude any rare TK gene fusions which have been reported to mimic MDS/MPN (91). Cytogenetic abnormalities can be identified in 30–50% of cases using conventional karyotyping with the most common abnormalities being +8, +9, -7, del7q, del20q, del13q and isochromosome 17q (92, 93). The detection of +8, abnormalities of chromosome 7 or a complex karyotype have been reported as poor risk in CMML (94). In the absence of a cytogenetic sample or failed result, single nucleotide polymorphism array (SNP-array) analysis should be considered which could increase the yield of

detecting an abnormality to 75% (95). FISH, in particular for chromosomes 7 and 8, should be performed as a minimum requirement (8).

- ***BCR-ABL1* should be excluded in all cases of suspected MDS/MPN, and rearrangements associated with MLN-eo should be excluded in cases with eosinophilia (GRADE 1B).**

Testing for additional somatic driver mutations with myeloid gene “panels”

(i) In patients with indeterminate morphology

Somatic mutations are consistently reported to occur in >90% of cases across the MDS/MPN overlaps (96-99). The high frequency of somatic mutations in these conditions means the presence of a mutation can provide supportive evidence of clonality and assist in difficult diagnostic scenarios. Concerns have however been raised regarding the use of mutational analysis in this setting, due to reports of frequent somatic mutations in aging healthy individuals (16-19). A recent study however, in patients investigated for possible CMML, confirmed that even in the absence of definitive morphological features, those patients with a somatic mutation had a clinical phenotype and genotype indistinguishable from those with disease, and comparably poor outcomes (100). A myeloid gene panel is therefore recommended in difficult diagnostic cases and the presence of two mutations, one of which has a high VAF (>20%) would support a diagnosis (8). The genes included in the current NGTD for suspected MDS/MPN overlaps are listed in Table 4 (see also NGTD test code M224.1) and the minimum genes recommended for the investigation of patients with suspected CMML in Table 5. It is accepted that the genes included in the NGTD panel are a minimum requirement and larger panels

may provide additional information, e.g. abnormalities of *NPM1* are uncommon in MDS/MPN but identify cases likely to transform rapidly to AML, whereas abnormalities of *FLT3* are potential therapeutic targets (8).

(ii) *In patients with a confirmed diagnosis of MDS/MPN.* The genes most commonly mutated in MDS/MPN are not specific for these conditions; however genotypic/phenotypic correlations have been identified which can assist in sub-classification. Mutations in genes with prognostic relevance can also be identified along with possible targets for therapy (*JAK2*, *IDH1/2*) with the latter likely to increase over time.

With respect to CMML, *SRSF2*, *TET2* and *ASXL1* are by far the most commonly mutated genes (96-98) and the combination of mutation in *TET2* and either *SRSF2* or *ZRSR2* is highly specific for a myelomonocytic phenotype (101). A diagnosis of aCML is supported by the presence of mutations in *SETBP1* and/or *ETNK1* which are reported in ~25–38% and ~10% of cases respectively (102-105). These genes are mutated less frequently in CMML and MDS/MPN-U although *SETBP1* is also mutated in CNL (102, 104). Patients with aCML also show a relative lack of MPN-phenotype driver mutations (*JAK2*, *CALR*, *MPL*) (92, 106) with the presence of these tending to exclude this diagnosis (1). In MDS/MPN-RS-T, mutations in *SF3B1* and *JAK2* are reported in up to 90% and 57% of cases, with *CALR* or *MPL* mutations in a small minority (107-109) and the detection of an *SF3B1* mutation in patients with 15% ring sideroblasts can help define the diagnosis (1). Co-mutation of these genes would strongly support a diagnosis of MDS/MPN-RS-T though is not a current requirement (1). Elevated tryptase and/or mast cell abnormalities in MDS/MPN

suggests SM-AHN, which is often underdiagnosed but may be supported by the finding of *KIT* D816V (110). The detection of *KIT* D816V in the context of a confirmed MDS/MPN should trigger review of bone marrow morphology for a possible co-existing mastocytosis.

Mutational analysis is now incorporated into prognostic scoring systems across these diseases. Four genes (*ASXL1*, *NRAS*, *RUNX1* and *SETBP1*) are independently associated with a worse overall survival (OS) in CMML and have been incorporated into the most recent CMML-specific prognostic scoring system (CPSS)-molecular and analysis of these is defined as mandatory for risk assessment (8). The number of mutations per patient has also been shown to correlate inversely with OS (96), and *ASXL1* and/or *NRAS* mutations are associated with worse survival after stem cell transplantation (111). *ASXL1* and *SETBP1* also infer a poor prognosis across other MDS/MPN with these genes being commonly co-mutated (99, 102, 104, 112). In atypical CML, *SETBP1* was associated with an adverse clinical phenotype and a significantly worse OS (102, 104) while both *SETBP1* and *ASXL1* were associated with poor survival in patients with MDS/MPN-RS-T and have been incorporated into a mutation enhanced prognostic model (112).

A targeted sequencing panel is therefore recommended in patients diagnosed with an MDS/MPN overlap disorder, particularly those being considered for active treatment or allogeneic transplantation (8, 93). Mutational analysis can also provide prognostic information and potentially identify therapeutic targets in patients not eligible for intensive treatment and analysis may therefore be considered even in those receiving supportive care (8, 93). There is strong concordance between

mutations detected in the peripheral blood and bone marrow, particularly in CMML, and mutational analysis has both a high positive and a high negative predictive value for a subsequent diagnosis (100). Mutational analysis is therefore a potential option in elderly patients or those unfit for a bone marrow biopsy to either confirm the presence of a clonal marker or eliminate the need for invasive testing.

- **Myeloid gene panel analysis and bone marrow cytogenetics or SNP array is recommended for patients diagnosed with MDS/MPN and for cases with suspected MDS/MPN but with indeterminate morphology (GRADE 1B).**

Future directions

The landscape of genetic and genomic testing is changing rapidly, with broad screening techniques such as large pan-cancer panels, whole genome sequencing and RNAseq beginning to impact on routine practice. Genomic, transcriptomic and epigenetic profiling of single cells are providing novel insights into the complexity and diversity of clonal disorders. Whilst these approaches clearly have huge potential, e.g. in chronic myeloid neoplasms, they will facilitate comprehensive prognostic modelling (48), detection of rare targetable gene fusions (113) and potentially cell type-specific assessment of measurable residual disease (114), it is currently unclear when or whether they will be cost effective compared to more diverse, targeted approaches.

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Review Process

Members of the writing group will inform the writing group Chair if any new pertinent evidence becomes available that would alter the strength of the recommendations made in this document or render it obsolete. The document will be archived and removed from the BSH current guidelines website if it becomes obsolete. If new recommendations are made an addendum will be published on the BSH guidelines website (www.b-s-h.org.uk).

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Table 1. Peripheral blood screening targets in suspected MPN

Presentation	Variant	Frequency	Reference
Erythrocytosis	<i>JAK2</i> V617F	96–97% PV	(115, 116)
	<i>JAK2</i> exon 12 mutations*	~3% PV	(116)
Thrombocytosis	<i>JAK2</i> V617F	50–60% ET	(117, 118)
	<i>CALR</i> exon 9 mutation	25–30% ET	(30, 119)
	<i>MPL</i> exon 10 mutation	3–11% ET	(120, 121)
	<i>BCR-ABL1</i> fusion	To exclude CML	
Suspected primary myelofibrosis	<i>JAK2</i> V617F	50–60% PMF	(119, 122)
	<i>CALR</i> exon 9 mutation	15–35% PMF	(30, 119)
	<i>MPL</i> exon 10 mutation	6–9% PMF	(119, 121)
Suspected chronic myeloid leukaemia	<i>BCR-ABL1</i> fusion	100% CML	

* Rare cases with a discrepancy between *JAK2* exon 12 mutant allele burden in bone marrow and peripheral blood have been reported, so testing of bone marrow may be considered if there is a high index of suspicion (123).

Table 2. Prognostic scoring systems for PMF, post-PV and/or post-ET MF incorporating cytogenetic and/or molecular information

Score	Disorder	Cytogenetic / molecular variable(s) included	HR	Reference
DIPSS+	PMF	Unfavourable karyotype*	2.4	(36)
MYSEC-PM	Post-PV/post-ET MF	<i>CALR</i> -unmutated	2.6	(124)
MIPSS70	PMF	Absence of <i>CALR</i> type 1/type 1-like mutation At least 1 HMR [†] mutation 2 or more HMR [†] mutations	1.89 1.77 3.95	(125)
MIPSS70+	PMF	Absence of <i>CALR</i> type 1/type 1-like mutation At least 1 HMR [†] mutation 2 or more HMR [†] mutations Unfavourable karyotype**	2.4 1.8 2.4 3.1	(125)
GIPSS	PMF	Very high risk karyotype*** Unfavourable karyotype*** Absence of <i>CALR</i> type 1/type 1-like mutation <i>ASXL1</i> mutation <i>SRSF2</i> mutation <i>U2AF1</i> mutation	3.1 2.1 2.1 1.8 2.4 2.4	(38)
MIPSS70+ v2	PMF	Very high risk karyotype*** Unfavourable karyotype*** 2 or more HMR mutations [†] 1 HMR mutation [†] Absence of type 1/type 1-like <i>CALR</i> mutation	5.9 2.5 2.6 1.8 2.1	(37)
Sanger multistage model	MPN	Up to 53 genomic features (single gene variant / copy number information)		(32)
MTSS	PMF/post-PV/post-ET MF	Absence of <i>CALR</i> or <i>MPL</i> mutation <i>ASXL1</i> mutation	2.4 1.42	(126)
FIM	PMF/post-PV/post-ET MF	<i>TP53</i> High risk mutations**** <i>ASXL1</i> only	8.68 3.24 2.45	(127)

[†] HMR = high molecular risk (*ASXL1*, *IDH1/2*, *EZH2*, *SRSF2*)

‡ HMR = high molecular risk (*ASXL1*, *IDH1/2*, *EZH2*, *SRSF2*, *U2AF1* Q157)

*Unfavourable: Complex karyotype or sole or two abnormalities that include +8, -7/7q-, i(17q), -5/5q-, 12p-, inv(3) or 11q23 rearrangement.

**Unfavourable: Any abnormal karyotype other than sole abnormalities of 20q-, 13q-, +9, chromosome 1 translocation/duplication, -Y or sex chromosome abnormality other than -Y

***Very high risk: single/multiple abnormalities of -7, i(17q), inv(3)/3q21, 12p-/12p11.2, 11q-/11q23, or other autosomal trisomies not including +8/ +9 (e.g., +21, +19); Favourable: normal karyotype or sole abnormalities of 13q-, +9, 20q-, chromosome 1 translocation/duplication or sex chromosome abnormality including -Y; Unfavourable: all other abnormalities.

**** ≥1 mutation in *EZH2*, *CBL*, *U2AF1*, *SRSF2*, *IDH1*, *IDH2*, *NRAS* or *KRAS*. *ASXL1*-only mutations had no or limited prognostic value, however *ASXL1* mutations conferred a worse prognosis when associated with a mutation in *TP53* or high-risk genes.

Table 3. Molecular abnormalities associated with eosinophilia

Category	Genes	Frequency	Reference
MLN-eo	<i>FIP1L1-PDGFR</i>	5–20% HE _{US} ; >80% MLN-eo	(70, 128)
	<i>Other PDGFR fusions</i>	rare	
	<i>PDGFRB fusions</i>	<10% MLN-eo	
	<i>FGFR1 fusions</i>	<5% MLN-eo	
	<i>PCM1-JAK2, BCR-JAK2</i>	<5% MLN-eo	
Tyrosine kinase gene fusions in CEL and eosinophilia associated with other MPN or MDS/MPN	<i>ETV6-ABL1</i>	?1–2% HE _{US} /MPN-eo	
	<i>FLT3</i> fusions	rare	
	Other <i>JAK2</i> fusions	rare	
	<i>NTRK3, RET, ALK</i> , others	very rare	
Other variants in CEL and eosinophilia associated with other MPN, MDS/MPN or SM	<i>JAK2</i> V617F	4% HE _{US}	(70)
	<i>JAK2</i> exon 13 indels	1–2% HE _{US}	(74, 129)
	<i>KIT</i> D816V	3% HE _{US}	(70)
	<i>STAT5B</i> N642H	2% persistent eosinophilia including MPN-eo and MDS/MPN-eo	(73)
	<i>DNMT3A, TET2, ASXL1, EZH2, SETBP1, CBL</i> other myeloid genes	11-21% HES/HE _{US}	(71, 72, 129)

HE_{US}, hypereosinophilia of undermined significance; HES, idiopathic hypereosinophilic syndrome

Table 4. Common abnormalities in CNL and MDS/MPN

Pathway	Gene	aCML	CNL	CMML	MDS/MPN- RS-T	MDS/MPN- U
Signalling	<i>KRAS</i>	3-10%	-	7-23%	-	4-5%
	<i>NRAS</i>	10-30%	10%	4-38%	-	8-12%
	<i>JAK2</i>	4-11%	8%	1-11%	37-78%	8-25%
	<i>CBL</i>	8-15%	5%	8-23%	3%	7-8%
	<i>KIT</i>	6%	-	0-3%	-	4%
	<i>FLT3</i>	5-7%	-	1-4%	-	3-4%
	<i>CSF3R</i>	0-25%	60-80%	2-4%	-	4-6%
	<i>SETBP1</i>	7-38%	14-56%	4-18%	1-6%	13-16%
	<i>SH2B3</i>	0-4%	-	0-5%	-	3%
	<i>MPL</i>	-	-	rare	4-20%	8%
	<i>CALR</i>	-	-	rare	17%	4%
	<i>ETNK1</i>	3-9%	3%	3-4%	3%	4%
	<i>PTPN11</i>	0-8%	~0%	3-5%	-	4-5%
	<i>NF1</i>	0-4%	-	6-10%	-	4%
Splicing	<i>SF3B1</i>	0-6%	3%	3-10%	97%	11-16%
	<i>SRSF2</i>	37-48%	44%	24-55%	4%	24-48%
	<i>U2AF1</i>	3-15%	15%	2-24%	-	8-19%
	<i>ZRSR2</i>	3-4%	3%	3-8%	-	0-6%
	<i>RUNX1</i>	6-20%	3%	8-28%	1%	4-17%
Transcription	<i>CEBPA</i>	4%	-	0-20%	-	4-8%
	<i>GATA2</i>	15-18%	13%	1-14%	3%	12-16%
	<i>NPM1</i>	4%	-	1-3%	-	0-3%
	<i>BCOR</i>	4%	-	3-7%	-	-
	<i>CUX1</i>	10-11%	5%	0-6%	4%	0-8%
	<i>TP53</i>	3%	3%	0-2%	3%	0-14%
	<i>STAG2</i>	11-15%	3%	3%	-	8-16%
Cohesin	<i>DNMT3A</i>	4-7%	5%	2-12%	18%	0-13%
DNA methylation	<i>TET2</i>	16-37%	21%	29-73%	21%	30-44%
	<i>IDH1/2</i>	0-3%	3%	1-7%	3%	0-10%
Histone modification	<i>ASXL1</i>	28-92%	57-77%	32-69%	0-11%	53-64%
	<i>EZH2</i>	13-33%	21%	5-13%	7%	10-25%

Data from (8, 76-78, 92, 99, 100, 103, 105, 109) and references therein. A dash indicates a mutation in that gene is rare or has not been reported. The NGTD also includes *CHEK2*, *NFE2*, *IKZF1* and *HRAS* but the prevalence of mutations in these genes is unknown for CNL and MDS/MPN.

Table 5. Recommended* minimal panel for targeted sequencing in CMML

<i>TET2</i>	<i>IDH2</i>	<i>NF1</i>	<i>FLT3</i>
<i>ASXL1</i>	<i>BCOR</i>	<i>JAK2</i>	<i>SRSF2</i>
<i>DNMT3A</i>	<i>CBL</i>	<i>RUNX1</i>	<i>SF3B1</i>
<i>EZH2</i>	<i>KRAS</i>	<i>SETBP1</i>	<i>U2AF1</i>
<i>IDH1</i>	<i>NRAS</i>	<i>NPM1</i>	<i>ZRSR2</i>

* see (8)

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